Male Reproductive Function

edited by

CHRISTINA WANG

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Male Reproductive Function

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CHRISTINA WANG, M.D.

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Preface

The purpose of this book is to provide the general endocrinologist and internist with up-to-date information on the recent developments in the understanding of molecular and cellular events in the testis, and their applications in clinical medicine. Some chapters are relatively brief, but they provide the current state of the art in the practice of clinical andrology.

I wish to thank my colleagues throughout the world who responded to my requests with chapters in each of their respective fields of expertise. The patience and help of my assistant, Sally Avancena, M.A., is gratefully acknowledged.

Christina Wang, M.D.

1 ENDOCRINE REGULATION OF MALE REPRODUCTION

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INTRODUCTION

The two functions of the testis are androgen production and spermatogenesis. The key role in the regulation of these functions is played by the two pituitary gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), as supported by a vast number of experimental and clinical data. These facts have recently been corroborated by findings on patients with inactivating mutations of the gonadotropin or gonadotropin receptor genes, and by transgenic and knock-out mouse models. Besides gonadotropins, a number of other hormones contribute to testicular regulation, and there is a plethora of paracrine and autocrine regulatory effects between and within the different testicular cell compartments (see Chapter 3). However, the physiological role of these regulatory mechanisms, so far mostly demonstrated *in vitro*, has only recently started emerging.

This brief review will only describe the key endocrine functions of the hypothalamic-pituitary-testicular (HPT) axis. In addition, some less clearly characterized and contentious topics of testicular endocrine regulation are discussed. For more comprehensive reviews of male reproductive endocrinology, other texts are recommended (e.g. Sharpe, 1994; Saez, 1994; Huhtaniemi and Toppari, 1995; Jégou and Pineau, 1995; Weinbauer et al, 1997; Griffin and Wilson, 1998).).

THE HYPOTHALAMIC-PITUITARY-TESTICULAR (HPT) AXIS

Structure-function relationships, and principles of function

The HPT axis is a classical example of an endocrine regulatory circuit, with cascades of forward and feedback regulatory events at multiple functional levels (Fig. 1). The highest level is at the hypothalamus, where the cells of specific nuclei synthesize the decapeptide gonadotropin-releasing hormone (GnRH), which is the

positive stimulus for gonadotropin secretion from the anterior pituitary. The same GnRH peptide is apparently responsible for the release of both gonadotropins, although evidence for a separate FSH releasing hypothalamic principle also exists (Yu et al, 1997). The axon terminals of GnRH neurons make contact in median eminence with the hypophyseal portal vessels, which transport the releasing hormone, secreted in pulses of 1-2 h intervals, to the anterior pituitary gland (Hotchkiss and Knobil, 1996).

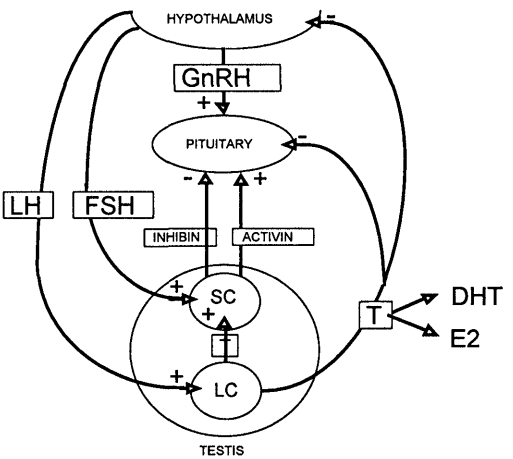

Fig. 1. The hypothalamic-pituitary-testicular axis. DHT, 5α-dihydrotestosterone; E2, estradiol; FSH, follicle-stimulating hormone; GnRH, gonadotropin-releasing hormone; LC, Leydig cell; LH, luteinizing hormone; SC, Sertoli cell; T, testosterone.

In pituitary gonadotroph cells, GnRH stimulates the synthesis and release of LH and FSH. The secretory peaks are more distinct with LH, due to its shorter circulatory half-life than that of FSH (Thorner et al, 1998). The target of LH action in the testis are the interstitial Leydig cells, whereas FSH regulates the Sertoli cells in seminiferous tubuli. Although gonadotropin receptors, especially for LH, have been detected in extragonadal tissues (Rao, 1996), convincing evidence for their function is still lacking. LH stimulates in mature Leydig cells their steroidogenesis, hence being responsible for the supply of testosterone (T) for the maintenance of spermatogenesis and for extragonadal androgen effects. FSH in principle maintains the functional capacity of Sertoli cells in the support of spermatogenesis.

The other part of the HPT axis is the negative feedback link of gonadal steroid and peptide hormones to the hypothalamic-pituitary levels, to maintain the functional balance of the regulation (Fig. 1)(Evans et al, 1996). Here T, at least partly after conversion to estradiol, suppresses GnRH secretion at the hypothalamic

level and gonadotropin synthesis in the pituitary gland. Although testicular steroids also have effects on FSH, the specific regulation of this hormone takes place at the pituitary level through the two Sertoli cell proteins, activin and inhibin, the former a stimulator, the latter an inhibitor of FSH secretion (Fig. 1).

Ontogeny of the HPT axis

The anatomical and functional maturation of the different levels of HPT axis take place in human during the first trimester of pregnancy. The hypothalamic GnRH neurons first appear in the nasal olfactory epithelium around 40 days of gestation (Schwanzel-Fukuda et al, 1996) from where they migrate backwards to their final locations in the hypothalamus. This process is disturbed in Kallmann's syndrome, a disease characterized by hypogonadotropic hypogonadism and anosmia. A mutation in the X-chromosomal gene of a cell adhesion molecule (KAL-X) prevents the normal development of olfactory bulbs and tracts, and prevents the migration of GnRH neurons backwards to the hypothalamus, thus explaining the molecular pathogenesis of the most common, X-linked form of this syndrome (Bouloux et al, 1992). Normally, GnRH neurons appear in the hypothalamus at week 8 of gestation, and vascular casts have demonstrated intact hypothalamo-hypophyseal portal system from week 11.5 onwards (reviewed by Huhtaniemi, 1996).

The gonadotroph cells appear in the anterior pituitary at the age of 8 weeks, and they first express the glycoprotein hormone common α-subunit gene (reviewed by Rabinovici and Jaffe 1990). The dimeric LH and FSH molecules can be detected around gestation week 10, and reach their maximum prenatal levels in the middle of gestation, declining thereafter towards term. As regards testicular differentiation, the Leydig cells appear on week 8 of gestation, and the first LH receptors have been detected on week 10. The Sertoli cell differentiation starts somewhat earlier, around weeks 6-7, and FSH receptors have been detected in fetal testis at 9-11 weeks of gestation (Huhtaniemi et al, 1987). When exactly the negative feedback regulation of fetal gonadotropin secretion starts is still open, because of confounding effect of the high placental steroid hormone production. They apparently bring about the suppression of fetal gonadotropin secretion during the second half of gestation.

The fetal testes produce two hormones, T and the anti-Müllerian hormone (AMH). T production starts as soon as the Leydig cells differentiate on week 8 of gestation (Huhtaniemi, 1996). The role of T in the fetal period is to stabilize the Wolffian ductal structures, thereby allowing the differentiation of the male internal genitalia. Conversion of T to 5α-dihydrotestosterone in the urogenital sinus and external genitalia is necessary for their differentiation. AMH is a Sertoli cell product, and its role is to induce Müllerian duct regression in the male.

In rodents, the fetal Leydig cell steroidogenesis starts independent of gonadotropins, since their secretion only starts after this period (El-Gehani et al. 1998). Human data are less clear, since the fetal circulation throughout gestation contains very high levels of choriongonadotropin (hCG), resembling LH in action. Since a male with inactivating mutation of the LHβ gene was normally

masculinized at birth, hCG apparently provides sufficient stimulus *in utero* (Weiss et al, 1992). In contrast, the near-complete lack of genital masculinization in males with inactivating LH receptor mutations indicates that stimulation of Leydig cells by LH is essential for male sexual differentiation (Latronico et al, 1996). After the peak around week 14 of gestation, fetal testicular steroidogenesis decreases, apparently due decreased hCG levels and the onset of negative feedback effects of placental steroids.

Gonadotropin secretion and testicular T production are temporarily activated during the first postnatal months (Forest et al, 1976). The physiological significance of this activity remains unclear, but it may represent an adaptational phenomenon to the rapid elimination of placental hormones at birth and to the postnatal increase in the proportion of T binding to sex-hormone binding globulin (SHBG)(Huhtaniemi et al, 1986).

The adult-type function of the HPT axis is established at puberty (see Chapter 5), and the male reproductive capacity matures soon thereafter. The endocrine regulation of the adult testis remains in principle unaltered for the rest of life. However, in reality there is a slow gradual decline of the HPT activity with advancing age, and some men eventually become hypogonadal. These aspects of testicular function are discussed in more detail in Chapter 7.

SYNTHESIS, SECRETION AND ACTION OF GnRH

GnRH is the key hypothalamic regulator of the HPT function (Fig. 1). The GnRH gene encodes a larger propeptide that is cleaved into a 24 amino acid signal peptide, the GnRH decapeptide and a 56 amino acid GnRH-associated peptide (GAP). GnRH and GAP are secreted in equimolar amounts from the GnRH neuron terminals in median eminence to the hypophyseal portal circulation. Some effects of GAP on gonadotropin and prolactin secretion have been demonstrated, but these findings are controversial, leaving the physiological role of this peptide still open. GnRH is distributed in the central nervous system in several locations, but those in the mediobasal hypothalamus and arcuate nucleus are most important for the regulation of gonadotropin secretion (Silverman et al, 1994; Hotchkiss and Knobil, 1996).

There is a multitude of neuronal connections from other brain areas to the GnRH neurons, and these inputs regulate the frequency and amplitude of the GnRH discharge. Excitatory signals are mediated by norepinephrine, neuropeptide Y, tachykinins, glutamic acid, nitric oxide (NO), transforming growth faction α and prostaglandin E2. The inhibitory signals include opioids, corticotrophin releasing hormone (CRH), vasopressin, γ aminobutyric acid (GABA) and inflammatory cytokines. These neurons also integrate the effects of various external signals, such as stress, metabolic and environmental influences into the modulation of the GnRH neuronal activity.

The secretion of GnRH occurs in pulses of varying amplitude, and in adult men at frequency of 8 to 14 per 24 h. The exact nature of the pulse generator responsible

for this type of GnRH release is not known, but it apparently represents an intrinsic functional features of the GnRH neurons or other structures of the mediobasal hypothalamus (Hotchkiss and Knobil, 1996). The pulse generator is under continuous tonic inhibition by peripheral steroids, which explains why after gonadectomy, both GnRH and gonadotropin secretion increase.

GnRH interacts in gonadotroph cells with a high-affinity receptor belonging to the G-protein associated seven-helix transmembrane receptor family (Chi et al, 1993). A specific structural feature of this receptor is the missing intracellular tail. After ligand binding, the GnRH-receptor complexes microaggregate and associate with the Gq protein, which results in increased production of inositol trisphosphate and increase of intracellular free calcium from intra- and extracellular sources (Conn, 1996). Both protein kinase C and Ca/calmodulin-associated protein kinases participate in the subsequent prosphorylation steps, resulting in acute gonadotropin release by exocytosis and more delayed increase in their synthesis. Pulsatile GnRH secretion is vital for the maintenance of GnRH responsiveness of gonadotropes, since tonic GnRH stimulation (e.g. during prolonged GnRH agonist treatment) results in down-regulation of GnRH receptors, blockade of the signal transduction, and suppression of gonadotropin synthesis and secretion.

In the male, the main hormone controlling GnRH secretion is T, but a part of these effects are due to its metabolite, estradiol. However, aromatization is not mandatory for the feedback action, since 5α-dihydrotestosterone is also effective. A part of the steroid feedback is directed to inhibition of gonadotropin synthesis at the pituitary level (Evans et al, 1997) The relative importance of the androgenic and estrogenic components of the steroid feedback, and whether they occur mainly at the hypothalamic or pituitary level, remain a matter of debate. The steroid effects on GnRH neurons are apparently indirect and mediated by inhibitory inputs from the neighboring neurons.

SYNTHESIS, SECRETION AND ACTION OF GONADOTROPINS

The two gonadotropins, LH and FSH, both with a molecular weight of about 30,000, belong together with the thyroid-stimulating hormone (TSH) and hCG to the family of glycoprotein hormones. LH and FSH are synthesized in gonadotroph cells of the anterior pituitary as heterodimers of the common α-subunit and specific β-subunit which confers the hormonal specificity.

Two N-linked carbohydrate side chains are coupled to the α-subunit, one to LHβ and two to FSHβ. The glycosylation occurs both co- and post-translationally, mainly in the rough endoplasmic reticulum and Golgi apparatus. The oligosaccharides contain a central branched mannose core, which is bound to an asparagine residue of the peptide chain through N-acetyl-glucosamine. The bi- or triantennary terminal extensions in LH are heavily sulfated (50%), in FSH mainly sialylated (Evans et al, 1996). This is the main reason for the longer half-life of FSH than LH in circulation, since a specific hepatic receptor for sulfated

glycoproteins accelerates the elimination of LH (Baenziger et al, 1992). There is considerable microheterogeneity in the carbohydrate moieties of gonadotropins. The different isoforms vary in bioactivity (Jaakkola et al, 1990), and their relative proportions are apparently hormonally regulated. However, the physiological significance of this phenomenon is still undetermined.

Both gonadotropins direct their actions to the specific gonadal target cells through G-protein-coupled 7-helix transmembrane receptors (Fig. 1), those of LH in Leydig cells, those of FSH in Sertoli cells.

Luteinizing hormone (LH)

A high intratesticular concentration of T is required for spermatogenesis. In the human it is about 100-fold higher than that of peripheral circulation (Hammond et al, 1977). LH is the key stimulus of Leydig cell steroidogenesis, which action is mediated through its binding to plasma membrane receptors (Segaloff and Ascoli, 1993). Cyclic (c) AMP is the main second messenger, but also other signal transduction systems are involved, i.e. intracellular free calcium and chloride, membrane phospholipids and prostaglandins (Leung and Steel, 1992). The steps after cAMP include activation of protein kinase A (PK-A) which then for instance catalyzes phosphorylation/activation of the enzymes involved in steroidogenesis (see below). Maximal steroidogenesis is evoked by very low levels of LH binding which hardly increases the cAMP production, and intracellular compartmentalization of cAMP may explain this. The involvement of other signal transduction mechanisms in LH action may provide channels for the numerous paracrine factors to modulate gonadotropin action. They may also be responsible for the pleomorphic LH effects on Leydig cells, including steroidogenesis, growth and differentiation.

The steroid response to LH stimulation is in principle very fast, but great species differences prevail in its magnitude. An injection of LH/hCG in the rodent evokes in less than an hour over 10-fold increase in serum T. In the human, in contrast, the same response is <50% and up to a 2-fold 2-4 days later (Huhtaniemi et al, 1983). The low response in man may be related to the nature of T transport in circulation. In the rodent, there is no specific circulatory androgen binding protein, whereas the majority of T in the human is bound to SHBG. This prolongs the half-life of T in circulation and buffers acute changes in its concentrations.

Hence, it is well established that LH stimulates testicular steroidogenesis and maintains the high intratesticular steroid concentration that is necessary for spermatogenesis. In contrast, it is not known how exactly the high intratesticular T regulates this process. Androgen receptors are present in Leydig cells, peritubular myoid cells and Sertoli cells, but apparently not in spermatogenic cells (Sharpe, 1994). The T effect on spermatogenesis must thus be mediated by testicular somatic cells. The primary evidence for this comes from studies of mouse chimeras in which sperm deficient of functional androgen receptor mature in the presence of Sertoli cells with functional androgen receptor (Lyons et al, 1975).

The contrasting views about the specific site of androgen actions within the testis are probably due to the fact that a number of hormones and paracrine factors are

needed for spermatogenesis, and none of them alone is sufficient. The synergistic and/or additive actions of all the regulators provide the physiological environment for spermatogenesis, and therefore elimination or supplementation experiments with single factors yield conflicting results. In addition, interactions between numerous testicular cell types are obviously needed.

Follicle-Stimulating hormone (FSH)

The same gonadotroph cells produce both gonadotropins, and only a minority of them are monohormonal. FSH secretion is also stimulated by GnRH, although its pulses are less distinct that those of LH, due to its longer half-time in circulation. Likewise, FSH exists in multiple isoforms with differing intrinsic bioactivities and circulatory half-lives, but still with unknown physiological significance. A wide body of evidence shows that Sertoli cells are the only target of FSH action in the testis.

The FSH receptor is structurally close to that of LH (Simoni et al, 1997). As with LH, the signal transduction of FSH occurs predominately via G_s-protein coupled activation of adenylyl cyclase, resulting in increased cAMP production and activation of PK-A. This enzyme phosphorylates specific functional and structural proteins, including the cAMP response element (CRE) binding proteins, CREBs, and their modulators (CREMs). These modulatory proteins are expressed in Sertoli and germ cells according to the hormonal status and functional stage, thereby influencing in oscillating fashion the cAMP responsive genes during the spermatogenic cycle (Montminy et al, 1990). CREB mRNA is expressed both in Sertoli cells and spermatogenic cells (Simoni et al, 1997). An activator isoforms of CREM ($CREM_t$) is specifically expressed in haploid spermatids and is necessary for spermatogenesis (Nantel et al, 1996). The repressor isoform of CREM (ICER) is a putative repressor of FSH receptor expression (Sassone-Corsi, 1998). The FSH-stimulated cAMP production is modulated by testicular phosphodiesterases (Conti et al, 1991), and adenosine also negatively modulates FSH-stimulated adenylyl cyclase activity through Gi-protein activation (Monaco et al, 1984).

Besides adenylyl cyclase, FSH is able to stimulate inositol trisphosphate (IP3) turnover in Sertoli cells, but the functional significance of this response remains open (P Manna and I Huhtaniemi, unpublished). Very little is known about the eventual involvement of protein kinase C in FSH actions. Calcium fluxes are regulated by FSH, and intracellular free calcium levels are able to modulate the FSH effects (Grasso and Reichert, 1990).

The FSH function in the testis depends on age. In prenatal and prepubertal period, it stimulates the proliferation of Sertoli cells (Orth, 1984). This influences fertility, since the Sertoli cell number correlates with total length of the seminiferous epithelium and sperm production capacity. Sertoli cells become terminally differentiated at puberty, and rarely divide thereafter. Thus, FSH action determines the spermatogenic capacity in adult age before the onset of puberty. In a hypothyroid state, the proliferative phase of Sertoli cells is prolonged, which leads to increased testicular size and daily sperm production in adulthood (Cooke et al, 1991). In addition to mitogenic effects on Sertoli cells, FSH stimulates the

proliferation of spermatogonia (Van Alphen et al, 1988). FSH has been considered essential for the initiation of spermatogenesis at puberty, but as will be presented below, the very recent data on mice with knock-out mutation of the FSHβ gene and men with inactivating mutation of the FSH receptor gene, provide evidence against this (see below).

Both testicular steroid and peptide hormones play a role in the feedback regulation of FSH secretion (Fig. 1). The two peptide hormones of Sertoli cell origin, inhibin and activin, are the main regulators of FSH secretion at the pituitary level. Inhibin is a heterodimer of an α subunit and a βA (inhibin A) or βB (inhibin B) subunit, and it inhibits the synthesis and secretion of FSH, whereas activin, a homodimer of two β subunits, has a stimulating effect. In addition, a third protein, follistatin, has inhibitory effects on FSH, by binding and inactivating activin. The inhibitory effects of androgen and estrogen on FSH action mainly occur at the hypothalamic level. The novel dimer-specific assays of inhibin have demonstrated inverse correlation between plasma inhibin B and FSH levels, thus proving the link between FSH action and inhibin production. Inhibin B is the Sertoli cell product with importance in the feedback regulation (Burger and Robertson, 1997).

TESTICULAR STEROIDOGENESIS

The main steroid hormones synthesized by the testis are androgens, in particular T, and the Leydig cells are the site of this synthesis. LH is the key regulator of steroidogenesis, and its action is modulated by numerous other endocrine, paracrine and autocrine factors, as has been discussed in detail by Saez (1994). The androgens are essential for all masculine functions of the body, including sexual differentiation, development secondary sex characteristics, spermatogenesis, the masculine features of the muscle-bone apparatus, and male sexual behavior. The mechanisms whereby a single regulatory step, i.e. the binding of androgen to its receptor, evokes this variety of structural and functional responses, are discussed elsewhere in this book (see Chapter 4). In the present chapter, we describe the key steps of testicular androgen formation, secretion and transport.

The synthesis of all steroid hormones starts from cholesterol, which in Leydig cells is either synthesized *de novo* from acetyl coenzyme A, or taken up from circulation by receptor-mediated endocytosis of LDL-cholesterol complexes, and followed by their degradation in lysosomes. Cholesterol is thereafter esterified and stored in intracellular lipid droplets.

The next step in the transport of cholesterol from lipid droplets to the outer mitochondrial membrane occurs by mechanisms that are still poorly understood. More is known about the cholesterol transfer from the outer to the inner mitochondrial membrane, where the crucial role in played by a recently discovered steroidogenic acute regulatory (StAR) protein, which is rate limiting and regulatory of testicular steroidogenesis (Clark and Stocco, 1996). The StAR protein is up-regulated by most of the

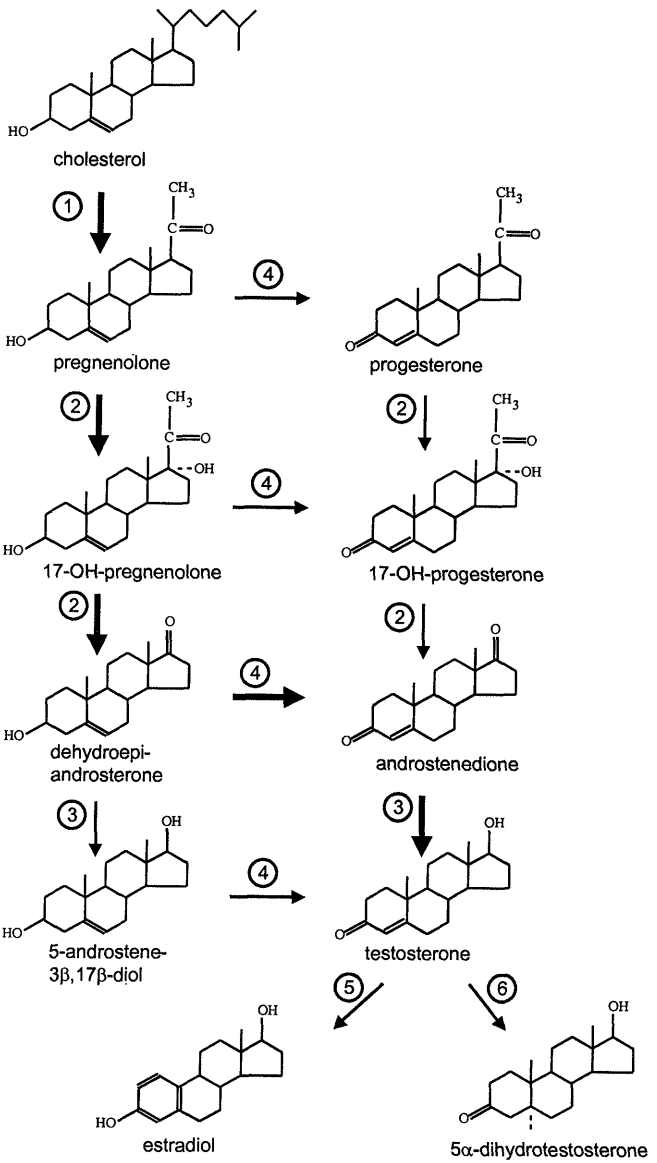

Fig. 2. Steroid biosynthesis in Leydig cells. Bold arrows depict the Δ5 pathway preferred in the human testis. The numbers indicate the enzymes used by the metabolic steps: 1, cholesterol side chain cleavage enzyme; 2, 17α-hydroxylase/17,20-lyase; 3, 17β-hydroxysteroid dehydrogenase; 4, 3β-hydroxysteroid dehydrogenase; 5, aromatase; 6, 5α-reductase.

stimuli of steroidogenesis, including LH, and inactivating mutations of its gene lead to congenital lipoid adrenal hyperplasia, a condition characterized by near complete blockade of steroidogenesis in adrenal gland and gonad (Bose et al, 1996).

The first step at the mitochondrial inner membrane is the conversion of cholesterol to pregnenolone, a step catalyzed by the cytochrome P450 cholesterol side chain cleavage enzyme (P450scc, CYP11A1). The following steps of androgen biosynthesis, from pregnenolone onwards, take place in the smooth endoplasmic reticulum. Depending on the order of enzymatic reactions, two alternative pathways, Δ5 or Δ4, are employed (Fig. 2). The former is more important in the human testis, and there pregnenolone is first converted by cytochrome P450c17 (CYP17) via 17-hydroxypregeneolone (17-hydroxylation step) to dehydroepiandrosterone (DHEA)(17,20-desmolase step). The same CYP17 enzyme catalyzes both enzymatic reactions. The next step is the conversion of DHEA to 5-androstene-3β,17β-diol by 17β-hydroxysteroid dehydrogenase (17β-HSD), and finally, the 3β-hydroxy-5-ene structure of the former steroid is oxidized by 3β-hydroxysteroid dehydrogenase (3β-HSD) to the 3-keto-4-ene structure, with formation of T. In the alternative Δ4 pathway, the first metabolic step after pregnenolone is the 3β-HSD-catalyzed conversion to progesterone, after which the metabolism proceeds further through 3-keto-4-ene intermediates to T (Fig. 2).

The human testes synthesizes 6-7 mg of T daily, but also a number of other steroid hormones are synthesized, which either are weak androgens themselves (e.g. androsterone) or are metabolized further in peripheral organs (androstenedione). Most of the other testicular steroids are intermediates of androgen synthesis along the Δ4 and Δ5 pathways, and are to a large extent sulfate-conjugated. Steroid sulfates have no known hormonal functions, and apparently represent storage and secretory forms of steroidogenesis. The testis also produces small amounts of 5α-dihydrotestosterone, which is the active molecular form in many extratesticular actions of androgens (see Chapter 4).

In circulation, only about 2% of T appears in free, i.e. non-protein-bound form, and the rest is bound to SHBG (44%), as well as albumin and other proteins (54%)(Griffin and Wilson, 1998). The affinity of albumin for T is only about 0.1% of that of SHBG, but its high concentration in circulation explains its overall importance in androgen transport. Due to the avid binding between T and SHBG, these complexes are unable to enter the androgen target cells. However, they may have some, as yet poorly characterized, functions at the cell membrane. In contrast, the binding of T to albumin is easily dissociable, and this fraction, together with free T, i.e. about 50% to total plasma T, is considered to form the so-called bioavailable fraction of androgen. The plasma level of SHBG is under endocrine regulation, decreased by androgen and increased by estrogen. If the HPT axis functions normally, changes in SHBG levels do not affect the androgen balance, since they are quickly compensated for by changes in the feedback regulation.

ARE BOTH LH AND FSH NEEDED FOR SPERMATOGENESIS?

The hormonal requirements for initiation of the first spermatogenic wave at puberty, for its maintenance in adult age, and for the reinitiation after transient suppression are apparently different. Androgen alone may not be able to drive spermatogenesis to completion beyond the early spermatid stage in the immature hypophysectomized rat (Chemes et al, 1979). In accordance, if a prepubertal animal is hypophysectomized, LH only partially prevents germ cell loss (Russell et al, 1987). In man, T alone seems to be able to initiate spermatogenesis and drives it further than in rodents (Chemes et al, 1982; Vicari et al, 1992).

Treatment of healthy men with T enanthate (200 mg/week i.m.) suppresses both gonadotropins and intratesticular T while maintaining peripheral T levels at normal to slightly elevated concentrations. As a result, spermatogenesis is severely suppressed – to the extent that this provides a successful means of male contraception. If these men are supplemented with injections of LH or hCG, their spermatogenesis recovers, due to the restoration of high intratesticular T (Matsumoto, 1989). However, the FSH level still remains suppressed. Although spermatogenesis recovers qualitatively, the sperm count remains suppressed (25-50 million/ml) from the pretreatment concentration (75-100 million/ml). This has been considered evidence that the reinitiation of spermatogenesis is possible with T alone, but its quantitative recovery also needs FSH. This was shown by addition of FSH to the above treatment regimen, which fully restored quantitative spermatogenesis.

The T-suppressed men were also treated with purified FSH (Matsumoto, 1989), and it was able to stimulate spermatogenesis, though not quantitatively. This was considered evidence that LH/T were not either absolutely necessary for spermatogenesis. However, it is possible that the FSH effect was actually a synergistic action of FSH and low intratesticular T. Hence, it still remains to be shown whether FSH alone, in the complete absence of androgen, is able to drive spermatogenesis. In this respect, a recent study of Gromoll et al. (1996) is of interest. They found a hypophysectomized man with undetectable gonadotropins, in whom full spermatogenesis persisted without T replacement therapy. It was hypothesized that the man must have a constitutively activating mutation of the FSH receptor gene. Such a mutation, indeed, was discovered in the 3rd intracellular loop of the receptor protein, and the case was considered proof that FSH alone, without concomitant androgen stimulation, is able to drive spermatogenesis in man. However, the subject had serum T concentrations of 5-8 nmol/l in the absence of replacement therapy, which is 5-10-fold higher than the castrate level. His residual T production could therefore have been significant. It therefore remains open whether the partially activated FSH receptor or the residual relatively high T production, or both, were responsible for the maintenance of spermatogenesis.

In a fully regressed hypophysectomy model, T is less effective in reinitiating spermatogenesis than in its maintenance (Santulli et al, 1990), but this finding may depend on the model studied. Hypophysectomized animals are more resistant to the

treatment than those where gonadotropins alone are suppressed, indicating that other pituitary factors are needed for full testicular function. In agreement, complete spermatogenesis can be induced in gonadotropin-deficient *hpg* mice by T treatment alone (Singh et al, 1995). In contrast, in hypophysectomized or hypogonadotropic men, T treatment alone does not restore full spermatogenesis (Matsumoto 1989; Weinbauer and Nieschlag, 1990), although there are sporadic cases where fertility of hypogonadotropic men was restored by hCG treatment alone (Vicari et al, 1992). Although the androgen deficiency of hypogonadal men is easy to treat with T replacement, this does not lead to fertility, due to the continued suppression of gonadotropins. When fertility is desired, treatment with gonadotropins, or pulsatile GnRH is needed to stimulate endogenous gonadotropins. If the patient is prepubertal, the initiation of spermatogenesis usually requires both FSH and LH (Weinbauer and Nieschlag, 1990). If the gonadotropin deficiency is acquired after puberty, LH/hCG alone is usually enough to initiate spermatogenesis (Finkel et al, 1985), but again, FSH is needed to restore it quantitatively. These findings point out that spermatogenesis shifts from FSH and T dependence to predominant T dependence during pubertal maturation.

The above contention has been recently challenged by findings on men with inactivating mutation of the FSHβ subunit and FSH receptor gene, and on mice with targeted disruption (knock-out) of the FSHβ subunit gene. Men with the nonfunctional FSH receptor gene have mildly to severely reduced testicular size, suppressed spermatogenesis, but, conspicuously, no azoospermia - some of the men even have children (Tapanainen et al, 1997). In accordance, the FSHβ knock-out mice have reduced testicular size, suppressed spermatogenesis, but are fertile (Kumar et al, 1997). These findings indicate that FSH is needed for the peripubertal proliferation of Sertoli cells (i.e. determination of the finite testis size), for maintenance of quantitatively and qualitatively normal sperm production in adult age, but not for the pubertal initiation of spermatogenesis.

There are two reports on men with inactivating mutations of the FSHβ gene (Lindstedt et al, 1997; Phillip et al, 1998). Both men have azoospermia and severely reduced testicular volume. On the basis of these two sporadic cases, it was proposed that FSH is an absolute requirement for spermatogenesis. However, it is more likely that some other condition, in addition to FSH deficiency, causes the azoospermia, since one of the men was resistant to FSH therapy, and the other had also low T and high LH, indicating primary Leydig cell failure.

It has been questioned, how much T is needed for the maintenance of spermatogenesis? Data on experimental animals indicate that intratesticular levels as low as 5%, in many cases 20-40% of normal, are sufficient (Cunningham and Huckins, 1979; Sharpe 1987; Weinbauer and Nieschlag 1990). Whatever the minimum level is, it is at least 10-fold compared with peripheral androgen levels, and, paradoxically, much higher than that needed for the saturation of androgen receptors. A common feature of these studies is that less T is required if gonadotropin secretion, and possibly also that of other pituitary hormones, are normal (hypophysectomy vs. selective suppression of gonadotropins). This again points out to the synergistic actions of T, FSH and other hormones/paracrine factors in normal spermatogenesis. One factor can to some extent compensate for the

missing one. The normal concentration of intratesticular T is clearly higher than the minimum needed for spermatogenesis, probably to assure this vital function. We can conclude that T is the "master switch" of spermatogenesis and other factors are needed to maintain it qualitatively and quantitatively normal.

TESTICULAR EFFECTS OF OTHER HORMONES

Besides LH and FSH, several other circulating hormones, such as prolactin (Prl), growth hormone (GH), insulin, glucocorticoids and thyroid hormone, have effects on testicular function. Since many 'extragonadal' hormones are also synthesized within the testis, it is difficult to delineate whether their testicular actions, shown *in vitro*, are *in vivo* due to a blood-born or intratesticular factor. We concentrate in the following on factors that most likely are of circulatory origin.

Prl augments Leydig cell steroidogenesis in response to LH in rodents, probably by maintaining the LH receptors (*e.g.* Huhtaniemi and Catt, 1981). The role of Prl is especially clear in seasonally breeding hamsters, in which short day is associated with low Prl and testicular involution (Bartke et al, 1980). Prl reverses the involution associated with darkness. Direct effects of Prl on human testicular functions are still controversial, since both positive and negative evidence exists for the presence of Prl receptors in human testis (reviewed in Huhtaniemi, 1993). Hyperprolactinemia in man has been related to testicular hypofunction, but this effect is most likely indirect through inhibitory action of Prl on gonadotropin secretion.

GH stimulates in the testis the formation of insulin-like growth factor (IGF) -1, which may be the mediator of its actions. In accordance, the testes of IGF-1 knock-out mice are hypoplastic (Baker et al, 1996), but it is unclear whether this is due to elimination of circulating IGF-1 or that of testicular origin. Evidence for testicular effects of pituitary GH comes from observations in rodents that GH deficiency or resistance are associated with delayed puberty and poor Leydig cell response to LH/CG (Chatelain et al, 1991).

Insulin receptors are found in Leydig cells, insulin and LH reciprocally up-regulate the receptors of each other, and insulin augments basal and LH-stimulated steroidogenesis of Leydig cells (reviewed by Huhtaniemi, 1993). Insulin at physiological (nanomolar) concentrations appears to act through its own receptor, whereas at pharmacological (micromolar) concentrations its action is mediated through insulin and IGF-1 receptors. Despite clear-cut effects *in vitro*, the physiological importance of insulin in testicular function remains unclear.

Leydig cell function is also modulated by steroid hormones. Since estrogens and androgens are Leydig cell products, their effects can be considered paracrine or autocrine. In contrast, glucocorticoids are of adrenal origin, and may contribute to the endocrine regulation of the testis. High systemic glucocorticoid levels, e.g. in Cushing's syndrome and during physical and mental stress, suppress testicular androgen production (Phillips et al, 1989). Testicular interstitial cells have glucocorticoid receptors, and these hormones suppress the conversion of cholesterol

to steroid hormones (Phillips et al, 1989). An interesting protective system is provided by the 11β-dehydrogenase activity in Leydig cells which can protect Leydig cells, by converting cortisol to the inactive 11-keto form, cortisone (Hales and Payne, 1989). Hence, protection of the testis from the inhibiting effects of high glucocorticoid levels is apparently physiologically desirable. In addition, we have recently found that intratesticularly formed progesterone may mediate in the LH stimulated down-regulation of its own receptors (El-Hefnawy et al, 1998).

We have recently established that thyroid hormones have an important role in the maintenance of Leydig cell steroidogenesis (Manna PR, Huhtaniemi IT, unpublished). They increase in additive fashion with LH the level of the StAR protein expression in Leydig cells. Without optimal thyroid hormone action the steroidogenic capacity of Leydig cells is severely compromised.

Despite the demonstration of direct testicular actions of numerous blood-born hormones, their physiological role remains obscure and the dogma still prevails that gonadotropins provide the driving force for testicular function. The role of the other regulators is likely to fine-tune the actions of gonadotropins.

FUTURE PERSPECTIVES

Numerous questions concerning the endocrine regulation of testicular function are still controversial. Their further study is important, on one hand for better understanding of the pathogenesis of male infertility, on the other hand for the development of novel strategies for male contraception. How much FSH is required for the maintenance of spermatogenesis is still a contentious issue needing further study, because it could provide further clues concerning the feasibility of a male contraceptive method based on inhibition of FSH action.

Despite the plethora of *in vitro* findings of various para- and autocrine regulatory mechanisms within the testis, next to nothing is known about their significance *in vivo*. Whether they really contribute to the physiological regulation of the testis is still uncertain. Since these factors are invariably produced outside the testis, it is difficult to address the significance of their intratesticular synthesis. One way of addressing these questions would be to produce testis-specific knock-out models for the paracrine factors.

Genetic aspects of the variability of testicular endocrine regulation will undoubtedly gain increasing attention in the future. One puzzling question has been the variable suppression to azoospermia in men during T treatment for contraception. Due to clear ethnic differences in this response it is likely that some key genes regulating spermatogenesis, *e.g.* the androgen receptor, contain critical polymorphisms that allow spermatogenesis either to proceed at lower androgen level, or independent of androgens. Finding out the cause of this variability would be an important step towards the development of a hormonal male contraceptive.

Recent knock-out data and human mutations, as well as the discovery of the novel estrogen receptor β, have shed new light on the role of estrogens in testicular physiology. It is likely that important new information of this topic will be obtained

in the near future. Concerning androgen action, it is still not understood why such a high androgen concentration, in comparison to that needed for the extragonadal androgen actions, is needed for the regulation of spermatogenesis. This fundamental question, among others, awaits to be answered in the future.

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2 SPERMATOGENESIS AND GERM CELL DEATH

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INTRODUCTION

The ability to produce functional gametes is essential for the survival of a species. Spermatogenesis is a dynamic process in which stem spermatogonia, through a series of events become mature spermatozoa and occurs continuously during the reproductive lifetime of the individual (Russell et al, 1990; Sharpe, 1994). Stem spermatoginia undergo mitosis to produce two types of cells: additional stem cells and differentiating spermatogonia which undergo rapid and successive mitotic divisions to form primary spermatocytes. The spermatocytes then enter a lengthy meiotic phase as preleptotene spermatocytes and proceed through two cell divisions (meiosis I and II) to give rise to haploid spermatids. These in turn undergo a complex process of morphological and functional differentiation resulting in the production of mature spermatozoa. Not all germ cells, however, achieve maturity, and such spontaneous death of certain classes of germ cells appears to be a constant feature of normal spermatogenesis in a variety of mammalian species, including the human (Allan et al, 1987; Russell et al, 1990; Sharpe, 1994). As will be addressed below, a growing body of evidence has now demonstrated that apoptosis is the underlying mechanism of germ cell death during normal spermatogenesis, and can be triggered by a wide variety of regulatory stimuli. In this chapter, we will focus on the role of programmed germ cell death in spermatogenesis, as well as highlight the similarities and differences in the pathways by which germ cell death is repressed or activated in various model systems. We will also address the recent advances in the intratesticular regulatory mechanisms that control germ cell apoptosis. It is not our intention to review sermatogenesis in depth. For the purpose of this chapter, it is necessary, however, to describe briefly the organization of spermatogenesis. The regulation of spermatogenesis is described in Chapter 1.

ORGANIZATION OF SPERMATOGENESIS

The mammalian testis has two basic compartments, the interstitial (intertubular) compartment and the seminiferous tubule compartment.The interstitial compartment is highly vascularized and contains Leydig cells clustered near or around the vessels. These cells are responsive to luteinizing hormone (LH) and secrete testosterone (T) which subsequently accumulates in the interstitium and the seminiferious tubules at relatively high concentrations. The formation of spermatozoa from stem spermatogonia (spermatogenesis), through a series events involving mitosis, meiosis, and cellular differentiation, takes place in the seminiferous tubule compartment which contains Sertoli cells and developing germ cells. Thus, the two major areas of activity within the testis center on steroidogenesis and spermatogenesis. A large body of literature provides evidence that LH (via stimulation of T) and FSH are the key regulators of spermatogenesis (Russell et al, 1990; Wang and Swerdloff, 1992; Sharpe et al, 1994).

The general organization of spermatogenesis (illustrated in Fig. 1) is essentially the same in all animals and can be divided into three main phases, each involving a class of germ cells (Russell et al, 1990; Kerr, 1992; Sharpe et al, 1994). The initial phase (also known as spermatocytogenesis) is the proliferative or spermatogonial phase during which stem spermatogonia undergo mitosis to produce two types of cells: additional stem cells and differentiating spermatogonia which undergo rapid and successive divisions to form preleptotene spermatocytes. In the rat, there are three types of spermatogonia: stem cell spermatogonia (Ais or A isolated), proliferarive (Apr or A paired and Aal or A alinged), and differentiating [A1, A2, A3, A4, In (intermediate) and B] spermatogonia. The stem cells, Ais, divide sporadically to replicate themselves as isolated entities and to produce pairs of Apr spermatogonia. The latter engage in a series of synchronous divisions leading to the formation of chains of Aal spermatogonia connected to each other by the intracellular bridges.The Aal spermatogonia do not divide but rather differentiate into A1 spermatogonia. The type A1 cells, however, divide to give rise more differentiating (A2, A3, A4, In, and B) cells. In man, mostly three different types of spermatogonia [the dark type A (Ad), pale type A (Ap) and B type] have been identified (Heller and Clermont, 1964). The Ap cells have the capacity to give rise to new Ap cells as well as to the more differentiated B spermatogonia and are considered to be the renewing stem cells. The Ad spermatogonia are reserve stem cells which normally divide only rarely. The precise mechanism by which stem spermatogonia transform into differentiating spermatogonia and simultaneously renew their own population is not known.

The meiotic or spermatocyte phase deals with the formation haploid spermatids from young primary spermatocytes and is traditionally divided into five sequential stages, including leptotene, zygotene, pachytene, diplotene, and diakinesis. The meiotic phase involves DNA synthesis in the youngest primary spermatocytes (preleptotene) entering into the long meiotic prophase and RNA synthesis in the

diplotene stage. Elaborate morphological changes occur in the chromosomes as they pair (synapse) and then begin to unpair (desynapse) during the first meiotic

Fig. 1. Schematic illustration of human spermatogenesis showing three major developmental phases (proliferative, meiotic and spermiogenesis), each involving a class of germinal cells. Abbreviations: Ad, dark type A spermatogonium; Ap, pale type A spermatogonium; B, type B spermatogonium, Pl, preleptotene spematocyte; L, leptotene spermatocyte, Z, zygotene spermatocyte; EP, early pachytene spermatocyte; MP, mid pachytene spermatocyte; LP, late pachytene spermatocyte; II secondary spermatocyte; Div 1 and Div2, first and second maturation divisions; N, nucleus; A, acrosome; C, centrioles; T, tail; m, mitochondria; RB, residual body.(Reprinted from Hermo and Clermont, 1995 with publisher's permission).

prophase. These changes include: 1) initiation of intimate chromosome synapsis at zygotene stage, when the synaptonemal complex begins to develop between the two sets of sister chromatids in each bivalent; 2) completion of synapsis with fully formed synaptonemal complex and occurrence of crossing over at pachytene stage; and 3) dissipation of the synaptonemal complex and desynapsing (allowing the chromosomal pairs to separate except at regions known as chiasmata) at the diplotene stage. Following the long meiotic prophase, the primary spermatocytes rapidly complete their first meiotic division to form two secondary spermatocytes, each containing 22 duplicated autosomal chromosomes and either a duplicated X or a duplicated Y chromosome. These cells undergo a second maturation division, after a short interphase with no DNA synthesis, to produce four spermatids, each with a haploid number of single chromosomes.

The spermiogenic phase (spermiogenesis) involves morphological and functional differentiation of newly formed spermatids into mature spermatozoa. Early in this transformation, the Golgi apparatus packages material that initiates acrosome formation. A flagellum forms from the centrioles and becomes associated with the nucleus.The nucleus progressively elongates as its chromatin condenses. During spermiogenesis the genome is re-packaged with protamines rather than histones, which is necessary to reduce the volume of the genetic payload from the relatively bulky round spermatids to the streamlined spermatozoa (compare the sizes of spermatozoa with round spermatids in Fig.1). Late spermatids are released almost simultaneously through the activity of the Sertoli cell. This process occurs without cell divisions and is one of the most phenomenal cell transformations in the body, and can be subdivided into many characteristics step. For example, this process can be divided into 19 steps (1 to 19) in the rat and 6 steps (Sa, Sb1, Sb2, Sc, Sd1, and Sd2) in man.

An intriguing feature of spermatogenesis is that the developing germ cells form associations with fixed composition or stages (Fig. 2) which constitute the cycle of the seminiferous epithelium (14 in the rat and 6 in the man). The fundamental organization and integrity of the seminiferous epithelium are provided by the Sertoli cells. These tall, irregularly-columnar cells span the distance from the base of the tubule into the tubular lumen and are elaborately equiped to support spermatogenesis. Adjacent Sertoli cells form contacts with each other at their lateral surfaces and near their base to effectively compartmentalize and separate the two population of germ cells. Thus, at each stage of the seminiferous epithelial cycle the germ cells are in intimate association with Sertoli cells in a predictable fashion, with the more immature cells (spermatogonia and young spermatocytes) located near the basal compartment and the advanced (most spermatocytes and spermatids) germ cells in the adluminal compartment. The interactions that occur between Sertoli cells and germ cells are described in Chapter 3. Each stage lasts for a fixed period of time at the end of which each germ cell type within that stage will progress into the next stage. For example, in the Sprague-Dawley (SD) rat, the progression of stage VII to stage VIII will take little more than 2 days. The time interval between the successive appearance of the same cell association at a given area of the tubule is known as the cycle of the seminiferous epithelium (which is about 12.9 days in

SD rats). The duration of the seminiferous epithelial cycle in man is about 16.0 days. However, it is pertinent to note here, that in humans, unlike rodents, individual tubular profile almost always contains more than one cell association or stage. Stages in human tubules may be mapped by drawing stage boundary lines among individual cell associations in a cross-sectioned tubule.

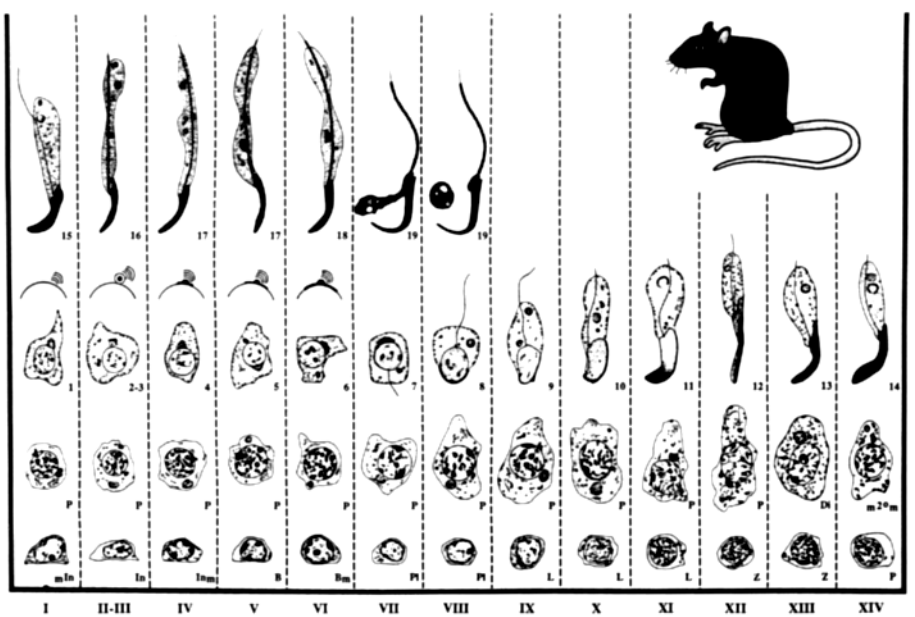

Fig. 2. Diagrammatic representation of the seminiferous epithelial cycle in the rat. The columns numbered with Roman numerals show the various cell types present at each cellular association, which are encountered in the various cross-sections of the seminiferous tubule. Different types of A spermatogonia are not indicated in the cycle map. Abbreviations: m In, dividing intermediate spermatogonium; In, intermediate spermatogonium; B, B spermatogonium; Pl, preleptotene; L, leptotene; Z, zygotene; P, pachytene; Di, diakinesis; m 2 m, dividing spermatocytes; 1-19, 19 steps of spermiogenesis. Cycle map from Russell et al, 1990.

Another intriguing feature of spermatogenesis is that there is a distinct ordering of cell associations along the length of the seminiferous tubule (segments) which has been called the 'wave of the seminiferous epithelium' (Perey et al, 1961).Thus, a wave encompasses all 14 segments that are composed of various cell associations. A segment is a portion of seminiferous tubule in its longitudinal axis occupied by a single cell association or stage. For example, a longitudinal portion of a seminiferous tubule occupied by stage VII is called segment VII. In freshly isolated unstained condition, the wave can be recognized by transillumination (Parvinen, 1993). This makes it possible to collect tubular segments representing various stages by microdissection for molecular and biochemical studies. However, in the longitudinal axis of the human seminiferous tubules, areas comparable to segments of the rat are found in patches and do not usually encircle the seminiferous tubule, thus giving the impression of a random pattern of germ cell development with no waves of the seminiferous epithelium. However, examination of serial testicular sections and computer modelling of the position of germ cells have revealed the existence of a wave in the human seminiferous tubule (Kerr, 1992). This wave appears to be organized in an 'Archimedian spiral' around the seminiferous tubule.

APOPTOSIS: DEFINITIONS AND SIGNIFICANCE

Essentially all animal cells have the ability to kill themselves by activating an intrinsic cell suicide program when they are no longer needed or have become seriously damaged (Steller, 1995; Jacobson et al, 1997). The execution of this program leads to a morphologically distinct from of cell death termed apoptosis (Kerr et al, 1972). Much of the progress has been made in understanding the regulation of apoptosis in various extragonadal cell systems (reviewed in Majno and Joris, 1995; White, 1996; Jacobson et al, 1997; Nagata, 1997; Peter et al, 1997) with little emphasis on programmed germ cell death in the testis (Huseh et al, 1996; Swerdloff et al, 1998; Sinha Hikim and Swerdloff, 1998). Apoptosis is now recognized as a genitically driven form of cell death that is either developmentally regulated, launched in response to specific stimuli (such as deprivation of survival factors, ionizing radiation and chemotherapeutic drugs, activation of various death factors and their ligands), or induced in response to various forms of cell injury or stress. It is now widely accepted that apoptosis serves as a prominent force in sculpting body parts, deleting unneeded structures, maintaining tissue homeostasis, defense mechanism to remove unwanted and potentially dangerous cells such self-reactive lymphocytes, cells that have become infected with viruses, and tumor cells (Thompson, 1995; Jacobson et al, 1997). The crucial role of apoptosis is also increasingly being recognized in pathogenesis of many diverse human diseases including cancer, acquired immunodeficiency syndrome, neurodegenerative disorders, atherosclerosis, and cardiomyopathy (Thompson, 1995; Hannun, 1997; Olivetti et al, 1997). The cellular and molecular characteristics of apoptosis have

been well documented in the literature (see Majno and Joris, 1995; Dini et al, 1996; Jacobson et al, 1997). We will provide a brief description of the identifying features of apotosis. These features include a reduction in cell volume, blebbing of the cell membrane, chromatin condensation and margination, formation of a distinctive DNA 'ladder pattern', translocation of phosphatidylserine from inner to outer surface of the plasma membrane, disruption of the mitochondrial membrane potential and opening of the large chanel. This mode of cell death affects individual or small group of cells and there is a rapid clearance of dead cells by 'professional' phagocytes or neighboring cells before they can lyase, spill their noxious contents and cause an inflammatory reaction.

PROGRAMMED CELL DEATH IN SPERMATOGENESIS

Germ cell death has long been recognized a significant feature of mammalian spermatogenesis (Roosen-Runge, 1977; Allan et al, 1987; Russell et al, 1990; Sharpe, 1994). There are several vulnerable steps at which germ cells spontaneously die such that the seminiferous epithelium yields less spermatozoa than theoretically possible. In adult rats, this loss is incurred mostly during spermatogonial development (up to 75%) involving primarily types A_2 and A_3 spermatogonia and to a lesser extent during maturation divisions of spermatocytes and spermatid development. Germ cell death can be induced at a accelerated rate by various regulatory stimuli (such as deprivation of survival factors, testicular hyperthermia, testicular toxins and chemotherapeutic agents). As will become apparent in the subsequent sections, a growing body of evidence now demonstrates that both spontaneous and induced germ cell death in adult rats occur almost exclusively by apoptosis.

Characterization of Germ Cell Apoptosis

Electron microscopy has long been used to detect programmed cell deaths, characterized by distinctive patterns of morphological changes, in unicellular and multicellular organisms (Kerr et al, 1972; Dini et al, 1996; Jacobson et al, 1997) and can also be reliably used to identify apoptotic germ cells (Russell et al, 1990; Henriksen et al, 1995; Blanco-Rodriguez and Martinez-Garcia, 1996; Sinha Hikim et al, 1997a; Ross et al, 1998). An electron micrograph of an apoptotic step 7 spermatid (Fig.3) in rat is shown here.

The presence of internucleosomal chromatin degradation in the majority of observations of apoptotic cells has resulted the use of DNA fragmentation as a reliable diagnostic tool for the occurrence of apoptosis. DNA 3'-end labeling is routinely being used to detect germ cell apoptosis in a large number of studies (Tapanainen et al, 1993; Billig et al, 1995; Sinha Hikim et al,1995; 1997b; Henriksen et al, 1995a and b; 1996; Blanco-Rodriguez and Martinez-Garcia, 1996;

Brinkworth et al, 1995). A ladder like DNA fragmentation can be seen in cells undergoing apoptosis.

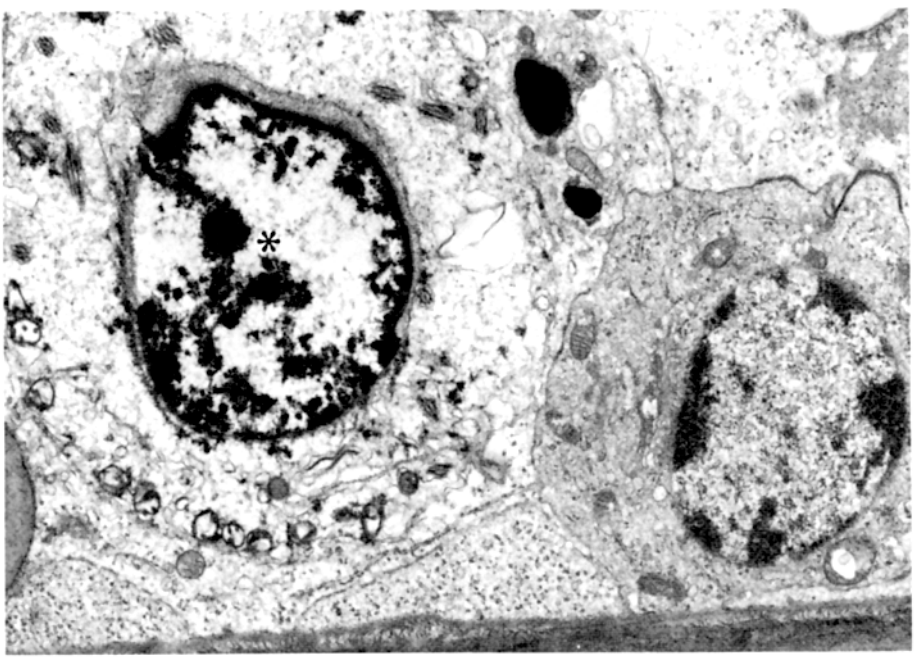

Fig.3. Electron micrograph of an apoptotic step 7 spermatid (asterisk) in the adult rat. Apoptosis of germ cells was induced by gonadotropin deprivation (Sinha Hikim et al, 1995). Magnification, x 13,500. (Reprinted from Sinha Hikim et al, 1997b with publisher's permission)

Assessment of apoptotic DNA in individual germ cells has proven to be of considerable need for the investigators from many disciplines. TdT-mediated dUTP nick end labeling (TUNEL) is routinely being used to detect apoptotic cells in various tissues. In recent studies (Lue et al, 1997; Sinha Hikim et al., 1997a and b; 1998) we have shown that the use of glutaraldehyde fixation provides improved TUNEL sensitivity and allows the superior structural preservation needed for the quantitative assessment of cell death involving various germ cells at different phases of their development. The labeling is also specific for apoptosis, as necrotic germ cells in cadimium-treated rats are clearly devoid of any specific labeling for

low MW DNA fragmentation (Sinha Hikim et al, 1997a and b). Flow cytometry has also been used to study germ cell apoptosis induced by complete testosterone (T) withdrawal after treatment with ethane dimethane sulphonate (EDS), a Leydig cell cytotoxin (Troiano et al, 1994). The presence of apoptotic cells was evaluated by measuring the reduced fluorescence of the DNA binding dye propidium iodide in the apoptotic cells. To ensure that the mode of cell death in a given system is apoptotic, one needs to consider both morphological and biochemical criteria for apoptotic cell death.

Spontaneous Germ Cell Apoptosis

Recent studies by us as well as by others have demonstrated that apoptosis is the underlying mechanism of germ cell death during normal spermatogenesis in various mammals, including the rat (Billig et al, 1995; Sinha Hikim et al, 1995; 1997 a and b), the hamster (Lue et al, 1997), the mouse (Mori et al, 1997; Sinha Hikim et al, unpublished data) and the human (Heiskanen et al, 1996; Sinha Hikim et al, 1998). Unlike rat, mouse, or hamster testes, human testes exhibit spontaneous occurrences of germ cell apoptosis involving all three classes of germ cells, including spermatogonia, spermatocytes, and spermatids (Sinha Hikim et al, 1998). Interestingly, the incidence of spontaneous germ cell apoptosis (apoptotic index) expressed as the number of apoptotic germ cells per 100 Sertoli cells, in the human varies along with ethnic background. For example, the incidence of spermatogonial as well as spermatid apoptosis was higher in Chinese than in Caucasian men. A higher incidence of spermatocyte apoptosis was noted for Chinese compared to Caucasian men, but the difference was not statistically significant. The triggering factors for spontaneous germ cell apoptosis during normal spermatogenesis are not known. It is also uncertain why there are ethnic differences in the inherent susceptibility of germ cells to programmed cell death. However, it should be noted that in testes, like many other tissues, the contribution of spontaneous germ cell apoptosis has been grossly underestimated due to their rapid and efficient clearance by professional phagocytes (Sertoli cells).

Induced Germ Cell Apoptosis

Withdrawal of Gonadotropins and/or Testosterone (T). Although the requirement of pituitary gonadotropins (LH and FSH) and testiclar T during initiation, maintenance, and restoration of spermatogenesis has been well documented, only recent studies have demonstrated the important roles of these hormones on the regulation of germ cell apoptosis (reviewed in Hsueh et al, 1996; Swerdloff et al, 1998; Sinha Hikim and Swerdloff, 1998). Tapanainen et al (1993), using a quantitative autoradiographic method for the detection of internucleosomal DNA cleavage, have shown induction of germ cell apoptosis in the testes of

immature rats after short-term (up to 4 days) hypophysectomy and its prevention by either gonadotropin or by T. However, hCG and T were not as effective as FSH in preventing the hypophysectomy-induced germ cell apoptosis. The specific germ cell types involved and the stages of their occurrence were not determined in that study. Furthermore, because FSH supplementation also increases LH receptor content and mRNA levels as well as the steroidogenic capacity of the Leydig cells in immature hypophysectomized rats (Vihko et al, 1991), the observed protective effect of FSH on germ cell apoptosis in these animals cannot be attributed with certainty to FSH alone. More recently, involvement of apoptosis in specific germ cells undergoing degeneration has been recognized in the adult rat after gonagotropin deprivation by gonadotrpin-releasing hormone antagonist (GnRH-A) treatment (Sinha Hikim et al, 1995). Treatment with GnRH-A, as early as, for 5 days resulted in selective activation of germ cell apoptosis involving preleptotene and pachytene spermatocytes, step 7 spermatids at stage VII, and step 19 spermatids at both stage VII and stageVIII; such apoptotic germ cells rarely if ever observed in a normal rat (Fig.4).

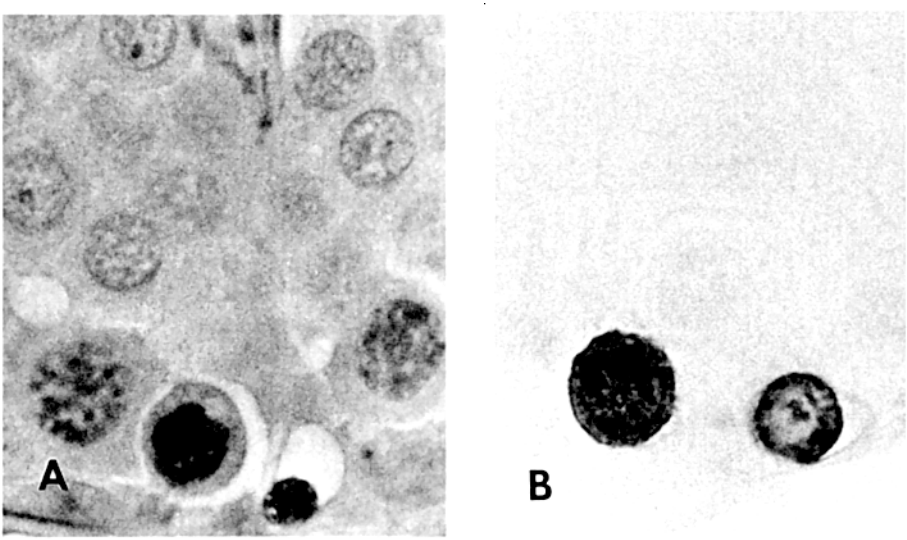

Fig. 4. *In situ* 3'-end labeling of DNA strand breaks in apoptotic germ cells, triggered by gonadotropin deprivation, in glutaraldehyde-fixed, paraffin- embedded testicular sections. A) Stage VII tubule of a rat treated with GnRH-A for 5 days, exhibiting an apoptotic pachytene (P) spermatocyte and a preleptotene (PL) spermatocyte among many nonapoptotic germ cells. Methyl green counter stain. B, Representative examples of immunostaining detected in the absence of a counterstain in germ cells undergoing apoptosis. Note complete absence of background staining. Magnification x 1400.

Similar stage-specific activation of germ cell apoptosis can also be found during early regression of spermatogenesis after complete T withdrawal following EDS treatment (Henriksen et al, 1995a). These studies suggests that apoptosis is an important determinant of hormone-dependent germ cell death in the testis. In a recent study (Sinha Hikim et al., 1997b), we further tested the possibility that apoptosis is the sole mechanism of germ cell death in the adult rat after selective withdrawal of gonadotropins and T by a potent GnRH antagonist (GnRH-A). To explore the possibility, we first investigated the temporal and stage-specific changes in the kinetics of germ cell death (characterized by the morphological criteria and also measured by TUNEL technique) in rats treated with GnRH-A for up to 2 weeks. We showed that comparison between the rates of apoptosis and cell degeneration measured at stages VII-VIII demonstrated an intimate association between apoptosis and overall germ cell loss. These results strongly support the concept that germ cell death after removal of hormonal support in the adult rat occurs almost exclusively via apoptosis. To determine the role and relative contribution of FSH and LH in regulating specific germ cell survival, we have examined the extent to which pure FSH or LH is able to prevent the GnRH-A induced germ cell apoptosis in the adult rat. A low incidence of germ cell apoptosis (expressed as numbers/Sertoli cell) was detectable at stages I, IX-XI, and XII-XIV in control rats. GnRH-A treatment for 1 week led to a significant increase in the mean incidence of apoptotic germ cells at stages VII-VIII and IX-XI in comparison

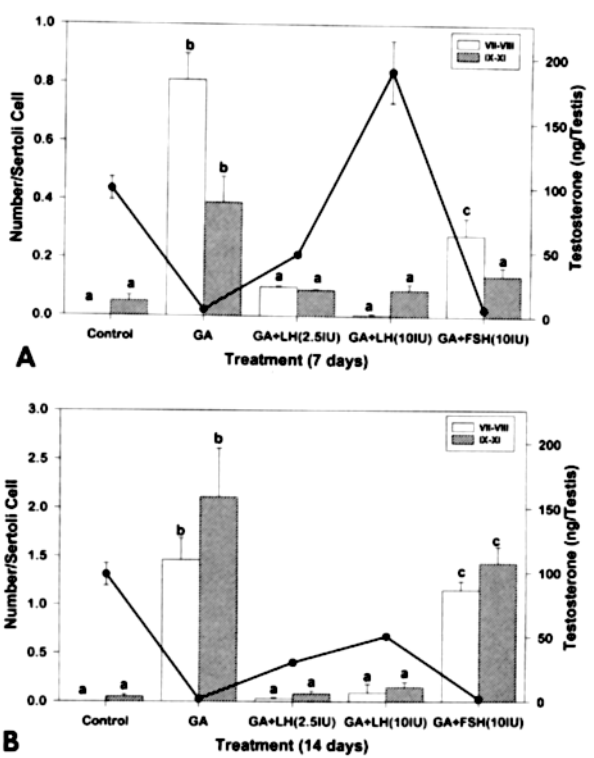

Fig.5. Gonadotropin rescue of GnRH-A (GA) induced germ cell apoptosis at stages VII-VIII and IX-XI. Adult rats were given sc injections of vehicle (control), GA, GA + LH (2.5 IU), GA + LH (10 IU), and GA + FSH (10 IU) for 1 (A) or 2 (B) weeks. Bars represent the mean number of apoptotic germ cells / Sertoli cell. Means with unlike superscripts differ significantly ($P < 0.05$). The solid line represents the changes in the intratesticular T levels in various treatment groups. FSH replacement to GnRH-A treated rats has no discernible effect on intratesticular T levels when compared to GnRH-A alone. From Sinha Hikim et al, unpublished data.

with the corresponding stages in controls. Concomitant administration of rhLH at both 2.5 and 10 IU doses fully attenuated the GnRH-A induced germ cell apoptosis at stages VII-VIII and IX-XI (Fig. 5A). Treatment with FSH also effectively suppressed the GnRH-A induced germ cell apoptosis at stages VII-VIII and stages IX-XI (Fig. 5A). As shown in Fig. 5B, significantly increased apoptosis specifically at stages VII-VIII and IX-XI was noted at 14 days after GnRH-A treatment. Similar to early time point, rh LH at both doses also completely prevented the GnRH-A induced germ cell apoptosis at these stages. In strking contrast, FSH replacement to GnRH-A treated rats only modestly suppressed the GnRH-A induced germ cell apoptosis at stages VII-VIII and stages IX-XI (Fig. 5B). Analysis of testicular internucleosomal DNA fragmentation further confirmed the preventive effect of either gonadotropin on GnRH-A induced germ cell apoptosis (Fig.6). These findings of gonadotropin rescue on GnRH-A

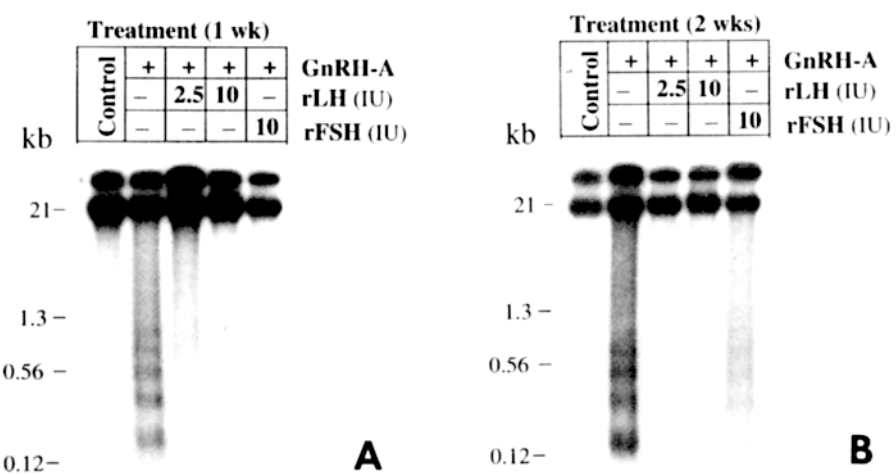

Fig.6. Gonadotropin rescue of GnRH-A induced testicular apoptotic DNA fragmentation after 1 (A) or 2 (B) weeks of treatment. From Sinha Hikim et al, unpublished observation.

induced germ cell apoptosis are further supported by experiments showing that specific immunoneutralization of either FSH or LH can also lead to apoptotic cell death in the adult rat (Marathe et al, 1995; Shetty et al, 1996). Furthermore, the observed beneficial effects of FSH on germ cell survival are most likely not due to the stimulation of the Leydig cell function (via paracrine interaction between the Sertoli and Leydig cells), because FSH addition to GnRH-A had no discernable effect on intratesticular or plasma T levels, accessory organs weight, and the total volume of the Leydig cells when compared with GnRH-A alone (Sinha Hikim and Swerdloff, 1995). These results demonstrate that concomitant administration of rhLH fully attenuates the GnRH-A induced germ cell apoptosis. This situation is very different from the immature rat, where hCG (LH surrogate) or T was unable to prevent the hypophysectomy induced germ cell apoptosis, even at earlier time (4 days) interval (Tapanainen et al, 1993). Recombinant human (rh) FSH also effectively prevents the early (1 week) GnRH-A induced germ cell apoptosis. The beneficial effects of FSH on germ cell survival, however, decline with time. Possible explanation of this difference is uncertain and cannot be explained by antibody formation in response to the administration of heterologous FSH, since antibody formation against rhFSH was not detected in any of the rats treated with GnRH-A + rhFSH for up to 4 weeks (Sinha Hikim and Swerdloff, 1995). The observed beneficial effects of FSH on GnRH-A induced apoptosis at stages VII-VIII and IX-XI are in contrast to earlier *in vitro* data showing a preferential effect of germ cell survival at earlier (I-IV) stages (Henriksen et al, 1996). Because FSH and androgen receptors are found almost exclusively in the Sertoli cells, it is likely that these hormones exert their stimulatory effects on Sertoli cells, which in turn results in stimulation of intratubular factor(s) essential for the survival of germ cells through a paracrine mechanism (Sharpe, 1994; Kierszenbaum, 1994). The idea that cells require an adequate supply of "survival factors" elaborated by other cells in order to survive is not new and has been elegantly demonstrated in other tissues (Raff, 1992). Collectively, these results suggest that: 1) pituitary gonadotropins LH (via stimulation of T) and, to a lesser extent, FSH are the important extrinsic regulators of germ cell apoptosis, and 2) hormonal regulation of germ cell apoptosis between immature and adult rats is decisively different.

Other studies also beginning to provide evidence that gonadotropins and T are critical germ cell survival factors. For example, selective deprivation of gonadotropin and testicular T by estrogen treatment in the adult rat is also followed by stage-specific activation of germ cell apoptosis (Blanco-Rodriguez and Martinez-Garcia, 1996; 1998). In a recent in vitro study (Erkkila et al, 1996), evidence was provided that incubation of segments of adult human seminiferous tubules under serum-free conditions induces, within 4 hours, apoptosis in germ cells, and this induction of germ cell apoptosis can be effectively suppressed by T.

Testicular Hyperthermia. In most mammals, including humans, the testis is maintained always at lower temperatures than that of the abdomen, and exposure of

the testis to body temperature or above results in accelerated death of germ cells (Allan et al, 1987; Mieusset and Bujan, 1995). Classical histological studies have shown that local heating of the testis or surgical induction of cryptorchidism in rats results in accelerated death of germ cells (Chowdhury and Steinberger, 1964; Collins and Lacy, 1969; Jones et al, 1977; Blackshaw and Massey, 1978). Mild testicular heating has been established as a safe and reversible treatment and is now suggested to be one of the methods for male contraception (Mieusset and Bujan, 1995). However, the cellular and molecular events underlying heat induced germ cell death remain unclear. In a recent study, we examined the underlying mechanisms of heat induced germ cell death (Swerdloff et al, 1998). Scrota of adult male rats were exposed to a temperature of 22C (control) or 43C for 15 minutes, and killed on day 1, 2, and 9 after heat exposure. Apoptosis was characterized by TUNEL and quantitated as number of apoptotic germ cells per Sertoli cell. Mild hyperthermia within 1 & 2 days resulted in a marked activation of germ cell apoptosis specifically at early (I-IV) and late (XII-XIV) stages with minimum effects on other stages, including the hormone dependent (VII-VIII) stages. By day 9, majority of the tubules were severely damaged and displayed only a few remaining apoptotic germ cells as most of these dead cells were eliminated (presumably) through phagocytosis by the Sertoli cells. The effect of heat on spermatogenesis is not only stage-specific but also cell-specific. Spermatocytes, including pachytenes at stages I-IV and XII, diplotene and dividing spematocytes at stages XIII-XIV, and early (steps 1-4) spermatids were most susceptible to heat. Differential spematogonial apoptosis, however, remained unaffected. Unlike the GnRH-A treated rat model, the initiation of germ cell apoptosis after heat stress was not preceded by a marked decrease in testicular T levels. These results suggest that testicular hyperthermia induces germ cell apoptosis possibly through different pathways than those involved after gonadotropin deprivation and intratesticular T plays an important role in protecting germ cells from heat induced apoptosis at hormone dependent (VII-VIII) stages.

Involvement of apoptosis in specific germ cells undergoing degeneration has also been recognized in adult rat (Henriksen et al, 1995b) and mice (Yin et al, 1997) after experimental cryptorchidism. In short-term (24 and 48 h) experimentally cryptorchid rat-model, a significant increase in the number of apoptotic germ cells was evident in all stages, except for VI and VIII. The most sensitive cells to increased temperature were early pachytene spermatocytes and spermatocytes at meiotic divisions, as well as early spermatids. Also, in mice a significant increase in the apoptotic DNA fragmentation was observed on day 6 in the cryptorchid testis (Yin et al, 1997). Germ cells most sensitive to heat stress were primary spermatocytes and roud spermatids. Stage-specific effects of cryptorchidism were not evaluated in that study.

The mechanisms by which testicular hyperthermia induces germ cell apoptosis are not known. It is likely that apoptosis will be controlled in a cell-type specific fashion, but the basic elements of the death machinery may be universal. A distinct genetic pathway apparently shared by all miulticellular organisms. The Bcl-2 family of proteins which contains both proapoptotic (such as Bax) and antiapoptotic (such as Bcl-2) family members constitutes a central checkpoint within this pathway

(Farrow and Brown, 1996; White, 1996; Knudson and Korsmeyer, 1997; Kromer, 1997; Allen et al, 1998). Bcl-2 and Bax have also been implicated as potential modulators of germ cell apoptosis (Knudson et al, 1995; Furuchi et al, 1996; Knudson and Korsmeyer, 1997; Rodriguez et al, 1997).

Testicular Toxins. Two-methoxyethanol (2-ME) is a ethynene glycol ether produces testicular lesions characterized by pachytene spermatocyte degeneration in rats and guniea pigs (Ku et al, 1994). 2-ME is primarily metabolized via alcohol and aldehyde dehydrogenases to 2-Methoxyacetic acid (MAA) and which has been shown in previous toxicological studies to produce selective and stage-specific damages of rat pachytene spermatocytes (Creasy et al, 1985; Bartlett et al, 1988). Recent *in vivo* and *in vitro* studies have now demonstrated that apoptosis is the underlying mechanism of ME or MAA induced spermatocyte death (Brinkworth et al, 1995; Ku et al, 1995; Li et al, 1996). The occurrence apoptosis in the germ cells has also recently been documented in the adult rats after exposure to cyclophosphamide (70 mg/kg BW), a commonly used chemotherapeutic agent (Cai et al, 1997). Spermatogonia and spermatocytes at stages I-IV and XI-XIV are most susceptible to drug induced apoptosis.

Molecular Mechanisms of Germ Cell Apoptosis

The most important insight into the intracellular mechanisms that control germ cell apoptosis came from studies using genetically altered mice either over expressing or harboring a null mutation of specific genes. The Bcl-2 family of proteins constitutes an important control mechanism in the regulation of germ cell apoptosis. Expression of high levels of Bcl-2 or Bcl-x_L proteins in the germ cells result hyperplasia within the spermatogonial compartment with subsequent disruption of spermatogenesis due to accelerated apoptosis of the mature germ cells (Furuchi et al, 1996; Rodriguez et al, 1997). The ablation of the Bax gene by homologous recombination also results in male sterility due to accumulation of atypical premeiotic germ cells but with accelerated apoptosis of mature germ cells leading to complete cessation of sperm production (Knudson et al, 1995). These results also suggest that a proper balance between cell proliferation and death is critical for normal germ cell development. Mutation in Bcl-w, another anti-apoptotic member of the Bcl-2 family, has also recently been reported to cause male sterility (Ross et al, 1998). Mutant animals have a block in the later phases of spermatogenesis and exhibits progressive depletion of germ cells through accelerated apoptosis to a Sertoli-cell-only phenotype by approximately six months of age followed by loss of Sertoli cells. Bcl-w is expressed in the enongated spermatids and in Sertoli cells (Ross et al, 1998). It is likely that death of late spermatids is due to the absence of Bcl-w function in those germ cells, whereas depletion of the entire germline in adults reflects the loss of Bcl-w function in the Sertoli cell.

Other regulators of germ cell apoptosis have also been identified. For example, the ubiquitin system is also required for spermatogenesis, since inactivation of the HR6B ubiquitin-conjugating DNA repair enzyme in mice results in male infertility associated with disturbance in chromatin remodeling and accelerated germ cell

apoptosis (Roest et al, 1996). Another protein that has recently been implicated in the regulation meiosis is the mouse DMC1, an *E. coli* RecA homolog that is specifically expressed in leptotene and zygotene spermatocytes (Yoshida et al, 1998). Targeted gene disruption of DMC1 (disrupted meiotic cDNA) results in failed meiosis, accelerated spermatocyte apoptosis, and male infertility (Yoshida et al, 1998; Pittman et al, 1998).

Surprisingly, null mutation of some genes which are merely overexpressed in the testis, and are also expressed elsewhere, can accelerate germ cell apoptosis and cause specific defects in spermatogenesis. Examples of these mutations that affects germ cell apoptosis are knockouts of the Hsp (heat shock proteins) 70-2 (Dix et al, 1996), CREM (cyclic AMP-responsive element modulator) gene (Blandy et al, 1996; Nantel et al, 1996), and Atm (ataxia telangiectasia mutated) gene (Xu et al, 1996). Consequently, future studies with these and other genetically manipulated mice will continue to impact on our understanding how various extrinsic factors (such as survival factors deprivation, chemotherapeutic drugs and testcular toxins, DNA damage, heat stress etc.) may mediate life and death signals in germ cells through activation or repression of cell death genes.

SUMMARY

In summary, this review has attempted to highlight the role of apoptosis in spermatogeneis, as well as to address the intratesticular regulatory mechanisms that control germ cell apoptosis. Essentially all animal cells have the ability to kill themselves by activating an intrinsic cell suicide program when they are no longer needed or become seriously damaged. The execution of this program leads to morphologically distinct form of cell death termed apoptosis. It is now widely accepted that apoptosis is a genetically driven form of cell death and plays a major role during normal development, homeostasis, and in many human diseases. In mammals, germ cell death is conspicuous during spermatogenesis and occurs spontaneously at various phases of germ cell development and can be induced at a accelerated rate by various regulatory stimuli (such as derivation of survival factors, testicular hyperthermia, testicular toxins and chemotherapeutic agent). Using spermatogenesis as a model system for studying the regulation of germ cell death, recent studies have provided evidence that both spontaneous and induced germ cell death occur via apoptosis. Apoptosis in germ cells is not random but is highly selective and occurs after different interventions in specific stages of the seminiferous epithelial cycles. Analysis of germ cell apoptosis confirm and extends earlier findings that pituitary gonadotropins LH (via stimulation of T) and, to a lesser extent, FSH are important extrinsic regulators of germ cell apoptosis. As would be expected from the vast amount recent literature, the control of germ cell apoptosis is complex and regulated by multiple genes that either inhibit or promote cell death. Future efforts toward improved fertility control and clinical management

of infertility with reduced sperm production are hampered by incomplete understanding of the process responsible for germ cell homeostasis. Elucidation of the mechanisms by which various proapoptotic and antiapoptotic genes control spermatogenesis will fill a major gap in our knowledge of this fundamental biologic process.

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3 PARACRINE CONTROL OF TESTIS FUNCTION

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INTRODUCTION

Two concepts have been put forward to explain the regulation of testicular function. Firstly, it has been suggested that testicular function is regulated primarily by pituitary gonadotropins (endocrine regulation). Secondly, it has been proposed that the "testis is not a mass of independently developing cells" (Roosen-Runge, 1952), so there are presumably local regulatory mechanisms to coordinate the activities of testicular cells (paracrine regulation). It is now generally accepted that there is no conflict between the concepts of peripheral control of testicular function and intratesticular control. The integration of these two concepts accounts for gonadotrophins regulating testicular paracrine activities and some paracrine factors regulating the testicular effects of pituitary hormone and exerting endocrine control over pituitary function .

Major progress has been made over the last decades towards understanding functioning of the pituitary-testicular axis because the communication network is simple: the pituitary and testis are located at large anatomical distances from one another. This makes possible their manipulation and the collection of blood and lymph transporting pituitary and testicular hormones. In contrast, progress towards deciphering the multiple elements of the intratesticular communication network has been very slow due to the structural complexity of the testis. However, the main elements of testicular paracrine regulation are beginning to emerge.

ORGANIZATION OF THE TESTIS

The testis consists of the convoluted seminiferous tubules embedded in a connective tissue matrix, the interstitium (Figure 1). The seminiferous tubule comprises the germ cells in various phases of development, the non-proliferating Sertoli cells, between or in which germ cells are embedded, and the peritubular cells, which

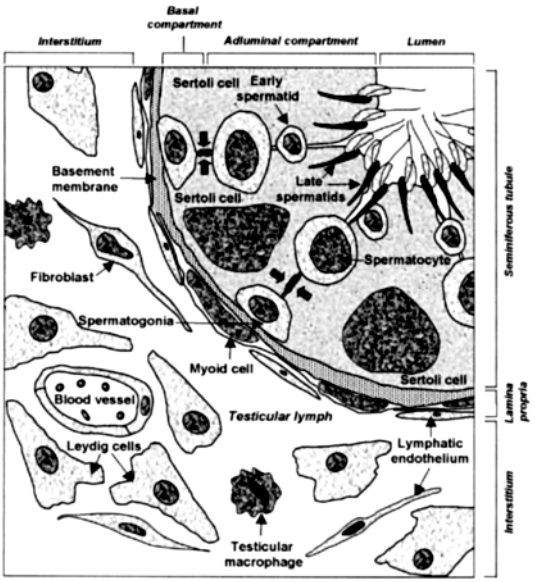

Figure 1. Schematic representation of the mammalian testis. The interstitial compartment, which is highly vascularized, contains principally the testosterone-producing Leydig cells, fibroblasts and macrophages. The seminiferous tubules, bounded by a basal membrane (lamina propria) contain the somatic Sertoli cells and the germ cells at various stages of development. The large arrows within the tubule indicate the tight junctions between Sertoli cells which delimit the basal compartment. This compartment contains the spermatogonia and newly-formed primary spermatocytes, which are not shown in this representation. The adluminal compartment contains mature spermatocytes and spermatids. This diagram is not drawn to scale.

surround the Sertoli and germ cells. Germ cells, which are continuously renewed, and Sertoli cells, which cease to divide during puberty, form the seminiferous epithelium. The seminiferous tubules are the site of spermatogenesis and account for 60 to 80 % of total testicular volume (Weinbauer et al, 1997). Spermatogenesis has three phases: (1) a phase of rapid mitotic division and cell replacement in the spermatogonia, often called the proliferative phase; (2) a meiotic phase involving the spermatocytes and (3) spermiogenesis, the process by which the spermatids produced by meiosis are transformed into spermatozoa. Spermatogenesis lasts 35 days in the mouse, 53 days in the rat and 74 days in man (Clermont, 1972).

The anatomical complexity of the seminiferous epithelium is entirely unique (Fawcett, 1975). Along the length of the seminiferous tubule, generations of germ cells simultaneously divide and differentiate, passing from the basal membrane to the lumen. The cells are in direct anatomical and functional contact with the Sertoli cells throughout this process.

Spermatogenesis is strictly coordinated both across the tubules, with germ cell differentiation occurring exclusively in the transverse direction with the progenitor cells on the basal side and the spermatozoa released into the lumen, and along the length of the tubules. Spermatogenesis across the tubules is controlled by a number

of germ cell associations of stable composition. Each type of association defines a stage in the cycle of the seminiferous epithelium. The cycle of the seminiferous epithelium occurs at all points along the tubule and involves a repeating series of associations that occur in a given chronological order. The duration of the cycle is the time interval between two phases of entry of spermatogonia into spermatogenesis at a particular point along the tubule (about nine days in mice, thirteen days in rats and sixteen days in humans) (Leblond and Clermont, 1952). Spermatogenesis is also coordinated along the length of the tubule, with the cellular associations occurring in the same order as the stages of the cycle. This succession is known as the spermatogenic wave (Regaud, 1901). It is linear in most mammals but may be helical in man and baboons and both segmented and helical in another primate, *Macaca fascicularis* (Jégou, 1993; Sharpe, 1994). However, a recent study has provided evidence that the spermatogenic wave in man may not be helical (Johnson, 1994). The extreme anatomical complexity of the association between germ cells and Sertoli cells is a clear indication of their total structural and functional interdependence.

The interstitium contains blood and lymph vessels, the Leydig cells, nerves, fibroblasts, macrophages, lymphocytes and occasional mast cells. The primary function of the Leydig cells is the production of testosterone, essential for differentiation of the embryonic male reproductive organs, the sexualization of the hypothalamus and brain, the formation of the accessory sex organs and spermatogenesis.

CELL TO CELL COMMUNICATION WITHIN THE INTERSTITIAL COMPARTMENT

The principal types of cell in the interstitium are: Leydig cells, macrophages, fibroblasts, lymphocytes and mastocytes. The proliferation of mast cells and lymphocytes is inhibited by Leydig cells (Born and Wekerle, 1982; Gaytan et al, 1990 ; Wang et al., 1994). Little else is known apart from aspects of the relationship between Leydig cells and macrophages.

Macrophages account for up to 25 % of all cells in the interstitium of sexually mature normal mammals (Fawcett *et al.*, 1973; Niemi *et al.*, 1986). The ratio of macrophages to Leydig cells is about 1:4 in the rat (Bergh, 1985). Structural and functional coupling of these two cell types has been observed.

The structural basis of Leydig cell – macrophage coupling

Projections of Leydig cell cytoplasm extend into neighbouring macrophages (Christensen and Gillim, 1969; Miller et al, 1983). In the normal testis, this coupling leads to small fragments of Leydig cells undergoing endocytosis by macrophages (de Kretser and Kerr, 1988), resulting in the passage of information between these cells (Hutson, 1992). These cellular digitations may also be used to anchor the cells. They appear in the rat at 20 to 30 days of age and consist of anything from simple tubular projections to complicated branched structures (Hutson, 1992). Subplasmalemmal linear densities were also observed at sites of contact between

macrophages and Leydig cells, and collagen is often detected in the area between macrophages and Leydig cells in rats younger than 30 days (Hutson, 1992). Macrophages engulf Leydig cells, which then undergo phagocytosis, in cases of impairment of testicular function by the cytotoxic drug ethane dimethanesulfonate (EDS) (de Kretser and Kerr, 1988).

The functional basis of Leydig cell – macrophage coupling

Macrophages and Leydig cells are also functionally coupled. Macrophage production is precociously induced in the testes of neonatal rat by stimulating Leydig cells with hCG (Raburn et al, 1991; Gaytan et al, 1994a). However, the identity of the Leydig cell factors responsible for the control of macrophage proliferation and activity is unknown. Gaytan et al (1994b) showed, in studies on the regeneration of Leydig cells after treatment with EDS, that macrophages are required for the differentiation of Leydig cells from mesenchymal precursors, and the proliferation of the newly formed Leydig cells. Furthermore, in experiments in which rat testicular macrophages were selectively depleted by intratesticular injection of liposomes containing dichloromethylene diphosphonate, macrophages were found to be required for Leydig cell proliferation and differentiation during postnatal development (Gaytan et al, 1994c). Gaytan and collaborators also showed that the proliferation of Leydig cells in response to hCG depends on the number of macrophages present. This suggests that the increase in the number of macrophages or their maturation during postnatal testicular development is a key factor determining the responsiveness of the Leydig cell to LH.

The mechanisms mediating the effects of macrophages on Leydig cells are unknown. In the adult testis, the effect of macrophages probably does not depend purely on to direct cell cell contacts. Macrophages produce factors *in vitro* that stimulate testosterone production by Leydig cells (Yee and Hutson, 1985). Consistent with this, previous studies have shown that TGFα and IL-1 β, two cytokines produced (not exclusively) by macrophages (Kern et al, 1995; Hedger, 1997), stimulate radioactive thymidine incorporation into immature Leydig cell DNA (Khan et al, 1992a,b). Testicular macrophage-produced TNF-α may also be involved in steroid production in Leydig cells (Moore & Hutson, 1994). TGF-α, IL-1 β and TNF-α receptors (or mRNA) have been detected in Leydig cells (Cunningham et al, 1992; Gomez et al, 1997; Mauduit et al, 1991; Sordoillet et al, 1991; Stubbs et al, 1990; Suarez-Quian et al, 1989; Takao et al, 1992)

The very close interdependence of testicular interstitial cells has also been demonstrated by experiments showing that the reappearance of Leydig cells in EDS-treated rats occurs at the same time as macrophage regeneration and the regeneration of blood vessels (de Kretser and Kerr, 1988).

CELL TO CELL COMMUNICATION WITHIN THE SEMINIFEROUS TUBULE

The structural basis of germ cell-Sertoli cell coupling

Sertoli cells and germ cells are connected *via* a unique set of structures of three types (Jégou, 1993) : (1) those involved in cell attachment, movement and shaping, such as desmosome-like and ectoplasmic specializations; (2) those that fulfill these functions but are also involved in the transfer of molecules and materials from Sertoli cells to germ cells or from germ cells to Sertoli cells. The spermatogonial processes, tubulobular complexes, spermatid processes and gap junctions belong to this category; (3) those exclusively involved in transfer, such as residual bodies, which are the portions of spermatid cytoplasm left behind when the mature sperm detaches from the Sertoli cell apex at spermiation. They then undergo phagocytosis by Sertoli cells. The location of these structures is indicated in Figure 2.

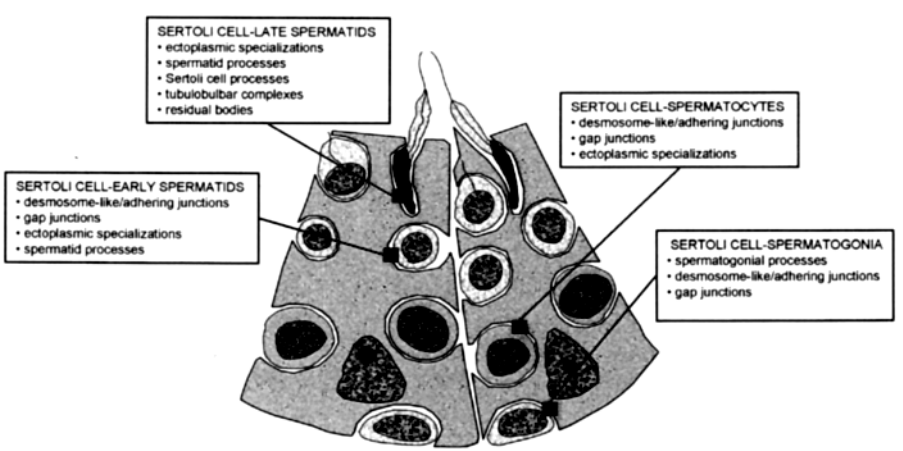

Figure 2. Structural basis of Sertoli cell/germ cell interactions (for references, see: Jegou, 1993).

In addition to the direct structural contacts between germ cells and Sertoli cells, specialized inter-Sertoli cell junction complexes play a key role in the control of spermatogenesis. The tight junctions that form between Sertoli cells during post-natal testicular development make up the Sertoli cell barrier (Ploën and Setchell, 1992), a key element of the so-called blood testis barrier, the function of which is to prevent various blood and lymph factors from entering the areas of the seminiferous tubules in which meiosis and spermiogenesis occur (Jégou, 1992). One spectacular example of the reciprocal control of Sertoli and germ cells is the synchronous breakdown and reconstruction of the Sertoli cell barrier, which occurs during the meiotic phase, when clusters of spermatocytes migrate from the basal to the adluminal compartment of the tubule (Russell, 1977).

The functional basis of germ cell-Sertoli cell coupling

Identifying the factors produced by the various tubular cells that are involved in cell to cell communication is an extremely difficult task due to the intricacy of the seminiferous epithelium cells. It is therefore not possible to collect these factors *in situ*, at their site of production and action. Tubule cells must be isolated and cultured to study and identify the factors they produce or to characterize the receptors they possess. However, very few germ cell types can be isolated intact with a high degree of purity (some spermatogonial types; pachytene spermatocytes and early spermatids) and, once isolated, these cells die very rapidly in culture. This is one of the best illustration of the dependency of germ cells on the structural and functional support of Sertoli cells.

Sertoli cell control of spermatogenesis. The position of Sertoli cells within the seminiferous tubules makes it possible for them to play a key role in the control of spermatogenesis. Sertoli cells supply the factors required for germ cell generation and probably also help synchronize the development of germ cells at all stages of the epithelium cycle, in the transverse axis of the tubule, and maintain the wave of spermatogenesis along the longitudinal axis.

Sertoli cells produce the tubule fluid, at least 100 peptides and proteins, and several steroids (Bardin et al, 1988; Jégou, 1992; 1993). The function of most Sertoli cell products is to assist germ cells through spermatogenesis. The tubule fluid is essential for the nutrition of germ cells, and for the transport of signals in the transverse axis of the seminiferous epithelium, and along the length of the tubule. It is also required for the transport of spermatozoa along the tubules, from the tubules to the rete testis and thence to the epididymis. We classified Sertoli cell substances into six categories: (1) - factors required for germ cell division and differentiation (Figure 3) ; (2) - transport or binding protein components; (3) - proteases; (4) - extracellular matrix components; (5) - energy metabolites and (6) - Sertoli cell membrane components and junction complexes (Table 1). The compartmentalization of the seminiferous epithelium, which results from the existence of the Sertoli cell barrier, leads to the bidirectional secretion of its products. Thus, most of the Sertoli cell substances preferentially secreted towards the apex of the cell and the tubule lumen are specifically required for

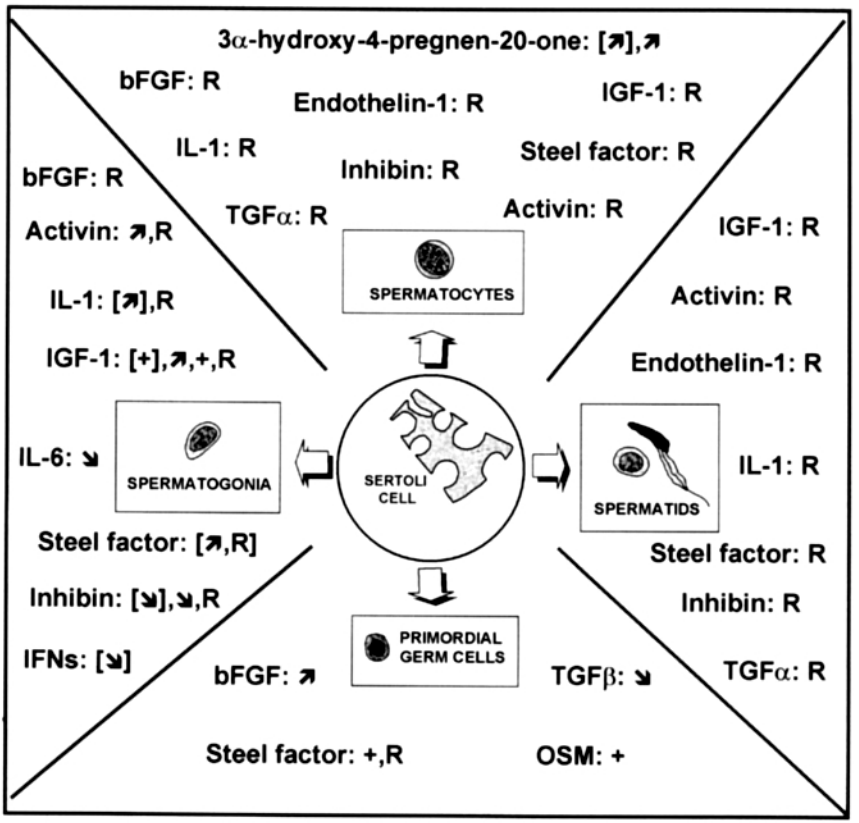

Figure 3. Sertoli cell factors with potential paracrine effects on germ cells. bFGF: basic fibroblast growth factor; IGF-1: insulin-like growth factor I; IL-1: interleukin-1; IL-6: interleukin-6; TGF α and β: transforming growth factor α and β; IFNs: interferons α/β; OSM: oncostatin-M ↗ : stimulates proliferation; ↘: inhibits proliferation; +: stimulates function; R: receptor or receptor mRNA detected; Abbreviations between brackets: effects also shown in vivo. TGFα: receptors detected on spermatozoa. Note that the identification of most, if not all, of the factors result from in vitro experiments. For references, see Gnessi et al, 1997; Piquet-Pellorce & Jegou, 1996b; see also Cudicini et al, 1997 a, b; Dejucq et al, 1997; De Miguel et al, 1996; Gomez et al, 1997; Kanzaki & Morris, 1998; Olaso et al, 1998.

spermatogenesis and maintenance of the newly formed spermatozoa. Early germ cells, myoid and Leydig cells may be the targets of other Sertoli cell factors secreted towards the basal compartment.

Germ cell control of Sertoli cell function. Changes in the composition of the germ cell complement, germ cell size and shape, division and migration, markedly affect Sertoli cell morphology and function in a cyclic pattern (Jégou, 1993). Several *in situ / in vivo* approaches have been used to explore these cyclical changes: - the "morphological approach", *in situ*, using histochemical and immunohistochemical

techniques, radiolabeling, and *in situ* hybridization ; - "transillumination-assisted microdissection", which is based on light absorption producing specific transillumination patterns for rat tubules at different stages, allowing their

Table 1 : Selection of Sertoli cell products thought to control germ cell activities.

Product	Putative function
Transport binding proteins	
Transferrin & ceruloplasmin	Important in iron and copper transport into germ cells
Androgen-binding protein (ABP)	Transport of androgens, including that to germ cells
Retinol-binding protein	Transport of retinol to postmeiotic germ cells
Sulfated Glycoprotein 1 (SGP1)/ prosaposin	Carrier of lipid precursors and specific fatty acids to germ cells
Sulfated Glycoprotein 2 (SGP2)/ clusterin	Delivery of lipids to germ cells and serum
α-macroglobulin	Binds and therefore regulates action of growth factors, cytokines, activin and inhibin
γ glutamyl transpeptidase (γ GTP)	Transport of glutathione to germ cells
Proteases and proteases inhibitors	
Urokinase , tissue-type plasminogen activators, type IV collagenase and other matrix metalloproteinases	May be involved in junction complex opening and closure, thus facilitating germ cell displacement. May also be involved in basement membrane remodelling .
Cyclic Protein 2 (CP2)/procathepsin L	Involved in sperm release
Cystatin C	Inhibitor of cathepsin L
Extracellular matrix components	
Types I and IV collagen, laminin, proteoglycans, fibulins	Required for the tubular cyto-architecture, for cell to cell com-munication and for the storage and action of various factors
Energy metabolites	
Lactate and pyruvate	Required for germ cells that are unable to metabolize glucose
Anti-oxidants	
Glutathione	Transferred to germ cells
Junction complex components	
Testins	Sertoli-germ cell junction complex proteins
Other membrane components	
γ GTP	See above
Liver-Regulating Protein (LRP)	Regulates Sertoli cell-primary spermatocyte interactions

microdissection. The tubule segments thus obtained are studied as extracts or inculture (Parvinen, 1993); - the "stage-synchronized" approach: in which vitamin A (retinol) supplements are given to vitamin A-deficient rats or mice in which spermatogenesis is at the spermatogonial level. This restores normal but synchronized spermatogenesis, making it possible to obtain testes from these animals at the desired stages of the cycle (Griswold, 1990). These three approaches have been used to demonstrate marked changes in Sertoli cell activity according to the germ cell associations made during the seminiferous cycle.

It is not possible to determine the contribution of a given germ cell type to the regulation of Sertoli cell activity in studies of the stage-related changes in Sertoli cell morphology and function. However, various agents known to be deleterious to the germ cell complement can be used to study the specific involvement of particular germ cell types in Sertoli cell control. These agents may be physical (irradiation, heat) or chemical (e.g. anti-mitotic drugs), and they cause selective destruction of a particular germ cell type or of a limited number of germ cell types. As the surviving germ cells differentiate, maturation-depletion results in a progressive and sequential decrease in the other germ cell types, making it possible to study Sertoli cell activity. The approach has led to the demonstration of the key role played by elongating and elongated spermatids in the control of Sertoli cell structure and function (Jégou et a., 1992; Jégou, 1993; Sharpe, 1993). This control is either positive (e.g. tubule fluid, ABP) or negative (e.g. testins, FSH-simulated cAMP production). Other germ cell types also control Sertoli cell function (Sharpe, 1993) and, at each stage of testicular development, Sertoli cell activity is may be regulated by the most advanced generation of germ cell within the seminiferous epithelium (Jégou, 1991; 1993). The only study in humans is that of Rodriguez-Rigau et al (1980) who showed that late spermatids positively control inhibin production in patients who have undergone varicocelectomy.

Cell separation techniques, cell cultures, and cocultures have also made it possible to obtain insight into the control of Sertoli cell function by germ cells. Cocultures have been set up using Sertoli cells isolated from rats of various ages, and pachytene spermatocytes, early spermatids or residual bodies/cytoplasts from elongated spermatids, from adult animals (mostly rats). Conditioned media have been prepared by incubation with the various germ cell preparations. Germ cell activity in coculture has been shown to depend on the germ cell type tested, the Sertoli cell characteristic considered, the age of the Sertoli cell donor, and the presence or absence of hormones in culture.

This *in vitro* approach demonstrated that spermatocytes and early spermatids together with hormones (FSH, testosterone) play a key role in Sertoli cell development (Jégou and Sharpe, 1993).

Various pathways for germ cells control over Sertoli cells have been suggested. Firstly, membrane molecules and morphoregulatory mechanisms have been implicated. Membrane molecules such as the Sertoli cell testins (Zong et al, 1994), which are components of junction complexes, and the liver regulating protein (Gérard et al, 1994), which is produced by both early primary spermatocytes and the Sertoli cells, are also involved in communication between germ and Sertoli cells.

Changes in Sertoli cell shape resulting from permanent changes in the nature and composition of germ cells probably result in the physical regulation of transcription,

via changes in the contacts between the cytoskeleton and the nuclear matrix of the Sertoli cell (Byers et al, 1993; Jégou et al, 1992). Secondly, the transfer of germ cell materials has been implicated. The transfer of parts of cells from spermatogonia and late spermatids to Sertoli cells is a specific feature of the germ cell – Sertoli cell

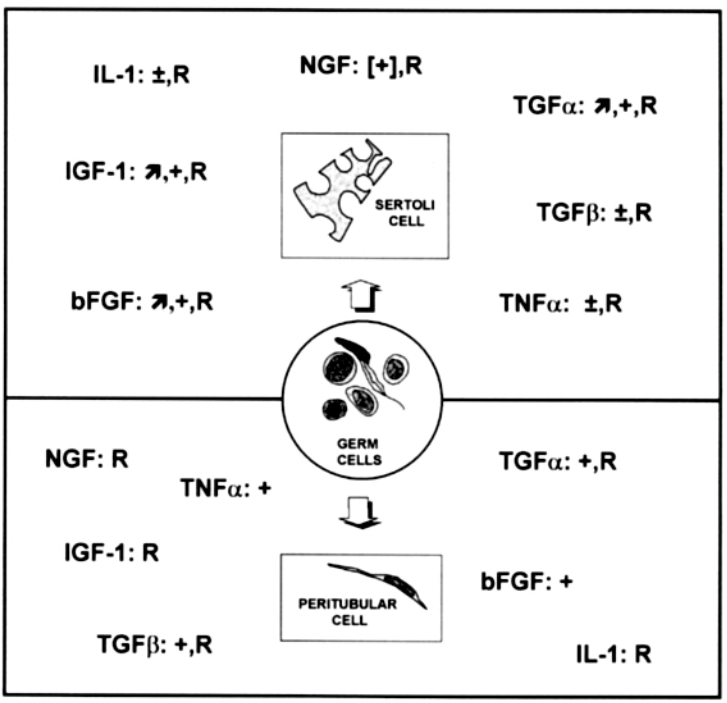

Figure 4. Germ cell factors with potential paracrine effects on Sertoli cells and peritubular cells. TNFα: tumor necrosis factor α; NGF: nerve growth factor; ±: stimulates or inhibits depending on the conditions. See legend to figure 3 for other abbreviations. TGFα also inhibits FSH-stimulated extradiol production. Note that the identification of most, if not all, of the factors result from in vitro experiments. For references, see Gnessi et al, 1997; Piquet-Pellorce & Jegou, 1996b; Gomez et al, 1997.

dialog (Jégou, 1993). The effect of spermatogonial processes on Sertoli cell function is unknown. There is evidence that the phagocytosis of residual bodies may trigger a cascade of events resulting in changes in Sertoli cell activity and, possibly spermatogenesis. Residual bodies activate Sertoli cell IL-1α mRNA and protein and Sertoli cell IL-1 receptor type I (Gérard et al, 1991, 1992; Wang et al, 1998). IL-1α stimulates IL-6 by an autocrine mechanism involving the lipoxygenase pathway (Syed et al, 1993; 1994). IL-1α and IL-6 have been implicated in the stimulation and inhibition of spermatogonial division and meiotic DNA synthesis (Parvinen et al, 1991; Hakovirta et al, 1995, respectively). Thus, the "message" sent by

elongated spermatids in the form of residual bodies probably regulates Sertoli cell function, with important consequences for germ cell division and differentiation.
Thirdly, germ cell soluble proteins are thought to be involved. Germ cells produce several proteins, "germins", that either stimulate or inhibit Sertoli cell activity (Jégou, 1993; Jégou and Sharpe, 1993). Attempts have been made to purify some of these proteins (Pineau et a., 1993; Onoda and Djakiew, 1993). There is now clear evidence that germ cells produce a number of molecules for which receptors are present on Sertoli cells. These molecules are therefore able to exert various effects on Sertoli cells (Figure 4).

The second messenger pathway for germ cell mediated-Sertoli cell regulation has been little studied. The cAMP pathway is not thought to play a key role (Fujisawa et al, 1992), but the inositol triphosphate/diacylglycerol pathway may be important (Welsh and Ireland, 1992).

COMMUNICATION BETWEEN PERITUBULAR CELLS AND THE SEMINIFEROUS EPITHELIUM

Communication between the peritubular myoid cells bordering the seminiferous tubules and the Sertoli cells begins during fetal development and continues during the postnatal development of the testis (Paranko et al, 1983; Bressler and Ross, 1972; Tung and Fritz, 1986a,b).

One of the most important functions of peritubular cells is to ensure the structural cohesion and contraction of the seminiferous tubule. They are also part of the blood testis barrier in rodents (Ploën & Setchell, 1992) and interact with Sertoli cells to produce the factors constituting the tubular *lamina propria* (basement membrane; BM) (Skinner et al, 1985; Dym 1994). The extracellular matrix secreted by peritubular cells and Sertoli cells that forms the tubule BM plays a key role in tubule cord formation, peritubular and Sertoli cell proliferation, Sertoli cell polarity and the control of peritubular and Sertoli cell differentiation (Jégou and Sharpe, 1993; Dym, 1994; Loveland et al, 1998). Gelatinase A, which is probably involved in remodeling of the testicular BM, is present at high concentrations in peritubular cells and at lower concentrations in Sertoli cells (Hoeben et al, 1996a). It has also been suggested that the early postnatal loss of fibulins (Ca^{2+} binding extracellular matrix proteins) and the appearance of collagen $\alpha 3$ (IV) chains in the seminiferous tubule BM stop peritubular and Sertoli cell proliferation as these cells differentiate and intervene in the initiation of germ cell differentiation, which involves the adhesion of gonocytes to the tubule BM (Loveland *et al.,* 1998). The effect that the tubule BM may have on spermatogonia located on the tubular BM is unknown.

A metabolic cooperation between peritubular cells and Sertoli cells (Hutson, 1983) has been demonstrated. Peritubular cells have been shown, *in vitro,* to stimulate the production of Sertoli cell total proteins (Hadley et al, 1985), ABP (Tung and Fritz, 1980; Hutson and Stocco, 1981) and transferrin (Holmes et al, 1984; Hadley et al, 1985). Peritubular cells regulate Sertoli cell enzyme activities (Cameron and Snydle, 1985) and markedly increase the response of Sertoli cells to androgens (Verhoeven, 1992).

The first attempt to identify a peritubular cell factor responsible for control of Sertoli cell function was made by Skinner and co-workers. They proposed that peritubular cell effects are mediated *in vitro* by a non-mitogenic factor called P-Mod-S (peritubular factors that modulate Sertoli cell function) (Skinner, 1991). However, the exact molecular nature of P-Mod-S has not been determined despite years of research and it now seems most likely that P-Mod-S activity results from a combination of factors, probably including heregulin-α and several cytokines (IL-1, IL-6, IGF-1, bFGF) (Hoeben et al, 1996b, Hoeben, 1997).

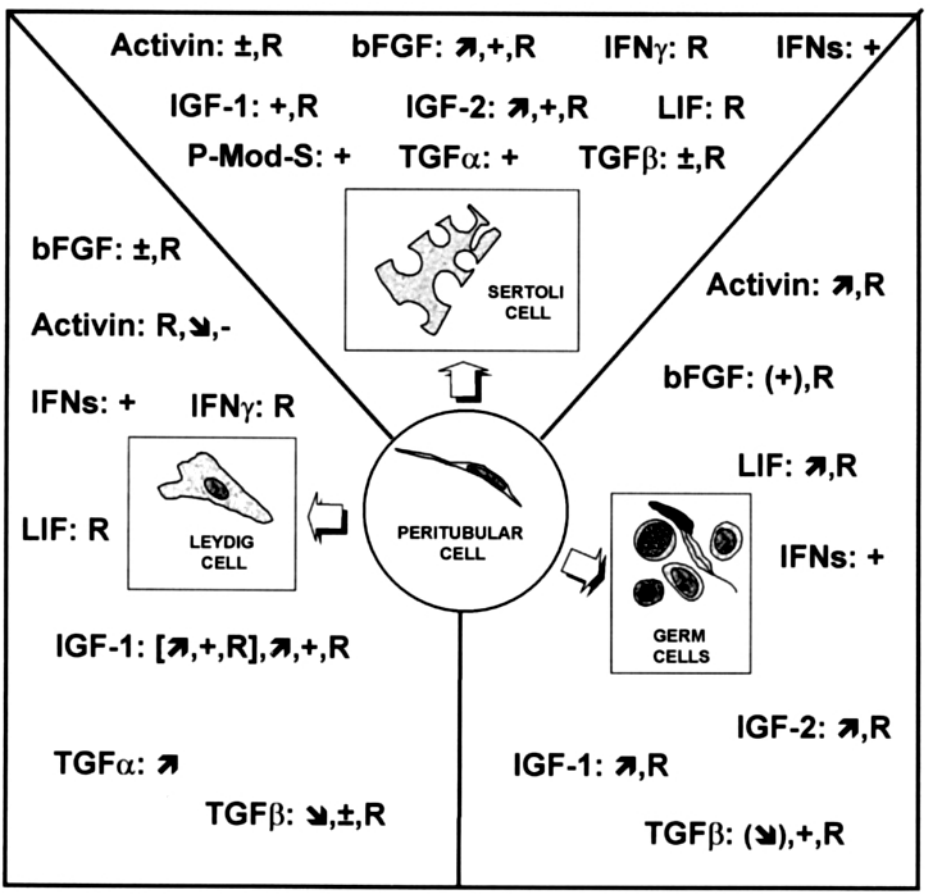

Figure 5. Peritubular cell factors with possible paracrine effects on Sertoli, germ cells and Leydig cells. LIF: leukemia inhibitory factor; IFN γ: interferon γ (↘): inhibits PGC proliferation; (+): stimulates PGC survival. See legends to previous figures for other abbreviations. Note that the identification of most, if not all, of the factors result from in vitro experiments. For references, see Gnessi et al, 1997; Piquet-Pellorce & Jegou, 1996b; Dejucq et al, 1997; Kanzaki & Morris, 1998; Piquet-Pellorce et al, 1998.

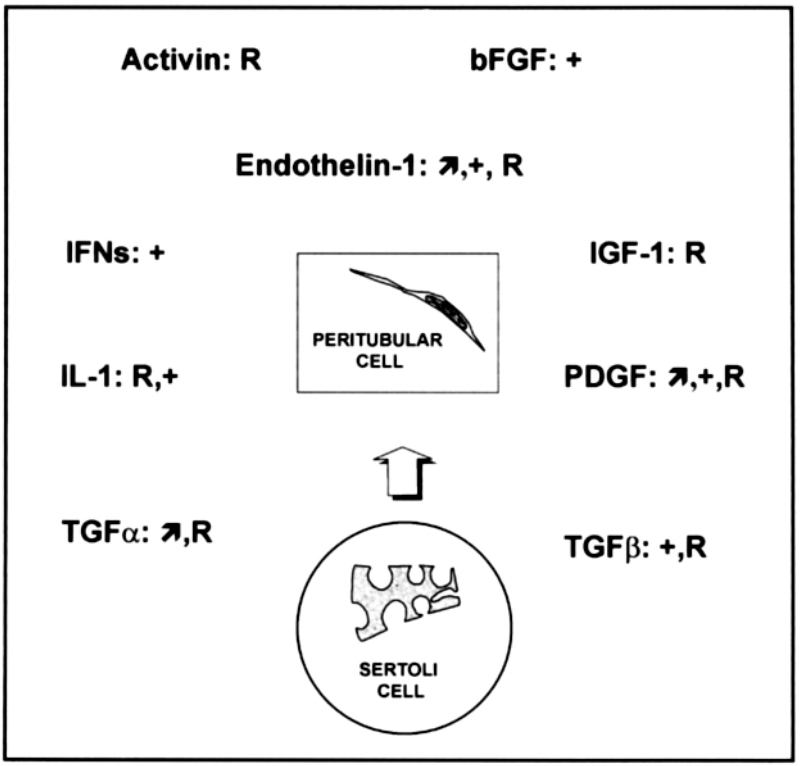

Figure 6. Sertoli cell factors with possible paracrine effects on peritubular cells. PDGF: platelet-derived growth factor. See legends to previous figures for other abbreviations. Note that the identification of most, if not all, of the factors result from in vitro experiments. IL-1 stimulates NO synthase activity in peritubular cells (Bauche, Touzalin & Jegou, unpublished results). For references, see Gnessi et al, 1997; Piquet-Pellroce & Jegou, 1996b; Dejucq et al, 1997.

Peritubular cells are the principal source of leukemia inhibitory factor (LIF) within the testis (Piquet-Pellorce et al, 1996a) and LIF receptors are present on Sertoli cells and spermatogonia (Piquet-Pellorce et al, 1998). Furthermore, LIF increases Sertoli cell and gonocyte survival (de Miguel et al, 1996), and the incorporation of thymidine into B spermatogonia *in vitro* (Piquet-Pellorce et al, manuscript in preparation). Germ cells may also regulate peritubular cell activity (Figure 4).

Other peritubular cell products that may be involved in the paracrine control of Sertoli cells and in germ cell activity are listed in Figure 5. Establishing that these products indeed regulate Sertoli and germ cells is however, masked by the production of many of these growth regulators by the Sertoli cell and germ cells themselves.

In turn, many Sertoli cell products are putative regulators of peritubular cells (Figure 6).

CELL TO CELL COMMUNICATION BETWEEN THE INTERSTITIAL COMPARTMENT AND THE SEMINIFEROUS TUBULE

The organization of the testis into two compartments, resulting in separation of the sites of gamete generation and steroid production, is highly conserved between species (Pilworth and Setchell, 1991; Loir et al. 1995). However, it is widely accepted that the steroidogenic interstitial compartment and the germinal compartment interact functionally.

Effect of Leydig cell on the seminiferous tubule cells

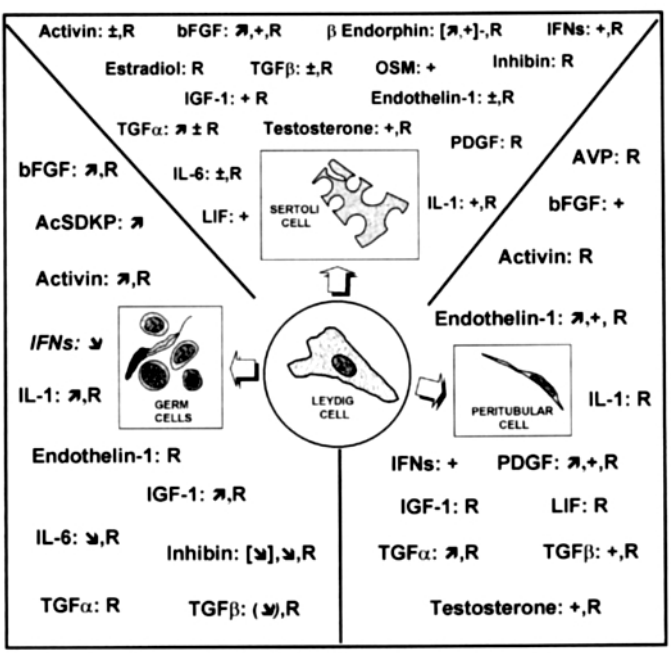

Figure 7. Leydig cell factors with possible paracrine effects on peritubular, Sertoli and germ cells. AcSDKP: N-acetyl-Ser-Asp-Lys-Pro; AVP: arginine vasopressin. See legends to previous figures for other abbreviations. In the interstitium, in addition to the Leydig cell factors, TNFα, IL-1 and IL-6 are produced by testicular macrophages. TNF receptor has been found on Sertoli and peritubular cells and found to stimulate activities of these cells. IL-1 and IL-6 receptors/mRNA have also been found on Sertoli and germ cells. Note that the identification of most, if not all, of the factors result from in vitro experiments. For references, see Gnessi et al, 1997; Piquet-Pellroce & Jegou, 1996b; Dejucq et al, 1997; Kern et al, 1995; Piquet-Pellorce et al, 1998; Stephan et al, 1996.

Testosterone was the first paracrine intratesticular factor to be identified and it is one of the few for which a function as a local regulator of spermatogenesis has been

clearly demonstrated in animals and humans. Much is known about its production, reception and action within the testis. For example, the elimination of Leydig cells by the chemical toxin EDS, results in the virtual disappearance of testosterone from the interstitial fluid and marked changes in spermatogenesis. High local concentrations of androgens, such those produced by a Leydig cell tumor, lead to fully active spermatogenic seminiferous tubules in contact with the tumor, whereas the tumor-free areas of the testis have only immature spermatogenesis (Chemes et al, 1982).

The precise targets of testosterone within the tubule are also well known. Androgen receptors have been detected in both peritubular and Sertoli cells (Verhoeven, 1992) and testosterone elicits a number of biological responses in both these cell types. In monkey, testosterone induces the production of α-smooth muscle action in peritubular cells (Schlatt et al, 1993) and, in the rat, it stimulates P-Mod-S activity which regulates Sertoli cell function (Skinner and Fritz, 1985; Verhoeven and Cailleau, 1988). Testosterone also directly stimulates Sertoli cell function (Jégou, 1992).

Leydig cells also produce a number of peptides and proteins with putative paracrine activity (Figure 7). Some may regulate aspects of tubule function because their receptors are present on various tubule cell types and several have been shown to affect the activity of tubule cells both *in vivo* and *in vitro*. Many of these factors are ubiquitous in distribution and action in the testis, so establishing that they are indeed involved in mediating Leydig cell–tubule cell interactions and determining their precise functions is very difficult.

Effect of tubule cells on the activity of interstitial cells

There is considerable, but mostly indirect evidence that the seminiferous tubules affect the function of the interstitial compartment. However, the precise nature of the factors involved is often difficult to determine.

Various approaches have been used to tackle this aspect of testicular paracrine regulation:

- *in situ* studies in the rat have shown that there are cyclic changes in the size and volume of the smooth endoplasmic reticulum (SER) of Leydig cells depending on the stage of the seminiferous epithelium of neighboring tubules (Bergh, 1982, 1985; Fouquet, 1987). For example, Leydig cell size and SER volume are highest at stages VII-VIII of the cycle, at which the tubule is the more androgen-sensitive (Bergh, 1982; 1985);
- *in vivo* and *in vitro* studies and observations in humans have demonstrated that FSH, for which there are receptors on the Sertoli cell, controls Leydig cell development (Kerr and Sharpe, 1985), increases the number of Leydig cells, and increases their responsiveness to LH and their steroidogenic activity (Johnson and Ewing, 1971; Teerds et al, 1989; Odell and Swerdloff, 1976). Sizonenko et al, (1977) have also shown that Leydig cell activity and the response to hCG depend on circulating FSH levels in hypopituitary patients and cryptorchid children;
- *in vivo* studies have shown that the disruption of spermatogenesis always affects the number of Leydig cells (but not of macrophages) (Hedger, 1997), their morphology and function. These changes are restricted to the affected testis

(unilateral treatment) or to the affected region of the testis (Verhoeven, 1992; Jégou and Sharpe 1993) ;

- *in vivo* experiments have demonstrated that Leydig cell regeneration after elimination by EDS treatment is more rapid in the vicinity of tubules lacking germ cells than in regions rich in germ cells (Sharpe et al, 1990). Thus, Leydig cell regeneration involves germ cell factors or germ cell-regulated Sertoli cell factors;

- *in vivo* experiments, with rat testes in studies of neonatal hypothyroidism-induced Sertoli cell proliferation, have shown that the more Sertoli cells there are, the more Leydig cells there are. The ratio between these two cell types remains constant (Kirby et al, 1992);

- *in vitro* culture and co-culture experiments have shown that Sertoli cells or Sertoli cell-conditioned media stimulate or inhibit Leydig cell testosterone production in the presence or absence of LH/hCG in pigs, rats, humans and mice (Jégou and Sharpe, 1993). A 70 kDa protein complex has been purified from rat Sertoli cell culture medium. This complex stimulated androgen secretion by isolated Leydig cells and by a mouse Leydig cell line (MA-10). The complex was formed from the 28 kDa tissue inhibitor of metalloproteinase-1 (TIMP-1) and the 38 kDa proenzyme form of cathepsin-L, also known as cyclic protein 2 (CP2) in the testis (Boujrad et al, 1995) ;

- *in vitro*, interstitial fluid from adult rat testis has been shown to contain factors that stimulate basal and LH/hCG-induced testosterone production by Leydig cells. These effects are far more marked than those observed with Sertoli-Leydig cell cocultures, and are greater if the fluid is collected from testes with disrupted tubular function or if testosterone is withdrawn. The exact nature of the factors involved in these effects is unknown, despite a number of investigations being carried out (Jégou and Sharpe, 1993).

The active factors in Sertoli cell-conditioned media and tubule interstitial fluid are extremely difficult to identify and purify. Therefore, another approach to the study of tubule-interstitium interactions has been used in which it was investigated whether there were receptors on interstitial cells for factors known to be produced by tubule cells (or their mRNA) or whether these factors have any effect on these cells *in vitro*.

As for Leydig cells, one of very first Sertoli cell factors identified and characterized as a putative Leydig cell regulator is a steroid, estradiol. Leydig cells have large numbers of estradiol receptors (Brinkmann et al, 1972; Fisher et al, 1997; Cobellis et al, 1998). Sertoli cells produce large amounts of aromatase before the Sertoli cell barrier is formed (15 to 18 days in the rat). After the formation of this barrier, estradiol is produced by the Leydig (Jégou, 1992) and germ cells (Nitta et al, 1991; Janulis et al, 1998; Levallet et al, 1998). Therefore, the effects of Sertoli cell estradiol on Leydig cell activity are probably restricted to the fetal and neonatal periods, at which time estradiol is known to be involved in the negative control of Leydig cell proliferation and perhaps also of differentiation (Jégou and Sharpe, 1993). Sertoli cells may also produce an aromatase inhibitor (Boitani et al, 1981), the concentration of which is strongly affected by the seminiferous epithelium cycle. This inhibitor may be also involved in the control of Leydig cell activity by Sertoli cells (Sharpe et al, 1992; Jégou and Sharpe, 1993).

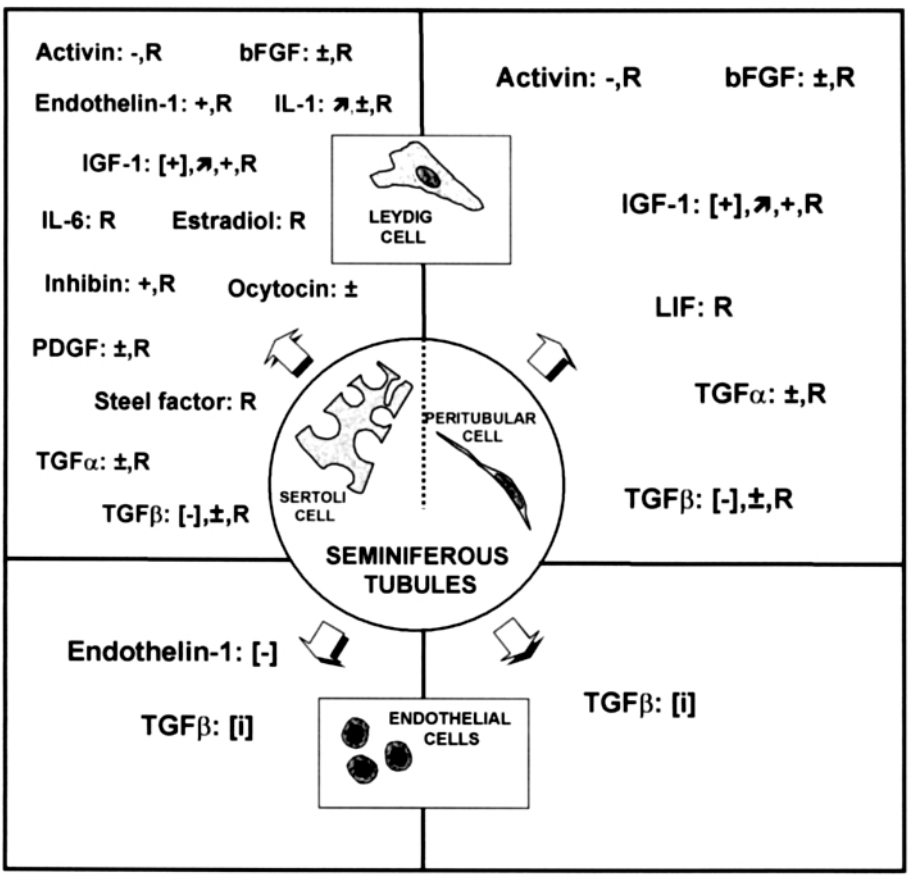

Figure 8. Seminiferous tubule factors with possible paracrine effects on Leydig and endothelial cells. I: inflammation; See legends to previous figures for other abbreviations. Note that the identification of most, if not all, of the factors result from in vitro experiments. For references, see Gnessi et al., 1997; Piquet-Pellorce & Jegou, 1996b; Piquet-Pellroce et al., 1998.; Rouiller-Fabre et al., 1998.

Despite substantial evidence that Sertoli cells affect Leydig cell activity, little is known of the identity of the factors mediating these effects. Figure 8 lists the Sertoli cell factors but also the peritubular cell factors thought to be involved in the control of Leydig cell activity. As for Leydig cell factors, these Sertoli and peritubular cell products are often present in both the interstitium and the tubular compartment, so it is often impossible to determine whether their putative action is paracrine or autocrine in nature (or both).

CONCLUSION

The concept that all testicular cell types communicate is as old as the study of testicular structure and function itself (Jégou et al, 1992; Jégou, 1993). There is now a large body of evidence demonstrating that these cells produce a very large variety of autocrine, paracrine and endocrine factors. Their also express many of the corresponding receptors. However, we are still for from a complete understanding of the intra-testicular regulatory system.

The extreme complexity of cell to cell communication networks within the testis reflects the complex structural organization of this organ. It is also a major obstacle to our understanding of the physiology and pathology of the male reproductive system and to the development of a male contraceptive pill. The complex testicular paracrine regulatory system successfully results in the daily formation of 100 to 200 millions spermatozoa in rats and man respectively (about 5 to 10% of the total cellular composition of the testis!). However, the disadvantage of such a complex system is that any disruption of any particular aspect of the system has negative consequences for the whole system. This is the main reason for the extreme vulnerability of the testis to the external environment, in terms of its physical (*e.g.*, temperature, irradiation), chemical (*e.g.*, antimitotic drugs, pesticides), biological (*e.g.*, viruses) or sociocultural (*e.g.*, stress) aspects. This vulnerability may account for the secular trend towards semen quality deterioration in some parts of the world (Auger et al, 1995; Swan et al, 1997). It also accounts for the great difficulty in determining the primary cause of male infertility and the fact that spermatogenic dysfunction is only rarely treatable.

ACKNOWLEDGEMENTS

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4 ANDROGEN METABOLISM AND ACTION

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INTRODUCTION

Androgen secretion by the testes during embryonic and neonatal life is responsible for the initial growth and differentiation of many organs of the male reproductive tract, such as the Wolffian ducts, urogenital sinus, and external genitalia primordia (Griffin and Wilson, 1998). Androgens also imprint regions of the central nervous system and determine the male pattern of gonadotropin secretion. During puberty, androgens promote the appearance of secondary male sex characteristics, including growth of the external genitalia, development of the prostate and seminal vesicles, distribution of body hair, and increase in muscle mass. These hormones initiate and maintain spermatogenesis, and they exert feedback control on the output of gonadotropins by the hypothalamic-pituitary axis. Although androgens act on the liver, kidneys, muscles, bones, and nervous and cardiovascular systems, it is within the male reproductive tract that the molecular mechanisms of androgen action are best understood.

Testosterone is the primary male sex hormone synthesized and secreted by the testes. Adequate levels of circulating testosterone are necessary for normal androgen biological activity; however, testosterone by itself is not sufficient to evoke the full complement of normal androgenic responses. The enzyme, steroid 5α-reductase, converts testosterone to an active metabolite, 5α-dihydrotestosterone (DHT), and the intracellular androgen receptor (AR) binds either steroid to act as a ligand-dependent transcription factor that directs the androgen to its site of action on DNA within the nucleus. In addition, the aromatization of testosterone to estradiol provides a source of estrogens which can bind to nuclear estrogen receptors (ER) and promote estrogenic actions on the hypothalamus-pituitary, bone, lipids and cardiovascular system. Human pathologic conditions, such as androgen insensitivity and 5α-

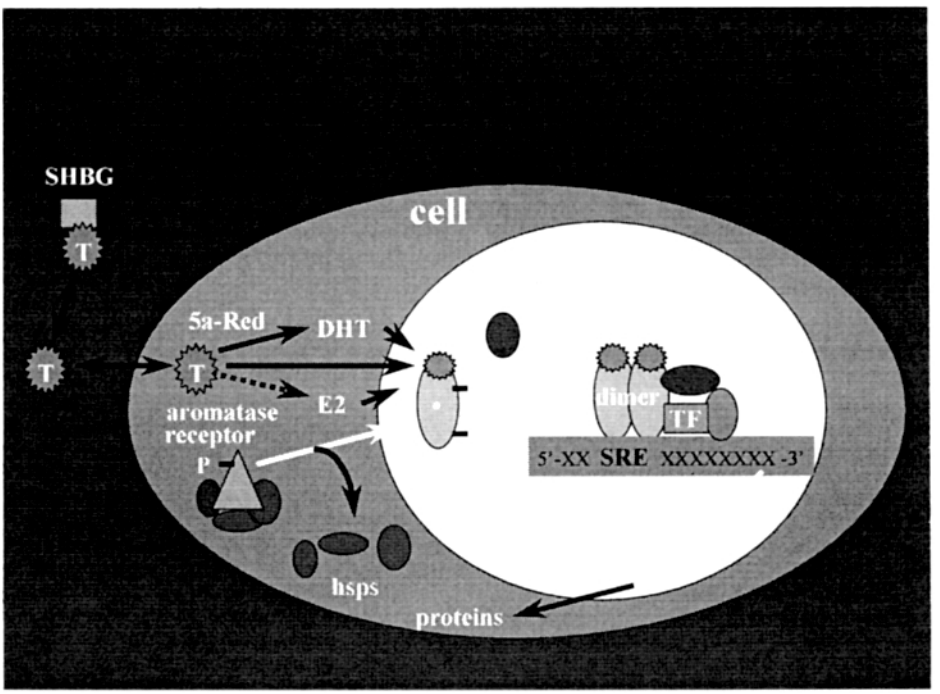

Figure 1: Mechanisms for target organ action of androgens. Testosterone circulates in the blood predominantly bound to carrier proteins, such as sex hormone-binding globulin (SHBG), and the free testosterone enters cells by passive diffusion. Within the target cell, testosterone may act itself or be converted to its active metabolites, dihydrotestosterone (DHT) by the 5α-reductase enzyme or estradiol (E2) by the aromatase pathway. The cytoplasmic androgen and estrogen receptors are phosphoproteins (-P) that form large macromolecular complexes with various proteins, including heat shock proteins (hsps). Upon binding of the appropriate steroid (androgen or estrogen), the androgen and estrogen receptors undergo a conformational change and hsps are released. The steroid-receptor complex undergoes further phosphorylation and acquires increased avidity for binding to DNA. The activated steroid-receptor complexes bind as dimers to specific steroid response elements (SRE) defined by DNA nucleotide sequences in regulatory regions of hormonally-regulated genes. The chromatin-bound receptors are complexed with other nuclear proteins that function as either coactivators or corepressors of gene transcription that may act to modify the chromatin structure and/or interact with the transcriptional initiation complex comprised of various transcription factors (TFs) and RNA polymerase II to facilitate or repress specific gene transcription of mRNAs and their subsequent translation into cellular proteins.

reductase deficiency, by virtue of their aberrant biochemical and developmental features, serve to reinforce and illustrate the recognized pathways for androgen action involved in the processes of male sex differentiation, development and reproduction (Grumbach and Conte, 1998). Cloning of the steroid 5α-reductase, aromatase, ER

and AR genes and the application of molecular biologic approaches have further defined the critical roles for these molecules in male physiology and reproduction. This chapter will discuss aspects of androgen metabolism and mechanisms of action in the context of normal human physiology and specific pathologic conditions.

MECHANISM OF ACTION

Overview

The daily production rate of testosterone by the testes is approximately 6 mg/day (Griffin and Wilson, 1998). Testosterone circulates in blood mainly bound to sex hormone-binding globulin (SHBG, also referred to as Testosterone-binding globulin, TeBG) and albumin, with only a small fraction of free hormone (Fig. 1). According to equilibrium kinetics, the passive diffusion of testosterone from the extracellular fluid into target cells results in the interaction of testosterone itself, or its 5α-reduced product, DHT, with a high-affinity AR located in the cytoplasm and the nucleus. The AR, like other steroid receptors, resides in the cytosol as part of a macromolecular complex that includes heat shock proteins which insure the proper folding of the receptor protein. Binding of androgen to its receptor produces a conformational (allosteric) change that results in the dissociation of accessory proteins and formation of an "activated" steroid-receptor complex with high affinity for DNA-binding sites. AR-steroid complexes bind as dimers to regulatory elements in DNA to activate (or repress) androgen-regulated genes and cause changes in gene transcription by RNA polymerase and in the levels of specific mRNAs. Translation of mRNAs on cytoplasmic ribosomes produces the appropriate proteins which can alter cell function, growth, or differentiation. In some cases, receptor-DNA interactions may serve to inhibit, rather than activate, gene transcription.

Sex Hormone-Binding Globulin

SHBG is a glycosylated 95 kDa β-globulin comprised of non-identical subunits synthesized in the liver and secreted into the blood (Hammond and Bocchinfuso, 1995). Several isoforms of SHBG, represented by differential post-translational glycosylation, appear normally in blood and a variant allele of the gene is present in a subpopulation of individuals. The level of SHBG in blood is increased by estrogens, but decreased by androgens. Each molecule contains one steroid binding site and can bind testosterone, DHT, or more weakly, estradiol (Dunn et al, 1981). Under normal conditions, about 2% of testosterone is free, with the remainder being bound to SHBG (40-50%) and albumin (50-60%). Although albumin has a 1000-fold lower binding affinity for testosterone than SHBG, the greater concentration of albumin equalizes the overall binding capacity of the two proteins (Pardridge, 1986). The bioavailable testosterone to tissues in vivo is actually equal to the free steroid and the lower

affinity, readily dissociable albumin-bound steroid, or about half of the total. In addition, recent studies demonstrate that SHBG-steroid complexes may also be biologically active via binding to cell surface receptors that evoke an increase in the intracellular cAMP level (Rosner et al, 1992). Androgen-binding protein (ABP) is a product of the same gene which is expressed in testicular Sertoli cells and secreted into the seminiferous tubules where it binds a significant proportion of the testosterone and/or DHT in the intratubular fluid of the testes and epididymides (Hammond and Bocchinfuso, 1995). This may explain, in part, the apparent requirement for high intratesticular levels of testosterone in the maintenance of spermatogenesis.

Androgen Metabolism

Testosterone itself is an active steroid with both androgenic and anabolic activities, as in the testes for spermatogenesis and in muscle, respectively. In sexual tissues such as

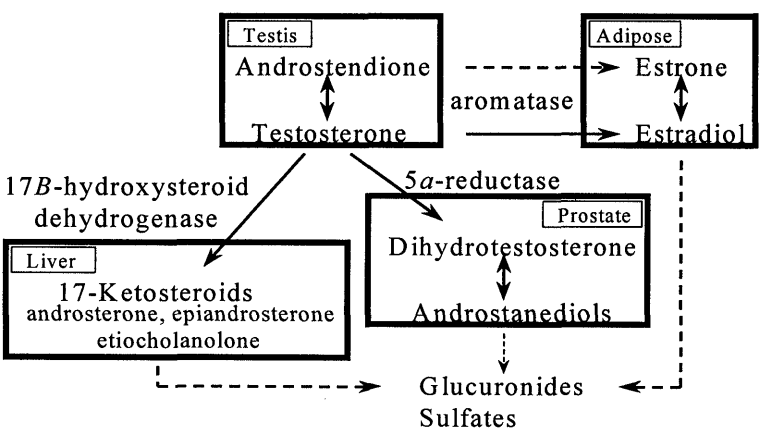

Figure 2: Pathways for androgen synthesis and metabolism. Through a series of enzymatic reactions, the testis synthesizes testosterone, with androstenedione serving as the final precursor. These androgenic steroids can be converted, primarily in adipose tissue, via the aromatase pathway to the estrogens, estrone or estradiol. In tissues such as the prostate, testosterone is converted to the active androgen, dihydrotestosterone by the 5 -reductase enzyme. Dihydrotestosterone can be further metabolized to 5 -androstanediols and these steroids can be conjugated to glucuronides for excretion. Within the liver, testosterone is catabolized to a series of ketosteroid derivatives, including androsterone, epiandrosterone and etiocholanolone, from which glucuronide and sulfate conjugates are formed for excretion.

skin or prostate, testosterone is irreversibly converted predominately to the active metabolite, DHT (Bruchovsky and Wilson, 1968) (Fig. 2). DHT is reduced to 5α-androstane-3α,17β-diol (3α-diol) and then further metabolized bysteroid conjugation, primarily glucuronidation to form 3α-diol glucuronide. These latter reactions are reversible so that 3α-diol can be converted back to DHT. Most of the DHT, 3α-diol and their glucuronides that appear in blood are derived from extrasplanchnic metabolism (Morimoto et al, 1981). Inactivation of testosterone occurs primarily in the liver and involves the formation of 17-ketosteroids via oxidation of the 17-OH group, reduction of the A ring, and reduction of the 3-keto group and the formation of polar metabolites which include diols, triols and their conjugates. The primary urinary products of androgens in humans are androsterone, etiocholanolone and the polar metabolites which are excreted in the form of glucuronide and sulfate conjugates (Brooks, 1975). Testosterone, and its synthetic precursor, androstenedione, can be aromatized to estradiol and estrone, respectively, by the cytochrome P-450 aromatase enzyme, designated CYP19 (Amarneh et al, 1994). The enzyme complex catalyzes a multi-step reaction leading to removal of the methyl group as formic acid and the rearrangement of the steroid A ring to an aromatic structure and requires NADPH as a cofactor for NADPH-cytochrome P-450 reductase to transfer reducing equivalents to the enzyme, and 3 moles of oxygen are consumed in the sequence of hydroxylation reactions. Approximately 60-70 μg of estrone and 40-50 μg of estradiol are formed each day in normal men, primarily within adipose tissue, and this serves to further diversify the biological actions derived from androgens at the tissue level.

Steroid 5α-Reductase Enzyme

The conversion of testosterone to a variety of 5α- and 5β-reduced metabolites was known prior to the discovery that DHT was actually the principal intracellular androgen concentrated within nuclei of many androgen target tissues, such as the prostate. DHT proved to be twice as potent as testosterone in bioassays and its physiological importance was confirmed by the abnormal sexual differentiation that occurred in human subjects with decreased concentrations of DHT due to genetic defects in 5α-reductase activity. Steroid 5α-reductase exists as two isoforms encoded by different genes, each containing 5 exons and having 50% identity of their nucleotide sequences (Russell and Wilson, 1994) (Table 1). The 28-29 kD enzyme proteins are localized to the endoplasmic reticulum and nuclear membranes, bind testosterone as a substrate and require NADPH as a cofactor. The human type 1 isoform is present at low levels in prostate, is encoded by a gene on the short arm of chromosome 5, has a optimal activity across a broad pH range from 6.5 to 8.0 and a high Km (1-5 μM) for testosterone and is relatively insensitive (Ki = 300-500 nM) to the 4-azasteroid inhibitor, finasteride. Type 1 isozyme is the major 5α-reductase present in skin and liver. The type 2 reductase isozyme is encoded by a gene on the

Table 1. Human 5α-Reductase Isozymes

	Type 1	Type 2
Amino acids (MW)	259 (29.5 kDa)	254 (28.4 kDa)
PH optima	6.5-8.0	5.0
Gene, chromosome	SRD5A1, 5p15	SRD5A2, 2p23
Gene structure	5 exons, 4 introns	5 exons, 4 introns
Substrate (testosterone)	K_m = 1-5 μM	K_m = 0.1-1.0 μM
Enzyme deficiency	None known	Various mutations
Tissue expression	Liver, skin	Urogenital tract
Prostate expression	Epithelium (low)	Stroma (high)
Finasteride inhibition	$K_i \geq$ 300 nM	K_i = 3-5 nM

short arm of chromosome 2, has an acidic pH (5.0) optimum and a low Km (0.1-1.0 μM) for testosterone, and is sensitive to finasteride inhibition (Ki=3-5 nM). As discussed later, molecular defects in the type 2 isozyme residing in the urogenital tract are responsible for the reduced serum and tissue DHT concentrations and inadequate virilization of the external genitalia in some infants with male pseudohermaphroditism.

Androgen Receptor

The AR, like other steroid receptors, is a member of the larger superfamily of ligand-dependent transcription factors (Zhou et al, 1994). The human AR gene is a single copy gene comprised of 8 exons and spanning over 90 kb of DNA in the q11-12 region of the X-chromosome (Lubahn et al, 1988). Expression of the gene is regulated by a single promoter region that contains 2 transcription initiation sites located within a 13-bp region. The AR promoter region does not contain a TATA-box or a CCAAT-box, but other regulatory sequences including a GC-box (recognition site for Sp1), a purine-rich region and a CRE element are present. Two mRNA species of 10.6 and 8.5 kb have been detected in various tissues. The 10.6 kb transcript consists of a 1.1 kb 5' untranslated region (UTR), a 2.7 kb open reading frame (ORF) and a relatively long 3' UTR of 6.8 kb. The shorter mRNA species contains the normal ORF and results from differential splicing in the 3' UTR. The various cloned human AR cDNAs encode an approximately 110 kD receptor protein containing a variable number of amino acids, from 910-919, due to the presence of two polymorphic stretches of amino acid repeats in the N-terminus. All references to amino acid number in this chapter will be to the human AR protein with 919 residues. The phosphorylated form of the AR protein migrates with an apparent MW of 112-114 kD. An 87 kD form of the receptor can be translated from an alternative initiation methionine codon, but its significance remains unknown. Like other nuclear steroid receptors, the AR has distinct functional domains for ligand- and DNA-binding and transcriptional activation, as well as a nuclear localization signal sequence (Jenster et al, 1991) (Fig. 3).

Transactivation Domain. The amino terminus, encoded by a single large exon, represents the least conserved region with the greatest variation in length among the steroid receptors, but is essential for gene transactivation by the human AR (Zhou et al, 1994; Lubahn et al, 1988; Jenster et al, 1991). Deletion of amino acids 142-239 in the N-terminal domain caused a decrease in target gene transactivation, whereas a deletion confined to amino acids 199-239 led to a significant increase in transactivation when compared to the wild-type AR. This region is referred to as the activating function-1 (AF-1) domain. Neither the location of AF-1 nor its amino acid composition is conserved among steroid receptors, suggesting that the N-terminus is

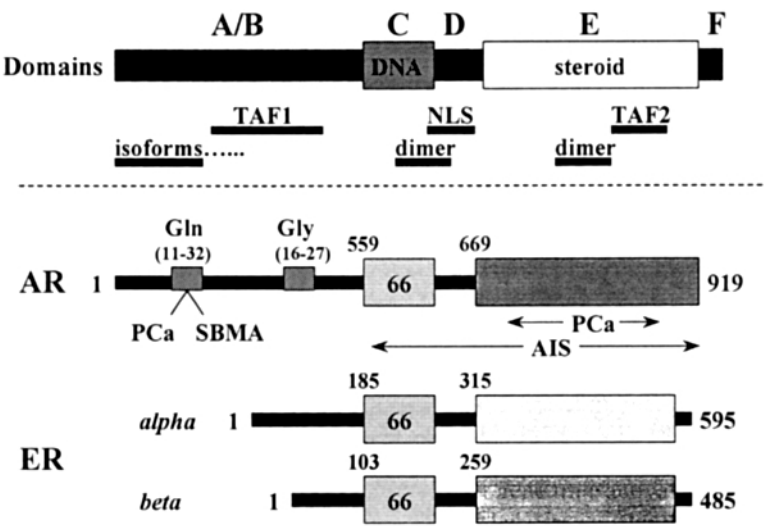

Figure 3: Structure and function of human nuclear steroid receptors for androgens and estrogens. Top: The receptors can be divided into functional domains. These domains include: 1) the A/B domain represents the transcriptional activation function (TAF) and varies in length according to specific classes and isoforms of receptors; 2) the C domain determines the DNA-binding specificity and includes a dimerization function; 3) the D, or hinge, region includes a nuclear localization signal (NLS) for these receptors; 4) the E domain primarily determines the specificity of steroid binding, but also serves an additional hormone-dependent transcriptional activation (TAF2) and dimerization function; and 5) some receptors, including that for estrogens, contain an additional carboxyl-terminal regulatory domain, F. Bottom: The comparative peptide lengths of the androgen (AR) and two isoforms (α and β) of the estrogen (ER) receptor are shown with their homologous functional domains. Regions of the androgen receptor associated with the pathologies of androgen insensitivity (AIS), spinal bulbar muscular atrophy (SBMA) and prostate cancer (PCa) are indicated.

involved in receptor-specific regulation of target genes. A second putative, highly conserved activation domain (AF-2) residing within the ligand-binding region of many steroid receptors. A functional interaction between the N-terminal activation and ligand-binding domains has been demonstrated for AR. A highly polymorphic poly-glutamine stretch encoded by (CAG)nCAA and a polymorphic poly-glycine stretch encoded by (GGN)n, are present in the AR amino-terminus, in addition to poly-alanine and poly-proline regions. In general, the glycine repeat region varies from 16-27 residues and the glutamine repeat ranges between 11 and 32 residues. Analogous acidic poly-proline and poly-glutamine sequence motifs are believed to function within transcriptional activation domains of various proteins. Indeed, recent findings related to prostate carcinoma and spinal bulbar muscular atrophy suggest that the poly-glutamine stretch may modify AR activity sufficiently so as to have significant pathologic implications.

DNA-Binding Domain. The central, cyteine-rich DNA-binding domain is the most highly conserved region within the steroid receptor superfamily (Zhou et al, 1994; Lubahn et al, 1988; Jenster et al, 1991). The AR DNA-binding domain, encoded by exons 2 and 3 of the AR gene, shares approximately 80% homology of amino acid sequence with the same domain of the glucocorticoid (GR) and progesterone (PR) receptors. By structural analogy derived from nuclear magnetic resonance and crystallographic studies of several other steroid receptors , the DNA-binding domain of AR consists of two zinc fingers incorporating two perpendicularly oriented α-helices in which four cysteine residues coordinate zinc in a tetrahedral array. Specific amino acids in the N-terminal α-helix directly contact the DNA in the major groove and define the specificity of the receptor for its steroid response element (SRE). The residues, gly577, ser578, and val581, within the proximal (P) box at the carboxyl-terminus of the first zinc-finger in AR, are conserved in GR and PR, and account for their recognition of a common SRE nucleotide sequence. The distal (D) box, consisting of five N-terminal amino acids (ala-ser-arg-asn-asp) within the second zinc finger, is thought to play a role in AR homodimerization that occurs coincident with binding to DNA, as previously shown for GR. The critical factor(s) that determine specificity of AR, GR or PR binding to DNA and hence their specificity in gene regulation remains to be elucidated.

Nuclear Localization Signal and Hinge Region. A bipartite nuclear targeting signal sequence overlaps the DNA-binding and hinge regions encoded at the junction between exons 3 and 4 (Zhou et al, 1994; Jenster et al, 1991). Two clusters of basic amino acids, separated by an additional 10 amino acids reside within this region, between residues 617-633, are required to shuttle the AR through nuclear pores. In the absence of ligand, the AR is distributed within the cytoplasm and nucleus. The presence of androgen induces a rapid migration of AR to the nucleus. Mutation in this region of AR cause an almost complete cytoplasmic localization of the receptor and loss of its transcriptional activity (Zhou et al, 1994).

Steroid-Binding Domain. The carboxyl-terminus of AR, encoded by exons 4-8, specifies the steroid binding properties of the receptor. Transactivation of genes by steroid receptors is repressed by the C-terminal steroid-binding domain in the absence of ligand (Zhou et al, 1994; Lubahn et al, 1988; Jenster et al, 1991). Interestingly, a deletion construct of AR lacking the steroid-binding domain possesses constitutive activity even in the absence of androgen. The binding of androgen to the receptor coincides with the dissociation of heat shock proteins, such as hsp 90 and hsp 70, and conformation induced activation of the steroid-receptor complex. The active conformation of the receptor is capable of forming homodimers and binding to SREs to promote gene transcription. Intramoleclar interactions between AR molecules involve the N-terminal transactivation and C-terminal steroid-binding domains in the presence of androgen, and this interaction may modulate receptor dimerization and DNA binding. A conserved activation function-2 (AF-2) region is present among many steroid receptors, including AR. The binding of transcriptional coactivators in the AF-2 region and the demonstration of an active ligand-dependent AF-2 function in AR that functions in concert with these coactivators, suggest that protein-protein interactions between AR and other cellular proteins may modulate transcriptional activity of AR through the steroid-binding domain. Androgen binds to both non-phosphorylated and serine phosphorylated forms of AR, but the impact of this post-translational modification on AR activity has not been defined.

Steroid Response Elements in DNA. SREs minimally contain a core recognition motif of 6 bp, but most consist of two core motifs (half-sites) separated by a spacer of variable length (Beato and Sanchez-Pacheo, 1996). The nucleotide sequence of the core motif is specific for subgroups of receptors; the AR, GR, and PR all bind to hexamer half-sites with the sequence, TGTTCT. This androgen response element sequence differs from that for the estrogen receptor (TGACCT) at positions 3 and 4 in both half-sites, which are critical for receptor specific recognition. In addition, the consensus SRE for AR is organized as an imperfect inverted or palindromic repeat of core motifs, although the AR has been shown to bind also to a direct repeat. The SRE is also characterized by the spacing of the two half-sites, which in the SRE for AR most often involves three non-specific nucleotides. The consensus SRE for AR binding is therefore represented by the nucleotide sequence, AGAACAnnnTGTTCT, but which also binds GR and PR.

Transcriptional Activation. Sequence specific transcription factors like AR, interact with other general transcription factors in the control of gene activation (Beato and Sanchez-Pacheo, 1996). These general factors in turn interact with the core promoter elements to induce basal transcription. RNA polymerase II and the general transcription factors assemble a transcription initiation complex along with the TATA-box binding proteins, TATA-binding protein (TBP) and TBP-associated factors ($TAF_{II}s$). Steroid receptors may enhance basal gene transcription, either by direct interaction with general transcription factors or with $TAF_{II}s$. In addition to these direct interactions between AR and the transcriptional machinery, other intermediary factors or coactivators may also be involved in regulation of transcriptional activity. AR interacts with other sequence specific DNA-binding

transcription factors, such as AP-1 and NFκB, that further modulate gene transcription.

Coactivators and Corepressors. Coactivators are cellular proteins that interact with the ligand-activated steroid receptor complexes to enhance transactivation of target genes (Horwitz et al, 1996). Coactivators may have several roles which include possession of intrinsic histone acetyltransferase (HAT) activity, recruitment of other proteins with HAT activity and as integrators which enable regulatory molecules to be recruited and assembled at sites of transcriptional activity. A number of coactivators, are either, directly or indirectly, involved in chromatin remodeling. These coactivators include *TIF1* and *2* (transcriptional intermediary factors 1 and 2), *SRC-1* (steroid receptor coactivator 1), and *GRIP1* (GR-interacting protein 1), that interact with the ligand activated AF-2 regions of different steroid receptors to enhance transcriptional activity in the presence of their ligands. *SRC-1*, in turn, interacts with *p300/CBP* (CREB binding protein) and *pCAF* (p300/CBP-associated factor), proteins that possess intrinsic HAT activity, with the overall effect being synergistic for transcriptional activation. ARA-70 (AR-associated protein) interacts in a ligand-dependent manner with AR to increase its transcriptional activity. By contrast, corepressors are cellular factors that appear to be tethered to DNA by interactions with steroid receptors in the absence of hormone, and thereby, act to repress basal promoter activity. Upon ligand-binding, the corepressor is released, allowing interaction of the receptor with coactivator proteins. Cloning of the cDNAs for two corepressor proteins, *N-CoR* (nuclear receptor corepressor) and *SMRT* (silencing mediator for retinoids and thyroid hormone), have provided some insights into the activities of corepressors. Initially, the presence of the repressor protein in association with the receptor on chromatin maintains a transcriptionally inactive structure by tethering of histone deacetylases to the DNA at sites close to the steroid responsive element of the receptors. Subsequently, binding of the steroid to its receptor causes the release of the corepressor and recruitment of acetyltransferases which disrupt the chromatin template. Finally, the interaction of the activating functions of both the receptors and the recruited coactivators with the basal transcription factors results in gene transcription. Steroid receptors are bound by a complex of heat shock proteins in the absence of steroid and are probably not involved in tethering of histone deacetylase activity to DNA. However, steroid receptors inhibit transcription when they are occupied by antagonists, suggesting that corepressors may play a role in this repression.

Estrogen Receptor

The recent discovery of a second estrogen receptor gene suggests that estrogenic activity is regulated by the tissue-specific expression of two different ERs, the classical ERα that has been studied for many years and the newer isoform, Erβ (Kuiper et al, 1997) (Fig. 3). ERα is expressed primarily in uterus, testis, pituitary, ovary, kidney, epididymis and adrenal, and ERβ in prostate, ovary, lung, bladder, brain, uterus and testis. The two ER isoforms are highly homologous, particularly in the DNA- and steroid-binding domains, but differ by truncation of the N-terminus of the ERβ protein with a molecular weight of 45 kDa, compared to the 66 kDa ERα

protein. The binding affinity of estradiol is four times greater for ERα than ERβ, and the ligand specificity for a number of other compounds differs between the two isoforms. The presence of two different ERs expands the diversity of potential responses to estrogenic and antiestrogenic compounds and suggests that ER homodimers or heterodimers may be binding to estrogen response elements to regulate gene expression (Fig. 1).

GENETIC ALTERATIONS IN TARGET CELL ANDROGEN ACTION

Steroid 5α-Reductase

Enzyme Deficiency. The enzyme steroid 5α-reductase is essential for conversion of testosterone to DHT. Both testosterone and DHT activate transcription from the AR, however, DHT binds to AR with greater affinity than testosterone and is a more potent activator of androgen-responsive genes *in vivo*. Conversion of DHT, and thus amplification of the androgenic response may be essential in peripheral tissues, where testosterone concentrations are lower. Alternatively, testosterone and DHT may be involved in the regulation of specific genes. As discussed previously, two 5α-reductase isoforms have been identified and each shows a tissue-specific pattern of expression. 5α-Reductase isoform 2 is involved in development of the male external genitalia and is expressed in many of the classic androgen target tissues. Mutations in the gene for 5α-reductase type 2 are responsible for male individuals with abnormal differentiation of the urogenital sinus and genital tubercle resulting in absence of the prostate and ambiguous or female external genitalia at birth (Wilson et al, 1993). Mullerian duct regression due to secretion of Mullerian Inhibiting Substance (MIS) and virilization of the Wolffian duct structures by testosterone is normal in affected subjects. These individuals are often raised as females but at puberty, increased testicular secretion of testosterone leads to deepening of the voice, increased muscle mass, growth of the penis, scrotal development, testicular descent and affected individuals may even ejaculate. These androgenic responses at puberty may be related to the increased concentrations of testosterone and/or to the residual type 2 and normal type 1 isoforms of 5α-reductase which produce sufficiently greater levels of DHT. A significant rise in the expression of 5α-reductase type 1 accompanies puberty in normal individuals.

Mutations in the 5α-reductase type 2 gene occur most often as point mutations that cause amino acid substitutions, splice-junction alterations, nonsense codons or small deletions. In most families, the mutations are homozygous, often due to consanguineous marriages within relatively isolated populations, but about a third are compound heterozygotes (different mutations on each of the two alleles). Deletion of the entire coding sequence for the 5α-reductase type 2 gene was found in members of a tribe residing in the New Guinea Highlands. In general, the mutations are distributed throughout the coding sequence and disturb enzyme function by one of

several mechanisms, including impairment of binding of testosterone or NADPH to the enzyme, formation of non-functional (deletions, premature termination codons, splice junction abnormalities) or unstable enzymes, and diminished levels of protein synthesis.

Not only is the disorder of 5α-reductase deficiency genetically heterogeneous, but its phenotypic expression is also variable. Over half of the affected subjects have a blind-ending vagina (pseudovagina), however in some sibships an affected sibling may have only a simple perineal orifice for the urethra. In a small percentage of subjects, the phallus is large enough at birth that the affected infant is identified as a male with hypospadias and is raised as a boy. While the anatomical features of the external genitalia are highly variable, the most consistent features are underdevelopment of the phallus and absence/hypoplasia of the prostate. The testes in all individuals are extraabdominal - present in the inguinal canals, labia majora, or scrotum. Spermatogenesis is uniformily absent or severely impaired due to either the direct effect of the mutation or the consequence of incomplete testicular descent. Interestingly, individuals with 5α-reductase deficiency within the Dominican Republic population were originally raised as girls but subsequently changed their gender role behavior to male at the time of puberty. This served to reinvigorate the argument as to the relative roles of biological determinants and psychological factors in the development of gender identity. By contrast, similar behavioral changes do not occur among subjects with androgen insensitivity due to mutations in the androgen receptor gene in which gender behavior conforms to the predominant anatomical development, and hence to gender assignment.

Allelic Polymorphism. A TA dinucleotide repeat polymorphism exists in the 3' untranslated region of the 5α-reductase type 2 gene (Davis and Russell, 1993). A hypothesis developed that longer TA repeat alleles might be associated with increased risk of prostate cancer in African-American men, the group with the highest incidence of prostate cancer. However, in a control group of Caucasian men, the TA genotype frequency for each allele was TA(0), 0.87; TA(9), 0.13; and TA(18), 0.01. Contrary to the original hypothesis, Caucasian men with prostate cancer did not have longer TA alleles and in fact, longer TA alleles were more prevalent in men without prostate cancer.

Androgenic Steroids

Tissues are exposed to serum levels of testosterone that are approximately ten-fold higher than that of DHT in the peripheral circulation. Higher testosterone concentrations in young black men than in young white men were reported to explain the underlying differences in the incidence of prostate cancer between these two racial groups. By comparison, serum testosterone concentrations of young Japanese men are not significantly different from those in young white or black men despite a lower incidence of prostate cancer. In one study, both black and white men had significantly higher serum levels of 5α-reduced androgenic products, androstanediol glucuronide and androsterone glucuronide, than were found in Japanese men (Ross et al, 1992). In a second study, both precursor androgens (dehydroepiandrosterone sulfate and androstenedione) and 5α-reduced androgen products (androstanediol and

androsterone glucuronides) were higher in serum of Caucasian men than of Chinese men (Lookingbill et al, 1991). These studies provide circumstantial evidence for genetic differences in androgenic hormone activity among men of different racial backgrounds that may have a role in specific androgen-related pathologies. However, the apparent genetic differences in androgen concentrations and androgen metabolism are small and variable between studies, such that environmental and dietary influences must also be considered as epidemiologic variables that influence the data and its interpretation.

Androgen Receptor

Androgen Insensitivity (AIS). AIS is caused by inactivating mutations of the AR gene that result in partial or non-responsive androgen target tissues (Quigley et al, 1995; Brown, 1995). In individuals with complete AIS due to total inactivation of the AR, testes are present and wolffian ducts are absent. The external genitalia exhibit a female phenotype and Mullerian ducts are absent as MIS activity in the fetus is normal. At puberty, androgen insensitivity results in high levels of LH and subsequently testosterone levels are also at or above normal values for men. Testosterone is aromatized to estradiol which is responsible for breast development and the typical female body habitus seen in complete AIS. Pubic and axillary hair is absent or sparse. The diagnosis in individuals with complete AIS is usually made in infants with inguinal hernias that contain a testis or at puberty because of primary amenorrhea. Partial AIS includes a spectrum of phenotypes ranging from a predominantly female appearance (external female genitalia and pubic hair at puberty, or with mild clitoromegaly, and some fusion of the labia) to ambiguous genitalia or even to individuals with a predominantly male phenotype (sometimes referred to as Reifenstein syndrome). Subjects in the latter group present with a micropenis, perineal hypospadias, and cryptorchidism. Wollfian duct derivatives may be nearly fully developed or rudimentary in partial AIS, dependent upon the residual androgenic activity. At puberty, elevated LH, testosterone and estradiol levels are observed but the level of feminization is less than that seen among individuals with complete AIS. Although severe forms of hypospadias represent a feature of AIS, AR mutations do not seem to be a frequent cause of isolated hypospadias. In several AIS subjects with the same AR gene mutation, genotype/phenotype variation has been observed. There are indications that receptor activity is influenced by the length of the poly-glutamine stretch in the transcriptional activation domain and additional factors such as the androgen level or expression of coactivators may account for modulation of receptor activity among individuals.

More than 100 different mutations have been identified in subjects with AIS and are primarily due to point mutations or small deletions or insertions. Some mutations have been found in multiple unrelated families, but no major hotspots for mutation have been observed, although CpG-dinucleotides are subject to a higher rate of mutation. Only 5% or less of the AR gene mutations causing AIS are due to deletions encompassing one or more exons. Amino acid substitutions due to single nucleotide substitutions in the AR gene are most frequently observed, and in general, conservative mutations appear to have less deleterious effects than non-conservative mutations. The majority of mutations occur in exons encoding the DNA-binding

domain and in exons 4-8 encoding the steroid-binding domain (Fig. 3). Mutations in these regions are responsible for the entire spectrum of phenotypic variation that occurs in both partial and complete AIS and no region is more or less represented in either form of AIS. Mutant receptors may have reduced specificity and/or affinity for androgen binding and for DNA binding, altered association and/or dissociation kinetics for steroid binding, and inherently decreased stability to proteolytic degradation.

The clinical and pathophysiologic features of AIS provide a human model for understanding the role of androgen and its receptor in the induction and maintenance of male sex differentiation and function. Upon inspection, one is immediately impressed by the diverse nature of the mutations involved in the phenotypic spectrum of AIS and the heterogeneous distribution of mutations throughout the coding sequence of the AR gene. Because of the large number and diverse array of these naturally occurring mutations and their associated clinical and biochemical phenotypes, there is great opportunity for understanding the structure-function relationships of AR from the in vivo expression of the mutant receptors in cells.

Spinal Bulbar Muscular Atrophy. Several polymorphic repeats are located in exon 1, which encodes the amino-terminus of the AR. An expansion of the polymorphic, poly-glutamine stretch, encoded by (CAG)nCAA is the molecular basis of spinal and bulbar muscular atrophy (SBMA; Kennedy's disease) (LaSpada et al, 1991). In normal individuals the (CAG)nCAA repeat contains 9-33 CAG triplets, whereas 38-75 CAG codons are associated with SBMA (Fig. 3). Disease severity is inversely correlated with length of this repeat. SBMA is characterized by progressive muscle weakness and atrophy with clinical symptoms manifest in the third to fifth decade of life. The pathology is associated with a severe depletion of lower motornuclei in the spinal cord and brainstem, and distal axonopathy of the dorsal root ganglion cells is observed. In addition, subjects with SBMA frequently exhibit endocrine abnormalities including testicular atrophy, reduced or absent fertility, gynecomastia and elevated FSH, LH and estradiol levels similar to observations in mild forms of AIS. Sex differentiation occurs normally and characteristics of mild androgen insensitivity appear later in life. This may be related to a reduced AR expression and reduced testosterone level in older men. SBMA is an X-linked disease and occurs only in men. At present it is not known whether disease progression involves the ligand-activated or ligand-free AR. In two cases, extended testosterone therapy had neither a beneficial nor harmful effect.

The molecular mechanisms underlying SBMA remain somewhat speculative at present. In fact, the disease appears to be a combination of the loss of normal AR function in androgen-dependent tissues coupled to a gain of function mechanism in motorneurons. A number of other similarly progressive neurodegenerative diseases, Huntington's disease, dentatorubal-pallidoluysian atrophy, and spinocerebellar ataxis are caused by an expanded CAG-repeat located in the coding region of the respective genes. Although the proteins encoded by each of these genes are widely expressed throughout the body, neuronal tissue is specifically affected. Theoretically, intragenic expanded CAG-repeats could be pathogenic at the DNA, RNA or protein level. Increased binding of RNA-binding proteins to RNAs containing expanded CAG repeats has been observed. These RNAs might disrupt normal transport in cells or

competitively affect the interactions of these proteins with other cytoplasmic proteins. Alternatively, the expanded polyglutamine stretch could serve as a better substrate for transglutaminase, an ubiquitously expressed enzyme that catalyzes coupling of glutamine and lysine residues. Poly-glutamine stretches might also function as polar zippers to form protein aggregates that are unable to be processed normally by the ubiquitin-mediated proteolytic degradation pathway and form intranuclear inclusions as seen in Huntington's disease. By comparison to a gain of function mechanism, the endocrine abnormalities present in subjects with SBMA reflect a loss of function for the AR. Because many transcription factors commonly contain polymorphic glutamine stretches, the question arose as to whether the length of the poly-glutamine stretch in AR might modify its transcriptional activity. In cotransfection studies, the length of the CAG-repeat was inversely proportional to transactivation function of AR (Trifiro et al, 1994). However, conflicting data has suggested that this was or was not related to reduced stability of AR mRNA and decreased expression of AR protein (Choong et al, 1996).

Prostate Cancer. Androgens, predominantly DHT, are involved in growth and development of the prostate, but also play a role in the evolution of prostate cancer. At the time of detection and surgery, tumors are often androgen dependent, and therefore endocrine abalation therapy that combines castration, antiandrogen and/or GnRH antagonist administration is often implemented. About 80% of subjects initially respond to endocrine ablation therapy but ultimately the majority of them show tumor recurrence and progression. Failure of endocrine therapy can be explained by several molecular mechanisms which include androgen-dependence or -independence.

Somatic mutation of the AR gene represents one potential mechanism for androgen independence where activation of AR occurs due to other steroids and antiandrogens. The first mutation (Ala877Thr) of this nature was detected in the human prostatic carcinoma cell line, LNCaP, that showed an increased growth rate in response to estradiol, progesterone and the antiandrogens, hydroxyflutamide and cyproterone acetate (Veldsholte et al, 1990). Additional somatic cell mutations were observed in primary tumors, but only at a very low frequency (Newmark et al, 1992). The majority of mutations have been identified in hormone refractory tumor samples and metastatic lesions (Culig et al, 1993; Taplin et al, 1995). These mutations most often involved amino acid substitutions in the steroid-binding domain that altered the steroid specificity for binding, although there are also reports of mutations occurring in the 5' UTR, as well as in the amino-terminal and DNA binding regions (Fig. 3). A large number of tumors have been screened for AR gene mutations, however the frequency of genetic alterations at this level appears to be quite rare.

Amplification of the AR gene was also observed in a number of hormone recurrent tumors, but not in primary tumors (Kovisto et al, 1997). AR gene amplification resulted in higher levels of AR mRNA and a corresponding increase in the levels of AR protein expression. The increase in AR levels within tumors might provide a growth advantage to cells when androgen levels are low such as after androgen ablation therapy. Although theoretical at the present, amplification of growth-related gene targets for AR activity or for genes of AR coactivators in prostate may also represent potential mechanisms for androgen-independent growth of tumors.

Recent studies have suggested that cross-talk between AR pathways and other signal transduction pathways involving growth factors and autocrine/paracrine peptides could lead to receptor activation in the absence of androgen (Culig et al, 1994). In addition, these alternative pathways could also potentiate the activity of AR in the presence of low concentrations of androgen. Whether these alternative pathways are initiated due to changes in kinase activity that affect the phosphorylation of AR or other phosphoproteins remains to be clarified.

A somatic variation in the polymorphic (CAG)nCAA-repeat in exon 1 of the AR gene was observed in a prostate tumor specimen and was suggested to have a role in prostatic carcinoma (Taplin et al, 1995). Studies related to subjects with SBMA showing that the increased length of the poly-glutamine stretch was inversely correlated with AR transactivation, prompted additional epidemiological studies among normal and prostate cancer patients to determine the relationship between the occurrence of prostate tumors and variation in (CAG)nCAA polymorphisms (Fig. 3) Several recent studies have shown a correlation between shorter CAG-triplet repeats in exon 1 and an increased risk for prostate cancer, and in addition, that these tumors tended to be more aggressive and were related to a younger age at diagnosis (Irvine et al, 1940; Giovannucci et al, 1997). Population distribution analyses have shown that African-Americans have a lesser mean number of CAG-triplet repeats in the AR gene than Caucasians, further suggesting a correlation between a shorter poly-glutamine stretch and the higher incidence of prostate cancer within the African-American population (Coetzee and Ross, 1994).

Breast Cancer in Males. Breast cancer occurs infrequently in males but clinical conditions of reduced androgen activity predispose subjects to gynecomastia, and possibly to further breast cell proliferation. Therefore, it was not surprising that several research groups reported mutations in the AR gene of male subjects with breast cancer in association with partial AIS (Wooster et al, 1992; Lobaccaro et al, 1993). However, further studies among men with breast cancer that was not associated with abnormal sex differentiation and AIS have failed to confirm a correlation mutations in the AR gene.

Male infertility. A reduction in AR binding in genital skin fibroblasts cultured from men with azoospermia and oligospermia, but otherwise normal virilization was reported to represent a mild form of AIS. However, each of these studies predated access to molecular techniques for AR gene analysis and the presence of molecular defects in AR have not been documented (Quigley et al, 1995). Infertility in a man with deletion of exon 4 in the AR gene was reported but remains an enigma since a gross dysfunction of this mutant receptor would be expected, but was never tested. A mutation in exon 5 was reported in one subject with severe oligospermia and a relatively long (CAG)nCAA repeat in the AR gene was also associated with impaired spermatogenesis. At present, evidence to support a role for mutations of the AR gene in subjects with isolated infertility remains inconclusive.

Aromatase

Aromatase deficiency has been described in only a small number of individuals and all postpubertal subjects have tall stature, delayed bone maturation and epiphyseal fusion, and osteopenia, suggesting that estrogens are essential for the prevention of osteoporosis in males and females and for normal skeletal maturation and proportion, but not for linear growth in men (Morishima et al, 1995). An affected male subject had normal sex differentiation and pubertal development, with macro-orchidism and elevated concentrations of FSH, LH and testosterone. He also had osteoporosis, insulin resistance and hyperinsulinemia, and an abnormal plasma lipid profile. By comparison, a rare cause of gynecomastia and delayed pubertal maturation in males is associated with an increase in rate of extraglandular aromatization of the androgen substrates, testosterone and androstenedione, to estradiol and estrone, respectively. In these subjects, testosterone production by Leydig cells is normal but the peripheral conversion of testosterone to estradiol is 10-50 times greater than normal, thus slowing pubertal progression. The fertility of affected subjects has not been described.

Estrogen Receptor

Although mutations in the estrogen receptor gene were thought to be lethal due to embryo implantation defects, the recent description of a man with estrogen resistance who had osteoporosis, unfused epiphyses and continuing linear growth in adulthood presented an alternative physiologic outcome (Smith et al, 1994). This was caused by a single base pair substitution in the estrogen receptor gene that created a stop codon for termination of estrogen receptor translation. The subject also had elevated serum estrogen concentrations, abnormal gonadotropin secretion, and no target tissue responses to estrogen therapy. These findings indicated that estrogen is important in the male, as in the female, for normal skeletal growth and development.

SUMMARY

Androgens play a key role in male sex differentiation and development, in maintenance of male reproductive function, and the effects of these hormones are an important component in the development of certain pathologic conditions, most prominently in prostate cancer. Testosterone and its 5α-reduced metabolite, DHT, are potent androgens that act upon target cells to initiate and maintain the masculine phenotype. Germ-line mutations in the androgen receptor and 5α-reductase genes that affect male sex differentiation and development have played a key role in elucidating the pathways of androgen action. Differences in serum testosterone levels and 5α-reductase activities between ethnic and racial groups have been implicated in the variable incidence of prostate cancer among certain populations. Androgen receptors transduce the steroid signal within cells, but attempts to correlate differences in receptor levels with prostatic disease have been unsuccessful. However, molecular studies of AR gene structure have recently provided new insights toward defining a molecular and genetic basis for the pathology associated with diseases - including

spinal bulbar muscular atrophy, breast carcinoma, and prostate cancer - affecting middle-aged and older men. In addition, genetic deficiencies in the aromatase enzyme and estrogen receptor has focused new attention upon the physiologic roles for estrogenic hormones in men. In summary, epidemiologic data on androgen biosynthesis, metabolism, and action of androgens and molecular genetic analysis of gene structure have led to a new understanding of the interrelationships between environmental and genetic factors that may impact on the incidence of certain pathologic conditions in men.

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5 MALE PUBERTY AND ITS DISORDERS

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INTRODUCTION

This chapter selectively highlights the major advances in the past three years. Consideration is given to those areas which have made a significant impact on our understanding of the mechanisms of pubertal regulation and influenced clinical management of male pubertal disorders. For a more comprehensive general coverage of this topic, the reader should refer to other recent reviews and monographs (Plant, 1994; Plant, 1995; Grumbach and Styne, 1998).

PHYSIOLOGY OF PUBERTAL TRANSITION

GnRH Pulse Generator

GnRH, the first key hormone of reproduction, is synthesised in the hypothalamus and released in a pulsatile manner into the hypophysial portal vessels to regulate pituitary gonadotropins, LH and FSH, synthesis and secretion (Conn et al, 1995). In primates, approximately 2000 GnRH-containing neurons (surrounded and isolated by glial lamellae) are scattered diffusely in the preoptic and arcuate areas (Silverman et al, 1994) projecting their axon terminals to the perivascular spaces of the median eminence to gain access to the vascular channels. Surprisingly, relatively sparing direct afferent synpatic inputs are found in only a small percentage of these GnRH cell bodies (Silverman et al, 1994; Goldsmith et al, 1983). Although immortalised murine GnRH neuronal cell lines are intrinsically "pulsatile" in vitro (Martinez de la Escalera et al, 1992; Wetsel et al, 1992), rat

retrochiasmatic (arcuate nucleus) hypothalamic explants containing predominantly GnRH axons and terminals also exhibit pulsatile GnRH secretion in vitro (Bourguignon and Franchimont, 1984). It remains uncertain whether the diffuse network of GnRH neurons or the functional circuit of neurotransmitters and neuropeptides synchronising GnRH axonal episodic discharge represent the in vivo pulse generator responsible for producing GnRH boluses in the portal circulation with concentrations varying from 200-1500pg/ml (Caraty et al, 1994).

Ontogeny of Pulsatile GnRH/LH Secretion

It is important to emphasise that GnRH and gonadotropin secretion is active in the fetus and neonate (Grumbach and Styne, 1998). Instead of proceeding immediately to adult reproductive activity, gonadal function in the primate is kept in abeyance between infancy and puberty by a markedly restrained GnRH pulse generator during a protracted quiescent juvenile hiatus (Plant, 1994). From the neuroendocrine perspective, reactivation or disinhibition (rather than initiation) of pulsatile GnRH secretion is the central event in the onset of puberty (Plant, 1994; Chongthammankun et al, 1993). Defining the developmental pattern of GnRH secretion during the transition from the juvenile to the pubertal mode of function is a prerequisite to understanding the mechanisms underlying the control and timing of the onset of puberty. GnRH has a very short half life of 2-4 minutes and its distribution is confined within the hypophysial portal circulation. Direct sampling of hypophysial portal and peripheral bloods simultaneously in animals has confirmed that hypothalamic GnRH pulses are faithfully mirrored by the pituitary-generated LH pulses in the systemic circulation (Clarke and Cummins, 1982; Caraty and Locatelli, 1988). Since it is not possible to study GnRH secretion in humans by hypophysial portal blood sampling, repeated fluctuations of pituitary hormones in the systemic circulation are usually employed as surrogates to model the operational characteristics, particularly the frequency activity, of the GnRH pulse generator during the onset of puberty. Methodological advances have greatly improved the capability and accuracy in this indirect monitoring of GnRH pulse generator activity during human puberty. These included (i) greatly increased sensitivity and precision of ultrasensitive immunofluorometric (IFMA) (Apter et al, 1989; Dunkelet al, 1990; Wu et al, 1991; Taylor et al, 1994) or immunochemiluminescent ICMA (Neely et al, 1995) assays which can accurately detect prepubertal levels of gonadotropins, (ii) appropriate frequency (every 5-10 minutes), timing (nocturnal) and duration of blood sampling (>6 hours) to reflect the half-life and diurnal rhythm of the hormones (LH, alpha submit, testosterone) of interest (Filicori et al, 1989; Hayes and Crowley, 1998) and (iii) statistically robust and biologically meaningful algorithms for the analysis of pulsatile hormone secretion (Veldhuis and Johnson, 1992) and its serial orderliness or regularity (Pincus, 1995).

Gonad-intact (Closed-loop Model): Clinical Studies in Boys

Largely consistent results have been obtained from several studies which took advantage of these methodological improvements. Thus in young (4-8 yr old) prepubertal boys, pulsatile GnRH secretion is clearly detectable albeit generating

only extremely low LH amplitudes which would ensure reproductive quiescence in childhood (Wu et al, 1991; Dunkel et al, 1990). The current weight of evidence (Wu et al, 1991; Goji and Tanikaze, 1993; Monasco et al, 1995;Dunkel et al, 1990; Wennick et al, 1988; Oerter et al, 1990) appears to indicate that diurnal modulation with sleep-associated augmentation of GnRH/LH is extant in the juvenile phase, although not all studies found this (Dunkel et al, 1992; Albertsson-Wikland et al, 1997; Clark et al, 1997).

During secondary sexual development, a significant increase in sleep-entrained GnRH secretion was first detectable some 2 years or so before the clinical onset of puberty (testicular enlargement >2mL) (Wu et al, 1991). Between midchildhood and late prepuberty, GnRH pulse frequency increased by 2-3 fold with the pulse interval decreasing from 130-180 to around 90 minutes (Wu et al, 1996; Albertsson-Wikland et al, 1997). Pulse frequency did not increase further during continuing development from early puberty to adulthood. Compared to this apparently modest and phase-restricted acceleration in pulse frequency, LH pulse amplitude (secretion rate) and mass of LH secreted per burst increased progressively throughout pubertal maturation by 20-30 fold respectively. The greatest percentage increase in LH, for both frequency and amplitude, was observed at the earliest stage between the childhood and late prepuberty. The physiological tempo of juvenile to pubertal transition seems to encompass an initial abrupt neuroendocrine switch involving an acceleration in GnRH pulse frequency followed by a more protracted and gradual amplification of the GnRH signal at the pituitary and testicular levels. This early acceleration in GnRH pulse frequency has also been confirmed by direct in vivo portal blood GnRH measurement in rhesus monkey (Watanabe and Terasawa, 1989) and in the incubation medium of hypothalamic explants of rat (Bourguinon et al, 1990). The consequent sex steroid secretion establishes a close-loop feedback circuit from the onset of puberty and may serve to dampen hypothalamic GnRH pulse frequency to around 90 minutes as well as moderating the LH pulse amplitude (Plant, 1986). The weakness of the accumulated clinical data is that pituitary GnRH response cannot be clamped so that the important question of whether GnRH pulse amplitude increases during puberty cannot be accurately assessed (Wu et al, 1989). However, direct measurement of GnRH concentrations in hypothalamic push-pull perifusates in female monkeys in vivo showed unequivocally that GnRH pulse amplitude increased progressively from prepuberty, to early and midpuberty (Chongthammankun et al, 1993;Watanabe and Terasawa, 1989).

Agonadal Model (Open-loop Model):Mainly Animal Studies in Primates

The agonadal model provides a unique opportunity to study the intrinsic neuroendocrine regulation of the GnRH pulse generator at puberty unsullied by the influence of gonadal feedback. Unfortunately, data from agonadal boys are extremely scanty. We have studied only three boys with congenital anorchia who had not previously been treated with testosterone (Wu, 1995). In the youngest (aged 11.0 yr), the LH (IRMA) was consistently undetectable clearly demonstrating the fully expressed CNS-mediated neuroinhibition of the GnRH pulse generator in the prepubertal human male and is similar to a younger agonadal boy (aged 7.5 yr)

studied by ultrasensitive IFMA showing extremely low amplitude LH pulses with pulse interval of 230 minutes (Dunkel et al, 1990). The profound inhibition of LH and FSH in young prepubertal agonadal boys contrasts with the partial suppression observed in girls with gonadal dysgenesis (see below). Two older agonadal boys (aged 11.3 and 13.3 yr), showed exuberant pulsatile excursions with grossly elevated levels of LH (Wu, 1995) and a circhoral pulse frequency typical of the adult castrate male (Matsumoto and Bremner, 1984). Taken together, the limited cross-sectional data in agonadal males appear to be compatible with a brisk switch to high frequency/high amplitude GnRH pulsatile discharge at the onset of puberty. This implies that gonadal and/or testosterone feedback, in addition to suppressing pulse amplitude, must also significantly restrain the frequency activity of the GnRH pulse generator during pubertal development. This probably accounts for the lack of a further increase in LH pulse frequency following the onset of puberty in the gonad - intact axis. A very different pattern however has been reported for girls with gonadal dysgenesis (Levine Ross et al, 1983; Nathawani et al, 1998) where only an increase in pulse amplitude (from a considerably higher prepubertal baseline compared to agonadal boys) with no apparent change in the subcirchoral pulse frequency (interval of 120 - 180 minutes) from age 2 yr to young adulthood was observed. This striking sexual dimorphism in GnRH pulse frequency between the agonadal juvenile male and female has also been described in neonatally gonadectomised monkeys (Plant, 1985; Pohl et al, 1995). There is evidence which suggest that prenatal exposure of the fetal hypothalamus to androgens may have an impact on the postnatal sex difference in modes of gonadotropin secretion in sheep (Kosut et al, 1997).

The tempo of the pubertal transition of GnRH secretion is more clearly defined in a longituidinal study of castrated juvenile male rhesus monkey whose pituitary response has been primed and clamped by intermittent exogenous GnRH infusion (Sutter, 1998). The onset of puberty was punctuated by an abrupt acceleration in GnRH pulse frequency and amplitude which presumably initiates the subsequent and more insiduous pubertal process. This accords with findings in the human male.

Summary

A pivotal but discrete acceleration (doubling) of GnRH pulse frequency at the onset of puberty abetted by a simultaneous increase in GnRH pulse amplitude, enhancing responsiveness to GnRH, triggers a major pituitary amplification of LH production rate in the subsequent stages of pubertal development. This pituitary priming action, effected by a relatively modest increase in GnRH pulse frequency and amplitude, appears to be necessary and sufficient for the developmental reaugmentation of gonadotropin secretion. The in vitro data (Haisenleder et al, 1991; Haisenleder et al, 1997; Haisenleder et al, 1998; Kaiser et al, 1995) showing that decreasing pulse intervals from 180 to 30 minutes can maximally stimulate LH beta, FSH beta and alpha subunit gene expression in gonadotrope-like cell lines lend strong mechanistic support to the in vivo information. Increasing cell surface GnRH receptor concentration may also play a part in priming the responsiveness to GnRH (Kaiser et al, 1997; Katt et al, 1985; and Albanase et al, 1996; Kaiser et al, 1997 for review).

NEUROBIOLOGICAL MECHANISMS TRIGGERING PUBERTAL ONSET

GnRH neurons originate from the olfactory placode in the nose and migrate along the organised paths of the terminal nerves to the fetal forebrain and hypothalamus during the first trimester of pregnancy (Schwanzel-Fukuda et al, 1996). All structural elements of the GnRH pulse generator and the neurovascular links with the anterior pituitary are established by 12 weeks of gestation. The brief neonatal gonadotropin and sex steroid secretion to levels comparable to those in adulthood (Plant, 1995; Grumbach and Styne, 1998) indicates that the hypothalamic-pituitary unit has differentiated to full functional capacity during fetal development. The GnRH pulse generator, pituitary, testes and end organs of reproduction are potentially functional in infancy and childhood, all capable of being activated by the appropriate experimental (chemical, electrical, or structural) and pathological stimuli leading to precocious puberty. Thus the brake in this system, applied from late infancy and maintained during childhood, must reside proximal to the GnRH neurons in the CNS. Knowledge on the mechanism(s) of CNS inhibition of GnRH secretion in childhood and its subsequent release is crucial to our understanding of the regulation of pubertal onset.

Many approaches have been used over the last two decades to characterize neuroregulation of the GnRH pulse generator during the onset of puberty. These included direct measurement of levels of neurotransmitters and GnRH in the stalk/median eminence by push-pull perfusion in vivo; assessing effects of putative neuroregulators on hypothalamic explants and GnRH neuronal cell lines in vitro; pharmacological manipulation using agonists, antagonists, immunoneutralization and antisense oligonucleotide to neurotransmitter receptors and enzymes for neurotransmitter synthesis; double-label in situ hybridisation for GnRH and neurotransmitters; structural remodelling of synapses and glial ensheathment in the neuronal network governing GnRH release.

It is now accepted that the pineal hormone, melatonin (Luboshitsky et al, 1995; Caballo, 1993; Caballo and Ritschel, 1996; Ozata et al, 1996 and Brezezinski, 1997 for review), and endogenous opioid peptides (Fraioli et al, 1984; Petraglia et al, 1986; Mauras et al, 1986) play no role in human/primate pubertal regulation. Although virtually all known neurotransmitters and neuromodulators can potentially exert stimulatory or inhibitory influences on GnRH secretion, recently, two candidates have emerged as potentially crucial players in the control of pulsatile GnRH secretion in the juvenile brain.

A considerable body of experimental evidence has accrued to implicate activation of the excitatory amino acid glutamate receptors of the n-Methyl-D-Aspartic acid (NMDA) and kainate subtype on the GnRH neurons in the developmental increase in pulsatile GnRH secretion at the onset of puberty (Urbanski and Ojeda, 1987; Plant et al, 1989; Wu et al, 1990; Bourguignon et al,

Bourguignon et al, 1995; Zamorano et al, 1998; Eyigor andJennes, 1997 and Brann and Mahesh, 1997 for review). Substantial experimental and some preliminary clinical evidence also indicate that gamma amino butyric acid (GABA) is a key neurotransmitter responsible for suppressing pulsatile GnRH release in the juvenile phase, the removal of which appears to be critical for the onset of puberty (Chongthammankun and Terasawa, 1993; Mitsushima et al, 1994; Terasawa, 1995; Bourguignon et al, 1997; Bourguignon et al, 1997; Bourguignon et al, 1997; Jung et al, 1998). The relative importance of the dual glutaminergic and GABAergic control of GnRH secretion at the onset of puberty has not been fully elucidated. It has been hypothesised that disappearance of retrochiasmatic inhibitory GABAergic interneurons may remove the prepubertal inhibition on the GnRH axons thereby allowing the glutaminergic drive to proceed unhindered (Bourguignon et al, 1997). It has also been suggested that a decline in glutamic acid decarboxylase (GAD 67), the catalytic enzyme responsible for converting glutamate to GABA, may lead to a fall in GABA and a simultaneous or subsequent increase in glutamate which could trigger the pubertal increase in GnRH release (Mitsushima et al, 1996; Terasawa et al, 1998).

In contrast to the paucity of synaptic inputs to GnRH neurons, ensheathment of the GnRH cell body by glial cells and axon terminals by modified ependymo-glial cells known as tanycytes is abundant (Silverman et al, 1991; Witkin et al, 1991). It has therefore been suggested that the secretory activity of GnRH neurons is regulated not only by transsynaptic inputs but also by tropic molecules of glial origin (Ma et al, 1994; Ojeda, 1997). Transforming growth factor alpha (TGF-alpha) and its distant congener neu-differentiation factor, NDF, are produced in hypothalamic astrocytes and stimulate GnRH release via a glial intermediacy (Ojeda, 1997).

The derepression of GnRH pulsatile secretion and its antecedent neurotransmitter/neuropepptide regulation is likely to involve some remodelling of synpatognesis and glial coverage. Thus structural plasticity in the GnRH neuronal network may represent a critical maturational event underlying the pubertal reaugmentation of pulsatile GnRH secretion. This is supported by the finding that polysialic acid neural cell adhesion molecule (PSA-NCAM), an embryonic marker for neuronal plasticity, is expressed in the region of the GnRH pulse generator of the pubertal monkey (Perera et al, 1993). However, the neuroanatomical correlates for the functional developmental changes have not shown major differences apart from an attanuation of synpatic density on the GnRH perikarya (Perera and Plant, 1997). The identity of the neurotransmitter at these synpatic sites are unknown and their functional significance to changes to GnRH secretion have not been established. To date, a fully integrated model of synpatological interrelationahips between the critical transmitter systems responsible for the characteristic changes in GnRH release during childhood and puberty remains to be established.

Leptin and Puberty

The identification of the obesity (*ob*) gene and its encoded protein product, leptin, as the circulating hormone produced by adipocytes for control of body weight (Zhang et al, 1994; Pollymounter et al, 1995; Halaas et al, 1995; Campfield et al, 1995) has stimulated a new area of research to determine its potential role as a key metabolic regulator of neuroendocrine function (Auwerx and Staels, 1998; Magoffin and Huang, 1998; Buchanan et al, 1998; Houseknecht et al, 1998; Flier, 1998 for review). Multiple functions of leptin include inhibition of food intake via increased anorexic (melanin concentrating hormone) and/or decreased orexic (NPY, galanin, orexin A & B) neuropeptides, stimulation of energy expenditure (via beta adrenergic stimulation and induction of uncoupling proteins, UCP2 & 3) and as a regulatory signal to the reproductive system (increases GnRH secretion) and the GH axis (increases GH). NPY initially seemed a possible mediator of the central actions of leptin (Stephens et al, 1995). However, reproductive function and starvation responses are preserved in the NPY knockout mouse (Erickson et al, 1997); this would argue against a critical role of NPY in mediating the leptin signal in the brain. How leptin exert its effects in the CNS and in particular the hypothalamus at present remains unclear.

The powerful impact of nutritional status on reproduction and puberty is well documented (Bronson, 1985; Bronson, 1986). The concept that puberty, at least in girls, only starts when a critical body mass or percentage body fat has been attained (Frisch and McArthur, 1974; Frisch, 1997) has been around for 25 years but was not supported by formal data. While it is acknowledged that the onset of puberty/fertility and energy availability is physiologically linked, the precise signal by which adipose stores inform the hypothalamus of the degree of energetic reserves was unknown. Because circulating leptin levels parallel changes in nutritional status and energy (fat) storage across a broad range from starvation to obesity (Maffei et al, 1995; Considine et al, 1996), it seems to be a plausible candidate to signal energy sufficiency in the growing child. The notion that circulating leptin may provide the missing somatic or metabolic signal for triggering or timing the onset of puberty has been examined in studies in animals and man.
Leptin treatment of normal prepubertal female mice advanced the onset of puberty compared with controls (Chehab et al, 1997; Ahima et al, 1997). However, more detailed studies which controlled for the effects of reduced food intake, dose and route of administration did not confirm the action of exogenously administered leptin in advancing pubertal onset in female rats (Cheung et al, 1997; Gruaz et al, 1998). Leptin administration prevents the decrease in pulsatile LH secretion during fasting in adult rats (Nagatani et al, 1998). The finding that leptin can reverse the effects of reduced food intake but cannot precociously advance onset of puberty is more in keeping with a permissive role as a metabolic gate which allows other critical rate limiting factors to control the precise timing of pubertal onset once an energy store threshold has been attained. Cross-sectional and longitudinal studies in male rhesus monkeys (Plant and Durrant, 1997; Urbanski and Pau, 1998) and rats (Flier, 1998) showed that normal initiation of puberty occurred in the absence of any preceding changes in plasma leptin levels. In contrast, a longitudinal study in 8 healthy boys showed a fleeting 2-fold increase in leptin just before the onset of detectable testosterone rise (Montzoros et al, 1997). Large cross-sectional studies in

healthy children however showed a progressive age and BMI-related increase in leptin in both boys and girls up to early puberty from whence a striking sexual divergence supervened. Leptin then showed a further increase in pubertal girls but a marked inflexion in boys with a decline to the nadir at Tanner stage G5 (Garcia-Mayor et al, 1997; Blum et al, 1997; Clayton et al, 1997). From the perspective of regulation of puberty, these studies are limited by problems inherent in interpreting cross-sectional developmental data, the imprecise definition of neuroendocrine pubertal onset and the indirect methods in assessing body composition. Nevertheless they consistently demonstrated the important sexual dimorphism in pubertal leptin changes suggesting that once the pubertal "threshold" has been reached, sustained or further increases of the adipocyte hormone is not required, to complete puberty or to maintain reproductive function. These results are consistent with animal data and support the view that progressively rising levels of leptin in late childhood signals the attainment of a safe threshold of energy reserve sufficient for reproductive expenditure rather than that leptin is the trigger which releases the neurobiological brake on the GnRH pulse generator. Thus, puberty may only be manifest when the capacity for switching on the GnRH pulse generator *and* the attainment of sufficient leptin are both achieved coincidentally. Clearly, more studies are required to clarify the mechanism by which the adipocyte-leptin and goandotropic axes interact during pubertal development.

GROWTH AT PUBERTY

Adolescent Growth Spurt (synergism between the gonadotropic and somatotropic axes)

Postnatal growth is directly correlated with GH and IGF-1 concentrations and in particularly with GH pulse amplitude. Puberty is characterised by an acceleration in linear growth followed by cessation of growth as the epiphyses fuse with the attainment of final height in early adulthood. Sex steroids, GH & IGF-1 increase contemporaneously with growth acceleration during puberty. 24 hr GH secretion and GH pulse amplitude increases 2-3 fold; this pubertal augmentation of GH is the direct consequence of sex steroid priming of the pituitary, i.e. increased transcription of GH gene in somatotropes.

Interactions between the gonadotropic and somatotropic axes during pubertal growth is best shown in various forms of hypopituitarism (Bourguignon, 1988). Patients with isolated GH deficiency have an attenuated but not absent growth spurt while GH treatment alone in pan-hypopituitary patients is not sufficient to induce the full pubertal growth spurt which requires the addition of sex steroids (Aynsley-Green et al, 1976; Tanner, 1976). Patients with isolated hypogonadotropic hypogonadism and intact GH axis have no pubertal growth spurt (low sex steroids and no pubertal augmentation of GH) but ultimately achieve excessive final height due to delayed epiphyseal closure (Uriate et al, 1992). These examples indicate that androgens, at low doses, exert a direct effect on chondrocyte proliferation and/or

IGF-1 production (Attie et al, 1990; Rogol, 1994), in addition to and independent of its facilitatory effects on pituitary GH synthesis and release. Normal adolescent growth therefore depends on the synergistic action between GH and sex steroids, each contributing approximately 50% of the total pubertal height gain. Sex steroids also determine the duration of the growth spurt by promoting epiphyseal closure at higher doses, thereby terminating further growth.

Role of Estrogens in Male Puberty

It is now well established that interactions between the gonadotropic and somatotropic axes during pubertal growth is dependent on aromatization of testosterone to estradiol. Thus, dihydrotestosterone (Keenan et al, 1993) and 17-alpha alkylated androgens such as oxandrolone (Metzger et al, 1994), which are non-aromatizable, do not increase GH despite their positive effects on growth presumably via direct action on the growth plate. Estrogen receptor blockade by tamoxifen decreased GH and IGF-1 in late pubertal boys (Metzger and Kerrigan, 1994). Priming with ethinylestradiol 40μg/m^2 for 2 days in prepubertal boys and girls increased GH response to three different provocative test (Marin et al, 1994). Patients with complete androgen insensitivity (46XY phenotypic females) have adolescent growth spurts at the appropriate time and final heights intermediate between normal men and women (Zachman et al, 1986; Quigley et al, 1995) suggesting that activation of the androgen receptor is not required for adolescent growth spurt if normal estrogen action (and GH/IGF-1) is preserved. In prepubertal boys estradiol and estrone levels are low but detectable by recombinant cell bioassay (Klein et al, 1996). Estrogens rise and fall progressively in puberty closely correlated with changes in growth velocity. Administration of low doses of estrogen, corresponding to a serum estradiol level of about 4pg/ml (15 pmol/l) caused a 60% increase over the prepubertal growth rate in both boys and girls (Cutler, 1997).

Closure of the epiphyses and accretion of bone mass in males, have also been shown to be critically dependent on estrogens. This unexpectedly pivotal role of estrogens in male puberty has been elegantly illustrated by experiments of nature (Bulun, 1996; Bacharach and Smith, 1996; McGillivray et al, 1998). Estrogen resistance due to an inactivating mutation in the estrogen receptor alpha gene (Smith et al, 1994) and estrogen deficiency caused by mutations in the aromatase gene (Morishima et al, 1995; Carani et al, 1997) give rise to a consistent phenotype in the 3 men. Despite elevated T levels, affected adult males are extremely tall with open epiphyses in the third/fourth decade with enuchoidal proportions and osteoporosis. In the aromatase deficient subject, estradiol but not testosterone treatment increased spinal bone mineral density and promoted complete epiphyseal closure after 9 months (Carani et al, 1997). These studies show unequivocally that cessation of growth and attainment of peak bone mass are critically dependent on estrogen receptor activation in male as well as female puberty. Estrogen treatment alone in patients with androgen insensitivity however cannot correct the subnormal bone mineral density (compared to male or female references). Direct androgen receptor mediated action therefore is also required for normal peak bone mass (Munuz-Torres et al, 1995).

In summary, pubertal growth spurt in both sexes is driven primarily by estrogen and not androgen. In boys as well as girls, estrogen has a biphasic effect on long bone epiphyses, with maximal stimulation of linear growth at low levels and epiphyseal closure at high levels.

DELAYED PUBERTY

This is a common clinical problem affecting boys who do not show signs of pubertal development at the age of 13.5-14 yr or 2.5 S.D. above the mean age at onset of puberty. Specific pathologies in the hypothalamic-pituitary-testicular axis are very rare but an increasing variety of conditions presenting with delayed puberty resulting from natural mutations of key genes in the reproductive axis are being described. They have yielded valuable new insights in the molecular pathophysiology of hypogonadism.

Etiology

GnRH deficiency with (Kallmann's syndrome) or without anosmia/hyposmia (idiopathic hypogonadotropic hypogonadism). Isolated gonadotropin deficiency is synonymous with GnRH deficiency. GnRH deficiency presents with failure of pubertal development in boys with appropriate height for age, enuchoidal body proportions who may also have cryptorchidism and micropenis. Fifty-five percent of all cases of GnRH deficiency is caused by the genetically heterogeneous condition Kallmann's syndrome where hypogonadotropic hypogonadism is associated with anosmia or hyposmia due to olfactory bulb dysgenesis. Kallmann's syndrome affects 1 in 10,000 males (and 1 in 70,000 females) occurring sporadically or with an autosomal or X-linked inheritance in familial cases (see Chapter 6).

X-linked Kallmann's syndrome is caused by mutations in the KAL gene located in region 22.3 of the X chromosome (Hardelin and Petit, 1995 and Ballabio and Zoghbi, 1995 for review). The candidate gene encodes a 680 amino acid putative extracellular matrix glycoprotein (anosmin-1) showing homologies with morphoregulatory neural adhesion molecules (Soussi-Yanicostas et al, 1996; Rugarli et al, 1996). Anosmin-1 is involved in the formation of the olfactory/terminal nerve fibre complex which forms the bridge along which GnRH neuron migrate from their origin in the olfactory placode in the nasal septum to the hypothalamus in early fetal brain development. Agenesis or malformation of the olfactory bulb and the absence of the olfactory/terminal nerve in X-linked Kallmann's syndrome, as a result of KAL gene mutation, probably accounts for the failure of GnRH neurons to migrate with the arrested neurons being lodged around the cribriform plate (Schwanzel-Fukuda et al, 1989). Deletions and disparate mutations in the coding regions (14 exons) of the KAL gene have been identified in only 40-48% of X-linked Kallmann's syndrome pedigrees (Hardelin et al, 1992; Hardelin et al, 1993; Quinton et al, 1996). While 34% of all cases of Kallmann's syndrome or idiopathic hypogonadotropic hypogonadism (IHH) are familial, the X-

linked mode of inheritance accounts for only one-third of familial cases, the rest being autosomal (Waldstreicher et al, 1996; Georgopoulos et al, 1997). It is highly probably that additional unidentified autosomal gene(s) (with sex-limited expression) are also involved in the pathogenesis of GnRH deficiency. The coexistence of Kallmann's syndrome and IHH in the same family (Waldstreicher et al, 1996) suggests that these two forms of GnRH deficiency should be regarded as members of a family of disorders resulting from mutations/malfunctions in a series of key interacting developmental genes regulating the ontogeny of GnRH neurons and/or pulse generator. This more expansive concept of GnRH deficiency should encompass a broader spectrum of developmental abnormalities in GnRH secretion including the atypical phenotypes such as arrested puberty after apparently normal onset (Spratt et al, 1987), normalisation of GnRH secretion in adulthood many years after Kallmann's syndrome was diagnosed - Bauman variant (Bauman, 1986), fertile eunuch syndrome (Smals et al, 1978), and adult-onset IHH (Nachtigall et al, 1997). Elucidating the pathophysiological basis of the various congenital forms of GnRH dysregulation will lead to identification of a likely cascade of gene products that orchestrate the onset of secondary sexual development.

In addition to anosmia, many other somatic abnormalities associated with Kallmann's syndrome have been reported: midline craniofacial defects, hearing deficit, colour blindness, abnormal eye movements, synkinesis, renal malformations, epilepsy, mental retardation and icthyosis. They are inconstant and variable in intensity even within a pedigree. However, upper limb mirror movements (85%), renal agenesis (38%), high arch palate and pes cavus deformity appear to be surrogate markers of KAL gene mutation rather than fortuitously associated or to have arisen from contiguous genes (Hardelin et al, 1992; Quinton et al, 1996). Careful identification of these are therefore useful for early diagnosis in childhood or in apparently sporadic cases.

X-linked congenital adrenal hypoplasia associated with hypogonadotropic hypogonadism. A rare X-linked variety of neonatal adrenal insufficiency, adrenal hypoplasia congenita (AHC), associated with delayed puberty due to hypogonadotropic hypogonadism has recently been recognised to be caused by deletion or mutation of the DAX-1 gene localised within the *D*osage-sensitive sex-reversal locus and the *A*HC locus on the *X* (Xp21) chromosome (Muscatelli et al, 1994;Guo et al, 1995; Burris et al, 1996). DAX-1 gene consists of two exons separated by a single 3.4 Kb intron and is expressed in the fetal and adult adrenal cortex, fetal gonad somatic supporting cells, hypothalamus and pituitary and encodes an orphan nuclear hormone receptor. The gene product contains an entire ligand binding domain at the C-terminal but differs from other classical members of the nuclear hormone receptor superfamily at the N-terminal where no canonical zinc fingers are found. Identity of the ligand for DAX-1 protein is unknown. All DAX-1 gene mutations described to date have been located in the C-terminal ligand binding domain with the vast majority being nonsense or frameshift mutations (Guo et al, 1995; Yanase et al, 1996; Peter et al, 1998).

Another orphan receptor, steroidogenic factor-1 (SF-1), is co-localised with DAX-1 in multiple endocrine tissues during early organogenesis (Ikeda et al, 1996). SF-1 knockout mice share some common phenotypic features with AHC (Luo et al,

1994). Formation of the ventromedial hypothalamic nucleus is defective in SF-1 deficient mice which may lead to abnormal GnRH pulse generator function (Ikeda et al, 1995). To date, SF-1 mutation has not been reported in humans. Accumulating data suggest that DAX-1 acts as a transcription silencing co-repressor of SRY - SF-1 activation of SOX-9 expression during early gonadal (Sertoli cell) differentiation (Swain and Lovell-Badge, 1997; Swain et al, 1998), inhibits SF-1 and StAR mediated transactivation (Ito et al, 1997; Lalli et al, 1997; Crawford et al, 1998) and antagonises synergy between SF-1 and WT1 in the developing testis (Nachtigal et al, 1998). Although the promotor region of DAX-1 harbours a candidate SF-1 binding site (Burris et al, 1995), DAX-1 expression is maintained in SF-1 knock out mice (Ikeda et al, 1996). These two transcription factors probably interact (? competing with other co-repressor) in a converging common pathway (Yu et al, 1998) to control expression of key developmental genes in multiple endocrine tissues including adrenal, gonads, pituitary gonadotropes and hypothalamus.

X-linked (cytomegalic) AHC is characterised by large vacuolated adrenal cells in a disorganised persistent fetal adrenal cortex. This is distinct from the sporadic or autosomal recessive variety (molecular basis unknown) in which adult zonal architecture is preserved in a miniature adrenal. X-linked AHC typically presents in the first months of life with adrenal insufficiency (failure to thrive, salt -wasting, hypoglycaemic convulsions and hyperpigmentation) and is commonly associated with failure of secondary sexual development with cryptorchidism. The isolated hypogonadotrophic hypogonadism can result from either a predominantly hypothalamic (Kletter et al, 1991) or pituitary deficit (Habiby et al, 1996). The exact mechanism underlying the impaired GnRH or gonadotropin secretion remains to be clarified. One Japanese infant with DAX-1 mutation (A300-V) showed normal neonatal activity in the hypothalamic-pituitary-testicular axis suggesting that the problem is a failure to release the GnRH pulse generator from childhood suppression (Takahashi et al, 1997). Though Leydig cells respond well to hCG/LH, induction of spermatogenesis is usually unsatisfactory (even in the absence of cryptorchidism) suggesting as yet unrecognised additional developmental defects in Sertoli or germ cells. X-linked AHC can occur as part of a contiguous gene syndrome (Xp22.1-p21.3) together with Duchenne muscular dystrophy, glycerol kinase deficiency and/or mental retardation (McCabe, 1995).

GnRH gene mutation. GnRH gene deletion in the hpg/hpg mouse leads to profound hypogonadotrophic hypogonadism (Mason et al, 1986). However, in patients with idiopathic hypogonadotrophic hypogonadism, GnRH gene structure is normal (Weiss et al, 1989; Weiss et al, 1991; Nakayama et al, 1990; Layman et al, 1997)

GnRH resistance. The human GnRH receptor gene has recently been cloned. The gene product is a typical G-protein coupled receptor except for the absence of the intracellular C-terminus (Kakar et al, 1992; Kakar, 1997 and Naor et al, 1998 for review). Compound heterozygous missense or homozygous mutations of the GnRH receptor gene has been described in 5 families with 13 individuals (6 males, 7 females) affected by "idiopathic" hypogonadotropic hypogonadism (de Roux et al, 1997; Layman et al, 1997; Kottler et al, 1998; Pralong et al, 1998) inherited as an

autosomal recessive trait. Heterozygote parents and sibs were phenotypically normal. Depending on the site and nature of the amino acid substitutions, functional disturbance in GnRH receptor are variable causing a range of phenotypes. Thus, some compound heterozygotes seem to have only partial gonadotropin deficiency with normal or slightly delayed puberty, normal gonadotropin response to pharmacological GnRH stimulation and even active spermatogenesis similar to the fertile eunuch syndrome. Females can present with either primary or secondary amenorrhoea. In one adult male patient, LH pulse frequency was reported to be normal but LH pulse amplitude was severely reduced (de Roux et al, 1997). Other cases had more severe gonadotropin deficiency and presented with complete failure of sexual maturation and resistance to pulsatile GnRH therapy (Pralong et al, 1998). These partial and variable loss of function mutation involving the GnRH receptor gene provides a good illustration of the importance of increasing GnRH pulse amplitude during physiological pubertal transition. At the diagnostic level, an intact response to pharmacological stimulation by exogenous GnRH bolus clearly does not preclude partially inactivating mutations in the GnRH receptor. Patients with familial hypogonadotrophic hypogonadism without stigmata of Kallmann's syndrome are therefore candidates for molecular screening to detect potential mutations in the GnRH receptor gene. Failure to response to increasing doses during pulsatile GnRH treatment is a further clue to the diagnosis. However, to date the incidence of GnRH receptor mutation appear to be low - 2% of all IHH probands (Layman et al, 1998).

Isolated LH deficiency. LH beta subunit homozygous gene mutation (single amino acid substitution Gln54-Arg) has so far only been identified in one hypogonadal man with elevated immunoactive but biological inactive LH (due to loss of receptor binding), Leydig cell aplasia, pubertal failure and infertility (Weiss et al, 1992). Male heterozygous relatives show a high incidence of infertility and low testosterone. In this patient, hCG treatment activated spermatogenesis and increased testosterone. The majority of cases of abnormal LH action are the results of LH receptor mutations leading to male pseudohermaphroditism (Kremer et al, 1995; Latronico et al, 1996) or male limited precocious puberty (see below).

LH polymorphism. Polymorphism in the LH beta subunit sequence arising from two single amino acid substitutions (Trp8-Arg and Ile15-thr) in the LH beta gene (Haavisto et al, 1995; Suganuma et al, 1996) can give rise to a variant form of LH distributed with varying frequencies in different ethnic groups (Huhtaniemi and Pettersson, 1998 for review) (see Chapter 1). The variant LH, containing an extra glycosylation site identical to hCG beta subunit, has increased bioactivity but shorter circulating half-life compared to wild type LH. Although, pubertal progression is slower, the timing of pubertal onset and adult male reproductive functions are normal (Raivio et al, 1996).

Isolated FSH deficiency. A few cases of presumed isolated FSH deficiency associated with variable hypospermatogenic infertility or normal fertility and normal testosterone have been described (Huhtaniemi and Pettersson, 1998). This clinical picture resembles the phenotype of five adult men with homozygous

inactivating mutations in the FSH receptor gene who are normally virilized but showed variable reductions in testicular size and sperm production (Tapanainen et al, 1997) and of FSH beta knock out male mice (Kumar et al, 1997). This suggests that FSH is not critical to male fertility and at most would exert only a quantitative effect on spermatogenesis. However, failure of sexual maturation with 1-2 ml testes, low testosterone, high LH, undetectable FSH and azoospermia in an 18 year old male have recently been described to be associated with a homozygous mutation in the FSH beta subunit gene (Phillip et al, 1998). A two-nucleotide deletion of codon 61 of exon 3 led to a frameshift followed by a premature stop codon. This mutation was identical to that reported in 3 female patients with primary amenorrhoea (Mathews et al, 1993; Mathews and Chatterjee, 1997; Layman et al, 1997). The truncated FSH beta chain is unable to dimerize with the alpha subunit to from bioactive FSH. The reason for the low testosterone and elevated LH indicating Leydig cell dysfunction in this patient is currently unclear. A further male with homozygous FSH beta gene mutation (Cys82-Arg) was reported to be normally virilized but infertile with small (3-4 mL) descended testes (Mathews et al, 1998). These newly-described cases require confirmation and further study before their significance in delineating the role of FSH action in the testes can be fully appreciated.

FSH resistance. The FSH receptor is a member of the G protein-coupled receptor family with seven transmembrane loops and a a very large extracellular domain. It is encoded by a gene localised to chromosome 2p21 (Simoni et al, 1997 for review). FSH receptor gene inactivating missense homozygous mutation (Ala189-Val) of exon 7 in the extracellular domain accounts for 29% of Finnish females with pure ovarian dysgenesis (Tapanainen et al, 1997;Aittomaki et al, 1995;Aittomaki et al, 1996) but has not been detected elsewhere (Layman et al, 1998). All affected females have primary or early secondary amenorrhoea. The male siblings' phenotype was discussed in the preceding paragraph. A French woman with hypergonadotropic secondary amenorrhoea from the age of 16 years was found to have an Ile160-Thr and Arg573-Cys compound heterozygous mutation in the FSH receptor gene (de Roux et al, 1998). Thus far, it appears that FSH receptor mutations are likely to remain a very rare cause of pubertal failure in either sex.

Diagnosis

The overwhelming majority of boys presenting with delayed puberty have constitutional or physiological delay and are destined to mature spontaneously later. However, delayed or failure of pubertal development is frequently the presenting complaint in patients with congenital hypogonadism. While hypergonadotropic hypogonadism is readily diagnosed by documenting high levels of gonadotropins, in the absence of clinical stigmata consistent with Kallmann's syndrome, differentiation between IHH and constitutional delayed puberty (CDP) is difficult but important to patient management. Even with third generation gonadotropin assays, it is not possible to distinguish normal prepubertal basal gonadotropin levels from those in hypogonadotrophic hypogonadism (Wu et al, 1991; Brown et al, 1996). There is also overlap in the response to bolus GnRH stimulation between the

two conditions (Wu et al, 1991). Being a variant of normal where the juvenile physiological hypogonadotrophic state is over-extended, it is not surprising that CDP is functionally similar to permanent hypogonadotropic conditions (IHH) except by the former's self-limiting nature. Thus the main distinguishing feature between the two is that CDP advances spontaneously to pubertal onset and progression while IHH typically remains unchanged with time.

Several new approaches have attempted to detect or anticipate the earliest evidence of spontaneous reactivation of the hypothalamo-pituitary-testicular axis in those with CDP. Mean nocturnal LH using sleep-entrainment as the physiological stimulus and third generation ultrasensitive assay (Brown et al, 1996), sequential urinary gonadotropin measurements over several years (Kulin et al, 1994), and pulsatile GnRH priming followed by assessment of gonadotropin (Smals et al, 1994) or free alpha subunit (Pralong et al, 1995; Lavoie et al, 1998) response to bolus stimulation of GnRH can all potentially differentiate CDP from IHH. However, their impracticality restricts wider and routine application. Superactive GnRH analogue stimulation (Ehrmann et al, 1989; Ibanez et al, 1994; Ghai et al, 1995; Zamboni et al, 1995; Lanes et al, 1997) is a more convenient test which combines priming and testing of the pituitary in a single injection. The peak LH but not FSH or testosterone response at 4 h post-injection was the most informative. Adopting a more physiological approach, we have shown that plasma testosterone in the early morning is a reliable in vivo bioassay of prior exposure to nocturnal GnRH priming/secretion in prepubertal boys which can predict the onset of spontaneous puberty (attainment of testes volume 4 mL) in the ensuing 12 (77% of subjects) to 15 (100% of subjects) months (Wu et al, 1993). Accordingly, a single "screening" morning plasma testosterone concentration above a threshold of 0.7 nmol/L can distinguish a more mature subgroup from those whose pubertal onset is likely to remain delayed. This information, especially if buttressed by follow-up measurements over several months of observation, facilitates early clinical decision to be made on the need for treatment and alerts the clinician to the possibility of IHH. It is interesting to note that during spontaneous pubertal development, inhibin B rises and plateaus early. The fastest rate of increase in was observed between Tanner stage I to II before any significant increase in daytime testosterone and, in some cases, testes volume (Crofton et al, 1997; Andersson et al, 1997). Inhibin B levels are low in IHH patients without evidence of spontaneous puberty but the variability in basal concentrations of this marker of Sertoli cell number and function may limit its utility in the differentiation from CDP. The diagnostic value of inhibin B in this context has not so far been critically assessed. Although no single measure at one time point can indubitably differentiate between IHH and CDP, when applied correctly and combined with serial testing, these investigations can usually provide helpful information which permits clinical decision to be made at an earlier stage than before. In reaching the correct clinical decision (i.e. to treat or to wait) it is prudent to emphasise the variable and diverse course of spontaneous pubertal development and the unfolding of permanent hypogonadotrophic hypogonadism (Kulin, 1996; Kulin et al, 1997). Partial gonadotropin deficiency and arrest of pubertal development after normal onset are examples. Even the most informative tests are therefore limited by the nature of an evolving process, the pace and pattern of which is often unpredictable (Kulin, 1996; Kulin et al, 1997).

Treatment

There are now many reports in the literature confirming that induction of secondary sexual characteristics and acceleration of growth can be safely accomplished using low doses of parenteral testosterone (Zachman et al, 1987; Richman and Kirsch, 1988), oral testosterone (Butler et al, 1992; Albanese et al, 1994), oxandrolone (Stanhope et al, 1988; Papadimitriou et al, 1991; Uruena et al, 1992; Bassi et al, 1993) and mesterolone (Strickland, 1993) in boys with CDP without undue advance in skeletal age or compromising final height. This has promoted a widely accepted management strategy which aims to induce and maintain the patient's secondary sexual development in line with the peer group norm and to prevent adverse psychosocial and physical sequelae of untreated delayed puberty. One of most persuasive arguments for early treatment is the realisation that males with a history of untreated delayed pubertal onset beyond 15 yr have significantly lower peak bone mass in adulthood even though spontaneous development eventually supervened (Finkelstein et al, 1992; Finkelstein et al, 1996).

As early short-term treatment becomes more accepted, it is less critical to make a definitive diagnosis from the outset in a patient presenting with delayed puberty. There is no reason to withhold or delay treatment if the patient is distressed or under pressure even if a diagnosis has not been established. Decisions on the need for long-term treatment can be deferred while the correct diagnosis will emerge eventually during follow-up. After the age of 18, if there is no clinical sign of pubertal development or increase in gonadotropins and testosterone after withdrawal of exogenous testosterone, the diagnosis of permanent IHH is probable.

Regardless of diagnosis, boys showing no signs of pubertal development by the age of 14 yr should be considered for induction or initiation of puberty. Testosterone is the androgen of choice since it can mimic the full spectrum of sex steroid actions during puberty including those mediated via aromatization to estrogen. The most commonly employed regimes are intramuscular testosterone enanthate 50mg monthly or oral testosterone undecanoate 40mg daily or alternate days for 6 - 12 months. If spontaneous progression in puberty and growth do not occur off treatment, a further course of testosterone is administered. There is no evidence that exogenous testosterone treatment accelerates the endogenous maturation of the GnRH pulse generator or the Leydig cells (Kulin et al, 1997; Brown et al, 1995). There is no place for hCG, menopausal gonadotropins or pulsatile GnRH in the induction of puberty; the complexity, low acceptability and high costs of these modes of treatment negates any perceived advantage of increasing testicular size. Therapeutic stimulation of the testes should be deferred until fertility is actively seeked. Testosterone treatment in adolescence does not impair adult gonadal functions (Lemcke et al, 1996).

PRECOCIOUS PUBERTY

Sexual precocity can be defined as the appearance of any sign of pubertal development at an age less than 2.5 SD of the mean age for normal pubertal onset. In European populations, this is usually under 9 years of age for boys.

The vast majority of cases are the result of premature reactivation of the GnRH pulse generator and pulsatile gonadotropin secretion. True (hypothalamic GnRH-dependent) precocious puberty is 5-6 times less common in boys than girls; the female excess being largely due to the "idiopathic" form. Thus in boys with true precocious puberty, the likelihood of recognisable pathologies, especially CNS tumours, is much higher (up to 50% of cases) (Grumbach and Styne, 1998). The much rarer pseudoprecocious puberty is independent of hypothalamic GnRH or pituitary gonadotropins (Cutler, 1993; Holland,1991). Excessive testosterone is associated with hCG secreting tumours, congenital adrenal hyperplasia, adrenal or gonadal tumours, testotoxicosis and McCune-Albright syndrome

Etiology

True Precocious Puberty (Central, gonadotropin-dependent). CNS or hypothalamic tumours or cranial irradiation may cause local injury-induced stimulation of the TGF alpha/ EGFR signalling system in the glial cells (Junier et al, 1991; Junier et al, 1993). This antagonises or abolishes the neuroinhibitory mechanisms normally holding the GnRH pulse generator in check during childhood and lead to premature pubertal development. Hamartoma of the tuber cinereum consists of a heterotopic mass of nervous tissues including GnRH positive neurons which function like an ectopic and unrestrained GnRH pulse generator (Judge et al, 1977; Hochman et al, 1981). High resolution CT and MR scanning in recent years have detected an increasing number of hamartoma in cases previously diagnosed as "idiopathic" (Cacciari et al, 1983; Pescovitz et al, 1986; Hahn et al, 1988). This is now the commonest cause of true precocious puberty. Hamartomata are malformations rather than true neoplasms and do not progress or enlarge with time (Mahachoklertwattana et al, 1993). They may however give rise to various forms of seizures. GnRH agonists treatment to arrest and reverse the precocious puberty is satisfactory and long-term outcome favourable (see below). Surgery is seldom indicated.

Autosomal dominant familial/sporadic male-limited true precocious puberty (FMPP, SMPP or testotoxicosis) is characterised by early presentation, under 3-4 years, of rapid growth, penile and bilateral testicular enlargement with active Leydig cell and spermatogenesis (Holland, 1991; Schedewie et al, 1981; Rosenthal et al, 1983; Wierman et al, 1985). Although testosterone is in the pubertal or adult range, gonadotropin levels and response to exogenous GnRH remain prepubertal. GnRH analogue treatment has no effect. The LH receptor is a member of the G protein-coupled receptor family. The LH receptor gene contains a uniquely large exon 11 which encodes for the entire transmembrane and intracellular domains (Dufau, 1995). Gain of function heterozygous mutation of the LH receptor gene leading to agonist-independent constitutively-active LH receptor is responsible for FMPP and SMPP (Shenker et al, 1993; Kremer et al, 1993). In vitro expression of mutated receptors in transfected cells show elevated basal levels of cAMP although response to ligand stimulation is preserved (Chan and Cutler, 1998). Thirteen

mutations have been identified to date. All are missense mutations within exon 11, the majority occurring in transmembrane helix 6 with the commonest Asp578-Gly substitution representing 63% of all identified mutations (Chan and Cutler, 1998). There are 13 kindreds of FMPP however in which LH receptor mutations have not been identified in exon 11. Malignant seminoma has been reported in a 35 year old FMPP patient (Martin et al, 1997) emphasising the importance of long-term follow-up.

Pseudoprecocious puberty (Gonadotropin-independent). McCune Albright syndrome (Danon et al, 1975; Ringle et al, 1996) is a sporadic condition characterized by the triad of irregularly-edged segmentally distributed cafe-au-lait spots, progressive polyostotic fibrous dysplasia and gonadotropin-independent precocious puberty which is more common in girls than boys. Early post-zygotic activating germline missense mutation in the gene (Arg201-cysteine or histidine) encoding the G protein subunit $G_S\alpha$ leads to loss of GTPase function, constitutively active adenyl cyclase and cAMP accumulation affecting cellular populations in a mosaic fashion in many tissues (Weinstein et al, 1991; Spiegel, 1996). This is responsible for protean clinical picture from autonomous hyperfunction and/or proliferation in a variety of tissues including gonads, thyroid, adrenal, pituitary and parathyroids. Mutant G protein subunit is presumably expressed in the active seminiferous tubules and hyperplastic Leydig cells (Giovanelli et al, 1978). The precocious puberty in boys in the McCune Albright syndrome share many common endocrinological features with FMPP.

Diagnosis

Gonadotropins levels (third generation assays), basally and in response to GnRH stimulation, gonadal steroids, adrenal steroids (including 17OH progesterone), CNS (MRI), adrenal (CT) and gonadal (ultrasound) imaging, hCG and alpha fetal protein detection usually point to the correct diagnosis. In gonadotropin-dependent precocious puberty in boys, it is essential to actively exclude CNS tumours by MR imaging.

Management

Potent GnRH analogue has revolutionised the treatment of gonadotropin-dependent precocious puberty since 1981 (Crowley et al, 1981; Boepple et al, 1986; Karten and Rivier, 1986; Conn and Crowley, 1994) and replaced progestational agents such as medroxyprogesterone acetate and cyproterone acetate. The superactive analogues desensitize the GnRH receptor signalling pathways (Conn et al, 1995) to produce reversible medical castration which is highly effective in arresting pubertal progression, decelerating linear growth rate and inducing regression of the pituitary-testicular axis back to a prepubertal state. Treatment is safe and increasing widespread use of long-acting monthly injectable depot preparations encourage acceptability and compliance. Plasma testosterone is maintained at <0.7 nmol/L until age 12-13. After discontinuation of treatment, subsequent sexual development proceeds normally (Schroor et al, 1995). Long-term outcome is favourable,

especially when treatment was started early, with significantly improved final height (Paul et al, 1995; Kletter and Kelch, 1994).

Treatment of FMPP and McCune Albright syndrome have largely been dependent on medroxyprogesterone acetate, ketoconazole (in USA) and cyproterone acetate (in Europe). Newer antiandrogens (flutamide, nilutamide, bicalutamide) (Mahler et al, 1998) together with an aromatase inhibitor (testolactone, letrozole, anastrozole) (Keloff et al, 1998) are currently being evaluated. The latter has been shown to be effective in reducing height velocity and skeletal maturation even in the face of elevated testosterone (Laue et al, 1989; Laue et al, 1996) in accordance with current views on the critical role of estrogen in pubertal growth (see above).

Secondary true precocious puberty commonly supervenes in treated patients with CAH and FMPP or any other forms of pseudoprecocious puberty when bone age has advanced to >11 yr. GnRH agonist treatment is then also required.

CONCLUSIONS

The onset of puberty involves a shift in balance between excitatory and inhibitory nuerotransmitter inputs to the GnRH neurons resulting in the derepression of the GnRH pulse generator . However, full details of synpatological interrelationahips between these transmitter systems remain to be established.

1. Concurrent activation and interaction of three functional axes (gonadotropic, somatotropic and leptin-adipocyte axes), primarily but not exclusively at the hypothalamus, is required for normal pubertal onset and progression.
2. Estrogen plays a critical role in male puberty.
3. An increasing number of single gene defects (familial or sporadic), leading to loss or gain of functions at each level of the hypothalmic-pituitary-testicular axis, present for the first time as disordered pubertal development
4. Concepts derived from basic and clinical research and technical improvements in hormone analyses and tissue imaging have significantly improved diagnosis and management of pubertal disorders.

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6 MALE HYPOGONADISM

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INTRODUCTION

Inadequate sexual development, gynecomastia, infertility and sexual dysfunction are the symptoms and signs which most often result in an evaluation for male hypogonadism. The evaluation begins with a careful medical history and physical examination as described in detail elsewhere (Clark, 1994). A detailed sexual history should also be performed preferably with the partner present. Psychochological and marital factors which contribute to sexual dysfunction can be ascertained. Questions about libido are important because a decline in libido is a frequent symptom of hypogonadism, whereas most adult men with neuro/vascular erectile dysfunction have a preserved libido, at least initially. Testicular size should be measured carefully with a ruler or orchidometer. Hypogonadism beginning before the onset of puberty is discussed elsewhere in this volume (Chapter 5). The clinical characteristics of hypogonadism in adult men are listed in Table 1.

Table 1. Clinical characteristics of hypogonadism in adult men.

Decreased libido	Soft smooth skin
Asthenia	Decreased beard, axillary and pubic hair
Erectile dysfunction	Decreased muscle mass and strength
Infertility	Decreased testicular size
Osteopenia/fractures	Gynecomastia
	Decreased prostate size

The laboratory evaluation for male hypogonadism begins with the measurement of the total testosterone level (Winters, 1994). Because variation in the level of the testosterone transport protein sex hormone binding-globulin (SHBG) directly infuences the total testosterone level, the free testosterone level, or the concentration

of testosterone not bound to SHBG (bioavailable-testosterone) is measured to confirm the diagnosis of hypogonadism, when the total testosterone level is borderline, or when the clinical findings and the plasma total testosterone level do not agree. The semen analysis serves as the primary assessment of male fertility. LH and FSH levels are measured if testosterone deficiency is found or if seminiferous tubular dysfunction is suspected because the testes are reduced in size or the semen analysis is abnormal. If LH and FSH are increased, the diagnosis is primary testicular failure. When testosterone deficiency is caused by impaired gonadotropin secretion, serum LH levels may be decreased or normal. Even though the diagnosis of gonadotropin deficiency has been facilitated by the development of sensitive, specific two-site assays, LH values in normal and hypogonadal men overlap because gonadotropin secretion is pulsatile. Therefore, the diagnosis of gonadotropin deficiency cannot be made by measuring LH alone, but rather a low serum testosterone level must be documented. Inhibin-B levels are also reduced in hypogonadal men, and inhibin-B may prove to be a sensitive indicator of the spermatogenic function of the testis.

GONADOTROPIN DEFICIENCY

Gonadotropin deficiency leads to reduced testosterone production and to hypospermatogenesis, hence the designation hypogonadotropic hypogonadism (HH). Gonadotropin deficiency may occur selectively or together with a deficiency of other pituitary hormones, and it may be congenital or acquired. HH may result from a disorders of the pituitary gland or hypothalamus, or from systemic factors which suppress GnRH secretion.

Congenital hypogonadotropic hypogonadism

Patients with congenital HH fail to enter into or progress normally through puberty. The disorder is clinically heterogeneous (Whitcomb et al, 1993). Some patients present as teenagers with complete sexual infantilism. In other patients, the testes enlarge somewhat, and testosterone levels increase slightly. A few men present later in life with incomplete masculinization, infertility or osteoporosis. Their testes may be normal in size. In most men with congenital gonadotropin deficiency the secretion of other pituitary hormones is normal, but gonadotropin deficiency also occurs together with GH, TSH, and/or ACTH deficiency. Patients with congenital HH are often divided into those with anosmia or other midline defects (Kallmann's syndrome), those in whom HH occurs with other disorders, and those with isolated gonadotropin deficiency (Table 2). Gonadotropin secretion is also variable in congenital HH. Frequent blood sampling is used to characterize pulsatile LH secretion which is used as a surrogate for GnRH secretion. In men with HH and infantile testes (< 3 ml volume), LH secretory episodes are usually absent. In partial HH, LH pulse amplitude may be diminished, or pulse frequency may be reduced. Stimulating the pituitary with a bolus of GnRH produces either an absent, attenuated or normal LH response which is proportional to basal LH secretion.

FSH is measurable in most patients with congenital HH suggesting partial independence from GnRH Long-term pulsatile stimulation with GnRH usually increases LH and testosterone levels indicating a defect in GnRH production or secretion, although some subjects do not respond.

Table 2. Syndromes associated with congenital hypogonadotropic hypogonadism.

Syndrome	Clinical Findings
Kallmann's syndrome	Anosmia and other midline defects
Congenital adrenal hypoplasia	Neonatal adrenal failure
Steroid sulfatase deficiency	Congenital ichthyosis, cataracts
Prader-Willi syndrome	Hypotonia, obesity, small hands and feet, retardation
Moebius syndrome	Oculofacial paralysis, seizures, limb anomalies, retardation
Lowe syndrome	Lenticular opacities, hypotonia, renal tubular acidosis
Multiple lentigenes (LEOPARD) syndrome	**L**entigenes (**E**KG conduction defects, ocular hypertelorism, **P**ulmonic stenosis, Growth **R**etardation, **D**eafness
Carpenter's syndrome	Obesity, acrocephaly, craniosynostosis, limb agenesis
Septo-optic dysplasia	Absent septum pellucidum, optic nerve hypoplasia
Partial- or panhypopituitarism	Cranial-facial abnormalities, GH, TSH and ACTH deficiency, diabetes insipidus, Birth trauma

Male hypogonadism with anosmia was first described in 1856 by Maestre de San Juan, and was called olfactogenital dysplasia. The disorder was described as a familial trait by Kallmann in 1944. Many other congenital abnormalities may be present, however (Table 3).

Table 3. Congenital abnormalities associated with hypogonadotropic hypogonadism.

Neurological abnormalities	Genital abnormalities	Somatic abnormalities
Anosmia	Microphallus	Cleft lip
Nystagmus	Cryptorchidism	Cleft or arched palate
Synkinesias		Malformed incisors
Sensorineural hearing loss		Renal aplasia
Cerebellar ataxia		Horseshoe kidney
Seizures		Pes cavus
Color blindness		Digital deformities

Most cases of Kallmann's Syndrome seem to represent sporadic mutations, although in 25-50% of patients there is a family history of either midline defects or hypogonadism (Waldstreicher et al, 1996). The kindred described by Kallmann appeared to have an X-linked recessive trait, but other kindreds suggest autosomal recessive or dominant inheritance with incomplete penetrance. Because the disease affects males far more often than females (6-9 fold), the X-linked form may be most common. Gonadotropin deficiency in X-linked Kallmann's syndrome sometimes results from a defect in the migration of GnRH neurons to the hypothalamus from their origin in the olfactory placode. This form of Kallmann's syndrome was recently mapped to chromosome Xp22.3, and several mutations have been described (Lutz et al, 1993). The candidate gene encodes a 680 amino acid protein, KAL, which is thought to function in axon and neuron migration and targeting during development in the CNS and other tissues, providing an explanation for the association of HH with somatic and neurological abnormalities. For example, anosmia results from hypoplasia of the olfactory nerves and tracts. The condition is only partly understood since anosmia and GnRH deficiency may be dissociated among members of an affected kindred. The coding sequence of the GnRH gene has been uniformly normal, and the cause of most cases of Kallmann's syndrome remains uncertain.

Congenital HH may occur in congenital adrenal hypoplasia (ACH), a rare association which provides insight into the control of gonadotropin subunit gene expression. ACH is characterized by neonatal hypotension, hyponatremia and hyperkalemia, and is generally fatal if untreated with glucocorticoids and mineralocorticoids. Congenital HH and ACH are related mechanistically (Habiby et al, 1996). ACH results from mutation of the gene for the transcription factor DAX-1. DAX-1 and a second transcription factor, SF-1, may form a nuclear protein complex which regulates GnRH receptor and LH subunit gene expression. GnRH production may also be impaired.

Congenital HH may occur with X linked recessive icthyosis, a disorder in which large dark dry scales develop on the trunk and limbs. Deficient arylsulfatase activity (STS) in keratinocytes leads to accumulation of steroid sulfates in the scaly skin. In the placenta, DHEA-S is inefficiently converted to DHEA, and heterozygote mothers may experience problems at childbirth. The association appears to arise from a deletion at chromosome Xp involving both the KAL and STS genes.

Mutations of the GnRH receptor gene have been reported in a few patients with congenital HH without midline defects. Males and females are affected with a pattern of autosomal recessive inheritance. These patients are GnRH resistant and respond poorly to low-dose GnRH therapy, although large doses of GnRH may overcome the defect..

Acquired Hypogonadotropic Hypogonadism

Gonadotropin deficiency may result from a pathological process within the sella which compresses or destroys the normal pituitary gland, from pathology in the suprasellar space that interrupts the axons transporting GnRH to the hypophysial-portal capillaries, or by other mechanisms. Various mass lesions, infectious and

infiltrative processes, head trauma and X-irradiation may produce HH (Table 4). Unique aspects of those disorders which produce hypogonadism are discussed in the following paragraphs.

Table 4. Causes of acquired hypogonadotropic hypogonadism

Pituitary adenomas	Acute and chronic systemic illness
Prolactinoma	Weight loss
Cushing's syndrome	Hemochromatosis
Suprasellar tumors	Autoimmune hypophysitis
Craniopharyngioma	Head trauma
Germinoma	X-irradiation, Post-surgery
Histiocytosis	Polyarteritis
Granulomatous diseases	Estrogen-producing adrenal and testicular tumors
Sarcoidosis	Idiopathic
Tuberculosis	

Men with prolactin-producing pituitary adenomas often present with a loss of libido and decreased sexual potency, or with tender gynecomastia. Prolactinomas in teenagers result in delayed puberty. Testosterone levels are reduced, and the pulsatile pattern of LH secretion is attenuated. In spite of high PRL levels, galactorrhea is rare, presumably because circulating estradiol levels in men are too low to stimulate mammary gland growth and development. With microadenomas, reduced LH secretion reflects GnRH deficiency because pulsatile GnRH treatment restores LH and testosterone levels to normal. Experimental hyperprolactinemia in rats reduces GnRH mRNA levels perhaps through a dopamine mechanism. Successful lowering of PRL secretion with dopamine agonists generally normalizes testicular function, and cabergoline may do so more rapidly than bromocriptine (De Rosa et al, 1998). Unfortunately, men with prolactin-producing pituitary tumors often present late in the course of their disease with headaches and visual disturbance. Gonadotrophs have been destroyed by these macroadenomas necessitating testosterone replacement, or gonadotropin treatment to restore fertility. There is a suggestion that rapidly growing invasive prolactinomas are more common in men than in women.

Hypogonadism is common, and may be the initial complaint, in male Cushing's Syndrome. Serum testosterone levels are low, and basal and GnRH-stimulated LH levels are reduced with either ACTH-producing pituitary tumors or with adrenal adenomas. Hypercortisolemia is the cause of the gonadotropin deficiency since adrenalectomy, or treatment with the steroidogenesis inhibitor mitotane, or the glucocorticoid receptor antagonist mifepristone (R1881) can restore LH and testosterone secretion. Furthermore, high dose glucocorticoid treatment of men with normal testicular function also suppresses gonadotropin secretion. Experimental studies with GnRH-producing hypothalamic cell lines reveal that activated glucocorticoid receptors bind to the promoter region of the GnRH gene, and repress its transcription.

Lymphocytic adenohypophysitis is an autoimmune disorder of the anterior pituitary that occurs most often in women. Approximately 10% of cases have been in men who have presented with headaches and HH, other anterior pituitary hormone deficits and diabetes insipidus, and were found to have pituitary enlargement by imaging. The disorder cannot be distinguished readily from a pituitary tumor, but is suggested by the presence of other autoimmune endocrine disorders such as thyroiditis. The mass may resolve spontaneously or with corticosteroid treatment.

Gonadotropin deficiency may occur with estrogen producing tumors of the adrenals or gonads. Presenting complaints include gynecomastia, infertility, decreased libido and impotence, or a testicular or adrenal mass. Testicular tumors may be palpable, or detected by ultrasonography. Adrenal tumors are usually identified by CT scan. Estradiol and/or estrone levels are usually elevated, and testosterone, LH and FSH levels may be reduced. Androgen-producing tumors may go undetected in adult men, but produce precocious puberty in boys. Sertoli cell tumors may occur with the Carney complex. Tumor overexpression of a Gs-protein a-subunit may activate adenylate cyclase and stimulate steroidogenesis. Stimulation with ACTH or hCG may increase the production of estrogens by adrenal or testicular tumors, respectively. The tumors may be benign or malignant, and treatment is surgical

Rarely, men present with adult onset HH but no explanation is found. Careful follow-up of these men is necessary since tumors and infiltrative lesions of the hypothalamus may be occult. Some patients with adult onset idiopathic HH give a history of remote head trauma.

Selective Deficiency of LH or FSH

Gonadotropin deficiency generally involves both LH and FSH because GnRH stimulates the synthesis and secretion of both gonadotropins, and both hormones are produced by gonadotrophs. Newer sensitive two-site assays reveal that FSH secretion is less dependent on GnRH stimulation than is LH, however. Thus, men with complete GnRH deficiency, absent LH, and plasma testosterone levels in the castrate range, produce some FSH. Similarly, the level of FSH often exceeds LH in plasma of patients with GnRH deficiency due to PRL-producing pituitary microadenomas. The preferential secretion of FSH may be explained by studies in rats which indicate that pituitary activin selectively sustains FSH production. Normally virilized infertile men with low plasma FSH levels but normal LH and testosterone have been reported. Most of these cases were identified using older double antibody radioimmunoassays in which plasma FSH levels in normal men were also sometimes undetectable. With current assays very few infertile men appear to have low FSH levels. Finally, LH and testosterone deficiency with elevated FSH levels suggests an FSH-producing pituitary tumor.

Mutations of the gonadotropin subunit genes have been identified (Conway 1996). An adult male homozygous for a missense mutation of the coding region of the LH-β gene presented with sexual infantilism, a low plasma testosterone and an elevated plasma LH level. The LH produced was bioinactive. A second kindred with a similar, but incompletely evaluated, syndrome has been reported. Up to 3.6%

of the Finnish population produce an LH molecule which was not detected by a two-site immunometric assay. In these subjects, among whom reproductive function is normal, point mutations of the LH-b gene are believed to alter the LH carbohydrate structure, obscuring the epitopes detected by the highly specific two-site assay used since other two-site assays recognized the variant LH. Women with primary amenorrhea and FSH deficiency due to mutation in the coding region of the FSH-β gene have been reported.

Patients with mutations of the LH and FSH-receptor genes have also been identified. Missense mutations of the LH receptor in males cause Leydig cell hypoplasia with ambiguous genitalia whereas activating mutations cause gonadotropin-independent precocious puberty. Men homozygous for inactivating mutations of the FSH receptor have small testes, oligospermia and poor sperm motility, but are not azoospermic, challenging the notion that FSH is an essential factor for spermatogenesis. It is unclear whether these men are completely unresponsive to FSH, however. Plasma inhibin-B levels were reduced (but detectable), and FSH was increased in these men (see Chapter 1). Activating mutations of the FSH receptor may produce a normal phenotype in men since the one case so far reported was of a man with hypopituitarism following hypophysectomy and radiotherapy for a pituitary adenoma whose sperm count was unexpectedly normal in the absence of measurable levels of LH or FSH during treatment with testosterone.

PRIMARY TESTICULAR FAILURE

Disorders which damage the testes can also be classified as inherited or sporadic, and congenital or acquired (Table 5). The symptoms and signs of testicular failure are gradual in onset with the exception of orchitis, which like orchidectomy or GnRH-analog treatment, can produce the vasomotor symptoms of the female menopause. The most constant physical finding in primary testicular failure is small testes. Whereas men with primary testicular failure have been uniformly infertile, testicular aspiration followed by intracytoplasmic sperm injection into an oocyte (ICSI) now permits fertilization and pregnancy even when there are few or no sperm in the ejaculate (see Chapter 14).

Table 5. Causes of primary testicular failure.

Congenital	Acquired
Klinefelter's syndrome and variants	Orchitis: e.g. mumps, leprosy
Cryptorchidism	Trauma
Noonan's syndrome	Torsion
Laurence-Moon-Bardet-Biedl syndrome	Spinal cord injury
Congenital Anorchia	Retroperitoneal fibrosis
Myotonic dystrophy	Cancer chemotherapy
Sickle cell disease	X-irradiation
Immune polyglandular endocrine failure	

Plasma level of FSH and LH are characteristically elevated in men with primary testicular failure, and the testosterone concentration is usually reduced. The total testosterone level is sometimes normal, however, because SHBG is increased by androgen deficiency. The bioavailable or free testosterone level should next be measured if androgen deficiency is suggested clinically, and is usually reduced. Because the seminiferous tubules are more sensitive to damage than are Leydig cells, some men have tubular dysfunction but normal testosterone production. Normal testosterone levels may be sustained by increased LH production, or both LH and testosterone may be normal, and FSH levels may be increased selectively. Decreased testosterone production leads to increased circulating gonadotropin concentrations because the number and amplitude of LH (and presumably GnRH) secretory episodes is increased, indicating that testosterone affects the GnRH pulse generator. Results in experimental animals indicate that testosterone deficiency also increases GnRH receptors. Inhibin is produced by the Sertoli cells of the testis, and selectively regulates FSH secretion by decreasing FSH-β gene expression. Experiments in monkeys and cross-sectional studies in normal and hypogonadal men have shown that reduced plasma inhibin-B levels correlate with and are responsible for the rise in plasma FSH in seminiferous tubular failure (Anawalt et al, 1995).

Klinefelter's Syndrome

Klinefelter's syndrome (KS) results from the presence of an extra X-chromosome which causes seminiferous tubular sclerosis and Leydig cell insufficiency. KS occurs in 1 per 500 to 1 per 2000 live male births. Meiotic nondysjunction results in 24,XY sperm or to 24, XX ova, and fertilization produces a 47, XXY karyotype. The additional X may be of maternal or paternal origin, and advanced maternal age is a risk factor for the development of the syndrome. Nearly 20% of KS patients are 46,XY/47,XXY mosaics among whom the clinical abnormalities are less pronounced. Other reported sex chromosomal abnormalities include 48,XXYY, 48,XXXY and 49,XXXXY. These men are often short, dysmorphic and mentally retarded as well as hypogonadal.

The clinical features of KS are summarized in Table 6. KS is sometimes diagnosed in teenagers who present with incomplete pubertal development, gynecomastia or small testes, and the remaining cases are detected in adulthood because of infertility or androgen deficiency. The testes are usually 1-2 ml in volume or <2 cm in length, and are somewhat firm. Body hair is often reduced, especially if the patient is compared to his male siblings, but hirsutism and frontal balding may occur. Gynecomastia is present in 30-75% of cases. The phallus may be slightly reduced in size but is normally formed, and cyptorchidism is uncommon.

Table 6. Clinical features of Klinefelter's Syndrome

Childhood	Adulthood
Small testes and penis	Small testes
Increased leg and arm length	Infertility
Decreased head circumference	Gynecomastia
Learning and behavioral problems	Long extremities
	Reduced body hair

Virtually all men with KS are azoospermic. If sperm are present in the ejaculate, the karyotype is usually 46,XY/47,XXY. Testicular biopsy reveals that most seminiferous tubules are sclerotic, but a few tubules may contain Sertoli cells. The testis of the newborn with KS is believed to be normal, but the mean seminiferous tubule diameter and germ cell number is reduced slightly in prepubertal boys with KS. Extensive testicular damage occurs when gonadotropin production rises in puberty. Although there is an appearance of Leydig cell hyperplasia, the Leydig cell mass is normal. The cause of the Leydig cell dysfunction remains uncertain.

Men with KS are at increased risk for many pathological conditions (Table 7) . There is a tendency to develop testicular or extragonadal germ cell tumors especially in the mediastinum (Hasle et al, 1995). Accordingly, a chest X-ray should be performed if pulmonary symptoms occur. Immune disorders such as systemic lupus and scleroderma occur more often in KS patients. Hormonal factors may be responsible for this predisposition since immune disorders are also more common in women than in men. Varicose veins and leg ulcers may occur in young adults. Platelet hypercoagulability or increased plasminogen activator inhibitor have been proposed to cause leg ulcers in KS. Bone density is reduced in KS. Reduced levels of osteocalcin and increased urinary hydroxyproline to creatinine ratio suggest that bone formation is reduced, and absorption is increased. A few cases of hypopituitarism in Klinefelter's syndrome and have been reported. Pituitary hyperplasia may occur with long standing untreated primary testicular failure, simulating a pituitary tumor, and massive obesity may decrease gonadotropin secretion.

Table 7. Disorders associated with Klinefelter's syndrome.

Germ cell tumors: mediastinum, retroperitoneum, brain, testis
Immunological disorders: scleroderma, lupus, rheumatoid arthritis
Varicose veins and leg ulcers
Diabetes mellitus
Restrictive lung disease
Osteopenia
Hypopituitarism

Cryptorchidism

Cryptorchidism is a common congenital anomaly. Its prevalence is 2.5-5% at birth but declines to 1% by age 1 year because of delayed testicular descent. Approximately 10% of cryptorchid testes are intra-abdominal, 20% are within the inguinal canal, and the remainder are high in the scrotum. Many high scrotal testes are retractile, i.e. a reflex pulls them out of the scrotum due to cold temperature or other stress, but they are scrotal when the patient is relaxed. The disorder is bilateral in 10-15% of cases. Cryptorchidism occurs in androgen deficient men with GnRH deficiency or with androgen biosynthetic defects, and in androgen resistance syndromes. The persistent mullerian duct syndrome is sometimes associated with undescended testes as are structural anomalies of the abdominal wall, and neurological defects such as meningomyeolocele. However, most cases are idiopathic. This heterogeneity of pathophysiology and gonadal location leads to variable testicular function.

The most important complications of cryptorchidism are infertility and malignancy (Cilvers et al, 1986). Gonocytes in the cryptorchid testis degenerate in early childhood rather than undergo transformation to type A spermatogonia. In an effort to preserve fertility, as well as for psychological and cosmetic reasons, orchidopexy is usually performed in childhood, although there is no apparent relationship between the age of orchidopexy and adult testicular size or fertility (Lee 1993). Plasma FSH levels are generally increased in those men with severe oligospermia and small testes. Plasma LH and testosterone levels are usually normal, but mean basal and GnRH-stimulated LH levels are slightly higher than normal suggesting subtle Leydig cell dysfunction. The percentage of married men with a history of unilateral cryptorchidism who report fathering children has ranged from 60-90%, whereas only 13-60% of men with bilateral cryptorchidism are fertile.

Cryptorchidism is the most important risk factor for testicular germ cell carcinoma with approximately 10% of testicular cancer patients reporting a history of an undescended testis. Estimates of the risk of developing testicular cancer with unilateral cryptorchidism have ranged from 2.7-5.8 fold above that of the general population, whereas the cancer risk with bilateral cryptorchidism is substantially greater. Approximately 80% of the testicular cancers occur before age 40 years. From another perspective, however, only 1% of patients who undergo orchidopexy subsequently develop a testicular neoplasm. Interestingly, the tumor sometimes develops in the scrotal testis, and extra-gonadal germ cell cancers may occur. Orchidopexy below age 10 years may reduce, but testicular biopsy at the time of orchidopexy may increase the risk of cancer.

Germinal Aplasia

Germinal aplasia is a histological finding which was first described by Del Castillo in 1947 in azoospermic men. The subjects were virilized, healthy men with scrotal testes which were reduced in size. Testicular biopsy may reveal foci of spermatogenesis, although by definition most tubules contain only Sertoli cells. Germinal aplasia is a syndrome with multiple etiologies (Table 8), but the cause is

most often cancer chemotherapy. Approximately 10% of men with germinal aplasia have mutations of genes on the long arm of the Y chromosome which are required for normal spermatogenesis (see Chapter 13). Serum inhibin is reduced and FSH levels are almost always elevated, but LH and testosterone levels are generally within the normal range (Wallace et al, 1997).

Table 8. Causes of Germinal Aplasia

Cancer chemotherapy	Androgen insensitivity syndromes
Testicular irradiation	Chronic renal failure
Y chromosome mutations	Idiopathic
Cryptorchidism	

Miscellaneous Causes of Testicular Failure.

Testicular failure involving both the seminiferous tubules and Leydig cells occurs in patients with orchitis. Clinical infection of the testis occurs in 15-35% of adult men with mumps. In severe cases, distension of the testes with leukocytes leads to necrosis and atrophy. Epididymo-orchitis may occur with gonorrhea infection. Leprosy, tuberculosis, brucellosis, nocardia, salmonella, schistosomiasis, filiarisis and syphilis may affect the testes as well. Torsion of the testes in adolescents, and testicular trauma, may result in testicular atrophy. Autoimmune testicular failure may occur in polyendocrine deficiency types 1 and 2. Testicular failure has also been reported in idiopathic retroperitoneal fibrosis and Acleroderma (see Chapters 12 and 14).

SYSTEMIC DISORDERS PRODUCING HYPOGONADISM

Acute and Chronic Illness

Hypogonadism is common in critical illness (Turner, 1997). Surgery with general anesthesia, head trauma, burn trauma and myocardial infarction are each associated with low plasma LH and testosterone levels within 24 h, and the extent of suppression is related to disease severity. Among intensive care unit patients, a very low testosterone level is a predictor of mortality. Chronic illnesses such as cancer, AIDS, inflammatory bowel disease and anorexia nervosa also cause hypogonadism in men.

The mechanism by which reproductive function is suppressed in sick patients is unclear. Caloric deprivation may be a pivotal event since fasting for 48 h lowers plasma testosterone and LH levels in normal men. GnRH secretion is suppresssed since the LH response to GnRH stimulation is preserved, and pulsatile GnRH treatment prevents fasting induced HH. Pain and medications may also contribute to gonadotropin suppression. Candidate CNS mediators of GnRH suppression are CRF, opioid peptides, PRL, and neuropeptide Y (NPY) which stimulates feeding and gonadotropin secretion. Peripheral factors such as cortisol, leptin., gut peptides

such as cholecystokinin, as well as glucose and insulin may also convey a nutritional signal to GnRH release. The testicular steroidogenesis acute regulatory protein (StAR), which transports cholesterol to the inner mitochondria for conversion to pregnenolone, is rapidly decreased following endotoxin injection, providing an additional testicular mechanism for the low testosterone levels in acute illness.

Exercise

Intense exercise is an established cause of reproductive dysfunction in women, but abnormalities also occur in men. Short-term intensive exercise increases testosterone levels because of hemoconcentration and decreased testosterone clearance. Several hours after heavy exercise the serum testosterone level declines. Decreased GnRH production is likely, because the fall in testosterone can be prevented by GnRH analog treatment With prolonged intensive physical exercise with weight loss and sleep deprivation, very low testosterone and LH levels are observed. On the other hand, testosterone levels are generally normal in trained althletes, although men who develop an abnormally low body mass index (< 20 kg/m^2) may develop HH (Bagatell et al, 1990). Clomiphene may normalize testicular function in exercise-related HH.

Obesity

Testosterone levels are often low in obese men (Giagulli et al, 1994). Much of the decrease is due to low SHBG, but free and non-SHBG bound testosterone concentrations are also reduced with massive obesity. LH levels are normal in moderate obesity, but may be low in the massively obese. The limited information available suggests that sperm production is normal in obese men. Hyperinsulinemia due to insulin resistance is believed to suppress SHBG production, but the explanation for gonadotropin deficiency is less certain. With dieting and weight loss, estrone levels in obese men decline as testosterone levels increase, suggesting that increased circulating estrogen may suppress gonadotropins, although individual cases have not demonstrated an inverse relationship between testosterone and estrone. Because testosterone treatment reduces triglyceride production, and may decrease the waist-to-hip ratio and the cardiovascular risk, some authors advocate testosterone replacement for massively obese men.

Thyroid Disease

Hyperthyroid men sometimes develop gynecomastia, depressed sperm count and/or sperm motility, and loss of libido and potency (Winters et al, 1997). Thyroxine stimulates SHBG gene expression, and therefore hyperthyroxinemia increases plasma SHBG levels. Elevated SHBG may produce hypertestosteronemia. Plasma LH levels also are elevated, and FSH levels may rise. Increased production of SHBG may produces a transient decline in circulating bioavailable (free + albumen-bound) testosterone which increases LH secretion. With this resetting of the negative feedback control mechanism, increased LH drive restores the bioavailable

testosterone level to normal. Alternatively, elevated gonadotropins in hyperthyroid men could reflect Leydig cell dysfunction.

The increased LH drive in hyperthyroid men also stimulates the aromatase gene in Leydig cells to increase estradiol production producing plasma estradiol levels typical of women in the mid-follicular phase of the menstrual cycle. Peripheral aromatization of androstenedione to estrone, and testosterone to estradiol, is also increased in hyperthyroid men. Abnormal values normalize when euthyroidism is restored.

Testicular dysfunction may occur more often among men with primary hypothyroidism than in the general population. Pituitary enlargement, hyperprolactinemia and hypogonadism has been described in men with severe long-standing hypothyroidism, although most cases are in women. The doses of radioiodine used to treat thyroid cancer may damage the seminiferous epithelium.

Liver Disease

Hypogonadism is common in men with chronic alcoholic liver disease (Villalta et al, 1997). Common symptoms and signs are a decline in libido and potency, infertility, reduced body hair, testicular atrophy, oligo- or azoospermia, and gynecomastia. The hypogonadism of alcoholic liver disease is multifactoral. Serum levels of testosterone are reduced, and LH and FSH are generally elevated, indicating primary testicular failure which has been attributed to ethanol testicular toxicity. SHBG levels are elevated, further reducing the bioavailable testosterone level and contributing to the rise and LH and FSH. Feminization occcurs because estradiol secretion is increased from excessive LH stimulation of testicular aromatase, and from increased production of CRF-ACTH which stimulates adrenal androstenedione which is converted to estrone and estradiol by peripheral tissues. Sickness and poor nutrition may cause superimposed GnRH deficiency, and hyperprolactinemia may occur. Cessation of drinking or liver transplantation improve the endocrine status of these men.

Cirrhosis subsequent to viral hepatitis also results in primary testicular failure and increased SHBG challenging the notion that testicular failure in alcoholic cirrhosis is due solely to alcohol toxicity (Kaymakoglu et al, 1995). Testicular dysfunction in hemochromatosis is characterized by low testosterone and blunted pulsatile LH secretion indicating hypothalamic-pituitary dysfunction.. Decreased libido is an early symptom of hemochromatosis which may precede skin changes, diabetes mellitus, cardiomyopathy or liver disease. Iron deposition in the hypothalamus and pituitary is the presumed cause of HH. SHBG and estradiol levels are usually normal, and gynecomastia is uncommon. Intensive phlebotomy may reverse the hypogonadism of hemochromatosis, but results are conflicting, and partial improvement occurs following liver transplantation.

Chronic Renal Insufficiency

Testicular dysfunction is common in men with chronic renal insufficiency (CRI) even with effective dialysis. The extent of the hypogonadism is variable, and the disorder is multifactoral. Erectile dysfunction, infertility, testicular atrophy,

hypospermatogenesis and gynecomastia occur often, and puberty is usually delayed. Chronic illness and medications contribute to the endocrine disturbance. Primary testicular failure is suggested in some men by low testosterone and elevated LH and FSH concentrations. Decreased gonadotropin clearance may contribute to these findings, however. Selective elevation in LH levels may have reflected cross-reactivity of gonadotropin α-subunit in double antibody LH assays. Free gonadotropin a-subunit and inhibin a-subunit accumulate in plasma in CRI because these small proteins are cleared by renal mechanisms. In other men, reduced total and free testosterone and normal LH levels, or spontaneous LH secretory episodes of reduced amplitude, suggest impaired GnRH secretion. Hyperprolactinemia may be present. Hypogonadism can be reversed by successful transplantation, but prolonged dialysis and chemotherapeutic drugs may cause permanent hypospermatogenesis .

Diabetes Mellitus

Erectile and ejaculatory dysfunction are common with diabetes mellitus, and are thought to be due to neuropathy and to vascular disease related to impaired production of nitric oxide. The majority of men with well-controlled insulin dependent diabetes mellitus (IDDM) have normal endocrine testicular function, although a few men have elevated FSH and LH levels. Low testosterone levels in men with IDDM during ketoacidosis, which presumably reflect the stress of acute illness, normalize within a few days of insulin treatment. Because insulin suppresses plasma SHBG, men with NIDDM, or hyperinsulinemia and normoglycemia, often have low total testosterone and SHBG levels (Haffner et al, 1996). In some studies, free testosterone is also reduced however. Secondary diabetes and hypogonadism coexist in hemochromatosis, polyglandular endocrine failure, Prader-Willi syndrome, Laurence-Moon-Bardet-Biedl syndrome, Acromegaly, Cushings's syndrome, myotonic dystrophy, and alcohol-related pancreatitis.

Congenital Adrenal Hyperplasia

Deficiency of steroid 21-hydroxylase leads to increased androgen production by the adrenal cortex. Some men with inadequately treated CAH have presented with infertility, small testes, oligo- or azoospermia, and reduced circulating LH and FSH levels. Chronic suppression of gonadotropin secretion by adrenal androgens is one explanation for this condition. Adequate replacement with gluco- and mineralocorticoids may restore spermatogenesis to normal. Interestingly, other male patients with 21-hydroxylase deficiency who receive inadequate hormone replacement maintain normal sperm production.

Bilateral testicular tumors may occur with inadequate treatment of 21-hydroxylase deficiency. These tumors are multiple irregular hard nodules, measuring 1-2 cm in diameter that develop from adrenal rests within the testes. The normal architecture of the testis may be destroyed causing the plasma FSH level to rise. Bilateral testis tumors have also been reported in men with ACTH-producing pituitary adenomas following bilateral adrenalectomy (Nelson's syndrome).

Neurological Disorders

Myotonic muscular dystrophy is an autosomal disorder characterized by weakness, myotonia, frontal balding, cataracts, cardiac arrhythmias, insulin resistance and primary testicular insufficiency. Serum FSH and LH levels may be increased or normal. The disease results from an expansion of CTG trinucleotide repeats in the 3' untranslated region of the myotonin protein kinase (Mt-PK) gene on chromosome 19 leading to reduced Mt-PK gene expression. Mt-PK is presumed to participate in a signal transduction pathway, but the link between this abnormality and impaired testicular function remains unknown.

Spinal and bulbar muscular atrophy (Kennedy disease) is an X-linked disorder in which men present with progressive muscular fasciculations, weakness, and muscle atrophy at age 20-40 years. Although fertile as young adults, affected men develop gynecomastia, hypospermatogenesis and androgen deficiency as they grow older (Ferlini et al, 1995) The finding that both serum LH and testosterone levels were elevated suggested androgen resistance. Affected patients have an expansion of the CAG trinucleotide repeat, which codes for glutamine, in the first exon of the 5' flanking region of the androgen receptor gene. Normal alleles have 17-26 CAG repeats whereas those with SMBA have 40-52 repeats. The expansion of the glutamine region of the AR amino terminus leads to impaired transactivation of androgen responsive genes (see Chapter 4). AR are present in spinal and bulbar motor neurons, but the mechanism for the neurological disease is uncertain.

Adrenomyeloneuropathy is a peroxisomal disorder in which very long chain fatty acids accumulate in plasma and in various tissues including the spinal cord and peripheral nerves, and in endocrine cells. The disorder is X-linked, and presents in late adolescence or early adulthood usually with spasticity. Primary adrenal failure and testicular failure may occur.

Respiratory Failure

Obstructive sleep apnea, characterized by narrowing of the hypopharynx with episodes of nighttime breathing cessation followed by heavy snoring, occurs in obese men, and is associated with sexual dysfunction. Several studies found low testosterone but normal LH levels, and concluded that the hormonal findings cannot be explained by obesity alone. Although the link between hypoxia and testosterone deficiency is unclear, continuous positive airway pressure (CPAP) treatment may improve testosterone deficiency not only in men with sleep apnea, but also in men with chronic obstructive pulmonary disease of other causes. There is a suggestion, however, that testosterone treatment worsens sleep apnea by increasing oxygen consumption or reducing hypoxic respiratory drive.

Delayed sexual maturation is common in patients with cystic fibrosis (CF) in whom hypoxia, poor nutrition, and hypercortisolemia may decrease GnRH secretion. Obstructive azoospermia, due to congenital bilateral agenesis of the vas deferens (CBAVD) is common in adult men with CF. Genetic testing of healthy men with CBAVD may also reveal cystic fibrosis (see Chapter 13). The CF transmembrane regulator gene (CFTR), which codes for a phosphorylation activated calcium channel, is expressed in the epithelium of the epididymis and vas deferens,

and in germ cells, but the cause for the male genital tract damage is presently unknown. Infertility also occurs in patients with mucociliary transport disorders producing bronchitis and bronchiectasis (Young's syndrome) and in the immotile cilia (Kartagener's) syndrome.

GYNECOMASTIA

Gynecomastia results from an increase in glandular tissue, stroma and fat (Braunstein 1993). Transient gynecomastia occurs in newborn and pubertal males as a small firm subareolar mass which may be tender. Breast enlargement is also relatively common among elderly men, but is painless. Gynecomastia is most often bilateral but asymmetrical. Unilateral breast enlargement in older men could represent a malignancy, and a biopsy should be performed. Breast enlargement occurs if estrogens are increased, or with androgen deficiency, even if estrogens are normal. The conditions associated with gynecomastia are listed in Table 9.

The gynecomastia of adolescence, which usually resolves, sometimes persists into adulthood without apparent cause, and is termed pubertal or idiopathic gynecomastia. A few patients have a mild defect in testosterone biosynthesis or an

Table 9: Conditions associated with gynecomastia

Physiological	**Medications**
newborn	Hormones:estrogens, androgens, hCG, hGH
puberty	Antiestrogens:clomiphene, tamoxifen
aging	Androgen antagonists or inhibitors flutamide, spironolactone, ketoconazole, finasteride
Neoplasms	Antiulcer drugs:cimetidine, *omeprazole*
steroid producing (testis and adrenal)	Cardiovascular drugs: amiodorone, *captopril,* digitoxin, *diltiazem, enalapril, methyldopa, nifedipine, reserpine*
hCG-producing (germ cell, hepatomas)	Chemotherapeutic agents: alkalyating agents, methotrexate
Primary testicular failure	Metaclopramide
Hypogonadotropic hypogonadism	Psychoactive drugs: *haloperidol, phenothiazines, tricyclic antidepressants*
Chest wall injury	Street drugs: *opiates, marijuana,* alcohol
Testosterone biosynthetic defects	
Androgen resistance syndromes	
True hermaphroditism	
Systemic Disorders	
thyrotoxicosis	
alcoholic liver disease	
hepatitis	
chronic renal insufficiency	
Idiopathic	

For drugs in italics the link to gynecomastia is incompletely established

androgen insensitivity syndrome leading to an imbalance between estrogens and androgens, but without ambiguous genitalia. Obesity often contributes to pubertal gynecomastia because fat tissue expresses the aromatase gene. Some authors have proposed that exposure to environmental toxins with estrogenic or antiandrogenic properties leads to pubertal gynecomastia.

Medications often cause gynecomastia. Some drugs such as hCG, clomiphene and antiandrogens increase estrogen production. Antineoplastic agents, which damage the testis and increase gonadotropin secretion, may produce gynecomastia via this mechanism. Antiandrogens also directly block the effect of androgens to suppress breast growth. Other drugs have estrogenic properties or contain estrogens as a contaminant, such as marajuana or lotions used by morticians. Ketoconazole reduces testosterone production. Case reports describing breast enlargement with a variety of drugs have been published, but the mechanisms are uncertain.

Treatment of long standing gynecomastia which is embarrassing to the patient is surgical. The results of plastic surgery using liposuction are generally excellent with no skin depression or nipple deformity. Medical management of recent-onset gynecomastia using antiestrogens, the progestin danazol, the aromatase inhibitor testolactone, or the non-aromatizable dihydrotestosterone may be effective, but reports are conflicting.

TREATMENT OF HYPOGONADAL MEN

Androgen Replacement Therapy

Patients with androgen deficiency from either primary testicular failure or HH should be replaced with testosterone. Androgen replacement should begin at the time of diagnosis in teenagers with congenital HH since since delayed initiation of treatment results in reduced bone mineral density which may not normalize with treatment in adulthood. These data suggest that there is a critical period in the skeletal response to sex hormones (see Chapter 8). In HH, testosterone is changed to hMG/hCG or to pulsatile GnRH when the patient and his partner wish to conceive.

Gonadotropin Therapy

The usual approach to stimulate sperm production in gonadotropin-deficient men is with human chorionic gonadotropin (hCG) alone or in combination with human menopausal gonadotropins (hMG) as a source of FSH (Burgues et al, 1997). There are few large series reported, and fewer controlled studies, so that treatment guidelines are empirical. Because of the higher cost and need for frequent injections with hCG, virilization is usually accomplished with testosterone, and hCG is used when fertility is desired. Prior treatment with testosterone does not adversely affect responsiveness to hCG.

hCG stimulates intratesticular testosterone and potentially other Leydig cell products required for spermatogenesis. hCG, rather than LH, is used because it has a longer circulating half life. The preparations of hCG in clinical use have been purified from pregnancy urine, although recombinant hCG is currently being evaluated. Doses of 1000-1500 U two or three times weekly subcutaneously or intramuscularly usually maintain the serum testosterone levels within the range of normal. Higher doses increase plasma estradiol levels and cause gynecomastia. Therefore, the plasma level of estradiol as well as testosterone is monitored. Some patients find sc administration to be less painful. Side effects such as acne and weight gain are similar to those of testosterone. Rarely, an allergic response to hCG occurs, or neutralizing antibodies develop. To prevent these complications, it is preferable to avoid intermittent treatment.

hCG stimulates testicular growth, and careful monitoring of the testicular size, as well as the serum levels of testosterone and inhibin-B predict the spermatogenic response to treatment. Selected patients produce sperm, and successfully impregnate their wives when treated with hCG alone, including men with adult onset HH (e.g. pituitary tumor patients), and those with congenital partial GnRH deficiency (pretreatment testis size > 4 ml). hCG may also maintain spermatogenesis following initiation with the combination of hCG and FSH. If the patient remains azoospermic after 12 months of hCG treatment, FSH should be added to the regimen.

Most men with complete HH (pretreatment testis size < 4 ml) require the combination of hCG and FSH. hCG is begun first to increase the intratesticular level of testosterone and Leydig cell proteins. Generally, if after six months of hCG treatment the size of the testes remains < 10 ml and the subject remains azoospermic, FSH is added to the regimen. The usual beginning dose of FSH is 75 IU every other day. To minimize the number of injections, hCG and FSH can be mixed in the same syringe. FSH alone does not stimulate spermatogenesis. Although FSH has been produced from the urine of post-menopausal women (hMG), recombinant human FSH is now available in selected markets. Because the maturation of spermatogonia to sperm takes about 70 days, sperm generally do not appear in the ejaculate for 3-6 months. But pregnancies may occur with a sperm count as low as 1-5 million/ml (Burris et al, 1988). If azoospermia persists after 6 months of combination treatment, the dose of FSH can be increased to 150 IU. FSH is generally well tolerated. The mixture of hCG and FSH may produce higher plasma levels of testosterone and estradiol than does hCG alone, permitting a reduction in the dose of hCG. Recombinant hLH will be available for the treatment of HH in the near future.

The sperm output among hCG/FSH-treated men is variable ranging from 1-60 million/ml with an average value of about 5 million/ml. Lower values are observed in men with complete GnRH deficiency. The inability to reproduce quantitatively normal spermatogenesis in these patients has been proposed to result from gonadotropin deficiency during the newborn and prepubertal periods of life resulting in impaired Sertoli cell development. The sperm quality (motility and morphology) is usually normal during treatment with hCG/FSH. If not, a second cause for infertility may be present. Overall the pregnancy rate ranges from 50-80% with gonadotropin therapy, but patients with a history of cryptorchidism tend to

respond less well. If pregnancy occurs, FSH can be discontinued, but hCG is maintained until delivery since miscarriage could occur. Thereafter if the couple does not want to conceive additional children, testosterone replacement is resumed. Otherwise hCG treatment is maintained.

Gonadotropin-Releasing Hormone

Pulsatile GnRH therapy can be used to stimulate spermatogenesis in men with GnRH deficiency and normal pituitary function (Schopohl, 1993). This approach is more physiological because it reproduces the normal pulsatile pattern of LH and FSH release from the pituitary. A starting dose of 4 μg per pulse delivered every 2 hrs into the subcutaneous tissue of the abdomen is often used, with increases of 2 μg every 2 weeks if LH secretion does not rise, up to a maximum dose of 20 mg per pulse. Serum testosterone levels usually normalize within 1-2 months, and the testes increase in size within 3-6 months after beginning therapy. Up to 2 years of treatment may be needed before sperm appear in the ejaculate, however. Similar to the results with hCG/hMG therapy, final testicular volume and sperm count are greater, and the time to first appearance of sperm in the ejaculate is less, among men with partial than with complete GnRH deficiency. The sperm density generally increases to 1-10 million/ml in complete and to 50-100 million/ml in partial GnRH deficiency. GnRH-treatment may stimulate spermatogenesis more rapidly than does hCG/hMG, but the maximum sperm output appears to be similar for both therapies. Elevated estradiol levels and gynecomastia are less common with pulsatile GnRH than with hCG. Given the complexity and inconvenience of pulsatile GnRH, it is recommended as initial therapy only for highly motivated patients, and is reserved for hCG/FSH non-responders.

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7 MALE SENESCENCE

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INTRODUCTION

There is no "andropause" that corresponds to the female menopause, but normal aging in men is accompanied by a gradual decline in serum levels of testosterone and changes in the hypothalamic-pituitary-gonadal axis. Although there is a great deal of variability among individuals in this regard, and the process can be impacted by disease and medications, there has been a growing interest over the past decade in whether declining testosterone levels result in a significant number of older men becoming hypogonadal and whether replacement therapy might be beneficial. It is the purpose of this chapter to review the changes that occur with normal aging in the male reproductive axis and to discuss the issues that surround androgen replacement therapy in the older man.

MALE REPRODUCTIVE CHANGES WITH AGING

Testicular changes

In general, testicular volumes and weight decrease slightly with age (Johnson et al, 1984a), but this change is neither large nor consistent (Stearns et al, 1974). Some studies suggest that the number of Leydig cells (Kaler and Neaves, 1978; Neaves et al, 1984), Sertoli cells (Johnson et al, 1984b), and interstitial cells (Neaves et al, 1985) all decrease with age. It is estimated that a 20 year old man will have about 700 million Leydig cells in his testes, and on average, will lose about 6 million to 7 million of these cells per year thereafter (Neaves et al, 1984).

Changes in spermatogenesis with normal aging are variable. A study comparing single ejaculates from men 60 to 80 years of age to those from men 24 to 37 years of age showed no difference in total sperm counts between the two age groups (Nieschlag et al, 1982). Sperm motility in the older men was lower, but the ability of the sperm to fertilize an egg in the heterologous ovum penetration test was similar in the two groups. Other studies have shown a significant decline in daily sperm production with age and a higher prevalence of azoospermia in elderly men compared to young men (Neaves et al, 1984; Johnson, 1986; Paniagua et al, 1987).

Sex Steroids

All components of serum total testosterone decline with normal aging. Conflicting data pertaining to this point were found in the early medical literature, where studies were not always controlled for overall health, smoking, obesity, or time of sampling. However, when these variables are considered, there is overwhelming evidence that men experience a slow, but continuous decline in average serum testosterone levels after about age 30 years, as has been shown in both cross-sectional and longitudinal studies (Figure 1; Vermeulen, 1991; Gray et al, 1991a; Morley et al, 1997). It is not known if the age-related decline in serum testosterone

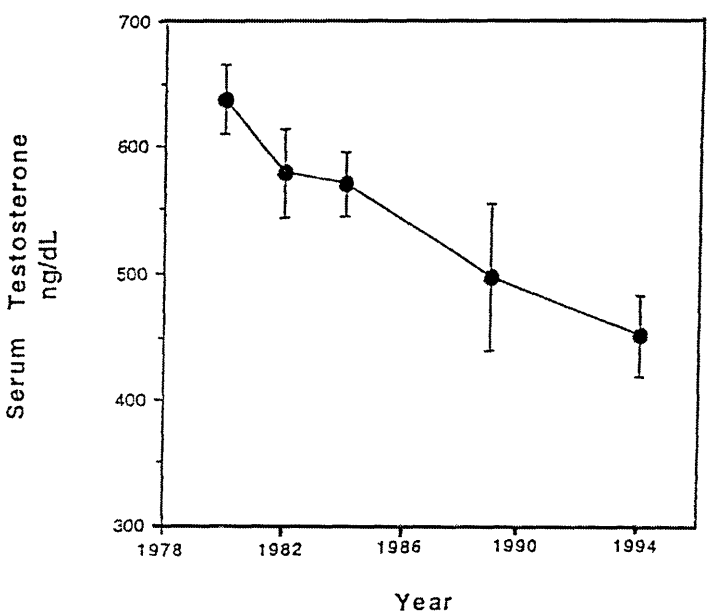

Figure 1. Fifteen year longitudinal changes in serum total testosterone in normal men ages 66-87 years at study entry (adapted from Morley et al, 1997)

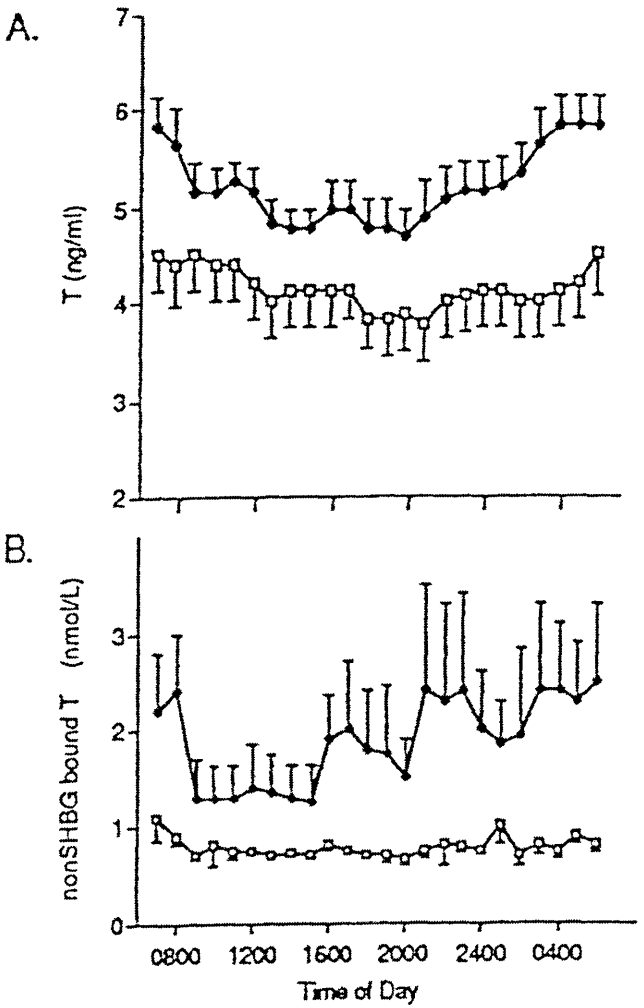

Figure 2. (A) Twenty-four hourly mean total serum testosterone levels (± SEM) in 20 young (open markers) and 14 elderly (closed markers) normal men (Adapted from Tenover et al, 1988); (B) Twenty-four hourly mean non-SHBG-bound testosterone levels (± SEM) in 10 young (open markers) and 10 elderly (closed markers) normal men (Adapted from Plymate et al, 1989).

in men is universal. Almost all available data are from studies of Caucasian men of Western European descent; little data are available for other ethnic populations. It also should be remembered that testosterone levels can decline in older men for a number of reasons other than normal aging. Concomitant disease can have a significant impact. In the Massachusetts Male Aging study, low serum testosterone levels were seen most frequently in men with diabetes mellitus and cardiovascular

disease (Gray et al, 1991b). The presence of sleep apnea (Santamaria et al, 1988) or severe obesity (Gaiguilli et al, 1994) also are associated with low testosterone levels. Medications such as cimetidine, ketoconazole, and glucocorticoids have all been reported to lead to a decrease in serum testosterone (Pont et al, 1984; Lardinois and Mazzaferri, 1985; MacAdams et al, 1986).

Nearly all of the testosterone circulating in blood is bound to proteins, either sex hormone binding globulin (SHBG) or albumin, with only about 1-2% of total testosterone circulating totally "free". The affinity of testosterone for SHBG is about 1000-fold higher than its affinity for albumin (Pardridge and Landaw, 1985) and because of the tight binding of testosterone to SHBG, the portion of serum total testosterone not bound to SHBG (free plus albumin-bound) has often been called "bioavailable" testosterone. Originally, only free testosterone was believed to be the "active" portion available to tissues (Vermeulen and Verdonck, 1972). Later data suggested that the non-SHBG-bound testosterone was the "bioavailable" portion (Manni et al., 1983). As more data are collected, it appears that the portion of serum testosterone available to a particular tissue may depend on the characteristics of that tissue and its blood supply (Sakiyama et al, 1988).

The concentration of all serum components of testosterone (total, free, and non-SHBG-bound) decline with normal aging. Since serum levels of SHBG tend to increase with age (Baker et al, 1976), the decline in levels of non-SHBG-bound testosterone with age often are much greater than the decline in levels of total testosterone (Stearns et al, 1974, Nankin and Calkins, 1986; Tenover et al, 1987). In addition, young men demonstrate a circadian rhythm in serum levels of total and non-SHBG-bound testosterone, while in older men both of these circadian rhythms are blunted or lost (Figure 2; Bremner et al, 1983; Tenover et al, 1988; Plymate et al, 1989). Whether the loss of a circadian rhythm in serum testosterone with age has any physiological consequences, other than contributing to lower 24 hour serum total testosterone level, is not known.

The decline in serum testosterone with age is due to a decrease in testosterone production; testosterone clearance slows with age (Vermeulen et al, 1972). The physiological causes for the decline in production are multifactorial, but the predominant change appears to be at the level of the aging testis. As noted earlier, Leydig cell numbers decrease with age. More importantly, there is an age-associated decline in the activity of enzymes in the metabolic pathway governing testosterone production (Takahashi et al, 1983), as well as a decreased ability to increase testosterone production in response to human chorionic gonadotropin (hCG) (Figure 3).

There appears to be no consistent effects of age on serum levels of dihydrotestosterone (DHT). Some studies have shown a small age-related increase in serum levels of DHT (Horton et al, 1975; Harman and Tsitouras, 1980), some have shown a decrease (Pirke and Doerr, 1970; Giusti et al, 1975), and some have found no change (Pirke and Doerr, 1975; Gray et al, 1991b). There also are conflicting data concerning the levels of serum estrogens as a function of age in

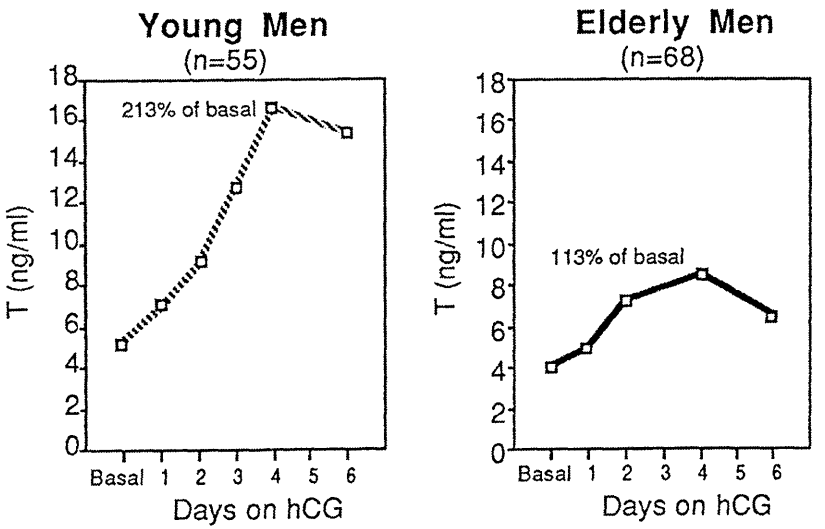

Figure 3. Serum testosterone levels in response to exogenous hCG administration in healthy young (n=55) and elderly (n=68) men (composite of five studies: Longcope, 1973; Mazzi et al, 1974; Rubens et al, 1974; Harman and Tsitouras, 1980; Nieschlag et al, 1982).

men. Some studies have shown that estrogen levels are similar in young and elderly men (Harman and Tsitouras, 1980; Nieschlag et al, 1982; Tenover et al, 1987), while others have reported higher levels in aging men (Pirke and Doerr, 1970; Rubens et al, 1974; Baker et al, 1976). Since the amount of body fat tends to increase with age, some of the increase in serum estrogens seen in older men may be the result of increased peripheral conversion from testosterone in adipose tissue (Hemsell et al, 1974). Whether estrogen levels increase with male aging or not, because of the declining testosterone levels, the testosterone-estrogen ratio decreases with age.

Gonadotropins and Hypothalmic Function

Declining testosterone levels with age in men usually are accompanied by moderate increases in immunoassayable serum gonadotropin levels (Figure 4; Stearns et al, 1974; Mazzi et al, 1974; Rubens et al, 1974; Moroz and Verkhratsky, 1985; Gray et al, 1991b; Morley et al, 1997). In general, follicle stimulating hormone, FSH, increases with age at a slightly more rapid rate than does luteinizing hormone (LH; Gray et al, 1991b). However, these increases in gonadotropins with age are not usually robust, and many older men, even those with quite low serum testosterone levels (< 7.0 nmol/L) often have both LH and FSH levels than are in the upper

normal range for young adult men, making these older men relatively hypogonadotropic.

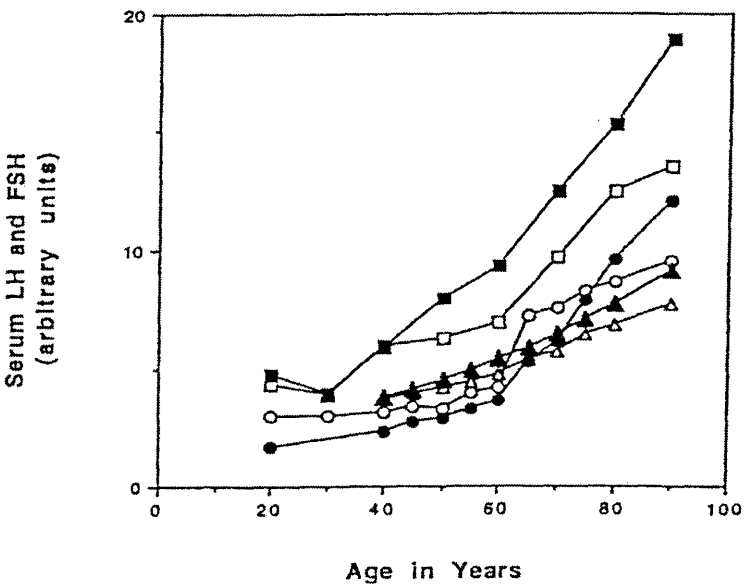

Figure 4. Serum immunoassayable LH (open markers) and FSH (closed markers) as a function of age in men from three studies (adapted from references Stearns et al, 1974; Moroz and Verkhatsky, 1985; Gray et al, 1991b).

Evaluations of the bioactivity of gonadotropins in young versus older men have shown either similar (Tenover et al, 1987; Urban et al, 1988) or slightly decreased (Marrama et al, 1984; Warner et al, 1985) bioactivity of these glycoproteins in the older men. In general, however, the overall quantity of bioactive gonadotropins which circulate in the blood of older men is very similar to that in normal young adult men and can not account for the decreased testosterone production seen in the older men.

Other evidence that suggests that there are changes with aging in the hypothalamic-pituitary component of the gonadal axis include age-related changes in luteinizing hormone (LH) pulse amplitude (Veldhuis et al, 1992), alterations in

LH 24-hour pulsatile rhythms (Tenover et al, 1988), increased sensitivity to sex steroid-negative feedback regulation (Muta et al, 1981; Winters and Troen, 1982), and decreased response to administration of gonadotropin-releasing hormone (GnRH) or clomiphene citrate (Rubens et al, 1974; Snyder et al 1975; Harman et al, 1982; Winters and Troen, 1982; Marrama et al, 1984; Tenover et al, 1987). Figure 5 is a composite of five studies demonstrating that older men, in response to GnRH, can not increase their LH production over the baseline level to same extent as that of younger men.

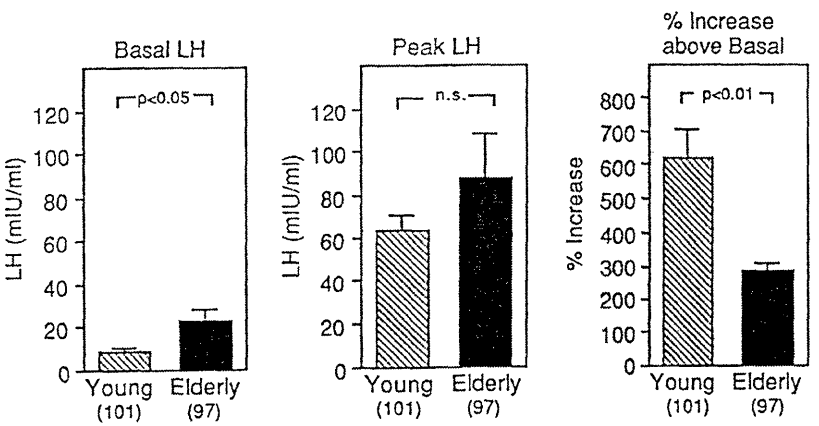

Figure 5. Effects of GnRH administration on serum LH levels (mean±SEM) in young and elderly men (composite of five studies; Rubens et al, 1974; Snyder et al, 1975; Harman et al, 1982; Winters and Troen, 1982; Marrama et al, 1984).

AGE-RELATED HYPOGONADISM

Testosterone Deficiency in Older Men

Since testosterone levels decline with normal male aging, the question arises as to whether there are a significant number of older men who can be considered "hypogonadal" and, therefore, might be candidates for male hormone replacement therapy. At this time, the diagnostic criteria for defining hypogonadism in the older man have not been delineated. There are a number of reasons for this. First, there is no straightforward target organ change, physiological finding, or symptom that can be used to define testosterone deficiency in older men. Second, as mentioned previously, using elevated gonadotropin levels to assist in defining the hypogonadal state in older men will greatly underestimate those men who are testosterone deficient, because gonadotropin levels often are not abnormally high, even when testosterone levels are quite low. If the serum total testosterone level of an older man is less than 7.0 nM, then he is clearly hypogonadal and replacement therapy

should be considered. However, this represents less than 5% of men over the age of 60 years.

Lacking a firm guideline, most investigators in the area of male hormone replacement therapy have used the lower range of normal for the young adult male (two standard deviations below the mean) in serum levels of total testosterone to define the level below which an older man might be considered "testosterone deficient". Using this type of definition, the prevalence of testosterone deficiency in generally healthy older men has been reported to be between 11% and 36% (Tenover, 1998). If the level of non-SHBG-bound testosterone is used as the criterion to define older male testosterone deficiency, then the prevalence in men over the age of 60 years may be as high as 50%.

At this time it may not be reasonable to determine if there is one serum assay measurement that can define an older man as being hypogonadal. Androgen target organs may vary in the threshold responses to testosterone replacement therapy in the older man and there also may be significant inter-individual variability in these responses. Until more is learned about androgen target end-organ responses to replacement therapy in this older age group, it may be more important to select men for therapy who have one or more specific androgen target organ deficiencies and who also have a serum testosterone level which is low enough that meaningful changes in serum testosterone can be made using physiological replacement doses.

Accompanying aging are a number of clinically detrimental physiological changes in organs and functions which, at least in the younger adult hypogonadal male, can be positively impacted by testosterone replacement (Table 1). Among these age-related changes are a decrease in muscle tissue mass and decline in muscle strength

Table 1. Androgen Target Organ Changes with Male Aging and with Testosterone (T) Replacement Therapy in Young Adult Men

Target Organ/Function	Change With	
	Aging	T Replacement
Muscle mass	▼	▲
Muscle strength	▼	▲
Fat mass	▲	▼
Bone mass	▼	▲
Libido	▼	▲
Erectile dysfunction	▲	▼
Sense of well being	NC/▼	▲

▼ = decrease; ▲ = increase; **NC** = no change

(Forbes and Reina, 1970; Reed et al, 1991); increase in body fat mass, particularly intra-abdominal fat (Shimokata et al, 1989); decline in bone mass and increased

incidence of osteoporosis and minimal trauma fractures (Riggs et al, 1981; Stanley et al, 1991; Orwoll and Klein, 1995); decline in quality and quantity of sexual thoughts and enjoyment, increased erectile dysfunction, and problems with decreased sense of well-being (Davidson et al, 1983; Mulligan et al, 1988; Feldman et al., 1994; Panser et al, 1995). These areas are the ones which have been targeted when evaluating whether testosterone replacement therapy might be beneficial for older men.

Androgen Replacement Therapy in Older Men

The data on testosterone replacement therapy for the older man are somewhat limited at this point. Replacement trials that have been reported often involve small numbers of participants, short terms of treatment (the longest is five years, most are six months or less), are not always placebo controlled or double- blinded, use various modes of androgen therapy, and often are reported in abstract form only. Nonetheless, by looking at the various study results in terms of target organ outcome, it is possible to get an overview of the current knowledge.

Muscle and Strength. To date there have been at least five trials of testosterone therapy in older men which have evaluated body composition changes; four of these, along with an additional three trials, have evaluated some aspect of strength (Tenover, 1992; Morley et al, 1993; Marin et al, 1993; Haddad et al, 1994; Sih et al, 1995; Urban et al, 1995; Katznelson et al, 1996; Sih et al, 1997). Table 2 is a

Table 2. Effect of testosterone replacement therapy on body composition and strength in older men

Length of Treatment (months)	Study N	Body Composition Change		Strength Change	
		Fat Mass	Lean Mass	Grip	LE*
3	13	NC	▲	NC	-----
6	8	▼	▲	-----	▲
9	31	▼	NC	-----	-----
18	29	▼	▲	-----	-----
12	17	NC	-----	▲	-----
6	14	-----	-----	▲	-----
24	10	-----	-----	▲	-----
1	6	-----	-----	-----	▲

*LE, lower extremity strength; ▼ = decrease; ▲ = increase; NC = no change

summary of the body composition and strength changes that have occurred in these trials. Consistently, there is some change in body composition with testosterone therapy, either a decline in body fat, increase in lean body mass, or both. In terms of testosterone therapy effects on muscle strength in older men, the studies also have

demonstrated consistent results. In six of the seven studies, muscle strength increased in the testosterone treated group. Generally the magnitude of the changes seen in these studies, which involve only testosterone therapy without any other modality such as exercise, are smaller than those seen with testosterone replacement in young hypogonadal men. Whether the magnitude of these changes in body composition and strength which occur with testosterone therapy will have significant clinical relevance and lead to maintenance or improvement in overall function has not yet been demonstrated.

There have been several double-blinded, placebo controlled studies of testosterone therapy in older men which have evaluated participants' responses on self-rated scales relating to energy or level of fatigue (Tenover, 1992; Marin et al, 1993). These studies reported an increase in energy level and/or a decline in fatigue that was significantly better in those men treated with testosterone as compared to placebo.

Bone. There have been at least eight studies which have reported on the effects of testosterone therapy on bone mineral density and/or biochemical parameters of bone turnover in older men (Jackson et al, 1987 Greenspan et al, 1989; Oppenheim and Klibanski, 1989; Tenover, 1992; Morley et al, 1993; Tomasic et al, 1994; Katznelson et al, 1996; Ellyin et al, 1998). These studies, some on which did not involve men who were osteoporotic, have lasted from 3 to 60 months, with the shorter term studies evaluating only bone turnover parameters. Table 3 gives an overview of these studies, which consistently have shown an increase in bone mineral density and a slowing of bone degradation with testosterone therapy.. Since testosterone is converted to estradiol *in vivo*, and since older men who are replaced with testosterone often show an increase in serum estradiol levels as well, it is unclear if the effect of testosterone therapy on bone is a direct result of testosterone or due to the increased estradiol levels. From the point of view of the use of testosterone therapy for prevention of osteoporosis in the older man, however, this argument is moot. What is important, but not yet known, is whether these testosterone induced changes in bone mineral density can be sustained over long periods of time, whether the increase in bone mineral density will eventually reduce the risk of non-traumatic fractures in the older men, and what level of testosterone replacement is necessary to achieve maximal effects on bone.

Sexual Function and Mood. Testosterone administration to young hypogonadal adult men has been shown to increase libido, frequency of sexual activity, and erectile function (Skakkeback et al, 1981; Kwan et al, 1983). Some studies have shown that testosterone replacement also improves sense of well being or mood in hypogonadal young men (Skakkeback et al, 1981; O'Carroll et al, 1985), although this finding has not been universal (Davidson et al, 1979; Salmimies et al, 1982). There have been no clinical trials which have evaluated the effect of testosterone therapy on aspects of sexual function in healthy older men with low or low normal testosterone levels. There are, however, some studies which have evaluated the effects of raising serum testosterone levels in older men with various types of sexual dysfunction. In general, men with low libido have shown improvement, while

Table 3. Testosterone therapy effects on bone in older men

Treatment Length (mths)	Study N	Parameters of Bone Turnover Formation	Degradation	Bone Density L-spine	Other
3	13	NC	▼	-----	-----
3	8	▲	------	-----	-----
12	4	-----	------	▲	-----
60	9	NC	NC	▲	-----
18	29	▼	▼	▲	-----
12	4	-----	-----	▲	▲
6	6	-----	-----	▲	-----
7-14	3	▼	▼	-----	-----

▼ = decrease; ▲ = increase; NC = no change

erectile dysfunction only occasionally is improved (O'Carroll and Bancroft, 1984; Carani et al, 1990; Guay et al, 1995). Several studies in older men which have evaluated the effect of testosterone therapy on mood, in a blinded, placebo-controlled manner, have demonstrated a positive impact (Tenover, 1992; Marin et al, 1993).

Risks of testosterone therapy in older men. Table 4 lists the most significant of the potential risks of testosterone therapy in older men. Liver toxicity is on the list because of the potential of the oral methylated androgens to increase hepatic enzymes, cause cholestasis, peliosis of the liver and liver tumors (Ishak and Zimmerman, 1987). The methylated testosterones are not recommended for use in men and none of the studies involving replacement therapy in older men have employed these oral agents; no problems with liver toxicity has been reported in these replacement trials. Modest fluid retention is possible with testosterone replacement, especially within the first few months of therapy. For most men, the small amount of fluid retention (transient weight gain of several kilograms) is not harmful; no cases of development of peripheral edema have been reported with testosterone therapy in older men. Tender breasts or gynecomastia do occur in a small number of older men on testosterone therapy. Sleep apnea has been shown to contribute to low serum testosterone levels, but testosterone therapy also has been shown to exacerbate sleep apnea (Sandblom et al, 1983). Screening for this condition, at least by history, prior to testosterone therapy should not be overlooked.

Most studies of testosterone replacement in older men have shown a significant increase in red blood cell mass, hemoglobin levels, and hematocrit with the therapy. The increases reported are much larger than those usually seen when hypogonadal young men are given testosterone replacement; in some cases it has been necessary with the older men to either terminate therapy or decrease the dose of testosterone given due to the development of polycythemia (Sih et al, 1997; Tenover, 1997).

Table 4. Potential or reported adverse effects of testosterone therapy in older men

Liver toxicity
Fluid retention
Breast tenderness or gynecomastia
Exacerbation of sleep apnea
Development of polycythemia
Exacerbation of benign or malignant prostate disease
Increased risk of cardiovascular disease

While the co-existence of sleep apnea and elevated body mass index may play a role for some men, this has not been the case for many of the men (Krauss et al, 1991; Drinka et al, 1995). The dose and method of testosterone replacement may affect the magnitude of the effect on hematopoeisis (Jockenhovel et al, 1997).

Both benign prostatic hyperplasia (BPH) and prostatic adenocarcinoma are androgen dependent diseases and both are responsive to androgen ablation therapy (Henderson et al, 1982; Horton, 1984). Therefore, there is concern that testosterone replacement therapy in the older man could exacerbate these pre-existing prostate diseases. The dose-response of the prostate to serum androgens, however, is not known, and if the prostate is maximally stimulated at quite low serum testosterone concentrations, then further increases in testosterone levels may have only minimal effects. There have been at least eighteen testosterone replacement trials in men aged 40 to 89 years of age in which prostate specific antigen (PSA) or other prostate measurements were made (for review see Tenover, 1996). A composite analysis of these studies, which represent just about 700 man-years of total observation, reveals that 15 of the 18 studies (83%) reported no change in PSA with testosterone treatment, while the three other studies reported small, but statistically significant, changes in PSA with therapy. All six of the studies which measured prostate size, maximum urine flow rates, or prostate symptom scores reported no changes in these parameters with therapy. If PSA production is viewed as a general measure of prostate stimulation, then these results suggest that, in the short term, testosterone therapy does not appear to appreciably stimulate most older men's prostates. Since both prostate cancer and BPH are diseases with long natural histories, however, the experience with testosterone therapy in older men is too limited to determine long term prostate safety.

Cardiovascular system. Compared to premenopausal women, men have a higher incidence of cardiovascular disease and related mortality. Whether this sexual dichotomy is due largely to the protective effects of estrogens in women, or whether androgens also have a detrimental impact on the male cardiovascular system is not yet known. Epidemiological studies have demonstrated that low, rather than high, serum testosterone levels are associated with an increased risk of cardiovascular disease (Bagatell and Bremner, 1995), but this does not address the issue of changes in an individual's cardiovascular risk with testosterone therapy. Cardiovascular risk factors that may be affected by sex steroids include serum lipoprotein levels,

vascular tone, platelet and red blood cell clotting parameters, and atherogenesis. There are no data as yet on the effects of testosterone therapy in older men on most of these parameters, except for serum lipoprotein levels. In general, parenteral testosterone therapy in older men leads to a decrease in total and low-density lipoprotein cholesterol levels, with no change, or a small decrease, in high-density lipoprotein-cholesterol levels (Bagatell and Bremner, 1995). These changes in serum cholesterol with testosterone therapy are generally modest and the ultimate impact on cardiovascular disease risk is unknown.

SUMMARY

The aging process in men is accompanied by significant decreases in serum total and bioavailable testosterone levels. Concomitant disease and medications can exacerbate this age-related decline, which, although not universal, may result in testosterone deficiency for as many as 25-50% of men 65 years and older. The decline in testosterone production with age is multifactorial, with a major component being primary testicular decline, but it also involves changes at the hypothalamic-pituitary level. As of yet, there is no agreement on what hormonal levels would indicate that an older man is truly testosterone deficient and should be considered for testosterone replacement therapy. The presence of clinical symptoms or findings that could relate to androgen target organ deficiency and the presence of a low normal to below normal range serum testosterone level might lead one to consider testosterone replacement therapy for an older man. Studies to date, although limited, suggest that testosterone therapy in older men may be able to improve body composition, strength, bone mineral density, energy, mood, and libido. Large clinical studies are needed to confirm these concepts and to help delineate the real risks of such therapy. Consideration of the potential risks of testosterone therapy in the older man needs to be a part of any decision on replacement and should impact both the pre treatment evaluation and monitoring during therapy.

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8 ANDROGEN REPLACEMENT THERAPY, RISKS AND BENEFITS

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INTRODUCTION

The primary goal of androgen replacement therapy is to develop and maintain male sexual characteristics and sexual function, as well as to preserve anabolic effects on nonreproductive organs such as maintenance of muscle and bone mass (Bhasin et al, 1995; Wang and Swerdloff, 1997; Nieschlag and Behre, 1990,1998). Testosterone (T) formulations are predominantly used for androgen replacement therapy for hypogonadal men. Occasionally, supraphysiological (pharmacological) doses of androgen are administered for their anabolic effects. The use of androgens in the absence of medical indications (androgen abuse) for sports and body building purposes are discussed in Chapter 11.

In the body, T acts either directly on the target tissue or as a precursor to be converted by the 5α reductase enzyme to dihydrotestosterone (DHT) in some tissues such as the prostate, external genitalia and skin. The androgenic effects of T and DHT are mediated through the nuclear androgen receptor (Chapter 4). Testosterone is also aromatized to estradiol which acting via the estrogen receptor may have effects on multiple tissues including hepatic production and metabolism of lipoproteins, bone resorption and brain function. Modifications of T steroid ring structure may result in derivatives which may not be aromatized to estradiol or 5α-reduced to DHT.

INDICATIONS FOR ANDROGEN REPLACEMENT THERAPY

Androgen replacement therapy is indicated for the correction of androgen deficiency in hypogonadal men (Table 1). It is not useful in the stimulation of spermatogenesis and generally does not restore fertility in hypogonadal men. Hypogonadism is diagnosed by symptoms and signs of androgen deficiency (see Chapter 6) and confirmed by a low serum testosterone level. Serum T levels are measured in the morning and a level less than 250 ng/dL (8.5 nmol/L) is generally accepted as low. Levels between 250 to 300 ng/dL are borderline and diagnosis may be substantiated with abnormalities in serum LH (in primary testicular disorders) and/or clinical evidence of hypogonadism. It must be noted that different commercial laboratories use different assays systems to measure serum T. The values obtained from a patient must always be interpreted with the knowledge of the normal adult male range quoted by the laboratory.

In boys with delayed puberty, androgen therapy is initiated with small doses of a T ester and gradually increasing the dose to allow male puberty to proceed mimicking the normal event. Use of androgens in prepubertal boys must be carefully monitored to ensure attainment of full growth potential and avoiding the premature closure of the epiphysis limiting the final adult height. For treatment of micropenis of the neonate, T is administered for a short period of time to increase penile growth. Elderly men with low testosterone levels and clinical evidence of hypogonadism or with osteoporosis should be administered androgen replacement which may prevent frailty and decrease morbidity due to falls and fractures in the older men (see Chapter 7).

Table 1. Indications for androgen replacement therapy

Definite	**Probable or Under Investigation**
Androgen Deficiency	Enhance Muscle Mass/Strength
Male hypogonadism	Chronic infection
Elderly men (low serum T levels)	Cancer cachexia
Delayed puberty	Wasting syndrome associated with
Micropenis	HIV infection
Hereditary Angioneurotic Edema	Male contraception (Suppression of Spermatogenesis)
Stimulation of Erythropoiesis	
(second line drug)	Replacement in postmenopausal
Aplastic anemia	women, in addition to estrogen and
Chronic renal failure	progestagens
Myelodyplasia	
	Elderly Men with borderline low serum testosterone levels

In the past, androgens have been used in pharmacological doses to stimulate erythropoietin formation by the kidneys. Many trials have shown the benefits of androgen therapy in bone marrow failure such as aplastic anemia and myelodysplasia and in chronic renal failure. The use of androgens in these diseases has been largely replaced by the availability of recombinant erythropoietin (Besa, 1994). Hereditary angioneurotic edema is due to the absence or deficiency of the

first component of complement. Orally active modified androgens are effective and weak androgens appear to be as effective as potent androgens. The beneficial effect of androgens is most likely due to the increased the synthesis of the first component of complement by the liver (Sheffer et al, 1977; Gould et al, 1978).

Recent studies using improved methods have documented that androgens increased muscle mass and strength in hypogonadal men (Katznelson et al, 1996; Bhasin et al, 1997; Wang et al, 1996). Moreover, it has also been shown that supraphysiological doses of androgens will also increase muscle mass and strength in eugonadal men (Bhasin et al, 1996). In androgen deficient men with AIDS wasting syndrome, administration of testosterone leads to increased lean body and increased muscle mass and quality of life (Grinspoon et al, 1998). The benefits of androgens in eugonadal men with AIDS wasting have not been documented. Other studies have showed no effect. There are ongoing multicenter clinical trials which will clarify the risk versus benefit ratio of androgen therapy together with caloric supplementation and resistance training in these wasting states (Muurahainen and Mulligan, 1998).

When T is administered to normal men at or above the physiological production rate, serum gonadotropins are suppressed and spermatogenesis is inhibited. Based on this principle, large multicenter trials have shown that T in a supraphysiological dose will suppress spermatogenesis with acceptable contraceptive efficacy (see Chapter 16). Ongoing studies are examining whether addition of another gonadotropin suppression agent such as a progestagen or a gonadotropin-releasing hormone antagonist to testosterone will lead to more rapid and safer suppression of sperm production.

In postmenopausal women, androgen deficiency persists despite hormonal replacement therapy with estrogens and progestagens. Androgens may have additional benefits of increasing bone mass, enhancing libido and improving quality of life (Sherwin, 1998; Davis and Burger, 1997; Sands and Studd, 1995). Large, long term, placebo controlled, multicenter trials are ongoing and which may provide answers to some of these questions.

Over 50 percent of elderly men (over 65 years) may have serum testosterone in the lower limit of the normal range (see Chapter 7). These low T levels may be partly responsible for the frailty associated with aging. Several long term studies are currently near completion. Preliminary published results indicate that there may be benefits of T replacement on bone and muscle mass, fat mass, sense of well being and quality of life. These benefits must be weighed against the long term risks (see below and Chapter 9). Elderly men with borderline low serum T levels often present to the clinician with erectile dysfunction. Unless these subjects have other symptoms and signs of hypogonadism and abnormalities in serum LH levels, they are frequently not androgen deficient. Replacement T therapy in most instances should not be expected to help their erectile dysfunction. Elderly men with erectile dysfunction may suffer from a primary penile vasodilatory defect which responds to pharmacologic vasodilators (i.e.,Sildenafil, prostaglandin E_2) but be left with impaired libido due to low serum testosterone levels. These men may benefit from combined treatment to stimulate erectile capacity, improve sexual drive and enhance general well being. More data are required to test this hypothesis.

CONTRAINDICATIONS FOR ANDROGEN REPLACEMENT THERAPY

Androgens must not be used in patients with carcinoma of the prostate or the breasts. Both cancers are hormone dependent and androgens may lead to growth and proliferation of the cancer cells (see Chapter 9). In patients with symptoms of benign prostate hyperplasia (BPH), medical or surgical treatment of the condition should be undertaken before androgen replacement. Androgens increase the hematocrit usually by 3 to 5% depending on dose and method of administration. Use of androgens in subjects with elevated hematocrit (e.g., over 53%) is contraindicated because of the risks of hyperviscosity and thromboembolism. Obese, elderly subjects and patients with chronic obstructive airway disease must be monitored for sleep related breathing disorders. Androgens can cause sleep apnea especially in high risk patients. Dyslipidemia is not a contraindication to androgen replacement. The dose (e.g., smallest dose) and, the route (parenteral) of administration may be adjusted to minimize the changes in the HDL-cholesterol levels induced by T replacement.

TYPES OF ANDROGENS AND METHODS OF REPLACEMENT (Table 2)

Testosterone administered by mouth is rapidly cleared by the liver and only a small proportion reaches the systemic circulation. At the present to achieve effective androgen therapy and attain physiological levels in circulation, T has to be administered as a slowly absorbed form (transdermal patch, T microspheres, T implants) or as a chemically modified androgen. The modifications decrease the rate of absorption or metabolism so that effective circulating and target tissue levels of T can be maintained. The modifications include esterification of the 17 β hydroxy group (e.g., T enanthate, cypionate, undecanoate, propionate and buciclate); alkylation at the 17 α position (methyl testosterone, fluoxymesterone, methandrostenolone, danazol); or modification of the steroid ring structure (e.g., mesterolone). Esterification of the T at the 17 β position makes the steroid soluble in oil and suitable for use as IM injections with a slow release of T into the circulation (Griffin and Wilson, 1998).

The 17α alkylated androgens are orally active because they are absorbed into the portal system and are very slowly metabolized by the liver. These 17α alkyl groups are not removed and act as such within the cells. They are termed anabolic steroids but in fact they are androgens and act to the best of our knowledge through the androgen receptor. Used in appropriate doses they can be administered to hypogonadal men as androgen replacement. These modified androgens can cause liver toxicity, elevations of serum alkaline phosphotase and bilirubin levels, which are rare to non existent with administration of the T esters (de Lorimier et al, 1965; Arias 1962). When very large doses are used in aplastic anemia, oral 17 alkylated androgens have been reported to cause peliosis hepatis (cysts with blood) and hepatoma (Sweeney and Evans, 1976). It is uncertain however if the primary

Table 2. Methods of Androgen Replacement

Currently Available	In Clinical Trials or Under Development
Injections	
T enanthate or cypionate 200 to 250 mg IM every 2 to 3 weeks (100 mg IM every 7 to 10 days)	T undecanoate (6 to 8 weeks) T buciclate (20 to 24 weeks) T microspheres (8 to 10 weeks)
Oral/buccal/Sublingual	
T microcrystals T undecanoate 40 to 80 mg bid or tid	T cyclodextrins Buccal T Nonsteroidal androgen receptor modulators
Transdermal	
Scrotal patch (4 or 6 mg T per day) Nonscrotal patch 2 patches delivering 5 mg T per day 1 patch delivering 5 mg T per day	T gel DHT gel Other patches
Implants	
Crystalline T pellets (600 mg every 4 to 6 months)	7 α methyl 19 nortestosterone (MENT)

disorders render the patient susceptable to these complications. 17α alkylated androgens are not converted to estradiol. After oral administration, they are absorbed into the portal circulation, and then to the liver. Because of these two reasons these agents cause more marked decreases in HDL-cholesterol levels than T esters (Friedl et al, 1990). 17α alkylated androgens are not recommended as androgen replacement therapy. The weak androgen Danazol is used for the treatment of angioneurotic edema.

Injections

The most common method of androgen replacement therapy is T enanthate in oil (Snyder and Lawrence, 1980). Deep intramuscular injections of 200 mg (or 250 mg in Europe, Asia) are administered usually into the buttock. Some patients may prefer the lateral thigh muscles or deltoid for ease of self administration. The patients are taught how to give their own injections. Serum T levels peaked at about 1 to 3 days and then gradually decreased reaching pretreatment levels in around 14 days (Sokol et al, 1982). T cypionate has the same pharmacokinetic profile as T enanthate. The commonest complaint after T enanthate administration is pain and bruising at the injection site. In elderly men, the dose is frequently lowered to 150 mg every two to three weeks. In some patients, variations of mood and sexual

dysfunction may be associated with the peaks and troughs of serum T levels after IM T enanthate injections.. In these patients, increasing the frequency of T enanthate injections to 100 mg once every 7 to 10 days may reduce symptoms. Only very rarely, suboptimal clinical response may require an increase in dose. Administration of higher doses of T enanthate does not prolong the duration of action but results in higher circulating levels of serum T (Wang and Swerdloff, 1995). Persistent inadequate responses with TE injections may indicate that the symptoms are not due to androgen deficiency and further increase in doses is not warranted.

For induction of puberty in hypogonadotropic hypogonadism or constitutional delayed puberty in boys, treatment begins with 50 mg T enanthate per month, increasing every 3 to 6 months. The goal is to take the boys through pubertal development over a few years. In naive patients, the injections of T enanthate may lead to frequent erections and the patients should be warned about this side effect. For treatment of micropenis in the neonate, T enanthate is administered as 25 mg injections monthly for 3 months.

In Europe, Asia, and other parts of the world a mixture of T esters (propionate, phenyl propionate, isocaproate, decanoate) is available at 250 mg in 1mL and is administered at 2 to 3 weeks interval. The serum T levels achieved by this mixture of esters are similar to that of T enanthate.

T undecanoate in oil is manufactured in China and approved for parenteral use in hypogonadal men (Zhang et al, 1998). A similar preparation is being tested in Europe. Preliminary results indicate that a single intramuscular injection of T undecanoate 1000 mg in 4 mL oil administered to hypogonadal men will result in serum T levels within the physiological range for 6 to 8 weeks (Behre HM, personal communication). Another even long acting ester under development is T buciclate. A single injection of 600 to 1000 mg of T buciclate will maintain serum T levels at the low normal adult range for 12 to 20 weeks (Behre et al, 1992). In contrast to other esters, this preparation of T buciclate is formulated in an aqueous suspension and not in oil.

Biodegradable microspheres loaded with T delivering 6 to 9 mg of T per day have been studied in men (Bhasin et al, 1993). The early T microspheres preparations, upon storage, result in leaching out of T and changing pharmacokinetic profiles. Stable preparations of T microspheres have to be developed before clinical applications are possible.

Oral, Sublingual, Buccal Pills

T undecanoate can also be administered as an oral preparation (40 to 80 mg taken 2 or 3 times per day). T undecanoate is absorbed into the intestinal lymphatics. It has a short duration of action of about five hours and a very variable oral bioavailability (Nieschlag et al, 1975; Cantrill et al, 1984). Despite the short duration of action and tresultant mean T levels generally are in the lower normal adult male range, clinical effects and restoration of sexual function have been reported to be satisfactory. Long term studies have demonstrated its safety and efficacy (Gooren et al, 1994). T undecanoate has been shown to be effective in inducing puberty without affecting growth velocity in boys with delayed puberty (Butler et al, 1992).

T cyclodextrin has a carbohydrate doughnut structure with T inside and when administered sublingually, the T is absorbed into the buccal mucosa capillaries. Peak T levels are achieved within 20 to 30 minutes and then decrease gradually to baseline levels by 4 hours. Despite the peaks and troughs, a short term study showed a moderate effect on sexual function, mood, muscle mass and strength (Wang et al, 1996). T cyclodextrin is not yet available for patients' use in the U.S. Acute buccal administration of T resulted in high, supraphysiological peak T levels in 30 minutes which returned to baseline in 4 to 6 hours (Dobs et al, 1998).

Transdermal Delivery

The first transdermal patch applied to the scrotum delivers 4 or 6 mg T per day. The scrotal skin has a rich capillary network which provides adequate absorption of T to provide stable levels of T lasting for 24 hours (Findlay et al, 1987; Cunningham, 1989). The size of the patch may not be suitable for men with poor scrotal development and clipping of hair may be necessary for the patches to adhere. The scrotal patches results in an increase in serum DHT up to 30 or 40% of serum T. The presence of high serum DHT levels however do not cause additional side effects (e.g., prostate, serum lipid levels) in long term studies of the scrotal transdermal patches. The newer non-scrotal skin patches can deliver 5 mg T per day either by one or two patches. The smaller Androderm® patches cause skin irritation in up to 30 percent of men and lead to discontinuation in about 10 percent because of wheals, blisters and vesicles (Meikle et al, 1992). The larger Testoderm TTS® patches have much less skin irritation but may have problem of poor adhesion. The T levels attained after application of the patches are in the low normal range. Depending on the time of application, the serum T levels may mimick the physiologic circadian T rhythm of normal men. The clinical significance of maintaining the circadian rhythm of T is unknown. Unlike the scrotal patches, these non-scrotal patches do not lead to high serum DHT levels. In general, despite the relatively low T levels achieved, these T patches have been shown to be effective in restoring sexual function as well as maintaining bone mass in hypogonadal men (Behre et al, 1994).

In France and Belgium, a 2.5 hydroalcoholic DHT gel (250 mg DHT in 10 g gel) applied to the skin is available for treatment of hypogonadal men. The gel does not lead to skin irritation. DHT given as an androgen replacement will avoid the side effect of gynecomastia (Schaison et al, 1990). Contrary to expectation, when administered to older men for androgen replacement, DHT gel application resulted in a small decrease in prostate volume (De Lignieres, 1993). This could be due to the suppression of endogenous estradiol or to a reduction in intraprostate DHT levels despite high circulating DHT levels. Both these hormones have been shown in animal models contribute to development of BPH. A newly formulated DHT gel (0.7%) is currently in clinical trials in the US (Wang et al, 1998) to examine whether DHT gel has the same beneficial effects as T but with less effect on prostate volume. Using a similar formulation, T in a gel is also being tested in clinical trials in hypogonadal men.

Implants

Testosterone implants are very popular as androgen replacement therapy for men in Australia and United Kingdom. The biodegradable implants are fused cylindrical pellets of 200 mg of T each. Usually three pellets are inserted under local anesthesia into the abdomen wall with a trocar and cannula. The operator must be trained for this minor surgical procedure. The pharmacokinetics showed maintenance of serum T levels in the physiological range for 4 to 5 months after 600 mg T implants (Handelsman et al, 1990). The main problem of T implants is pellet extrusion which occurs in about 10% of the procedures and depends on the skill of the operator. Pain and minor bruising may occur after implants but infection and bleeding are uncommon.

A synthetic androgen, 7α-methyl-19-nortestosterone (MENT) is also being developed as an implant that will last for a year or longer. The steroid does not undergo 5α reduction but can be aromatized. MENT is about 10 times more potent than T on suppression of gonadotropins in castrated rats and in restoring mating behavior, but only 4 times more effective on stimulation of ventral prostate weight (Kumar et al, 1992). Thus MENT may have a prostate-sparing effect when administered as androgen replacement for hypogonadal men. Long term clinical trials of MENT will be possible after completion of long-term toxicological testing.

Non-steroidal androgen receptor modulators

It is now possible to design non-steroidal molecules that may selectively bind to the steroid hormone receptors. This has been described for the estrogen and progesterone receptors (Zhi et al, 1998; Edwards et al, 1998; McDonnell and Norris, 1997). These selective steroid receptor modulators may possess either agonist or antagonist effects or both. Using a similar strategy of direct high throughout screening of a defined chemical library using human androgen receptor co-transfection cell based assays, a new nonsteroidal androgenic pharmacophore has been discovered. The androgen receptor antagonists is characterized by a linear, triayclic 1.2-dihydropyridone [5, 6-g] quinoline core (Hamann et al, 1998). Molecules based on a similar series of compounds can be developed into androgen receptor agonists. These compounds are orally active and may display tissue selectivity similar to the nonsteroidal progestational or estrogenic compounds. Thus it may be possible to develop androgen receptor modulators that have no significant effects on the prostate and serum lipidsbut with all the other beneficial effects on the reproductive tissues, bone, muscle and brain.

RISKS VERSUS BENEFITS

The effects of androgens on reproductive and non-reproductive organs must be considered during androgen replacement therapy. This chapter

will describe the potential benefits and some of the side effects. The following chapter (Chapter 9) will focus on the effects of androgens on the prostate gland and cardiovascular risk factors.

Benefits

Development and maintenance of secondary sex characteristics. The goals of androgen replacement therapy are to restore or induce secondary sexual characteristics. Androgen administration to hypogonadal men will result in development of a beard, body, axillary and pubic hair. In prepubertal subjects naïve to androgen therapy, the administration of small doses of androgens will result in penile growth, darkening and coarsing of scrotal skin, deepening of voice, appearance of secondary sex characteristics within the first year. Androgens will also lead to a pubertal growth spurt. With continued administration and a gradual dose increase, the treatment results in normal pubertal development over several years. It should be noted that for induction of puberty oral replacement with testosterone undecanoate or other newer oral delivery systems may have the advantage of ease of administration and when used carefully will not result in accelerated epiphyseal closure.

Sexual Function and Mood. The major effect of androgens are on sexual desire, day dreams, fantasy and other motivation parameters. Studies on hypogonadal men showed that nocturnal and spontaneous erections are increased with androgen replacement (O'Carroll et al, 1985; Burris et al, 1992; Salahian et al, 1995). It should be noted that return of sexual function requires relatively low physiological levels of testosterone. Increasing the dose of testosterone to the mid or high physiological range does not appear to further enhance libido and erectile function (Gooren et al, 1987; Salahian et al, 1995).

The effect of T replacement on mood are less well understood. Anecdotal information and correlation data based on population studies have suggested to some clinicians that T may increase aggression, hostility, anger and depression in men (see below). In hypogonadal men T replacement reduces depression, anger, fatigue and confusion (Burris et al, 1992). In our study, T replacement in hypogonadal men improved energy, sense of well being and friendliness. At the same time, anger, nervousness and irritability decreased (Wang et al, 1996b). Before T replacement, when the men were hypogonadal, serum androgen levels showed a direct correlation with positive mood parameters and a negative correlation with negative mood parameters. The relationships disappeared when serum T reached the normal range. The data suggest that once a minimally adequate serum androgen is achieved, further increases in serum T or DHT levels will not contribute to further improvement in mood. The improvement in mood parameters is correlated with increases in sexual function.

Nitrogen Balance, Muscle Mass and Strength, Body Fat. It is known that androgens will cause a positive nitrogen balance when administered to men and women (Griffin and Wilson, 1998). Clinical experience indicates the T replacement to hypogonadal men results in weight gain presumably due to increase in muscle

mass. In recent years, with modern techniques, it has been shown in a number of studies that T replacement to hypogonadal men increases body weight, muscle mass, muscle size and strength (Katznelson et al, 1996; Wang et al, 1996a; Bhasin et al, 1997). The increase in muscle mass is due to enhanced muscle protein synthesis (Brodsky et al, 1996). Associated with this increase in lean body mass that may be decreased in total body fat (Katznelson et al 1996). This is most evident in the decrease in visceral fat in older obese men and is due to the inhibition of triglyceride assimilation in the intraabdominal fat deposits (Marin et al, 1996). The significance of this finding is that visceral fat may be related to cardiovascular disease and noninsulin-dependent diabetes.

Bone Mineral Density. It is well know that osteoporosis occurs in hypogonadal men. Progressive loss of bone mineral density occurs in men after bilateral orchiectomy (Stepan et al, 1989). Testosterone replacement in men with hyperprolactinemic hypogonadism and hypogonadotropic hypogonadism results in improved bone mineral density as assessed by single and dual energy x-ray absorptiometry. In these studies, the increase was more marked in cortical bone of men with open epiphysis suggesting that the increase in bone density is due to a process of bone accretion similar to puberty (Finkelstein, 1995). Long term effect of androgen replacement in hypogonadal men showed that bone mineral density can be normalized and maintained (Behre et al, 1997). The mechanism of how androgens affect bone density is not clear. Androgen replacement in short term studies decreases bone resorption in hypogonadal men (Katznelson et al, 1996; Wang et al, 1996) and may increase bone formation. Androgen receptors are present in osteoblasts. However, androgens may also mediate its effect on bone via estrogens. Support for the latter comes from studies in men with mutations of the estrogen receptor (Smith et al, 1994) and aromatase enzyme (Morishima et al, 1995). Both mutations resulted in men who manifested severe osteoporosis. It must be noted these men had congenital absence of estrogen action and the interaction might be quite different in men with partially suppressed estrogens.

Brain Function and Cognition. Studies in men have shown positive correlations between serum T and visuospatial ability and negative relationship between serum T and verbal ability (Christiansen and Knussman, 1987b). In older men with low T levels, T administration resulted in enhancement of visuospatial ability (Janowsky et al, 1994). In a recent report from our center showed that T replacement in hypogonadal men improved verbal fluency (Alexander et al, 1998). More studies are clearly needed in this area to show whether androgens have any consistent and significant effects on cognition in hypogonadal men.

Potential Side Effects

The common, initial side effect of androgen replacement therapy include weight gain due to fluid retention. This is usually mild and requires no treatment except in elderly patients with renal or heart failure. Gynecomastia may occur with

testosterone replacement because concomitant increases in serum estradiol with T administration. The gynecomastia is usually mild and require no specific treatment. The use of a non-aromatizable androgen such as DHT will not result in gynecomastia. Many patients may experience increased sebaceous gland secretion resulting in acne and oiliness of skin and are treated symptomatically. This side effect appears to be dose related and occurs when the T replacement results in high serum concentration of T. Use of delivery systems without peaks and troughs of serum T and reduction of the dose, may sometimes help patients with severe acne. Testosterone and its esters do not lead to liver dysfunction. In a large multicenter study, administration of T enanthate did not result in significant changes in liver function tests (Wu et al, 1996).

Hematocrit and Hemoglobin. Androgens affect the hematopoietic system by increasing erythropoietin production from the kidneys and also directly acting on the stem cells. Prepubertal boys and hypogonadal men have low hematocrit. As boys undergo puberty, gradual increases in hematocrit are observed. Similarly hypogonadal men show small but significant increases in red cells indices after T replacement therapy. In older men, smokers and patients with chronic obstructive airway diseases, testosterone replacement therapy may, though uncommonly, lead to marked increased in red cell mass and decrease in plasma volume. This may be associated with increased risks of ischemic attacks and stroke (Krauss et al, 1991). Thus subjects with high risks should be monitored with hematocrit and hemoglobin checks. Before administration of testosterone to men with baseline hematocrit of over 50%, one must carefully consider the risks of thrombosis against the possible benefits.

Sleep Apnea. Sleep apnea occurs predominantly in men. Testosterone administration in hypogonadal men may result in sleep related breathing disorders. In some studies, hypoxic ventilatory drive was decreased with T treatment. In other studies hypercapneic ventilatory drive was affected by T. In a small study, when T enanthate was administered to hypogonadal men, one out of 5 subjects developed sleep apnea and symptoms worsened in another (Matsumoto et al, 1985). Thus sleep apnea may be a potential risk of T replacement therapy. Subjects at high risk for sleep related breathing disorders such as those who are obese, chronic smokers, high hematocrit, and with medical illnesses prone to sleep apnea should be screened by symptoms of loud snoring, irregular breathing during sleep, and day-time somnolence. If present, such high risks patients have to be monitored during T replacement for deterioration of symptoms or by sleep studies.

Aggressive Behavior. The role of androgens in human aggression is much less clear and convincing than in animals (Archer 1991). The evidence available from human which suggests that T may have an effect on aggressive behavior, is usually based on correlation studies in special populations (inmates, military delinquents, athletes) or self ratings in responses to specific situations (Dabs, 1995; Scaramelli et al, 1978; Christiansen and Knussman, 1987a). These studies may not reflect response of normal or hypogonadal men. In normal men, when T enanthate was

administered at supraphysiological doses, there were no changes in irritability, anger or readiness to fight (Anderson et al, 1992; Bagatell et al, 1994). These studies were ascertained using self-reporting of emotional behavior and did not include response to specific situations nor observation from the female partner. In pubertal boys when T was given in a randomized, double-blinded, placebo-controlled, crossover design, aggressive behavior assessed by an aggression inventory showed that significant hormone effects on physical aggressive behaviors but not in verbal aggressive behaviors (Finklestein et al, 1997). Studies using pretested, validated hormone responsive instruments to measure aggressive behavior in double blind, placebo control trials are currently ongoing in adult eugonadal and hypogonadal men to determine whether T has any effect on aggressive behavior.

MONITORING ANDROGEN REPLACEMENT THERAPY

During the first few months after the initialization of androgen replacement, serum T levels are usually measured just prior to the next dose . This may be used as a guideline to assess whether T levels are in the normal range. Serum gonadotropin levels are not useful for as a guide of androgen treatment. Once the patient is on long term therapy, frequent monitoring of serum T level is unnecessary. The best guide to the adequacy of androgen is the patient's self assessment of response with improvement in libido and sense of wellbeing. However, restoration of sexual funciton requires a low threshold for androgen action. Effects on bone, muscle and other organs may require higher T levels and a long period of treatment to have optimal benefits. In older men who present with sexual dysfunction, erectile function may not be restored. As discussed in Chapter 15 relative androgen deficiency is an uncommon sole cause of erectile dysfunction. The use of T together with sildenafil or a similar vasodilator may be considered. At the initiation and at regular intervals (6 to 12 monthly) hematocrit, liver function tests, fasting lipid profile, PSA and digital rectal examination should be monitored. In men with low bone mineral density, follow-up with DEXA may be done at yearly intervals to ensure adequate response (WHO, 1992).

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9 THE SAFETY OF ANDROGENS: PROSTATE AND CARDIOVASCULAR DISEASE

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INTRODUCTION

Traditionally, the major concerns regarding the safety of androgen therapy are the possibility that cardiovascular and prostate disease might be initiated or pre-existing diseases aggravated. These concerns arise in relation to all forms of androgen therapy but are increasingly prominent in relation to the growing use of androgen supplementation in ageing men (Tenover, 1996) where these strongly age-related conditions are already common. This chapter reviews the basis for such concerns, aiming to evaluate the likely benefits and risks of androgen therapy, particularly for ageing men. Similar considerations arise among users of hormonal male contraception and androgen replacement therapy for classical androgen deficiency. In these clinical situations the younger population with low background rate of cardiovascular and prostate diseases together with the therapeutic imperative to maintain strictly physiological androgen levels makes these safety concerns more remote. Pharmacological androgen therapy (Liu et al., 1998) and androgen abuse (Jin et al., 1996; Handelsman et al., 1997) involve additional toxicological and safety issues beyond the scope of this review.

ANDROGENS AND PROSTATE DISEASE

The prostate is a classically androgen-dependent organ both during its prenatal development (Cunha et al., 1987) and maintenance during maturity (Isaacs, 1994). The prostate is analogous in many respects to the breast for which the hormonal dependence and its consequences are better understood. In adults, both prostate and breast undergo major atrophy following castration demonstrating the continuing hormonal dependence of the mature organs on gender-specific sex steroids.

Prolonged exposure to cyclical endogenous estrogen is the major factor in the causation of breast cancer (Bernstein et al., 1993). In contrast, use of exogenous estrogen for oral contraception or post-menopausal estrogen replacement, has modest, if any, additional effects on rates of breast cancer, particularly if prolonged, high dose usage is discounted (Hoover, 1995). Furthermore, benign breast disease is prevented by administration of exogenous estrogen in oral contraceptives (Ory, 1982). By analogy with estrogen therapy and breast disease, androgen therapy may prevent benign prostate disorders while the additional effect on background rate of prostate cancer may be minimal if net androgen exposure does not exceed natural exposure to endogenous androgens.

Epidemiology Of Prostate Disease

The best established predictors of prostate disease are increasing age, familial/genetic factors and early life androgen exposure (Guess, 1992; Boyle, 1994). Numerous other environmental factors such as religion, marital status, sexual activity as well as various dietary, toxic and environmental exposures have been identified inconsistently by epidemiological studies. Among other factors, major reasons for these inconsistencies may be both imprecise case definition for prostate disease and recall bias. Case definition for BPH is particularly difficult with histological, macroscopic and surgical criteria all being variously utilised (Guess, 1992). For prostate cancer, case definition based on the relatively objective death rates from invasive prostate cancer have become increasingly blurred by the wider use of screening in the context of the vast pool of undiagnosed in-situ prostate cancer (Whittemore, 1994). Recall bias arises with diagnosed cases having motivation to recollect many more incidental facts than healthy controls. Characteristically, such false positive findings may be sensitive to arbitrary details of questionnaire design and often involve factors that cases may retrospectively re-interpret in the lay imagination as suspiciously harmful (Guess, 1992; Boyle, 1994).

The importance of genetic factors in prostate disease was first suggested by familial clustering (Gronberg et al., 1996) and twin studies (Gronberg et al., 1994; Partin et al., 1994). While these methodologies highlight genetic factors, they cannot exclude the influence of early shared environment which may be an important limitation if prenatal and childhood environmental factors are relevant in the origins of prostate diseases. Direct confirmation of a genetic causation would be provided by the characterisation of susceptibility genes for prostate cancer in the familial prostate cancer syndromes for which chromosomal localisation has been identified (Smith et al., 1996) although even then non-genetic factors are still likely to have a decisive influence in the pathogenesis of disease. Genetic cofactors such as the CAG repeat polymorphism in the exon 1 of the androgen receptor have also been suggested as modifying the pathogenesis of prostate cancer (Giovannucci et al., 1997; Ingles et al., 1997; Stanford et al., 1997).

Environmental factors are also clearly important in the origins of prostate disease. The geographical distribution provides some interesting clues about the pathogenesis of prostate diseases. Dramatic geographical variations exist in age-adjusted death rates from invasive prostate cancer (Parkin et al., 1993). This differs substantially from BPH (Guess, 1992) and even in-situ prostate cancer (Breslow et

al., 1977; Yatani et al., 1982) where the age-adjusted prevalence differs little between populations. This geographical variability in death rates from invasive prostate cancer is likely to be determined by environmental rather than genetic factors since migration studies indicate that individuals moving from low to high risk countries adopt slowly over decades the increased susceptibility to invasive prostate cancer associated with the geographical area of their residence rather than of their origins (Haenszel et al., 1968; Yatani et al., 1982; Haenszel, 1985; Muir et al., 1991; Yu et al., 1991; Hanley et al., 1995). Among the environmental factors considered in this context, dietary mechanisms have attracted most interest although their importance relative to non-dietary factors remains unclear. Ecological and correlational evidence have suggested that dietary differences contribute to the different susceptibility to prostate and other cancers that exhibit major geographical variability in prevalence. In particular, high vegetable and/or low fat intake have been considered protective against prostate cancer but convincing prospective evidence is lacking. Recent suggestions that the high dietary intake of plant extracts ("phytoestrogens") may protect against prostate and other hormone-dependent diseases are interesting (Murkies et al., 1998) since so-called phytoestrogens may also have androgenic activity (East et al., 1949; Adams, 1979). The precise mechanism(s) for the geographical variability in rates of invasive and in-situ prostate cancer however remains to be fully explained.

Hormonal Factors in Prostate Development and Disease

A basic tenet of the hormonal epidemiology of prostate disease is the dependence of late-life prostate diseases on early-life androgen exposure. It is often written that prostate disease does not develop among men who were androgen deficient early in life. While plausible, this familiar assertion has little empirical substantiation (Moore, 1944), is now difficult to verify and counterexamples are reported (Deming et al., 1935; Kretschmer, 1935; Deming et al., 1939; Scott, 1953; Sharkey et al., 1960; Yokoyama et al., 1989; Uno et al., 1998). The best available evidence for this conjecture is a study of elderly Chinese eunuchs castrated during adolescence among whom the prostate was barely or not palpable (although 86% had nocturia) when examined more than 40 years later (Wu et al., 1987). Consistent supportive evidence is provided by experiments of nature in genetic males with congenital defects in androgen action which lead to maldevelopment of the prostate. For example, a non-functional androgen receptor which causes the clinical phenotype of complete androgen resistance (Quigley et al., 1995) is associated with nondevelopment of the prostate. Other mutations leading to partial defects in androgen action such as partial androgen insensitivity (Quigley et al., 1995) or 5α-reductase deficiency (Wilson et al., 1993) render prostate development vestigial (Imperato-McGinley et al., 1992). Although such rudimentary prostate development may reduce the likelihood of late-life prostate diseases, this remains to be verified. The prevention of prostate disease by prostate underdevelopment may be considered analogous to the relatively rarity of breast cancer in men. Whether disease risk is simply proportional to reduced glandular mass rather than gender or any additional effects of sex hormones is unclear. Potential protective effect of androgen deficiency early in life against prostate diseases might still be estimated in future

large epidemiological studies of prostate disease and epidemiological evaluation would be of interest. It appears most plausible that, as with breast cancer, prolonged exposure to eugonadal levels of androgens after puberty may be a crucial factor in the development of late-life prostate disease. A corollary of the importance of early-life androgen exposure in determining late-life prostate disease is that variations in mid-life androgen exposure within the range that eugonadal men habitually experience may also have minimal effects on the occurrence of prostate disease if prolonged, high dosage exposure is avoided.

In elegant embryological tissue recombination experiments, Cunha has shown that prostatic epithelial differentiation requires indirect androgen action (Cunha et al., 1987). The primary site of androgen action in prostate development is the prostatic mesenchyme that has functional androgen receptors while prostatic epithelium only comes to express androgen receptors when fully differentiated. In the mature prostate, functional epithelial-mesenchymal interactions remain important (Hayward et al., 1997) as exemplified by paracrine mediators such as keratinocyte growth factor (KGF, also known as FGF7) whose synthesis is androgen-dependent and limited to the prostatic stroma whereas its receptors are restricted to the overlying prostatic epithelium (Yan et al., 1992). Recent epidemiological evidence has identified important long range influence of fetal hormonal and other exposures on the subsequent development of various degenerative diseases decades later in life (Scrimshaw, 1997). The long term impact of hormonal effects during fetal prostate development on late-life prostate disease have yet to be evaluated. Experimental rodent studies have however demonstrated that neonatal hormonal exposure has persistent effects on mature prostate morphology and sensitivity to androgens (Rajfer et al., 1978; Prins, 1992). Evidence that prenatal influences determine long-term ovarian function (Creswell et al., 1997) support preliminary evidence that perinatal factors influence occurrence of prostate disease decades later (Tibblin et al., 1995; Ekbom et al., 1996). An interesting test of this proposition would be whether the cohort of boys exposed prenatally to DES (Wilcox et al., 1995) has differences in development of prostate disease. Prenatal estrogen exposure might modify the occurrence of prostate disease (Henderson et al., 1988) perhaps by altering subsequent hormonal sensitivity to androgen action of the mature prostate gland as predicted by rodent experiments (Rajfer et al., 1978; Prins, 1992; Santti et al., 1994).

Androgen Amplification in the Prostate

The molecular mechanism of action of testosterone (T) on the prostate involves both the direct effects of T on the androgen receptor (AR) as well as two types of indirect effects. The indirect effects arise from T's two active metabolites, 5α-dihydrotestosterone (DHT) formed by the enzyme 5α-reductase and estradiol formed by the enzyme aromatase. These two active metabolites act upon the androgen and estrogen receptors, respectively, which serves to both amplify and widen the biological action of T. DHT is a non-aromatisable and more potent androgen than T so that 5α reduction of T provides an amplification of its androgenic potency. Although the net whole body metabolic conversion rates of T to its active metabolites by 5α-reductase (~4%) and aromatase (0.1-0.2%) are

quantitatively small, this triad mechanism of T action allows for and explains some tissue variability in androgen action. In the prostate, the uniquely avid conversion of most incoming T to DHT by the type II 5α reductase enzyme constitutes with aromatisation a powerful local androgen amplification system. The physiological, pharmacological and pathological consequences of this prostatic androgen amplification system are still not fully appreciated. While medical or surgical castration results in maximal involution of the prostate (Oesterling et al., 1986), this inevitably produces significant concomitant androgen deficiency which limits its application to palliative treatment of inevitably fatal prostate cancer. More selective blockade of androgen action on the prostate relative to other tissues is feasible by neutralising the prostatic androgen amplification system. This can be achieved by inhibition of the 5α-reductase enzyme but the blockade of prostatic androgen action could be further enhanced by adding an aromatase blocker. Indeed both properties could be incorporated within a single one synthetic steroid since the structure-activity requirements in the steroidal A ring to inhibit 5α-reductase and aromatase may be convergent.

In common with all tissues derived from the urogenital sinus, prostate development requires adequate supply of not just testosterone (T) but its 5α reduced metabolite 5α-dihydrotestosterone (DHT). The precise molecular basis for the unique tissue requirement however remains unknown. Since DHT is a more potent, pure androgen than T, it is likely that the requirement for 5α reduction indicates a distinctive tissue-specific difference in the androgen receptor or post-receptor sensitivity. This suggests that the androgenic threshold to support masculine urogenital sinus differentiation may be higher than for other androgen-dependent tissues. The suggestion that the urogenital sinus derivatives have a more stringent androgen requirement is consistent with the observation that even relatively severe maternal or fetal virilisation rarely produces fully masculine urogenital differentiation (Forest, 1995). The biological mechanisms involved, however, remains to be clarified.

The relatively high androgenic threshold requirement for androgen action on prostate cells may also be present in the mature gland. Considerable debate has centred around the potential biological significance of low potency adrenal androgens on the basis that they may maintain androgenic support of prostate cancer cells after castration (Labrie, 1995). This supposition, which assumes that prostate cells are very sensitive to androgens, led to the development of a therapeutic regimens based on attempts to block residual androgen action beyond that achieved by medical or surgical castration. The bulk of evidence from biological (Oesterling et al., 1986), epidemiological (Anonymous, 1995) or controlled clinical studies (Zalcberg et al., 1996) suggests however that maximum androgen blockade is no more effective than castration and that therefore adrenal androgens are insufficient to stimulate prostate cells. This would be consistent with the embryological observation of a stringent requirement for androgenic support for prostate development from the urogenital sinus. The androgenic threshold for support of the mature prostate may therefore be as relatively high during adult as it is in fetal life.

In addition to the fundamental importance of androgen action on the prostate, there is also evidence for the importance of estrogens in the origins and evolution of prostate disease (Mawhinney et al., 1979; El Etreby, 1993; Santti et al., 1994). In

particular, aromatisation of T entering the prostate to estradiol has multiple effects on the prostate to cause stromal growth and epithelial squamous metaplasia. Few studies of estrogen effects on the normal human prostate have been reported (van Kesteren et al., 1995; Jin et al., 1996) so the importance of aromatisation and estrogens on human prostatic structure and pathology have remained largely speculative. Since DES treatment for advanced prostate cancer was recognized to cause excess mortality from arterial thrombosis (VACURG, 1967) and GnRH analogs supplanted estrogen therapy for medical castration, estrogens have fallen into disfavour for treatment of prostate cancer. More recent interest has focussed on estrogen deficiency due to blockade of aromatase (El Etreby, 1993; Habenicht et al., 1993). The first controlled clinical trials to evaluate the effects of an oral aromatase inhibitor as a sole agent for treatment of BPH have been negative (Gingell et al., 1995; Radlmaier et al., 1996). Since aromatisation is most likely subsidiary in importance to prostatic 5α-reduction of T in the prostatic androgen activation system, the hindrance to androgen action of an aromatase inhibitor alone might be weak and it would still be of interest to examine the efficacy of a combination of a 5α-reductase inhibitor with a potent aromatase inhibitor (Habenicht et al., 1993), particularly a synthetic steroid having both biochemical properties.

Another consequence of the intraprostatic androgen amplification system is the opportunity for chemoprevention of prostate cancer. A large chemoprevention study involving 18,000 men randomised to finasteride (an oral 4-azasteroid which inhibits prostatic type II 5α reductase) or placebo for 7 years to determine if prolonged 5α reductase blockade can prevent prostate cancer should be completed by 2002 (Feigl et al., 1995). Finasteride is well suited to this study as it has a reasonable safety record and its partial blockade of androgen action does not lead to general androgen deficiency. The chemoprevention study will determine if such partial blockade of T action (ie only the indirect pathway of AR activation via metabolism of T to DHT) is sufficient to prevent prostate disease without requiring either more complete blockade of androgen action and/or concomitant blockade of prostatic aromatisation of T to estradiol. If chemoprevention by 5α reductase blockade is feasible, this would greatly heighten interest in the development of synthetic, selective androgens (designer androgens) with tissue selectivity based on the tissue-specific metabolic activation pathways for T by 5α reduction and/or aromatisation. At present, however, the putative advantages of selective androgens remain speculative (Sundaram et al., 1996).

Androgen Effects and Aging

Prostate diseases become more prevalent during aging when testicular androgen secretion is gradually declining (Tenover, 1996; Zmuda et al., 1997). The modest reduction in androgen secretion due to aging together with the relatively high androgenic threshold to support the prostate make it most likely that the decline in androgen secretion and onset of prostate disease are co-incidental but unrelated features of aging. Less likely is the possibility that gradual progressive reduction in androgen secretion might promote or inhibit the onset of prostate diseases. This latter possibility will be tested by studies observing the incidence of new prostate disease during use of androgen therapy in aging men. Studies administering T

(Lesser et al., 1955; Tenover, 1996) or DHT (de Lignieres, 1995) to older men have so far reported no excess of prostate disease related to treatment; however, controlled studies of longer duration are required to resolve this issue.

Attempts to elucidate any short-term relationship of sex steroids to prostate disorders have generally proved unrewarding. There appears to be no consistent relationship between blood or tissue hormone levels at the time of diagnosis of prostate diseases when samples are readily obtainable (Nomura et al., 1991) or in cross-sectional or correlational studies relating prostate size to ambient circulating hormone levels (Meikle et al., 1997). Even prospective analyses of stored sera have been unable to find any consistent relationship between blood hormone levels as predictors of the development of prostate cancer over the subsequent decade (Nomura et al., 1988; Gann et al., 1996; Guess et al., 1997). More limited studies have examined whether intraprostatic tissue concentrations of hormones are related to the development of prostate diseases. With the most careful methods, there also appears to be no direct relationship between blood hormone levels at the time of diagnosis with the onset of prostate disease. These negative findings are, however, still consistent with the androgen dependence of the prostate and not surprising if early life androgen exposures - such as fetal and perinatal influences and/or prolonged decades-long exposure to eugonadal male androgens - are the decisive influences determining the development of late-life prostate diseases. Consistent with this reasoning, the only study of the prostate in androgen abusers (Jin et al., 1996) showed that even prolonged exposure to grossly excessive androgen doses does not increase total prostate size or blood PSA concentrations whereas the central zone of the prostate was moderately increased (~30%) in size but without overtly symptomatic clinical consequences. These changes occur in the region of the prostate with highest hormonal sensitivity (Habenicht et al., 1988) and where BPH originates (McNeal, 1978). Whether they are reversible with cessation of androgen abuse or have any relationship to BPH remains unknown.

Overview

Prostate diseases in later life cause considerable morbidity and mortality among men who live out their full life expectancy. Currently no prevention strategies or modifiable precipitating factors have been clearly identified. In addition to aging and genetics, prolonged exposure to endogenous androgens at eugonadal levels appears to predispose to prostate disorders. It is neither clear nor inevitable that exogenous androgen therapy in otherwise eugonadal men would increase these risks. Indeed based on the analogy with breast disorders, either neutral or preventive effects are equally credible especially for benign prostate disease. The strong age-dependence of prostate disorders such that they have very high prevalence only at advanced age means that studies with adequate placebo controls and long-term surveillance is required to evaluate the occurrence of these effects. In the meantime the applications of androgen therapy in otherwise healthy men should not be precluded by unbalanced concerns about potential detrimental effects which may be less likely than beneficial effects.

ANDROGENS AND CARDIOVASCULAR DISEASE

The other major safety concern about androgen therapy is the possibility that cardiovascular disease might be increased in incidence or severity. Even small increases in mortality from the most common cause of male death could negate any morbidity benefits of androgen therapy. Hence the effects of androgen therapy on vascular disease assume great significance in considering its wider application in the general community.

The Gender Disparity in Cardiovascular Disease and its Mechanisms

The main reason for suspecting that androgen therapy may initiate or aggravate cardiovascular disease is the higher age-specific prevalence and incidence of atherosclerotic cardiac and other vascular disease among men compared with women (Godsland et al., 1987). This gender disparity appears consistent among different populations although its magnitude varies. Such disparity may reflect gender differences due to genetic, hormonal, lifestyle or interactive factors in rates of aging. Hormonal differences have been the overwhelming focus of research compared with other possible mechanisms such as genetic and/or lifestyle differences. The main hormonal explanations have been global hypotheses that estrogens are protective and/or androgens deleterious for cardiovascular disease in both men and women.

Direct evidence to test the hypothesis that masculine testosterone levels increase cardiovascular disease can be obtained from considering the life expectancy and/or mortality experience of (a) men after castration or (b) women treated with male doses of androgens. Data regarding the former are available from studies of castrated men which show no alteration of life expectancy. The best controlled study shows that, with careful age-matching, orchidectomy even before puberty (to preserve singing voice) does not alter life-expectancy (Nieschlag et al., 1993). An older case-control study of mentally retarded dwellers in an institution has purported to show that castration prolonged life expectancy although in that study the apparent excess mortality among the non-castrated men was not due to cardiovascular causes (Hamilton et al., 1969). In the Hamilton study, however, the selective use of post-pubertal castration to pacify behaviorally difficult inmates introduced a major confounding bias since life expectancy among such inmates is best predicted by independent mobility (Eyman et al., 1990). Hence the more mobile patients with longer life expectancy would have been more likely to undergo castration. This bias explains the unusually short life expectancy observed in the intact men whereas the castrate had normal rather than prolonged life expectancy. Data concerning the second test of the hormonal hypothesis are provided by the mortality experience of female-to-male transsexuals. One follow-up study of genetic females taking testosterone to replicate androgen exposure of eugonadal men reported no excess cardiovascular disease or mortality among 293 female-to-male transsexuals during 2418 patient-years of exposure to masculine levels of testosterone (van Kesteren et al., 1997). The same study also observed 816 male-to-

female transsexuals for 7734 patient-years during treatment with pharmacological estrogen doses without any modification in cardiovascular disease. Although the latter study has limitations in the relatively young age and short surveillance period, it is unlikely that major cross-sex steroid effects on cardiovascular disease were overlooked. Further evidence for or against the role of androgens in the gender disparity in cardiovascular disease would be provided by the life expectancy and cardiovascular consequences of complete androgen resistance. Such patients with male genetic and gonadal sex but otherwise normal female phenotype due to a mutated, non-functional androgen receptor (Quigley et al., 1995) would be informative if mortality or cardiovascular disease followed male or female patterns. Data on the prevalence of cardiovascular disease or life expectancy in patients with complete androgen resistance, however, appears to be currently lacking.

The hypothesis that estrogen protects against cardiovascular disease may similarly be tested by whether (a) there is an acceleration of age-specific female cardiovascular disease at menopause and (b) estrogen therapy reduces cardiovascular disease after menopause. Empirical evidence suggests the former implication is incorrect as the age-specific incidence of cardiovascular disease decelerates rather than accelerates at the time of menopause (Heller et al., 1978; Godsland et al., 1987). Evidence on the latter criterion however does support the hypothesis as estrogen therapy after menopause consistently reduces cardiovascular disease although not to premenopausal levels (Stampfer et al., 1991). By contrast, in men high dose oral estrogen (DES) causes excess cardiovascular (and cerebrovascular) arterial thrombotic events (VACURG, 1967). Whether or not lower dose oral DES or parenteral estradiol are free of the adverse cardiovascular effects of high dose oral DES, there is no evidence for any protective effects of estrogen therapy in men. Taken in concert, these observations suggest the cardiovascular preventive effects of estrogens are genuine but confined to estrogen-deficient women and even then substantially overshadowed by the effects of age itself.

The gender disparity also influences other forms of vascular disease such as peripheral vascular and cerebrovascular disease which have a fundamentally similar pathogenesis as for cardiovascular disease. Epidemiological studies have shown that low testosterone concentrations are associated with acute ischemic stroke (Jeppesen et al., 1996). Similarly androgens have neither beneficial nor deleterious effects on peripheral vascular disease (Liu et al., 1998). Whether androgen therapy would have beneficial effects or not on non-cardiac vascular disease remains to be established by prospective surveillance studies of adequate power and duration.

Hormonal Determinants of Cardiovascular Disease in Men

Whatever hormonal or other mechanism creates the gender disparity in cardiovascular disease, the hormonal influences on cardiovascular disease within genders are dramatically different from those between genders. Gender-restricted findings in coronary artery flow physiology have been reported (Collins et al., 1995). This is hardly surprising for gender-specific exposure to sex hormones is radically different quantitatively and qualitatively. Men have 20-50 fold higher androgen exposure and only 5-20% concentrations of estradiol compared with

younger women. Despite estradiol being cardioprotective in women, it may not have similar effects in men either due to inefficacy or, even if effective, being present at insufficient levels. Conversely even if life-long androgen exposure to levels 20-50 fold higher in men does cause the male excess of cardiovascular disease, variations in androgen levels between men may still not be predictive of cardiovascular disease. Indeed, as reviewed in detail elsewhere (Alexandersen et al., 1996; Barrett-Connor, 1996), the epidemiological findings within men are quite consistent that low blood testosterone is consistently associated with a higher risk of cardiovascular events in both cross-sectional (Alexandersen et al., 1996; Barrett-Connor, 1996) as well as longitudinal (Zmuda et al., 1997) studies. At face value these consistent findings predict that androgen therapy would have a protective or neutral effect on cardiovascular disease in men. Nevertheless empirical evaluation by surveillance in the course of longer-term studies remains of paramount importance to evaluate these predictions.

The Risks of Risk Factor Epidemiology

The pathogenesis of any chronic disease involves complex, protracted and sequential series of pathophysiological steps the nature of which along with their interrelationships may remain incompletely understood. Logically, any intermediary step, viewed in artificial isolation, may be considered a risk factor for the chronic disease. This artifice is valuable in creating reliable surrogate variables that telescope the very delayed, albeit genuine, biological end-points into a more convenient time-frame. Due to complex interdependencies, not every intermediary variable has the necessary unambiguous and predictable relationship with the ultimate biological end-point to be a suitable predictor of outcome despite their undoubted involvement in the pathogenesis of disease. For example, while bone density is an accepted surrogate marker for osteoporotic fractures, biochemical markers of bone turnover are not. Similarly for cardiovascular disease, plasma total cholesterol has been proven as a valid surrogate marker for cardiovascular disease events since several of the statin class of cholesterol lowering agent have reduced cardiovascular disease end-points in large prospective, placebo-controlled trials. In this context, androgens either lower or leave unchanged plasma total cholesterol (Alexandersen et al., 1996; Barrett-Connor, 1996) and hence androgen therapy might be expected to be protective or neutral for cardiovascular events to the extent that pharmacological effects of androgens replicate the effects of HMG-CoA reductase inhibitors.

Numerous other intermediary variables arising from mechanistic or epidemiological findings and which, considered in artificial isolation, might be construed as risk factors, have yet to be accepted as valid independent surrogate variables for cardiovascular disease. These include other lipid fractions (HDL or LDL cholesterol, triglycerides, apolipoproteins), vasoactive factors (endothelin, nitric oxide, ANP, prostaglandins), blood vessels (flow, endothelial cell & smooth muscle function), clotting/fibrinolytic factors (thrombosis, platelet function, fibrinolysis, hyperviscosity), metabolic (insulin resistance, body fat and its distribution, sodium & fluid retention) and lifestyle (smoking, occupational) factors. Although each of these variables plays a role in the pathogenesis of cardiovascular

disease, for most the effects of androgens are poorly or not understood. Nor would it be feasible to integrate such fragmentary information meaningfully without integrative studies of genuine clinical endpoints. For example, androgens influence both thrombotic (Ajayi et al., 1995) and thrombolytic (Winkler, 1996) mechanisms so the net effects from conflicting mechanistic studies are difficult to evaluate. In this situation, despite conflicting evidence from mechanistic studies, there is no evidence for any association between androgens and thromboembolism unlike estrogens. Ultimately the significance of such intermediary variables can only be properly evaluated with long-term prospective surveillance utilising genuine clinical endpoints to provide decisive data.

Among this plenitude of potential risk factors, observational and clinical studies aiming to infer the effects of androgens on cardiovascular disease have focussed almost exclusively on lipids. Such studies are easy to conduct but hard to interpret. Androgen effects on lipids are related to dose, type of androgen (Friedl et al., 1990) and route of administration (Thompson et al., 1989). The observed effects are most often no effect or lowering of total, LDL and/or HDL cholesterol (Alexandersen et al., 1996; Barrett-Connor, 1996). Most studies have utilised either oral 17-α alkylated androgens which, being hepatotoxic are considered clinically obsolete, or injectable testosterone esters in oil vehicle in which the pharmacokinetic limitations create unphysiological peaks and troughs in blood testosterone (Behre et al., 1990) which in turn create artefactual pharmacological effects not observed with more stable, depot T formulations. Using blood SHBG levels as a sensitive marker of hepatic androgen overdosage (Conway et al., 1988), when physiological testosterone replacement levels are maintained consistently as indicated by stable blood SHBG concentrations, lipid changes are usually absent. The significance of lowered HDL cholesterol concentrations by supraphysiological doses and/or unphysiological testosterone concentrations (Bagatell et al., 1995) when total cholesterol is concurrently lowered remains unclear. In summary, the significance of pharmacological androgen therapy on cardiovascular risk factors are probably uninterpretable in isolation although the most persuasive evidence concerning the only well established surrogate variable, total cholesterol, suggests that androgen therapy may have no adverse effect or even reduce progression of coronary heart disease.

Effects of Androgens in Controlled Studies

A limited number of prospective placebo-controlled intervention studies have examined the effects of androgens on cardiovascular disease using objective clinical end-points. The first randomised 50 men with symptomatic and cardiographic evidence of cardiac ischemia to receive weekly im injections of either testosterone cypionate (200mg) or oil vehicle for 8 weeks (Jaffe, 1977). Testosterone significantly reduced (32-51%) postexercise ST segment depression compared with placebo (<2%). These findings were confirmed by another placebo-controlled, cross-over study of 62 elderly men with established ischemic heart disease who were randomised to commence treatment with either testosterone undecanoate (120mg per day for 2 weeks followed by a maintenance dose of 40mg per day for another 2 weeks) or placebo and then crossed-over to the other treatment after a 2

week washout period (Wu et al., 1993). Testosterone ingestion produced marked improvement in cardiac ischemia in subjective anginal symptoms and objective cardiographic criteria (ECG, Holter monitor) but no change in cardiac function (echocardiography). Suboptimal design of the latter study (Wu et al., 1992), however, undermines its confirmatory value. At face value these findings suggest that testosterone may cause coronary vasodilatation in man. This would be consistent with experimental findings that testosterone facilitates acute coronary vascular dilatation (Adams et al., 1994; Yue et al., 1995; Chou et al., 1996; Costarella et al., 1996) as well as increasing coronary vessel diameter in androgen-treated female non-human primates (Adams et al., 1994; Obasanjo et al., 1996) consistent with increased chronic coronary flow. Two older studies which included placebo control treatment also demonstrated apparent benefits of testosterone propionate injections on symptomatic angina (Sigler et al., 1943; Lesser, 1946) although the ad hoc use of placebo controls in these studies limited their persuasiveness.

Overview

From the global hormonal hypotheses that estrogens are beneficial and/or androgens harmful regardless of gender, more recent evidence has allowed a refinement of these hypotheses (Barrett-Connor, 1996). These may be reformulated into the unified hypothesis that sex hormone deficiency is harmful and that sex steroid replacement is beneficial but the benefits of hormone replacement are gender-appropriate rather than unselective. That is, androgen replacement for men and estrogen replacement for women should provide optimal cardiovascular health and disease prevention. These considerations support the safety of further placebo-controlled studies of androgen therapy in aging as well as in other applications of androgen therapy. Given the small effect sizes that are most likely and relevant, thorough quantitative evaluation of the effects of androgen therapy on cardiovascular disease will require large-scale, placebo-controlled studies and prolonged surveillance to have sufficient power to estimate the small risks and benefits.

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10 ANDROGENS AND BEHAVIOR IN MEN

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INTRODUCTION

In most mammalian species, androgens play an important role in the development and maintenance of sex-typed behavior. Sex steroids acting during fetal life are critical for sexual differentiation of the brain and development of sex-appropriate internal and external genitalia (Wilson et al, 1981). Exposure to androgens secreted by the testes during critical periods of fetal development is necessary for the masculinization and defeminization of brain areas (Arnold and Gorski, 1984), most notably in the preoptic area and ventromedial nucleus of the hypothalamus (Goy and McEwen, 1980; MacLusky and Naftolin, 1981). One area of hormone-behavior research is focused on this "hardwiring" of the brain substrates of sexually dimorphic behavior, termed "organizational effects". A second area of hormone-behavior research is focused on sexually dimorphic behavior patterns in postnatal development, which usually depend to some degree on circulating levels of gonadal steroids acting on mediating brain areas and possibly also peripheral effects of sex hormones, termed "activational effects" (Young et al, 1964).

In mammals, including nonhuman primates, most sexually dimorphic behavior patterns are a function of both organizational and activational effects of hormones (Feder, 1984). However there are interspecies differences in the extent to which gonadal hormones are critical for behavior and the degree to which these mechanisms influence human behavior has yet to be fully elucidated. Studies of the organizational effects of androgens on human behavior typically depend on clinical populations, where prenatal androgen levels are inconsistent with the genotype of the fetus (e.g., congenital adrenal hyperplasia, androgen insensitivity syndrome) (for review see Collaer and Hines, 1995). Not all sex-typed behaviors organized by androgens in early development depend on steroid levels in later life (e.g., rough and tumble play) (for reviews see Beatty, 1984; Meaney, 1989). Therefore, evidence of organizational effects of androgens on behavior does not directly address the existence of any activational effects of androgens on men's behavior. Activational effects of androgens on human behavior are typically determined by measuring behavior following androgen administration or by measuring the relationship between current behaviors and current androgen levels in non-clinical

populations, where sex-appropriate prenatal androgen levels are assumed. The evidence supporting a role for current levels of androgens on sexual behavior, mood, and cognitive abilities in men is summarized below.

SEXUAL BEHAVIOR

It is very likely that the vast proportion of the variance in human sexual behavior can be attributed to social factors. The methods of attracting a sexual partner and the stimuli that are capable of sexually arousing individuals are known to be culturally dependent (Ford and Beach, 1951). Cultural and social influences also affect the types and frequencies of both sexual activity and sexual dysfunction (Kinsey et al, 1953). Moreover, for as long as castration has been reported to reduce or abolish male sexual behavior, there have been paradoxical observations that some castrated men remain sexually active (Kinsey et al, 1953; Tauber, 1940). Given the apparent overwhelming influence of social learning and the lack of empirical data supporting biological determinants of human sexual behavior, it is not surprising that past researchers suggested that androgens were not necessary for any component of male sexuality (Kinsey et al, 1953). Subsequent well-controlled investigations have shown this conclusion to be premature.

Androgen deficiency and sexual behavior

Although testosterone replacement therapy for testicular failure in men was a common medical practice, it was not until 1979 that the results of the first double blind, placebo-controlled investigation of the effects of exogenous testosterone on the sexual behavior of hypogonadal men were reported (Davidson et al, 1979). The consistent replication of the results of that study in subsequent research of similar design has provided unequivocal confirmation of the hypothesis that androgens modulate important aspects of sexual behavior in men. This research demonstrates that hypogonadal men administered testosterone report increases in a wide range of sexual behaviors, including frequencies of sexual activities, sexual daydreams and sexual thoughts, and feelings of sexual desire (Bancroft and Wu, 1983; Davidson et al, 1979; Skakkeback et al, 1981). Although androgen replacement therapy enhances sexual activity, changes in sexual desire show better concordance with rising and falling plasma levels of testosterone than do changes in sexual activity (Skakkeback et al, 1981). This research indicates that the primary effects of androgens are on sexual interest or sexual motivation.

Testosterone replacement therapy also increases spontaneous erections, episodes of nocturnal penile tumescence (NPT) (Kwan et al, 1983) and both the duration and the maximum level of penile rigidity associated with erectile response to erotic visual stimuli (Carani et al, 1995). However, other aspects of erectile response to erotic visual stimuli (e.g., maximal increase in penile circumference) are not influenced by androgen deficiency or by androgen replacement - which may explain how coitus occurs in hypogonadal states (Bancroft and Wu, 1983; Kwan et al, 1983). In other words, androgens clearly enhance men's desire to engage in sexual

activities but appear to have less influence on men's ability to engage in sexual activities.

Studies of androgen replacement therapy suggest that there is a dose-response relationship between sexual motivation, defined as level of sexual interest or sexual arousal to sexual thoughts, and dosage of exogenous testosterone (Davidson et al, 1979; Salmimies et al, 1982; O'Carroll et al, 1985). However, a dose-response relationship between androgens and sexual behavior is not found in hypogonadal men administered testosterone replacement therapy when resulting plasma levels are within the normal range of testosterone values (Gooren, 1987). The general interpretation of these and other similar data is that restoration of sexual motivation in hypogonadal men is achieved at a low threshold of serum androgen (Salehian et al, 1995)

Androgen-sexual behavior relations in eugonadal men

A testosterone-sexual behavior relationship in men with normal testicular function is more equivocal. No relations between individual differences in serum testosterone levels and self-reports of sexual behavior in healthy men have been reported (Brown et al, 1978; Kraemer et al, 1973; Raboch and Starka, 1973). When serum testosterone levels were pharmacologically varied in men, sexual behavior measures such as subjective sexual interest and physiological measures of NPT were similar in groups of men with very high normal levels or very low normal levels of testosterone (Buena et al, 1993). Similarly, daily records of sexual activity were unchanged in studies of eugonadal men administered weekly injections of pharmacologic doses of testosterone as a contraceptive (Anderson et al, 1992; Bagatell et al, 1994). This research is consistent the results from studies of androgen deficiency suggesting that, once the threshold level of testosterone required for normal sexual behavior is achieved, higher levels of testosterone have no further enhancing effect on sexual behavior (Bancroft, 1988).

Other research suggests that increasing levels of circulating testosterone within and beyond the normal range of values for men may alter some aspects of sexual behavior. Compared to placebo, exogenous testosterone enhanced frequencies of sexual thoughts in eugonadal men complaining of low sexual desire but not in men with erectile dysfunction (O'Carroll and Bancroft, 1984). An effect of testosterone on subjective sexual response was also observed in an investigation of testosterone administered in a contraceptive trial (Anderson et al, 1992). In that study, men received 200 mg of testosterone enanthate (TE) intramuscularly for 8 weeks or received placebo for 4 weeks followed by hormone treatment for 4 weeks. In addition to daily diaries of behavior, men in both groups completed a questionnaire measure of sexual arousability to stimuli, such as erotic literature, prior to treatment and at post-treatment (4 weeks and 8 weeks). Interestingly, exogenous testosterone enhanced men's perception of sexual arousability in that study, but this increased responsiveness to nonphysical (e.g., visual or imaginal) sexual stimuli was not associated with higher frequencies of sexual behavior or greater sexual interest, as documented by the daily records. An enhancing effect of exogenous testosterone on sexual arousability is consistent with recent correlational research showing that eugonadal men administered 200 mg of TE for 6 weeks or more reported increased

sexual arousal and sexual enjoyment to a sexually explicit audiotape (Alexander et al, 1997). Moreover, eugonadal men in that study were more distracted by sexual stimuli presented in a dichotic listening task, suggesting that men with supraphysiological levels of testosterone may be more aware of sexual stimuli in their environment.

Conclusions

Descriptive and well-controlled studies of androgens and human sexuality have provided an increasingly clear understanding of the aspects of sexual behavior that are influenced by androgens. In hypogonadal men, androgens appear to have their primary effects on sexual motivational variables, such as sexual interest or sexual desire. Sexual motivation decreases in men with androgen deficiency and is increased following androgen replacement. However, increasing levels of androgens beyond a low-normal amount have no effect on most aspects of psychological or physiological sexual behavior. An exception to this general finding is the apparent linear relationship between sexual arousal to erotic stimuli and androgen levels in men. A linear relationship between sexual arousal and androgen levels is noteworthy given other evidence that measures of physiological sexual arousal (penile rigidity and duration of maximum erectile response) are also androgen dependent.

MOOD

In humans, androgen levels in perinatal development are associated with aggressive behavior in adulthood (Collaer and Hines, 1996). Evidence that androgen levels in later development influence aggressive behavior in human and nonhuman primates is more equivocal (for reviews see Archer, 1991; Dabbs, 1996). In fact, recent research suggests that androgens may actually enhance men's mood and general well-being

Androgen deficiency and mood

Testosterone replacement therapy generally improves mood in hypogonadal men (Burris et al, 1992; O'Carroll et al, 1985; Skakkeback et al, 1981; Stuenkel et al, 1991; Wang et al, 1996). Daily records of behavior and retrospective reports of mood using measures with good reliability and validity show androgens increase positive mood states (e.g., vigor) and decrease negative mood states (e.g., aggression or irritability). However, treatment conditions resulting in very different therapeutic levels of testosterone produce no differential effects on men's ratings of a variety of mood states (Alexander et al, 1997; Wang et al, 1996; Buena et al, 1993). Again, once serum levels of testosterone are within the normal range, higher serum testosterone levels appear to have no measurable effect on men's behavior.

Not all researchers have replicated the mood enhancing effects of exogenous testosterone in some groups of hypogonadal men (Davidson et al, 1979; Salmimies

et al, 1982; Morales et al, 1994). Compared to hypogonadal men not showing improved mood following testosterone replacement, hypogonadal men showing improved mood may have more profound deficiencies in testosterone and report relatively less positive and more negative mood states (Alexander et al, 1997). These data suggest that the threshold of serum testosterone required for restoration of positive mood and well-being is minimal - and may even be lower than that required for normal sexual function.

We recently showed that peripheral administration of testosterone produces a conditioned place preference (CPP) in male rats, indicating that testosterone has rewarding affective properties (Alexander et al, 1994; DeBeun et al, 1992; Packard et al, 1997) that may be relevant to understandings of the mood enhancing effects of testosterone. The nucleus accumbens is known to mediate the rewarding properties of drugs, such as amphetamine (Reicher and Holman, 1977; White et al, 1987), and natural rewards, such as food (Papp, 1989). Consistent with the hypothesis that testosterone may enhance sexual motivation by interacting with brain reward systems (for review, see Everitt, 1990), intra-accumbens injections of testosterone produce a CPP in male rats (Packard et al, 1997) that is mediated, in part, by the mesolimbic dopamine system (Packard et al, 1998).

A relationship between testosterone and brain structures implicated in sexual motivation and reward is noteworthy given the suggestion that, in males, androgens may increase sexual motivation secondary to an enhancement of the rewarding quality or pleasure inherent in sexual interactions (Davidson et al, 1982; Michael and Wilson, 1974). Although hypogonadal men's ratings of sexual satisfaction appear not be androgen dependent (O'Carroll et al, 1985), the magnitude of their improvement in sexual motivation is correlated with the decrease in negative mood (anger, nervousness, and irritability) and the increase in positive mood (i.e., friendliness, energy, alertness) (Wang et al, 1996). These findings suggest that testosterone, sexual motivation, and mood relations in hypogonadal men warrant further examination.

Androgen-mood relations in eugonadal men

In contrast to studies of testosterone replacement and mood, in eugonadal men administered testosterone, the effects of androgens on mood states have been generally absent. Studies of androgen and mood in eugonadal men typically have measured the mood effects of 200 mg of TE administered weekly (Anderson et al, 1992; Alexander et al, 1997; Bagatell et al, 1994; Tricher et al, 1996) or biweekly (Schiavi et al, 1997) for a duration of 6 to 8 weeks. Measures of mood have included general mood inventories, daily ratings, and, less frequently, questionnaires designed to measure multiple dimensions of anger or aggression. In all, the general finding is that high-dose administration of androgens does not influence men's mood states, at least in the short term. This finding is consistent with the effects of a replacement dose of exogenous testosterone on mood states of older men (Janowsky et al, 1994). In that study, men's ratings and, importantly, spousal ratings of men's positive and negative moods were not influenced by testosterone administration. In contrast to these data, a high-dose (250 mg) of methyltestosterone administered to eugonadal men for three days resulted in

increased irritability, hostility and mood swings (Su et al, 1993). Clearly, the possibility that increasing testosterone levels in men may increase negative mood states merits continued assessment in studies of testosterone administration because of the potentially maladaptive consequences of these effects.

Conclusions

Androgen deficiency appears to have a negative effect on mood that is reversed by androgen replacement. Although there is a justifiable concern that exogenous androgens may enhance aggressive behavior in hypogonadal men and eugonadal men, most existing data are not consistent with that possibility. However, it may be premature to conclude that exogenous androgens do not enhance negative mood states because all dimensions of anger or aggression (Caprara and Pastorelli, 1989) have not been adequately measured in most research of testosterone and mood. The recent findings that androgens act on brain areas mediating reward is consistent with the general finding that mood is enhanced in hypogonadal men following testosterone administration. Significantly, the animal data are also consistent with the proposal that androgens may have addictive properties and possible abuse potential in humans (Kashkin and Kleber, 1989).

COGNITIVE BEHAVIOR

The role of androgens in cognitive behavior is a relatively recent area of hormone-behavior research. A growing body of research supports the hypothesis that perinatal levels of androgens influence sex-typed cognitive abilities. In rodents, estrogens aromatized from androgens in the brain organize male patterns of spatial function (Williams and Meck, 1993; Roof and Ravens, 1992) and the development of neural substrates of spatial ability, such as the hippocampus (Roof and Ravens, 1992). In infant rhesus monkeys, perinatal androgen levels organize transient sex differences in cognitive abilities (i.e., object reversal, concurrent discrimination) by influencing the development of cortical areas (Clark and Goldman-Rakic, 1989). In humans, there are consistent sex differences in some cognitive abilities. Men generally outperform women on visuospatial tasks and women generally outperform men on verbal fluency tasks, measures of perceptual speed (Halpern, 1992; Linn and Petersen, 1985; Maccoby and Jacklin, 1974; Ekstrom et al, 1976) and spatial memory, defined as memory for object locations (Silverman and Eals, 1994). Studies of individuals with endocrine disorders (for review see Collaer and Hines, 1995) support the hypothesis that human sex differences in cognitive abilities may result in part from sex differences in gonadal steroid levels during critical periods of human perinatal development.

The hypothesis that androgen levels in adulthood may also contribute to intra-individual and inter-individual differences in cognitive abilities is based, in part, on the evidence that other sex-typed behaviors, such as sexual behavior, depend on current levels of sex steroids. Based on this model of sexual differentiation of behavior, it is hypothesized that sex-typed cognitive abilities that favor males (i.e.,

visuospatial ability) are dependent on male-typical androgen levels during perinatal and, possibly, during postnatal development. In addition, the general finding that androgen deficiency in men is associated with decreased frequencies of sexual fantasies and sexual thoughts that are increased following androgen replacement has suggested that androgens may facilitate cognitive processes underlying sexual fantasies, namely visuospatial ability (Bancroft, 1980). For these different reasons, most research of androgen-cognitive ability relations has focused on the role of androgens in visuospatial ability.

Androgen deficiency and cognitive behavior

Evidence that androgen deficiency influences visuospatial ability in men is equivocal. For example, androgen replacement therapy did not reverse visuospatial ability deficits in men with idiopathic hypogonadotropic hypogonadism (Hier and Crowley, 1982) or enhance visuospatial ability in hypogonadal men without visuospatial deficits (O'Carroll, 1984). These data are consistent with recent findings of no association between endogenous or exogenous androgen levels and visuospatial ability in hypogonadal men (Alexander et al, 1998). However, other research suggests that androgen deficiency may produce visuospatial ability deficits in some groups of men. In older men with low serum levels of androgens, exogenous testosterone enhanced their performance of a block design task (Janowsky et al, 1994). Sex reassignment and associated treatment with anti-androgen and estrogen depressed visuospatial abilities in men (Van Goozen et al, 1994). Research of potential factors underlying visuospatial abilities (e.g., attention or motivation) and their relationship to testosterone levels may clarify these apparent contradictory results.

It is also possible that cognitive abilities other than visuospatial ability may be influenced by androgen deficiency in adulthood. We recently found that deficits in verbal ability (a composite score based on three measures of verbal fluency) were reduced in hypogonadal men following androgen replacement (Alexander et al, 1998). These data suggest that verbal fluency may covary with serum levels of testosterone in hypogonadal men. Brain imaging studies indicate that verbal fluency is associated with activation of the frontal and temporal lobes (Parks et al, 1988). An effect of exogenous androgen on cortical areas involved in verbal fluency is consistent with the identification of androgen receptors in the human temporal cortex (Puy et al, 1995; Sarrieau et al, 1990)

Androgen and cognitive behavior in eugonadal men

It is variously hypothesized that androgen levels in adulthood may influence cognitive ability in men through steroid dependent effects on the general activation of the central nervous system (Broverman et al, 1968), on right-hemisphere functioning and/or left-hemisphere functioning (Gouchie and Kimura, 1991; and see, Mead and Hampson, 1996), or on sexually dimorphic nuclei in the brain (Hampson, 1990). Consistent with the existence of activational effects of androgens on cognitive behavior, higher testosterone levels were associated with better performance on tasks measuring visuospatial ability in groups of young European

men (Christiansen and Knussman, 1987) and African Bushmen (Christiansen, 1993). However, other researchers have reported that testosterone levels in adulthood are unrelated to visuospatial ability (Kampen and Sherwin, 1996; McKeever et al, 1987; Alexander et al, 1998) or are negatively associated with men's performance on some visuospatial tasks (Gouchie and Kimura, 1991; Kimura and Hampson, 1994; Moffat and Hampson, 1996). The nature of the association between testosterone levels and visuospatial ability in men appears unrelated to visuospatial measures used in the research or the sample characteristics, although some researchers suggest that testosterone and visuospatial ability relations are influenced by time of day or handedness (Moffat and Hampson, 1996). Alternatively, correlations between testosterone and visuospatial ability may not indicate any causal relationship between the two variables. Correlations between testosterone and visuospatial ability may indicate that other factors, such as perinatal hormone levels, equally influence both testosterone levels and visuospatial ability in adulthood (Christiansen and Knussman. 1987). Variable associations between testosterone and visuospatial ability may occur because of variables that alter endogenous testosterone levels (e.g., disease, age) or variables that alter visuospatial abilities (e.g., training).

To date, there are only a few studies measuring the effects of exogenous androgens on cognitive behavior in eugonadal men. In a double-blind trial comparing high-dosage testosterone injections with placebo, eugonadal men complaining of low sexual interest or erectile dysfunction showed no enhancement of spatial ability following 6 weeks of hormone administration (O'Carroll, 1984). In a recent study of healthy, eugonadal men administered exogenous testosterone in a contraceptive trial, we found no relationship between androgens and sex-typed cognitive abilities, including visuospatial ability, verbal ability, and perceptual speed (Alexander et al, 1998). However, both studies included only a small number of men and a small number of cognitive tests. Further research is clearly required before rejecting the hypothesis that androgens activate some aspects of cognitive behavior in men.

Conclusions

Evidence from correlational studies suggesting that androgen levels in adulthood enhance cognitive function is equivocal. Exogenous testosterone administered to hypogonadal and eugonadal men appears to have little influence on a variety of sex-typed cognitive abilities, including visuospatial ability. Whether there are underlying processes (e.g., attention, motivation) that may be androgen dependent or whether long-term administration of androgens is required to influence cognitive abilities are empirical questions that may be useful to address in future research.

CURRENT ISSUES

Adrenal androgens and behavior?

The adrenal androgen dehydroepiandrosterone (DHEA) and its sulfate (DHEA-S) are the most plentiful circulating steroid hormones in the human body (Baulieu, 1995). Recently, researchers have argued that serum levels of testosterone may not be sensitive measures of the level of biologically active androgen because peripheral tissues are capable of converting inactive precursor steroids, such as DHEA, into active androgens without changing extracellular levels of androgens (LaBrie, 1991; LaBrie et al, 1997). This possibility suggests that serum levels of DHEA may influence androgen-dependent human behavior.

Lower levels of DHEA are associated with both the incidence and the risk of physical illnesses, suggesting a protective effect of DHEA against age related disease (for review, see Bellino et al, 1995). At birth, levels of DHEA and DHEA-S exceed those in adulthood but fall rapidly until adrenarche (DePeretti and Forest, 1976). Following adrenarche, DHEA levels increase markedly until the third decade of life, and then decrease with age to very low amounts (e.g., Orentreich et al, 1984). Evidence that age related changes in DHEA levels may influence the development of human behavior includes an association between increased DHEA production at adrenarche (i.e., prior to puberty) and the emergence of sexual attraction in sexually immature boys and girls (McClintock and Herdt, 1996). Similarly, serum androgen levels, sexual activity and libido decrease with age in elderly men (Davidson et al, 1983; Tsitouras et al, 1987; Korenman et al, 1990) coincident with the known decrease in adrenal androgen production. Given evidence that decreasing DHEA levels are associated with physical decline, one hypothesis is that age dependent decreases in psychological variables such as visuospatial ability, memory (Poon, 1985) and sexual functioning (Kinsey et al, 1945) may also reflect, in part, decreasing adrenal production of DHEA. Correlations between DHEA and well-being (Cawood and Bancroft, 1996) and cognitive function (Berkman et al, 1993; but see Barrett-Conner and Edelstein, 1994) are consistent with this possibility. The suppressing effects of physical or mental illness on DHEA may attenuate correlations between adrenal androgens and some behaviors, contributing to some of the inconsistent research findings. In addition, although suggestive, correlation data cannot address the possible benefits of DHEA replacement.

In laboratory animals, DHEA administration has been associated with memory enhancement (Flood et al, 1992) and memory impairment (Fleshner et al, 1997), effects that may depend on dose, steroid administration regimen, and task requirements. Ongoing research in our laboratory is consistent with the memory enhancing effects of DHEA on memory for spatial locations in male rats (Jensen et al, unpublished data). However, animal research of the behavioral effects of DHEA may be of limited applicability to understanding relations between age related declines in DHEA and behavior, given that DHEA/DS is not secreted by the adrenals of small laboratory animals (Baulieu, 1995).

Direct evidence of a possible role of DHEA in psychological health is provided by the results of a placebo-controlled study of DHEA replacement to aging women and men (Morales et al, 1994). In that study, a majority of men administered DHEA for 6 weeks reported increased well-being, as defined by improved sleep, feelings of relaxation, increased energy, and increased ability to handle stress. DHEA administration had no influence on men's retrospective assessment of sexual

behavior, a measure that may be less sensitive to behavioral changes than are prospective assessments of sexual behavior. In a more recent placebo-controlled investigation, a replacement dose of DHEA administered to older men daily for 2 weeks had no effects on cognitive measures of memory, attention and concentration. In addition, DHEA administration did not enhance retrospective ratings of mood (Wolf et al, 1997). However, like research of androgen-cognitive relations in men, the studies of DHEA and behavior included only a small number of men and a small number of behavioral measures. Therefore, it seems premature to conclude that DHEA administration will have no beneficial effects on men's sexual behavior, cognitive behavior, or mood.

Individual differences

Not all hypogonadal men report enhanced well-being following androgen replacement (Alexander et al, 1997). Some eugonadal men complain of increased irritability following administration of high-dose, exogenous testosterone (Bagatell et al, 1994). Increased understanding of the factors associated with individual differences in response to exogenous and endogenous androgens may be useful in resolving the existing contradictions in the literature. An interesting example of individual differences in behavioral response to sex-steroid deficiency is provided by a study of the effects of ovarian suppression on cognitive function in women (Varney et al, 1993). In women, menstrual cycle studies suggest a relationship between changes in ovarian steroid secretion and performance on measures of sex-typed cognitive abilities (Hampson, 1990). Further, there is a growing body of literature suggesting that estrogens enhance memory (e.g., Packard, 1998). Nonetheless, acute ovarian suppression produced only modest effects on average measures of women's cognitive performance (Varney et al, 1993). However, for a small number of women in that study, the hypoestrogenic state was associated with marked disorientation, confusion, and cognitive impairment. The existence of such individual differences in behavioral responsiveness suggests a more idiographic perspective may be useful in future research of androgen-behavior relations.

SUMMARY

Androgens activate important aspects of sexual behavior and mood in men. Androgen deficiency in men is associated with decreased sexual motivation and decreased well-being. For both variables, it appears that only a minimal amount of androgen is required to maintain normal behavior function. This conclusion does not preclude an effect of higher levels of androgens on more circumscribed aspects of behavior. For example, sexual arousal to erotic stimuli and, perhaps, attention to sexual stimuli appear to increase as serum testosterone levels increase in men. Therefore, it seems very possible that some aspects of mood may show a similar relationship to serum androgen levels. In contrast to research of sexual behavior and mood, there are no clear data indicating androgens activate visuospatial ability in men. However, androgen, sexual behavior and mood relations have been

elucidated by descriptive and experimental research of both hypogonadal and eugonadal men, suggesting that more of these types of studies are required to better understand the role of androgens in men's cognitive behavior.

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11 ANDROGEN ABUSE IN SPORT: INTERNATIONAL AND NATIONAL ANTI-ANDROGEN PROGRAMS

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INTRODUCTION

The misuse of anabolic androgenic steroids for the purpose of enhancing performance continues to be a difficult problem for major sport organizations. After nearly fifteen years of education, research, and testing there is still ample evidence that the problem has not been effectively solved. The roots of the problem lie somewhere in the psychology, sociology, and economics of sport. Athletes are not drug users by nature and the point of the drugs is not to obtain a high, it is to enhance performance and win. Athletes are placed in an approach-avoidance conflict, the public expects them to win and rewards the winners, and they are expected to play fair. Many do play fair, but the ones that choose to cheat erode the fundamental value of sport.

Unfortunately, the anabolic androgenic steroids (AAS) do enhance performance in some sports. For those that do use drugs the disciplines of pharmacology and chemistry play key roles. Knowledge of dose, administration patterns, pharmacokinetics, and adverse effects are vitally important to the user. Some advisors are extremely knowledgeable on these matter and there is an abundant supply of books, articles and Internet advice on how to use AAS - without getting caught.

In recent years there has been a shift in usage patterns from exogenous or xenobiotic steroids to endogenous steroids (Catlin and Murray, 1996). While it is not difficult to detect endogenous steroids in urine, it is very difficult to prove that the source of the steroid is pharmaceutical rather than endogenous. Further, the shift places great emphasis on knowledge of endocrinology and particularly on steroid metabolic pathways and the hypothalamus-pituitary-gonadal axis. With the use of growth hormone already entrenched sport, and numerous synthetic releasing

factors on the market or in the pharmaceutical pipeline, the role of endocrinology and endocrinologists in doping control will continue to grow.

This review summarizes the major programs instituted by sport to deal with the issue of AAS abuse, the organization and characterization of testing activities, and the analytical approaches to detecting AAS in urine.

INTERNATIONAL ANTI-STEROID ACTIVITIES

The International Olympic Committee (IOC) promotes drug-free sport through the activities of its Medical Commission, particularly via the Subcommission on Doping and Biochemistry. It is the Subcommission on Doping that recommends additions or deletions to the list of prohibited substances. In addition the Subcommission elaborates analytical procedures, assists in the development of new IOC accredited laboratories, and conducts an annual reaccreditation of the laboratories (IOC Medical Code, 1995). During the Olympic Games the Subcommission supervises the laboratory and makes recommendations to the Medical Commission on the disposition of positive cases. The Medical Commission reports through it chairperson to the IOC executive board, who makes the final decision. The IOC sponsors numerous meetings throughout the year dealing with various aspects of doping, research, and coordination of policy among International Sports Federations. Harmonizing policies among the 34 International Federations, or IF, is key as these federations are responsible for the adjudication of cases in the Olympic context

Currently, the IOC has dedicated approximately $1,000,000 toward an extensive research program aimed at detecting the use of growth hormone. They have also sponsored research into the detection of erythropoietin. The IOC has sponsored some grants for research into steroid detection and at least one IF has funded research related to identifying steroids in urine and blood.

The linchpin of the IOC program is the laboratory accreditation system. To become accredited, a laboratory must demonstrate competence in chemistry and related disciplines, possess a wide variety of laboratory instruments, and have a substantial infrastructure of support from national sport authorities. Expertise in several types of chromatography and mass spectrometry is a prerequisite as all positive cases must be confirmed through mass spectrometry. Although the bulk of the work is still performed with bench-top quadruple gas chromatograph coupled with quadruple mass spectrometers (GC/MS), since 1997 the IOC has required the laboratories to obtain instruments with the capability of detecting 2 ng/mL of several steroids. About one third of the IOC laboratories are meeting this requirement with high resolution mass spectrometers (GC-HRMS), the others use MS/MS techniques or ion trap MS (Bowers, 1997).

One of the difficulties faced by the IOC laboratory system is uniformity in analytical criteria for positive cases and related laboratory activities. Currently, there are 26 IOC accredited laboratories in the world. While the majority are in Europe, the United States, and Scandinavia, the number of laboratories in Asia and the Pacific Rim is expanding, with Thailand likely to have an accredited facility by

the end of 1998. Unfortunately, Central and South America still do not have a laboratory. While it is heartening to see an expanding consciousness toward doping, the variety of national standards, funding, and philosophies result in differing laboratory capabilities and practices. In 1997, the IOC took an important step toward standardization by requiring all IOC laboratories to hold accreditation from the International Organization for Standardization (ISO) by January 2000. This move will substantially improve the laboratory system because it will harmonize all technical aspects of laboratory affairs.

Together the IOC laboratories tested approximately 96,500 urine samples for anabolic steroids in 1996 (Catlin and Murray, 1996). Ten years earlier the annual output was 33,000. From 1986 through 1991, the annual growth rate of urine sample tests was greater than 20%, while in the last five years it has been less than 5%. Given that doping is still a critical problem for sport, the plateau in testing numbers shown in figure 1 seems related to priorities of the organizations that

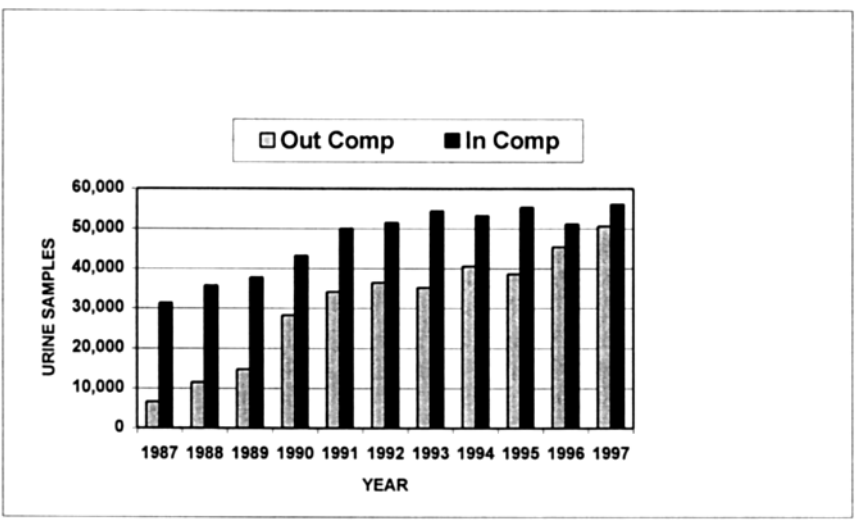

Figure 1. Sport samples tested by IOC-accredited laboratories. Samples collected at competition (In Comp) and out-of competition (Out Comp)

provide the funding. Ten years ago the majority of samples were collected at the time of competition. Now nearly 50% of samples are collected out-of -competition. The year-round testing strategy is the most effective means of deterring the use of AAS. The percent positive for AAS has stabilized at ~ 1.0%; however, there is so much variability in how these data are collected that it is difficult to draw meaningful conclusions. The IOC does not directly fund the testing programs.

Recently, the IOC has stepped up its legal support for athletes accused of taking steroids or other drugs. The vehicle for this activity is the Court of Arbitration for Sport (CAS). Athletes with positive urine tests may, after first appealing to their IF, appeal to CAS. A substantial portion of the funding for CAS infrastructure is borne by the IOC.

NATIONAL ANTI-STEROID ACTIVITIES

The United States Olympic Committee (USOC), National Collegiate Athletic Association (NCAA), the National Football League (NFL), as well as many colleges and universities operate anti-steroid programs. The educational programs include sponsoring meetings, posters, speakers bureaus, videotapes, and related activities. The NCAA program includes provisions for each campus to have drug education specifically targeted to student athletes. Similarly each team in the NFL and many sports in the Olympic family have steroid education programs.

Together the two U.S. laboratories process about 30,000 urine samples from these programs. About another 10,000 samples are estimated to be analyzed in commercial laboratories. The number of positive steroid cases in the USOC, NCAA, and NFL programs ranges from 1-3 per 1000 samples per year (Catlin and Murray, 1996). While there is ample anecdotal evidence that the number of positive cases is far less than the number of users, it is very difficult to estimate the number of actual users. Factors that contribute to the underestimation are: lack of year-around testing or testing only during the season, minimal risk of being called to a test, advance notice of testing, analytical instrumentation, the use of epitestosterone (E) to rapidly lower an elevated testosterone to epitestosterone ratio (T/E), and the use of titrated doses of T to remain under the cutoff of T/E ratio of 6.

Concerning the extent to which positive cases are acted on, the NCAA program is completely transparent, they publish the outcome of all the testing in some detail. The NFL also has an effective mechanism for tracking and seeing each case to a conclusion. USOC cases are more difficult to track because the USOC shifts the responsibility for action to the National Governing Bodies for the sport in question. In turn the NGB must report their finding on to the IF who bear the final responsibility for acting on a case. The IOC accreditation program requires that the laboratories simultaneously report cases involving Olympic athletes to the national authority, the IF, and to the IOC. This step helps to track cases.

EXOGENOUS STEROIDS

The features and characteristics of steroid use in the athlete community are well known and have remained fairly constant over time (Committee on the Judiciary, 1972; 1988; Wilson and Griffin, 1982; Wilson, 1988; Council on Scientific Affairs, 1988; Buckley et al, 1988; Catlin and Hatton, 1990). Similarly the basis of detection continues to be identification of the drug or its metabolites by mass spectrometry. The principal metabolites of each steroid are well known (Schänzer, 1996; Schänzer et al, 1990; Schänzer et al, 1992), although new ones are reported on regular intervals. In most cases, the additional metabolites do not add to the efficacy of testing because they are present in small amounts and are rapidly cleared from the body. One exception is the unusual and interesting finding that 17β-methyl-5β-androst-1-ene-3α, 17α-diol and 18-nor-17,17-dimethyl-5β-androsta-1,13-dien-3α-ol, metabolites of methandienone, are not present for the first few

days after administration (Schänzer et al, 1996). They first appear several days after administration and it remains detectable for several days and possibly weeks. This is an important finding because it extends the period of detection well beyond the 3-4 days that methandienone and its main metabolites are present.

Most of the AAS that are administered by mouth (orals) are detectable in urine for a week or less either as the parent compound or its metabolites (Schänzer, 1996; Schänzer and Donike, 1992; Schänzer et al, 1990; Schänzer et al, 1996). In contrast, the injected steroids are typically detected for 1-2 weeks, and the metabolites of nandrolone decanoate are detectable for several weeks. In cases of high dose nandrolone decanoate administration for several weeks the metabolites are detectable for months. Long lasting drugs have essentially disappeared from urines collected from Olympic and NFL athletes. They do still appear from time to time in the college program. At the international level nandrolone cases accounted for over 50% of the positive cases until recently. In 1990 nandrolone metabolites constituted 60% of the positive AAS cases, by 1994 the percent was down to 52, and in 1997 nandrolone cases constituted 45% of the positive cases.

ENDOGENOUS STEROIDS

Testosterone

Testosterone/Epitestosterone Ratio. The analytical difficulties of detecting testosterone usage have been described for several years (Kicman et al, 1990; Cowan et al, 1991; Catlin and Cowan, 1992; Catlin et al, 1996, 1997; Donike et al, 1994; Garle et al, 1996). The essential issue is, whereas the proof of use of an exogenous steroid is identification of that steroid or its metabolites in urine, the evidence of T use is an elevated ratio of testosterone to epitestosterone (T/E). Thus the T/E is an indirect test which depends on the concentration of two endogenous steroids. While T administration does result in an increased excretion of T in urine the relationship between dose and concentration is not well understood in part because only ~ 1% of a dose of T is excreted in urine as T glucuronide or T sulfate (Horton et al, 1963; Camancho and Migeon, 1964). The situation is further complicated by the fact that T administration results in suppression of E production and lower levels of urinary E (Dehennin and Matsumoto, 1993). Thus the T/E increases after T administration because of an increase in T and a decrease in E.

In 1982 the IOC proposed a reporting threshold for T/E of 6 (IOC Medical Commission, 1982). At that time it was thought that no person who had not used T could achieve a T/E >6. When it became apparent that a few people could normally have T/Es of >6 (Dehennin, 1994; Namba et al, 1989; Raynaud et al, 1992), the reporting threshold was changed to 10. Subsequently it became more apparent that since the median T/E for a male was ~1, the threshold of 10 provided to great a margin to take T and remain under the reporting threshold. The situation is further complicated by the desire of sport administrators to have an unambiguous report

that states that 'the sample is positive for testosterone', an outcome that laboratories simply cannot provide.

The T/E Profile. In 1993 the IOC acted to alleviate the dilemma by lowering the threshold back to 6, but stating that a ratio > 6 requires further investigation by the relevant sport authority. The sport authority was required to come to a conclusion after collecting and reviewing 'additional data'. The simplest additional data to collect was longitudinal data on the T/E, although other types of clinical investigation were encouraged. Profiling urine only required analyzing additional urines or retrospective analysis of prior urines. Thus the notion of monitoring the T/E 'profile' of an athlete over weeks or months became popular. Various other steroids and ratios were also monitored but none have been found which add additional information to the T/E itself. Fortunately the T/E of an individual is very stable for years (Donike et al, 1993; Baenziger and Bowers, 1994; Catlin, 1995; Catlin et al, 1997).

Currently the next task is to define the details of what constitutes an alteration in the T/E and over what period of time must it be monitored. Consensus is developing that three (or more) urine samples separated in time by at least one month with a pattern of one T/E greater than 6 and two with T/Es of ~ 1 constitute the minimal criteria for usage of T. Further, it has been suggested that the CV of urinary T/E in normal subjects does not exceed 30%. (Donike et al, 1994). In view of the infinite number of values for the numerator and denominator in a T/E ratio, that some individuals have very low urinary E, and the experience of others (Garle et al, 1996), we favor a more conservative CV of 60%, and taking all data into consideration.

When cases of T/E are adjudicated, the most common defenses are that the test is flawed because sexual activity, ethyl alcohol, the menstrual cycle and oral contraceptives raise the T/E. In fact there are no data to support the sexual activity hypothesis, and limited data on the influence of alcohol (Falk et al, 1988; Karila et al, 1996), the menstrual cycle (Geyer et al, 1995), and oral contraceptive on the T/E. With the possible exception of very large doses of ethyl alcohol (Falk et al, 1988; Karila et al, 1996), there is very little to support the hypothesis that other factors can lead to a T/E >6 in a healthy athlete.

Testosterone / Luteinizing Hormone Ratio. The IOC rule change in 1995 also encouraged research to find additional markers of T administration. The one that has gained the most support is the ratio of urinary T to urinary luteinizing hormone (T/LH) (Brooks et al, 1979; Cowan et al, 1991; Perry et al, 1997). This ratio is based on the well know effect of T on the pituitary. There are two difficulties with the T/LH test. First, it requires a rigorous method for estimating the concentration of LH in urine, and second the test will not become positive until several suppressing doses of T have been administered. Currently, therefore the T/LH ratio is considered corroboratory evidence of T administration, but a negative test does not eliminate T administration.

Ketoconazole Challenge Test. The ketoconazole challenge test (Oftebro, 1992, 1994; Kicman et al, 1993) gained some support in the early 1990s but it has not

been widely utilized because it requires, or coerces, the athlete to take a drug to prove his/her innocence. The test is based on the well studied inhibitory effect of ketoconazole on enzymes in the pathway from cholesterol to T, thus in the normal male the urinary T declines, the E is unchanged and the T/E decreases (Oftebro et al, 1994; Kicman et al, 1993). In the case of T administration the T/E is unaffected or increases after ketoconazole, while individuals with naturally elevated T/Es experience a decrease in T/E. While the available data are limited, the ketoconazole test does seem to provide good discrimination power. Nevertheless the test is not widely employed internationally and very rarely in the U.S. sport authorities are understandably reluctant to recommend application of the test.

Serum Testosterone / 17-OH-progesterone Ratio. Another test with potential merit but practical problems is the use of the ratio of 17-OH-progesterone (17-OHP) to T in blood (Carlstrom et al, 1992). This test is based on the inhibition of 17-OHP production by T. At this time blood samples are not approved by the IOC. While this is likely to change in the future, there is still the difficulty of the retrospectivity of the T/17-OHP test. Typically at least a week has elapsed before the T/E of a urine sample is reported and by that time the ratio will likely have reverted to normal.

Carbon Isotope Ratio Test. This promising and novel approach is based on a difference between the $^{12}C/^{13}C$ (^{13}C) of natural and pharmaceutical T (Southan et al, 1990). Although most carbon atoms in nature are ^{12}C, about 1.1% are the naturally occurring isotope ^{13}C. The ratio of these two isotopes of carbon can be determined with great precision with a gas chromatograph coupled in series with a combustion oven and a mass spectrometer (GC-C-IRMS).

All carbon atoms in pharmaceutical and natural T are ultimately derived from plant sources. These atoms are derived from atmospheric carbon dioxide fixed by plants during photosynthesis. The ^{13}C values of plants vary because of isotopic fractionation during physical, chemical, and biological processing. The ^{13}C of natural human testosterone represents the integral sum of the ^{13}C of the plants and animals ingested. Most if not all pharmaceutical T is derived from a single plant source - soy, thus the ^{13}C of synthetic testosterone reflects that of soy plants. Measurements of ^{13}C of various synthetic testosterones are ~ -30.0 (Shackleton et al, 1997a), that is, lower than that of natural human testosterone (~-26.0), therefore by measuring the ^{13}C of urine testosterone it may be possible to determine whether it is derived from natural or synthetic sources.

The ^{13}C of various urinary steroids has been measured in urines collected from subjects participating in testosterone administration studies. In general the data show that following T administration the ^{13}C of T and T metabolites 5α-androstane-3α, 17β -diol (5α-Adiol) and 5β-androstane-3α, 17β-diol (5β-Adiol) are lower while the ^{13}C of T precursors, cholesterol, 5-androstene-3β, 17β-diol, remain unchanged (Becchi et al, 1994; Aguilera et al, 1996; Shackleton et al, 1997a; Shackleton et al, 1997b). Unpublished data suggests that the ^{13}C of T in the urine of subjects with naturally elevated T is low.

Epitestosterone

Epitestosterone is the 17 epimer of T and it is present in urine in concentrations similar to T. T and E differ chemically only in the configuration of the hydroxyl group on C-17. The biological role of E is unknown, but it is known that T is not metabolized to E (Donike et al, 1983). In the present context E is important because self administration is an effective way to rapidly reduce an elevated T/E (Dehennin 1994). E is not available as a pharmaceutical but it is available from chemical companies and it has been found by police agencies investigating trafficking.

The IOC Medical Commission classified E as a urine manipulating agent, set a level of 200 μg/L (520 nmol/L) as the threshold for reporting cases. In our opinion the threshold should be higher since we often encounter cases in the 150-200 μg/L (520-693 nmol/L) zone. In the future we expect that the carbon isotope ratio technique might be useful for detecting epitestosterone administration. The two highest E concentrations we have encountered in urine samples were 1200 (4.16 μmol/L) and 1550 μg/L (5.20 μmol/L).

Dehennin (1996) pointed out that the ratio of production rates of T and E is ~25:1, thus administering T and E in this ratio might be a useful doping agent. In theory this might produce a T/E of 1:1 and therefore produce false negative test results while allowing administration of T. In practice it is likely to be difficult to achieve this ideal due to pharmacokinetic differences between T and E. Moreover Dehennin (1996) proposes to detect this scheme by measuring the ratio of T and E to 5-androstene-3β,17α-diol, a precursor of E.

Dihydrotestosterone

Although there had been rumors that dihydrotestosteone (DHT) was in use for several years, it was not until the Asian Games in 1994, when many cases of doping with DHT were discovered, that criteria for use were advanced (Donike et al, 1995). Since then an extensive DHT administration study was carried out which further established criteria (Kicman et al, 1995). The criteria are based on the ratio of the DHT or metabolites of DHT with the 5α configuration, which are expected to rise after DHT, to E, LH, and 5α- metabolites of T. The ratio DHT/E in urine appears to be the most sensitive and specific marker of DHT administration (Kicman et al, 1995; Donike et al, 1995; Coutts et al,1997). Other useful ratios are 5α-androstane-3α,17β-diol (5α-Adiol)/E, 5α-Adiol/LH, 5α-Adiol/5β-androstane-3α, 17β-diol, DHT/etiocholanolone, and androsterone/etiocholanolone (Kicman et al, 1995; Donike et al, 1995; Coutts et al, 1997).

Relatively little is known about the duration of the ratio changes. The DHT/E ratio remained elevated for 4 days following oral administration of 250 mg of DHT for 4 days (Kicman et al, 1995), and following 250 mg of DHT heptanoate intramuscularly the primary ratios remained elevated for 10-14 days (Coutts et al, 1997).

STEROIDS MARKETED AS FOOD SUPPLEMENTS IN THE U.S.

One outcome of enactment of the Dietary Supplement Health and Education Act of 1994 was that steroids such as dehydroepiandrosterone (DHEA), androstenedione, 19-norandrostenedione, and others could be marketed over-the-counter as food supplements. This created difficulties and questions for the sport organizations, particularly those that operate exclusively in the U.S.

There are no studies yet on the question of whether or not these steroids enhance athletic performance. Likewise, the adverse effects have not been systematically investigated. While there are a number of articles on the endocrine effects of androstenedione and DHEA in man, none have focussed on the side effects. Athletes often take larger than recommended doses of drugs, therefore when such studies are performed it will be important to administer large doses.

The IOC laboratories report that during adjudication procedures a number of athletes with elevated T/E ratios explain that they did not take T but admit to ingesting androstenedione, DHEA, or both. Given that androstenedione and DHEA are metabolic precursors of T (Longcope, 1996; Horton and Tait, 1966), it would not be surprising to find that they do influence T/E. The only relevant study showed little or no influence of DHEA (50 mg, single dose) on T/E (Dehennin et al, 1998).

Recently we discontinued reporting T/E cases to the Department of Defense (DOD) because, unlike sport organizations, DOD does not prohibit the use of OTC steroid supplements. In our opinion there was sufficient information to indicate that androstenedione or DHEA, or both, in the doses that were being used, might elevate the T/E, therefore we have temporarily suspended reporting T/E cases to DOD.

The IOC provides a list of drugs that are considered AAS. In addition, to prevent the use of designer AAS that are not specifically named, the regulation also states that "... related substances" are also prohibited. Thus, androstenedione and DHEA have always been prohibited by the IOC rules. Nevertheless, late in 1997 the IOC specifically named androstenedione and DHEA as prohibited steroids. The reason for this action was to avoid the legal expense of adjudicating the interpretation of the phrase "and related substances".

We have analyzed the steroid content of many different bottles of 19-nor-androstenedione and androstenedione purchased from health-food stores. Several types of labeling errors were discovered. For example, one bottle labeled 19-nor-androstenedione actually contained only androstenedione, and another bottle labelled androstenedione contained 19-norandrostenedione.

Currently we are investingating the metabolism of these steroids and their effects of on other urine steroids. We have identified three urinary metabolites of 19-norandrostenedione. Two of these (19-norandrosterone and 19-noretiocholanolone) are also metabolites of 19-nortestosterone (nandrolone), therefore a person who ingests 19-norandrostenedione could be reported to have the metabolites of nandrolone in their urine. At this time we do not have a good way to differentiate between the use of 19-norandrostenedione alone and 19-norandrostenedione plus 19-nortestosterone.

SUMMARY

Sport authorities have developed educational and testing programs designed to combat the use of anabolic steroids to enhance athletic performance. An extensive but somewhat maldistributed network of IOC accredited laboratories now performs nearly 100,000 doping controls annually. About one half of these tests are carried out during times when the athlete is not attending a competition. The extensive knowledge of metabolism of xenobiotic AAS makes their detection routine and uncomplicated, but their detection times are relatively short. Despite these efforts it is evident that AAS remain a serious issue in sport. The reason is that drug taking behavior has shifted from the xenobiotic steroids to the endogenous steroids such as testosterone and DHT. The difficulties of proving that T or DHT was administered are due to the use of indirect testing methods based on ratios or T or DHT to E. Further E may be used to rapidly lower an elevated T/E. Despite these difficulties the technique of longitudinal monitoring of the T/E, carbon isotope ratio methods, and analytical approaches offer the prospect of achieving better control of the problem in the future. To achieve greater efficacy it will be necessary for the sport organizations to dedicate additional funds to research and method development.

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12 MALE INFERTILITY CAUSES AND DIAGNOSIS

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INTRODUCTION

Male infertility is a heterogeneous disorder. A variety of factors may adversely impact sperm production and function and impair fertility. An algorithmic approach based on information collected during a careful history, physical examination and the results of the semen analyses and hormone evaluation can assist the clinician to categorize the diagnosis of the patient's infertility into: 1) hormone disorder, 2) anatomic abnormality, 3) idiopathic.

HISTORY

History of Medical/Endocrine Disorders

A careful history is taken to uncover any underlying medical or endocrine disease. Past and present illnesses, surgery to the brain and the genito-urinary system (i.e. orchiopexy, pelvic or retroperitoneal surgery, herniorrhaphy, vasectomy): infectious disease (venereal disease, mumps, TB, epididymitis); drugs and medication; alcohol use; and occupational or environmental chemical exposures can contribute to male factor infertility. A history of recurrent respiratory infections suggest cystic fibrosis (associated with congenital absence of the vas deferens), ciliary defects (Kartagener's Syndrome) associated with sperm motility abnormalities, and Young's Syndrome which causes epididymal inspissation.

A detailed account of the patient's developmental history, failure of testicles to be descended at birth, age of puberty, congenital abnormalities of the urinary tract

or CNS, history of anosmia, gynecomastia, changes in libido and potency headaches, visual field changes, and fertility history is ascertained. Symptoms consistent with hypogonadism are elicited (fatigue, depression, erectile dysfunction, muscle weakness, osteopenia and fractures, see Chapter 6).

History of toxic exposure

Toxic exposures which result in infertility include chemotherapeutic agents, radiation therapy, prescription and nonprescription drugs, recreation drugs, and environmental/occupational toxicants.

Chemotherapeutic agents and radiation therapy: Cytotoxic damage to the testicular germinal epithilium by cancer treatment with chemotherapy and/or radiation is well documented. A review of the literature suggests that the severity of germ cell damage is related to the category of chemotherapeutic agent, and the dose and duration of therapy. Radiation effects on spermatogenesis depend on total dose received and the developmental stage of the germ cell at the time of exposure.

Prescription and non-prescription drugs: A variety of prescription drugs can interfere with the reproductive axis, sperm motility, sperm function, or potency. Digoxin, cemtidine, and spironoloactone act as competitive inhibitors of the estrogen receptor. These patients present with gynecomastia, impotence, and infertility. Ketoconazole disrupts testicular steroidogenesis, lowering serum testosterone levels, and impairing fertility. Anti-hypertensive agents adversely affect fertility either by disrupting motility, interfering with the ability of sperm to penetrate and fertilize the ova, or by causing decreased libido and or impotence. A number of popular agents advertised to promote health and prolong life may interfere with fertility. These include chromium, DHEA, and melatonin. There are no controlled studies evaluating the toxic effects of these drugs on the male reproductive system.

Recreational drugs: Alcohol, marijuana, cocaine and heroin have all been implicated as reproductive toxicants. All are thought to disrupt hormone secretion at more than one level of the reproductive axis. The degree of risks dependent on the amount of drug take, the frequency of use, and the amount of active ingredients and contaminants ingested.

Environmental/occupational toxicants: Although numerous chemicals have been reported to alter the male reproductive axis, few have been extensively studied. However, groups of toxicants have been identified as leading to reproductive dysfunction in males. These include the heavy metals (lead, boron, mercury, cadmium, arsenic, manganese, and perhaps chromium); the agricultural chemicals such as DBCB and the organophophates. Compounds which are estrogen-like or estrogenic are toxicants which can disrupt the male reproductive system by interfering with the normal feedback regulation of the HPT axis. Environmental estrogens include estrogenic substances in plats as well as synthetically produced estrogenic compounds such as DES and O, P-dichlorodepplenyl trichlorethane DDT. Environments which are elevated in temperature can also lead to abnormal spermatogenesis and impaired fertility (see Chapter 17).

The diagnosis of toxicant induced infertility is determined by the history.

PHYSICAL EXAMINATION

A complete physical examination is performed in order to determine if the patients infertility is due to an endocrine disorder or an underlying medical condition or anatomic abnormality. Physical findings consistent with hypogonadism are listed in Table 1.

Table 1. Physical Findings Associated with Hypogonadism

Eunuchoid proportions
Decreased facial and body hair
Absence of male pattern balding
Female body habits
Gynecomastia
Poor muscle development
Abnormal external genitalia
Small testes
Small prostate

The examiner can frequently ascertain if a patient's hypogonadism presented prior to or following puberty based on specific physical findings. Inadequate Leydig cell function or androgen action during embryogenesis may manifest itself by the presence of hypospadias, cryptorchidism, or microphallus. Leydig cell failure that occurs prior to puberty, will disrupt normal sexual maturation resulting in the failure of androgen - induced closure of the long bones, or eunuchoidism. Eunochoidism is defined as an arm span more than two inches longer than the height, and a lower body segment (pubic to heel) more than two inches longer than the upper body segment (crown to pubic). Other findings associated with the prepubertal onset of hypogonadism include sparse body, pubic and facial hair; poor development of skeletal muscles; absence of male patterns baldness, infantile genitalia with small firm testes (less than 15cc); failure of voice to deepen; and, on occasion gynecomastia (Sokol, 1997).

Because onset of Leydig cell failure which commences after puberty is insidious, the patient with late onset hypogonadism presents with more subtle physical findings. Physical signs include a female body habitus with female fat distribution, decrease in skeletal muscle mass, gynecomastia, a decrease in facial hair, excessive facial wrinkling and small soft testes. Patients with isolated germ cell failure present with a normal physical exam except for small testes. Patients with spermatogenic arrest or obstruction have normal sized testes.

General medical conditions which may contribute to infertility include CNS tumors, thyroid disease and liver abnormalities. Visual field changes are associated with CNS tumors. Thyroidmegaly, eye changes, hyper or hyporeflexia may reflect thyroid disease. Hepatomegaly and/or gynecomastia are associated with alcoholic liver disease or other disorders of abnormal steroid metabolism. Anatomic abnormalities associated with infertility include varicocoele, congenital absence of

the vas deferens, or an epididymal obstruction. The latter conditions are identified by careful examination of the scrotal contents.

SEMEN ANALYSIS AND ENDOCRINE EVALUATION

Laboratory investigation of male infertility begins with basic screening tests. The cornerstone of the work up is the semen analysis. Baseline reproductive hormonal testing is indicated by the history and physical exam. More specialized tests are performed to isolate specific abnormalities in selected patients (Sokol, 1997).

Semen Analysis

A normal semen specimen is one that has an adequate number of spermatozoa, the majority of which are motile and morphologically normal. Because there is a marked variability in sperm density, motility, and morphology among multiple semen samples from an individual man, interpretation of a semen analysis must occur in the context of this marked variability for a given individual (Schrader et al, 1988). Collection of three to six samples over 2-3 months increases the reliability of the mean values calculated for the semen parameters recorded for those samples. Standardization of abstinence time and evaluation of the sample within one hour of collection improves the reliability of the interpretation of the results.

Initial evaluation of the semen sample includes measurement of the semen volume and pH. The color and viscosity of the semen are noted and the time for liquefaction is recorded. Motility is assessed after the semen has liquefied. An aliquot of semen is examine with phase contrast microscopy and the percentage of motile sperm and the quality of movement are recorded. Sperm concentration is usually measured using a sperm counting chamber. The hemocytometer method is recommended by the WHO and is outlined in their manual (WHO, 1987, 1993, 1998). Other methods include the Makler Chamber, or a MicroCell.

Morphologic assessment of spermatozoa can be performed by using a variety of staining techniques. These include the Giemsa method, a modified Papanicolaou stain, a Bryan Leishman stain, or a prestained slide. After staining, 100 sperm cells are evaluated for size of head, presence and normalcy of the acrosome, nucleus and tail. The number of immature germinal cells are reported and differentiated from the number of leucocytes identified. The percentage of normal sperm forms is reported using either WHO criteria or "STRICT" morphology. The rationale behind the latter system is that the stricter the criteria, the less the inter-technician variation (Kruger et al, 1988). Borderline normal sperm are considered abnormal, with >14% normal sperm forms considered as normal. Success in IVF correlates with a normal sperm morphology of greater than 4% (Kruger et al, 1988).

An alternative to the standard microscopic assessment system of sperm parameters is computerized semen analysis (CASA). These systems are more efficient, but also more expensive. Whether they improve accuracy is uncertain (Davis, 1992).

Clinical interpretation of the semen analysis is baseded on the comparison of the sample to be evaluated with the published results of studies evaluating fertile and non-fertile male populations. Although there is much overlap between the semen analyses of fertile and infertile men, values below certain established threshold values help identify men whose fertility is likely to be compromised. Table 2 lists the most commonly used reference ranges of normal semen analyses as established by the WHO (WHO, 1993, 1999).

Table 2. Normal Semen Values: WHO Guidelines

Volume: >2.0 mL
pH: 7.2-7.8
Sperm concentration:>20 million sperm/mL
Motility: >50% with forward progression
Normal morphology: >15%*

* Based on data from in-vitro fertilization studies

Sperm Function Tests

Because the semen analysis does not definitively predict fertility potential, a number of sperm function tests were developed. In general, these assays test different steps in the cascade of biologic steps that the spermatozoon must pass through before fertilization takes place (Sokol, 1997). These tests include: The hypo-osmotic swelling test (HOS), which tests sperm plasma membrane integrity; the acrosome reaction (AR), which tests the ability of the spermatozoon to undergo the acrosome reaction; the hemizona assay (HZA), which tests for the ability of the spermatozoa to bind the zona pellucida; the human spermatozoa-zona-free hamster ova in vitro penetration assay (SPA), which assesses the ability of the spermatozoon to undergo capacitation and the acrosome reaction, fuse with the oolemma, and decondense in the ooplasm; and the measurement of sperm creatine phosphokinase M-isoform ratios (CK-MM), which is the key enzyme in the synthesis and utilization of energy in spermatozoa. None of these tests have proven to definitively predict fertility (Sokol, 1997).

Microbiology

The presence of white blood cells (WBC's) in the patient's semen may indicate the presence of an infection. If a specific pathogen is identified, the patient and his partner are treated with the appropriate antibiotic (Greendale et al, 1993; Hellstrom and Neal, 1992). The efficacy of empiric treatment with doxycycline is controversial (Branigan and Muller, 1994).

Endocrine Evaluation

The endocrine status of the reproductive hormonal axis (hypothalamus, pituitary, testes) is assessed by measuring serum LH, FSH and testosterone. A single sample

of 10 ml of whole blood collected in the morning to minimize diurnal variation is usually adequate.(Baum et al, 1988) However, because of the pulsatile nature of hormone secretion in man, single random serum levels may not accurately reflect the mean concentration of LH, FSH and testosterone over a prolonged period of time.(Santen and Bardin, 1973) By pooling three samples, a more integrated measure of basal hormone secretion is obtained.(Santen and Bardin, 1973) If an abnormal result is obtained on a single sample, the patient should be re-evaluated with a collection of multiple samples (three samples collected through an indwelling cannula at 20 minute intervals).

The measurement of free (unbound) testosterone is a more accurate marker of physiologically available testosterone than is total testosterone levels when conditions of altered SHBG concentrations or binding exist.(Vermeulen et al, 1960) In general, the proportion of testosterone that is "free" is inversely related to the SHBG concentration. Reduced SHBG levels occur in association with obesity, acromegaly and hypothyroidism. Increased SHBG levels occur in association with cirrhosis and hypogonadism. In general, estrogens stimulate and androgens inhibit the biosynthesis of SHBG.(Pugeat et al, 1981) Many natural and synthetic steroids alter the binding of SHBG.

Estradiol levels are measured if the patient presents with gynecomastia, a testicular mass, or a history consistent with exogenous estrogen exposure. Prolactin measurement is included if the man has a relevant drug history, or presents with impotence and/or evidence for a CNS tumor. The measurement of dihydrotestosterone (DHT) is indicated when a disorder of testosterone conversion to DHT is suggested by the clinical presentation, although such enzymatic defects are rare (Cai L-Q et al, 1994).

Dynamic tests available to determine the physiological state of the hypothalamic-pituitary-testicular axis include stimulation tests with GnRH and Human Chorionic Gonadotropin (HCG). The GnRH test evaluates the functional capacity of the gonadotrophs to release LH and FSH. (Snyder et al, 1979) The ability of the testes to secrete testosterone is conventionally tested with the administration of hCG. For a more detailed discussion, see Chapter 5 and 6.

DIAGNOSTIC CATEGORIES

Hormone Disorders

Testicular Failure. Men with irreversible infertility can be subdivided into two major groups: 1) Men with spermatogenic failure who present with severe oligospermia, elevated serum FSH levels, normal LH and testosterone levels, and small testes (1^{o}germ cell failure); and 2) men who present with classic hypergonadotropic hypogonadism identified by elevated gonadotropins, low testosterone, and severe oligospermia or azoospermia. Klinefelter's Syndrome (47XXY) occurring in approximately 0.2% of adults is the most common cause of hypergonadotropic hypogonadism (Ratcliffe, 1982). Causes of both types of

testicular failure include orchitis, trauma, radiation, drugs, toxicants, auto-immunity, granulomatous diseases and defects associated with systemic diseases.

Hypogonadotropic Hypogonadism . A small number of infertility patients carry the diagnosis of hypogonadotropic hypogonadism (Sokol, 1997). These men present with diminished LH and FSH, low testosterone levels, azoospermia or oligospermia. The etiology is either congenital or acquired. The former, Kallmann's Syndrome or idiopathic hypogonadotropic hypogonadism, is an abnormality of the secretion of gonadotropin-releasing hormone (GnRH). Acquired causes include tumor, infection, infiltrative diseases and autoimmune hypophysitis.

Androgen Resistance or Insensitivity. Androgen resistance may cause infertility with or without underandrogenization. These men present with oligospermia or azoospermia, an elevated testosterone level, and a borderline elevated LH value (Aimen et al, 1979). A small number of men lack the enzyme 5α reductase which results in a deficiency of DHT. They present with ambiguous genitalia, azoospermia, and low levels of DHT (Cai L-Q et al, 1994).

Anatomic Defects

Varicocele-Associated Infertility. A varicocele is a dilatation of the scrotal portion of the pampiniform plexus/internal spermatic venous system that drains the testicle. Since the 1950's, the varicocele has been implicated as a cause of male infertility (Howards, 1992). Support for this notion derives from the reportedly increased incidence of varicocele in men evaluated for infertility (20 to 40%) as compared to the incidence found in the unselected male populations (2-15%). These data are derived from men with clinically palpable varicoceles. The role that "subclinical varicoceles" play in male infertility is an even more controversial topic. There are no reliable methods available to document the presence of the "subclinical varicocele"; nor do published studies suggest that repair of a "subclinical varicocele" improves pregnancy rates (Jarow, 1994).

The pathophysiology of varicocele - induced infertility remains undefined. Proposed mechanisms include elevated testicular temperature, hormone imbalance, hypoxia secondary to venous stasis, and reflux of adrenal or renal metabolites. Animal studies support the theory that varicoceles do damage to the testes.(Saypol et al, 1981; Rajfer et al, 1987) Recent animal studies suggest improved pregnancy rates following surgical correction (Sofikitis and Miyagawa, 1992). The clinical studies which report improved semen parameters and pregnancy rates after varicocele ligation were uncontrolled studies.(Howards, 1992) One crossover study doses suggest improvement in pregnancy rates (Madgarn et al, 1995). Prospective controlled studies report no improvement in pregnancy rates with varicocele ligation (Baker et al, 1985; Vermeulin et al, 1986; Nieschlag et al, 1995; Nilsson et al, 1979). More data is needed before the conclusion can be made that varicocelectomy definitely improves pregnancy rates.

Obstructive Azoospermia. The causes of obstructive azoospermia include vasectomy, inadvertent ligation or transection of the vasa during herniorrhaphy or pelvic surgery, and epididymal obstruction secondary to acute infection. A small group of men are born without vasa deferens, a condition associated with cystic fribrosis (Chillon et al, 1995). Men with obstructive azoospermia and congenital absence of the vasa present with normal hormones, normal sized testes, and azoospermia.

Of those conditions, vasectomy reversal yields the most successful results. Following first time reversal, 86% of men have sperm present in their ejaculate and 52% of the couples establish a pregnancy. An inverse correlation exists between patency and pregnancy rate with obstruction interval (Belker et al, 1991).

If the etiology of the azoospermia is not a vasectomy, or congenital absence of the vasa, a testicular biopsy is performed to differentiate between obstruction and spermatogenic arrest. If normal spermatogenesis is noted, a vasogram may be performed at the time of the surgery, and microsurgical repair of the obstruction is attempted as indicated at the time of surgery. Vasograms should not be performed before surgery because of the risk of causing an obstruction. An alternate approach to microsurgery is to perform transepididymal sperm aspiration (Nagy et al, 1995) and intracytoplasmic sperm injection (see Chapter 14).

Partial Obstruction. Abnormalities of the ejaculatory duct can rarely cause partial obstruction and oligospermia (Carson, 1984; Hellerstein et al, 1992; Weintraub et al, 1993). Patients present with low or variable semen volume and oligospermia. Transrectal ultrasonography of the seminal vesicles and ejaculatory ducts to identify the obstruction should precede testicular biopsy. When indicated, patients are treated with transurethral resection of the ejaculatory ducts (Weintraub et al, 1993).

Retrograde Ejaculation. Disruption of the innervation of the vasa deferentia and bladder neck may result in retrograde ejaculation. Diabetes mellitus complicated by peripheral neuropathy, multiple sclerosis, medical therapies interfering with sympathetic tone, transurethral resection of the prostate, bladder neck surgery, retroperitoneal lymph node dissections, and extensive pelvic surgery may lead to retrograde ejaculation.

Patients with retrograde ejaculation report absent or near-absent ejaculate following orgasm. The diagnosis is confirmed by identification of large numbers of sperm in a post-ejaculate urine specimen. Patients with medical causes for their condition may respond to treatment with sympathomimetic drugs. If medical therapy is not indicated, the semen is alkalinized and sperm can be recovered from the urine and washed in Hams F10 tissue culture medium with albumin prior to insemination (see Chapter 14).

Idiopathic Infertility

Patients with idiopathic infertility present with normal gonadotropins, normal testosterone and oligospermia. Occasionally patients with long-standing infertility will present with normal sperm counts, but abnormal sperm function tests. By definition, this is a diagnosis of exclusion. A subset of these men present with an

abnormality of motility or sperm function. If no toxicant exposure is uncovered in the history, the abnormality is categorized as idiopathic. (Please see chapter 13 for the genetic causes of male infertility).

SUMMARY

A alogorithmic approach to the differential diagnosis of infertile men presenting with azoospermia and oligozoospermia is depicted in figures 1 and 2, respectively (Wang and Swerdloff, 1995). The diagnosis is based on the history, physical examinations, semen analysis and reproductive hormone levels.

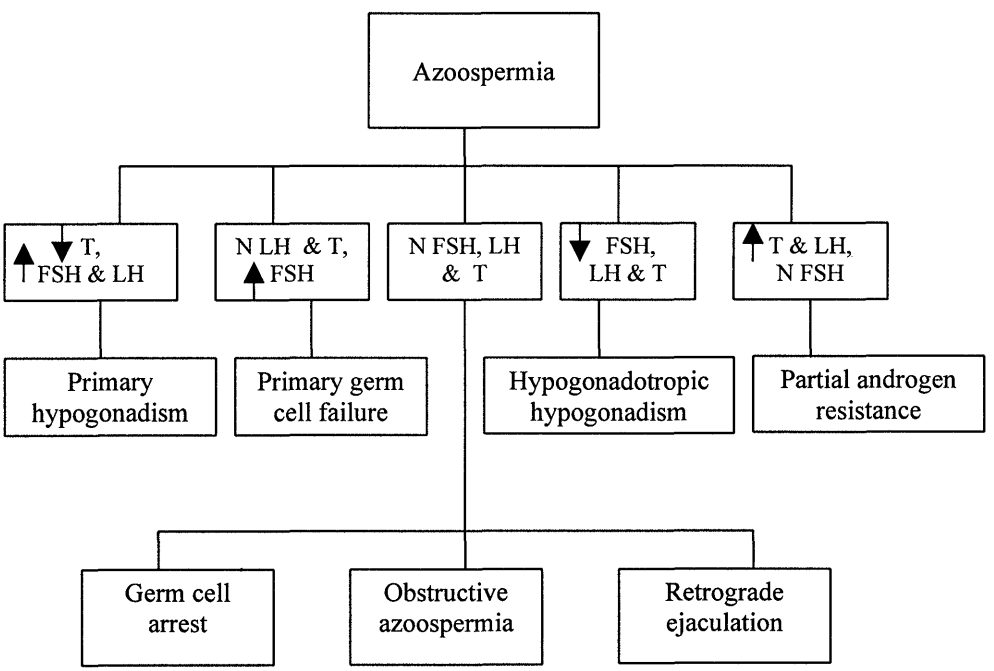

Figure 1. Algorithmic approach to the diagnosis of the causes of azoospermia (n=normal)

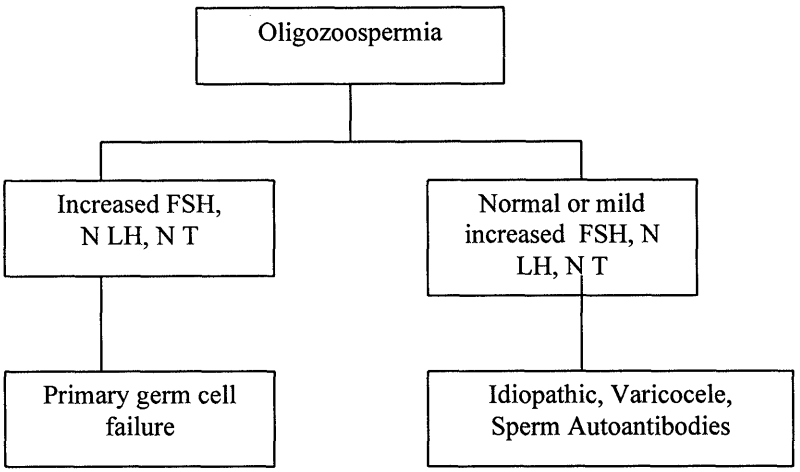

Figure 2. Algorithmic approach to diagnosis of the causes of oligozoospermia (n=normal)

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13 THE GENETICS OF MALE INFERTILITY

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While a multitude of acquired causes can impair spermatogenesis, there is reason to believe that a genetic basis exists in a majority of infertile men (Bhasin et al, 1994; De Kretser et al, 1972; Lamb and Niederberger, 1994; Jaffe and Oates, 1994; Skakkebaek et al, 1994). The occurrence of these genetic defects in infertile men has significant implications for assisted reproductive technologies, particularly intracytoplasmic sperm injection (ICSI) (Bhasin et al, 1994). Because intracytoplasmic sperm injection may allow partners of these infertile men to become pregnant, it is possible that these genetic defects may be transmitted to the male offspring. This raises issues of informed consent and ethical concerns. Similarly, the widespread use of assisted reproductive techniques to induce pregnancy may result in accumulation of genetic defects in the population; these defects would have been otherwise weeded out because of infertility. Substantial prevalence of Y deletions and other known and unknown genetic defects in infertile men and the potential risk of transmitting this genetic disorder to their offspring provide a compelling rationale for genetic screening of infertile men prior to ICSI. The couples undergoing ICSI should be counseled about the potential risk of transmitting this genetic disorder to the offspring. Long term monitoring of ICSI babies for genetic disorders including Y deletions is warranted.

The process of spermatogenesis takes place in the seminiferous tubules of the testis, and is divided into three major stages (Clermont, 1970; Heller and Clermont, 1964): spermatogonial replication, meiosis, and spermiogenesis. During the first stage, the spermatogonial stem cells divide mitotically several times to give rise to successive generations of spermatogonia of which there are at least three main types in the human tubules, the dark type A, pale type A, and type B (Clermont, 1970). The type B spermatogonia proliferate to give rise to primary spermatocytes at the preleptotene stage of meiosis, in which DNA is actively synthesized. The second stage is the process of meiosis, which consists of two successive divisions of the spermatocyte accompanied by only one duplication of chromosomes. At the

completion of meiosis, four spermatids are produced, each containing a single, or haploid, set of chromosomes. The final stage of spermatogenesis is called spermiogenesis, which involves a complex process of structural transformation and differentiation of the spermatid. During spermiogenesis, the chromatin of the spermatid condenses into a compact mass of dense granules, and the nucleus becomes invested by a membranous derivative of the Golgi apparatus, the acrosome, which contains enzymes that will digest a path for the sperm to penetrate the outer vestments of the egg. The cytoplasm elongates and surrounds the flagellum sprouted from a centriole. At the time of sperm formation most of the cytoplasm is cast off in the form of a residual body. The spermatid completes its metamorphosis into a spermatozoon by forming a complex tail by the axonemal complex of two inner singlet and nine outer doublet microtubules. In man, the total duration of spermatogenesis is 74 days (Heller and Clermont, 1964).

The normal spermatogenesis requires complex interactions between germ cells and various somatic such as Sertoli and Leydig cells (Griswold , 1988), and the synergistic actions of the pituitary gonadotropins luteinizing hormone (LH) and follicle stimulating hormone (FSH). LH after binding to its G-protein coupled receptor stimulates the production of testosterone by the Leydig cells; high intratesticualr testosterone concentrations are essential for the initiation and maintenance of spermatogenesis within the testis. FSH initiates function in immature Sertoli cells by stimulating the formation of the blood-testis barrier and the secretion of a wide range of proteins and growth factors, such as androgen binding protein, inhibin, activin, stem cell factor, plasminogen activator, transferrin, sulphated glycoproteins, and lactate (Griswold, 1988).

Failure of spermatogenesis can result from impaired secretion or action of LH and FSH (Crowley et al, 1991) or from intrinsic defects in spermatogenesis within the testis. This review will highlight the known genetic syndromes associated with infertility in man, mouse and drosophila. It is worth emphasizing that the molecular defects that result in infertility in a majority of infertile men remain unknown. We will not discuss disorders of sexual differentiation and androgen action. Although the number of human genes implicated in the pathophysiology of infertility is small, a significantly larger database exists in the mouse and Drosophila. For instance, 2400 Drosophila loci have been implicated in male sterility! Undoubtedly there is reason to quibble with this number, however, a careful study of the Drosophila and mouse sterility loci can provide useful clues to candidate genes that are associated with defects of germ cell replication, meioisis or spermiogenesis in man.

GENETIC DISORDERS ASSOCIATED WITH INFERTILITY IN MAN

Genetic Disorders Associated with Impaired Gonadotropin Secretion or Action

Kallmann's Syndrome. Idiopathic hypogonadotropic hypogonadism occurs in both sporadic and familial forms; the familial cases account for about a third of all cases of idiopathic hypogonadotropic hypogonadism (Crowley et al, 1991; Waldstreicher et al,

1996). Both X-linked and autosomal patterns of inheritance have been described (Waldstreicher et al, 1996). The murine model of idiopathic hypogonadotropic hypogonadism has revealed a large deletion involving exons 3 and 4 of the GnRH gene. However, mutations in the GnRH gene have not been detected in humans with this disorder (Weiss et al, 1989). In a subset of patients with the X-linked form of the disease, deletions of a neural cell adhesion molecule like protein, Kalig-1 (Kallmann-interval 1 gene), have been described (Legouis et al, 1991;Bick et al, 1992). Kalig-1 gene plays some poorly understood role in the orderly migration of the GnRH neurons from the region of the olfactory apparatus to the pre-optic region of the hypothalamus. Thus, Kallmann's syndrome can be viewed as a developmental migratory disorder that results from failure of the GnRH neurons to migrate into their proper location in the hypothalamus.

Family studies by Waldstreicher et al. (Waldstreicher et al, 1996) suggest that at least two additional autosomal loci may be implicated in subsets of idiopathic hypogonadotropic hypogonadism. Several pedigrees with hypogonadotropic hypogonadism due to mutations of the GnRH receptor gene have been described (Layman et al, 1998; De Roux et al, 1987); however, these mutations are an uncommon cause of idiopathic hypogonadotropic hypogonadism (Layman et al, 1997).

The clinical manifestations of idiopathic hypogonadotropic hypogonadism depend upon the severity of gonadotropin deficiency. These patients often present with delay or absence of the pubertal development; those with less severe defects may present in adulthood with infertility, impaired virilization, sexual dysfunction, and osteoporosis. A number of somatic abnormalities including anosmia or hyposmia, congenital sensorineural deafness, horseshoe shaped kidneys, cleft lip, cryptorchidism, color blindness and other midline defects have also been reported in association with Kallmann's syndrome. The genetic basis of these associated congenital anomalies in patients with idiopathic hypogonadotropic hypogonadism is not known; some of the anomalies may be due to deletion of genes that are contiguous to the IHH gene.

Mutations of LH and FSH Beta Genes. Inherited mutations of the LH beta and FSH beta genes are uncommon (Conway, 1996; Jameson, 1996; Albanese et al, 1996; Simoni et al, 1997). Inactivating mutations of the FSH beta gene have been reported to produce male hypogonadism and delayed puberty in boys (Layman et al, 1997; Phillip et al, 1998). FSH-deficient male mice are fertile although they have small testes and subnormal spermatogenesis (Kumar et al, 1997).

A 46, XX patient, homozygous for the FSH beta point mutation, presented with primary amenorrhea and infertility, and low serum FSH levels (Jameson, 1996). Analysis of FSH beta gene revealed a two-nucleotide deletion that resulted in a frame shift of subsequent codons and premature termination. A relative of the index case was post-menopausal and had subnormal FSH levels. Female mice deficient in the FSH beta subunit, produced by the embryonic stem cell technology, are infertile due to a block in folliculogenesis prior to antral follicle formation (Kumar et al, 1997).

A single patient with mutation of the LH beta subunit gene has been reported; this individual presented with delayed pubertal development (Jameson, 1996, Tsigos et al, 1997). The patient had increased serum immunoreactive LH levels but decreased bioactive LH concentrations. The mutant LH had a homozygous substitution of Gln

54 with Arg and had decreased receptor binding activity. The male individuals in the family who were heterozygous for this mutation had lower testosterone levels. Heterozygous females had regular menstruation and were fertile. A polymorphic variant of LH has been reported in Finland and Japan (Raivio et al, 1996). The variant LH has two amino acid substitutions, W8R and I15T, that are associated with increased bio-activity and a reduced serum half-life. The clinical significance of this polymorphism is not known.

Decreased gonadotrope viability and hypogonadotropic hyopogonadism has been reported in association with mutations of the SF-1 and DAX-1 genes.

Mutations of LH and FSH Receptor Genes. A number of families with resistance to LH action due to inactivating mutations of LH receptor have been reported (Tsigos et al, 1997; Themmen et al, 1997; Laue et al, 1996; Latronico et al, 1996; Laue et al, 1995). Men with LH receptor mutations present with a spectrum of phenotypic abnormalities ranging from feminization of external genitalia in 46, XY males to Leydig cell hypoplasia, primary hypogonadism, and delayed sexual development (Tsigos et al, 1997; Themmen et al, 1997; Laue et al, 1996; Latronico et al, 1996; Laue et al, 1995). The female member of the kindred with LH receptor mutation revealed normal development of secondary sex characteristics, increased LH levels and amenorrhea.

Activating or gain-of-function mutations of LH receptor are associated with gonadotropin independent, sexual precocity in boys, but do not produce a discernible phenotype in females (Conway, 1996;Simoni et al, 1997). Only a single case of activating mutation of the FSH receptor is on record; this patient was fertile even after surgical hypophysectomy that had lowered his FSH immunoreactivity to undetectable levels.

Prader Willi Syndrome. Prader Willi syndrome, a disorder of genomic imprinting, most commonly results from deletions of proximal portion of paternally derived chromosome 15q (Cassidy et al, 1997; Gunay-Aygun et al, 1997; LaSalle et al, 1998), and is associated with constitutional obesity, mental retardation, and hypogonadotropic hypogonadism. The maternally derived copies of genes responsible for the Prader-Willi syndrome in proximal 15q are normally silent (Cassidy et al, 1998). Therefore, the deletion of the paternally derived copy of the normally active genes produces the disease. Prader-Willi syndrome can also result if both copies of the gene are derived from the mother because the maternal copies are inactivated presumably by DNA methylation (LaSalle et al, 1998); this condition is known as uniparental disomy. Structural abnormalities of the imprinting center can also produce the Prader-Willi syndrome. The genes responsible for the Prader-Willi syndrome have not been identified. Allele-specific methylation at locus D15S63 can be detected by a PCR method and has been used as a diagnostic test for this syndrome (LaSalle et al, 1998).

Other Congenital Disorders Associated with Hypogonadotropic Hypogonadism. Hypogonadotropic hypogonadism is observed in association with the multiple lentigenes syndrome, Laurence-Moon and Bardet-Biedl syndromes, Cohen syndrome,

Borjeson-Forssman-Lehmann syndrome, congenital icthyosis, Rud's syndrome, cerebellar ataxia, optico-septal dysplasia, and Mobius syndrome (Gunay-Aygun et al, 1997; Rimoin and Schimke, 1971). The pathophysiology of hypogonadotropic hypogonadism in these disorders is not known and the diagnosis is made by recognizing the specific somatic abnormalities associated with these syndromes; these somatic features are described in detail in a monograph by Rimoin and Schimke (Rimoin and Schimke, 1971).

Primary Defects of Spermatogenesis Associated with Major Chromosonal Disorders

Sex Chromosome Disorders. Approx. 5% of infertile men carries chromosome abnormalities; of these, a majority involves sex chromosomes (4% on average), and 1% involves the autosomes (Zuffardi and Tiepolo, 1982; Kjessler, 1966; Chandley, 1979; Koulischer and Schoysman, 1975). The prevalence of sex chromosomal and autosomal abnormalities in infertile men is 15 and 6 times higher than in general population (Jacobs et al, 1974; Hamerton et al, 1975).

Klinefelters' Syndrome. Klinefelter's syndrome is the most common chromosomal disorder associated with male infertility and is found in 1:500 to 1:1000 live-born males (Jacobs et al, 1974; Hamerton et al, 1975). The most frequent karyotype in men with Klinefelter syndrome is 47, XXY (93%), but 46, XY/47, XXY; 48, XXXY; 48, XXYY and 49, XXXXY karyotypes have also been found (Fryns et al, 1983).

The XXY chromosomal constitution has been described in other mammals such as mouse (Cattanach, 1961), Chinese hamster (Ivett et al, 1978), cat (Centerwell and Benirschke, 1973), dog (Clough et al, 1970), sheep (Bruere et al, 1969), ox (Rieck, 1970), and pig, and is associated with sterility. The testes of 47, XXY animals are completely devoid of germ cells (Huckins et al, 1981; Paulsen et al, 1968; Gordon et al, 1972).

Most men with Klinefelter's syndrome are diagnosed in adulthood (Paulsen et al, 1968); in one study, 73% of patients were diagnosed after age 18, and only 4% were diagnosed before puberty. Klinefelter's syndrome is characterised by small testes, hyalinisation and fibrosis of the seminiferous tubules, azoospermia and underdeveloped secondary sex characteristics. The syndrome may present at puberty with gynecomastia, delayed sexual development, and small testes. In Danish study of 25000 school boys aged 15-16 years, 16 were found to have previously undiagnosed Klinefelter's syndrome. Comparison of these boys with normal controls indicated that on average patients with Klinefelter's syndrome had increased height, reduced weight, impaired hearing, slightly lower intelligence, poor school performance and increased incidence of behavioral problems. Adult men often present with infertility. Azoospermia is the rule in men with Klinefelter's syndrome who have 47, XXY karyotype. In one study 39 of 40 patients were azoospermic, and the only remaining man had severe oligozoospermia.

Long legs are the hallmark of Klinefelter's syndrome. Long lower extremities are not related to androgen deficiency because they are present even before puberty. The

patients have eunuchoidal proportions although the difference between the arm span and height is usually less than 10 cm.

Leg ulcers due to venous stasis, prognathism, corpus callosum atrophy, hemihypertrophy have been reported occasionally in men with Klinefelter syndrome. There are reports of systemic lupus erythematosus in men with Klinefelter syndrome responding favorably to androgen replacement therapy.

The men with Klinefelter's syndrome have a higher prevalence of breast cancer, mediastinal germ cell tumors (Bandmann et al, 1984), and lymphoma/leukemia. Endocrine diseases associated with Klinefelter's syndrome include diabetes mellitus, hypothyroidism, empty sella syndrome, hypoparathyroidism, and precocious puberty in association with hCG producing germ cell tumors.

It is a common misperception that men with Klinefelter's syndrome have mental retardation. In one study only 12 of 397 patients with 47, XXY, and 3 with 46, XY/47, XXY karyotype were mentally retarded. However verbal IQs are lower in men with the classical Klinefelter's syndrome (Bandmann et al, 1984). The boys with Klinefelter' syndrome have been reported to have poor school performance and a higher frequency of behavioral problems. Mental retardation may be associated with a higher number of X-chromosomes. In contrast of classical Klinefelter's syndrome with 47, XXY karyotype, the diagnosis of X chromosome polysomy is made before puberty in 50 percent of prepubertal children due to mental retardation.

Hypogonadism in Klinefelter's syndrome is hypergonadotrpic with high level of FSH and LH. Bone mineral density in the forearm is lower in Klinefelter's patients than healthy controls. Serum osteocalcin levels are decreased in Klinefelter's syndrome and hydroxyl proline/creatinine ratio higher, reflecting decreased bone formation and increased bone resorption.

Testicular histology in men with Klinefelter's syndrome shows hyalinization of seminiferous tubules and absence of spermatogenesis (Paulsen et al, 1968; Gordon et al, 1972). Patients with mosaicism may have normal size testis and spermatogenesis at puberty. However, progressive degeneration and hyalinization of seminiferous tubules takes place after puberty. In some men, the tubular dysgenesis is patchy; degenerating tubules are interspersed with apparently normal tubules. The Leydig cells appear to be increased although their function is impaired.

The 47, XXY karyotype in patients with Klinefelter's syndrome results from nondysjunction during the first meiotic division in one of the parents. Nondysjunction of maternal chromosomes is the cause of 47, XXY karyotype in two-thirds of affected men. Advanced maternal age is a risk factor for nondysjunction. The mechanism by which an extra X chromosome renders patients infertile is not known. In male germ cells inactivation of the single X chromosome in primary spermatocytes of heterogametic males is necessary for spermatogenesis to proceed through meiosis. The necessity for X inactivation in male germ cell differentiation in heterogametic species is not clearly understood, however, inactivation of the single X may be necessary for normal sex chromosome pairing or to prevent expression of some X-linked genes that are detrimental to spermatogenesis.

Noonan's Syndrome, The Male Turner's Syndrome. These patients have 46, XY karyotype, male external genitalia, and clinical stigmata of Turner's syndrome (Sharland et al, 1992; Chaves-Carballo and Hayles, 1966). The clinical features may

include webbed neck, short stature, low-set ears, ptosis, shieldlike chest, lymphedema of the hands and feet, cardiovascular anomalies, and cubitus valgus. The testis size is reduced and the Leydig cell function is impaired. Sterility and cryptorchidism are common.

XYY Syndrome. There is a higher frequency of 47, XYY karyotype in men with tall stature and nodular cystic acne, among prisoners and mental hospital patients (Schiavi et al, 1978; Santen et al, 1970). The mean intelligence quotient and educational level are lower in 47, XYY individuals than age-matched healthy controls. It is not clear whether the high incidence of convictions for criminal behavior in 47, XYY men is due to the diminished intellectual function or to the effects of the extra Y chromosome on aggressive behavior.

Mixed Gonadal Dysgenesis (45, X/46, XY). The patients with mixed gonadal dysgenesis usually have a 45, X/46, XY karyotype, and typically a testis on one side and a streak gonad on the other (Davidoff and Federman, 1973). Some degree of ambiguity in the genitalia is usual including varying degree of fusion of the labial folds, and the presence of urogenital sinus, phallic enlargement, and hypospadias. The phenotypic males with mixed gonadal dysgenesis often have abdominal testis with normal Leydig cells but without any germ cells (Davidoff and Federman, 1973). The dysgenetic gonad is at a high risk for neoplastic degeneration into a gonadoblastoma or seminoma/dysgerminoma.

XX Males. These patients have a male phenotype and normal looking testis, but are azoospermic and have high LH and FSH levels (Page et al, 1985 Page et al, 1985; Perez-Palacios et al, 1981). The presence of the testis and the male phenotype suggests that the genes required for the testis determination, including the Sry gene, are present in the genome of these patients (Page et al, 1987 Page et al, 1985). However, because they lack other Y-specific genes required for spermatogenesis, they are sterile. The portion of the Y chromosome that contains the Sry may be translocated onto the X chromosome or an autosome; a few patients may be mosaics and carry some 46, XY cell lines.

Other Chromosomal Disorders Associated with Gonadal Dysfunction. Men with Down's syndrome have mild testicular dysfunction characterized by varying degree of reduction in germ cell number or spermatogenic arrest, and elevated LH and FSH levels (Swersie et al, 1971).

The Y Chromosome Microdeletion Syndrome

Tieopolo and Zuffardi (Tiepolo and Zuffardi, 1976) examined the karyotypes of 1,145 men and found 6 that had large deletions of the long arm of the Y chromosome; all of these men were azoospermic. Based on these observations, Tiepolo and Zuffardi (Tiepolo and Zuffardi, 1976) correctly predicted that deletions of this region would lead to infertility. Vergnaud et al. (Verganaud et al, 1986) developed a seven-interval

map of the Y chromosome using widely spaced hybridization probes. The Page laboratory (Vollrath et al, 1992) developed the first detailed sequence-tagged site (STS) and yeast-artificial chromosome (YAC) map of the Y chromosome; this made it possible to detect and map the Y chromosome deletions with much higher resolution.

Large deletions of the Y chromosome that could be seen under the microscope in late prophase and hence detectable on routine karyotype are uncommon in infertile men. However, submicroscopic deletions of the long arm of Y chromosome, that are not detectable on karyotype and hence called microdeletions, are present in 5%-15% of azoospermic men (Vogt et al, 1992; Nagafuchi et al, 1993; Najmabadi et al, 1996; Henagariu et al, 1994; Reijo et al, 1995; Ma et al, 1992). These microdeletions can be detected by polymerase-chain reaction (PCR)-based sequence-tagged site mapping or by Southern hybridization. Most initial studies had focused on infertile men with severe defects of spermatogenesis i.e. those with azoospermia. However, more recent studies have shown that Y deletions are also present in oligozoospermic men (Reijo et al, 1996, Bhasin, Ma, Mallidis, and De Kretser, unpublished data). Most infertile men with Y deletions have severe defects of spermatogenesis i.e. they have either azoospermia or severe oligozoospermia (Najamabadi et al, 1996, unpublished data). Although the total number of infertile men with Y deletions that have been studied in detail is small, most of these patients have had testicular volumes of less than 15 ml and elevated FSH levels. The testicular histologies in the small number of reported cases of Y deletions have revealed either Sertoli Cell Only or germ cell arrest phenotype. The limited number of patients in whom testicular histology has been examined has not allowed a correlation between the location and size of the deletion and the histologic phenotype. However, Vogt et al. (Ma et al, 1993) have reported that three loci can be identified in Yq, termed AZFa, AZFb, and AZFc, wherein deletions cause specific histopathologic features in the testis.

Two Y-specific candidate gene families have been cloned by deletion mapping of infertile men with Yq deletions and proposed as candidates for the putative AZF locus, the RBM (*R*NA *B*inding *M*otif containing) gene family (Najmabadi et al, 1996; Eberhardt et al, 1996), and the DAZ (*d*eleted in *az*oospermia) gene family (Reijo et al, 1996). Both are multiple-copy gene families (Saxena et al, 1996; Cooke et al, 1996) that contain the RNA binding motif. The RBM gene family has more than 30 copies spread throughout the Y chromosome, most of the copies are located in deletion intervals 6A and 6B. At least two members of the RBM gene family, RBM-1 and RBM-2 are expressed in the testis (Najmabadi et al, 1996). The presence of the RNA-binding motif in the predicted protein sequence suggests that these genes play a role in RNA processing; however, the precise role of the RBM protein/s in germ cell development remains unclear.

The DAZ gene family is also a multiple-copy gene family (Saxena et al, 1996). The mouse and Drosophila homologs of the DAZ have been mapped to chromosomes 17 and 3, respectively (Eberjardt et al, 1996; Cooke et al, 1996). An autosomal homolog of the DAZ has also been identified in the human and mapped to chromosome 3 (Reijo et al, 1996; Saxena et al, 1996). In Drosophila, mutations of the DAZ homolog *boule* are associated with meiotic arrest and azoospermia (Eberhardt et al, 1996). Thus, in Drosophila, *boule* plays a role in the regulation of meiosis. In

infertile men with DAZ deletions, both meiotic arrest and Sertoli Cell only phenotype have been described; it is possible that germ cell degeneration may occur secondarily.

The precise physiologic function and role of the RBM and DAZ gene families in human spermatogenesis remains unclear. The RNA molecules that are the targets of these RNA binding proteins have not been identified. It is also not clear how deletions of one or two copies could explain infertility when there are multiple copies of these genes elsewhere on the Y chromosome.

Although deletions involving the DAZ gene/s appear to be the most frequent, a large proportion of Y deletions are outside the DAZ region; some of these involve the RBM gene. A significant proportion of infertile men with DAZ deletions is oligozoospermic and not azoospermic. Furthermore, only 10-15% of infertile men has Y deletions. These data suggest that additional Y-specific and/or autosomal genes may be involved in other infertility phenotypes.

Autosomal Gene Defects and Male Infertility

Bilateral Congenital Absence of Vas Deferens and the CFTR Mutations. There is a high prevalence of bilateral congenital absence of vas in men with cystic fibrosis. Mutations in the coding region of the cystic fibrosis conductance regulator gene may result in congenital absence of vas without causing the classical pulmonary disease (Aguiano et al, 1992). Fifty to seventy percent of men with congenital absence of the vas deferens harbor mutations of the cystic fibrosis transmembrane conductance regulator gene. About 50% are homozygous for the common cystic fibrosis gene abnormality such as F508, and some have compound heterozygosity (i.e. they have two separate mutations on the two alleles). We do not know the precise role of the CFTR gene in the development of the Wolffian structures.

Gonadal Dysfunction Associated with Sickle Cell Disease and Beta-Thalessemia. Sickle cell disease is an autosomal recessive disorder that results from a point mutation in the beta-globin gene. The mutation produces excessive amounts of hemoglobin S in the red cells. A significant proportion of men with sickle cell disease has low testosterone levels. A majority of men with sickle cell disease that has low testosterone levels suffers from primary testicular dysfunction (Abbasi et al, 1976; Landfeld et al, 1993; Dada and Nduka, 1980). It is assumed that testicular dysfunction results from micro-infarcts in the testis because of the vaso-occlusive disease. However, hypogonadotropic hypogonadism due to hypothalamic-pituitary dysfunction has been reported in men with sickle cell disease (Landfeld et al, 1993).

The pathophysiology of hypogonadism in thalessemic disorders is different from that in sickle cell disease. The pituitary and gonadal dysfunction occurs in thalessemia due to iron deposition in these tissues (Kletzky et al, 1979; DeSanctis et al, 1988). Multiple transfusions and ineffective erythropoeisis result in iron overload and parenchymal damage. In contrast to patients with sickle cell disease, hypogonadotropic hypogonadism is the predominant form of androgen deficiency syndrome in men with thalessemia and can be treated effectively with gonadotropin replacement therapy. Pituitary and testicular overload and the resulting hypogonadism can be prevented by prophylactic iron-chelating therapy.

Testicular Dysfunction in Myotonic Dystrophy. Myotonic dystrophy is an autosomal dominant disorder associated with CTG repeats in the dystrophin gene. Testicular atrophy occurs in 75% of these men primarily due to degeneration of the seminiferous tubules. Although Leydig cells are preserved, serum testosterone levels are low in many patients (Takeda and Ueda, 1977). Most men with myotonic dystrophy and androgen deficiency have high LH and FSH levels consistent with primary testicular failure (Takeda and Ueda, 1977). The degree of androgen deficiency correlates with the length of the CTG repeat (Mastrogiacomo et al, 1994).

Miscellaneous Disorders Associated with Infertility. Men with diabetes mellitus may experience infertility if they have retrograde ejaculation due to autonomic neuropathy or if they are in poor glycemic control. Impotence is common in men who have had diabetes for more than 10 years. Males with myelodysplasia often suffer from ejaculatory disorder due to their neurologic defects (Jaffe and Oates, 1994). Others have also reported impairment of spermatogenesis in some patients with this form of neurospinal dysraphism.

MALE STERILE MUTATIONS IN DROSOPHILA (Table 1)

The male-sterile mutations occur frequently in natural populations of *Drosophila*. The spermatogenesis in *Drosophila* is extremely sensitive to many metabolic stresses, leading to high frequency of mutations that can cause sterility by pleiotropic effects. For instance, 30% of temperature-sensitive lethal mutations do not actually cause death but render males sterile at restrictive temperature after development at permissive temperature (Lindsley and Tokuyasu, 1980). It has been estimated that the number of male-sterile mutations in *Drosophila* could be as high as 2400 (Shellenbarger and Cross, 1977; Lin et al, 1996). This number seems very high and the assumptions behind this estimate have been questioned (Erdelyi, 1997).

Sterility in male flies can result from abnormal testicular development, reduced number of germ cells, meiotic arrest, defects in post-meiotic differentiation, as well as problems in mating behaviour.

Drosophila Mutations Associated with Reduced Number of Germ Cells.

Mutations in the *Chickadee* and the *Diaphanous* genes are associated with reduced number of germ cells in the testis.

Drosophila Mutations Associated with Meiotic Defects.

Defective expression of several genes can result in meiotic arrest. For instance, mutations of *Always Early Mutation*, *Cannonball*, *Meiosis Arrest 1*, and *Spermatocyterren* result in failure of the germ cells to enter into meiosis (Horowitz,

Table 1. Naturally Occurring Sterile Mutants in Drosophila

Infertility due to Defects in Gonadogenesis and Germ Cell Migration		
Serpent, Hucklebein		Both of these mutations are associated with abnormal germ cell migration
Columbus, heartless		Abnormal mesoderm migration
AbdominalA, abdominal B	*Abd-A, abd-B*	Abnormal germ cell association with gonadal mesoderm
Zfh-1		Attachment of germ cells to somatic cells
Bocce	boc	In males, they have small testes and variable nuclear size in spermatocyte cysts. They are infertile. Females are semi-sterile; number of eggs laid is reduced.
Cueball	cue	In males, testis is short with detective sheath. Also, spermatocytes contain cytoplasmic abnormalities. Females are semi-sterile; ovaries are small and misshapen.
Hephaestus	heph	Tip of the testes is enlarged in circumference to approx. twice that of wild type.
Abnormalities of Male Sexual Differentiation		
Sex-lethal	*sxl*	Male lethality due to inadequate X-dosage compensation
Transformer, transformer-z	*Tra, tra-z*	Aberrant tra mRNA splicing leading to female differentiation
RBP1		Aberrant splicing of mRNA of the SR gene
intersex	*ix*	XX female intersex mutants
Double sex	*Dsx*	Intersex mutants, female repressor
Infertility Due to Reduced Germ Cells		
Chikadee	*Chic*	Results in testes with reduced

		germinal content; the males are sterile.
Diaphanous	*Dia*	Males are infertile; those that are at least five days old have empty testes. In females, ovaries have reduced egg chambers, rendering them semi-sterile.
Infertility due to Meiotic Defects		
Always early mutation Cannonball Meiosis Arrest Spermatocyt-rren	*Aly* *Can* *Mia* *Sa*	All of these mutations result in meitotic arrest. These genes are required for entry into the first division and progression into spermatogenesis. The post meitotic stages of spermatogenesis are absent. Mutations result in infertility in males.
Degenrative spermatocyte	*Des*	Failure to initiate meiotic chromosomal condensation
Twine (cdc25 homolog), cdc2		Meiosis is skipped and spermatid differentiation proceeds abnormally
Pelota	*Pelo*	Males are infertile because of meiotic arrest.
Defects in Post-Meiotic Differentiation		
Fumble	*Fbl*	In males, testes contain degenerating spermatids with large nebenkerne and micronuclei at onion stage.
l(2)26Ab	*l(2)26ab*	Testes are short, and filled with cysts of 16 cell spermatocytes, which degenerate prior to completion of growth phase, resulting in infertility in males.
Boule	*Bol*	Some 16-cell cysts resemble pelota; others contain nuclei in addition to the abnormally large nebenkerne.
Shank	*Shk*	Spermatids contain 2 or 4 nuclei associated with a single large nebenkerne, resulting in infertility in males. In addition, females are semi-sterile.
Cashews Dispersed Effete	*Cas* *Disd* *Eff*	In the males of these Drosophila mutants, the elongated spermatid nuclei are dispersed.
Halley	*Hal*	In males, spermatid nuclei fail to

		elongate, and the mutation is lethal.
Scattered Thousand points of light	*Sat* *Tho*	Elongated spermatid nuclei are dispersed.
Scratch	*Stc*	Needle-shaped crystals accumulate through developing germline resulting in male-sterility.
Doublefault	*Dbf*	In males, spermatid nuclei fails to change shape, rendering them infertile.
Infertility Due to Behavioral Defects		
Cuckold	*cuc*	Males are semi-sterile due to failure to court females.
Fruitless	*fru*	Males are infertile because they court both females and males, but fail to mate because abdominal muscle is reduced.
Pointless	*ptl*	Males are semi-sterile with wild types levels of motile sperm in seminal vesicle. Little or no sperm is transferred to females.
Ken and Barbie	*ken*	External genital structures are absent in some males and females. Aristae are sparse and unpigmented. Breakdown in courting. Mutation is semi-lethal, and both males and females are semi-sterile.

1996); therefore, the post-meiotic stages of spermatogenesis are completely absent. In flies with mutations of the *boule* gene, the Drosophila homolog of the human DAZ gene (Eberhardt et al, 1996), the germ cells enter the meiosis, but the meiotic division does not progress to completion resulting in the production of cysts that are suspended at the 16-cell stage. The *che caos* (chec) mutation is associated with a dramatic disruption of male meiosis: cytological analysis of homozygous testes shows alterations in spindle structures and chromosome separation. *Diaphanous (dia)* and *four wheel drive* are required for cytokinesis. With a mutation in *fireworks (fir)* gene chromosome segregation is highly impaired in the second meiotic division. Several genes on Drosophila chromosome 1 *including ms(1)RD15, ms(1)RA40, ms(1)244,* and *ms(1)202,* are required for meiosis although their precise function is not known.

Drosophila Mutations Associated with Defective Postmeiotic Differentiation.

Defects in post-meiotic differentiation have been described with mutations of *Capon (cap), cashews (cash), dispersed (dis),* and *emmenthal (emm). Pelota* gene encodes for a protein that is required for meiotic cell division (Bhat, 1996). Deficient function of this protein is associated with abnormally large nebenkern in late spermatocytes. Drosophila with mutations of the *ms(3)72D* have sickle shaped nuclei in postmeiotic cells. *Bobble* mutation result in cysts that contain spermatids of an abnormal size (Tomkiel, 1995). *Doublefault* mutations render the spermatid unable to change shape (Li et al, 1998).

Drosophila Mutations Associated with Abnormal Mating Behaviour.

Another category of genes that includes *Fruitless, Cuckold,* and *Pointless* mutants leads to infertility because of a breakdown in courtship between males and females (Ahmad, 1998; Bridges, 1916).

Y Chromosome Abnormalities in Drosophila.

The Y chromosome plays a key role in regulating spermatogenesis in *Drosophila.* The lack of the Y chromosome in XO males of *D. melanogaster* leads to meiotic arrest at or before metaphase I (Lin et al, 1996; Kiefer, 1966; Lifschytz and Hareven, 1977; Lifschytz and Meyer, 1977; Stern, 1929). The male *D. melanogaster* with X-Y translocations carrying either the long arm, XY^{L}/O, or the short arm, XY^{S}/O, of the Y chromosome are sterile. This led Stern (Stern, 1929) to propose that each arm of the Y chromosome carries a complex of fertility genes (Kl or Ks) that are essential for male fertility. Brosseau (Brousseau, 1960) induced male-sterile mutations on free Y chromosomes and tested different Y chromosomes (Y^{i}, Y^{j}) for complementation in an $X/Y^{i}/Y^{j}$ constitution. In additional experiments, the sterile Y chromosomes were complemented by different X-Y translocations. He then produced a genetic map for the Y-chromosomal, male-fertility genes in D. melanogaster, in which the fertility complexes were subdivided into seven fertility loci, five (kl-1 to kl-5) on the long arm and two (ks-1, ks-2) on the short arm. Brosseau (Brousseau, 1960) suggested that the mutation of any single male-fertility gene would sterilize the males as effectively as the loss of the complete Y chromosome. Indeed, the deletions of the regions containing kl-5, kl-3 and ks-1 prevent the formation of the outer dynein arm of the axoneme. The deletion of ks-2 disturb the proper apposition of axoneme and nebenkern and lead to a complex phenotype with nuclear crystal formation and abnormal meiosis (Hulsebos et al, 1984; Hess, 1970).

A lampbrush-loop like structure is formed from the Y chromosome in the primary spermatocyte of *D. melanogaster* and all other Drosophilids studied (Hess and Meyer, 1968; Beermann et al, 1967). Each of the five pairs of lampbrush loops observed in *D. hydei,* has a specifically defined morphology, is ordered linearly on the Y chromosome, and is essential for male fertility (Bonaccorsi et al, 1988). The inactivation of one or more loops renders the males carrying them sterile (Hess and Meyer, 1968).

The fertility genes in *D. hydei* are mainly composed of complex, locus-specific repetitive DNA sequences which are transcribed stage-specifically in the primary spermatocyte nucleus as continuous long transcription units with a length ranging from 260 kb to up to 4000 kb (Vogt and Hennig 1986). The fertility locus on the short arm of Y chromosome of *D. hydei*, known as locus Q which forms the lampbrush loop nooses, reveals no open reading frames (Hennig, 1987). However, these Y-specific sequence shows a high capacity to form secondary structures due to repeats, leading to speculation that these DNA sequences may be involved in DNA-protein interaction (Erdelyi, 1997). Different parts of this sequence share homology with the autonomously replicating sequences of yeast, the α and β sequences which promote the amplification of the chorion genes of *D. melanogaster,* and to some enhancers of transcription. The role of these repetitive sequences in the function and evolution of the lampbrush loop is not known (Hennig, 1987). Sequences homologous to the *Drosophila* fertility gene sequences have been mapped to deletion interval 6 of the human Y chromosome. It is notable that Y chromosome sequences pY6HP35, pY6HP52 and pY6BS65/E, that have homology to the Drosophila fertility gene sequences were deleted in four of our severely oligospermic or azoospermic men with a cytologically normal Y chromosome (Ma Kun, unpublished data).

MOUSE MODELS OF STERILITY (TABLE 2)

In mice, all the described reciprocal X-autosome translocations arrest spermatogenesis at the pachytene/metaphase I (MI) stage of the primary spermatocyte and render males sterile (Lyon et al, 1964; Russell, 1983; Eicher et al, 1991; Searle, 1974; Pilder et al, 1993; Quack et al, 1988). The Y-autosome translocations in mice are often but not always male-sterile (Lyon and Meredith, 1966; de Boer, 1976) with a breakdown at diakinesis or MI (Searle, 1974). Approximately 20% of purely autosomal reciprocal translocations in the mouse are associated with male sterility. Many cases of human infertility as a consequence of autosomal chromosome aberrations, including translocations and insertions, have been reported (Searle, 1974; Quack et al, 1988; Lyon and Meredith, 1966; de Boer, 1976), although chromosomal translocations are not a common cause of infertilty in man.

The mouse ortholog of the human X-linked gene A1S9 encodes the ubiquitin-activating enzyme E1 (Sutcliffe and Burgoyne, 1989). Two copies of this gene, A1s9Y-1 and A1s9Y-2, are present on the mouse Y chromosome. The deletion of the functional copy, A1s9Y-1 is associated with sterility, leading to speculation that A1s9Y-1 may be a candidate for the spermatogenesis gene, Spy, which has been mapped to this region of the Y chromosome.

Mice with inactivating mutations at the *W* and *Sl* loci are sterile in addition to having defects in hematopeisis, neural development, and skin pigmentation (Zsebo et al, 1990; Godin et al, 1991). The testicular histology in these mice is similar to that in men with Sertoli cell only syndrome. Stem cell factor plays a critical role in primordial germ cell proliferation, migration, and survival (Godin et al, 1991). The demonstration that mice with W and *Sl* locus abnormalities have deletions of the *c-kit*

and SCF genes has led to speculation that mutations of the SCF gene or its receptor might be responsible for at least a subset of men with the Sertoli cell only phenotype.

Table 2. Mutations Affecting Fertility in Male Mice

Gene symbol	Gene name	Chromosome	Effects and References
Hpg	Hypogonadotropic mouse		A large deletion of exons 3 and 4 of GnRH gene resulting in deficiency of LH and FSH and hypogonadotropic hypogonadism
Bc	Blind-sterile	--	Abnormal spermiogenesis (Sotomayor & Handel, 1986)
$_c3H/_c6H$	Albino-deletion heterozygotes	--	Abnormal spermiogenesis (Lewis et al, 1978)
Hop	Hop-sterile	--	Polydactyly. Sperm tails absent or aberrant (Johnson and Hunt, 1971)
Hpy	Hydrocephalic polydacty	6	Polydactyly. Sperm abnormal and immotile (Hollander, 1976)
Olt	Oligotriche	--	Azoospermia (Moutier, 1976)
*p*bs	*p*-Black-eyed sterile	7	Coat colour diluted. Sperm abnormality (Handel, 1987)
p^{6H}, p^{25H}, p^{s}	Pink-eyed, sterile	7	Abnormal spermiogenesis (Johnson and Hunt, 1971)
Pcd	Purkinje-cell degeneration	--	Abnormal spermiogenesis (166)
Qk	Quaking	17	Defects of myelination (Handel, 1987) and of spermiogenesis (Bennett et al, 1971)
$_t{}^x/_t{}^x$	t-haplotypes	17	Abnormal spermatids, few spermatozoa (Dooher and Bennett, 1974)
$T^x/_t{}^y$	t-haplotypes	17	Spermiogenic defects, failure of sperm function (Lyon, 1981)
Sl locus mutation	SCF mutations		Anemia, fur pigmentation defect, infertility
W locus mutation	c-kit mutation		Anemia, fur pigmentation defect, infertility
An (Hertig's anemia)			Anemia, infertility
At (atrichosis)			Decreased hair density, infertility
Wr	Wobbler		Abnormal spermiogenesis (Handel, 1987)

Mice homozygous for the M. spretus allele of a *t* complex gene called *Hst-1* have abnormalities of sperm flagellar curvature and are sterile (Pilder et al, 1993). The gene (s) responsible for flagellar curvature have not been cloned. The principal morphological abnormality appears to be the failure to form an axoneme. Defects of *t* haplotype are also associated with defective sperm egg interaction. DNA sequences homologous to the mouse *t* locus have been described on human chromosome 6. However, human genes or cDNAs that subserve similar function have not yet been characterized.

Mouse Sterility Associated with Insertional Mutagenesis.

Introduction of a transgene into the genome of the host animal can occasionally disrupt the expression of a functional gene. The insertional mutations that are associated with sterility are of interest because they provide clues to the genes that are essential for spermatogenesis.

Russell et al. (Russell et al, 1991) described a line of transgenic mice in which the developing spermatids form multinucleated syncytia (symplasts) and do not undergo maturation into sperm. These symplasts undergo degeneration and are phagocytosed by Sertoli cells. This mutation has been mapped to mouse chromosome 14, in proximity to the gene encoding esterase-10 gene.

In a transgenic mouse line (Magram and Bishop, 1991), carrying the HCK protooncogene, the males hemizygous for the mutation are sterile although they can mate normally. The spermatogenesis is normal but the resulting sperm have abnormally shaped nuclei. This insertional mutation, called *lacking vigorous sperm* (*Lvs*), is transmitted as a dominant trait.

Germ cell deficient (*gcd*) is a recessive mutation characterized by sterility in both males and females due to depletion of the germ cells in the gonads (Pellas et al, 1991). The somatic cells are normal. Deficiency of germ cells is apparent as early as embryonic day 11.5.

Yokoyama et al. (Yokoyama et al, 1993) reported reversal of left right symmetry in a family of transgenic mice. This insertional mutation was mapped to mouse chromosome region 4 between *Tsha* and *Hxb* loci. However, these mice with *situs inversus* do not have defects of ciliary motility, abnormalities of dynein arms, or infertility and are, therefore, not a good model for the Immotile Cilia Syndrome.

A line of transgenic mice carrying the human epidermal growth factor transgene is sterile because of axonemal abnormalities in their sperm tails. The testicular sperm have normal ultrastructure, but the epididymal sperm have only 5 or 6 microtubular doublets instead of the usual complement of nine.

Over-expression of some gene products in the testis can adversely affect spermatogenesis. The transgenic male mice that overexpress human growth hormone have larger testes than wild type mice, but these animals have a higher incidence of infertility (Bartke et al, 1992). The male mice that overexpress interferon gene product in the testis experience degeneration of spermatogonia and atrophy of their seminiferous tubules (Hekman, 1988). Over-expression of the murine interleukin-2 is also associated with testicular atrophy and spermatogenic arrest (Ohta et al, 1990).

Null Mutations of Many Genes Are Associated with Infertility in Knock-out Mice (Table 3).

With the availability of stem cell technology, investigators have produced null mutations in a number of genes. The phenotype resulting from the "knockout" can provide useful clues to the function of the gene. Sometimes the gene knockout produces no discernible phenotype presumably because the function of the disrupted gene is taken over by another gene product. In addition, the presence of sterility in knockout mice produced by the embryonic stem cell technology does not necessarily establish a role for its gene product in spermatogenesis. Although we assume that the embryonic stem cells are totipotent and can differentiate into all cell types in the body including germ cells, these cells after multiple passages may lose their ability to differentiate into germ cells leading to the absence of germ cells in the testis. Therefore, the results of the knockout experiments using the embryonic stem cell technology should be viewed cautiously within the context of the naturally occurring mutations.

Table 3. Targeted Gene Mutations in Knock-out Mice Associated with Male Sterility

GENE NAME: (REFERENCE)	THE EFFECT OF THE TARGETED MUTATION/PHENOTYPE:
MUTATIONS ASSOCIATED WITH DEFICIENT GONADOTROPIN SECRETION	
CSF-1 (Colony Stimulating Factor-1) (Cohen et al, 1997)	Reduced testosterone levels due to low serum LH levels Disruption of the normal testosterone negative feedback response of the hypothalamus Reduced mating ability and low sperm numbers
MUTATIONS ASSOCIATED WITH IMPAIRED LEYDIG CELL FUNCTION AND REDUCED TESTOSTERONE LEVELS	
HNF-1 alpha (Hepatocyte Nuclear Factor 1 alpha) (Lee et al, 1998)	Reduced testosterone levels and sterility Growth retardation, and non-insulin dependent diabetes mellitus (NIDDM)
IGF-1 (Baker et al, 1996)	Failure of androgenization due to reduced testosterone levels Testes reduced in size, spermatogenesis sustained at only 18% of normal Impaired mating behavior and sterility Dwarf (growth retardation)

Sp4 (Supp et al, 1996)	Reduced testosterone levels leading to impaired mating behavior Growth retardation 2/3 die within first days of birth
MUTATIONS ASSOCIATED WITH ABNORMAL GERM CELL DEVELOPMENT AND/OR DEFICIENCY OF GERM CELLS IN THE TESTIS	
TIAR (Beck et al, 1998)	Reduced survival of primordial germ cells that migrate to the genital ridge around embryonic day 11.5; failure of development of spermatogonia
Zfx (Luoh et al, 1997)	Reduction in number of primordial germ cells prior to gonadal sex differentiation; subfertile due to reduced sperm count Dwarf; less viable
FAC (Whitney et al, 1996) (Fanconi Anemia Complementation)	Reduced number of germ cells Normal neonatal viability and gross morphology Cells have chromosome breakage and DNA cross liner sensitivity; progenitor cells hypersensitive to interferon gamma
Bmp 8B (Zhao et al, 1996)	Failure or reduction of germ cell proliferation; in adults significant increase in programmed cell death of spermatocytes leading to sterility
MUTATIONS ASSOCIATED WITH MEIOTIC DEFECTS	
Mlh 1 (Baker et al, 1996)	Spermatocytes exhibit high levels of prematruely separated chromosomes and arrest in first division of meiosis; microsatellite instability
Pms 2	Abnormal chromosome pairing in meiosis; microsatellite instability Predisposition to tumors
A-myb (Toscani et al, 1997)	Germ cells enter meiotic prophase and arrest at pachytene Growth defects

ATM (Xu et al, 1996) (Ataxia-telangiectasia)	Meiosis arrested at the zygotene/pachytene stage of prophase 1 due to abnormal chromosomal synapses and chromosome fragmentation Growth retarded Majority develop thymic lymphomas and die before 4 months of age
Hsp70-2 (Dix et al, 1996)	failure of meiosis concident with dramatic increase in spermatocyte apoptosis; lack post meiotic spermatids and mature sperm
MUTATIONS ASSOCIATED WITH POST MEIOTIC DEFECTS IN SPERMIOGENESIS	
Bclw (Ross et al, 1998)	Spermatogenesis is blocked during late spermatogenesis in young adults; gradual depletion of all stages of germ cells by 6 months of age. Later, Sertolic cells are lost from the seminiferous tubules and the Leydig cell population is reduced
RXR beta (Kastner et al, 1996)	Failure of spermatid release within germinal epithelium and epididymis contained few Spermatazoa; the spermatozoa exhibit abnormal acrosomes and tails 50% die before or at birth; the 50% that survive are sterile
Sprm-1 (Supp et al, 1996)	Sperm functionally compromised due to interruption of the regulatory function of haploid Spermatids; subfertile compared to heterozygous and wild types Normal testes morphology; normal sperm production
hHR6B (Roest et al, 1996)	Derailment of spermatogenesis during post meiotic condensation of chromatin in spermatids
CREM (Cyclic AMP-responsive Element modulator) (Hendy et al, 1996)	Developing spermatids fail to differentiate into sperm; post meiotic gene expression in the testis declines dramatically; no sperm production

MUTATIONS ASSOCIATED WITH POST-TESTICUALR DEFECTS IN SPERM MATURATION, FERTILIZATION, OR EMBRYONIC DEVELOPMENT	
Testis ACE (Angiotensin-Converting Enzyme) (Hagaman et al, 1998)	Sperm show defects in transport within oviducts and binding to zona pellucidae; reduced fertility
Apo-B (apolipoprotein B) (Huang et al, 1996)	Sperm show abnormal binding to the egg after fertilization; reduced sperm motility, survival time, and sperm count; reduced fertility
C-ros (Sonnenberg-Riethmacher et al, 1996)	Defective epididymis; defective sperm maturation and fertilization; reduced fertility
PC4 (Mibikay et al, 1997)	In vivo fertility of spermatazoa of males severely impaired in absence of evidence of spermatazoa abnormality; egg fertilized by sperm fails to grow to the blastocyst stage
INSUFFICIENT INFORMATION AVAILABLE TO ACCURATELY CLASSIFY THE CAUSE OF INFERTILITY	
DAZLA(Deleted in Azoospermia) (Ruggiu et al, 1997)	Loss of germ cells and complete absence of sperm in homozygous mice; heterozygotes have few sperm that are abnormal. Phenotype different from the Drosophila *boule* that shows meiotic arrest.
Bax (Knudson et al, 1995)	Disordered seminiferous tubules with an accumulation of atypical premeiotic germ cells, but no mature haploid sperm; hyperplasia or hypolasia
Bmp 8A (Bone Morphogenetic Protein) (Zhao et al, 1998)	Disruption of spermatogenesis; degeneration of germ cells and epididymal epithelium
ER (Estrogen Receptor) (Eddy et al, 1996)	Disruption of spermatogenesis and degeneration of seminiferous tubules ; reduced mating frequency; low sperm numbers; defective sperm function

Mouse Mutations Associated with Androgen Deficiency. Disruption of some genes can be associated with impaired testosterone secretion. Thus mice null for the IGF-1 expression have impaired mating behavior due to lower testosterone levels (Baker et

al, 1996). Targeted mutations of hepatocyte nuclear factor-1 alpha (Lee et al, 1998), and Sp4 (Supp et al, 1996) also result in decreased testosterone secretion and failure to copulate. Mice with inactivating mutations of the colony stimulating factor-1 (Cohen et al, 1997) have reduced LH levels and consequently are androgen deficient.

Mouse Mutations Associated with Reduced Number of Germ Cells. Disruption of the *FAC* (Fanconi anemia complementation gene), the DAZ gene, and the TIAR gene is associated with a reduced number or absence of germ cells in the testis of these animals (Beck et al, 1998; Ruggiu et al, 1997; Hsieh-Li et al, 1995). The TIAR mutation is associated with reduced survival of primordial germ cells that migrate to the genital ridge around embryonic day 11.5; consequently, spermatogonia fail to develop (Beck et al, 1998). Mice with null mutations of the DAZLA gene experience loss of germ cells and complete absence of gamete production (Ruggiu et al, 1997). The FAC knockout mice suffer chromosomal breakage, have reduced number of germ cells in the testis and are sterile (Whitney et al, 1996).

Hoxa 11 knockout disrupts spermatogenesis due to failure of testicular descent and malformation of the vas deferens (Hsieh-Li et al, 1995).

Mouse Mutations Associated with Defects in Meiosis. Targeted mutations of *A-myb, Bclw, Bmp 8A*, ataxia-telangiectasia, and *Hsp70-2* genes are associated with meiotic defects (Ross et al, 1998; Zhao et al, 1998; Toscani et al, 1997; Xu et al, 1996; Dix et al, 1996). The testis of mice, null for the *A-myb* gene, show germ cells entering the meiotic prophase but arresting at pachytene. Knock-out of the ataxia-telangiectasia gene results in meiotic arrest at the zygotene or pachytene stages due to abnormal chromosomal synapses and fragmentation (Xu et al, 1996). The failure of meiosis in *Hsp70-2* mutant mice is associated with increased spermatocyte apoptosis; these mice lack post-meiotic spermatids and sperm (Dix et al, 1996)

Mouse Mutations Associated with Defects in Post-meiotic Differentiation. Mutations of the *hHR6B* (Roest HP et al, 1996) and *Sprm-1* (Pearse RV et al, 1997) genes are associated with defects of post-meiotic differentiation. There is failure of post-meiotic chromatin condensation in mice mutant for the hHR6B gene (Roest HP et al, 1996). *Sprm-1* mutations result in production of functionally compromised sperm (Pearse et al, 1997).

Some mutations in are associated with sterility although they produce no apparent abnormality in spermatogenesis.

SYNOPSIS

Infertility in men is a common, but heterogeneous syndrome. The pathophysiology of spermatogenic failure in a majority of infertile men remains unknown. Therefore, it is likely that a multitude of genes and loci will be implicated in different infertility subsets. Although a large number of genes and loci in experimental animals are

associated with sterility, the human homologs of most of these genes have not yet been cloned.

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14 MODERN MANAGEMENT OF MALE INFERTILITY

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About 15% of couples do not achieve a pregnancy within 12 months of trying to have a child. The man may have a disorder that accounts for, or contributes to the low fertility in up to 50% of these couples (Baker 1995a). Investigation or infertility should, from the outset involve both the man and the woman. Some treatable or reversible conditions cause male infertility but most often nothing can be done to the man to increase the chance of natural conception. Assisted reproductive technology, particularly intracytoplasmic sperm injection (ICSI) with in vitro fertilization (IVF) or donor insemination is particularly helpful for these otherwise untreatable types of male infertility.

INFERTILITY INVESTIGATIONS

Medical history, clinical examination and semen tests are usually sufficient to develop a plan of management. General health, previous genital tract disorders or surgery, symptoms and signs of androgen deficiency and careful palpation of the scrotal contents are particularly important (see Chapter 12 for discussion).

Semen analysis

The most important laboratory investigation in male infertility is semen analysis and this should be performed in a laboratory using accepted procedures (WHO 1999). Several semen tests are performed because the results may vary considerably from day to day (Mallidis et al 1991). Because there is no semen pattern characteristic of

sperm autoimmunity all men being evaluated for infertility should to be tested for sperm antibodies. The immunobead test is used either directly on sperm or indirectly to assess sperm antibodies in blood or seminal plasma. The sperm mucus penetration test could be used as an alternative method of screening for sperm autoimmunity. Leukocytospermia is detected by staining peroxidase positive cells or using monoclonal antibodies to pan-leukocyte or specific subset antigens can be used. More than 1 million/mL polymorphs in semen is considered abnormal. There is often a poor relationship between leukocytospermia and other features of genital tract inflammation such as variations in semen volume, viscosity, pH and sperm agglutination and the results of culture of semen (Aitken and Baker 1995). Routine cultures of semen are not warranted except for sperm donors. Patients with clinical evidence of recurrent bacterial prostatitis require urological management.

New tests of sperm function

Other sperm tests are now available. These include assessments of the acrosome with fluorescent dyes or antibodies, computer assisted analysis of sperm motility and morphology, and sperm-oocyte binding and penetration tests (Liu and Baker, 1992,1994; Garrett and Baker, 1995). The human sperm-zona free hamster oocyte penetration test assesses the ability of sperm to capacitate, acrosome react, fuse with the oolemma and undergo nuclear decondensation in the ooplasm (WHO, 1999). Using human oocytes that fail to fertilize in vitro it is possible to check for defects of the major phases of sperm-oocyte interaction: zona pellucida binding, zona induced acrosome reaction, zona penetration and oolemma fusion (Liu and Baker, 1992, 1994). Patients with a specific defect of sperm zona penetration because of disordered zona pellucida induced acrosome reaction have been discovered using these tests (Liu and Baker, 1994). The sperm-zona binding ratio and the proportion of zonae penetrated are powerful predictors of fertilization rates with standard IVF procedures and may be used in patients with borderline semen defects, particularly of sperm morphology, to decide whether to use IVF or ICSI (Liu and Baker, 1992).

Other tests

Blood tests may be needed to check for sperm antibodies, hormonal abnormalities, and genetic or chromosomal disorders. Ultrasound examination of the testes and prostate, testicular biopsies and exploratory operations are also performed occasionally to check sperm production and look for blockages.

Psychological Factors

Emotional reactions to the diagnosis of infertility are common including denial of the problem, anger with the partner and medical attendants, resentfulness of the need to participate in infertility tests, depression, and temporary sexual problems. These feelings are essentially normal initial psychological aspects of grief and decrease with time as an understanding of the infertility is achieved. The investigating physician should anticipate and allow for these reactions. Some couples may be helped to adjust by discussions with their doctor or other infertile couples in self-help groups.

Specialist infertility counsellors are also available to assist couples with the emotional reactions and to explore the medical and social options.

TYPES OF MALE INFERTILITY

The results of a physical examination and tests help determine whether or not a treatable condition exists (Baker, 1995a). In Western societies approximately 12% of men being seen with have untreatable sterility and 13% have treatable conditions. The other 75% have disorders of sperm production or function that do not usually have clearly defined effective treatments (Table 1).

Table 1 Clinical classification of causes of male infertility

Untreatable male sterility	**12%**	
Primary seminiferous tubule failure		12%
Treatable conditions	**18%**	
Gonadotropin deficiency		0.5%
Obstructive azoospermia		10%
Sperm autoimmunity		7%
Disorders of sexual function		0.5%
Reversible toxin effects		0.02%
Untreatable subfertility	**70%**	
Oligozoospermia		35%
Asthenozoospermia and teratozoospermia		30%
Normozoospermia with functional defects		5%

Untreatable Male Sterility

About 12% of patients have primary seminiferous tubule failure and untreatable sterility because there are no sperm in their semen or in the testes. This may be caused by chromosomal or genetic defects such as Klinefelter syndrome, androgen receptor defects and microdeletions in the long arm of the Y chromosome, previous inflammation of the testes, or treatment with cytotoxic drugs and radiotherapy (Bhasin et al, 1994). Previous bilateral undescended testes may also be associated with severe primary seminiferous tubule failure in the adult even although orchidopexies were performed in early childhood. Despite the increase in understanding of genetic causes of spermatogenic defects, the cause of the failure of sperm production remains unknown in over 50% of patients. Clinical features vary from a normal male phenotype to a man with small testes and marked features of androgen deficiency. Gynecomastia may also be present. Usually, but not always, serum FSH levels are increased and testicular volumes are reduced.

As it is now possible to attempt ICSI with just a few sperm, the absence of sperm production needs to be demonstrated carefully in this group of patients. Repeated

semen tests should be done, for example one a week for several weeks, in a laboratory that can cryopreserve semen if live sperm are seen. If no sperm can be found in the centrifuged deposits of the semen, the collection sequence could be repeated at intervals, as sperm production may be intermittent. This approach has been successful in some men with extremely severe spermatogenic disorders such as Klinefelter syndrome (Bourne et al, 1997). If there is persistent azoospermia, testicular biopsies still may reveal some spermatogenesis that might allow extraction of sperm for ICSI (Tournaye et al, 1995). While successes have been reported using testicular sperm from men with severe spermatogenic disorders there are many failures and adoption or donor insemination are required for many of these couples.

Some men with severe primary spermatogenic failure are also androgen deficient or will become so as they get older. Their general health and sexual performance are improved by replacement therapy with testosterone (see Chapter 8).

Treatable Conditions

A number of specific disorders cause infertility that can be treated to improve fertility but these are individually rare and the treatments are not invariably successful.

Gonadotropin Deficiency. (See Chapter 6) Gonadotropin deficiency is a rare cause of male infertility. Most patients have Kallmann syndrome or idiopathic combined LH and FSH deficiency that has been diagnosed in adolescence because of delayed puberty. Some have acquired gonadotropin deficiency from pituitary tumors, head injury or hemochromatosis. Starvation, excessive exercise and male anorexia nervosa and some drugs may cause functional gonadotropin suppression. Hyperprolactinemia, usually from a macroadenoma, is a very uncommon cause of infertility in men. Almost invariably men with these conditions have symptoms and signs of severe androgen deficiency. While most are azoospermic a few have sperm present in the semen. In the latter, the semen tests usually show low volume and poor sperm motility that is attributed to the androgen deficiency. The sperm concentration varies from severe oligozoospermia to normal. This is called the fertile eunuch syndrome and may be caused by partial gonadotropin deficiency, LH deficiency without FSH deficiency or, possibly, a constitutively active FSH receptor (Gromoll et al, 1996).

Gonadotropin suppression is treated by correction of the defect of food or drug intake. Hyperprolactinemia is managed by surgery and dopamine agonist drugs. Replacement therapy with hCG (for LH) and rhFSH (recombinant human FSH) can be used successfully in the management of other patients (see Chapter 6). Often patients with testicular volumes greater than 4 mL, the fertile eunuch syndrome and those who acquired gonadotropin deficiency after completing puberty respond to hCG alone. It is standard to give all gonadotropin deficient patients hCG for 6 months before starting FSH. The dose of hCG: 1500-2000U once a week, should be increased to 3000U and the frequency of administration up to 3 times a week to achieve adequate testosterone production. The response is judged clinically: the man should report adequate androgen effects on libido, hair growth and muscular strength. In patients who will respond to hCG alone testicular volume increases and sperm appear in the semen by 6 –12 months. In the others testicular volumes may

increase slightly in the first 2-3 months but thereafter there is no change. FSH should be added at a dose of 37.5 – 75 IU three times a week. More frequent administration has not proved better. Monitoring of the response should be every 3 months. There should be a progressive rise in testicular volume and sperm often appear in the semen when the testes reach 8-12 mL volumes. If testicular volumes do not increase over a 6-month period the dose of FSH should be doubled. Some patients respond very slowly and it may be necessary to treat them for more than 2 or 3 years before sperm appear. At least 50% of the partners of men conceive by natural sexual intercourse during treatment of gonadotropin deficiency, but the treatment often needs to be given for many months. The semen quality is often poor at the time of the conceptions (Burger and Baker, 1984). If a pregnancy does not occur after sperm have been present in the semen for 6 – 12 months, subfertility in the female partner should be suspected and ICSI considered. Occasionally adequate sperm can be collected during the first course of treatment and stored frozen for later use avoiding the need for further courses of gonadotropin treatment.

Obstructive Azoospermia. Approximately 10% of infertile men have blockages in the genital tract causing absence of sperm in the semen. There are four major groups of causes of male genital tract blockages: disorders of development of the epididymis, vas and seminal vesicles, postinflammatory epididymal obstructions (especially from gonorrhea), vasal obstructions (vasectomy) and ejaculatory duct obstructions. In general men with genital tract obstructions have normal sized testes, FSH levels and spermatogenesis and there is usually a history of urethritis, epididymitis, or surgery and signs such as inability to palpate normal vasa that suggest the diagnosis. Low semen volume (<1.5 mL) and low pH (<6.5) or fructose (<6mmol/L) indicate a lack of seminal vesicle contribution to the semen and suggest bilateral congenital absence of the vasa or ejaculatory duct obstruction. Sperm antibodies are commonly present with postpubertally acquired obstructions. Spermatogenic disorders may also be associated with genital tract obstructions (Baker, 1995a).

Surgery may be offered for genital tract obstructions. The results depend on the level of the blockage, being poor with blockages close to the testis. This is partly because the sperm are immature, having bypassed parts of the epididymis in which maturation usually occurs. With blocks in the tail of the epididymis or vas, sperm appear in the semen of up to 80% of men after surgery, although less than half of these produce pregnancies within one year. It is possible to obtain sperm by a surgical operation from the tubes above the obstruction, by aspirating the epididymis or epididymal cysts through a needle or from a testicular biopsy (Harari et al, 1995, Bourne, 1995a, Watkins et al, 1997). These sperm can be used for ICSI. This approach is particularly useful for absence of the vasa deferentia, high epididymal obstructions for which the results of bypass surgery are predicted to be poor, and failures of vasectomy reversal or other operations. About 70% of the oocytes fertilise and 40% of the couples obtain a continuing pregnancy (Harari et al, 1995).

Disorders of development of the epididymis, vas and seminal vesicles.

Bilateral congenital absence of the vasa (BCAV) in which the structures derived from the Wolffian ducts do not develop or degenerate in fetal life affects about 1 in 4000 men. Usually the testes are of normal size and enlarged heads of the epididymides are present but there is no body or tail of epididymis and no vasa. Absent or very small seminal vesicles can be demonstrated by rectal ultrasound. Occasional men with this condition have partial defects for example the body and tail of the epididymis may be present or the vas may appear normal in the scrotum but become atrophic in the pelvis. In Caucasians about 70% of men with BCAV have a common cystic fibrosis gene mutation detectable on one allele and some of these have another mutation on the other allele (compound heterozygote) (Anguiano et al, 1992). It is presumed that these men have defective production of the cystic fibrosis transmembrane regulator at a critical stage that causes the degeneration of the Wolffian duct structures but the deficiency is not severe enough to cause severe cystic fibrosis with respiratory and intestinal defects. The partners of men with BCAV must be screened for CF mutations and if positive a strategy for screening the embryos or pregnancy developed before commencing ICSI (see Chapter 13).

Unilateral congenital absence of the vas is occasionally seen with genital tract obstruction from another cause on the other side. Frequently there are ipsilateral renal tract abnormalities associated with unilateral congenital absence of the vas. Other malformations of the epididymis causing obstructions are very rare. Reconstructive surgery may be possible in come cases but sperm aspiration and ICSI is now commonly used for all obstructions for which surgery does not have a good prognosis or has failed.

Postinflammatory epididymal obstructions. While common in the developing world, postinflammatory epididymal obstruction of the tails of the epididymides is becoming rare in developed societies, probably because of prompt and effective treatment of urethritis. Some patients may not admit the history of sexually transmissible disease. Usually the tails of the epididymides are enlarged and hard and there is persistent azoospermia. These men can be treated by microsurgery, by joining the epididymal tube the above the blockage to the vas deferens with results similar to those for vasectomy reversal.

Epididymal obstructions from other nonspecific or specific (tuberculosis) infections may occur. These often affect large sections of the genital tract. In the past a number of patients were seen with a combination of chronic sinopulmonary disease, usually bronchiectasis, and caput epididymal obstructions. This condition is called Young syndrome and may have been caused by mercury poisoning in infancy (Hendry et al, 1993). It has become rare possibly because mercury containing teething powders were banned in the mid 1950s.

Vas obstructions. The requirement for vasectomy reversal has reached epidemic proportions in many countries with high divorce rates. Between 5 and 10% of patients seen for infertility have had a vasectomy or a failed reversal procedure. While vasovasostomy is relatively successful in re-establishing sperm in the semen, many do not produce a pregnancy in the year following surgery. An alternative approach is to use ICSI with sperm obtained by needle aspiration from the testes or epididymides.

Prevasectomy semen cryopreservation would largely prevent the need for these expensive and incompletely successful treatments.

Ejaculatory duct obstructions. While ejaculatory duct obstructions are rare they may be under diagnosed. The main causes are utricular cysts and inflammatory obstructions from sexually transmissible diseases or other infections sometimes associated with prolonged catheterization of the bladder. The obstruction may be partial or intermittent. Thus the possibility of distal genital tract obstruction should be considered if the semen volume is low. Ultrasound of the prostate may reveal a cyst or enlarged seminal vesicles. In some cases it may be possible to relieve the obstruction by probing the openings of the ejaculatory ducts or unroofing a cyst at panendoscopy.

Sperm Autoimmunity. Antibodies to sperm develop in many men after vasectomy and may interfere with fertility after vasectomy reversal operations. Antibodies are also found in about 7% of other infertile men, some of whom have had injuries to the genital tract that may have caused the immunization against sperm. There may be other autoimmune diseases, particularly of the thyroid, in the patient or his family suggesting a genetic predisposition. However in most, the reason why the sperm antibodies develop is not obvious.

Sperm antibodies may reduce fertility at several levels: such as interference with sperm production, causing clumping together of sperm, reducing sperm motility, preventing sperm from swimming through the liquid in the female genital tract including cervical mucus, and interfering with sperm binding to the oocyte and undergoing the acrosome reaction (Liu et al, 1991). While defective sperm motility, sperm agglutination and the shaking phenomenon (non-progressive sperm motility) in cervical mucus are features of sperm autoimmunity, these are not seen in all cases and semen quality can vary from azoospermic to normal. Therefore it is necessary to screen all patients for sperm autoimmunity. The immunobead test involves mixing polyacrylamide beads with rabbit antihuman immunoglobulin G and A antibodies coupled to the surface with washed sperm. Sperm with antibodies bound to their surface are indicated by the attachment of immunobeads. This is a good screening test for sperm antibodies. However low level positivity for sperm antibodies is common and probably unrelated to infertility unless more than 50-70% of motile sperm have beads attached to the heads. The Kremer test (cervial mucus capillary tube test) of the ability of sperm to penetrate midcycle cervical mucus can be used to assess the clinical significance of positive sperm antibody tests and to monitor treatment. Men with sperm antibodies and sperm that will not penetrate normal midcycle cervical mucus are severely infertile and rarely produce pregnancies without treatment (Baker et al, 1983). On the other hand if sperm mucus penetration is not completely blocked pregnancies often occur without treatment suggesting that the sperm antibodies do not contribute significantly to the infertility. Patients with these findings should not be diagnosed as having sperm autoimmunity and other causes for the infertility should be sought. The results of the Kremer test also relate to the results of IVF. If the sperm antibody levels are high and mucus penetration is blocked standard IVF usually fails where as if sperm

penetration of mucus is possible reasonable fertilization rates can be achieved with standard IVF.

Treatment of men with sperm autoimmunity with glucocorticoids in immunosuppressive doses (Prednisolone 50mg/day) for several months will reduce the antibody levels, increase sperm motility and mucus penetration and also increase fertility (Baker et al, 1983). The latter has been shown by controlled therapeutic trials (Hendry et al, 1990). Negative trials have also been reported but these were small and patient selection did not differentiate sperm autoimmunity from low level sperm antibody positivity (Baker, 1998). Overall, approximately 25% of female partners conceive while the man is being treated. Other pregnancies may follow artificial insemination or IVF with sperm stored during the treatment. As well as the relatively low success rate there can be unpleasant and serious side effects from glucocorticoid treatment, particularly aseptic necrosis of the head of the femur. ICSI is an alternative approach with a live birth pregnancy rate of 20% per attempt (Clarke et al, 1997).

Disorders of Sexual Function. In a small number of couples disorders of sexual function are the only reason for the infertility (Baker, 1995a). Impotence, failure of ejaculation, retrograde ejaculation and low frequency or mistiming of intercourse may have organic, psychological or unknown causes. Occasionally these conditions respond to treatment with cholinergic drugs to increase bladder neck tone or to sex behaviour psychotherapy, but most often they do not. Sperm may be prepared for insemination from the urine of men with retrograde ejaculation (Baker, 1995a). The development of procedures for treating men with spinal cord injuries has improved the management of these problems (Lim et al, 1995). If masturbation, nocturnal emission, vibroejaculation or electroejaculation can obtain adequate sperm, artificial insemination of the woman may be successful in producing a pregnancy. If the semen quality is reduced ICSI may be a more efficient method of treatment. ICSI is also used if sperm need to be obtained surgically or by testicular biopsy.

Reversible Conditions. Sometimes there are obvious factors contributing to the poor results of sperm tests: incorrect sperm collection techniques, such as too short or too long an interval since previous ejaculation, recent illness, such as influenza, acute inflammation in the genital tract such as epididymitis or prostatitis, heavy alcohol or social drug consumption, obesity, frequent hot spa baths or saunas, use of anabolic steroids, social drugs and certain medical treatments (salazopyrin, nitrofurantoin, psychotropic agents). Recovery from illness or removing the cause results in improvement of semen quality (Baker, 1995a,b) For example temporary impairment of sperm production may occur with many acute illnesses particularly those associated with fever. It may take 3-4 months for the semen quality to recover. Chronic illnesses such as hepatic cirrhosis and chronic renal failure may be associated with poor sperm production. Semen quality may recover after organ transplantation.

Untreatable Subfertility

The majority of men investigated for infertility have sperm present in the semen, but lower numbers than normal (<20 million/mL) - oligozoospermia (35%); or adequate

numbers, but with reduced motility (<50% total, or <25% progressive), - asthenozoospermia (35%), reduced normal morphology (<15-30% - teratozoospermia, or both (30%) (WHO, 1999). A few (5%) have normal semen tests – normozoospermia, but there are abnormalities that impair fertilizing ability of the sperm such as disordered zona pellucida induced acrosome reaction (Liu and Baker, 1994). If specialized sperm function tests are available, the latter condition should be screened for using sperm-oocyte interaction tests in patients with idiopathic infertility and the patients directed to ICSI as they are severely infertile and have low or zero fertilization rates with standard IVF (Liu et al, 1997).

While defects are often combined isolated or predominant abnormalities of sperm number, motility or morphology occur. Severe asthenozoospermia (<5% progressive motility) with normal sperm concentration may require evaluation with electronmicroscopy for specific ultrastructural defects of the axoneme. Men with absent dynein arms and other less common axonemal defects may have general cilial disorders and associated bronchiectasis, situs inversus, and colour blindness. ICSI may be successful with these defects. Necrospermia is important to distinguish from other types of asthenozoospermia because some patients with necrospermia produce pregnancies despite the low sperm motility. There are usually some motile sperm (<20%) but very poor progressive motility (<5%) and the Eosin Y test for sperm vitality is low (<30%) indicating a high proportion of dead sperm. Characteristic of necrospermia is an improvement of sperm motility with increased frequency of ejaculation (Wilton et al, 1988).

Abnormalities of sperm shape are usually of mixed types but specific universal abnormalities such as absent acrosomes (globospermia) or headless tails (pinhead defect) are occasionally seen. Methods of sperm morphology assessment are controversial and not standardized (WHO, 1999). However there is a strong relationship between sperm morphology and the results of fertilization in vitro using standard techniques. Patients with very low proportions of normal morphology (<5%) should be offered ICSI.

At the present, very little is known about the mechanisms by which sperm shape and function are reduced either in men with an obvious cause such as previous undescended testes or inflammation of the testes, or in men who have no apparent cause for the problem. There are also men in general community with poor sperm test results who have no major problems producing pregnancies.

Other Conditions Which May Be Associated With Infertility

Varicoceles are often present (20-40%). The value of treating varicoceles remains controversial. There are now results from controlled trials: some suggest an increase in fertility, others do not (Hargreave, 1995). Whatever the truth, a return of normal semen quality and fertility after surgery is not seen in a high proportion of men (Baker et al, 1985). The benefits claimed from reports of uncontrolled series of varicocelectomy probably resulted from regression to the mean (Baker, 1998). Also not uncommon in men with oligozoospermia or asthenoteratozoospermia are: previous testicular injuries, minor hormone disorders, surgery for torsion of the testis or undescended testes, inflammation of the accessory sex organs and previous sexually transmissible diseases. These conditions may cause or contribute to the poor

semen quality, but it has not been proved that treatments improve the semen test results and increase fertility.

Factors often suspected as being significant such as tobacco smoking, moderate alcohol intake, quality of diet, exercise, exposure to environmental toxins, mental stress and anxiety are of uncertain relevance as causes of disorders or sperm production or function. Changing lifestyle may be important for good health in the long term, but there is usually no consistent marked change in the semen test results. Exposure to heat occupationally or as a result of wearing tight underpants, particularly with other abnormalities such as varicocele may be relevant as normally the testes are maintained several degrees lower than core body temperature. A temperature effect might act through increased oxidative stress in the testes and this could impair spermatogenesis and sperm function (Aitken et al, 1989). Oxidative stress might also cause mutation of mitochondrial DNA which in turn could impair sperm production and function (Cummins et al, 1994). Heating the testes to body temperature even for a short time will result in deterioration in semen quality that may take weeks to months to recover (Brown-Woodman et al, 1984). Studies of intrascrotal temperature in men with infertility tend to show higher temperatures than in fertile men (Zorgniotti & Sealfon , 1988). However this may be more related to smaller testicular size than to the spermatogenic defect. Examination of semen in men wearing either tight or loose underwear suggests the former reduces semen quality, however further studies are needed to confirm this (Tiemessen et al, 1996). In the past methods of reducing testicular temperature have been promoted as part of the management of men with infertility but no adequately controlled trials have been conducted.

Usually the men do not have any significant medical or family history. Physical examination may be normal or there may be testicular atrophy and the associated disorders mentioned above. Semen analysis results may show considerable variation from day to day often for no obvious reason. Hormone tests and testicular biopsies are not usually necessary. FSH levels are normal or high and LH testosterone and prolactin levels are normal. Testicular biopsies usually show hypospermatogenesis consistent with the degree of oligozoospermia. However some patients have partial germ cell arrest. The latter information does not assist in management of the patient. There are some rare genetic causes of reduced sperm production that may need investigation and genetic counselling about the risks of having children with similar infertility or other health problems. Sex chromosomal disorders and balanced autosomal translocations are more common in oligozoospermic men than in the general community. Thus it is a policy of some groups to perform karyotypes in all infertile men with sperm concentrations below a certain limit such as 5 million/mL. Patients with balanced autosomal translocations need counselling about the risks of miscarriage and having a child born with serious defects. Sex chromosomal abnormalities do not appear to be transmitted. It is possible that microdeletions of the long arm of the Y chromosome and other yet to be discovered mutations, may be responsible for many of the more severe defects of sperm production or function and that these defects may be transmitted directly to sons or to future generations (Vogt et al, 1992; Reijo et al, 1995; Najmabadi et al, 1996) (see Chapter 13).

MANAGEMENT OF UNTREATABLE MALE INFERTILITY

As indicated above many types of male infertility do not have treatments of proved effectiveness to increase semen quality and natural fertility. In these cases, after excluding treatable or reversible conditions, management depends on estimating the prognosis for natural fertility and explaining the availability of other options such as assisted reproductive technology, including ICSI and donor insemination or adoption and accepting childlessness.

Factors Affecting Management

Combination of Male and Female Disorders. The female partners of men with reduced sperm numbers or function often have disorders such as irregular ovulation, endometriosis or tubal blockages that contribute to the infertility. As might be expected in infertile couples, problems with the woman are found more often when the man has less severe abnormalities. Thus both partners of the infertile couple must be investigated in detail unless the prognosis for a natural pregnancy is so low the only option is ICSI. In other situations abnormalities in the woman should be corrected where possible even although there is no effective treatment for the semen abnormality. The timing of intercourse to coincide with ovulation should be encouraged vigorously including ways to recognise the fertile period (Baker, 1995a).

Ineffectiveness of Treatment. Many treatments have been used in attempts to remedy reduced sperm output or function in the past (Howards,1995; Hargreave, 1996; Leifke and Nieschlag, 1996; Baker, 1998). There are problems in assessing the success of treatment of infertile men. First, semen test results are very variable from day to day within the one man so that an apparent increase in sperm number (for example, from 3 to 20 million/mL) may result from a chance fluctuation that has nothing to do with the treatment the man happens to be taking at the time. Second, these patients are not sterile; pregnancies occur, but at a lower rate than normal. Thus if a pregnancy occurs during treatment, it also may not necessarily be due to the treatment. Placebo controlled trials are needed to demonstrate that a treatment is effective: it must be shown that semen tests improve more often and pregnancy rates are higher than with similar men given no treatment (Baker, 1998). Meta analyses of several treatment approaches have shown that strictly randomised trials of antiestrogens (clomiphene or tamoxifen) were negative but pooled results suggest a significant positive effect of antiestrogens on pregnancy rates (O'Donovan et al, 1993). A large randomised trial of tamoxifen versus placebo also showed a beneficial effect on semen quality (Kotoulas et al, 1994) Recombinant human FSH had no significant effect on semen quality or fertilisation rates in vitro in two as yet unpublished controlled trials of men with primary spermatogenic disorders. Stimulation of multiple ovulation and artificial insemination with husband's semen (AIH) may increase pregnancy rates, but multiple pregnancies are common (Crosignani and Walters,1994). IVF is preferable because the embryo numbers can be controlled to reduce the risk of high multiple pregnancy.

Thus although a number of prospective controlled clinical trials of gonadotropins, treatment of varicoceles, AIH, and antioxidant and other medications have been conducted and some are positive, none normalise fertility in high proportions of patients (Baker, 1998). Additional controlled trials are necessary before any of these treatments can be regarded as established.

Prognosis for Fertility. In the general community, average pregnancy rates are about 20% per month. That is, of women trying to conceive, about one in five is successful in the first month, one in five of the remainder successful in the second month, one in five of the remainder successful in the third month etc. However, the rate drops with time, so that approximately 50% couples conceive within four or five months, and 85% within one year.

When couples in which the man has poor semen tests are followed over several years, a proportion conceive naturally whether or not they have been treated. In a large group of infertile men seen in Melbourne who had at least some motile sperm in their semen and whose wives were not sterile, the pregnancy rate was approximately 4% per month for the first few months (Baker et al, 1985, Baker, 1995). Overall, 30% of the female partners conceived in one year and 45% by two years. Factors, which were related to the pregnancy rates, were as follows:

Sperm number - the higher the sperm concentration in semen the higher the pregnancy rate

Length of time the couple had been trying to produce a pregnancy - the longer the period of infertility, the lower the pregnancy rate.

Age of wife - the older, the lower the pregnancy rate

Previous pregnancy in the couple (same woman and man) is associated with a higher pregnancy rate than in couples with no previous pregnancies.

Similar results have been reported by others (Duleba et al, 1992). Pregnancy rates can be predicted from these factors so that patients can be advised about their chances of producing a pregnancy. This should enable a couple to make plans regarding the length of time they would like to try themselves before changing to other alternatives such as IVF or donor insemination. Other factors being equal, those with better chances of conceiving spontaneously should try longer than those with little chance of having a child of their own. However, this must be balanced against the added stress of further waiting and hoping for a pregnancy where the chances of success are low.

Assisted Reproductive Technology

In-Vitro Fertilization (IVF). IVF produces moderately good results for couples with male infertility. Standard IVF procedures produce good fertilization rates unless the sperm morphology, motility or concentration is very low or there is a specific defect of the fertilization process. For severe semen abnormalities, for example, if there are less than 2 million normal motile sperm in the semen, sperm morphology is >95% abnormal or sperm are obtained from the above described obstructions in the genital tract there is a substantial chance of obtaining poor fertilization rates with standard IVF and ICSI is used to reduce the risk of failure of fertilization (Harari et al, 1995).

The live birth pregnancy rate following IVF declines with increasing numbers of transfers but averages 5 -12 % per pre-embryo transferred either fresh or after storage frozen. Usually 1 or 2 embryos are transferred at a time to reduce the risk of multiple pregnancy. The cumulative chance of a successful pregnancy is about 13% after 1 transfer, 22% after 2 transfers, 31% after 3 transfers and 50% after 6 transfers. Other factors affecting the success of IVF include female age; declining after 35 years, quality and number of cells in the embryos and previous successful IVF treatments.

Some clinics use GIFT (Garnete intrafallopian transfer) and ZIFT (Zygote intrafallopian transfer) in which sperm and oocytes or pre-embryos are placed in the Fallopian tubes, for male infertility. These procedures do not give better pregnancy rates than IVF.

Intracytoplasmic Sperm Injection (ICSI). ICSI is now the method of choice for treating severe sperm problems. With this technique a single sperm is injected into the substance (cytoplasm) of each oocyte with a fine glass needle. This procedure may also be applied to situations where fertilization could fail because of sperm antibodies or certain specific disorders of sperm motility, shape or function. Provided that a live sperm can be found for each oocyte good results can be obtained with average normal fertilization rates the same as those for normal semen in standard IVF. Sperm with absent acrosomes and sperm not known to be alive produce lower fertilization rates (Harari et al, 1995; Bourne et al, 1995b; Watkins et al, 1997).

Despite the major improvement in fertilization achieved with ICSI, there is still the problem of the low pregnancy rates after embryo transfer and thus a number of couples with failures of fertilization or implantation will still have to accept the infertility and consider other alternatives such as donor insemination, adoption or child free living.

Donor Insemination. In countries where donor insemination is accepted it is estimate conservatively that over 1 in 500 births are the results the medical use of donor semen. Because of the risk of transmitting HIV and hepatitis viruses cryopreservation and quarantining of semen is used. Efficient semen cryobanking was made possible by the discovery of effective cryoprotectants. Extensive experience indicates the rate of pregnancy wastage and major congenital abnormalities are similar to those of natural pregnancies. The best pregnancy rates reported for cryopreserved semen are the same as are achieved with artificial insemination with fresh semen and range from 10% to 20% per month or cycle of treatment (Clarke et al, 1997).

The social effects of donor insemination are receiving increasing attention and infertility counselling has become a specialty in many countries. While carefully screened anonymous donors are used most commonly donors known to the recipient couple: relatives or friends can be used. Legal regulation of sperm banks and donor insemination services varies widely from country to country. For example, anonymity of donors is legally required in some whereas donor children are to be given access to the identity of the donor when they attain legal age in several places in Australasia and Europe. In some cultures and religions the use of donor gametes in the treatment of infertility is proscribed as it is viewed as equivalent to adultery.

Since 1992 the number of patients requesting donor insemination or donor IVF has decreased significantly because men with severe oligozoospermia can achieve pregnancies through ICSI. In addition, many men with untreatable obstructive azoospermia can use ICSI with sperm obtained from the genital tract. Donor insemination is now used for primary seminiferous tubal failure with complete absence of elongated spermatids and where patients do not achieve a pregnancy with ICSI or cannot afford ICSI. It is also used rarely for hereditary disorders and recurrent abortion.

Semen cryopreservation is also used to store semen for subsequent use for artificial insemination with husband's semen (AIH), for example as insurance before vasectomy, treatment with cytotoxic agents or radiotherapy and following a successful treatment or procedure for infertility. It is also useful as a backup for ICSI in men with severe oligozoospermia and only intermittent presence of motile sperm in the semen. Sperm obtained from the male genital tract by aspiration or testicular biopsy can also be cryopreserved for ICSI.

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15 MALE SEXUAL DYSFUNCTION

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INTRODUCTION

Life can be defined as an autonomous self-replicating system that evolves by natural selection. The penile erection is a requisite for the propagation of the human species. One could say that when one loses the ability to attain an erection, one loses life.

The above statement mirrors the sentiment of sexual dysfunction patients. They seek restoration of their sexual function and restoration of their life as a man. The immense psychological and social impact of sexual dysfunction can only be grasped by direct contact with the individuals who suffer.

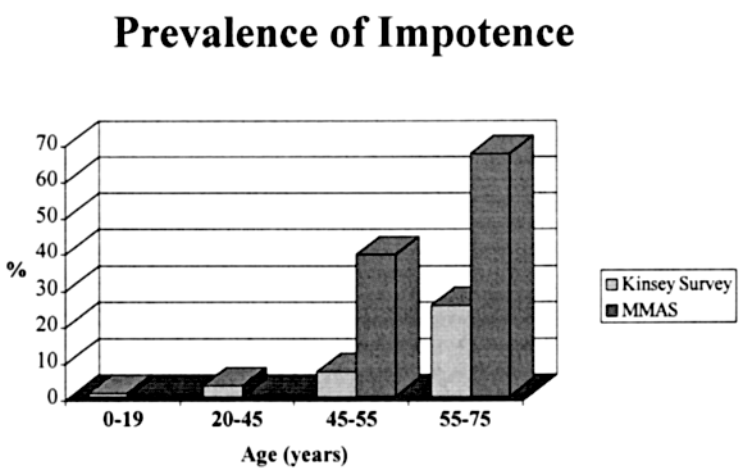
EPIDEMIOLOGY OF ERECTILE DYSFUNCTION

Male sexual dysfunction can be defined as the inability of a man to obtain penile rigidity sufficient to permit coitus of adequate duration to satisfy himself and his partner. The personal and private nature of this problem has hindered accurate estimates of its true prevalence in the general population. Current estimates suggest that 20 to 30 million American males suffer from erectile dysfunction (VandenBerg and Hellstrom, 1997).

For five decades the Kinsey survey remained the most comprehensive population-based study on male sexual behavior in the United States (Kinsey et al, 1948). The Kinsey survey was based on structured interviews of 12,000 males, stratified by socioeconomic and demographic variables to represent the general population. It demonstrated an age-dependent increase in sexual dysfunction.(Figure 1).

From 1987 to 1989, a multidisciplinary community-based epidemiological study was conducted in Massachusetts (Feldman et al, 1994). Structured interviews evaluated health status, socioeconomic, psychosocial and lifestyle characteristics. Hormonal profiles were obtained, and a 23-question self-administered survey of

FIGURE 1



sexual activity was completed by each participant. A total of 1290 men participated in the study. The Massachusetts Male Aging Study (MMAS) reported that 52% of men aged 40-70 experienced some degree of erectile dysfunction (Figure 1). The MMAS also reported that with advancing age there were progressive declines in libido, sexual thoughts, and frequency of nocturnal or morning erections and intercourse. However, sexual satisfaction did not decline, suggesting that men accommodate for age-related changes in sexual capacity by altering expectations.

ANATOMY AND PHYSIOLOGY OF THE PENIS

Anatomy

The penis is a tubular appendage consisting of three distinct cylindrical compartments, each of which is encased by connective tissue. The ventral compartment is the corpus spongiosum, containing the bulbar and penile urethra. The paired dorsal compartments are the corpora cavernosa, or erectile bodies of the penis, covered by a thick fascial layer called the tunica albuginea. The tunica albuginea in the midline septum is permeable to blood flow, which allows free exchange of nutrients and pharmacological agents between the corpora cavernosa. The corpora cavernosa originate under the pubic arch as the crura of the corpora

cavernosa. The crura are firmly anchored to the periosteum of the ischial rami. These crura converge beyond the pubic arch and fuse for the distal three-fourths of the corporal length (Hinman, 1993). A condensation of Buck's fascia forms the suspensory ligament of the penis, anchoring the penis to the pubic symphysis.

Penile arterial supply originates in the internal pudendal artery, a terminal branch of the internal iliac artery. The internal pudendal artery becomes the common penile artery, which then divides into the paired dorsal penile arteries and the paired cavernosal arteries. The dorsal penile arteries supply the corpus spongiosum through the circumflex arteries, while the cavernosal arteries supply the corpora cavernosa through the helical arteries. The helical nature of the helical arteries allows them to maintain their internal diameter during elongation of the penis.

The penis is under autonomic neural regulation. The parasympathetic visceral efferent fibers arise from S2-S4 to supply the pelvic plexus located on the lateral wall of the rectum, 5-11 cm from the anal verge. Sympathetic fibers arise from T12-L3 and travel to the pelvic plexus via the hypogastric nerve. The cavernous nerves leave the pelvic plexus and travel in the lateral pelvic fascia on the posterolateral surface of the prostate gland to supply the corpora cavernosa of the penis. Parasympathetic stimulation is responsible for erections. Sympathetic stimulation is responsible for seminal emission by inducing contraction of the prostate and seminal vesicles while simultaneously resulting in closure of the bladder neck. Somatic neural stimulation is important for contraction of the bulbocavernosus and ischiocavernous skeletal muscles during the final stages of erection and ejaculation.

Physiology

The three neuroeffector pathways which coordinate the smooth muscle tone in the corpora cavernosa are the adrenergic, cholinergic and non-adrenergic, non-cholinergic (NANC) (Hellstrom et al, 1994). Penile flaccidity is maintained by the adrenergic-mediated sympathetic tone of the cavernosal smooth muscle. Penile erection can be considered to occur in five phases. During the first phase, an increase in arteriolar inflow and a lesser increase in venous outflow result in passive dilation of the lacunar spaces. This phase is triggered by stimulation of a dual-innervated neuronal pathway involving both cholinergic and NANC mediators which results in relaxation of the smooth musculature of the cavernosal arteriovenous bed and trabecular network (Hellstrom et al, 1994). The primary mediator of penile erections is nitric oxide, originating from NANC neurons, cavernosal smooth muscle cells, and cholinergic-stimulated endothelial cells. Nitric oxide synthase generates nitric oxide from its precursor, L-arginine, and acts on the enzyme guanylate cyclase to increase cGMP levels. Cyclic GMP is the active second messenger responsible for smooth muscle relaxation that initiates the first phase of erections.

The tissue characteristics of the tunica albuginea permit the distension of the corpora cavernosum during erection. Undulating bundles of collagen fibers intertwined with elastin allow the penis to distend under appropriate stimuli. During the second phase, the emissary veins and subtunical venules are compressed against the fibroelastic tunica albuginea by the expanding lacunar spaces, and the axillary veins elongate, resulting in venous pooling in the sinusoidal bodies and increased

tumescence. As the intracavernosal pressure rises, the third phase of erection is reached, where both the arterial inflow and venous outflow decrease and a steady condition is achieved. The fourth phase of erection involves the contraction of the bulbocavernosus and ischiocavernosus muscles, which results in the rigidity of the erection. Finally, the fifth phase of erection commences with a sympathetic stimulation that leads to seminal emission, ejaculation, and contraction of the lacunar space, and detumescence with return of the basal adrenergic-mediated cavernosal smooth muscle tone. Endothelin-1 is a potent vasoconstrictive mediator of penile function and is believed to be important in maintaining a flaccid tone. Imbalances between endothelin-1 and nitric oxide levels may therefore play a role in erectile dysfunction (Saenz de Tejada et al, 1991; Dohi et al, 1995).

CAUSES OF ERECTILE DYSFUNCTION

Psychological

Any patient who reports diminished sexual function has a component of psychological disease. Once an erection is suboptimal, the next sexual situation will be clouded by the patient's fear and anxiety that his performance will again be dysfunctional. Each subsequent unsatisfactory sexual encounter adds a layer of psychological frustration and fear. Therefore, while an organic component to impotence can be identified in over 50% of men, one should consider that practically all men have some degree of psychogenic overlay.

Anxiety, depression, and stress can have a profound effect on sexual function and performance. These emotions may stem from sexual problems, social difficulties, or medical conditions. Physical handicaps or difficult socioeconomic situations may alter the patient's self-image as well as the receptiveness of the partner. Physical disfigurements can diminish self-esteem and nurture fears of rejection by their partner. The psychological strength of both the individual and the couple in adapting to these emotions will determine the degree to which their sexuality is affected.

Endocrine

Testosterone is the primary sex hormone in men. Low testosterone levels have a strong correlation with a decrease in libido, which suggests a major central effect for androgens on sexual function. Approximately 98% of testosterone is bound to sex hormone-binding globulin and albumin. Bioavailable testosterone is the sum of free and albumin-bound testosterone. Recent studies suggest that with advancing age, the albumin-bound fraction decreases, while the SHBG fraction increases and the free testosterone fraction remains unchanged (Diver et al, 1997; Morley et al, 1997). Bioavailable testosterone levels correlate significantly with nocturnal penile tumescence (Schiavi et al, 1993).

Studies have demonstrated a significant decline in nitric oxide synthase (NOS) activity in castrated animals that can be reversed by androgen supplementation. Increases in NOS mRNA have been demonstrated with androgen supplementation. (Weiner et al, 1994; Chamness et al, 1995). These studies suggest a biochemical pathway for the influence of androgens on erectile function and dysfunction.

Hyperprolactinoma. Hyperprolactinemia decreases the secretion of GnRH, resulting in low testosterone levels (VandenBerg and Hellstrom, 1997). Erectile dysfunction (88%) and decreased libido (80%) are the most common presenting symptoms of hyperprolactinemia (Buvat and Lemaire, 1997).

Disruption of the Hypothalamic-Pituitary-Gonadal Axis. Malignancies that impinge on the central or peripheral nervous system or alter the pituitary-hypothalamic-gonadal axis may directly affect sexual function by altering endocrine function. GnRH release from the hypothalamus is suppressed by stress-induced increases in catecholamines, prolactin, corticotropin-releasing factor, and opiates.

Patients undergoing cranial irradiation for brain tumors may develop irradiation-induced hypothalamic dysfunction with hyperprolactinemia. This might manifest as erectile dysfunction and galactorrhoea. Oral bromocriptine can normalize prolactin levels and restore normal erections. Patients undergoing cranial or pituitary surgery or irradiation may develop hypogonadotrophic hypogonadism, thereby requiring hormonal supplementation with exogenous GnRH (see Chapter 6).

Systemic Illness

Hepatic Disorders. Patients with chronic liver disease commonly have low testosterone levels with elevated LH levels. Estradiol and prolactin levels are also often elevated. All of these endocrine changes associated with liver disease can predispose to impotence.

HIV-Related. Erectile dysfunction is common in patients with HIV disease. Testosterone levels are often low, and supplementation is indicated to reverse their wasting syndrome as well as erectile function. HIV patients may also experience peripheral neuropathies, with viral myelitis and myelopathy being late causes of impotence (Kwan and Lowe, 1995).

Renal Failure. Patients on dialysis report extremely high incidences of erectile dysfunction (80%) and complete impotence (55%) (Mellinger and Weiss, 1992). Part of this may be related to co-morbidities, such as hypertension, diabetes and vascular disease. However, renal transplantation can occasionally improve potency in these men, suggesting that the uremia and dialysis do contribute to impotence. A central cause for erectile dysfunction is suggested by the efficacy of dopaminergic agents in some of these uremia patients (Eardley, 1998).

Hypertension. Impotence occurs in 25% of men treated for hypertension. Prescription medications play an important role in the development of impotence in

these men. However, withdrawal of anti-hypertensives often fails to restore erectile function (Mellinger and Weiss, 1992).

Diabetes. Diabetes is a very common etiology of erectile dysfunction. Approximately 4 million American men have been diagnosed with diabetes in the United States, and there are an estimated additional 4 million cases undiagnosed (Konen et al, 1996). Within five years of onset of diabetes mellitus, 60% of patients experience some erectile dysfunction (Mellinger and Weiss, 1992). Diabetic impotence may be related to somatic and autonomic neuropathy, large and small vessel vascular disease, or direct damage to the cavernosal tissue.

Vascular. A vascular component for the etiology of erectile dysfunction exists in up to 60% of patients (Feldman et al, 1994). The term vascular impotence refers to a spectrum of diseases from arterial insufficiency to arteriosclerosis to venous leak. Arterial disease related to impotence is most commonly a manifestation of generalized peripheral vascular disease or cardiovascular disease. LeRiche's syndrome includes impotence and buttock claudication caused by vascular insufficiency in the internal iliac arteries. Occasionally, localized lesions in the genital arterial system can be related to trauma.

Venous leak refers to a dysfunction in the normal veno-occlusive function of the tunica albuginea on the emissary veins. This can be due to decreased distensibility of the corpora cavernosa or inherent abnormalities in the tunica albuginea. Elastin concentration in the tunica albuginea and trabeculae of impotent men is decreased (Raviv et al, 1997). Collagen type I appears to be increased in the tunica albuginea of patients with arterial impotence, while collagen type IV is increased in patients with venous impotence (Raviv et al, 1997).

Neurological. Disorders affecting the brain, spinal cord, or peripheral nerves can affect erectile function. Neurologic dysfunction may be secondary to congenital defects, spinal cord injury, or surgery. Alcoholism, diabetes, and vitamin deficiencies can also contribute to neurologic impotence.

Demyelinating diseases are commonly associated with impotence. Erectile dysfunction has been demonstrated in 43-71% of men with multiple sclerosis (Berger et al, 1994). These patients also report decreased penile sensation and decreased libido. Abnormally elevated sensory thresholds to vibratory and electrical stimulation have been observed in aging men (Rowland et al, 1989). This suggests that sensory uncoupling of the sensory erectile reflex may be a factor in erectile dysfunction.

Iatrogenic

Pelvic Surgery. Pelvic and retroperitoneal surgery can damage the autonomic nervous system that controls the physiology of penile erection and ejaculation. Resection of the cavernosal nerves during radical prostatectomy for localized prostate cancer and radical cystoprostatectomy for bladder cancer will lead to impotence in over 90% of men. Preservation of one or both neurovascular bundles can preserve potency in 70% of men (Walsh, 1987). In bladder cancer patients,

results are comparable, unless a urethrectomy is required by involvement of the prostatic urethra. Preservation of the neurovascular bundles must be individualized to each patient, based on preoperative clinical staging and intra-operative findings. Patients with high-grade cancers, high serum markers (prostate specific antigen), and palpable suggestion of bulky disease may require wide surgical excision, making a nerve-sparing procedure impossible. Results following nerve-sparing radical prostatectomy correlate with the patient's age and preoperative sexual function. Patients older than age 70 have only a 20% chance of preservation of sexual function with bilateral nerve-sparing procedures, while in men younger than age 55 preservation of one neurovascular bundle may be adequate (Quinlan et al, 1991). Early institution of adjunctive therapies, such as vacuum erection device or intracavernosal injection therapy one-month following prostatectomy, has been demonstrated to significantly improve the chance that erections will return following nerve-sparing procedures (Montorsi et al, 1997). Such therapy may increase cavernous oxygenation during the initial postoperative period, minimizing hypoxia-induced changes in the penile tissue.

Damage to the pelvic plexus may occur during surgery for colorectal cancer. The incidence of erectile dysfunction is 30% with low anterior resections and 50% with abdominoperineal resections (Hojo et al, 1991). Nerve-sparing procedures have been less successful in colorectal surgery and may compromise local disease control and cancer-specific survival. Retrograde ejaculation and anejaculation may result from damage to the sympathetic component of the pelvic plexus.

Radiation. Radiation for prostate cancer can lead to fibrosis and stenosis of the pelvic arteries and accelerate existing arteriosclerosis, leading to vascular impotence. Patients with normal pretreatment sexual function tend to do better, with 27% developing erectile dysfunction as compared to 54% in patients who had some degree of pre-existing dysfunction (Banker, 1988). Radiation for prostate cancer may also decrease libido in up to 75% of men and ejaculatory volume in 90% of men. Fifty percent of men reported a decrease in orgasmic pleasure following external beam radiotherapy. New techniques using conformal radiation technology and brachytherapy under ultrasound guidance may prove advantageous in preserving sexual performance. Interstitial radiotherapy for prostate cancer may preserve potency in 50-85% of men, however long-term cancer efficacy remains undetermined (Kaye et al, 1995; Porter and Forman, 1993).

Drug-Related

Pharmaceutical side effects can contribute to sexual dysfunction in up to 25% of men (Benet and Melman, 1995). A wide variety of classes of medications can affect erectile function in a variety of ways. (Table 1) Centrally acting agents such as phenothiazines, opiates, tricyclic antidepressants, and H_2-antagonists can cause erectile dysfunction by increasing prolactin levels or decreasing dopaminergic stimulation. Anti-hypertensive agents may lower blood pressure below the level needed to generate and maintain an erection in patients with atherosclerotic disease in the arteries supplying the penis. The anti-hypertensive agents that have the least effect on erectile dysfunction include α-adrenergic antagonists, calcium channel

antagonists and angiotensin converting enzyme inhibitors (Mellinger and Weiss, 1992).

Table 1: Medications Affecting Erectile Function

- Diuretics
- Antihypertensives
- Cardiac Medications
- Tranquilizers
- Antidepressants
- H_2 Antagonists
- Steroids/Hormones
- Anticonvulsants
- Chemotherapeutics
- Anticholinergics
- Anti-Inflammatory Agents
- Tobacco
- Alcohol
- Amphetamines
- Opiates
- Neuroleptics

Alcohol is the most common cause of decreased bioavailable testosterone. Excessive alcohol intake is the most common identifiable factor in men aged 45-55 with secondary impotence (Mellinger et al, 1992). Alcoholic impotence can be related to liver dysfunction, psychological disturbances, and alcoholic autonomic neuropathy (Berger et al, 1994).

Tobacco can significantly alter penile arterial hemodynamics. The incidence of impotence in elderly smokers (current or ex-smokers) is 81% (Mellinger et al, 1992). The MMAS reported a 41% risk of complete impotence in people who smoked, compared with 14% for non-smokers.

Hormonal ablation therapy using LHRH agonists or estrogens to reduce circulating testosterone to castrate levels is the mainstay of therapy for metastatic prostate cancer. This leads to a decrease in libido and erectile dysfunction. However, 15-20% of men with castrate levels of testosterone are able to achieve normal erections. Recently, investigators have reported that intermittent androgen ablation, tailored to responses in serum prostate specific antigen levels, may maintain efficacy of therapy while allowing for drug-free periods during which sexual function resumes and quality of life improves (Peeling, 1989).

Aging

Epidemiological studies such as the MMAS and the Kinsey survey strongly suggest an age-dependent increase in the prevalence of impotence. Certainly, many of the above considerations, such as decreased testosterone bioavailability, increasing vascular disease, multiple medications, and increased systemic co-morbidities, contribute to this exponential increase in prevalence.

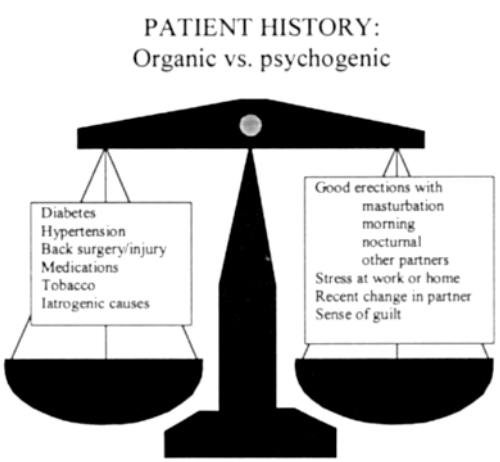
Cellular changes associated with aging also contribute to decreased erectile function. Cellular senescence results in increased deposition of a less compliant collagen subtype in the corpora cavernosa and tunica albuginea. This can lead to venous-occlusive dysfunction and decreased neuronal transmission to the cavernosal smooth muscle (Padma-Nathan et al, 1990). Aging also is believed to result in altered endothelial function, which manifests in decreased basal nitric oxide release and increased basal endothelin-1 (Dohi et al, 1995). There is evidence

for upregulation of endothelin-1 mRNA with aging (Kumazaki et al, 1994). Correlations have been demonstrated between androgen levels and mRNA levels for nitric oxide synthase (Weiner et al, 1994).

CLINICAL EVALUATION OF ERECTILE DYSFUNCTION

A complete history and physical examination is the cornerstone to the evaluation of the impotent man. Special attention in the history is placed on the known risk factors of organic impotence: diabetes, hypertension, tobacco use, concomitant medications, back surgery or spinal cord injury, genital trauma, or possible iatrogenic injury. Focus is also placed on indicators of significant psychological components: good erections with masturbation, normal morning or nocturnal erections, significant stresses in the work or home environment, recent change in partner, or guilt. Such observations allow the clinician to weigh the contribution of organic causes against psychological causes and taper the degree of intervention appropriately (Figure 2).

FIGURE 2



The physical examination focuses on potential organic causes of erectile dysfunction. The secondary sexual characteristics are evaluated: body habitus and muscular development, body hair distribution, and vocal tone. Gynecomastia may be noted. Peripheral and femoral pulses are examined for signs of potential vascular compromise. A focused neurologic examination will evaluate the patient's sensory threshold in the genital region, including examination of the bulbocavernosal reflex

(contraction of the anal sphincter with squeezing of the glans penis). The genital examination checks for gross penile deformities, as well as size, location, and consistency of the testes. A prostate examination is important to evaluate for evidence of asymmetry, induration, or nodularity suggestive of malignancy.

Hormonal Studies

As previously mentioned, the form of testosterone active in supporting erectile function is the bioavailable testosterone, which represents the sum of the albumin-bound and free testosterone fractions. The most common clinical values available today for total and free testosterone do not change significantly with age (Diver et al, 1997; Morley et al, 1997). However, patients with low levels of measurable testosterone should be offered testosterone supplementation. Studies demonstrate that 10% of men with erectile dysfunction have low testosterone levels. However, with repeat testing, 40% of these men have normal levels (Buvat and Lemaire, 1997). The prevalence of persistent hypogonadism has been reported as 4% in patients younger than age 50 and 9% in patients older than age 50. This study suggests that serum testosterone levels should be evaluated in all men over age 50, but only in men under age 50 who demonstrate decreased libido or physical signs suggestive of hypogonadism (See Chapters 6 and 7).

Prolactin levels should be checked in patients with low testosterone levels. Prolactin levels should also be checked in patients with gynecomastia, significantly decreased libido, visual field disturbances, or severe headaches (Lewis and Barrett, 1995). Persistent elevations of prolactin are demonstrated in only 0.2-1.8% of impotent men, and only 20-30% of these will have a prolactinoma identified on imaging studies (Buvat and Lemaire, 1997).

Patients with erectile dysfunction should undergo a screening serum prostate specific antigen (PSA) screening if they are age 50 to 70 (age 40 to 70 in high-risk patients (e.g., African-American or family history)). Serum PSA levels should always be documented prior to initiation of testosterone supplementation therapy.

Additional Studies

Sophisticated studies are usually not required for the goal-directed therapy of the impotent man. Such studies should be reserved for those select patients in whom primary therapies are unsuccessful and for those patients who choose surgical intervention.

Duplex ultrasonography can be utilized to measure peak flow velocity rates in the cavernosal arteries; normal peak flow velocities are 20 cm/sec in the erect penis and 25 cm/sec in the erect penis with pharmacological and visual erotic stimulation. Duplex ultrasonography can differentiate between arterial insufficiency, veno-occlusive dysfunction, or mixed vascular disease. Penile angiography and dynamic infusion cavernosography are invasive tests that should be reserved for those patients who are considering vascular surgery for arterial or venous disease. Intracavernosal vasodilators and visual sexual stimulation can be used to augment

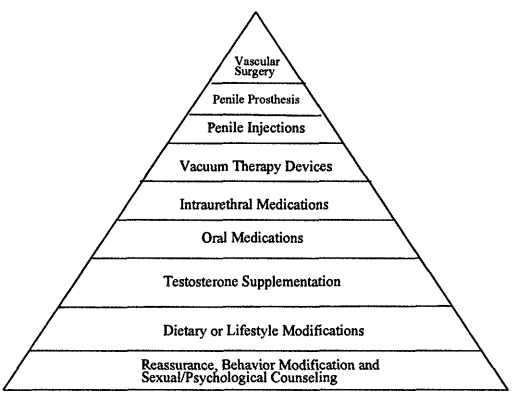
ultrasonographic or radiographic modalities in the evaluation of inflow and outflow of blood.

CLINICAL MANAGEMENT OF ERECTILE DYSFUNCTION

Patient-Directed Therapy

Patient-directed, goal-oriented therapy requires discussion of all the therapeutic options available to the patient in the impotence pyramid Figure 3). As a patient ascends the impotence pyramid, the invasive nature and inherent risks involved in the therapies increase, resulting in fewer patients reaching each tier. A detailed discussion of the pros and cons involved in each therapy usually requires at least a 30- minute office visit, supplemented by educational brochures and videotape presentations.

FIGURE 3: THE IMPOTENCE PYRAMID



When patient-directed therapy is indicated, the clinician's responsibility is to steer the patient towards therapies that would best suit his underlying pathology as well as his current social situation. For example, patients with significant psychogenic components to their erectile dysfunction would be encouraged to try the lower three tiers of the pyramid. Likewise, a patient who is single, with multiple sexual partners, would most likely not choose a vacuum erection device. All patients would be encouraged to accept at least one lower tier before proceeding to the highest tier, surgical intervention.

Behavioral Modification, Sexual Counseling and Diet

Stress reduction, adequate rest, and adequate exercise are general behavior modifications that improve overall health and sexual function. If social problems in the relationship with the patient's partner exist, joint counseling with a social worker, marital counselor, clergyman, or psychiatrist may be considered. Patients should be instructed on techniques of sensate focusing and increased sexual foreplay to enhance arousal. Both the patient and partner should be encouraged to broaden their sexual dialogue and to share wants, needs, and preferences.

Dietary supplements have been suggested as effective in erectile dysfunction. For example, dehydroepiandrosterone (DHEA), an adrenal androgen, is marketed at health food stores as a nutritional supplement. However, clinical trials in the use of DHEA have not been conducted, and high oral doses of DHEA can actually suppress serum testosterone levels (Roberts and Fitten, 1990). Concerns exist as to similarities in potential side effects with other androgen supplementations.

L-arginine is the precursor for nitric oxide, and one preliminary study has suggested efficacy of large oral doses of L-arginine in treating erectile dysfunction (Konen et al, 1996). Other dietary supplements have included Spanish fly, rubaceae extracts, ginseng, rhinoceros horn powder, and deer antler powder. The patient must weigh the risks and benefits of trying these remedies without the support of medical studies.

Testosterone Supplementation

Testosterone supplementation to improve libido and potency is indicated in patients with low testosterone levels. Androgen supplementation has the additional benefits of preserving bone mass, lean body mass, and musculature. However, it can exacerbate sleep apnea, cause polycythemia, decrease HDL, and potentiate the risks of cerebrovascular accident (VandenBerg and Hellstrom, 1997). Serum cholesterol, lipid profiles and hemoglobin determinations should be monitored during the initial three months of therapy, then yearly. Different authorities propose different regimens of follow-up (See Chapter 8).

Testosterone supplementation results in an increase in serum prostate specific antigen and prostate volume when administered to hypogonadal men (Tenover, 1992; Behre et al, 1994). For this reason, patients should be counseled that pre-treatment voiding patterns may be altered by androgen supplementation. Prostate screening by digital rectal examination and serum prostate specific antigen should be performed prior to initiation of androgen supplementation and repeated on a yearly basis while on therapy.

The oral formulations of androgens available in the United States are methyltestosterone and fluoxymesterone. Both of these agents undergo significant first-pass hepatic inactivation, which decreases efficacy and increases the risk for hepatotoxicity and altered lipid metabolism. For these reasons, the majority of urologists do not use them for these reasons (Lugg and Rajfer, 1996). Testosterone undecanoate has been used outside the United States with more effective results; it is absorbed through the intestinal lymphatics, bypassing the hepatic circulation (VandenBerg and Hellstrom, 1997; Morales et al, 1997).

The parenteral formulations of androgens available in the United States are testosterone enanthate and testosterone cypionate. These are effective and

inexpensive modes of androgen supplementation. However, dosing intervals of every two to three weeks are required to maintain adequate serum levels and clinical response. Dosage and dosing intervals should be tailored to patient serum levels while on therapy. Implanted crystalline pellets capable of sustained serum testosterone levels for four to six months are under investigation (VandenBerg and Hellstrom, 1997).

Transdermal testosterone systems are available: Testoderm® (Alza) and Androderm® (Smith-Kline Beecham). Each of these formulations is now available for non-scrotal, once-daily application. Transdermal application bypasses hepatic inactivation and approximates physiologic diurnal testosterone levels (VandenBerg and Hellstrom, 1997). The main limiting side effect is local skin irritation (See Chapter 8).

While many studies have documented restoration of serum testosterone levels with androgen supplementation, few have evaluated the efficacy in the management of erectile dysfunction. One study reported significant improvements in frequency, duration, and rigidity of nocturnal penile tumescence with testosterone supplementation (Arver et al, 1996). Sexual desire and arousal were also significantly improved. However, only 35-60% of men can expect a measurable improvement in sexual performance with restoration of normal serum testosterone levels (Buvat and Lemaire, 1997; Behre et al, 1994).

Oral Medications

Yohimbine. Yohimbine is an indolalquinolonic alkaloid derived from the bark of the Central African Pausinstalia yoimbe tree. Yohimbine has central-acting effects, including α-2 adrenergic blockade, and cholinergic and dopaminergic stimulation (Ernst and Pittler, 1998; Teloken et al, 1998). A recent placebo-controlled study demonstrated complete response in 14% and partial response in 55% of patients using a single 100 mg dose regimen. However, this was not statistically significant when compared to placebo (Teloken et al, 1998). Another recent meta-analysis of randomized clinical trials suggests a 3.85 odds ratio that yohimbine is superior to placebo. The most common dosage regimen was 5.4 mg three times daily, and response rates ranged from 34-73%. Adverse side effects were rare and mild, including sweating, anxiety, tachycardia, and hypertension (Ernst and Pittler, 1998). However, yohimbine should not be used in patients with significant cardiovascular disease or hypertension (Lugg and Rajfer, 1996).

Trazodone. Oral trazodone can improve sexual performance in up to 30-60% of patients (Kurt et al, 1994). Trazodone possesses both serotonin and α-2 antagonist properties (Eardley, 1998). Randomized placebo-controlled studies suggest durable responses to combinations of trazodone and yohimbine in 35% of patients (Montorsi et al, 1994). The main side effect of trazodone is sedation (Lugg and Rajfer, 1996).

Sildenafil. Sildenafil received FDA approval for marketing in March 1998. It has a unique mechanism of action, involving the competitive and selective inhibition of cyclic GMP specific phosphodiesterase V, the primary phosphodiesterase in

cavernosal tissue. Inhibition of phosphodiesterase V results in an increase in cGMP levels, with a corresponding increase in cavernosal tumescence and rigidity. Sildenafil has been reported to be effective in over 60% of men and has additional beneficial effects, such as anti-thrombotic properties and coronary vasodilatory properties. Most initial studies were performed in patients without a definable organic cause for impotence, and success rates approached 90%. A recent placebo-controlled study in which 73% of patients had identifiable organic impotence reported improved erections in 79% of patients (Eardley, 1998). Other reported side effects include headache (2.5-11%), dyspepsia (0-8%) and diarrhea (0-8%) (Lue and the Sildenafil Study Group, 1998). Reverse color blindness has been reported. Sildenafil is used on-demand about 20-60 minutes prior to intercourse, resulting in significant improvement in the ability to achieve and maintain an erection satisfactory for intercourse (Eardley, 1998; Lugg and Rajfer, 1996; Lue and the Sildenafil Study Group, 1998).

Apomorphine. Apomorphine is a selective dopamine receptor agonist that stimulates the central nervous system, generating an arousal response that includes a penile erection. A sublingual formulation has been developed to treat erectile dysfunction while minimizing side effects (Heaton et al, 1995). Phase III placebo-controlled clinical trials have confirmed efficacy of this agent, with reported side effects including nausea, dizziness, and hypotension occurring at higher dosing regimens. Patient satisfaction is reported as high as 70%, with nausea decreasing with subsequent doses and sometimes managed by the co-administration of anti-emetics. Apomorphine is used on-demand 15-30 minutes prior to intercourse.

Phentolamine. Phentolamine is an α-adrenergic antagonist that also has anti-serotoninergic actions and acts as a direct smooth muscle relaxant (Fallon, 1995). Oral phentolamine has been investigated for several years; however, a number of studies suggest efficacy comparable to placebo (Eardley, 1998). The drug is administered 15-30 minutes prior to intercourse. Side effects are minor, including nasal congestion and tachycardia.

Bromocriptine. Bromocriptine is a dopaminergic agent that is indicated in the treatment of erectile dysfunction secondary to hyperprolactinemia. Success with bromocriptine correlates with the degree of prolactin elevation, with 66% of patients responding when the prolactin was greater than 35 ng/ml, and only 41% responding when the prolactin is lower (Buvat and Lemaire, 1997). Bromocriptine may also be of benefit to patients on hemodialysis (Eardley, 1998). Side effects (including nausea, vomiting and hypotension) are common causes of treatment cessation.

Topical Medications

Transdermal medications utilized to treat male erectile dysfunction include nitroglycerin, nitroprusside, minoxidil, prostaglandin, and vasoactive intestinal peptide (Lugg and Rajfer, 1996). The thin penile skin makes this an attractive approach; however, all agents must still penetrate the thick, impermeable tunica

albuginea. Lack of efficacy and severity of side effects have prevented the commercial feasibility of these approaches.

Intraurethral Medications

Alprostadil. MUSE®, released by Vivus in 1997 for the treatment of erectile dysfunction, is an intraurethral delivery system developed for the administration of an intraurethral prostaglandin E_1 suppository. However, as absorption through the ventral side of the tunica albuginea lining the corpora cavernosum is required and some medication is absorbed systemically through the well-vascularized corpus spongiosum, high concentrations of PGE_1 must be used to maintain efficacy. This causes some discomfort in up to 30% of patients. Initial randomized, placebo-controlled studies suggest 65% success rates, determined as having at least one successful sexual intercourse during a three-month study period (Sexton et al, 1998). Overall, approximately one-third of patients attain a complete erection with this intraurethral therapy alone (Lugg and Rajfer, 1996). Further investigation into a venous constricting band (Actis) system is currently undergoing multi-center evaluation.

Intracavernosal Medications

Intracavernosal injection is a mainstay of therapy for erectile dysfunction. Vasodilatory medications are injected into the corpora cavernosum by use of a 27 or 30 gauge needle. The primary limitation in clinical use is the patient's fear of a needle inserted into the penis. Pain is the most common side effect reported by patients, frequently resulting in discontinuation of therapy (Sexton et al, 1998). The addition of sodium bicarbonate or lidocaine to the formulation has been utilized to decrease the discomfort.

Patients with a neurogenic cause of impotence often have an enhanced response to these agents, and 25% of the usual dosage should be given as a test dose. As with the vacuum erection device, intracavernosal injection therapy should be carefully used in patients on anti-coagulation therapy (Lewis and Barrett, 1995). Patients require adequate manual dexterity and visual acuity to perform the injection properly (Lugg & Rajfer, 1996). Long-term studies with intracavernosal injection therapy report patient and partner satisfaction rates of 70% and 67%, respectively, but only 41% of patients continue to use this therapy beyond five years follow-up (Sexton et al, 1998).

Prostaglandin E_1. Two commercial forms of prostaglandin E_1 are available: Caverject® (Upjohn) (Figure 4) and Edex® (Schwarz). Prostaglandin E_1 is a smooth muscle relaxant, binding to receptors on cavernosal tissue and resulting in increases of cAMP and an efflux of intracellular calcium. This induces relaxation of the trabecular smooth muscle and vasodilation of the cavernosal arteries. Significant pain is reported by over 30-40% of patients using prostaglandin E_1.(Lugg and Rajfer, 1996) The initiation of erection satisfactory for intercourse is

reported in over 70% of patients. (Fallon, 1995)

FIGURE 4: The Caverject® intracavernosal injection system. (Photo provided by Pharmacia & Upjohn Co., Kalamazoo, MI)

Papaverine. Papaverine is a smooth muscle relaxant derived from opium, and it is the most effective single agent for intracavernosal injection therapy. It inhibits phosphodiesterase degradation of cAMP, resulting in smooth muscle relaxation (Lugg and Rajfer, 1996). Single-agent therapy results in satisfactory erections in 35-55% of patients (Fallon, 1995). Significant fibrosis and plaque formation with intracavernosal injection therapy is rare but is more commonly associated with papaverine than with other agents (Lugg and Rajfer). In addition, elevations in liver enzymes are more common with the use of papaverine (3-10%), and levels should be checked if high doses of papaverine are used (Lugg and Rajfer, 1996).

Phentolamine. Phentolamine is a competitive α_1 and α_2-adrenergic antagonist that also acts as a direct smooth muscle relaxant (Fallon, 1995). It has minimal efficacy as a single-agent but potentiates the effects of papaverine and prostaglandin.(Lugg and Rajfer, 1996; Fallon, 1995).

Tri-Mix. In an attempt to maximize efficacy while minimizing side-effects, most urologists have their own combination of PGE_1, papaverine and phentolamine, which allows the use of lower doses of each agent. A common mixture is papaverine, 120 mg (4 ml of 30 mg/ml); phentolamine, 6 mg; and PGE_1, 120 μg (6 ml of 20 mg/ml) to make a total volume of 10 ml. The patient titrates his intracavernosal dosage from 0.2 to 0.5 cc.

Preliminary studies suggest that investigational agent forskolin may prove beneficial in up to 61% of patients unresponsive to tri-mix (Mullhall et al, 1997). Forskolin is a plant-derived alkaloid that directly activates adenylate cyclase to

increase cAMP levels. Its mechanism of action differs, therefore, from prostaglandin E_1 which indirectly activates adenylate cyclase through receptor-mediated events (Mullhall et al, 1997). Side effects may prevent its clinical use.

Priapism. Any physician prescribing intracavernosal injection therapy should be prepared to treat priapism. Brethine, 5mg orally, can be self-administered by the patient in the event that an erection lasts longer than one hour. If priapism continues, any adrenergic agent can be used to induce detumesence. For example, one ampule of neosynephrine (1000 mg/cc) can be mixed in 9 cc normal saline. Vital signs are monitored and an injection of 0.3-0.5 cc (300-500 mcg) is administered intracavernosally. If this fails, the urologist must attempt a variety of aspiration and irrigation maneuvers before considering surgical reversal.

Constrictive Bands and Vacuum Therapy

Constrictive bands can be used in patients with adequate tumescence and rigidity when their primary problem is early detumescence. These patients often have strong psychological overlays to their impotence. Patients with mild to moderate veno-occlusive dysfunction also can benefit from constrictive bands.

Vacuum erection therapy (Figure 5) is a highly successful therapy for select

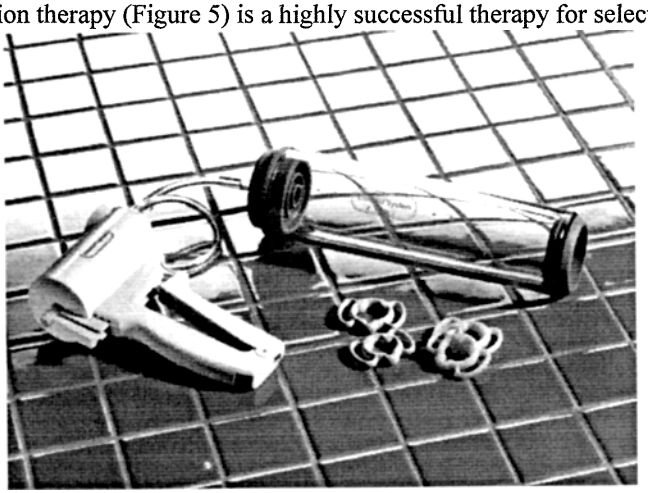


FIGURE 5: The Osbon vacuum erection device. (Photo provided by Imagyn, Augusta, GA)

patients. A cylinder is placed over the penis, and vacuum pressure of greater than 100 mm Hg is generated by use of a hand-pump or battery-operated pump.(Nadig, 1994) Improved rigidity is obtained by a "double-pump" technique- applying a vacuum for two minutes, then releasing and reapplying. Passive engorgement of the penis accounts for tumescence, in conjunction with a transient increase in cavernosal arterial flow. Once a good erection is attained, a constrictive band is placed at the base of the penis and the vacuum is released. The main limitation for

vacuum erection is the variance of its adaptability to the individual's sexual relationship. As it is difficult to conceal the use of the vacuum device, it is often more successful in a stable relationship with a supportive partner where its use can be incorporated into sexual foreplay. Rare side effects include penile ecchymosis or hematoma; the use of a vacuum erection device is cautioned in the anticoagulated patient. Some patients complain of penile cyanosis and diminished sensation. Partners may complain of lowered phallic temperatures. Another limitation is that rigidity extends proximally only to the level of the constrictive device; more proximally the corpora is flaccid, therefore the crura of the corpora cavernosa is not stabilized at the pubic bone.

Penile Prosthesis

The insertion of a penile prosthesis is a significant step for a patient with erectile dysfunction. Patient concerns include the relative irreversibility of the procedure and the artificial nature of the erection. Significant surgical risks include hematoma formation, infection, and device malfunction. Surgical infection requires removal of the artificial material from the patient. Re-operation can be anticipated in 5 to 10% of patients (Lewis and Barrett, 1995). However, recent product modifications have improved the reliability of penile prostheses.

Malleable and inflatable models are available. (Figures 6 and 7) Patient preference, body habitus, and co-morbidities are factored into the decision of which device to implant.

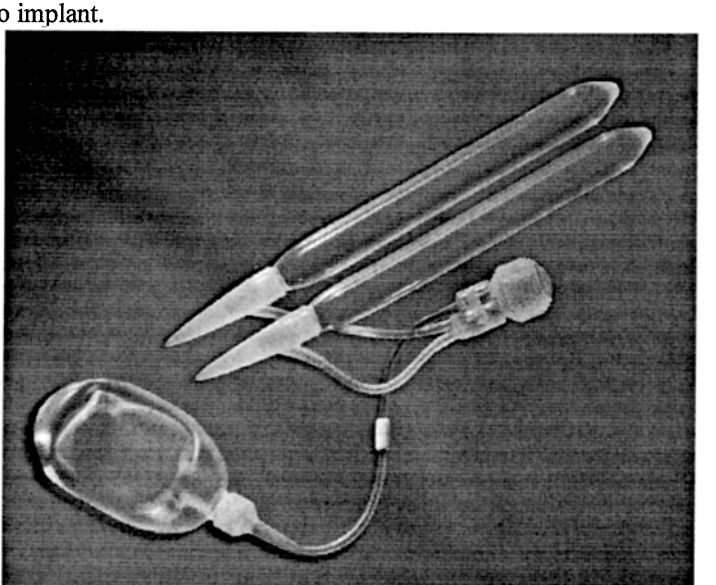


FIGURE 6: The Mentor three-piece inflatable penile prosthesis. (Photo provided by Mentor, Santa Barbara, CA)

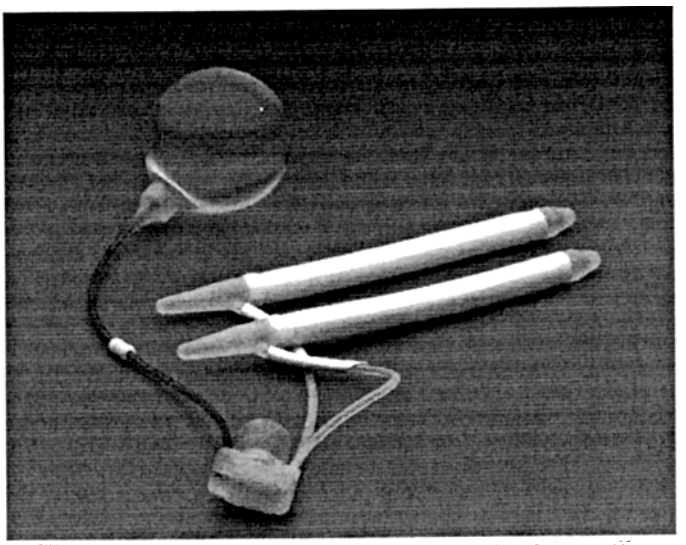


FIGURE 7: The AMX 700 Ultrex™ Plus inflatable penile prosthesis. (Photo provided by American Medical Systems, Inc., Minneapolis, MN)

Long-term follow-up of penile prosthesis patients report patient and partner satisfaction rates of 77% and 79%, respectively (Sexton et al, 1998). Inflatable penile prostheses fare better than malleable penile prostheses, with patient satisfaction rates of 88% versus 45%, respectively, and partner satisfaction rates of 87% versus 55%, respectively (Sexton et al, 1998).

Vascular Surgery

Arterial revascularization of the penis can be performed by anastomosing the inferior epigastric artery to the superficial dorsal artery, the cavernosal artery, the cavernosal body, or the deep dorsal vein of the penis. Venous leak surgery can be performed by ligation or resection of the dorsal and deep dorsal veins of the penis. However, long-term success rate for these procedures is less than 50%. In properly selected patients, such as younger individuals with a history of perineal trauma, long-term success rates can be as high as 65% (Lewis and Barrett, 1995).

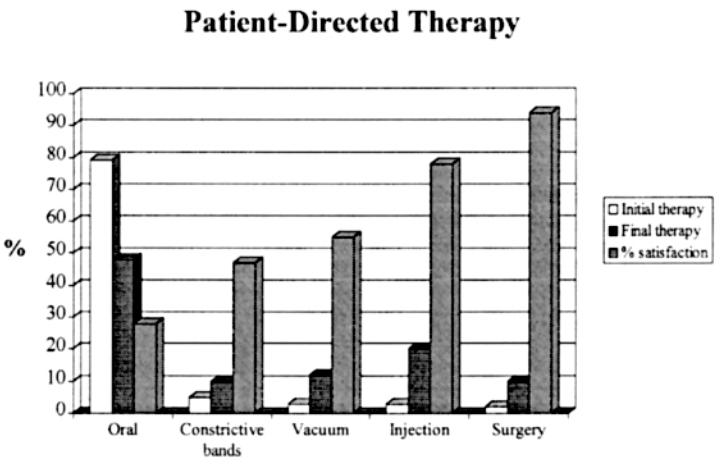
Continued Follow-Up

Long-term studies demonstrate that many patients move up and down the impotence pyramid. The average patient may try two treatment modalities; however, ultimately only 40% of patients achieve long-term satisfaction with such goal-directed therapy (Jarrow et al, 1996). The main limitation to improved results is the patient's reluctance to chose more effective but more invasive therapeutic modalities.

The results of patient-directed therapy are summarized in Figure 8. A generalized shift occurs with time from the lower-tiered therapies to more invasive

therapies. Patients overwhelmingly choose oral medications as the initial mode of therapy; however, only 48% of patients continue to use oral therapy at one to three year follow-up (Jarrow et al, 1996). Patient satisfaction correlates directly with the invasiveness of therapy selected.

FIGURE 8



A five-year study reported that 70% of patients continued to achieve sexual satisfaction with the use of their penile prosthesis, while only 41% of patients using intracavernosal therapy continued to do so (Sexton et al, 1998). Over 60% of patients who discontinued injection therapy chose an alternative therapy - most commonly penile prosthesis or vacuum erection device. The main reasons for discontinuation of injection therapy included inadequate erections, lack of spontaneity, and side effects. However, 31% of patients who discontinued injection therapy either lost sexual interest or did not have a sexual partner.

CONCLUSION

Erectile function is a complex coordination of central and sensory stimuli, neural reflexes, vascular relaxation, and muscular contraction - all orchestrated by a psychological maestro. The slightest miscue can bring the performance to a halt. Once the confidence of the maestro is shattered, reassurance and practice are required to restore the quality of function once again.

The importance of psychosocial counseling in the management of erectile dysfunction cannot be over-emphasized. Prompt intervention and selection of

appropriate adjunctive therapies will optimize results. Whenever possible, patient-directed therapy and incorporation of the partner's feelings, expectations, and preferences should be encouraged.

The restoration of life and sexual function can be very rewarding, to physician and patient alike.

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16 MALE CONTRACEPTION IN THE 21ST CENTURY

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INTRODUCTION

World population growth is a major problem of modern civilization. At present there are 6 billion people in the world and most of them live in developing countries. The rate of population growth is about 1 billion per decade. Studies have shown that about 50% of couples in the reproductive age do not have access or choose not to use modern contraceptive methods. In the female, the available methods include the oral contraceptive pills, injectables, implants, intrauterine devices, cervical caps, diaphragm, female condom and tubal ligation. In the male, the paucity of methods persists. International agencies, national governments, women's health groups and communities recognize the need and are supporting research and development in contraceptive methods for men (World Health Organization, 1998). We believe that a variety of methods of male contraception will be available in the twenty-first century.

AVAILABLE MALE METHODS

Coitus interruptus and periodic abstinence are termed male methods. In the latter, participation of the female partner is necessary when combined with natural family planning. The acceptance of these methods is very low and failure rates are very high.

Condoms are used by 40 to 50 million men. The typical failure rate is about 15% (Trussel and Kost, 1987). A major advantage of condoms is that they protect against transmission of sexually transmitted disease, a property not shared by any of the male methods under development. Non latex condoms which may be more

esthetically pleasing (cause less reduction in sexual pleasure) are yet not widely available.

Vasectomy is a safe and effective method but is offered to couples when the family size is completed. Acceptability of vasectomy varies from country to country. Most of the 45 million men who had vasectomy reside in the United States, United Kingdom, Australia, the Netherlands, China, Korea and Thailand. The failure rate of vasectomy is extremely low but efficacy may be delayed to allow for sufficient time to clear the spermatozoa from the ejaculatory system. Vasectomy acceptance has been improved in many countries with the "no scalpel" surgical method (Li, 1980; Li et al 1991). Intravasal injections of vas occlusive agents, cured-in-place elastomors or silicone have been studied in some countries (China, Indonesia) with variable success rates reported (Li, 1980; Zhao et al 1992, Chen et al, 1992). The major disadvantage of vasectomy is its poor functional reversibility. While reanastomosis of the vas when done by a skilled operator has very high technical success rate (reappearance of spermatozoa in the ejaculate in over 90% of subjects) but pregnancy in the female partner is reported in only about 50% of the couples. This is believed to be due to the development of antisperm antibodies in the reproductive tract and circulation of vasectomized men. If fertility is not achieved after successful vasovasostomy, microsurgical aspiration of spermatozoa from the epididymis followed by in vitro fertilization of the spouse's eggs by intracytoplasmic injection of spermatozoa and embryo transfer is possible but not available to all (see Chapter 14).

METHODS DIRECTLY ACTING ON THE TESTIS

In the 1970s, **gossypol**, extracted from cottonseed oil was extensively studied in China. Over 8000 men received gossypol for varying periods of time and essentially all men became azoospermic and infertile. The action of gossypol is directly on the testis, affecting the germ cells without significant action on Leydig cell steroidogenesis. Gossypol works through disruption of oxidative phosphorylation (National Coordinating Group, 1978). The main problem of gossypol is irreversibility which is dose dependent. Permanent sterility occurs in up to 25% of men after using gossypol for a few years (Meng et al, 1988). The other problem is renal loss of potassium resulting in hypokalemia and occasionally hypokalemic periodic paralysis. Moreover, animal toxicological studies in several species including the non-human primate showed additional toxic effects of gossypol (Waites et al, 1998). Other agents that have been studied include **sulphasalazine** and 3 **indazolecarboxylic acids**. These agents have multiple actions on cellular events are not testis specific, and may cause irreversible destruction of the germ cells.

Physical agents such as **heat** have been shown to decrease spermatogenesis in animals and men (Kandeel and Swerdloff, 1988). Germ cells are highly susceptible to the effects of heat. They are protected from the damage that would occur at core body temperature by the location of the testes in the scrotum. In men, when the testes are pushed back in the inguinal canal and held in that position to increase

testes temperature , significant suppression of sperm counts and motility are noted (Mieusset and Bujan, 1995). Our studies in rodents show that the effects of short term testicular warming (43°C for 15 minutes) induces reversible loss of testicular germ cells by stage and germ cell specific accelerated apoptosis (Lue et al, unpublished data).This is likely to be true in humans also. However, when the increase in the scrotal temperature is only about 0.8 to 1°C (achieved by men wearing polyester lined athletic support) no suppression of spermatogenesis was observed (Wang et al, 1997).

EPIDIDYMIS AS THE TARGET OF MALE CONTRACEPTIVE AGENTS

An agent acting on the epididymis would not affect the hypothalamic-pituitary-testis axis, and have fast on and off action. Efforts have been undertaken to identify unique functions of the epididymis which can be disrupted. The epididymis, like the testis, is not readily accessible to pharmacologic intervention because of the blood-epididymis barrier. The most well studied agents are **α-chlorohydrin** and **6-chloro-6 deoxy sugars** (Ford et al, 1978) which act on the epididymal spermatozoa. They impaired sperm metabolism and affect sperm motility and cause reversible infertility in rodents. They are, however, neurotoxic in the non-human primates.

Tripterigium Wilfordii multiglycosides, isolated from the plant Tripterygium Wilfordii,, have been reported to have antifertility effects both in the rat and the human (Qian 1987; Qian et al, 1995). This plant extract is used as a traditional medicine in China for the treatment of dermatologic or immunologic diseases (e.g., psoriasis and rheumatoid arthritis). Studies in the rat showed that administration of this multiglycoside resulted in marked reduction in sperm motility within 5 weeks of administration suggesting that the action may be on post meiotic sperm or sperm in the epididymis. Based on bioassay-guided isolation of active agents, triptolide has been identified as one of the active components from the plant extract. Studies performed in our laboratory with purified triptolide showed that administration for 35 days resulted in moderate decreases in sperm motility, continued administration for 70 days led to marked decrease in motility and fertility (Lue et al 1998). Unpublished studies from our demonstrated that when triptolide was administered for over 80 days, spermatogenesis is severely affected with decreased testis volume, marked reduction in testicular sperm and irreversible fertility. Studies are ongoing to determine the mechanism of action of triptolide and whether reducing the dose but administering for longer periods of time will result in selective post-meotic effect without affecting spermatogenesis. Of note, all the above contraceptive agents were discovered as a toxic side effect when these agents were administered for other medicinal indications.

HORMONAL METHODS

Hormonal methods are based on reversible suppression of the hypothalamic-pituitary axis resulting in reduction in intratesticular testosterone and suppression of spermatogenesis (Figure 1) and see Chapters 1 and 2. Hormonal deprivation in

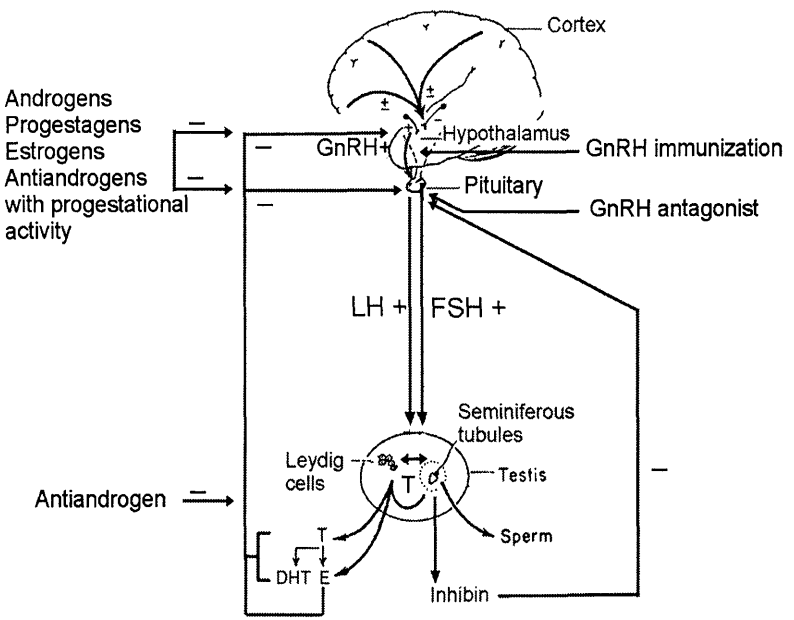

Figure 1. The hypothalamic-pituitary-testis axis showing the sites of actions of hormonal methods of male contraception in clinical testing (T-testosterone, E=estradiol, DHT=5α dihydrotestosterone)

the testis results in accelerated germ cell apoptosis (Sinha Hikim et al, 1995) which is the cellular mechanism resulting in reduced sperm production. Many hormonal methods have been tested in clinical trials and represent the most promising lead with the hope of a product ready to be introduced in the next decade (Waites, 1993; Cummings and Bremner, 1994; Wang et al, 1994). The hormonal methods include androgen alone and androgen plus progestagens, GnRH agonists/antagonists, antiandrogen and estrogen. The primary goal of hormonal methods is to provide safe, effective, reversible contraception to couples in stable relationships.

Androgens Alone

Testosterone enanthate (TE) was the hormonal prototype contraceptive that was first tested in the 1970s. When administered at a weekly dose of 200 to 250 mg IM, azoospermia resulted in 40 to 60% of normal men. Oligozoospermia (sperm concentration <5 million/mL, arbitrarily defined) occurred at 90 to 95% of the treated subjects (Swerdloff et al, 1978, Cunningham et al, 1978; Mauss et al, 1978; Paulsen et al, 1982; Patanelli, 1978). If the frequency of TE injections were decreased to 2 weekly intervals, effective suppression of sperm production was decreased (45 to 65% oligozoospermia). If suppression was initially induced by weekly TE injections and then spacing of injections changed to 2 to 4 weekly intervals, sperma counts increased (Patanelli, 1978). In all instances, sperm concentration and serum hormone levels returned to normal after stopping TE injections.

In a protocol based on the earlier studies, the World Health Organization (WHO) organized two consecutive, multicenter studies to determine whether TE induced azoospermia (first study) and oligozoospermia (second study, oligozoospermia redefined as <3 million/mL) would provide effective contraception. These studies are novel in that the primary end point was pregnancy rates instead of the suppression of sperm counts. TE 200 mg IM administered at weekly intervals was chosen for the initial studies because of its known efficacy, safety and reversibility. In the first study (WHO, 1990), 271 couples participated in 10 centers from seven countries. After a pretreatment phase of 4 to 6 weeks, the subjects were treated with TE 200 mg IM/weekly for 6 months. If by the end of 6 months, sperm was still present in the ejaculate, the subject was discontinued from the study. If the subject became azoospermic (defined as three consecutive semen samples showing no spermatozoa in the ejaculate), the couple then entered 12 months of efficacy where no other contraceptive methods were used. Overall, 64% of them developed azoospermia. The suppression of spermatogenesis to azoospermia occurred in 50 to 60% in the non-Asian centers compared to over 90% in the Asian centers. There was one pregnancy during 1846 months of efficacy exposure. The contraceptive efficacy rate (Pearl rate) was 0.8 per 100 person years (95% confidence interval 0.02 to 4.5). This Pearl rate is better than most of the existing methods (WHO 1990). The second study was different from the first in that the threshold of entry into the 12 months efficacy was sperm concentration less than 3 million/mL in three consecutive semen samples. The answer to this question is very important since, to date, none of the hormonal methods of spermatogenesis suppression resulted in universal azoospermia in all men. 399 couples from 15 centers in nine countries participated in this second study. At 6 months of TE weekly injections treatment, 97.8% reached severe oligozoospermia and entered the efficacy phase. The results are shown in Table 1.

No pregnancy occurred in the 230.4 person-years of observation in those who reached persistent azoospermia. Four pregnancies resulted from the 49.5 person-years of exposure in couples whose male partner had sperm concentrations between 0.1 to 3 million/mL. The contraceptive efficacy rate was 1.4 per 100 person-year (95% confidence interval of 0.4 to 3.7). The pregnancy rate was directly related to the sperm concentration in the ejaculate (WHO, 1996). Once azoospermia is

reached, rebound of sperm concentration to above 3 million/mL occurred only in four out of 749 men. These studies demonstrated conclusively that if a hormonal

Table 1. Contraceptive efficacy (rate per 100 person years) of Testosterone Enanthate induced azoospermia or severe oligozoospermia

Sperm Concentration million/mL	Exposure person-years	Pregnancies	Rate per 100 Person-years
0	230.4	0	0 (0.0 to 1.6)
0.1 to ≤ 3	49.5	4	8.1 (2.2 to 20.7)
0 to ≥ 3	**279.9**	**4**	**1.4 (0.4 to 3.7)**

Values in parentheses are 95% confidence interval (WHO, 1996)

method renders most of the men azoospermic and the remainder severely oligozoospermic, then such a method would provide efficacious contraception for the population.

Despite the positive results of the above studies, many people believed that weekly injections of TE would not be a practical or acceptable method for most men. Furthermore, the pharmacokinetic profile of TE with peaks and troughs may not be ideal for contraception and that steady level of hormones may be associated with better efficacy and less side effects (see Chapter 8). In the two WHO sponsored studies there were no severe adverse effects (Wu et al, 1996).

Other T delivery systems (see Chapter 8) have been or are being developed to provide more acceptable and hopefully more efficacious delivery of androgen based hormonal contraception. Crystalline **(T implants)** (1200 mg) which maintained serum T levels at the high normal range, provided the same suppression of spermatogenesis as TE weekly injections (Handelsman et al, 1992). When less T implants (600 and 800 mg) were inserted, the efficacy rate decreased (Handelsman et al, 1996). **Testosterone bucilate**, a long acting ester of T, when administered as an aqueous suspension at the dose of 1200 mg IM resulted in azoospermia in three out of eight men (Behre et al, 1995). A single 1200 mg dose resulted in serum T concentration in the low normal range in normal men for up to 20 weeks. This preparation is undergoing formulation modifications to allow optimization of the particle size and stability of T buciclate in aqueous suspension. A third formulation of a long acting testosterone ester in oil (**testosterone undecanoate** 1000 mg/per month) is being tested in China for contraceptive efficacy. Similar studies are beginning in Germany.

To circumvent some of the potential side effects of androgens on the prostate, a non-5α reducable androgen has been developed and is being tested in animals and human volunteers. It is hypothesized that such an androgen will be prostate sparing yet provide satisfactory anabolic activity on other end organs such as muscle and bone. 7a methyl-19-nortestosterone (**MENT**) is ten times more potent than T in gonadotropin suppression but only four times more potent in stimulating prostate growth in castrated rats (Kumar et al, 1992). MENT has been formulated into a one year implant for contraceptive development and clinical trials will begin once toxicological studies are available. Other new androgens may be on the horizon.

Molecular receptor drug modeling techniques may produce non-steroidal androgens with selective antagonistic and/or agonistic actions (Hamann, 1998).

Androgens and Progestagens

Combined steroid treatment with androgens and progestagens may enhance contraceptive efficacy and allow greater safety by lowered dosage of androgens. Progestagens when given alone to normal men are effective in suppressing gonadotropins secretion, circulating T levels and spermatogenesis but androgen replacement is necessary to prevent the symptoms and signs of hypogonadism. The combination of androgens and progestagens are synergistic in suppressing gonadotropins and inducing azoospermia. The dose of androgens used in these combination regimens are usually lower than those of T alone. Early studies employed a number and variety of androgen-progestagen combinations in small clinical trials supported by the WHO and Population Council (Schearer et al, 1978; Paulsen et al, 1982; WHO, 1992 to 1995) showed variable degrees of suppression of spermatogenesis. The most promising agent identified in these early studies was **depot medroxyprogesterone acetate (DMPA).** Table 2 shows the more recent studies of androgen and progestagen combinations as potential male contraceptive agents. The progestagens used all have intrinsic androgenic activity.

Combinations of DMPA with TE or **19 nortestosterone** (19NT) were studied in German and then Indonesian men (Knuth et al,1989; WHO, 1993). Suppression of spermatogenesis to azoospermia occurred in about 60% of white men and in 97% of Asian men (Table 2). Sexual function and well being were well preserved but main side effects included weight gain, gynecomastia and suppression of HDL-cholesterol levels. More recently, the use of androgen and progestagens versus androgens alone were compared in terms of efficacy of suppression of spermatogenesis (Bebb et al, 1996; Anawalt et al, 1997). These investigators showed that addition of 125, 250 or 500 μg of **levonorgestrel** administered orally to TE 100 mg IM weekly injections significantly increased the suppression of sperm concentration to azoospermia or severe oligozoospermia. Moreover the combination regimens appeared to induce a more rapid suppression (Table 2). The main side effects noted were weight gain and lowering of HDL levels by about 20%. When **desogestrel** was used in the combination with TE (Wu et al, 1998, personal communication) results were similar to levonorgestrel (Table 2). Desogestrel-TE combination resulted in equivalent suppression of serum HDL-cholesterol levels. These groups of investigators are currently studying lower doses of levonorgestrel and desogestrel as well as pursuing other orally available progestagens with less androgenic potential (e.g., 17 hydroxy-derivatives).

Other combinations include IM injections of DMPA with crystaline T implants (800 mg) resulted in a more rapid and efficacious suppression of sperm counts to azoospermia (Handelsman, 1996) (Table 2). Ongoing clinical trials are studying the combination of **Norplant II** (levonorgestrel implants) with transdermal T (or DHT) to test the hypothesis whether attaining stable levels of both androgens and progestagens can reduce the dose requirements of both agents and the adverse

Table 2. Androgen plus progestagens as potential methods of male contraception

Progestagen	Androgen	Azoospermia %		Oligozoospermia %		Reference
DMPA 250 mg every 6 weeks	19 NT (200 mg every week x 6 /7wks, then 200 mg/3 or 4 weeks)	67 98	(W) (A)	92 99	(W) (A)	Knuth et al (1987); WHO (1993)
	TE (200 mg/IM every week x 6/7 wks, then 200 mg/4 weeks)	59 96	(W) (A)	91 96	(W) (A)	
DMPA 300 mg	T implant (800 mg)	90	(W)	100	(W)	Handelsman et al (1996)
Levonorgestrel (oral)						
500 µg/day	TE (100 mg/ week IM)	67	(W)	94	(W)	Bebb et al (1996)
250 µg/day	TE (100 mg/ week IM)	78	(W)	89	(W)	Anawalt et al, (1997)
125 µg/day	TE (100 mg/ week IM)	61	(W)	94	(W)	
Desogestrel (oral)						
300 µg/day	TE (100 mg/ week IM)	81	(W)	94	(W)	Wu et al (1998)
150 µg/day		100	(W)	100	(W)	
	TE (50 mg/ week IM)	73	(W)	100	(W)	
		57	(W)	78	(W)	

W=Whites, A= Asians, DMPA = depotmedroxyprogesterone acetate,
TE=testosterone enanthate, 19 NT = 19 nortestosterone hexyloxyplenylpropionate

effects on lipid profile. Other studies are planned for T undecanoate (injectable) with DMPA as a 3 weekly contraceptive regimen or T buciclate with the long acting levonorgestrel butanoate in aqueous suspension. It is anticipated that androgens plus progestagen combinations are the most likely candidates for hormonal contraceptive preparations for men within the next 10 years. Similar to the selective estrogen receptor modulators, a number of new quinolones have been synthesized to have selective interaction with the progesterone receptor without any actions on the androgen receptor (Edwards et al, 1998; Zhi et al, 1998). These orally active progesterone agonists may replace the steroidal compounds currently in use.

Androgens and Antiandrogens

Cyproterone acetate (an antiandrogen with progestational activity) unlike flutamide (a pure antiandrogen) when administered to men led to suppression of gonadotropins and endogenous T production as well as having antagonistic effects on the androgen target tissues (see Chapter 4). Use of cyproterone acetate (CPA) alone resulted in moderate suppression of sperm production but was accompanied with marked decrease in sexual function (Wang and Yeung, 1980). Subsequent studies in India (Roy, 1985) using oral CPA with TE injections achieved of azoospermia in most subjects without impairment of sexual function or symptoms of androgen deficiency. This concept was validated in more recent studies where CPA (50 or 100 mg) plus TE 100 mg IM/week resulted in azoospermia or near azoospermia in all subjects. Notably there was no change in lipid profile and CPA might have a theoretical protective effect of the prostate agonist the concomitant androgen administration. A dose-dependent suppression of hematocrit and hemoglobin was noted (Meriggiola et al, 1996, 1997). However, when TE was substituted by oral T undecanoate in an effort to develop a male "pill", spermatogenic suppression was markedly reduced and azoospermia was observed in two out of eight subjects. This suggests that the balance between the antiandrogenic and progestational effects of CPA and the androgenic properties of the administered T may be critical for optimizing spermatogenesis suppression with these agents. Studies are planned for extending the CPA plus TE combination in multiple centers and substituting TE injections by T undecanoate 1000 mg IM in oil once every 8 weeks.

GnRH Analogs and Androgens

Initially **GnRH agonists** were studied because of their effects on down regulation of LH and FSH receptors and suppression of intratesticular T. D-Trp^6, Buserelin and Nafarelin were administered with TE in 12 clinical studies involving over 100 white men (Cummings and Bremner, 1994). In general, combinations of GnRH agonist administered SC with varying doses of TE failed to completely suppress spermatogenesis to azoospermia in most men. Even when the GnRH agonist was administered as a continuous infusion (500 μg/day), the decrease in sperm count to zero in the ejaculate was uncommon (Bhasin et al, 1984; 1985 a, b; Pavlou et al, 1986). The failure of adequate suppression of spermatogenesis may be due to 1) the dose of GnRH agonist used, 2) tendency to escape from serum FSH levels suppression, and 3) partial direct testosterone stimulation of spermatogenesis even with complete suppression of pituitary gonadotropins (LH and FSH). The latter possibility may be supported by data in rodents, primates and humans suggesting that exogenous T might reinitiate or inhibit the complete suppression of spermatogenesis by GnRH analogs (Bouchard and Garcia, 1987; Behre et al, 1992). In contrast, data from other studies indicated that synergism between T and GnRH agonists may exist (Heber and Swerdloff 1980; Bhasin et al, 1984). A recent study administered even higher doses of agonist (1 or 3 mg/day SC D-Trp^6 GnRH) with TE 100 mg IM once every two weeks but failed to significantly improve the rate of

suppression of spermatogenesis to azoospermia (Swerdloff et al, unpublished studies). GnRH agonists have no reported side effects in men in these studies.

GnRH antagonists competitively inhibit binding of native GnRH to its receptor resulting in rapid and complete suppression of gonadotropins, serum and intratesticular testosterone and spermatogenesis. Studies in men showed that GnRH antagonist plus testosterone led to rapid and efficacious suppression of spermatogenesis (Pavlou, 1987; Tom et al, 1992; Bagatell et al 1993). The GnRH antagonists used in the above studies caused local side effects at the site of injection with pain, redness and wheals. Recently a newer GnRH antagonist, Cetrorelix, when used in men showed no local side effects (Behre et al, 1992, 1997).

The authors and their collaborators at the University of Washington have tested the hypothesis that once azoospermia is induced by GnRH antagonist plus T administration, withdrawal of GnRH antagonist will not result in rebound of spermatogenesis (Swerdloff et al, 1998). The results showed that when azoospermia or severe oligozoospermia was achieved with the concurrent administration of Nal-Glu GnRH antagonist (10 mg/day SC) together with TE (100 mg per week IM) for 12 weeks, spermatogenic suppression could be maintained by the same dose of TE alone. The results of this study showed that once near complete suppression of spermatogenesis occurred, continued suppression might require less amount of hormone. Despite their apparent effectiveness, GnRH antagonists may be less practical at present, than steroids for contraceptive purposes since they are expensive to synthesize and long-acting preparations are not yet available.

Under development are non-peptide GnRH antagonists designed by molecular drug-receptor modeling and GnRH vaccines which has been tested only in men with prostate cancer.

Androgens and Estrogens

Studies initiated by Ewing et al (1978) showed that estradiol implants in addition to T implants led to more suppression of spermatogenesis in rats and monkeys without adverse effects on sexual function. Estrogens would have the advantage to neutralize the adverse effects of androgens on lipids. However, the synergistic effects of estrogens androgens on prostate growth have to be studied. A clinical study in a single center has been initiated to examine the efficacy of T plus estradiol implants in suppression of gonadotropins and sperm production in man.

Selective Inhibition of FSH

Selective inhibition of FSH would have the advantage of not requiring androgen supplementation because of the sparing of the LH-T axis. Non human primates **immunized with FSH** resulted in incomplete and not persistent suppression of spermatogenesis (Murty et al, 1979; Nieschlag 1985). **Recombinant inhibin or selective FSH antagonist** have been synthesized and tested in animals but not in man. Recent reports of inactivating mutations of the human FSH receptor in family studies showed variable alteration of spermatogenesis in men with the mutations. These men could be fertile or have small testes and decreased sperm counts (Tapenainen et al, 1997). Such naturally occurring genotypic and phenotypic

aberrations suggest that selective inhibition of FSH may not be a viable method of male contraception. The controversy of whether FSH is essential for human spermatogenesis has not be settled.

ETHNIC DIFFERENCES IN SPERMATOGENIC SUPPRESSION

As indicated above, administration of TE alone or DMPA plus TE or 19 NT resulted in marked differences in the suppression of spermatogenesis between Asian versus non Asian men. The Asian men appeared to be more susceptible to exogenous administration of steroids resulting in over 90% of men being suppressed to azoospermia when the similar regimen led to azoospermia in about 60% of non-Asian men. There are multiple possible mechanisms for the ethnic/geographical difference in the suppression of spermatogenesis. It has been demonstrated that the persistently oligozoospermic subjects in the WHO-TE study had higher levels of 5α reduced steroids in their serum suggesting that the small amounts of active androgenic steroids can maintain some spermatogenic activity (Anderson et al, 1996). We have shown that Asian men are more susceptible to suppression of serum levels of gonadotropins and sperm counts, when T is infused in a ramped dose paradigm, Asian men showed more rapid suppression of LH secretory pulse amplitude and frequency than non-Asian men (Wang et al, 1998). Moreover, we have compared spermatogenic capacity from autopsy testicular samples and showed that the daily sperm production rate per man was lower in Asian when compared to Hispanic or white men (Johnson et al, 1998). Furthermore, in these samples, the spontaneous apoptic rate of germ cells was higher in Asian versus white men (Sinha-Hikim et al, 1998). These studies indicate that different hormonal regimens of male contraception may have to be designed for different geographical/ethnic groups depending on genetic, environmental, social and acceptability factors.

IMMUNOCONTRACEPTION

Immunocontraception can be directed to the spermatozoa in the epididymis or during sperm-egg interaction. The spermatozoa as they pass through the epididymis acquire a number of proteins on their surface. It has been shown in animal experiments specific antibodies against these proteins will block fertilization in vitro and in vivo (Perez Martinez et al, 1995; Ellerman et al, 1996). Recent studies have found a human epididymal protein homologous to the rat proteins that block sperm-egg interaction (Hayashi et al, 1996; Kratzschmen et al, 1996). There are some conceptual problems with this approach including difficulty in directing the antibodies access the blood-epididymis barrier to act on the spermatozoa made the epididymal lumen. Nevertheless, some studies have indicated that this barrier may

be semipermeable to immunoglobulins making an epididymal, immunological approach possible.

Presence of sperm antibodies on the sperm surface may interfere with sperm-egg interaction, leading to failure of fertilization, and is a cause of infertility in about 5% of male infertility. Such infertile men have no other autoimmune disease. Presence of sperm autoantibodies in the female also causes infertility. Sperm specific proteins such as SP10, PH20, Fertilin, SP17, or LDH-C4 have been identified. Initial experiments of immunization of male guinea pigs with PH20 resulted in infertility which was reversible one year after injection of the antigen (Primakoff et al, 1997). Immunization agonist LDH-C4 suppressed fertility in mammalian species including primates (female baboons) (O'Hern et al, 1996). The contraceptive effect was gone one year after last immunization in the female baboons. Studies are in progress with other sperm specific proteins. Immunocontraception using antisperm antibodies will, in principle, work in both men and women (Herr, 1996; Dickman and Herr, 1998).

CONTRACEPTIVE DEVELOPMENT INTO THE 21ST CENTURY

For hormonal methods of contraception, the development of GnRH-non-peptide antagonists will allow the progress required in this approach. Other mutations of the FSH receptor and studies on the FSH antagonist or analogs of inhibin will help to establish whether FSH is essential for spermatogenesis in the human. Selective modulators of the androgen and progesterone receptors may develop into compounds that will suppress spermatogenesis, maintain normal sexual function, bone and muscle mass, without exerting adverse effects on the prostate and lipid metabolism. Such agents may enhance reproductive health in addition to contraception.

Understanding the molecular factors that induce germ cell proliferation or apoptosis may lead to the discovery of master switches that can direct germ cells to apoptic death and to proliferate on demand. Meiosis is a specific event of germ cells. Identification of germ cell specific meiosis initiation and regulating factors would provide insights for infertility and future contraceptive development. Many proteins have been identified which are required for the completion of the spermatogenic cycle. These proteins should be testis specific. Genes encoding these proteins can be cloned and their functional role in controlling mammalian spermatogenesis determined in gene knock-out, targeted gene disruption, or gene over expression experiments.

Epididymal proteins and transcription factors that are important to sperm maturation have to be identified. Interference of these proteins or factors at the epididymis may lead to impaired sperm function (Hinton et al, 1995;Orgebin Crist, 1996). Acidification of the epididymal fluid environment is required to suppress sperm motility (Hinton and Palladino 1995). A proton pumping (H+) ATPase has recently been shown to be important to generate this acidic environment (Breton et al, 1996). Agents that disrupt this acidification process may interfere with sperm motility and infertility. Though attractive as a male contraceptive approach because

of its non-hormonal based action, epididymal interfering agents must pass through the blood-epididymis barrier and have effects specific to the epididymis.

Other specific events prior to fertilization occur while the spermatozoa are in the female reproductive tract. These include opening of calcium channels in the outer sperm membrane, induction of the acrosome reaction, and exposure of sperm docking proteins (e.g., PH20) that allow the attachment to the reciprocal zona pellucida protein (e.g., ZP3). The molecular basis and regulation of these processes are under extensive investigation. Agents can be developed that will inhibit these interactions which may be functional for both men and women. The reversibility of all these methods must also be demonstrated.

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17 ENVIRONMENT AND MALE REPRODUCTIVE FUNCTION

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Environmental impact on male reproductive function has gained increased attention because of adverse trends in male reproductive health. In some geographical areas, the incidence of testicular cancer has increased up to 3-fold, while mean sperm counts in healthy men appear to have declined by up to 50%. Further, the rate of congenital malformations of the male reproductive system has increased. Environmental rather than genetic factors must be invoked as explanation for the observed changes, because these have appeared within 1-2 generations, although genetic susceptibility could well confer an increased sensitivity to certain exogenous exposures.

Male reproductive function is sensitive to environmental exposures, including life-style factors, chemical pollutants, drugs, radiation, etc. However, current knowledge of etiological factors in male reproductive disorders is very limited, and most of the information concerning adverse effects originates from experimental animal studies. A small number of incidents have vividly demonstrated that human reproductive function can be adversely affected by environmental factors. Thus, DES (diethylstilbestrol) prescribed to pregnant women both reduced the sperm counts and increased the prevalence of reproductive system abnormalities of their sons (Gill et al, 1978; Gill et al, 1979). Occupational DBCP (1,2-dibromo -3-chloropropane) exposure was found to decrease semen quality and fertility of production workers (Whorton et al, 1977; Potashnik et al 1978). In a population residing in an area polluted by TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin), the male to female birth ratio was considerably reduced (Mocarelli et al, 1996).

The impact of the environment on male reproductive health may appear as structural, congenital alterations in infants and as functional alterations in adolescents and adults. Adverse effects incurred during fetal development can result in permanent changes in the organization of the reproductive system, whereas adverse effects occurring in adult organs are more likely to be reversible.

PRENATAL AND CHILDHOOD EXPOSURE

Male genital and reproductive disorders are often referred to different medical specialists. Pediatricians deal with cryptorchidism, urologists and oncologists treat testicular cancer, and andrologists attend to fertility problems. The overall perspective may therefore not be that apparent within single specialty groups. However, cryptorchidism, urogenital malformations, testicular cancer and infertility appear to share pathogenetic factors during fetal and perinatal development. Thus, male reproductive disorders are interrelated. The effects of intrauterine exposure to diethylstilbestrol offers a good example of disrupted fetal development.

Diethylstilbestrol (DES)

DES was widely used for more than 25 years to prevent abortions and pregnancy complications (Palmlund et al, 1993). However, a double-blind placebo-controlled study of 840 pregnant women given DES and 806 women given placebo treatment showed that DES was not efficacious for the indications for which it was used (Dieckmann et al, 1953). Later, semen samples from 88 sons of DES-treated and 85 sons of the non-treated women from the same study revealed that sperm concentrations of the DES-exposed men were significantly lower than those of the non-exposed men (83 mill/ml vs. 123 mill/ml); the total sperm count, the total number of motile sperms and the number of morphologically normal sperms were also reduced (Gill et al, 1978). Similar results on sperm parameters have been detected in other studies. However, the sperm concentration of the DES exposed men were above what is considered levels of subfertility, and the fertility of these men was reported to be normal (Wilcox et al, 1995).

Gill et al. (Gill et al, 1979) included 308 DES-exposed men and 307 controls from the Dieckmann et al. study (Dieckmann et al, 1953) in a follow-up study and reported that 31.5% of the exposed men had an abnormality of their reproductive tract versus only 7.8% in the non-exposed group. Cryptorchidism (Stillman, 1982), testicular cancer (Toppari et al, 1996), varicocele, epididymal cysts, and anorchia (Stenchever et al, 1981) seem to be more frequent in men exposed to DES in utero, especially if they were exposed before gestational week 11 (Wilcox et al, 1995). These observations emphasize the sensitivity of organogenesis to chemical exposure.

Normal and abnormal genital development

At the beginning of the fourth week of development, germ cells begin to migrate via the yolk sac through the gut and into the mesentery, ending in the coelomic epithelium of the gonadal ridges (Witschi, 1948). The indifferent gonad is composed of three cell types: germ cells, supporting cells which in male fetuses give rise to Sertoli cells, and stromal (interstitial) cells. The first sign of gonadal differentiation is the development of the Sertoli cells and their aggregation into

primitive seminiferous cords during the eighth week of development (Jirasek, 1976). The differentiation of the gonad into a testis rather than an ovary is

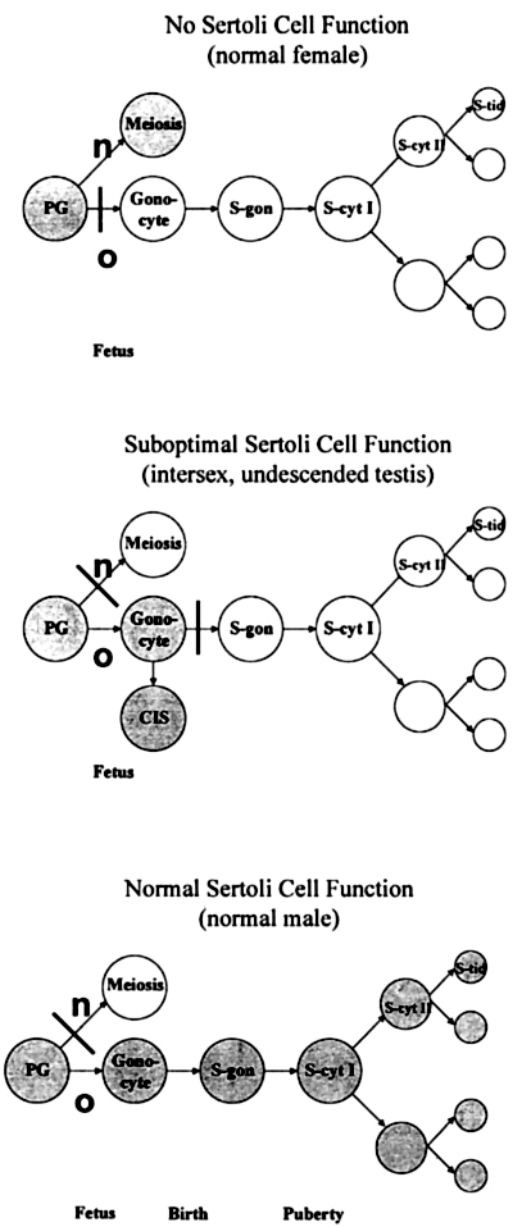

Figure 1: Th development of germ cells is fundamentally different in the two sexes. I the normal male where Sertoli cells develop, the primordial germ cells differentiate and mature into spermatogonia through a gonocyte stage dependent on adequate stim ulation by the Sertoli cells. I the female, the germ cells ente meiosis due t lack of meiosis inhibiting factors from the Sertoli. In cases of sub optimal Sertoli cell function decreased inhi bition from the Sertoli cells may be crucia for development of CIS and/or later reduced sperm count.

genetically dependent, e.g., on the SRY gene (the gene of the sex determining region of the Y chromosome) which is expressed by testicular cells (Sertoli cells) (Gubbay et al,1990) and the SOX9 gene (Foster et al, 1994; Ikeda et al, 1994).

Sertoli cell multiplication. Sertoli cell multiplication occurs during fetal, and to a relatively smaller extent during neonatal and prepubertal life (Cortes et al, 1987). The final number of Sertoli cells reached during development has important consequences in adult life, because these cells are responsible for the regulation of spermatogenesis, and each Sertoli cell can only support a limited number of germ cells (Russell and Peterson, 1984; Orth et al, 1988). Thus, the number of Sertoli cells essentially determines the maximum attainable sperm output. FSH (follicle stimulating hormone) secreted by the pituitary gland stimulates the multiplication of Sertoli cells, and a decreased FSH secretion may therefore lead to a reduced number of Sertoli cells and thereby a subsequently reduced sperm production.

Germ cells. Germ cells in the newly differentiated testis tend to enter meiosis, unless they are inhibited by the Sertoli cells. Thus, it is possible that decreased Sertoli cell function, especially during the first trimester, triggers the development of testicular carcinoma in situ (CIS) cells, which later develop testicular cancer in adults (Skakkebaek et al, 1998). The phenotype of CIS cells is very similar to that of fetal germ cells (Skakkebaek et al, 1987; Jergensen et al, 1995) and CIS cells can be detected in the neonatal period (Muller and Skakkebaek, 1984; Muller et al, 1985). No evidence is available to suggest that CIS cells should arise in adulthood, and the assumption that CIS cells arise before adult life as a result of a prenatal carcinogenic change of the primordial germ cells (Skakkebaek et al, 1987) (Fig. 1) is generally accepted (Damjanov, 1991), although it is difficult to prove experimentally.

Since the clinical development of most testicular germ cell tumors occurs after puberty, the transformation of CIS cells into invasive tumors is thought to be dependent on hormonal factors such as gonadotropins and/or testicular steroids (Skakkebaek et al, 1987; Rajpert et al, 1993). The life-time incidence of CIS and of subsequent invasive cancer is less than 1% in the general Danish male population. However, men with a history of cryptorchidism (maldescent of the testis) have a risk of 2-3% of harboring CIS (Giwercman et al, 1989). Men treated for unilateral germ cell cancer have a prevalence of approximately 6% of harboring CIS in the contralateral testis (Berthelsen et al, 1982; von der Maase et al, 1986). Patients with gonadal dysgenesis and a karyotype including a Y chromosome have a risk of 25-30% to develop gonadal neoplasia (Manuel et al, 1976). CIS has also been described frequently among patients with androgen insensitivity syndrome (Muller and Skakkebaek, 1984; Muller, 1984). Infertile men appear to have an increased risk of CIS (Nüesch-Bachmann and Hedinger, 1977; Skakkebaek, 1978; Pryor et al, 1983), although the magnitude of this risk still is unclear.

Secondary sex characteristics and testicular descent. The secondary sex characteristics are dependent on the hormones produced by the newly formed gonad. Testosterone is secreted by the fetal Leydig cells and, acting via the

androgen receptor, this hormone is responsible for the differentiation of the Wolffian duct (the mesonephric duct) into epididymis, the vas deferens, the ejaculatory duct and the seminal vesicle (Wilson and Lasnitzki, 1971). Testosterone is converted to 5-α-dihydrotestosterone at the bipotential external genitalia and stimulates formation of the penile urethra, the penis and the scrotum. Decreased testosterone secretion may lead to disturbances of these structures, e.g., hypospadias. Testicular descent can be divided into two phases; the intraabdominal descent is quite complex and its regulation is not quite settled, while the descent through the inguinal canals and into the scrotum depends on stimulation by an adequate testosterone concentration (Wilson et al, 1995). The onset of testosterone synthesis may be independent of stimulation by gonadotropins, because the placenta synthesizes and secretes large amounts of chorionic gonadotropin with potent LH activity during the time of embryonic sexual differentiation. Later, when sexual differentiation is more advanced, testosterone synthesis is probably regulated by fetal pituitary LH-secretion (Wilson et al, 1995). Testosterone then may exert its feed back response to the pituitary after aromatization to estradiol.

Exogenous compounds

The sexual differentiation of animal fetuses can be disturbed by exposing them to compounds that possess sex steroid activity or to compounds that alter the endogenous hormone levels. Compounds with estrogen activity may reduce the gonadotropin secretion from the pituitary gland, and thereby imitate the testosterone feed back, thus resulting in lowered androgen action. Furthermore, the testosterone effects may be reduced by compounds that possess an anti-androgenic effect (Kelce and Wilson, 1997). The effect of such compounds is to demasculinize/feminize the fetus. Thus, unbalanced androgen-estrogen ratio can disturb sexual development and lead to congenital malformations, but it may also reduce the potential of future spermatogenesis.

Experimental demonstration that sex steroids may affect the physiology of testicular descent and urethral development point to the possibility that environmental factors that interfere with these steroids could cause developmental abnormalities. Many environmental toxicants are now known to act by interfering with the hormonal balance. A widely accepted definition of endocrine disruptors is: an exogenous substance that causes adverse health effects in an intact organism, or its progeny, consequent to changes in endocrine function (Bergman et al, 1996). Endocrine disruption can occur at several levels: synthesis, transportation, metabolism or excretion of hormones can be altered. However, most attention has been paid to the direct hormone agonist or antagonist actions of environmental agents. In addition, several new extra-nuclear modalities of signal transduction are emerging for steroid hormone effects, including MAP-kinases, cAMP and calcium (Katzenellenbogen et al, 1996; O'Malley et al, 1995), and interference with these functions may also play a role.

EXPOSURES IN ADOLESCENTS AND ADULTS

As described in Chapters 2 and 3, the regulation of spermatogenesis relies not only on endocrine control involving the hypothalamic-pituitary-testicular axis, but it also depends upon autocrine and paracrine interactions between Sertoli cells, germ cells, Leydig cells, peritubular cells, interstitial macrophages, and endothelial cells of the interstitial vessels. Experimental data suggest that several agents can adversely affect the spermatogenesis, and compared to small laboratory animals spermatogenesis in humans may be more vulnerable to toxic damage (Mattison and Thomford, 1987). The susceptibility of adult spermatogenesis to environmental chemicals is best illustrated by an incident where factory workers were exposed to a chemical that was not thought to be toxic to the male reproductive system as described below.

1,2-dibromo-3-chloropropane (DBCP)

DBCP is a highly effective nematocide and fumigant that was widely used in agriculture between 1955 and 1977. In the 1970s, it was recognized that production workers in California (Whorton et al, 1977) and Israel (Potashnik et al, 1978) had become infertile.

Initially, severe impairment of spermatogenesis was found in 18 (78%) of 23 workers exposed to DBCP. Twelve had azoospermia, and the duration of exposure varied between 100 and 6,000 hours. Six workers with exposures between 34 and 95 hours had oligozoospermia. Testicular biopsies showed selective atrophy of the germinal epithelium, intact Sertoli cells and normal appearance of Leydig cells. After exposure had ceased, sperm counts recovered more frequently in oligozoospermic than azoospermic men. Elevated serum concentrations of FSH were associated with permanent impairment of sperm production (Potashnik and Porath, 1995; Whorton and Milby, 1980; Olsen et al, 1990). Serum concentrations of LH and testosterone were normal at the initial investigation. Later on, LH concentrations increased steadily, indicating a delayed toxic effect of DBCP to the Leydig cells (Leroith et al, 1981; Potashnik and Yanai-Inbar, 1987).

The prevalence of male infants conceived during paternal exposure to DBCP dropped to 16.6% for men who later became oligo-or azoospermic. For all exposed men, including those with sperm counts >20 mill/ml, the proportion of male infants was 35.2% during the exposure period. Prior to exposure, the prevalence had been normal at 52.9% (Potashnik and Porath, 1995). In the post-exposure years, the male birth rate rose among couples with men showing recovering sperm counts. Otherwise, follow-up of the children conceived during the exposure period revealed no abnormalities (Potashnik and Abeliovich, 1985).

Animal experiments published as early as 1961 showed DBCP to be moderately toxic to the male reproductive system after a single respiratory exposure and highly toxic at repeated exposures even at very low concentrations. The effects included severely reduced testicular size, reduction of spermatogenesis, and increased number of morphologically abnormal sperm cells (Torkelson et al, 1961). In retrospect, the animal experiments should have been taken into account in the

original risk assessment of DBCP, and adequate preventive measures should have been applied in production and use.

DBCP is now known to reduce lactate production by Sertoli cells (Miller et al, 1985), it may decrease the oxygen consumption, and it may also induce single-strand DNA breaks, especially in round spermatids, but also in spermatocytes and Sertoli cells (Bjørge et al, 1995). Further, round spermatids appear to be the cells most vulnerable to DBCP-induced acute toxicity. The effect of DBCP is related to cell proliferation activity and possibly the differentiation of the testis (Shemi et al, 1988). Thus, the testes are likely to be sensitive to DBCP also during the antenatal/perinatal period (Sod-Moriah et al, 1990).

Results of postnatal exposures

Prolonged dosing with any testicular toxicant, regardless of its mechanism of action, will almost invariably result in germ cell degeneration or loss. The reason is that the germ cells are completely dependent on the coordinated functioning of all other cell types and processes within the testis. Although Sertoli cells and Leydig cells are also very sensitive to damage, these cells generally respond with biochemical disturbances rather than cell death.

Sertoli cell damage will disturb their structural and metabolic support of the germ cells, resulting in germ cell degeneration or exfoliation into the tubular lumen. The release of the mature spermatids is a function of Sertoli cells mediated by cell-to-cell junctions between the Sertoli cells and the spermatids. Some studies have shown that this morphological abnormality may be the only one associated with infertility (Ku et al, 1993; Sharpe et al, 1995).

Decrease of testosterone output from the Leydig cells may not be associated with any visible changes or may be associated with Leydig cell hyperplasia. In laboratory animals, a decreased testosterone level may be associated with a stage-specific degeneration of spermatids and/or spermatocytes. The implications for human spermatogenesis is less clear, but presence of testosterone is essential for the spermatogenesis (Chapter 1).

Changes in testicular blood flow induced by vasoactive compounds or by chemicals that may damage the vascular endothelium are likely to reduce the oxygen and nutrient supply to the interstitial fluid. The germ cells will be the first cells to suffer as they are metabolically active and farthest from the blood vessels. Under normal conditions, the germ cells are in a state approaching anoxia, and they are therefore very vulnerable to changes in the oxygen supply.

SECULAR TRENDS AND GEOGRAPHICAL DIFFERENCES

It follows from the previous sections that semen quality can be affected by compounds that act prenatally and/or postnatally. The mentioned malformations and testicular cancer appear to result from disturbed prenatal sexual differentiation. The recent concern for the environmental impact on male reproductive function is based

on epidemiological evidence that incidence rates for several types of the reproductive system pathology have increased drastically in the recent decades.

Semen quality

In 1992, a possible decline in sperm density during a 50-year period was reported (Carlsen et al, 1992). This metaanalysis was based on 61 studies published between 1938 and 1990. Numerous editorials and critical comments followed. Recently, Swan et al. published a detailed reanalysis of the same data supplemented with further studies from the same period. Taking the critical remarks into consideration, the authors allowed for the effects of duration of abstinence, age, proven fertility, specimen collection method, study goal and geographical location (Fig. 2).

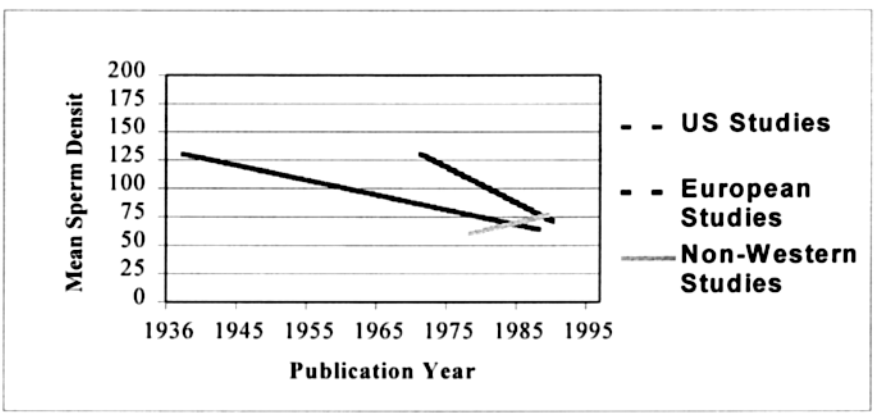

Fig. 2: Mean sperm densities in millions/ml shown as fitted regression lines for USA, Europe and non-Western countries at different (publication) years. Adapted from Swan et al. (56) with permission. See text for further explanation.

Significant declines in mean sperm densities were seen both in the USA (1938-1988, slope = -1.50) and Europe (1971-1990, slope = -3.13). These decreases were greater than originally reported by Carlsen et al. (1992). However, no decrease was detected in non-Western countries during a shorter time period (1978-1989, slope = +1.56) (Swan et al, 1997).

In addition to temporal differences in sperm density, large geographical differences seem to exist both in Europe and the USA (Auger et al, 1995; Irvine et al, 1996; Vierula et al, 1996; Fisch et al, 1996; Paulsen et al, 1996). In this regard, Finland represents a unique case, where sperm density is comparatively high and has remained unchanged during a 28-year period (Vierula et al, 1996). In other areas, decreasing sperm density seems to reflect a general trend within the affected population. French and Scottish data have shown that sperm density continues to deteriorate, with sperm densities in adult men declining with more recent years of birth (Irvine et al, 1996; de Mouzon et al, 1996). This finding is in agreement with the concept of increasing exposures in early life to substances that affect subsequent development of sperm production.

Testicular cancer

The incidence of testicular germ cell tumors, which constitute more than 95% of testicular cancers, has increased in many countries during the last 40-50 years (Toppari et al, 1996; Adami et al, 1994; Forman and Meller, 1994). Testicular cancer is a malignancy predominantly affecting otherwise healthy young men aged 25-40 years, and in this the disease differs from other malignancies that predominantly arise in elderly people. The outcome of testicular cancer is lethal if undiagnosed or untreated. In most Western countries reliable cancer registries exist, and the changes over time do not reflect increased reporting. In Denmark, the incidence has increased 3-4-fold between 1943 and 1982 (Østerlind, 1986) and is among the highest in the world. In countries with a lower incidence, e.g., Scotland (Boyle et al, 1987), the USA (Brown et al, 1986) and Finland (Hakulinen et al, 1986), an increasing incidence has also been observed. Currently, the incidence of testicular cancer is five times higher among Danish men than among Finns, who, at the same time, also have a higher sperm concentration than Danish men. Interestingly, Danish men born during the occupation period of the Second World War have a lower risk of developing germ cell tumors than expected from the overall trend for the birth cohorts (69), thus indicating that exogenous compounds associated with affluent society may be of importance.

Cryptorchidism

Cryptorchidism appears to have become more common. Comparisons of prevalence of cryptorchidism are difficult, because classification has not been standardized (e.g., inclusion of boys with retractile testes will increase the numbers). The reported prevalence rates have varied between 0.03% and 13.4% during the first year after birth, according to hospital chart surveys or central registries, and between 0.16% and 13.3% in surveys of school children, military recruits, etc. (Toppari et al, 1996) (and references therein).

A recent British study from the John Radcliffe Hospital of 7,441 boys (1984-1988) detected a higher rate of cryptorchid testes (Ansell et al, 1992) than a study in the late 1950s of more than 3,500 boys (Scorer, 1964). Thus, at 3 months of age, 5.16% vs. 1.74% of the boys with a birth weight less than 2,500g and 1.61% vs. 0.91% of boys with a birth weight above 2,500g had maldescended testes. In both studies, the same standardized examination technique and classification method were used, i.e. as described in the Scorer study(Scorer, 1964). Although both cohorts were examined at birth and at 3 months age, Scorer did not exclude boys with congenital malformations other than cryptorchidism, while the John Radcliffe Hospital study did. Thus, the 77% increase at 3 months for babies weighing over 2,500 g at birth might be an underestimation.

Hypospadias

Older prevalence figures of hypospadias range from 0.37 to 41 per 10,000 infants (Hohlbein, 1959; Sweet et al, 1974). Inclusion of minor forms and different

methods of ascertainment contribute to the reported differences. However, available evidence suggest that the rate may have increased (WHO, 1991).

Few longitudinal studies have been performed. The National Register of congenital malformations in England and Wales, showed an increase in hypospadias during 1964-83 from 14.65 to 35.68 per 10,000 births. An increased rate also occurred for cryptorchidism and hydrocele. During the same period, the prevalence rate for abnormalities of the vagina and external genitalia of females has been decreasing (Matlai and Beral, 1985). Thus, it seems unlikely that the observed trends are due to changed reporting practices or improved diagnostic methods.

Two population-based surveillance systems in the USA, the nation-wide Birth Defects Monitoring Program (BDMP) and the Metropolitan Atlanta Congenital Defects Program revealed an increased incidence of hypospadias during 1968-1993 in all regions of the country (Paulozzi et al, 1997). The incidence doubled in the Metropolitan Atlanta area, and the trend was statistically significant. In addition, the ratio of severe to mild forms of hypospadias increased 3-5-fold. According to BMDP, the incidence of hypospadias increased from 20.2 per 10,000 births in 1970 to 39.7 per 10,000 in 1993, and all regions showed parallel trends. The ratio of severe to mild cases also increased over the study period. These observations cannot be explained by improvements of the surveillance system or changes in ascertainment or reporting.

Male/Female ratios

Reproductive hazards associated with male occupations could possibly decrease the male/female ratio of the offspring (James, 1996). Although the evidence in this area is still limited, a similar effect may be caused by environmental exposures, such as TCDD.

In 1976, an industrial accident in the town of Seveso, Italy, caused widespread contamination with TCDD. From April, 1977 (9 months after the accident), to December, 1984, the prevalence of males born decreased to 35,1% among couples living in the most contaminated area. Analysis of serum samples drawn in 1976 from the future parents showed that high TCDD levels were associated with a low male/female birth ratio. During 1985-1994, the prevalence of newborn boys had increased to 48.8%, no longer significantly different from the ratio prior to the accident (Mocarelli et al, 1996).

Up to the 1950s, the male proportion of Danish births increased from 51.2% to more than 51.5%, but it then decreased again to about 51.3%, a difference which is statistically significant (Meller, 1996). The initial increase in the male proportion is mainly a consequence of decreasing stillbirth rates and the decreasing male excess among stillbirths. The recent decrease in the male proportion is more difficult to explain. Hormonally induced ovulation will decrease the male/female birth ratio, but this treatment was not introduced in Denmark until the mid-1980s and will barely have influenced the trend through 1985. Similar decreases have been detected in the Netherlands (van der Pal-de Bruin et al, 1997), Germany (Bromen and Jöckel, 1997), Canada and to a lesser degree in the USA (Allan et al, 1997). Canadian figures emphasize that geographical differences may occur, as the greatest

decrease was detected in the eastern part of Canada. Data from Latin America are less clear, but a decrease is possible (Feitosa and Krieger, 1992).

CAUSATIVE AGENTS

Thousands of man-made chemicals are distributed in the environment, but most of them have not been evaluated for their endocrine-disruptive effects. Several agents are known to cause functional changes in the reproductive system: pesticides (e.g. DBCP, chlordecone), organic solvents (e.g. carbon disulfide, ethylene- and methylene glycol), metals (e.g. lead, cadmium, chromium, manganese, mercury), physical agents (e.g. heat, ionizing radiation, microwaves) and other exposures. Comprehensive databases concerning toxicity, including reproductive effects, are available, e.g. U.S. EPA's Integrated Risk Information System (IRIS Database). Specific data on reproductive toxicity of individual chemicals can also be found in recent large reviews (Toppari et al, 1996; Tas et al, 1996; Bonde and Giwercman, 1995; Hass et al, 1991). Most chemcals have not yet been investigated for reproductive toxicity. The number of agents that may cause these adverse effects is therefore likely to be much higher than it appears from these reviews.

Many chemicals have already been banned in Western countries but still exist in the environment due to their abundant previous use, their persistence, bioaccumulation and due to atmospheric dissemination from continued use. Organic pollutants, such as the PCBs, are pertinent examples. Polybrominated biphenyls are currently used for the same purposes as PCBs, and our knowledge of their reproductive effects is very limited.

One of the key questions in discussions on reproductive toxicity is whether a given compound presents a threat for human health at the dose levels that we are exposed to and whether there is any safe level of exposure. Traditional regulatory guidelines have heavily relied on dose-effect relationships, but molecular mechanisms of tumorigenesis suggest that there may not be any safe level for most carcinogenic compounds. Overt adverse effects on reproductive functions have appeared in many studies only with high doses, but emerging new data on late effects of fetal exposure to chemicals may force a reappraisal of many compounds that were considered safe in the past. The findings that connect developmental endocrine disruption to permanent adverse effects necessitate new research on environmental effects on male reproductive function. Estrogenic components given to fetuses at low levels can induce effects opposite of those occurring at large doses, and permanent changes can then appear in adult reproductive organs at exposure doses that were previously considered safe (vom Saal et al, 1997).

RESEARCH METHODS

Occupational incidents and the secular trends in testicular abnormalities have inspired considerable epidemiological research, but a reasonable understanding of

the processes involved and the significance of environmental exposures requires cross-disciplinary research. The question is then how to approach this study area most efficiently.

Experimental studies are needed to further characterize the biochemistry and physiology of sexual development of the male fetus with a view to identifying different types of susceptibility to chemical exposures. Toxicology studies should then identify the chemicals that may cause such effects and their dose-response relationships. Although routine two-generation studies are now carried out as part of standardized toxicology tests, such studies may not necessarily address the issue of male fetal hypersusceptibility. Efforts are therefore needed to determine the most useful revisions of the experimental designs. Toxicokinetic studies may be useful as well to determine whether the placenta offers any barrier against the passage of toxicants from the maternal circulation to the fetus and whether another barrier exists for the protection of the testicles.

Given the species-related differences and the public health implications of the secular trends described above, the experimental studies must be complemented by epidemiological evidence to provide new understanding. Although the final and most important endpoint is obviously infertility, research is in particular needed to determine abnormalities in earlier stages of the pathogenesis. First, toxicokinetic studies are useful to determine to which degree toxicants reach the testicular cells. Furthermore, determination of relevant environmental pollutants in seminal fluid may be used as an exposure marker, especially for chemicals with a long biological half-life (Schecter et al, 1996; Xu et al, 1993). Early effects on testicular cells may be reflected in serum hormone concentrations, and small changes may become apparent in comparisons of men with different degrees of exposure. Still, direct toxic effects on the spermatogenic cell line may occur without major changes in circulating concentrations of testosterone or gonadotropins. Thus, determination of sperm quality will often be necessary. In epidemiological studies, archived semen samples, i.e., smeared, frozen, or videotaped, may be useful for documentation and later in-depth studies.

Studies of occupational groups may be very informative as high-level exposures occur, and because other causes of reproductive problems may be of limited importance (Bonde and Giwercman, 1995). Many studies have been carried out on fertility rates, but most of them are difficult to interpret, because fertility is dependent on numerous factors that may be difficult to ascertain in an epidemiological study. More sensitive and specific measures should be preferred, such as semen quality parameters and serum hormone concentrations. If semen is examined, the possibility of selection bias must be kept in mind, as the participation rate may be as low as 20%, and the volunteers may be far from representative of the background population. This problem is valid for the comparison (control) group as well. Further, a detailed understanding of the dose-effect relationship may require follow-up studies of the same subjects, e.g., with examinations before, during, and after cessation of exposure. In such cases, comparisons can be carried out for each individual, who then will serve as his own control. In this case, the important issue of reversibility may be examined as well. Case-control studies may occasionally be useful, although most studies of infertile men have suffered from problems of

comparability with the control subjects selected. Such studies would be most useful if nested within cohorts with detailed exposure information. Studies of occupational cohorts have documented several testicular toxicants, including lead, chromate, DBCP, chlordecone (mirex), ethylene dibromide, carbaryl, carbon disulfide, 2-ethoxyethanol, and chloroprene (Whorton et al, 1977; Potashnik et al, 1978; Toppari et al, 1996; Tas et al, 1996; Bonde and Giwercman, 1995; Hass et al, 1991). Given the difficulties in conducting such studies, these chemicals must be regarded mere examples of a group of exposures which probably includes a much larger number of compounds.

Whether cross-sectional or prospective, the studies of exposed workers are relevant only for exposures that affect the reproductive function of the adult male. For exposures that may cause damage to the development of the reproductive system, cohorts of exposed women need to be identified, and the effects should then be sought in their male children exposed during gestation (Toppari et al, 1996). In addition to the DES evidence, a recent study has shown that maternal smoking during pregnancy may affect the reproductive function of the male child. Still, very little is known about the effects of environmental exposures in this regard (Jensen et al, *in press*). Although such studies may require unique study settings, a substantial investment in this type of research would no doubt add greatly to our current knowledge.

One final comment is in place. Many published studies have a limited statistical power and therefore are unable to refute or confirm the apriori hypothesis with any confidence. While small-scale studies may be indicative, major progress will often require that small cohorts are merged and that long-term follow-up be conducted. The need for information in this field will therefore entail substantial funding requirements.

CONCLUSION AND PERSPECTIVES

The testicles may be more vulnerable to many environmental exposures than is any other part of the body, especially with regard to heat, microwave radiation, and ionizing radiation, but probably also to several chemical pollutants. Recent reports on decreasing sperm quality and increasing incidence rates for testicular cancer and abnormalities of the male reproductive system have attracted new attention to the possible effects of environmental exposures on the development of the male fetus. While these findings should not be extrapolated to suggest that sperm counts will reach zero at some future time, their health implications even at present would demand an increased effort to identify the reasons for these worrisome tendencies. This research must be based on a thorough understanding of the basic mechanisms involved in fetal development of the male reproductive tract and its functions. Different types of evidence suggest that disruption of hormone function may be an important toxic mechanism, along with direct sperm toxicity, but the details are unclear, and current methods for identifying etiologic agents are not very sensitive. The experience gathered during recent years has demonstrated that basic research on the development of the reproductive tract may provide keys to understanding the

epidemiologic evidence of abnormalities in male reproductive function, and vice versa. An improved insight into this very challenging area of biomedical research therefore requires a multidisciplinary approach, collaboration between research groups and mutual exchange of results. Given the discoveries made recently, the future of this research area seems to be bright. The implications for human health and modern society must at the same time be recognized, and the prevention needs of society must therefore be considered when setting priorities for this research effort.

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