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Chapter 17 – Estrogens and Male Reproduction

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Updated 8 April 2005

INTRODUCTION

The intriguing concept that a role for estrogens exists in male reproduction has recently been recognized in the field of endocrinology (1, 2). It has developed from observations and studies that have been performed over the last 10 years. In particular, the development of lines of male transgenic mice lacking functional estrogen receptors or a functional aromatase enzyme have shed new light on the role for estrogens in male reproduction (3). Concomitantly, the discovery of mutations in both the human estrogen receptor alpha (4) and aromatase (5) genes have reinforced the idea that estrogens play a key role in the human male reproductive system.

Previously a role for estrogen action in the male reproductive system was being proposed based on scattered data (6, 7) but recent advances have come from in vitro, in vivo and immunohistochemical studies which have begun to elucidate the mechanisms of estrogen action on the male reproductive tract (8-10).

PHYSIOLOGY

Estrogen biosynthesis and actions

In males, estrogens derive from circulating androgens. Aromatization of the C19 androgens, testosterone and androstenedione, to form estradiol and estrone, respectively, is the key step in estrogen biosynthesis, which is under the control of the aromatase enzyme. The aromatase enzyme is a P450 mono-oxygenase enzyme complex present in the smooth endoplasmic reticulum which acts through three consecutive hydroxylation reactions, whose final effect is the aromatization of the A ring of androgens (Figure 1). P450 aromatase is the product of the CYP19 gene, which consists of at least 16 exons and is located on chromosome 15 in humans (5, 11) (Figure 2).

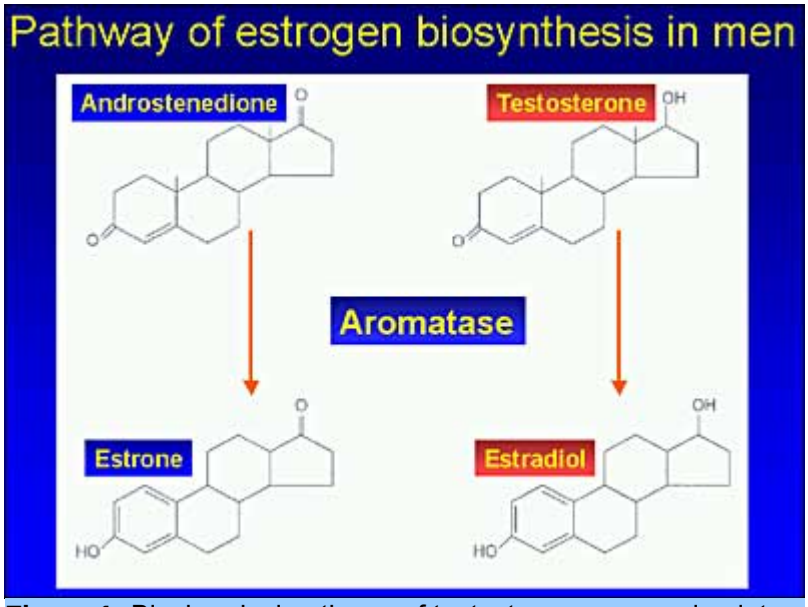

Figure 1: Biochemical pathway of testosterone conversion into estrogen in men.

P450 aromatase is the product of the CYP19 gene which consists of at least 16 exons and is located on chromosome 15 in humans (5, 11) (Figure 2).

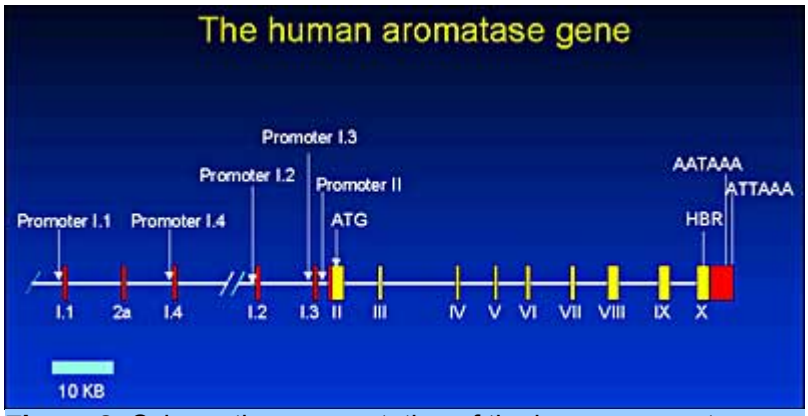

Figure 2: Schematic representation of the human aromatase gene.

In plasma, estrogens are reversibly bound to sex hormone binding globulin (SHBG), a β-globulin, and, to a lesser degree to albumin. Estrogen actions are mediated by binding to specific nuclear estrogen receptors (ERs), which are ligand-inducible transcription factors regulating the expression of target genes after hormone binding. Two subtypes of ERs have been described: estrogen receptor α (ERα) and the more recently discovered estrogen receptor β (ERβ). The human gene encoding for ERα is located on the long arm of chromosome 6, while the gene encoding for ERβ is located on band q22-24 of chromosome 14. The two ER (α and β) proteins have a high degree of homology at the amino acid level (Figure 3).

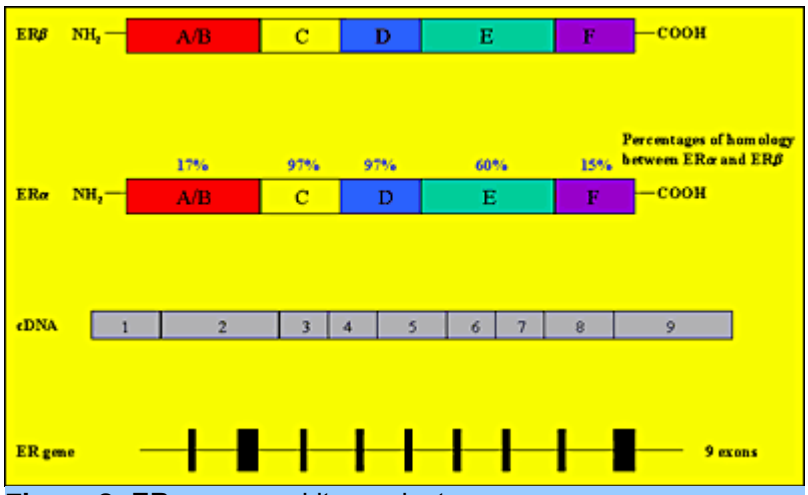

Figure 3: ERs gene and its products.

While it is clear that estrogens regulate transcription via a nuclear interaction after binding their receptors, a non-genomic action of estrogens has been recently demonstrated, suggesting a different molecular mechanism accounts for some estrogen actions. In vitro studies showed a very short latency time between the administration of estrogens and the appearance of biological effects. These actions are thought to be mediated through cell-surface receptors, which are not believed to act via a transcriptional mechanism (12). The different types of estrogen action are summarized in Table 1.

Table 1. Estrogen actions and related biomolecular pathways and mechanisms.

Estrogen Actions	Receptors	Mechanism	Final effect	Features
Genomic (nuclear actions)	ERa	Transcriptional: nuclear interaction with estrogen-responsive elements	Modulation of estrogen target gene expression.	Slow effects (minutes or hours)
	ERb	Transcriptional: nuclear interaction with estrogen-responsive elements	Modulation of estrogen target gene expression.	Slow effects (minutes or hours)
Non Genomic (cell membranes actions)	Estrogen receptors on cells membrane	Cells membrane changes	Changes in ionic transport through cell surface.	Rapid effects (seconds)

Distribution of ERs and aromatase in the male reproductive system

ERs and the aromatase enzyme are widely expressed in the male reproductive tract in both animals and humans, implying that estrogen biosynthesis occurs in the male reproductive tract and that both locally produced and circulating estrogens may interact with ERs in an

intracrine/paracrine and/or endocrine fashion (12). The concept of a key estrogen action in the male reproductive tract is strongly supported by the fact that male reproductive structures are able to produce and respond to estrogens (13). Here we summarize the distribution of both ERs and aromatase in the male reproductive tract of both animals and humans, accounting also for different developmental stages of maturation since both ERs and aromatase are widely expressed at all stages of testicular development (at least in rodents).

ERs and aromatase in fetal rodent testis

Aromatase and ERs are found at a very early stage of development in the rodent testis, thus suggesting a role for estrogens in influencing testicular development (13-16, 17).

ERα is expressed by Leydig cells in the rodent fetal testis at a developmental stage in which the androgen receptor is not yet expressed. The developing efferent ductules and epididymis also express ERα in the fetal rodent. By contrast, it is unclear whether ERα is present within the seminiferous tubules of the fetal testis, with variable results having been reported (13, 16). ERα is abundant in the developing efferent ductules, which are the first male reproductive structures to express ERs during fetal development (18).

ERβ is also found early in testis development in the gonocytes, Sertoli cells and Leydig cells, with the gonocytes showing the highest expression suggesting a role for estrogens in their maturation. In addition, ERβ is expressed by rat Wolffian ducts, the structures from which the efferent ductules and epididymis arise (13, 16).

Aromatase is expressed in both Leydig and Sertoli cells in the rodent fetal testis, but not in gonocytes and immature structures of seminal tract. ERs and aromatase distribution in the fetal testes is summarized in Table 2.

The finding of both aromatase and ERs in the developing fetal testis imply a possible involvement of estrogens in the process of differentiation and maturation of developing rodent testis from an early stage of morphogenesis (14, 15) (see also below: "Effects of estrogen excess or deficiency on male reproduction").

Table 2. ERs and Aromatase distribution in the rodent fetal testis.

	ERa	ERb	Aromatase
Leydig cells	++	++	+
Sertoli cells	-	++	++
Gonocytes	-	+++	-
Seminiferous tubules	+/-	+	+
Ducts	+	+	-

ERs and aromatase in postnatal immature rodent testis

In the postnatal immature rodent testis ERα expression does not occur in the seminiferous epithelium, remaining confined to the Leydig cells, rete testis, efferent ductules and (13) epididymis (Table 3).

In the neonatal rodent testis, ERβ is widely expressed by the rat seminiferous epithelium (Sertoli cells and germ cells) as well as by Leydig cells, efferent ductules and epididymis. At this stage ERβ seems to be the only ER in germ cells and is found in pachytene spermatocytes, round spermatids, and perhaps in elongated spermatids of rats and humans (13) (Table 3).

Aromatase is expressed by the dividing Sertoli cells and is stimulated by FSH, with the levels of aromatase declining with age. Fetal Leydig cells also have the ability to produce estrogens in response to LH, but aromatase in this cell type is expressed to a lesser degree than during neonatal life. Interestingly the neonatal testis continues to show a greater degree of aromatase expression in the Sertoli cells than in the Leydig cells (the latter only express aromatase to a greater extent in the adult rat testis when they become one of the major sources of estrogens under the influence of LH, 13) (Table 3). Germ cells in immature rats do not yet express aromatase.

Table 3. ERs and Aromatase distribution in postnatal immature rodent testis.

	ERa	ERb	Aromatase
Leydig cells	+	+	+
Sertoli cells	-	+	+++
Gonocytes	-	+	-
Seminiferous tubules	-	+	+
Ducts	+	++	(?)

ERs and aromatase in adult rodent testis

ERα is expressed in the Leydig cells of both adult rats and mice (19) but not in Sertoli cells. ERα expression in adult rodent germ cells remains to be confirmed, with its presence in pachytene spermatocytes and round spermatids being suggested by one study yet its absence demonstrated by others (see 13 for review) such that the prevailing view is that ERαo is absent in germ cells. Knowledge of the distribution of ERα is of great importance in understanding estrogen action on the male reproductive tract. ERα is highly expressed in the proximal reproductive ducts (rete testis, efferent ductules, proximal epididymis) and its expression progressively decreases distally (corpus and cauda of the epidydimis, vas deferens). The highest degree of ERα expression is seen in the efferent ductules of the rat (20) and accounts for one of the most well-documented estrogenic actions on male reproductive system, that of fluid reabsorption from the efferent ductules (see below in the text: "Role of estrogens in male reproduction"). It has to be remarked that the concentration of ERα in the male reproductive tract is opposite to that of ERβ, which is more concentrated in the distal tract (Table 4).

Table 4. ERs and Aromatase distribution in the adult rodent testis.

	ERa	ERb	Aromatase
Leydig cells	+/-	+/-	+++
Sertoli cells	-	+	+
Germ cells Spermatogonia	+/- -	++ +	++++ + (?)

Pachytene Spermatocytes Round Spermatids Spermatozoa	-/+ -/+ + (?)	+ + + (?)	+ ++ +
efferent ductules	++++	+	- (?)

ERβ is expressed in Leydig, Sertoli and germ cells in adult rodents (13, 16, 21, 22) and has also been detected in primate germ cells (23). There is now considerable evidence that germ cells contain both ERβ and aromatase (13, 23). It should be noted that there are some controversies in terms of ERβ localization, with immunohistochemical studies showing some discrepancies, possibly due to methodological differences (see 13 for review). It seems that the regulation of gonocyte multiplication, which is under the influence of growth factors and estradiol, may occur through the involvement of ERβ (3).

By adulthood, rodent Leydig cells show higher aromatase activity compared to every other age and in comparison to Sertoli cells (24). Aromatase is also expressed at high levels in germ cells throughout all stages of maturation, and its expression appears to increase as the germ cell becomes a mature spermatid. Aromatase mRNA and activity, in fact, are found in germ cells from the pachytene spermatocyte stage in both rats and mice, and during their subsequent maturation into round spermatids (24, 25, 26, 17). Aromatase seems to be present in higher levels in mature spermatids of the rat than in earlier germ cells (25, 26,17). It is of interest that aromatase mRNA expression and enzyme activity is higher in germ cells when compared with Leydig cells, suggesting that germ cells may be a major source of estrogen in adult rodents (25, 27, 26, 17).

When fully developed spermatids are released from the epithelium, aromatase remaining in the residual body is subsequently phagocyted by the Sertoli cell. Some aromatase activity remains in the cytoplasmic droplet that remains attached to the flagellum as the sperm make its way through the epididymis, suggesting that mature spermatozoa are able to synthesize their own estrogen as they traverse the efferent ducts (27, 28). The ability to synthesize estrogen gradually decreases as the droplet slowly moves to the end of the tail during epididymal transit until it's finally lost. The demonstration of aromatase in sperm is important as it suggests that the sperm itself could control the levels of estrogen present in the luminal fluid, directly modulating functions such as the reabsorption of fluid from the efferent ductules (20).

Distribution of ERs and aromatase in the human male reproductive system

Both ERs have been found in human testis and reproductive tract. In the male fetus ERβ expression is higher than ERα, the latter being absent or expressed at very low levels. In the human fetus ERβ immunoreactivity has been shown in the seminiferous epithelium (Sertoli cells and a few germ cells) and in the epididymissuggesting a role for ERβ in the prenatal development and function of male reproductive structures (29).

ERβ has been detected in rodent (21) as well as in primate germ cells (23). In adult men ERα was expressed only in Leydig cells, while ERβ .has been documented in both Leydig and Sertoli cells and in the efferent ducts (30). The presence of ERs in the human epididymis is still debated (26), even though recently ERa has been detected in the nuclei of epithelial cells of the caput of the epididymis (31). Both ERα and β have been detected in human pachytene spermatocytes and round spermatids with in situ hybridization (32, 33). These latter studies have been contradicted by more recent studies showing strong expression of ERβ in human testis but failing to find evidence for ERα using immunohistochemistry (34) and RT PCR (35), suggesting that ERβ is the primary mediator of estrogen action in the

human testis. Of particular interest is the demonstration of differential expression of wild type ERβ (ERβ1) and a novel human variant form of ERβ, arising from alternate splicing (ERβcx, or ERβ2), in the human testis (36). ERβ2, which may act as a dominant negative inhibitor of ER action, was highest in spermatogonia and Sertoli cells in adult men, suggesting that these cells may be "protected" from estrogen action by the expression of this variant. However wild type ERβ1 was highest in pachytene spermatocytes and round spermatids, which have been proposed to be estrogen sensitive (see 13 for review), yet was low in less mature germ cells (34).

As previously suggested by Durkee et al. (37), ERs are present in human sperm. In particular it has recently been documented by Luconi et al (9) that the sperm membrane contains an estrogen receptor-related protein able to bind steroid hormones that may act through a calcium-calmodulin dependent pathway and thus perhaps accounts for a well documented rapid non-genomic action.

Aromatase expression in the human testis is present in both somatic and germ cells from pachytene spermatocytes through elongated spermatids (38, 16, 25, 39). Aromatase is also expressed in both human Leydig and Sertoli cells (25, 38). Recently, the presence of aromatase has been demonstrated not only in immature germ cells (25, 39), but also in mature human spermatozoa (40, 16). In contrast to rodents, aromatase expression in human gametes is not lost during transit through the genital tracts since P450 aromatase was demonstrated in ejaculated human spermatozoa at three different functional levels: mRNA expression (40), protein and activity (8). Thus ejaculated human spermatozoa continue to express P450 aromatase and contain active aromatase, and thus sperm have to be considered a potential site of estrogen biosynthesis (8, 25, 17). These evidences support the concept that human spermatozoa should be considered a mobile endocrine unit since they are able to synthesize and to respond to estrogens. Again, the presence of functionally aromatase in human spermatozoa permits the conversion of androgens into estrogens throughout the whole transit of reproductive tract, an event that constantly provides free estrogens in the seminal fluid able to act on the cells of the reproductive ducts. Recently a role for estrogen as survival factor during sperm transit in the seminal way has been suggested (33).

In summary, the testes are able to synthesize and respond to estrogens throughout development. The localization of ERα, ERβ and aromatase suggests that estrogen action is likely to be important for testicular and efferent ductule function. The role of estrogens in the male reproductive system has become clearer in regard to animals, and the mapping of ERs and aromatase distribution in the human male reproductive system has led to the suggestion that estrogen plays a role in human male reproduction. As a consequence, a new field of research has evolved aimed at improving our knowledge of estrogen action on male reproduction and the molecular mechanisms involved in both animals and men. To date some estrogen actions on male reproduction have been well characterized but more research is in progress to further define the nature of estrogen action, as outlined in the following section.

Role of estrogens in male reproduction

Role of estrogens in animal male reproduction

In animals, a previously unsuspected physiological role of estrogens in testicular function was revealed by the creation of the ERα knockout (αERKO) mouse. Adult, sexually mature, male αERKO mice are infertile even though the development of the male reproductive tract is largely unaffected (3). Adult testicular histology shows an atrophic and degenerating

seminiferous epithelium, together with dilated tubules and a dilation of the rete testis (41). The disruption of spermatogenesis is progressive as the testicular histology is normal at ten days of age but starts to degenerate at twenty-thirty days. By about 40-60 days the tubules are markedly dilated with a corresponding significant increase in testicular volume while the seminiferous epithelium becomes atrophic (3). A severe impairment in tubule fluid absorption in the efferent ducts was demonstrated to be the cause of infertility in αERKO male mice, and this defect is partially mimicked also by the administration of an anti-estrogen in wild-type mice (20). In the male genital tract the highest concentration of ERα is found in the efferent ducts (42) and the estrogen-dependent fluid reabsorption in this site probably results from estrogen interaction with the ERα that seems regulate the expression of the Na(+)/H(+) exchanger-3 (NHE3). In fact, the disruption of ERα or the use of antiestrogens result in decreased expression of NHE3 mRNA, as well as in a decrease of other proteins involved in water reabsorption, such as aquaporin I (43, 44). The lack of fluid reabsorption in the efferent ductules of αERKO male mice and the consequent dilatation of these ductules induces a retroactive progressive swelling of the seminiferous tubules. The seminiferous tubule damage results from the increased fluid pressure and severely impaired spermatogenesis coupled with testicular atrophy as seen at the age of 150 days (20, 3). In addition, reproductive hormones profiles are abnormal in αERKO male mice as serum LH is significantly increased with a consequent elevated serum testosterone and Leydig cells hyperplasia, but FSH remains in the normal range (3). It is also worth noting that detailed investigations into the development of efferent ductules in αERKO male mice suggest that a congenital absence of ERα leads to developmental abnormalities in this tissue (45).

The recent production of both aromatase knockout (ArKO, 46) and ERβ knockout (βERKO, 47) mice supports the idea that in mice estrogen actions on the male reproductive tract are more complex than previously suggested on the basis of the αERKO mice (3). In fact, unlike αERKO mice, male ArKO mice are initially fully fertile (46), but fertility decreases with advancing age (48), and, conversely, βERKO mice are fully fertile and apparently reproductively normal in adulthood (47).

From seven months of age male ArKO mice are not able to sire any litters. Again histology of the testes of one-year-old ArKO mice shows a disruption of spermatogenesis at the early spermatid without significant changes in the volume of seminiferous tubule lumen, together with Leydig cell hyperplasia (48). Despite the phenotype of αERKO male mice, the mechanism involved in the development of infertility is different in ArKO male mice, since the early arrest of spermatogenesis suggests a failure of germ cell differentiation probably caused by the lack of estrogen action at the level of the seminiferous epithelium rather than a problem referable to impaired fluid reabsorption (9, 15). Recent findings from studies in which human germ cells were treated with estrogen in vitro suggest that estradiol may serve as a survival factor for round spermatids and that lack of estradiol may promote apoptosis with a resulting failure in elongated spermatid differentiation (33). Recently studies in mice deficient in both ER α and β (αβERKO mice) showed a male phenotype very close to that of αERKO mice with infertility and dilated seminiferous tubules (3). These findings, together with the observation that βERKO male mice are fully fertile (47), lead to the hypothesis that estrogen activity in the male reproductive tract differs with regard to both the type of estrogen receptor involved in the pathway of estrogenic action and the site of action through the male reproductive tract. Importantly, results from mice lacking functional ERs or aromatase point to an important role for estrogen in the maintenance of mating behaviour in male mice, and that infertility in αERKO, αβERKO and ArKO mice are at least in part due to reductions in various aspects of mating behavior from an early age (see 3, 13 for review).

The above studies support the concept that a functional ERα, but not ERβ, is needed for the development and maintenance of a normal fertility in male mice (3, 20, 41, 47). Clearly, further studies are needed to fully understand the precise role of estrogens and their

receptors in the establishment and maintenance of male fertility, and the importance of intracrine and paracrine pathways for these effects.

Role of estrogens in human male reproduction

The demonstration of abundant ERs in human efferent ducts and aromatase activity in human sperm, speaks in favor of the involvement of estrogens in male reproductive function. On the other hand, data from human subjects with congenital estrogen deficiency have provided conflicting and somewhat confusing results. The only man with estrogen resistance discovered up till now, a human equivalent of the ERKO mouse, had normal testicular volumes and a normal sperm count but with slightly reduced motility (4) (Table 5). The four adult men affected by congenital aromatase deficiency showed a variable degree of impaired spermatogenesis (49, 50, 51, 52, 53). The patient described by Carani et al., showed both a severely reduced sperm count and an impairment of sperm viability with germ cell arrest at the level of primary spermatocytes (5, 14, 49) (Table 5). A more recent patient had complete germ cell arrest on testicular biopsy but a semen analysis was not performed according to patient's religious views (50, 51) (Table 5). Data concerning the patient described by Morishima et al. are lacking since sperm counts were not analyzed (52) (Table 5). It should be remarked that a clear cause-effect relationship between infertility and aromatase deficiency is not demonstrable in the patient studied by Carani et al., since one of his brothers was infertile despite the absence of mutations in the aromatase gene, suggesting an alternate common cause for their infertility. (49).

Recently a new patient with aromatase deficiency has been described to have impaired fertility (53), confirming a possible association between congenital estrogen deficiency and infertility.

Table 5: Reproductive phenotypes of men with congenital estrogen deficiency.

SUBJECTS	REPRODUCTIVE HORMONES	EXTERNAL GENITALIA	SEMEN ANALYSIS
Estrogen resistance (Age: 28 yrs) (ref 5)	Increased serum LH. Increased serum FSH. Normal serum testosterone. Increased serum estradiol.	Normal male genitalia. Volume of each testis:20-25 mL.	Sperm count: $25x10^6$ / mL (normal > 20×10^6 / mL). Viability: 18% (normal > 50%).
Aromatase deficiency (Age: 24 yrs) (refs 52 and 68)	Increased serum LH. Increased serum FSH. Increased serum testosterone. Undetectable serum estradiol.	Normal male genitalia.Volume of each testis: 34 mL	Not performed.
Aromatase deficiency (Age: 38 yrs)	Normal-to-raised serum LH.Increased	Normal male genitalia.Volume of each testis: 8mL.	Sperm count: $1x 10^6$ / ml (normal > 20×10^6 / mL).

(refs 49 and 125)	serum FSHNormal serum testosterone. Undetectable serum estradiol.		Viability: 0% (normal > 50%). Testis biopsy: germ cell arrest at primary spermatocyte level.
Aromatase deficiency (Age: 28 yrs) (refs 50 and 51)	Normal serum LH. Increased serum FSH. Low-normal serum testosterone. Undetectable serum estradiol.	Cryptorchidism. Volume of each testis: 10-11 mL.	Not performed. Testis biopsy: complete germ cell arrest.
Aromatase deficiency (Age: 27 yrs) (ref 53)	Normal serum LH Increased serum FSH Increased serum testosterone Undetectable serum estradiol	Volume of each testis: 13-14 mL	Sperm count: 17.4 x 10^6 / ml (normal >20 x 10^6 / mL). Viability: reduced

The variable degree of fertility impairment in men with congenital deficiency of estrogen action or synthesis deficiency does not permit a firm conclusion about whether these features are a consequence of a lack of estrogen action or are only epiphenomena, even though a possible role of estrogen on human spermatogenesis is suggested by rodent studies. Recently, the administration of aromatase inhibitors to infertile men with an impaired testosterone to estradiol ratio resulted in an improvement of fertility rate (10), although in the absence of a placebo or control group, these findings need to be interpreted with great caution. Clearly our knowledge of a role for estrogen in human male reproduction is far from complete. The exposure to the excess of environmental estrogens has been proposed as a possible cause of impaired fertility. It is difficult to reconcile existing data about effects of both estrogen deficiency and excess on male reproductive function (1, 6, 54, 55, 56). These issues are discussed further below.

Regulation of gonadotropin feedback

The regulation of gonadotropin feedback is an important and well-documented action of estrogen in males. While testosterone has been classically considered the key hormone for the control of gonadotropin feedback in the male (Figure 4), a role for estrogens has become clear from studies performed in normal and GnRH-deficient men. Recently, the discovery of men with congenital estrogen deficiency has also provided further evidence for the relationship between estrogens and gonadotropin secretion in men (5).

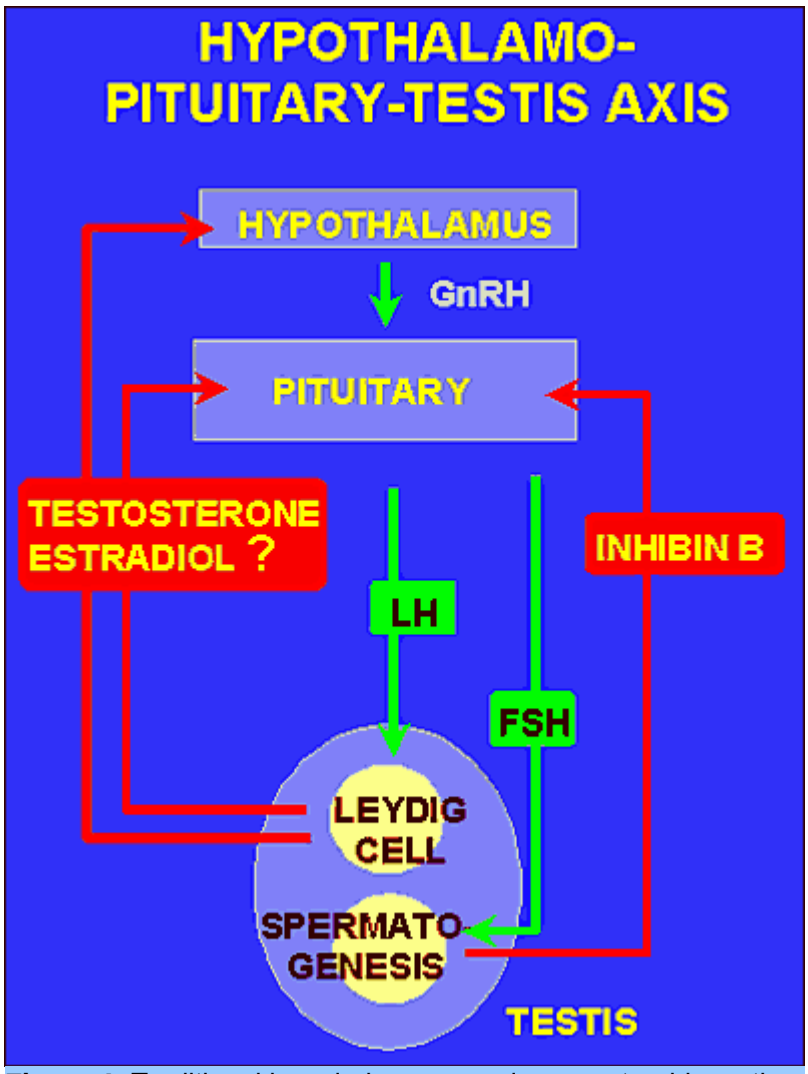

Figure 4: Traditional knowledge concerning sex steroids on the control of gonadotropin secretion.

The effects of estrogens on gonadotropin secretion have been investigated in GnRH deficient males whose gonadotropin secretion was normalized by pulsatile GnRH administration. In order to determine the precise role of sex steroids in the hypothalamo-pituitary-testicular axis, two studies were performed in which testosterone alone, testosterone plus testolactone (an aromatase inhibitor), or estradiol were administered (57, 58). When given testosterone alone, these subjects revealed a significant decrease in mean basal LH and FSH levels as well as LH pulse amplitude, demonstrating a direct suppressive effect on the pituitary of testosterone and its metabolites. Mean LH levels and LH frequency were suppressed to a greater extent in normal control subjects during testosterone administration suggesting also a hypothalamic site of action of testosterone in suppressing GnRH secretion. In order to discriminate the impact of testosterone as opposed to its aromatized products, both groups of subjects were administered with testosterone plus testolactone. The addition of the aromatase inhibitor completely prevented the suppression of gonadotropin secretion by testosterone in both normal and GnRH deficient men: in fact the mean LH levels increased significantly in both groups. The increase in mean LH levels was greater in the normal men who received testolactone alone compared to normal men who

received testosterone plus testolactone, thus revealing also a direct effect of androgens in normal men.

It is clear that the aromatization of testosterone into estradiol is required for normal gonadotropin feedback at the pituitary level (57). In fact, when the same experimental model was applied using estradiol administration, mean LH and FSH levels as well as LH pulse amplitude all decreased significantly during estradiol administration (58). This study demonstrates an important direct inhibitory effect of estradiol on gonadotropin secretion in both the GnRH-deficient and normal men (57, 58) and supports the concept that at least part of the inhibitory effect on gonadotropin secretion is mediated by the conversion of testosterone to estradiol (52, 53). In contrast it seems that the 5α-reduction of testosterone to DHT does not play an important role in pituitary secretion of gonadotropins (59).

More recently a hypothalamic site of action of estrogens has been demonstrated in men. In order to clarify the role of estrogen on the feedback regulation of gonadotropin secretion at the hypothalamic level, Hayes et al (60) conducted a study involving the administration of the aromatase inhibitor, anastrozole, to men affected by idiopathic hypogonadotropic hypogonadism (IHH), whose gonadotropin secretion had been normalized by long term pulsatile GnRH therapy. They observed that inhibition of estradiol synthesis led to an increase in mean gonadotropin levels in both normal and IHH men, but with a greater increase in the normal subjects suggesting a hypothalamic mode of action. The rise in mean LH levels in the normal subjects was shown to be due to anastrozole causing an increase in LH pulse frequency and amplitude. The authors concluded that estrogen acts at the hypothalamic level to decrease both GnRH pulse frequency and pituitary responsiveness to GnRH (60).

Accordingly, the effects of estrogen on gonadotropin secretion at the pituitary level has recently been demonstrated to operate from early- to mid-puberty (61, 62) into old age in men (63). The administration of an aromatase inhibitor (anastrozole 1 mg daily for 10 weeks) to boys aged 15-22 years (61) resulted in a 50% decrease in serum estradiol concentrations, an increase in testosterone concentrations and an increase in both LH and FSH values during the whole study protocol. These hormonal parameters returned to normal values after discontinuation of anastrozole treatment. Recently, administration of letrozole, another potent aromatase inhibitor, was shown to increase serum LH, frequency of LH pulse amplitude and the response of LH to GnRH administration in boys during early and mid-pubertal phases, indicating that estrogens acts at the pituitary level during early phases of puberty (62). The same mechanism continues to operate during adulthood and also during early senescence. In fact, in fifteen eugonadal men aged 65 years treated with 2 mg anastrozole for 9 weeks, serum FSH and LH levels increased significantly, in spite of an increase in serum testosterone levels (63).

Data suggests that estradiol may modulate GnRH receptors number and function at the pituitary level (64), although no ERs, (at least of the α type) were found in GnRH secreting neurons in monkeys (65). However ERβ is found in GnRH expressing neurons in male and female rats (66). The precise mechanism of estrogen action at both the hypothalamic and pituitary level in men remains unclear (67). It remains to be established whether estrogen receptors are involved at these two sites and/or whether non-genomic estrogen actions play a role in the control of the gonadotropin feedback. Further studies are needed to establish the contribution of both circulating and locally produced estrogens to gonadotropin feedback as well as the target cells involved in estrogen action within the hypothalamus. Nevertheless it is now well established that some androgens need to be converted to estrogens in order to ensure the integrity of the gonadotropin feedback mechanism in men, testosterone itself having a more minor role than previously thought (Figure 5).

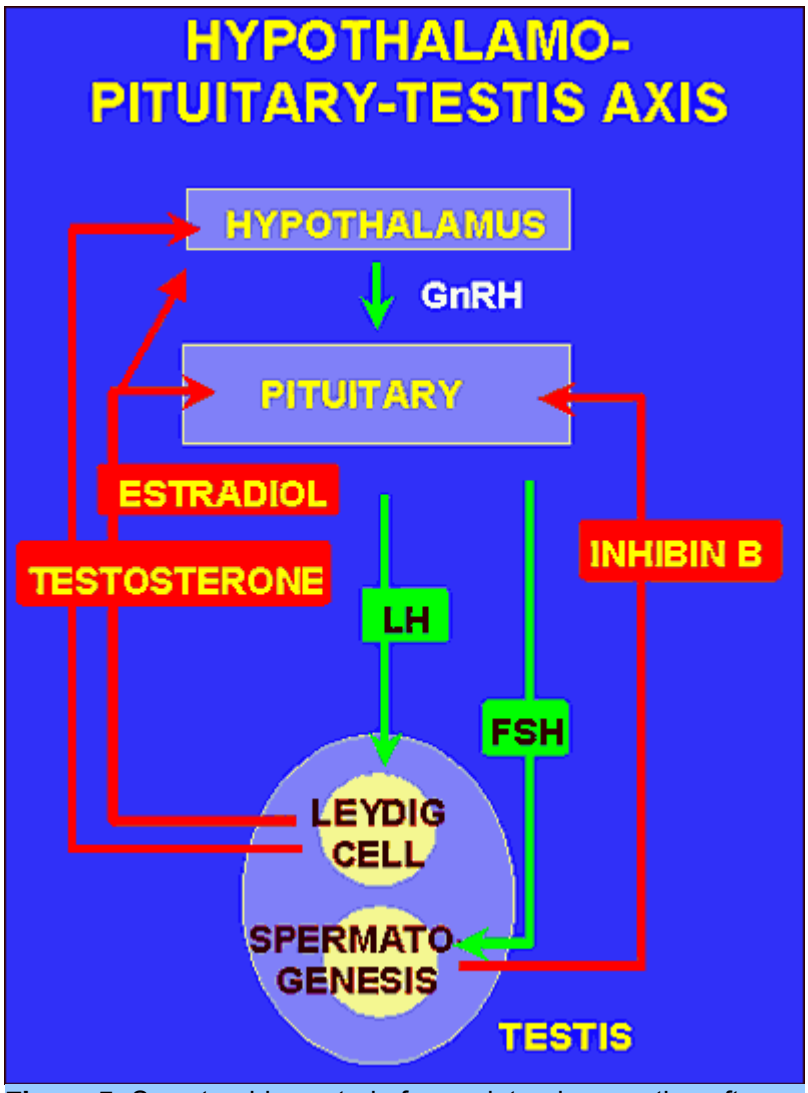

Figure 5: Sex steroids control of gonadotropin secretion after recent advances.

Our understanding of the role of estrogens in gonadotropin feedback has been enhanced through studies of men affected with congenital estrogen deficiency. The description of a man lacking a functional ERα (4) revealed a remarkable hormonal pattern consisting of a normal serum testosterone, high estradiol and estrone levels but increased serum FSH and LH concentrations (Table 5). Other important information about the role of estrogens in the human male has come from the discovery of naturally occurring mutations in the aromatase gene. To date five different cases of human male aromatase deficiency have been described, four of these males were discovered to be aromatase deficient during adulthood and one as a child (49-53, 68, 69). The four adult patients had an increase in basal FSH concentrations, while LH showed a more heterogeneous pattern, being elevated only in one subject (52, 68), high to normal in another (49, 70, 71) and normal in the third and fourth (50, 51, 53). Serum testosterone concentrations were also variable being elevated (52, 53, 68), normal (49, 70, 71) and low to normal (50, 51) in the four patients respectively. In all four patients estradiol concentrations were undetectable (Table 5). The demonstration of elevated gonadotropin levels in the presence of normal to increased serum testosterone levels in these men further highlights the important role for estrogen in regulating circulating gonadotropins in men.

A detailed study of the effects of different doses of transdermal estradiol on pituitary function in a man with congenital aromatase deficiency demonstrated that estrogens might control not only basal secretion of gonadotropins but also their responsiveness to GnRH administration. In this study, estrogen administration to a male patient with aromatase deficiency resulted in a decrease in both basal and GnRH-stimulated LH, FSH and a-subunit secretion with the response to GnRH administration being dose-dependent (71) (Figure 6). These results have been recently confirmed in the last case of aromatase deficiency described (53). However, a complete normalization of serum FSH during estradiol treatment was not achieved in the presence of physiological levels of circulating estradiol and supraphysiological levels of estrogens were necessary to obtain FSH normalization (49, 70), however this was attributed to the concomitant severe impairment of patient's spermatogenesis.

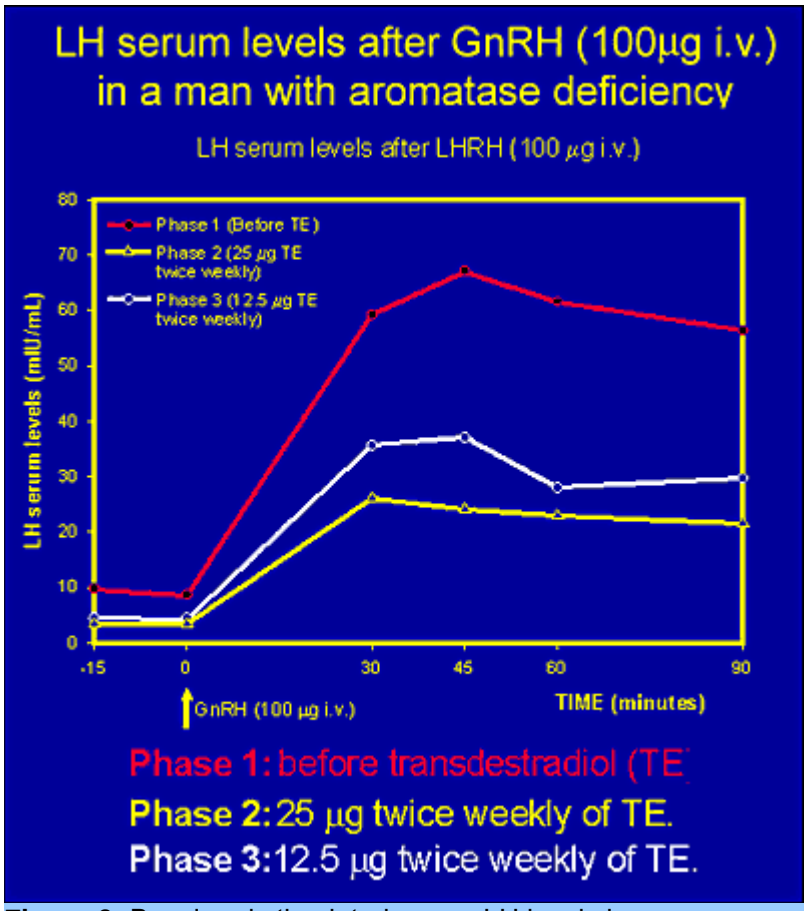

Figure 6: Basal and stimulated serum LH levels in a man affected with aromatase deficiency: effect of three different dosages of transdermal estradiol. (TE) (66)

Some difficulties remain with interpreting these data from men with congenital estrogen deficiency. For example, in the young patient with congenital aromatase deficiency, no abnormalities were found in either gonadotropin secretion nor in testis size; both testes were descended and the penis was normal (69). The presence of normal levels of gonadotropins raises the possibility that the role of estrogens in the hypothalamo-pituitary-testicular axis only become relevant in a later stage of life than infancy. Thus the control of gonadotropin feedback exerted by sex steroids during early infancy and childhood remains a matter of debate in the human male.

Clinical and therapeutical implication

On the basis of the certain role of estrogens on gonadotropic feedback inhibition (72, 73, 74), some clinical insights on the management of male infertility have been made.

Since 1960s antiestrogen agents have been used as an empirical treatment of male infertility (75), based on their modulation of the hypothalamic-pituitary testicular axis. The blockade of the negative feedback on gonadotrophins, in fact, which is obtained by the inhibition of estrogen action at hypothalamic and pituitary levels, excites LH and FSH secretion with a proposed consequent stimulatory effect on spermatogenesis, in the absence of clear evidence of direct effects of antiestrogens on spermatogenesis (74, 76, 77, 78). Accordingly, LH and FSH serum levels increase after aromatase inhibitor administration in infertile men (79 (79).

Clomiphene or tamoxifen have been the most used antiestrogen agents for the treatment of male infertility (77, 78, 80, 81, 82, 83); on the contrary the new generation of selective estrogen receptor modulators does not show significant changes in male fertility (84, 85). Tamoxifen represents the first line treatment for men affected by idiopathic oligozoospermia as recommended by WHO (2000) (76). However, the real efficacy of antiestrogens is far from being elucidated yet since other works (80, 81) provided opposite conflicting results (77, 82). Besides, it is a matter of debate whether the increase of sperm density induced by antiestrogens is actually related to a real improvement of both sperm fertility and pregnancy rate (78, 83). A possible explanation of uncertain results for what concerns antiestrogen efficacy in the treatment of male infertility is that patients with idiopathic oligozoospermic constitute an heterogeneous group, of which only a subgroup responds positively to therapy (86, 87). However till now all the studies failed to identify the characteristics of this subgroup and now physicians still do not know in advance who will improve sperm count during treatment and differences between responder and non-responder (86, 87).

Tamoxifen (20 mg/d) has been also used with testosterone undecanoate (120 mg/day) in men affected by idiopathic oligozoospermia. This combined treatment was efficacious in improving not only the sperm parameters (total sperm number, sperm morphology and motility) (82, 88), but also the pregnancy rate (88).

Few data are available about the effect of aromatase inhibitors in male infertility. A previous study failed in demonstrating the efficacy of testolactone in the treatment of idiopathic oligozoospermic infertility. However, when aromatase inhibitors (testolactone or anastrazole) are administered in a selected group of infertile men with abnormal baseline testosterone-to-estradiol ratio an improvement of fertility rate is obtained (10).

In conclusion antiestrogens, alone or in combination with testosterone, may represent a first line therapy for idiopathic oligozoospermia, as suggested by WHO, before using assisted reproduction techniques. However, further studies will be necessary to detect the real efficacy of antiestrogens treatment in improving the pregnancy rate or to identify the features of the responders to treatment.

EFFECTS OF EXCESS ESTROGEN OR ESTROGEN DEFICIENCY ON MALE REPRODUCTION

Exposure to excess estrogens in animals

In order to evaluate the effect of estrogen excess on the reproductive tract, several studies have been performed in various animal species treated with diethylstilbestrol, a synthetic estrogenic compound. In male mice, the critical period for Műllerian duct formation is day 13 post-coitus. Prenatal exposure of fetal male mice to DES caused a delay in Műllerian duct

formation by approximately two days as well as incomplete Műllerian duct regression with a female-like differentiation of the non-regressed caudal part (89). An increase in the expression of anti-Műllerian-Hormone (AMH) mRNA in male mice fetuses exposed to DES has also been demonstrated. This increase was not accompanied by a regression of the ducts. This data was interpreted to suggest that the asynchrony in the timing of Műllerian duct formation, with respect to the critical period of Műllerian duct regression, led to the persistence of Műllerian duct remnants at birth in male mice. Moreover DES exposure did not impair embryonal genetic development, but increased ERs number, and slightly prolonged the gestation time (cesarean sections were performed to rescue the litter and revealed no difference in size of fetuses from control and DES treated mothers). The timing of DES exposure is crucial to the induction of abnormalities of Műllerian duct development and regression (89).

Many studies in rodents suggest that inappropriate exposure to estrogen in utero and during the neonatal period impairs testicular descent, efferent ductule function, the hypothalamic-pituitary-gonadal axis, and testicular function (see 2 and 13 for review). The latter effect can be a direct consequence of exposure to excess estrogen, as well as a secondary effect due to perturbations in circulating hormones or the ability of the efferent ductules to reabsorb fluid. Some studies show that low dose estrogenic substances given during puberty can actually stimulate the onset of spermatogenesis, likely due to stimulatory effects on FSH (90), highlighting the fact that the effects of excess estrogen on male fertility are often complex. The effects of excess estrogen in the neonatal period can impact upon the testis into adulthood, with permanent changes in testis function and spermatogenesis evident (see 2 and 13 for review).

Aromatase over-expression in rodents

Recently a transgenic line of mice overexpressing aromatase enzyme (AROM+) has been developed (91, 92). These mice show highly elevated serum estradiol concentrations, with a reciprocal decrease in testosterone concentrations. The AROM+ males display several of the changes observed in males perinatally exposed to estrogens, such as undescended testes, testicular interstitial cell hyperplasia, hypoandrogenism, and growth inhibition of accessory sex glands. A disruption of spermatogenesis has also been observed which could be a consequence of multiple factors, including cryptorchidism, abnormal Leydig cell function, hypoandrogenemia or hyperestrogenemia. Estrogens are thought to inhibit Leydig cell development, growth and function, resulting in the suppression of androgen production (see 13 for review). The observation of numerous degenerating germ cells and the absence of spermatids within the seminiferous tubules of AROM+ mice suggest that germ cells development was arrested at the pachytene spermatocyte stage in the cryptorchid testes. Interestingly, the spermatogenic arrest occurred at a stage where P450arom is typically expressed. The spermatogenic arrest found in the AROM+ mice could be explained, at least partially, by the suppression of FSH action. The reduced serum FSH levels in AROM+ males are further evidence of the inhibiting actions of estrogens on FSH secretion in males. No significant differences in the LH concentrations were seen in AROM+ and wild type mice (91, 92).

Exposure to excess estrogens in humans

The clinical use of diethylstilbestrol (DES) by pregnant women in order to prevent miscarriage resulted in an increased incidence of genital malformations in their sons (93). In these individuals the presence of Műllerian ducts remnants was found indicating that fetal exposure to DES may have an effect on sex differentiation in men, as is the case in rodents (89). Moreover a large number of structural and functional abnormalities were found, the

most frequent being: epididymal cysts, meatal stenosis, hypospadias, cryptorchidism and microphallus (93). The frequency of abnormalities was dependent on the timing of estrogen exposure: in fact, men who were exposed to DES before 11th week of gestation (i.e. the time of Műllerian ducts formation) had a two fold higher rate of abnormalities than those who were exposed only later (93). This data supports the previously discussed hypothesis that the asynchrony between formation and regression of embryonal reproductive structures is determined by estrogen exposure.

Various reports have demonstrated that semen quality of men exposed to DES in utero is significantly worse than in unexposed controls (94, 95). However, the sperm concentrations of most of the DES exposed men were well above the limit at which subfertility occurs, and it is therefore not surprising that the fertility of these men was reported to be normal (7). The risk of testicular cancer among men exposed to DES in utero has been a controversial issue and several meta-analyses showed no increased risk (96). However more direct evidence will be necessary in order to fully understand this issue.

While various studies suggest that environmental estrogens affect male fertility in animal models, the implications for human spermatogenesis are less clear (97). It has been demonstrated that male mice whose mothers have consumed a 29 ng/g dose of bisphenol A for seven days during pregnancy had a 20% lower sperm production as compared to control males (98). Various abnormalities in reproductive organs have also been described in males exposed to bisphenols (i.e. a significant decrease in the size of the epididymis and seminal vesicles and an increase in prostate gland volume), suggesting that bisphenols interfere with the normal development of the Wolffian ducts in a dose-related fashion. Exogenous estrogens could interfere with the development of the genital structures if administered during early organogenesis, by leading to both an impairment of gonadotropin secretion and by creating an imbalance in the androgen to estrogen ratio, which may account for impaired androgen receptor stimulation or inhibition according to the dose, the cell type and age (93, 99, 100, 101).

An excess of environmental estrogens has been suggested as a possible cause of impaired fertility in humans (54, 55, 56). A progressive decline in sperm count has been reported in some Western countries during the past 50 years, suggesting a possible negative effect of environmental contaminants on male reproductive function (6, 55, 93, 99). Data concerning the role of estrogens in male reproductive structure development remains conflicting. Animal studies suggest that exposure to estrogen excess may negatively affect the development of reproductive male organs. These effects, however, are considered to be the result of an impaired hypothalamic-pituitary function as a consequence of estrogen excess and of the concomitant androgen deficiency (100, 101). Much of the knowledge on excess estrogen exposure and human fertility depends upon animal data and the validity of these concepts to humans has not been established.

Aromatase over-expression in humans

In 1996 a boy with aromatase excess syndrome was reported (102). His condition was presumably inherited in an autosomal dominant fashion with sex-limited expression as his father had a history of peripubertal gynecomastia, elevated serum estrogen levels and increased aromatase activity in vitro. The father was fertile and had a normal libido despite a small testicular volume (15 mL bilaterally), and a reduced testosterone level of 234 ng/dL (102). In the son, mild suppression of testicular growth and Leydig cell function probably reflected direct estrogen negative feedback on pituitary gonadotropin secretion. In general, the inhibitory effects of estrogen on reproductive function appear to be milder in males with aromatase excess syndrome than in patients receiving exogenous estrogens or with

estrogen-secreting tumors, probably because serum estradiol and/or estrone levels are lower in the former (102).

Estrogen deficiency: animal models

The study of transgenic mice lacking ERs or the aromatase enzyme demonstrated that a lack of estrogen was compatible with life and represented an interesting model to evaluate the physiology of estrogen in males. Congenital estrogen deficiency in mice leads to an impairment of male reproductive function to a variable degree, ranging from normal fertility with a fully male phenotype in βERKO mice to complete infertility in both αERKO and αβERKO mice. An intermediate pattern exists for the ArKO mice in which spermatogenesis is normal in young mice, but is progressively impaired during aging (3, 13, 20, 41, 46-48, 103, 104).

These data have been just described in detail in a previous section (see up in the text: "Role of estrogens in male reproduction") and are now only summarized (Table 6).

Table 6: Male mouse models of estrogen deficiency.

aERKO	bERKO	abERKO	ArKO
Infertility (41)	Fully fertile (47)	Similar to aERKO mice (3)	Normal fertility in young mice, infertility with advancing age (46)
Normal FSH Elevated LH Elevated testosterone Elevated estradiol	- - - -	- - - -	Normal FSH Elevated LH Elevated testosterone Undetectable estradiol
Germ cell deprivation with dilated seminiferous tubules	Normal testicular histology	Testicular histology similar to aERKO mice	Histology of the testis is disrupted with advancing age
Impairment of sexual behavior	Normal sexual behavior	Complete suppression of sexual behavior	Impairment of sexual behavior

The testes of infertile αERKO male mice show significant atrophy of the seminiferous epithelium and severe dilation of the tubule lumen. Interestingly these defects aren't present at birth, but they progressively become evident as the testicular phenotype of these mice worsens (3). When germ cells from αERKO mice are transplanted in wild type mice, they show a normal development (105). The αERKO mouse is also characterized by a reduced number, motility and fertilizing capacity of the sperm. The βERKO mice have normal testes and normal sperm count and they are fertile (see 3 for review).

Recently, the creation of ArKO mice has permitted to clarify the effects of the lack of estrogens on male reproductive function. Morphological studies revealed a progressive

disruption of spermatogenesis at one year of age, with a concomitant impairment of sperm count and motility (46, 48).

The function of the hypothalamo-pituitary-testicular axis is impaired in both αERKO and ArKO male mice, leading to elevated serum LH levels in presence of normal values of FSH, while, as expected, testosterone is augmented and estrogens are higher than normal or undetectable in αERKO and ArKO mice, respectively (3, 13). Thus negative effects on male reproduction are the result of estrogen deprivation directly in the reproductive structures or indirectly through changes in the regulation of sex steroid secretion.

It remains to be ascertained if congenital estrogen deficiency affects the development of male reproductive structures. In fact, even though the negative effects of excess estrogen exposure during fetal life are well documented, mouse models with congenital estrogen deficiency show normal male reproductive structure, suggesting that congenital estrogen deficiency does not alter the development of male reproductive tract in animals (3, 13). It should be noted that some defects in the development of the efferent ductules in αERKO mice are thought to be a consequence of a congenital absence of estrogen action (45). Estrogen deficiency is not associated with abnormalities of testicular descent in transgenic mouse models. However, in αERKO male mice a defect in cremaster muscle development has been demonstrated (106), whereas ArKO mice developed normally, likely as a consequence of the presence of circulating maternal estrogens during fetal life (46, 48).

Estrogen deficiency: human models

Effect of estrogen deficiency on gonadotropin feedback

The role of estrogens in human male physiology has become better understood as a result of the description of a man lacking a functional estrogen receptor alpha (4). This patient presented with tall stature, continuing linear growth during adulthood, unfused epiphyses and osteoporosis. He had normal serum testosterone, high estradiol and estrone levels, and increased FSH and LH concentrations. He had normal male genitalia with bilaterally descended testes each of 20-25 mL volume and a normal prostate volume. No further studies were performed on the reproductive system of this male (Table 5). The only data available are those on sperm characteristics, which revealed a reduced viability of sperm (Table 5).

Other important information about the role of estrogens in the human male came from the discovery of naturally occurring mutations in the aromatase gene. To date only five different cases of human male aromatase deficiency have been found, four of these males were discovered to be aromatase deficient during adulthood and one of them as a child (49-53, 68, 69,).

The hormonal pattern of the four adult patients affected by aromatase deficiency is summarized in Table 5. The study of men with aromatase deficiency shows that estrogens modulate gonadotropin feedback by regulating both basal secretion of gonadotropins and their responsiveness to GnRH (49, 53, 68, 70, 71).

In the young patient with congenital aromatase deficiency no alterations were found either in gonadotropin secretion or in testis size, both testes were descended and the penis was normal (69). The presence of normal levels of gonadotropins raises the possibility that the role of estrogens in the regulation of the hypothalamo-pituitary-testicular axis becomes relevant in a later stage of life than infancy. This is in contrast to aromatase deficient female patients in whom an elevation of FSH and LH is seen even in childhood (107), demonstrating

the importance of estrogens in the feedback regulation of gonadotropin secretion in girls during every stage of life.

Effect of estrogen deficiency on the human testis

Two of the adult males affected by aromatase deficiency showed a decreased testicular volume, one had normal testes, while the other had large testes. The histological study performed in only two of the four patients showed profound alterations in germ cell development, in particular one of the two patients had germ cells arrest at primary spermatocytes and the other had complete depletion of germ cells. Sperm analysis of one of these two patients showed severe oligozoospermia and astenozoospermia (Table 5). It remains unclear as to whether their disordered estrogen physiology accounts for the spermatogenic defects.

Effect of estrogen deficiency on the development of reproductive structures

Bilateral cryptorchidism was present in one patient with aromatase deficiency (50, 51), suggesting a possible role of estrogen also in testis descent, although this was not seen in the transgenic mice models (see above). The presence of a unique case of cryptorchidism among men with aromatase deficiency does not permit any conclusions to be drawn concerning a possible relationship between estrogen deficiency and the occurrence of abnormalities in testis development and descent.

ESTROGEN AND MALE SEXUAL BEHAVIOR

Gender-identity and sexual orientation

Sex steroids and particularly testosterone are able to affect adult male sexual behavior in mammals (108). In non-primate mammals, androgen exposure during late fetal and early neonatal development in the male accounts for the sexual dimorphism of the central nervous system (CNS), probably as a result of testosterone aromatization in the brain (109-114). Prenatal and perinatal estrogen action in the brain is believed to be responsible for the establishment of a male brain (115). Paradoxically, male rat brain is exposed to a greater amount of estradiol than female brain, since ovaries release less estrogen than testes at this stage of development (obviously in male estrogens derives from the conversion of testosterone produced by testis). Furthermore, estrogens are inactivated in the female fetus by various biochemical mechanisms, such as binding to alpha-fetoprotein (116). As a consequence, a sexual dimorphism of hypothalamic structures develops in rodents and the same mechanism seems to be involved also for the establishment of differences in hypothalamic structures between men and women (114, 117-119).

The role of sex steroids and of testosterone aromatization in the determination of the imprinting of sexual behavior has been considered of primary importance for the determination of both adult sexual orientation and sexual behavior in both animals and humans (114, 118-120). It was suggested a possible role of prenatal hormonal exposure on sexual orientation (113, 121) also on the basis of some differences in hypothalamic structures which have been found between heterosexual and homosexual men (117, 121). In particular, prenatal androgen deficiency and the lack of its estrogenic metabolites was proposed? to be responsible for male homosexuality (113, 122). In fact, the lack of estrogen action on the developing brain, in males, was believed to be strictly related to both dimorphism of hypothalamic structures, and sexual orientation development (113, 117, 121, 122).

Recently, the concept of a possible role of estrogen on sexual orientation and gender identity arise from the demonstration that the aromatase expression in the hypothalamus, the volume of hypothalamic nuclei, and partner preference are associated in animals (123).

Anyhow data concerning a possible strict linkage among anatomic correlates, prenatal and perinatal hormonal exposure, and sexual orientation in humans have been widely criticised (124) and debated (122).

Aromatase deficiency in men accounts for the absence of aromatase activity in the brain during prenatal and perinatal period, constituting a unique experimental model to study the role of estradiol on human male sexual behavior modulation.

Recently, a detailed study of a man with aromatase deficiency did not reveal any abnormalities of both gender-identity and sexual orientation (125). Based on this study the patient was categorized as masculine, his gender identity was male and the psychosexual orientation was heterosexual. Also data from the other men with aromatase deficiency don't show any association between congenital aromatase deficiency and gender-identity or sexual orientation disturbances (4, 50-53, ,) (Table 7). Since aromatase deficient patients would be subjected to maternal estrogens in utero, it is also possible that such estrogen exposure would be sufficient for sexual behavior development.

These data suggest that congenital aromatase deficiency does not affect psychosexual orientation and gender-identity in humans and that, in contrast to animals, human psychological and social factors may be the most relevant determinants of gender role behavior in men, with hormones having a minor role. Hormones, in fact, may affect sexual differentiation and sex assignment at birth and, only indirectly, psychosexual development in men (101).

Table 7: Sexual behavior in men with congenital estrogen and aromatase deficiency.

SUBJECTS	SEXUAL FUNCTION	GENDER IDENTITY	PSYCHOSEXUAL ORIENTATION
Estrogen resistance (Age: 28 yrs) (ref 5)	Libido: normal. Morning erections: normal. Nocturnal emissions: normal. Ejaculations: normal.	Male	Heterosexual
Aromatase deficiency (Age: 24 yrs) (refs 52 and 68)	Libido: modest. Morning erections: normal. Nocturnal emissions: normal. Ejaculations: normal.	Male	Heterosexual
Aromatase deficiency(Age: 38 yrs) (refs 49 and 125)	Libido: normal. Morning erections: normal. Ejaculations: normal.	Male	Heterosexual
Aromatase deficiency (Age: 28 yrs) (refs 50 and 51)	Morning erections: normal. Libido and sexual activity have not been investigated according to the religious	Male	Heterosexual

	thinking of the patient.		
Aromatase deficiency (Age: 27 yrs) (ref 53)	Libido: normal. Morning erections: normal. Ejaculations: normal.	Male	Heterosexual

Sexual behavior

In mammals, adult male sexual behavior is at least partially dependent on the presence of testosterone. Androgens are also necessary for male sexual behavior during adult life (126-128). In fact, the lack of testosterone frequently produces loss of libido and erectile dysfunction (127, 128). At the same time, testosterone replacement therapy increases sexual interest and improves sexual behavior (108, 127). By contrast, the role of aromatization in the establishment and maintenance of male sexual behavior has been characterized only recently.

Congenital aromatase deficiency and estrogen action blockade result in a severe impairment of sexual behavior in rodents. ArKO mice (46) exhibit a significant reduction in mounting frequency and a significantly prolonged latency to mount when compared with heterozygous and wild-type animals (129). Also the sexual behavior of αERKO mice is characterized by a reduction of intromissions, an increase in the latency to first intromission and a lack of ejaculation, despite the presence of a normal motivation to mount females. The same sexual behavior pattern occurs in αβERKO male mice (see 3, 13 for review). On the contrary, βERKO mice showed all three components of sexual behavior including ejaculation (Figure 7). These findings suggest that at least one of the ERs (ERα) is required for the expression of simple mounting behavior in male mice and, as a consequence, that activation of the androgen receptor alone is not sufficient for a fully normal sexual behavior, confirming that aromatization of androgens is also required.

However, novel evidence suggests that this issue may be more complex than expected. Genetic background may affect sexual behavior in some lines of inbreeded α-knock out mice. Accordingly, some selected genetic backgrounds restored sexual behavior (particularly intromission and ejaculation) in αERKO mice offspring (130).

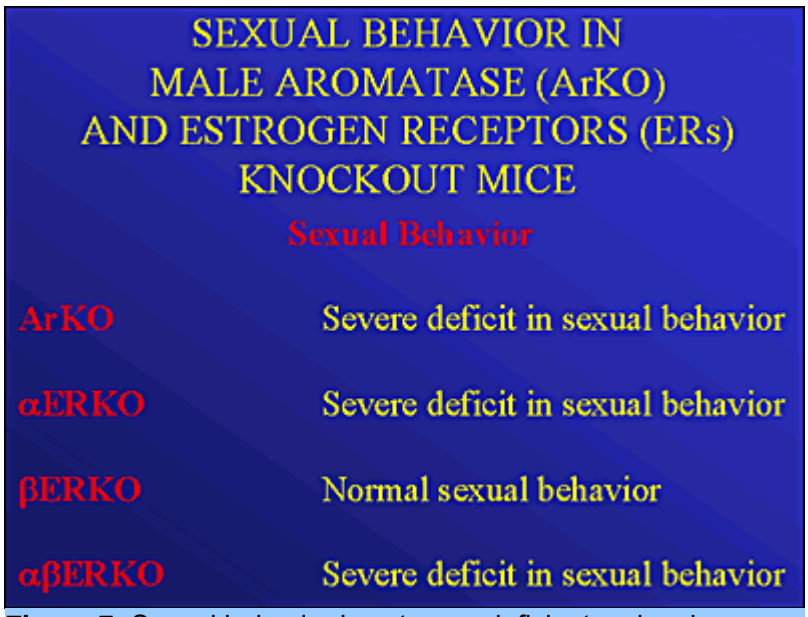

Figure 7: Sexual behavior in estrogen deficient male mice.

The role of estrogens in male sexual behavior is confirmed by studies in gonadectomized rats treated with testosterone (131). Vagell and McGinnis showed, in fact, a complete inhibition of male sexual behavior in gonadectomized rats when the aromatase inhibitor fadrozole was administered in addition to testosterone, demonstrating that this inhibition disappeared when estrogen administration was added (131).

Much less is known about the role of estrogens in sexual behavior in the human male, particularly the degree to which the effects of testosterone ought really be ascribed to its conversion into estradiol. Previous studies aimed at addressing this issue have provided conflicting results both in support (132, 133) and against (134, 135) an important role of estrogen on human male sexuality. In order to evaluate the role of estrogens in human male sexual behavior, sexual activity has been investigated in a man with aromatase deficiency, before and during testosterone or transdermal estradiol treatment. When the patient received his physiological dose of estrogens (i.e. 25 µg transdermal estradiol twice weekly) he experienced an increase of all the parameters of sexual activity (the frequency of masturbation, sexual intercourse, erotic fantasies and libido) (Table 7, Figure 8), without change during testosterone treatment. Recently, sexual behavior was investigated in one of the other three men with aromatase deficiency (51). In this patient, also affected by hypogonadism, sexual function was unaffected by testosterone or estradiol treatment alone, while the associated treatment induced a great increase in libido, in frequency of masturbation and in sexual fantasies with a concomitant normalization of testosterone and estradiol serum levels (51)

In men with congenital estrogen deficiency it seems that estrogen may play a role in adult sexual behavior, even if it's not possible to exclude that the improvements observed were the result of an improvement in well being and mood related to the estrogen replacement therapy. Recently, estrogen receptors have been identified in the corpus cavernosum suggesting also a possible involvement of estrogen in the local mechanism of erection (136). Thus, a possible indirect effect of testosterone through the activation of the aromatase pathway may be possible also in the penile tissue. This is confirmed by data obtained in rodent in which reproductive behaviour is severely impaired when estrogen action is absent (3, 129, 137).

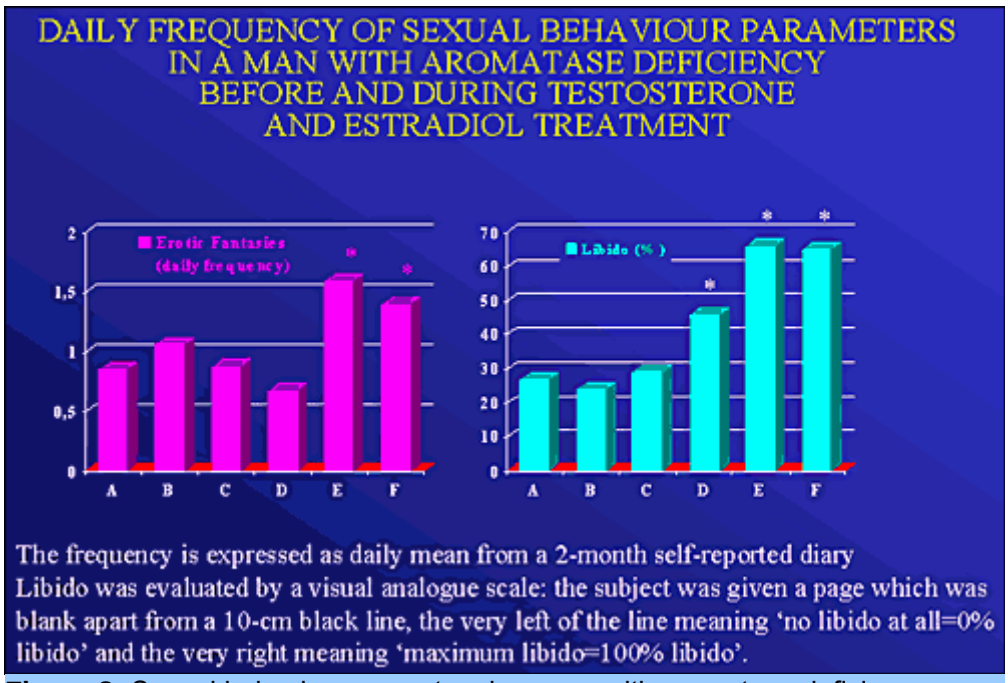

Figure 8: Sexual behavior parameters in a men with aromatase deficiency: effect of testosterone and estradiol treatment.

- PHASE A: before testosterone treatment.
- PHASE B: during testosterone treatment (testosterone enanthate): 250 mg every 10 days i.m for 6 months.
- PHASE C: before estradiol treatment.
- PHASE D: during transdermal estradiol treatment: 50 mg twice/week transdermal estradiol for 6 months.
- PHASE E: during transdermal estradiol treatment: 25 mg twice/week transdermal estradiol for 9 months.
- PHASE F: during transdermal estradiol treatment: 12,5 mg twice/week transdermal estradiol for 9 months.

These findings from transgenic mice and humans deficient in aromatase suggest that physiological levels of estrogens could be required for completely normal sexual behavior.

CONCLUSIONS

Sex steroids account for sexual dimorphism because they are responsible for the establishment of primary and secondary sexual characteristics, which are under the control of androgens and estrogens in male and female, respectively (Figure 9).

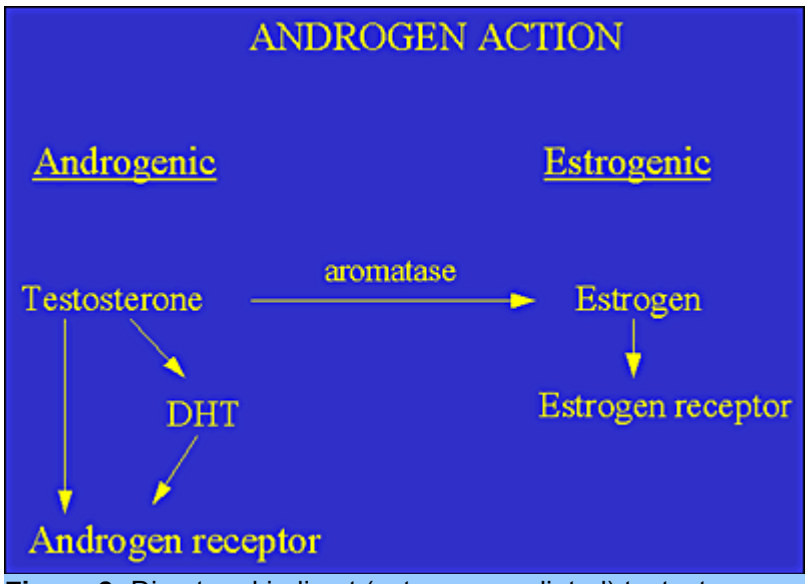

Figure 9: Direct and indirect (estrogen-mediated) testosterone action.

Advances in the understanding of the role of estrogens in animal and human models suggest a role for this sex steroid in the reproductive function of both sexes. The fact that both estrogen excess and estrogen deficiency influence male sexual development, testis function, the hypothalamic-pituitary-testis axis, spermatogenesis and ultimately male fertility highlight the importance of estrogen action in the male. From an evolutionary perspective this provides an example of the parsimony operating in biological events that are crucial for the evolution of the human species such as growth and reproduction.

This chapter has been concerned with the reproductive effects of estrogens in males but there are emerging roles for estrogens in non-reproductive tissues. In particular, while traditionally, testosterone has been considered the sex hormone involved in bone maturation and growth arrest in men, but recently the key role of estrogens on growth has been emphasised (5, 49). In men and women, in fact, epiphyseal closure and growth arrest are not achieved without estrogens, underlining the fact that estrogens on human growth are highly conserved in both sexes. Thus it is clear that testosterone might act directly or through its conversion into estrogens (124). This aspect of estogen action is discussed in Chapter 2 and Chapter 3 in this section.

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