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·*Review*·

Estrogens in males: what have we learned in the last 10 years?

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Abstract

This review focuses on the role of estrogen in men, mainly in male reproduction. The continuing increase in data obtained, and recent discoveries in this area will enable a better understanding of male physiology; these, in turn, will have important clinical implications. *(Asian J Androl 2005 Mar; 7: 3–20)*

Keywords: estrogen; male sexual behavior; male infertility; gonadotropin feedback

1 Historical background

Traditionally the adult male reproductive function was considered to be controlled by both gonadotropins and androgens, and it was testosterone [1], which for many years was considered the "male hormone". In 1934 the conversion of the male hormone into the female one in male stallions was postulated, leading to the first step in considering testosterone as a prohormone for estrogens [2, 3]. This may be considered the ancestor for the development of a field of research focusing on the biological significance of estrogens in men. Nevertheless, testosterone was not considered a prohormone for estradiol with certainty until the 1950s [4–8] and detailed studies on circulating sex steroids were provided later in the 1960s [9]. Well-established evidence for testosterone conversion into estradiol in the human male was not provided till the 1970s, when MacDonald *et al.* described estrogens synthesis in peripheral tissues of normal men [10]. A major step forward was also made in the 1970s when it was demonstrated that the testes are a source of estrogens in men [11, 12]. In the meantime, further research on this issue has demonstrated that both immature and mature germ cells and spermatozoa, are all able to produce estrogens [13–16].

The development of testicular paracrinology between 1980s and 1990s played a significant role in the study of estrogens in the male reproductive function [17–19]: the interest in understanding in detail the regulatory role of each paracrine substance in the complicated hormonal network in the testicular milieu, as well as the "*mystery*" of cell-to-cell interaction in the testis, provided strong stimuli to researchers. In this view, starting from biochemical studies on aromatization of androgens to estrogens [20–29], the following immunocytochemical studies on both aromatase [30–33] and estrogen receptors (ER) [34–39] directed the interest of biologists to the significance of estrogens signaling the pathway in the male reproductive system. Progress in molecular biology leaded to gene cloning for aromatase [40–42], ERα [43–45] and ERβ [46–48] gene cloning, this being a crucial step for the following deep investigation of estrogens physiology in the male. In fact the studies on aromatase [42] and ER functions [49, 50], together with corresponding gene-expression studies on aromatase [51–54], on ERα [55] and on ERβ [55, 56] have progressively clarified the relationships between estrogens and male reproductive function.

For a long time, data on the effects of estrogens on

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the male reproductive system have been limited to the prenatal period, as the developing testis was considered to be responsive to estrogens [57, 58]. A direct cause-effect relationship between the exposure to high doses of estrogens or diethylstilbestrol (DES) and malformation of male reproductive structures was known in both animals [59] and humans [60–62] from the 1950s onwards. However, the role played by estrogens in male reproduction became known only in the late 1990s [63, 64]. The revelations started with the development of lines of male transgenic mice lacking functional ERα [65, 66] or β [67] or a functional aromatase enzyme [68]. This shed new light on the role of estrogens in male reproduction [69]. Concomitantly, the discovery of mutations in both the human ERα [70] and aromatase [71] genes have reinforced the idea that estrogens play a key role in the human male reproductive system. Accordingly, since the 1980s it was known that seminal fluid contains both sex steroids: testosterone and estrogens [72–75].

Previously a role for estrogen action in the male reproductive system had being proposed based on scattered data [61, 62], but recent advances came out from *in vitro*, *in vivo* and immunohistochemical studies that have begun to elucidate the mechanisms of estrogen action on the male reproductive tract [16, 76, 77].

2 Introduction

Today the concept that estrogens are essential for bone maturation and mineralization in both men and women is well established [71, 78]; however, physicians still do not accept the idea that estrogens may also regulate human male reproduction.

It seems paradoxical that estrogen, the "female hormone", may play a critical role in human male reproduction, even though a clear demonstration of the need of estrogen for normal fertility has been obtained in rodents [69, 79, 80]. Accordingly, the discovery of mutations in both the human ERα [70] and aromatase [71] genes fits largely with data obtained from several lines of estrogen deficient mice. Anyhow, at present, a certain cause-effect relationship between congenital estrogen deficiency and abnormal fertility in men has not yet been determined, and it remains a hard issue to transpose to the human male what we have learned from the animal. Surely in the future, increasing cases of congenital estrogen deficiency in men will help to elucidate whether or not the congenital lack of estrogenss is related to reduced fertility in men. At the moment, the increasing body of evidence on the importance of estrogens on male reproductive function have led to the appearance of comprehensive chapters about estrogens and male reproduction in some textbook of endocrinology [81, 82], underlining the impact of this issue on both experimental studies [80] and clinical practice [77].

3 Estrogens and the male reproductive system

The immunocytochemical studies performed on the male reproductive structures of rodents and men revealed the areas in which ERs and aromatase enzymes are expressed and are functionally active. A different pattern exists between rodents and human males [69, 80, 81].

The site of ERs and aromatase expression varies widely during development in rodents and both aromatase and ERs are expressed at a very early stage [81, 83]. ERα is abundant in the developing efferent ductules, as well as in the mature ones [15, 80, 81], leading to the idea that ERα may be crucial for lifelong male reproduction in rodents. Even in the rodent testes, ERα is expressed early by Leydig cells when the androgen receptor is not yet expressed. In contrast, ERβ expression prevails during fetal life, suggesting a major role for ERβ in the development and function of male reproductive structures until birth [83]. However β-ERKO mice display normal fertility and reproductive structures nothwistanding non-functioning ERβ, thus leaving partially unknown the significance of ERβ during the development of male reproductive system [67, 69]. Probably ERβ may be the target when the fetus is exposed to a supraphysiological amount of estrogens and may be enrolled leading to negative effects on reproductive structures. Accordingly, it was known as early as the 1930s that prenatal exposure to estrogens interferes with the normal development of testes and the reproductive structures of some species [57]. In the seminiferous epithelium (Sertoli cells and a few germ cells) and in the epididymis of the male fetus, ERβ expression is higher than ERα, the latter being absent or very low [69, 84].

ERβ is involved in estrogen-related apoptosis of germ cells and as a consequence in the blockade of germ cell lineage growth during fetal and neonatal life [85]. Thus, ERβ may take part in the process through which exposure to environmental estrogens produce negative effects on male reproduction. Finally, the finding of both aromatase and ERs in the developing fetal testes implies a possible involvement of estrogens in the process of differentiation and maturation of rodent testes during prenatal life starting from an early stage of morpho-

genesis, albeit ERβ playing a more significant role than ERα.

In the adult male reproductive system, ERα is highly expressed in the proximal reproductive ducts (rete testis, efferent ductules, proximal epididymis), and its expression progressively decreases distally in rodents (corpus and cauda of the epididymis, vas deferens). The highest degree of ERα expression is seen in the efferent ductules of the male rat. The presence of abundant ERα in the efferent ductules constitutes the prerequisite for one of the most well-documented estrogenic actions on male reproductive system: the fluid reabsorption from seminal plasma in the efferent ductules [79]. Both ERs have been found in human testis and reproductive tract. Conversely, in humans, ERα are confined to Leydig and germ cells [15, 80, 81, 86]; this suggests that they play a minor physiological role.

Aromatase expression in human testes occurs in somatic and germ cells from pachytene spermatocytes through elongated spermatids [15, 87–89], as well as in human Leydig and Sertoli cells [88, 90]. Recently, the presence of aromatase has been demonstrated not only in immature germ cells [14, 90], but also in mature human spermatozoa [13, 15, 16]. This is in contrast to what it happens in rodents. Aromatase expression in human gametes, in fact, is not lost during the transit through the genital tracts as in mice; this is demonstrated by the fact that P450 aromatase was found in ejaculated human spermatozoa [13, 16]. Likely both ERs are present in human sperm [91], and sperm membrane contains an ER-related protein that accounts for a well-documented, rapid, non-genomic action [76]. Thus, sperm has to be considered at the same time a site of estrogen biosynthesis and a target for estrogen action because ejaculated human spermatozoa continue to express P450 aromatase lifelong and contain active aromatase; in addition, spermatozoa express classical and non classical ERs [76]. Particularly mature spermatozoa are able to synthesize estrogen as they traverse the efferent ducts and this ability gradually decreases as they move during epididymal transit. This suggests that the sperm itself could control the levels of estrogens in the luminal fluid, directly modulating functions such as the reabsorption of fluid from the efferent ductules [15, 76].

A detailed map of the distribution of ERs and aromatase enzymes in the human male reproductive system is summarized in Figure 1.

The wide expression of both ERs and the aromatase enzyme in the male reproductive tract of animals and humans suggests 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 [64]. Thus, if male reproductive structures are able to produce and to respond to estrogens [69], the female hormones necessarily play a minor or major role in male reproduction. However, we do not know in detail the molecular mechanisms involved in estrogen action and the degree of importance of estrogens in the reproductive system of men. To date, some estrogen actions on male reproduction have been well characterized, but more estrogen actions and related mechanisms remain

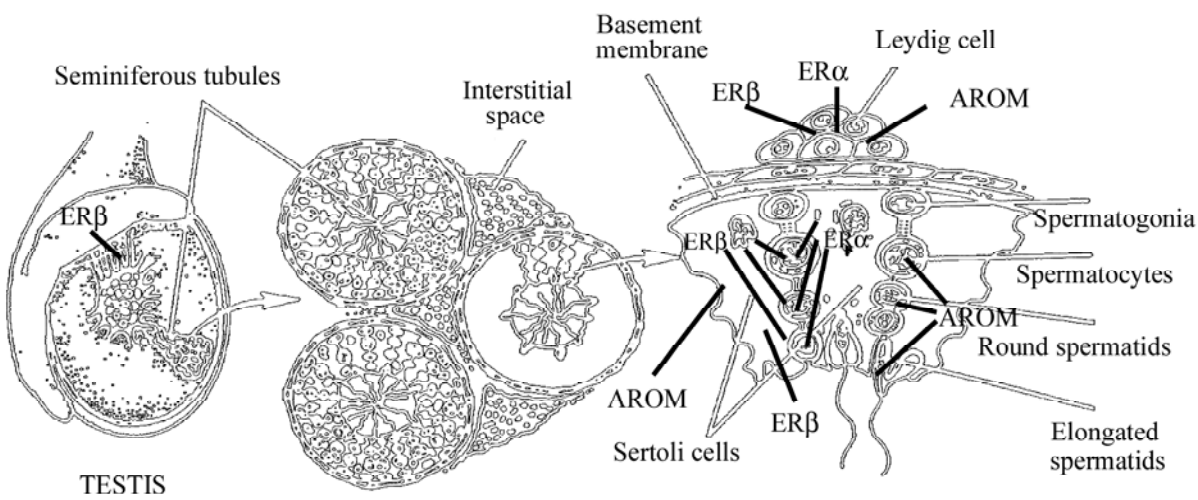

Figure 1. Distribution of estrogen receptors and aromatase enzyme in the human male reproductive system.

to be elucidated in detail.

4 The role of estrogens on male reproduction

The previously unsuspected physiological role of estrogens in the testicular function of animals was revealed by the creation of estrogen-deficient mice. Lines of estrogen-deficient mice represent a useful experimental model obtained by genetic manipulation which consists in the knock-out of a single gene, resulting in a non functioning product (enzyme or receptor) in the offspring. Gene inactivation generated four different lines of estrogen-deficient knock-out mice (Table 1). The knock-out of genes encoding for ERs led to the following lines of estrogen-resistant mice: 1) the α-estrogen receptor knock out (ERKO) mice, in which the gene encoding for the ERα is disrupted; 2) the β-ERKO mice, with an inactivated ERβ; and 3) the αβ-ERKO mice, in which both receptors α and β are non-functioning. The α-ERKO, β-ERKO and αβ-ERKO mice provide helpful information regarding the loss of ERs function (estrogen resistance). The fourth line is that of aromatase knock-out (ArKO) mice in which the gene encoding for the aromatase enzyme is knocked-out with undetectable circulating estrogens from birth, provides an experimental animol model useful for studying the effects of the congenital lack of both circulating and locally produced estrogens. The reproductive phenotype of estrogen-deficient knock-out mice is summarized in Table 1.

Adult, sexually mature, male α-ERKO mice are infertile even though the development of the male reproductive tract is mainly unaffected [69]. Seminiferous epithelium is atrophic and degenerating, while tubules and rete testes are both dilated [92]. Testicular histology is normal at birth and starts to degenerate when the mouse is 20–30 days old. At 40–60 days, testicular histology shows very dilated tubules, an increase in testicular volume and atrophy of the seminiferous epithelium [69]. In α-ERKO mice, fluid absorption is reduced at the level of the efferent ducts [79] and a defect partially mimicked also by the administration of anti-estrogens in wild-type mice with a similar effect on testicular histology [79, 93–95]. In the male genital tract, the highest concentration of ERα is found in the efferent ducts [94], and the estrogen-dependent fluid reabsorption in this site probably results from estrogen interaction with the ERα during prenatal development [79, 92, 96]. The lack of fluid reabsorption in the efferent ductules of α-ERKO male mice and the consequent dilatation of these ductules induces a retroactive progressive swelling of the seminiferous tubules. The damage of seminiferous tubule is due to increased fluid back-pressure and it leads to severely impaired spermatogenesis, coupled with testicular atrophy, as clearly seen at the age of 150 days [69, 79] (Table 1). In addition, the pattern of reproductive hormone profiles is peculiar in α-ERKO male mice: serum Luteinizing hormone (LH) is increased and as a result, serum testosterone is higher and Leydig cells hyperplasia is present, but with normal Follicle-stimulating hormone (FSH) [65, 66, 69] (Table 2).

Table 1. Reproductive phenotypes of ERKO and ArKO mice.

Estrogen deficient knock-out male mice	Fertility pattern	Testicular histology	Mechanism of induction of infertility
α-ERKO	Infertility starting at 30 days of age	At birth: normal in adult mice: germ cell deprivation with dilated seminiferous tubules and atrophy of the seminiferous epithelium.	Reduced fluid absorption at level of the efferent ducts. Impaired expression of the Na^+ transported NHER
β-ERKO	Normal fertility	Normal testicular histology at birth and in adult mice. Increased number of germ cells at birth.	–
α-βERKO	Similar to αERKO Mouse	Similar to αERKO mice	–
ArKO	Normal fertility until 7 months, fertility decreases with advancing age. Infertility at the age of 1 year.	At birth: normal until 14 wk. In adult mice (age > 1 year): impaired spermatogenesis with arrest of spermatogenesis	- Failure of germ cell differentiation; - Need of estrogens for sperm matu ration through the reproductive tract; - Temporary compensatory effect of estrogens in diet.

ERKO: estrogen receptor knock-out; ArKO: aromatase knock-out.

Studies performed on α-ERKO mice established not only the role of estrogens on male reproduction, but also highlighted a previously unknown physiological function of efferent ductules. Thus, efferent ductules, other than providing an anatomic connection between rete testes and the epididymis, which is useful for sperm transit they constitute also a functional structure in which about 90 % of sperm fluid is reabsorbed. In this view, efferent ductules regulate sperm concentration, which becomes higher in the ducts prior to entry into the epididymis [97]. As a matter of fact, the histology of the efferent duct is very close to that of the proximal tubules of the kidney [98]. It is likely that the fluid reabsorption in the efferent ductules is mediated through the Na^+ transporter, named NHE3; the disruption of ERα or the use of anti-estrogens resulted in decreased expression of NHE3 mRNA, as well as in a decrease of other proteins involved in water reabsorption, such as acquaporin I [99, 100].

Data from the study of ArKO [68] and β-ERKO [67] on male mice supports the idea that estrogen actions on the male reproductive tract are more complex than previously thought on the basis of the only knowledge of α-ERKO mice physiology [69]. In fact, unlike α-ERKO mice, male ArKO mice are initially fully fertile [68], but fertility decreases with advancing age [101], conversely, β-ERKO mice are fully fertile and apparently have normal reproductivity also in adulthood [67] (Table 1). ArKO mice show an abnormal pattern of circulating gonadotropins according to the absence of the estrogen-dependent inhibitory effect at the pituitary level (Table 3), while hormonal pattern in β-ERKO mice is less clear (Table 2). The reproductive phenotype of αβ-ERKO mice is very close to that of α-ERKO mice and it is characterized by infertility and enlarged seminiferous tubules [69] (Table 1). The modification of the testicular histology of the testes in male ArKO starts at 7 months and, after 1 year, a complete arrest of spermatogenesis is evident at the level of early spermatid and Leydig cell hyperplasia, without significant changes in the volume of seminiferous tubule lumen [101] (Table 1). Surely, the mechanism involved in the development of infertility is different in ArKO if compared with α-ERKO male mice, because the early arrest of spermatogenesis suggests a failure in germ cell differentiation, probably due to the lack of estrogens in the testicular environment in the first, while reduced fluid reabsorbion occurs in the second. These findings, together with the observation that β-ERKO male mice are fully fertile [67], lead to the hypothesis that estrogen activity in the male reproductive tract differs, with regard to both the types of ERs involved in the pathway of estrogenic action, and the site of action through the male reproductive tract [69]. Accordingly, in ArKO male mice, the failure of germ cell differentiation that is probably related to the lack of estrogen action on seminiferous epithelium while αER disruption and related arrest of fluid reabsorption take place in the efferent ductules of α-ERKO mice [102]. In very young ArKO mice spermatogenesis is preserved because a small amount of estrogens, such as those introduced with the diet, probably is sufficient to promote germ cell maturation for a brief period. Thus, the degree of infertility is less severe in ArKO mice than in α-ERKO; since ligand independent ER pathways remains functionally active [69] in ArKO mice. Later, the continuous lack of estrogens causes sperm abnormalities with advancing age in ArKO mice, since estradiol is probably necessary to maintain spermatogenesis and promote normal sperm maturation, both in the seminiferous epithelium and through the repro-

Table 2. Reproductive phenotype in estrogen-receptor disruption: a comparative analysis between mice and men.

	α-ERKO	β-ERKO mice	Estrogen resistance in men (ERα)
Testis	Germ cells loss; enlarged seminiferous tubule	Normal	Normal volume (20–25 mL)
Germ cells	Normal development of germ cells when transplanted in the WT	Not described	Not described
Sperm characteristics	Reduced number; motility and fertilizing capacity	Normal sperm count	Normal sperm count ($25\times10^6\cdot mL^{-1}$) Reduced viability (18 %)
Fertility	Infertile	Fertile	Fertile?
Hormonal Pattern	LH ↑	LH =	LH ↑
	FSH =	FSH =	FSH ↑
	T ↑	T =	T ↓ =
	E_2 ↑	E_2 =	E_2 ↑

T = testosterone; E_2 = estradiol

Table 3. Reproductive phenotype in aromatase deficiency, a comparative analysis between mice and men.

	ARKO mice	Aromatase deficiency in men			
	Simpson *et al.* 1997[53]	Morishima *et al.* 1995[117]	Carani *et al.* 1997[118]	Herrmann *et al.* 2002[120]	Maffei *et al.* 2004[116]
Testis	Normal at 14 wk	Increased Volume 34 mL	Normal Volume 8 mL	Normal Volume 14 mL	Normal Volume 10-11 mL Cryptorchidism
Germ cells	Disruption of spermatogenesis at 1 of age	Not described	Germ cell arrest at spermatocyte level	Not described	Complete germ cell depletion
Sperm characteristics	Reduced sperm count and decreased viability at 8 month of age	Not studied	Severe oligozoospermia absent motility	Oligo-asteno Zoospermia	Not studied
Fertility	Infertile at 1 year	Not studied	Infertile	Fertile?	Infertile
Hormonal pattern	LH ↑ FSH = T ↑ E_2 Undetect.	LH ↑ FSH ↑ T ↑ E_2 Undetect.	LH = ↑ FSH ↑ T = E_2 Undetect.	LH = FSH ↑ T ↑ E_2 Undetect.	LH = FSH ↑ T ↓ = E_2 Undetect.

T = testosterone; E_2 = estradiol; Undetect. = undetectable

ductive tract [15, 90, 101]. Accordingly, recent findings from *in vitro* studies on human germ cells treated with estrogens, suggest that estradiol may serve as a survival factor for round spermatids and that the lack of estradiol may promote apoptosis with a resulting failure in elongated spermatid differentiation [103]. Again, ERα is not present in the seminiferous epithelium, and the presence of ERα in Sertoli cells does not impair normal development, as shown in β-ERKO mice which are fully fertile [69].

The above studies support the concept that a functional ERα, but not ERβ, is needed for the development and the maintenance of a normal fertility in male mice [67, 69, 79, 92]. Recently, another estrogen function has been postulated on the basis of the finding that estrogens probably regulate cell-to-cell adhesion in the testis and may play a role in the establishment of Sertoli-germ cell structural connection [104]. Clearly, further studies are needed to fully understand the precise role of estrogens and their receptors on both spermatogenesis and function of seminal way as well as the importance of intracrine and paracrine pathways for these effects. It has to be remarked, however, that results from mice lacking functional ERs or aromatase enzyme point to an important role for estrogens in the maintenance of mating behavior in male mice, and that infertility in α-ERKO, αβ-ERKO and ArKO mice are, at least partially, due to weekness of various aspects of mating behavior just at early age [69, 80]. Sexual behavior, in fact, is strictly linked to reproductive functions, and if estrogens modulate mating behavior, they also necessarily affect reproductive outcomes in an indirect way.

Many studies involving rodents suggest that inappropriate exposure to estrogens *in utero* and during the neonatal period impairs testicular descent, efferent ductule function, the hypothalamic-pituitary-gonadal axis, and testicular function [57, 60, 62, 64, 69]. Hence, a role for estrogens in the development of male reproductive structures has been largely supported by several studies. In both rodents and various animal species, prenatal exposure to diethylstilbestrol, a synthetic potent estrogenic compound, led to an abnormal development of male reproductive structures. It seems that both a delay in Müllerian duct formation or an incomplete Müllerian duct regression, with a female-like differentiation of the non-regressed caudal part may account for abnormal sex structures at birth, after estrogens excess in prenatal life [105]. Accordingly, an increase in the expression of anti-Müllerian-hormone (AMH) mRNA, which is not accompanied by a regression of the ducts, may be involved in male mice fetuses exposed to diethylstilbestrol (DES). Certainly, the timing of DES exposure is crucial for the induction of abnormalities of Müllerian duct development and regression [57, 105]. In animals, exposure to estrogens excess in the neonatal period leads topermanent changes in testis function and spermatogenesis, with resulting reduced fertility into adulthood [64, 80]. The concept that estrogens excess may impair fertility has been extended also to men, and an excess of environmental estrogens has been related to impaired fertility [61, 106]. A decline in the sperm count of men in Western countries has coincided with a progressive increase

in environmental estrogens [61, 106–108]. In the past, uncorrect clinical use of DES by pregnant women increased the incidence of genital malformations at birth [107]. The most frequent structural and functional abnormalities reported were: epididymal cysts, meatal stenosis, hypospadias, cryptorchidism and microphallus [106-111]. The frequency of abnormalities was dependent on the timing of estrogen exposure, and it was higher when DES was taken before the 11th week of gestation (i.e. the time of Müllerian duct formation) [109–111]. These data support the previously discussed hypothesis that the asynchrony between formation and regression of embryonal reproductive structures is determined by estrogen exposure (e.g. the presence of Müllerian duct remnants) [105].

In the past, various reports have also demonstrated that the quality of the semen of men exposed to DES *in utero* is significantly worse than that of unexposed controls [110, 111], although no clear condition of subfertility or clinical infertility has been evident [62]. While various studies suggest that environmental estrogens affect male fertility in animal models, the implications for human spermatogenesis are less clear [112]. Exogenous estrogens could interfere with the development of the genital structures if administered during early organogenesis, by both leading to an impairment of gonadotropin secretion and an imbalance in the androgentos to estrogens ratio, which may account for impaired androgen receptor stimulation or inhibition, according to the dose, the cell type and the age [107, 108, 113, 114].

The role of estrogens in male reproductive structure development remains conflicting. Animal studies suggest that exposure to excessive amounts of estrogen may negatively affect the development of male reproductive organs. However,these effects are considered to be the result of an impaired hypothalamic-pituitary function, as a consequence of estrogen excess and of the concomitant androgen deficiency [113, 114]. Much of the knowledge on estrogen overexposure and human fertility is inferred from animal data, and the validity of these concepts has not been established in men.

The negative effects of estrogen excess during fetal life are well documented, but we do not know if congenital estrogen deprivation may affect the development of male reproductive structures. Mouse models of congenital estrogen deficiency show a normal male reproductive structure, suggesting that congenital lack of estrogen activity does not affect the development of male reproductive organs in animals [69, 80]. Anyhow, some defects in the development of the efferent ductules in α-ERKO mice are thought to be a consequence of a congenital absence of estrogen action [93], such as a defect in cremaster muscle development [115]. Bilateral cryptorchidism was present in one patient with aromatase deficiency [116], suggesting a possible role of estrogens in testis descent, although this was not seen in the transgenic mice models. The presence of a unique case of cryptorchidism among men with aromatase deficiency, does not permit to draw any conclusions about a possible relationship between estrogen deficiency and the occurrence of abnormalities in testis development and descent.

Congenital estrogen deficiency in men is the result of naturally occurring inactivating mutations of both the aromatase gene (aromatase deficiency) [71] or of the ERα gene [70]. To date, five subjects with aromatase deficiency have been described (four adult men and one male infant) (Table 4) [116–120], and only a unique case of estrogen resistance is still known [70]. Many clinical aspects are shared by both the estrogen-resistant man and the four adult men with aromatase deficiency, but the possible occurrence of infertility has not been reported in all of them [71, 81, 116–118, 120–121]. The demonstration of abundant ERs in human efferent ducts and aromatase activity in human sperm, indicates the involvement of estrogens in the reproductive function of men. On the other hand, data from human subjects with congenital estrogen deficiencies have provided conflicting and somewhat confusing results. Even though it is hard to transfer what we have learned from models of estrogen-deficient mice to men, the comparison of rodents and human reproductive phenotypes (Tables 2, 3) may be helpful in resolving questions regarding the role of estrogens in male reproductive systems. Again the reproductive phenotype of rodents seems to resemble, at least in part, that of patients with naturally occurring mutations in their ERα or aromatase gene (Tables 2, 3).

The only man know to be estrogen resistant had reduced sperm motility [70], but normal sperm count; however, α-ERKO mice show an impairment of both sperm count and sperm viability (Table 2). The four adult men affected by congenital aromatase deficiency showed a variable degree of impaired spermatogenesis [121]. A severely reduced sperm count and an impairment of sperm viability with germ cell arrest at the level of primary spermatocytes, was found in one subject [71]. A complete germ cell arrest was shown at the testicular biopsy in a second subject, whose semen analysis was unfortunately not available: the patient refused the analysis, according to his religious believe [116] (Table 3). A third patient had a slightly reduced sperm count and reduced

Table 4. Human aromatase deficiency: summary of the 5 described cases.

Author	Age (years)	Location	Affected exon	Mutation
Morishima *et al.*, 1995[117]	24	New York	IX	Arg375-Cys
Carani *et al.*, 1997 [118]	31	Modena	IX	Arg365-Gln
Deladoey *et al.*, 1999 [119]	<1	Bern	V	Leu157XDC-stop
Maffei *et al.*, 2001 [116]	28	Buenos Aires	V	Nucleotide 628 G to A insertion of 10 aa and stop
Herrmann *et al.*, 2002 [120]	27	Essen	VI	C to A in splicing acceptor site

sperm viability [120] (Table 3). There is no relevant data concerning the fourth patient described because the sperm count was not obtained [117]. It should be noted that impaired sperm motility is the main feature in both α-estrogen resistant man and mice, and that germ cell arrest is the main feature in both aromatase-deficient men and mice (Table 2, 3). Thus, the possible association between the lack of estrogen activity and infertility in men–which is suggested by the constant finding of abnormal spermatogenesis in men with congenital estrogen deficiency, together with reproductive abnormalities in estrogen-deficient mice – discloses the important role played by estrogens on male reproductive function.

The effects of estrogen replacement treatment on spermatogenesis are available only in two of the four adult men with aromatase deficiency. In both, estrogen administration did not improve neither sperm count nor motility (Table 5) [71, 118, 120]. In the patient described by Herrmann *et al.*, estrogen treatment resulted in a decline in the sperm count and a decrease in the testis volume, probably as a consequence of LH and FSH inhibition [120], but these data should be interpreted with caution.

The variable degree of fertility impairment in men with congenital deficiencies of estrogen action or synthesis means that there is uncertainly as to whether these features are a consequence of a lack of estrogen action or only epiphenomena, even though a possible role of estrogens on human spermatogenesis is suggested by rodent studies. Our knowledge on the role of estrogens in human male reproduction is far from complete, and the issue is more complex, if we consider that excessive exposure to environmental estrogens is a possible cause of impaired fertility [61, 106]. Thus, it is difficult to reconcile existing data about effects of both estrogen deficiency and excess on male reproductive function [61, 63, 106, 122].

The recent discovery of an inactivating mutation of the aromatase gene in a male infant [118], together with new cases of infant or adult men with congenital estrogen deficiency will shed new light on this controversial issue. Certainly, better comprehension of the natural history of human estrogen deficiency will improve our knowledge about the role of estrogens in male fertility.

5 Regulation of gonadotropin feedback

Animal and human models of congenital estrogen deficiency provide further evidence on the role of estrogens on gonadotropin secretion [71, 78], thus confirming that aromatization of testosterone is required for the normal functioning of the hypothalamic-pituitary testicular axis.

Previous data obtained from gonadotrophin-releasing hormone (GnRH)-deficient males treated with testosterone alone, testosterone plus testolactone (an aromatase inhibitor), or estradiol [123, 124], are available. Since longtime, these studies showed that the addition of the aromatase inhibitor completely prevented the suppression of gonadotropin secretion classically induced by testosterone, in both normal and GnRH-deficient men, thus revealing a direct and an indirect effect (through aromatization) of androgens. These studies demonstrated an important direct inhibitory effect of estradiol on gonadotropin secretion in both the GnRH-deficient and normal men, and support the concept that at least part of the inhibitory effect on gonadotropin secretion is mediated by the conversion of testosterone to estradiol at the pituitary level [123, 124]. In contrast, it seems that the 5α-reduction of testosterone in DHT does not play an important role in the pituitary secretion of gonadotropins [125].

More recently, a hypothalamic site of estrogen action 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.* [126] showed that the aromatase inhibitor, anastrozole, led to an increase in the mean gonadotropin levels, in both normal men and men affected by idiopathic hypogonadotropic hypogonadism (IHH); the increase was

Table 5. Effect of estradiol treatment on fertility in the two of the four adult men with aromatase deficiency that performed semen analysis before and during estrogen treatment.

Carani *et al.* 1997 [118]			
Time	0-6 months	6-15 months	15-24 months
Treatment (Transdermal estradiol)	Estradiol 50 μg twice weekly for 6 months	Estradiol 25 μg twice weekly for 9 months	Estradiol 12.5 μg twice weekly for 9 months
Testicular volume			
Right	8 mL	→	8 mL
Left	8 mL		8 mL
Sperm analysis			
Density	1×10^6/mL	1×10^6/mL	1×10^6/mL
Motility	0 %	0 %	0 %
Vitality	0 %	0 %	0 %

Herrmann *et al.* 2002 [120]			
Time	0 month	3 months	6 months
Treatment (Transdermal estradiol)	Estradiol 50 μg twice weekly for 3 months		Estradiol 25 μg twice weekly for 3 months
Testicular volume			
Right	14 mL	→	10 mL
Left	13 mL		9 mL
Sperm analysis			
Volume	4	3	1.5
Density	17.4×10^6/mL	23.1 - 10^6/mL	1.11×10^6/mL
Motility	10 %	5 %	0 %
Morphology	46 %	48 %	10 %
Vitality	55 %	45 %	70 %

greater in the normal subjects, suggesting a hypothalamic mode of action. The authors concluded that estrogen acts at the hypothalamic level to decrease both GnRH pulse frequency and pituitary responsiveness to GnRH [126]. Subsequently, it was postulated that estrogens play a major role in FSH regulation; this was due to the fact that anastrozole had a more pronounced effect on FSH than LH [127].

Accordingly, the effects of estrogens on gonadotropin secretion at the pituitary level have recently been demonstrated to operate from early- to mid-puberty [128, 129] as well as into old age in men [130]. The administration of an aromatase inhibitor (anastrozole 1 mg daily for 10 weeks) to boys aged 15–22 years [128] resulted in a 50 % decrease in serum estradiol, with a corresponding increase in testosterone, LH and FSH. Recently, 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, just during early and mid-puberty, indicating that estrogens act at the pituitary level during early phases of puberty [129]. It seems that the same mechanism continues to operate during adulthood and old age [130].

Obviously, lack of estrogen activity leads to a rise in serum gonadotropins in men with congenital estrogen deficiency (Tables 3, 4). In the two tables references for all patients are reported: four adult patients with aromatase deficiency presented high gonadotropin levels in presence of normal-to-increased serum testosterone, thus highlighting the importance of estrogens for the control of circulating gonadotropins in men.

A detailed study of the effects of different doses of transdermal estradiol on the pituitary function in men with congenital aromatase deficiency demonstrated that estrogens might control not only the basal secretion of gonadotropins but also their responsiveness to GnRH administration. In this study, estrogen administration to a male patient with aromatase deficiency reduced in both basal and GnRH-stimulated LH, FSH and α-subunit secretion in a dose-dependent manner [131]. These re-

sults have been recently confirmed in the last described case of aromatase deficiency described [120]. However, a complete normalization of serum FSH during estradiol treatment was not achieved in presence of physiological levels of circulating estradiol and supraphysiological levels of estrogens were necessary to obtain FSH normalization [117, 132]; Higher serum FSH was undoubtely related to the concomitant severe impairment of patient's spermatogenesis [118, 132].

Some dilemmas still remain when we interprete these data from men with congenital estrogen deficiency. For example, in the infant with congenital aromatase deficiency, no abnormalities were found in either gonadotropin secretion or testes size [119]. The presence of normal levels of gonadotropins raises the possibility that the role of estrogens in the hypothalamo-pituitary-testicular axis becomes relevant post-infancy, probably in the peripubertal period [128, 129]. Thus, the control of gonadotropin feedback exerted by sex steroids, during early infancy and childhood, remains a matter of debate.

The precise mechanism of estrogen action at both the hypothalamic and pituitary levels in men remains unclear [109, 133–135]. Whether the respective role of each ER at these two sites and/or whether non-genomic estrogen actions play a role in the control of the gonadotropin feedback remains to be determined.

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, having testosterone itself a less significant role than previously thought.

6 Clinical and therapeutical implication

On the basis of an undisputed role of estrogens on gonadotropic feedback inhibition, some clinical insights on the management of male infertility have been made [109, 134, 135].

Since the 1960s antiestrogen agents have been used as an empirical treatment of male infertility [136], which was based on their effect of modulation of the hypothalamic-pituitary testicular axis. The blockade of the negative feedback on gonadotrophins by the inhibiting estrogen action at the hypothalamic and pituitary levels, stimulates LH and FSH secretions with a consequent improvement of spermatogenesis, in the absence of clear evidences of direct effect of antiestrogens on testicular spermatogenesis [135, 137–139]. Accordingly, aromatase inhibitors administration improves fertility rate in infertile men with an impaired testosterone to estradiol ratio [77].

Clomiphene or tamoxifen have been the most used antiestrogen agents for the treatment of male infertility [138–143]; on the contrary the new generation of selective ER modulators does not show significant changes in male fertility [147, 148]. Tamoxifen represents the first line treatment for men affected by idiopathic oligozoospermia as recommended by the World Health Organization (WHO) (2000) [137]. However, the real efficacy of antiestrogens is far from being elucidated yet since other published reports [140, 141] desceibed opposite conflicting results [138, 142]. Also, it is a matter of debate whether the increase of sperm density induced by antiestrogens is actually related to a real improvement in both sperm fertility and pregnancy rate [139, 143]. 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 [144, 145]. 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 [144, 145].

Tamoxifen (20 $mg \cdot day^{-1}$) has been also used with testosterone undecanoate (120 $mg \cdot day^{-1}$) 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) [142, 146], but also the pregnancy rate [146].

In conclusion, as indicated by WHO, anti-estrogens, alone or in combination with testosterone, may represent a first line therapy for idiopathic oligozoospermia, to produce the use of 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.

7 Estrogens and male sexual behavior

7.1 Gender-identity and sexual orientation

Sex steroids, mainly testosterone, modulate adult male sexual behavior in mammals [149]. In non-primate mammals, sexual dimorphism of the central nervous sys-

tem (CNS) has classically been ascribed to androgen exposure in male during late fetal and early neonatal development; thus testosterone aromatization to estradiol was considered to be the key step towards the establishment of a masculine brain [150–155]. According to Dorner's hypothesis [155], a prenatal and perinatal estrogen exposure of the brain may be responsible for the establishment of a male brain [156], an event that paradoxically occurs in the male brain rather than the female one. In rodents, ovaries release less estrogen than testes at this stage of development, and estrogens are inactivated in the female fetus by various biochemical mechanisms, such as binding to alpha-fetoprotein [157].

The role of prenatal sex steroids in the determination of both the volume of some hypothalamic structures and adult sexual preference has been suggested in several studies on different species [153, 154, 156, 159] and it has been applied also to humans [150–152, 160, 161]. Recently, the role of local hypothalamic aromatase activity and expression in partner preference has been confirmed in rams [162, 163]. In this study, sexual partner preferences were strictly linked to the volume of the ovine sexually dimorphic nucleus (oSDN) (i.e. a larger oSDN for female-oriented rams, a smaller oSDN for male-oriented rams) and the oSDN was associated with a different pattern of aromatase expression (a higher aromatase expression for female-oriented rams having a bigger oSDN) [162, 163]. This study demonstrated for the first time that aromatase expression, brain structure and partner preference may be all involved in the determination of adult sexual behavior [163], and behavioral aspects of partner preference. If we consider also that differences in aromatase expression in the brain between male and female rodents develop early [164], and that males have more neurons containing aromatase mRNA than females at birth [165], it is possible that estrogen-related precocious changes in brain structures will determine sexual behavior during adulthood.

All these studies indicate that it is reasonable to postulate that aromatization in the CNS may be a prerequisite for the development of a male brain (female-oriented males) in animals. However, a clear cause-effect relationship has not been established and different patterns of aromatase expression in the hypothalamus are only associated with differences in the volume of hypothalamic structures and partner preferences [162–165]. In addition, it should also be noted that a different pattern of hormonal status or differences in volume of brain structure may be the results of a different behavior rather than a condition which precedes behavioral features.

In the last two years a lot of data highlighted the importance of non genomic actions of estrogens in the brain. In this regard it seems that not only aromatase expression but also aromatase activity may be modulated by estrogens, via a rapid non genomic pathway, through plasma membrane receptors [159, 166]. Thus, non genomic actions of estrogens in the brain may be involved in the control of sexual behavior [166] and in the regulation of hypotalamic-pituitary axis [167].

Sexual dimorphism of hypothalamic structures develops in rodents as a consequence of early estrogen exposure in males or early lack of estrogens in females, and the same mechanism seems to occur in men and women [150, 155, 161, 168]. Particularly, it was thought that testosterone deficiency, and the lack of its estrogenic metabolites during the early phases of development could affect sexual orientation [155, 161, 168]. Anyhow, the lack of a clear demonstration that sexual orientation depends on both early estrogen exposure and the volume of oSDN, makes it difficult to establish if the same mechanisms operate in humans too.

In humans, the relationship between anatomic structures of the brain, sex steroid exposure and sexual orientation are more complex. LeVay suggested that the third interstitial nucleus of the anterior hypothalamus (INAH3), the human analog of the sexually dimorphic nucleus in the preoptic area (oSDN-POA), is smaller in women and homosexual men than in heterosexual men [161]. Previously, INAH3 resulted larger in men than in women [150, 160]; but there have been conflicting results regarding the link between INAH3 and sexual preference in humans [169] – other studies have found no difference in the INAH3 volume of homosexual and heterosexual men [151, 152, 169].

Starting from a proven association between early estrogen exposure and brain structure on one hand, and brain structure and partner preference on the other hand, the role of sex steroids and of testosterone aromatization on sexual preference has been considered of primary importance for the determination of both adult sexual orientation and sexual behavior in both animals and humans [150, 151, 155, 168, 170, 171].

Recently, a detailed study of a man with aromatase deficiency did not reveal any abnormalities in gender identity and sexual orientation [172]. Based on this study, the patient was categorized as masculine, his gender identity was male and the psychosexual orientation was heterosexual. Data obtained from the other men with estrogen resistance or aromatase deficiency, confirmed

the absence of changes in gender identity or sexual orientation in men with a congenital lack of estrogen activity [70, 81, 116, 117, 120]. These results contribute new and important information regarding the effects of estrogen deprivation on human male psychosexuality; these results conflict with the data obtained from animal studies.

Surely, aromatase deficient patients would be subjected to maternal estrogens *in utero*, and it is also possible that such estrogen exposure would be sufficient for normal sexual behavior development, but the fact that congenital estrogen aromatase deficiency in men does not affect psychosexual orientation and gender identity, suggests that estrogen does not have a significant role in the establishment of some aspects of sexual behavior during early prenatal and perinatal life in men. Thus, in humans psychological and social factors probably remain the most relevant determinants of gender-role behavior [78, 170–172], with hormones playing a minor role. Evidence does exist that a man with complete androgen insensitivity syndrome presents female gender identity and female heterosexual orientation, notwithstanding normal early estrogen exposure and a male karyotype [114]. Obviously, hormones may affect sexual differentiation and sex assignment at birth and, only indirectly, psychosexual development in men [114].

Rare syndromes of congenital deficiency of sex steroid synthesis or function in men disclose some important differences in the sexual behavior of males of different species and reinforce the complexity of the relationship between anatomic correlates and behavior in humans [173].

However, the cause-effect relationships among sex steroid exposure, brain structures and partner preferences remain to be established.

7.2 Sexual behavior

In mammals, adult male sexual behavior is at least partially dependent on the presence of testosterone, which is the main hormone involved in male sexuality [174–176]. In men testosterone deficiency frequently produces loss of libido and erectile dysfunction [175, 176]. At the same time, testosterone replacement therapy increases sexual interest and improves sexual behavior [149, 175]. In contrast, the role of aromatization in the establishment and maintenance of male sexual behavior has been characterized only recently. In rodents estrogens are necessary for normal male sexual behavior. Congenital aromatase deficiency and estrogen action blockade result in a severe impairment of sexual behavior in rodents. ArKO mice [177], αβ–ERKO male mice and α-ERKO mice exhibit a significant reduction in mounting frequency and a significantly prolonged latency to mount when compared with heterozygous and wild-type animals [69, 80, 177]. On the contrary, β-ERKO mice did not show any defect in the components of sexual behavior, including ejaculation. 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 in rodents, confirming thus 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 imbreeded a-knock out mice. Accordingly, some selected genetic backgrounds restored sexual behavior (particularly intromission and ejaculation) in α-ERKO mice offspring [178].

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 may be ascribed to its conversion into estradiol. Some data speak in favour of a possible role of estrogens on the sexual behavior in men [179, 180], but other studies did not show estrogen to have any positive effects on male sexuality [181, 182]. A detailed sexual investigation of a man with aromatase deficiency, before and during testosterone or transdermal estradiol treatment, showed an increase in all the parameters of sexual activity (frequency of masturbation, sexual intercourse, erotic fantasies and libido), without significant changes during testosterone treatment [172].

In men with congenital estrogen deficiency it seems that estrogens may play a role in adult sexual behavior, even if it is not possible to exclude the possibility that improvements observed were the result of an adjustments in well being and mood, which were produced by the estrogen replacement therapy.

These findings from transgenic mice and aromatase-deficient men suggest that the physiological levels of estrogen could be required for completely normal sexual behavior, although androgens are the main sex steroid involved in controlling male sexual behavior [149].

Recently, ERs have been detected in the penile tissue of corpora cavernosa [183, 184] and increasing evidence suggests that estrogens play an important role in the endothelial function also in men [185]. Thus, it will be not surprising if in the future it is revealed that estrogens has a role in erectile function in men[186].

8 Conclusion

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 men and women, respectively. A previously unsuspected role of estrogens on male reproduction changed our knowledge that reproductive functions of estrogen were confined to females. Table 6 summarizes the role of estrogens on male reproduction system in animals and humans. Recent studies on the role of estrogens in humans [71, 78, 88, 118] showed that a great number of estrogen actions are preserved in both sexes [131, 132], such as estrogens effects on bone and growth arrest [71, 118, 132]. From a biological perspective, this field of research discloses a new mechanism of parsimony, which has been selected by nature, according to a general conservative principle.

Surprisingly, data obtained from animals point the attentions of researchers to the role of estrogens on reproduction in men, a concept that, in the past was confined only to female reproduction.

Finally, differences on estrogen actions among species indicate how difficult it is to apply what we have learned from animal studies to human physiology, especially for what concerns behavioral aspects.

The importance of variation among species [187], the evidences that estrogens are the major sex steroid acting on some physiological functions in men [71], the emerging minor role on others physiological functions [81], the demonstration that at the same time some conservative biological estrogen actions are preserved among species [69] and between sexes [118] seem to be in contrast with the concept that estrogens ensure sexual dimorphism. Nevertheless it simply display a multiplicity of actions which demonstrates again the wonderful way in which Nature operates in assuring the uniqueness and variety of biological processes.

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Table 6. Summary of both well-established and supposed estrogen actions on male reproductive system.

Function		Animals	Humans
Spermatogenesis	Well-established	Fluid reabsorption in the efferent ductules (ERα)	Abnormal development of male reproductive structures after exposure to estrogen excess
		Sperm concentration (ERα)	
		Abnormal development of male reproductive structures after exposure to estrogen excess	
	Supposed	Growth control of germ cells proliferation during fetal life (ERβ)	Control of spermatogenesis and sperm maturation
		Germ cell differentiation	
		Inhibition of germ cell apoptosis (ERβ)	
		Control of cell adhesion (particularly on Sertoli cells)	
Gonadotropin secrection	Well-established	Inhibition of gonadotropin secretion at pituitary level	Inhibition of gonadotropin secretion at both pituitary and hypothalamic level
	Supposed	Inhibition of gonadotropin secretion at hypothalamic level	–
Sexual behavior	Well-established	Promotion of mating copulative behavior	No effects on gender identity and sexual orientation
	Supposed	Determinant for partner preference	Possible positive role on male sexual behavior

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