

Review

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## Aromatase expression and role of estrogens in male gonad : a review

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### Abstract

The ability of the testis to convert irreversibly androgens into estrogens is related to the presence of a microsomal enzymatic complex named aromatase, which is composed of a specific glycoprotein, the cytochrome P450 aromatase (P450arom) and an ubiquitous reductase. The aromatase gene is unique in humans and contained 18 exons, 9 of them being translated. In the rat testis we have immunolocalized the P450arom not only in Leydig cells but also in germ cells and especially in elongated spermatids. Related to the stage of germ cell maturation, we have shown that the level of P450arom mRNA transcripts decreases, it is much more abundant in pachytene spermatocytes and round spermatids than in mature germ cells whereas the aromatase activity is 2–4 fold greater in spermatozoa when compared to the younger germ cells. Using a highly specific quantitative competitive RT-PCR method we have evidenced that several factors direct the expression of the aromatase gene in Leydig cells, Sertoli cells, pachytene spermatocytes and round spermatids, and it is obvious that promoter PII is the main one but other promoters could be concerned.

In the bank-vole testis we have observed a positive correlation between a fully developed spermatogenesis and a strong immunoreactivity for both P450arom and estrogen receptor  $\beta$  not only in Sertoli cells but also in pachytene spermatocytes and round spermatids. Our recent data obtained from ejaculated human spermatozoa demonstrate the presence of aromatase both in terms of mRNA and protein, and in addition, we suggest that aromatase could be involved in the acquisition of sperm motility. Indeed in men the congenital aromatase deficiency is associated with severe bone maturation problems and sterility. Together with the widespread distribution of estrogen receptors in testicular cells these data clearly show that estrogens play a physiological role in the regulation of spermatogenesis in mammals.

### Introduction

The mammalian testis is a complex organ characterised by two main functions : synthesis of steroid hormones and production of spermatozoa. It is well known that normal

testicular development and maintenance of spermatogenesis are controlled by gonadotrophins and testosterone whose effects are modulated by locally-produced factors, and among them estrogens are obviously concerned [1].

Within the reproductive tract of the male the levels of estrogens are far higher than in the general blood compartment [2], therefore favoring a testicular source of estrogens [3].

The role of estrogens in the development and physiology of male reproductive tract of mammals is still a matter of debates even though there is a growing body of evidence suggesting that estrogens are playing a role via their specific receptors (ER $\alpha$  and ER $\beta$ ) which are distributed all along the genital tract [4–6].

### **Aromatase expression in male germ cells**

The cytochrome P450 aromatase (P450arom) is involved in the irreversible transformation of androgens into estrogens and is present in the endoplasmic reticulum of numerous vertebrate tissues. The P450arom is a microsomal enzymatic complex composed of two proteins: a ubiquitous NADPH-cytochrome P450 reductase and a cytochrome P450 aromatase, which contains the heme and the steroid binding pocket. In humans the P450arom is the product of a single gene called *Cyp19*, which belongs to the cytochrome P450 gene family, containing more than 500 members [7]. The *Cyp19* gene lies on more than 120 kb length with a coding region of 9 exons plus 9 untranslated exons I. The *Cyp19* gene expression is regulated by tissue-specific promoters producing alternate 5'-untranslated exons I that are then spliced onto a common 3'-splice acceptor site in the exon II upstream of the translation start site [8].

In the mammalian testis it's well known that aromatase is mainly localized in Leydig cells (for review [1]). In rodents, past efforts to determine the source of testicular estrogens have been a considerable subject of interest and led to the assertion that Leydig cells synthesize estrogen in adults, whereas Sertoli cells are the major source in immature animals. However it has been shown that germ cells from adult male rat represent a new source of estrogens [4]. Indeed, the amount of P450arom transcripts is higher in pachytene spermatocytes than in round spermatids and obviously than in testicular spermatozoa. Conversely the aromatase activity is more intense in haploid germ cells than in younger germ cells which was also confirmed by the immunolocalisation of the protein on testicular cells mainly elongated spermatids [9]. These observations have been corroborated by Janulis et al [10] who showed that the aromatase activity is localized in the cytoplasmic droplet and decreased according to the epididymal region, being higher in caput than in cauda. Moreover it has been reported the existence of P450arom transcripts in the epithelial cells of the rat epididymis [11].

In mouse [12] as well as in bank vole [13], the aromatase is present not only in Leydig cells and Sertoli cells but also

in germ cells. In the bank vole, a seasonal breeder, the P450arom is much more expressed (in terms of specific transcripts, activity and immunolocalisation) in pachytene spermatocytes and spermatids of animals bred in long day-light cycle than in animals in winter rest [13,14]. Indeed there is a synchronisation between the recrudescence of spermatogenesis and the levels of expression of aromatase and estrogen receptors beta. Recent observations made in our group showed that in seminiferous tubules of the stallion there was a positive immunoreactive signal for aromatase not only in Leydig cells but also in cytoplasm surrounding germ cells therefore suggesting strongly the presence of aromatase in Sertoli cells [15].

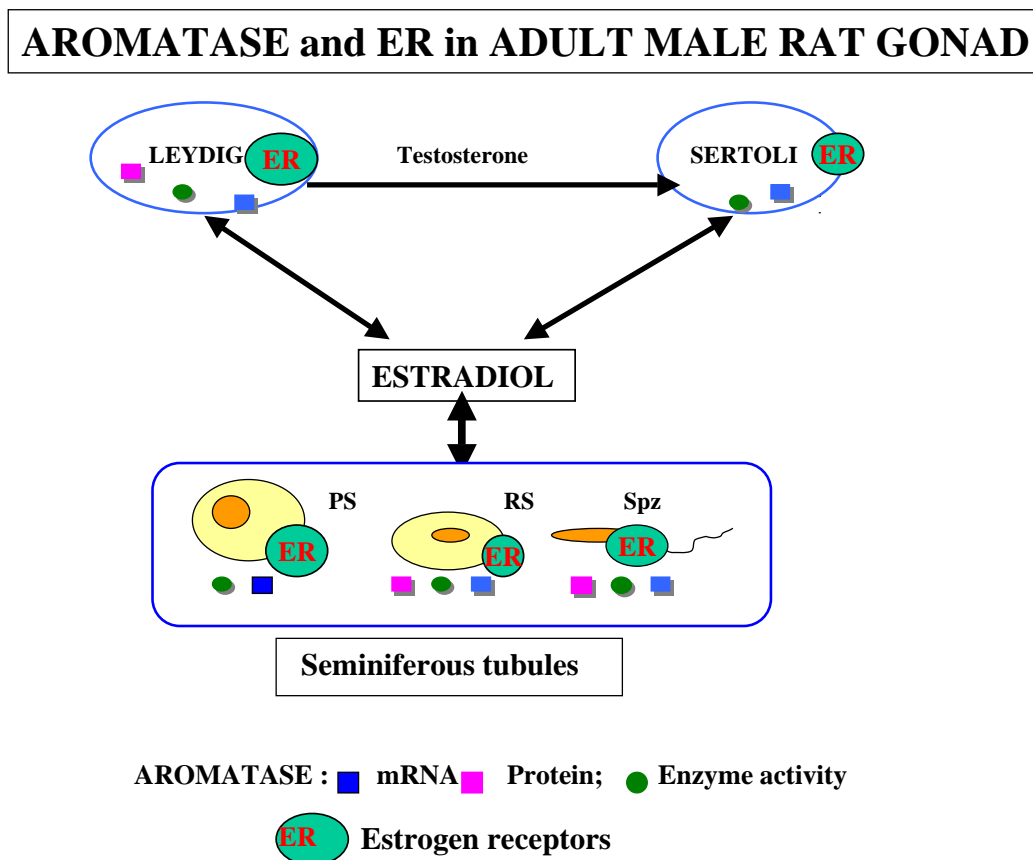
In the *Rhesus* monkey, it has been reported that testis and to a lesser extent epididymidis contained P450arom transcripts [16] and moreover, in the epididymal regions a discrepancy was observed between the amount of transcripts and the aromatase activity which was found more active in caput than in cauda although the P450arom mRNA levels were reversed. In humans the main source of estrogens is in Leydig cells; nevertheless the Sertoli cells are able to synthesize estradiol *in vitro* [17]. It has been also claimed that estrogens are present in spermatozoa [1,18]. From these observations we have examined the ability of human ejaculated spermatozoa to transform androgens into estrogens. When sperm RNA was used as template in RT-PCR we have shown the presence of P450arom transcripts; the sequences alignment from these PCR products and granulosa cells with published human P450arom gene were identical. Using Western blots and a specific monoclonal antibody against aromatase [19] we have evidenced the presence of aromatase in these sperm cells which was obviously more abundant in spermatozoa containing cytoplasmic droplets. These observations are in fitting with other recent data [20], and in addition we have demonstrated that the amount of P450arom transcripts was 30% lower in immotile than in motile spermatozoa [21].

In order to bring insights onto the role of estrogens in seminiferous tubules, it is first necessary to analyse the regulation of the *Cyp19* gene transcription. Thus we have evidenced using RACE-PCR that promoter II directs the expression of aromatase gene whatever the testicular cell type studied in the rat [22]. Germ cells are potent targets for growth factors and cytokines; indeed, pachytene spermatocytes (PS) and round spermatids (RS) produce TGF $\beta$  and TNF $\alpha$ ; but only TGF $\beta$  receptors are present in germ cells [23]. Accordingly in highly purified germ cells from adult rat (pachytene spermatocytes and round spermatids) we have demonstrated that TGF $\beta$  inhibits the expression of *Cyp19* in both germ cell fractions although TNF $\alpha$  exerts a stimulatory role in pachytene spermatocytes only; these data have been confirmed by the estradiol outputs

**Table 1: Aromatase deficiencies in men**

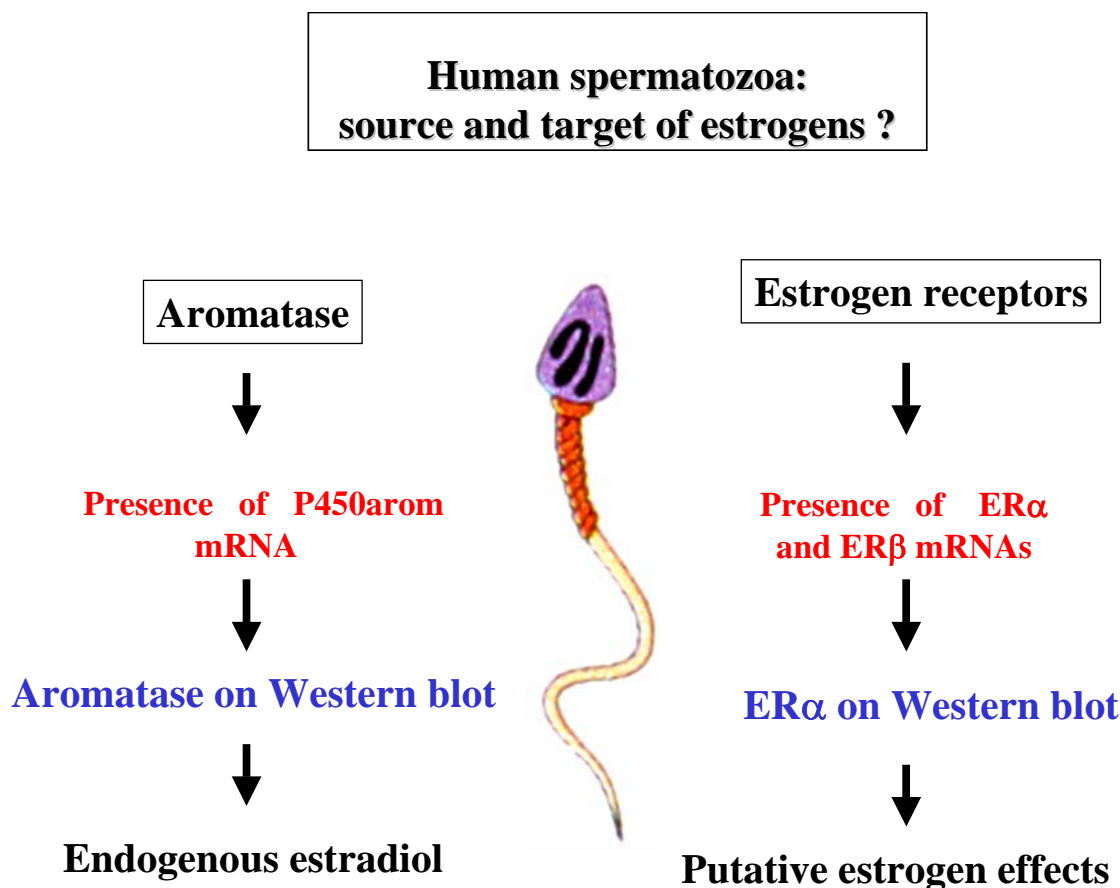
Authors	Affected Exon	Mutation
Morishima et al., 1995*	IX	Arg375Cys
Carani et al., 1997	IX	Arg365Gln
Deladoey et al., 1999*	V	Leu157XΔC-stop
Maffei et al., 2001*	V	Nucleotide 628 G to A Insertion of 10aa and stop
Kottler et al., 2002	IX	Insertion of T position 1058 and stop
Herrmann et al., 2002	V	C to A substitution position 3 and stop

\* see Carani et al [44] for details



**Figure 1**

**Aromatase and estrogen receptors (ER) in adult male rat gonad.** PS: pachytene spermatocytes, RS: round spermatids, Spz: spermatozoa. Aromatase has been demonstrated in terms of mRNA (RT-PCR), protein (Western blots) and enzyme activity (measurements of estradiol output in culture media) in the various testicular cells. ER : estrogen receptors localisation.



**Figure 2**  
**Human spermatozoa : source and targets of estrogens.** It has been shown that human ejaculated spermatozoa contain aromatase as revealed by the mRNA, protein on Western blots and the presence of endogenous estradiol. As far as estrogen receptors are concerned mainly ER alpha are present.

measured in germ cell culture media [23]. It is noteworthy that the effect of TNF $\alpha$  is amplified in presence of dexamethasone which is may be supported by the presence of an other promoter like promoter I.4 as shown in adipose tissue [24]. It is also obvious that other modulators direct the expression of the aromatase gene since in Leydig cells we have shown that the amount of P450arom transcripts are increased in presence of seminiferous conditioned medium [25]. Our data are likely in agreement with a recent work showing that the aromatase expression is controlled by three different promoters in mice that is to say that in the male gonad the *Cyp19* gene expression is directed by a testis specific promoter [26].

**Estrogens and testicular functions**

In order to exert a biological role, testicular estrogens should interact with estrogen receptors (ER) which in turn modulate the transcription of specific genes. Therefore considering the presence of at least two ER (ER $\alpha$  and ER $\beta$ ) in most of the testicular cells and the other parts of the genital tract, the physiological role of estrogens in male reproduction is now extensively revisited [4–6,27,28]. Spermatogenesis in rodents is in part under estrogens control, namely the stem germ cell number and the spermatid maturation [29,30]. In the bank the expression of androgen receptors, P450arom and ERs ( $\alpha$  and  $\beta$ ) in testicular cells is related to the length of the photoperiod. More precisely P450arom and ER $\beta$  are much more expressed in testes (especially in spermatocytes and elongated spermatids) of long photoperiod-reared animals in which

spermatogenesis is fully developed when compared to short-day length bred animals with regressed testes [13,31]. These observations are in fitting with previous reports [32,33] and the recent observations of Pak et al [34] demonstrating an improvement of the recrudescence of spermatogenesis in estradiol-treated hamster which have been confirmed by Bilinska et al [35] in bank vole.

Therefore estradiol is now considered as a survival factor for germ cells [36] that is consistent with data obtained in male monkeys treated with aromatase inhibitor in which a blockage of spermatid maturation has been observed [30]. In addition Shetty et al [37] have reported a negative role of androgens in the recovery of spermatogenesis in irradiated rats and in mice the sperm capacitation, acrosome reaction and fertilizing capacity are improved by estradiol and even more by phytoestrogens [38].

Today several experimental models of mice have been developed and shown helpful to clarify the estrogens role in vertebrates [39]. There is evidence from estrogen receptor gene knock out (ER $\alpha$  KO) mouse that estrogens are necessary for the achievement of fertility [40]. The male mice deficient in aromatase (ArKO) develop normally, the males are able to breed and to produce litters; nevertheless starting at the age of 5 months ArKO males start to have failures of spermatogenesis and by the age of one year all male mice are infertile [41].

In men it has been shown that the aromatase deficiency consecutive to a P450 aromatase gene mutation leads to sterility [42,43]; to our knowledge from the 6 reported (Table 1) cases 3 of them showed a low sperm counts number [44-46].

An inactivating mutation in the ER $\alpha$  gene (exon 2) has been reported by Smith et al [47] in an infertile man with a number of spermatozoa in the normal range although the viability was diminished. In addition a correlation between the amount of estradiol in the seminal plasma and the germ cell number has been shown [48] as well as a positive role for estradiol in stimulating the motility of human spermatozoa [49].

Durkee et al [50] have reported the existence of ER in human sperm and in addition, it has been demonstrated that the sperm membrane contains an estrogen receptor-related protein able to bind steroids (see for review [51]).

Obviously these membrane receptors would be connected with a signal transduction pathway involving quick answers such as the calcium channel and a calcium/calmoduline complex, known to be concerned for instance in sperm mobility and capacitation [52]. The demonstration of the production of nitric oxide in spermatozoa is now

considered as an alternative pathway for improving the sperm motility and capacitation [53]. Together with the existence of aromatase [21] the intracrine role of estrogens should be considered in spermatozoa.

## Conclusion

Today it is clear that not only testicular somatic cells but also germ cells represent an additional source of estrogens in several species of mammals including man. Germ cells (both meiotic and post-meiotic cells) do not only produce estrogens but since they contain estrogen receptors that would explain part of the role (intracrine and / or paracrine) of estrogens in male germ cell development. The mechanisms of action of estrogens in the reproductive organs of the male (Figure 1) remain to be clarified as well as the regulation of the aromatase gene expression, not yet fully understood especially according to the testicular development. Furthermore one should kept in mind that not only rodent spermatozoa but ejaculated human spermatozoa express a functional aromatase and together with ER these data open new considerations about the role of estrogens all along the male genital tract and may be also in sperm fertilizing ability (Figure 2).

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