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Aromatase and estrogens in man reproduction: a review and latest advances

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ABSTRACT

The mammalian testis is a complex organ which produces spermatozoa and synthesizes steroids. The transformation of androgens into estrogens is catalyzed by aromatase, an enzymatic complex encoded by a single copy-gene (*cyp19*) which contains 18 exons, 9 of them being translated. In man besides Leydig cells, we have demonstrated the existence of a biologically active aromatase in immature germ cells and in ejaculated spermatozoa. In addition the presence of estrogen receptors (ERα and ERß) in immature germ cells and in spermatozoa has been reported. Concerning aromatase, a 30% decrease of the amount of mRNA is observed in immotile compared to motile sperm fraction from the same sample. In asthenoteratozoospermic, teratozoospermic and asthenozoospermic patients, the aromatase gene expression is decreased respectively by 67%, 52% and 44%, when compared to normospermic controls. Statistical analyses between the sperm morphology and the aromatase/GAPDH ratio have revealed a high degree of correlation (r=-0.64) between that ratio and the percentage of abnormal spermatozoa (especially microcephaly). In men genetically deficient in aromatase diminutions of sperm number and motility have been published. Therefore besides gonadotrophins and testosterone, estrogens are likely playing a relevant role in spermiogenesis and human male gamete maturation.

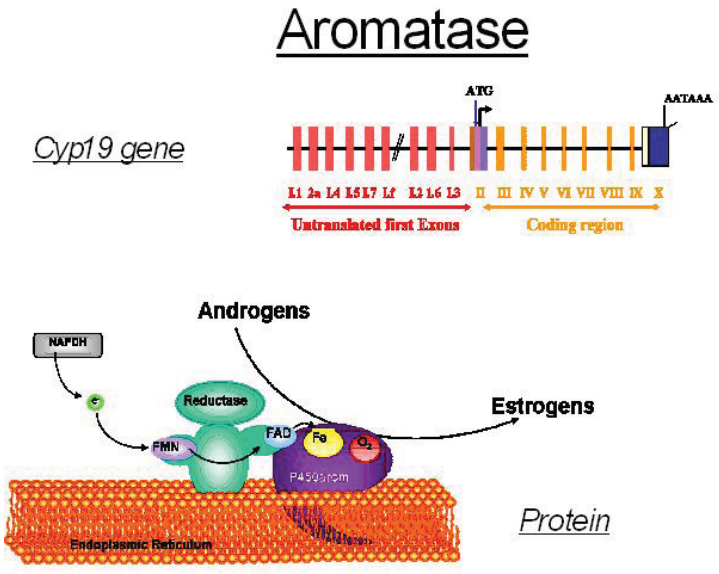
Key words: man, fertility, aromatase, estrogens, estrogen receptors, spermatozoa

INTRODUCTION

Aromatase is an enzymatic complex localized in the endoplasmic reticulum of numerous tissues which ensures the irreversible conversion of androgens into estrogens. Aromatase is involved in sexual differenciation, in lipid metabolism and bone structures but also in cancer development which therefore suggest a major role for that enzyme in humans. It is well known that the normal testicular development and the maintenance of spermatogenesis are controlled by gonadotrophins and testosterone whose effects are modulated by a complex network of factors produced locally and among them, estrogens are concerned. Aromatase is composed of two proteins: a ubiquitous NADPH-cytochrome P450 reductase and a specific cytochrome P450 aromatase (P450arom), which contains the heme and the steroid binding pocket. In humans the P450arom is the product of a single gene located on chromosome 15 and called *cyp19*, which belongs to the cytochrome P450 gene family. The *cyp19* gene lies on more than 123 kb length with a coding region of 9 exons (II-X) and 9 untranslated exons I (*Fig. 1*). 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 starting site (for reviews [1-3]. Therefore *cyp19* variants are numerous but the coding sequences are identical in humans giving rise to a unique protein of 55 kDa [4].

The estrogens role in male reproduction has been re-examined especially after the clinical and biological analysis of aromatase-deficient men [5]. Moreover from several epidemiological studies, decrease sperm counts and increase male reproductive tract disorders (cryptorchidism, hypospadia, testicular cancers) have been attributed to a deleterious effect of endocrine disruptors with either estrogenic or antiandrogenic actions [6,7]. An other interesting feature about estrogens in male is provided by studies showing that the prenatal

Figure 1. **Schematic presentation of the human aromatase.**



exposure to diethylstilbestrol induces abnormalities of the genital tract in the newborn male mice and in men [8]. All together the capacity of the testis to synthesize estrogens has been extensively studied since the last two decades and there is a growing body of evidence suggesting that estrogens play a role via their specific receptors (ERα and ERβ), which are present throughout the genital tract [9,10].

Aromatase in human germ cells (Tab. 1)

In humans, during several decades Leydig cells have been considered as the only source of estrogens [11,12]. However *in vitro* both Leydig and Sertoli cells produce estrogens [13]. Therefore taking into account the possible role of estrogens in the mammalian testes [10,14] we have looked for the expression of aromatase in motile and immotile spermatozoa from healthy donors. Using "nested PCR", P450arom transcripts have been detected in ejaculated spermatozoa from healthy men and the PCR products showed more than 98% identity compared to the human placenta aromatase sequence [15-16]. On Western blots we have evidenced the presence of aromatase in both immature germ cells and ejaculated sperm cells [15] and the intensity of staining was more abundant in spermatozoa containing cytoplasmic droplets as reported elsewhere [17]. Moreover we have observed that the amount of P450arom transcripts was 30% lower in immotile than in motile spermatozoa from several samples prepared individually by density gradient purification; in addition the aromatase activity was 50% greater in motile fraction compared to immotile spermatozoa [18]. Our observations showing that aromatase was expressed both in terms of transcript and of biologically active protein in spermatozoa from normal donors are in fitting with other reports [19]. According to our recent data collected from infertile patients and using Real-Time PCR, we have measured the amounts of aromatase mRNA

Table 1. **Aromatase and estrogen receptors transcripts in testicular cells of man.**

Cells	Aromatase	ERα	ERβ
Leydig	+	+	+/-
Peritubular	ND	-	+
Sertoli	+	-	+
Spermatogonia	ND	-	+
Spermatocytes	+	+	+
Spermatids	+	+	+
Spermatozoa	+	+	+

ND- not determined, + positive, - negative

in asthenospermic, teratospermic and asthenoteratospermic patients: they were decreased respectively by 44%, 52% and 67% as compared to controls. A high degree of correlation between the percentage of abnormal forms of spermatozoa and the aromatase/GAPDH transcripts ratio have revealed a high degree of correlation (especially head defects) (Saïd, Galeraud-Denis, Carreau, unpublished data). Moreover Carpino et al. [20] have immunolocalized aromatase in the epithelial cells of human efferent ducts and in the proximal caput epididymis suggesting an additional source of estrogens in the male genital tract. Similar observations have been published in the *Rhesus* monkey, in which it has been reported that testis contained two P450arom transcripts [21]. As to the regulation of the aromatase gene expression in human testis til now only the promoter PII has been reported [1].

Our recent data about the implication of aromatase in human spermatozoa have permitted to discuss new aspects of the estrogens role in male reproduction either as a diagnostic tool for evaluating male infertility or as a possible marker of spermiogenesis.

Signification of aromatase transcripts and Putative translational activity in spermatozoa

In spermatozoa the transcripts are believed to be remnants from post-meiotically active genes found mainly in spermatids [22-23]. However spermatozoa functions such as motility or sperm morphology could also be related to the mRNA profile of aromatase. We have also described differential mRNAs distribution between motile and immotile fractions of normospermic patients [18]. Analysis of spermatozoal mRNA including aromatase transcripts could represent the fingerprint for monitoring past events such as spermatogenesis and/or spermiogenesis. The relationship between .the active synthesis of estrogens in mature spermatozoa and the amount of aromatase transcripts must be further investigated.

The potential involvement of mRNAs in functional spermatozoal activities is actually subject to debate [24-27]. However a sperm endogenous reverse transcriptase activity [28] and the incorporation of foreign DNA sequences into sperm issuied from reverse transcription of RNA molecules suggests that reverse transcribed products are fully active in spermatozoa [29]. Moreover Pittoggi et al. [30] have demonstrated the correct outsplice of an intronic sequence incorporated into a DNA construct and Shaman et al. [31] have reported the existence of an active topoisomerase (TOP2B) associated with nucleases which thus are in favor of a less inert activity of sperm chromatin than previously evoked [24].

Estrogen roles in spermatogenesis (Tab. 1)

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 ERs (ERα and ERβ) in most of the testicular cells and in the other parts of the genital tract, the physiological role of estrogens in mammalian testes and especially in human reproduction has been extensively re-evaluated [8,32-33]. In sperm cells, we have found specific transcripts for ERs but only an ERα protein with a mol wt of 46 kDa was revealed [16]. That variant could be located on the membrane as reported elsewhere [34]. Aquila et al. [35], Solakidi et al. [36] have described the presence of ERα and ERβ proteins in human ejaculated spermatozoa with some discrepancies on their respective localisation. Recently Aschim et al. [37] reported the presence of several splice variants of ERβ in the human testicular cells and a putative relationship between two ERβ polymorphisms and man infertility has been suggested [38]. Indeed the effects of estrogens in human ejaculated spermatozoa are more and more obvious: besides the classical genomic effects, membrane estrogen receptors connected with numerous signal transduction pathways involving quick answers have been described [39]. In that respect Fraser et al. [40] have demonstrated that genistein improves the capacitation and acrosome loss of human spermatozoa. In addition, the existence of estrogen receptors in mitochondria a cytoplasmic organelle very concentrated in the mildpiece of spermatozoa [41] could be additionally relevant for a significant role of estrogens in male gamete motility.

Recently the presence of androgen receptor has been demonstrated in human spermatozoa [42] which thus could provide andogens for the aromatase activity since the human spermatozoa express a functional P450arom. Together with the presence of ERs new considerations about the role of estrogens all along the male genital tract and likely in sperm mobility and fertilizing ability should be taken into account [43]. The observations of decreased sperm motility in men with aromatase deficiency [5] which is a feature in common with the knockout models of mice [8,44] together with our data showing a significant decrease of aromatase expression in spermatozoa from infertile men likely suggest that aromatase is involved in the acquisition of sperm motility.

In relation to the variations of aromatase ARNm with teratozoospermia, the another event in which aromatase could be implicated is spermiogenesis. Indeed the generation of ArKO mice has provided evidence for an estrogen dependent acrosome formation during spermiogenesis because the blockage of germ cell maturation at the spermatid stage leads to a 50% decrease of both the number of round and elongated spermatids and then non apoptotic round spermatids showed an acrosomal dysgenesis [8].

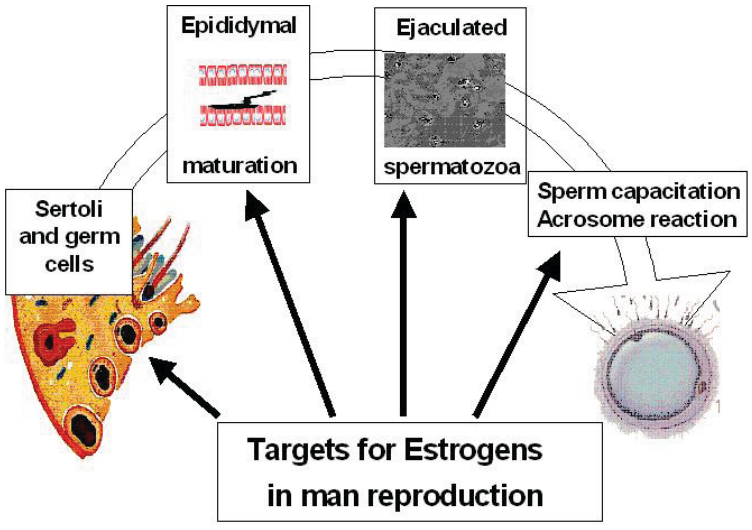
Besides the positive role for estrogens in male gamete quality we should also keep in mind the adverse effects of these female hormones in man testis. As a matter of fact, in leydigoma [45] and in seminoma [46-47] estrogens (and/or xenoestrogens) are responsible for the abnormal cell proliferation and thus play a major role in these deseases.

CONCLUSIONS (Fig. 2)

The development of biotechnologies in reproduction requires reliable parameters to characterize the best spermatozoa able to fertilize the oocyte. The analysis of spermatozoal mRNAs could represent the fingerprint to monitor the past event especially the development profile of genes expression during spermatogenesis and/or spermiogenesis. Our data suggest a potential function of some transcripts during capacitation and/or acrosome reaction. The aromatase is constitutively expressed not only in Leydig cells and Sertoli cells but also in germ cells whatever the stage of development in humans. As far as estrogens are concerned in male gamete maturation, not only the existence of estrogen sources but also the presence of ERs in man genital tract and especially in ejaculated sperm have been clearly demonstrated. Therefore studying mRNAs profile in man could be helpful not only as a diagnostic tool to assess male fertility but also as a prognosis value for fertilization and embryo development since these RNAs are delivered to the oocyte [48-49].

Male infertility is a World and Society problem and consequently, comparative studies of mRNA fingerprints in ejaculates between fertile and infertile men may contribute to elucidate some aspects of male reproductive pathology.

***Figure 2*. Putative roles for estrogens in man genital tract.**



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