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Estrogens: a new player in spermatogenesis

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Abstract: The mammalian testis serves two main functions: production of spermatozoa and synthesis of steroids; among them, estrogens are the end products obtained from the irreversible transformation of androgens by aromatase. The aromatase is encoded by a single gene (*cyp19*) in humans which contains 18 exons, 9 of them being translated. In rat the aromatase activity is mainly located in Sertoli cells of immature animals and then in Leydig cells of adults. Moreover rat germ cells represent an additional source of estrogens: the amount of P450arom transcript is 3-fold higher in pachytene spermatocytes (PS) compared to gonocytes or round spermatids (RS); conversely, aromatase activity is more intense in haploid cells. Male germ cells of mice, bank vole, bear and monkey express also aromatase. In man besides Leydig cells, we have shown the presence of a biologically active aromatase and of estrogen receptors (ERα and ERß) in ejaculated spermatozoa and in immature germ cells. Concerning aromatase, a 30% decrease of the amount of mRNA is observed in immotile compared to motile sperm fraction from the same sample; moreover the aromatase activity is also diminished of 34%. In asthenoteratozoospermic and teratozoospermic patients the aromatase gene expression is decreased by 67 and 52%, respectively 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 the ratio and the percentage of abnormal spermatozoa (especially microcephaly and acrosmome malformations). Alterations of sperm number and motility have been described in men genetically deficient in aromatase, which together with our data, suggest a likely role for aromatase/estrogens in the acquisition of sperm motility. Therefore besides gonadotrophins and testosterone, estrogens produced locally should be considered as a physiologically relevant hormone involved in the regulation of spermatogenesis and spermiogenesis.

Key words: Aromatase - Estrogens - Estrogen receptors - Spermatogenesis - Fertility - Man

Introduction

Aromatase is a terminal enzyme which transforms irreversibly androgens into estrogens and it is present in the endoplasmic reticulum of numerous tissues. Aromatase is involved in sexual differenciation, in lipid metabolism and bone structures but also in cancer development which therefore is in favour for a major role of that enzyme in humans. The mammalian testis is a complex organ which serves two important functions : the synthesis and secretion of steroid hormones, and the production of spermatozoa. 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. Estrogens have been for a long time considered as a specific female hormone; however, the presence of estrogens in the male gonad is now well documented since the publication of Zondek more than 70 years ago [52] demonstrating the exisence of estrogens in the stallion urine (for review [9]). Indeed the androgen / estrogen balance is essential for normal sexual development and reproduction in mammals. In the mammalian testis, maintenance of this balance is under a fine tuning via endocrine and paracrine factors, but is also related to the aromatase activity. 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

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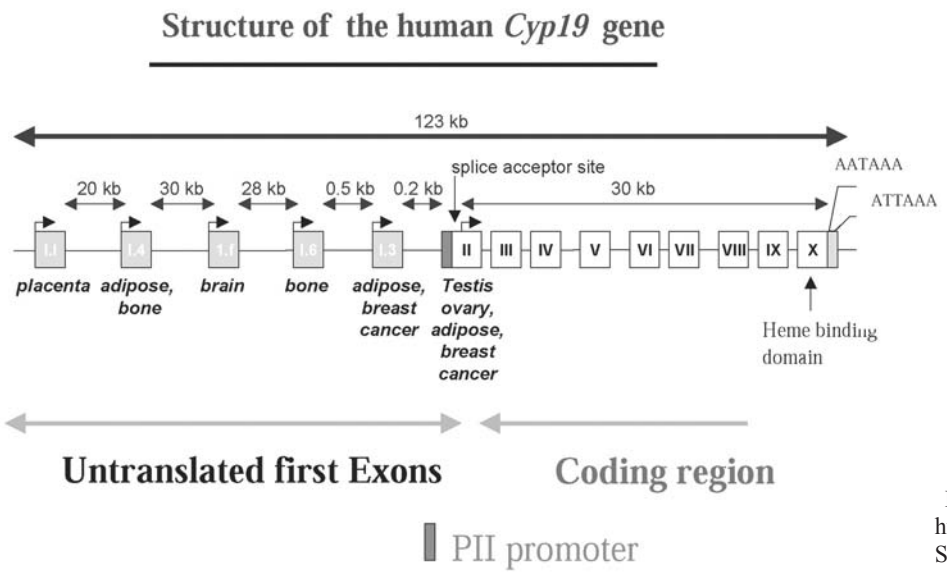

Fig. 1. Schematic presentation of the human aromatase gene modified from Sebastain & Bulun, (2002).

spliced onto a common 3'-splice acceptor site in the exon II, upstream of the translation starting site (for reviews [6,15, 42-43]. Therefore there is generation of *cyp19* variants with different 5'UTRs giving rises to different mRNAs; however the coding sequences are identical and give rise in humans to a unique protein with a molecular weight of 55 kDa (review [13]).

Besides the well-known negative effect exer the in testis and appear to be important not only in women but also in men as demonstrated in patients deficient in aromatase (for review [36]). Moreover from several epidemiological studies, decrease sperm counts and increase male reproductive tract disorders (cryptorchidism, hypospadia, testicular cancers) in men have been attributed to a deleterious effect of endocrine disruptors with either estrogenic or antiandrogenic actions [44,48]).

An other interesting feature about estrogens in male is provided by studies showing that the prenatal exposure to diethylstilbestrol, a synthetic estrogen, induces abnormalities of the genital tract in the newborn male mice and in the man [30]. All together the capacity of the testis to synthesize estrogens has been extensively studied since the last two decades but nevertheless, until recently little data have been dealing with an estrogen effect the in male reproductive tract, even though 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 (reviews [11,21]).

Expression of aromatase in germ cells

As abundantly documented in the literature, it is difficult to find a tissue completely devoid of aromatase gene expression [43]. Despite the great number of experiments realized both in vitro and in vivo, in different species, an agreement on the precise localization of aromatase activity at different ages has not always been evident. Indeed, studies investigating the testicular site of aromatization of androgens in rat testicular tissues and / or in isolated cells have shown that Leydig cells express aromatase. In the rat there is an age-related change in the cellular distribution of aromatase activity being mainly in Sertoli cells of immature animals and then in Leydig cells of adults [9,16]. Nevertheless and for the first time in 1993, Nitta et al [29] reported the presence of aromatase in male germ cells of the mouse. During the same period we have demonstrated the existence of an additional source of estrogens in purified pachytene spermatocytes (PS), round spermatids (RS) and spermatozoa of adult rat [5,27]. The amount of P450arom mRNA is higher in younger compared to mature rat germ cells ; conversely, the aromatase activity is greater in spermatozoa than in PS or RS [13]. Recently we have extended our studies and showed that preleptotene spermatocytes and spermatogonia contain aromatase whereas peritubular cells are devoid of P450arom [13, 41]. It is noteworthy that epididymal cells of the rat are also equipped of aromatase [46,51]. Similarly in the bank vole, aromatase has been demonstrated in germ cells and its expression is photoperiod-dependent [4]; identical observations have been reported in the bear [49].

Aromatase in human germ cells

In humans, Leydig cells have for a long time been considered as the only source of estrogens [16,31] ; however in vitro both Leydig cells and Sertoli cells produce estrogens (for review [8]) and spermatozoa can

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

Cells	Aromatase	ERα	ERβ
Leydig	+(a)	+(g)	+(g)
Peritubular	ND	-(c,g)	+(c)
Sertoli	+(a)	-(c,g)	+(c,g)
Spermatogonia	ND	-(c)	+(c,d)
Spermatocytes	+(b)	+(b,e)	+(b,c,d,e)
Spermatids	+(b)	+(b,e)	+(b,c,d,e)
Spermatozoa	+(b,f)	+(b,f,h,i)	+(b,f,h)

ND: not determined; +: positive; -: negative;
a: Carreau *et al.* (2003; 2007); b: Lambard *et al.* (2003; 2004); c: Saunders *et al.* (2001; 2002); d: Makinen *et al.* (2001); e: Pentakainen *et al.* (2000); f: Aquila *et al.* (2002; 2004); g: Pelletier *et al.* (2000); h: Solakidi *et al.* (2005); i: Durkee *et al.* (1998);

convert pregnenolone into estrogens [19]. We have detected the presence of P450arom transcripts in both immature germ cells and ejaculated spermatozoa from healthy men and the PCR products showed more than 98% identity when compared to the published sequence of human placenta aromatase [21-22]. On Western blots we have evidenced the presence of aromatase in both immature germ cells and ejaculated sperm cells and the intensity of staining was more abundant in spermatozoa containing cytoplasmic droplets. Moreover we have demonstrated 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. 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 data [1]. These observations are correlated with the immunolocalisation of aromatase in cytoplasm surrounding elongated spermatids in man [50]; aromatase has been also revealed in cytoplasmic droplets of ejaculated human spermatozoa [35]. In addition, we have amplified aromatase mRNA by Real-Time PCR in teratospermic spermatozoa from infertile men and recorded a 52% decrease of the amount of transcripts as compared to controls ; it is of note that the levels of P450arom transcripts was much lower (67%) in asthenoteratospermic spermatozoa (Said, Galeraud-Denis, Carreau, unpublished data).

Carpino *et al.* [7] 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 and to a lesser extent epididymis contained two P450arom transcripts, one being truncated [33].

Regulation of aromatase gene expression

In order to bring insights onto the role of estrogens in male reproduction and especially within seminiferous tubules, it is necessary to study the regulation of the *cyp19* gene (for review [12]). Thus we have evidenced using RACE-PCR that the promoter II directs the expression of the aromatase gene whatever the testicular cell type studied in the rat [25]. Besides FSH and LH we have also shown that the LRH-1 (SF-1 homologue) which is present both in Leydig cells and germ cells, but not in Sertoli cells, increases the P450arom gene expression in a mouse Leydig cell line [34]. Numerous functional motifs have been identified in PII [43]; in that context it is worth noting that in pachytene spermatocytes and round spermatids of the adult rat, truncated transcripts of P450arom gene giving rise to putative inactive proteins have been described [26]. Moreover it is now clear that not only PII drives aromatase gene in rat testis but two additional promoters PI. f (brain promoter) and a new one that we called PI.Tr (testis rat; [41]) more expressed in pachytene speramtocytes, are concerned. The identification of the specific *trans*-activating factors should bring some enlightenments to understand the regulation of the three promoters in germ cells according to their stage of maturation.

We have also evidenced that the nutritional status of fetuses [47] and aging [20] could modulate the aromatase gene expression in male rat. In mice it has been shown that the aromatase expression is controlled by three different promoters among them there is a testis specific promoter [18]. In human gonads til now only the promoter PII has been reported to control the aromatase gene expression [6].

Estrogen roles in spermatogenesis

In order to exert a biological role, testicular estrogens should interact with estrogen receptors (ERs) which in turn modulate the transcription of specific genes. Until 1996, the only data on estrogen receptor concerned ERα however with the discovery of a novel estrogen receptor designed ERß, the localization of ERs has been reexamined, and it has been shown that the α and β forms are not always present in the same cells (or are present in different amounts) within the male genital tract. The distribution of the mRNAs coding the two types of ERs α and β in the male rat gonad has been studied [22] and Saunders *et al.* [37] have shown the presence of ERß in pachytene spermatocytes and sper-

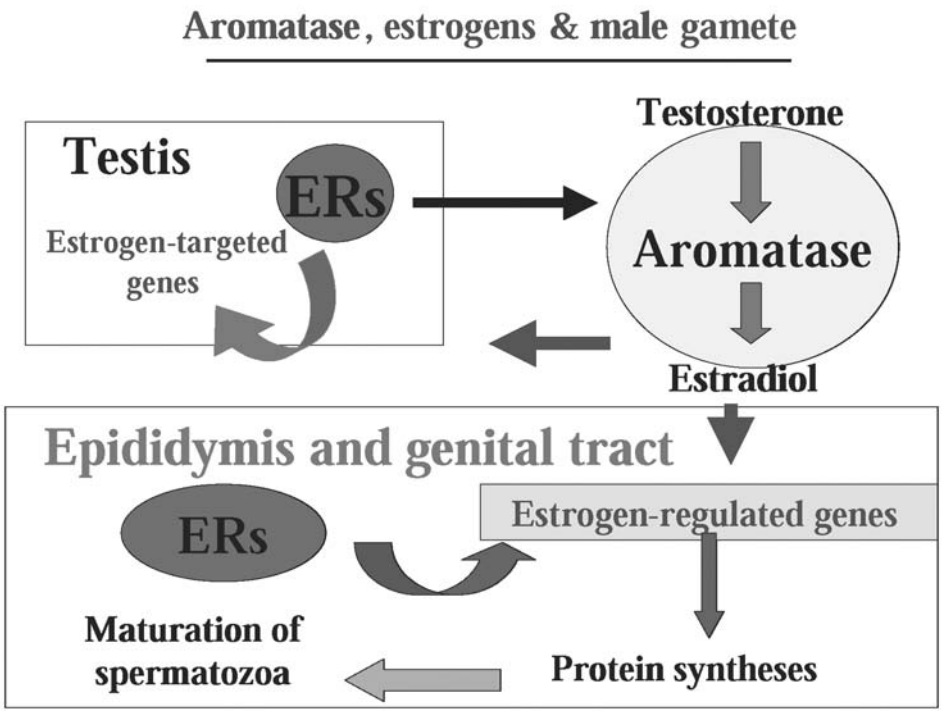

Fig. 2. Putative estrogen roles in male genital tract.

matids. In rats an age-related decrease of the aromatase and of ERs (α and β gene expression has been observed but low caloric diet is beneficial for spermatogenesis and likely improves the protection of the cells via an increase of the cellular antioxidant defense system [20]. In immature germ cells of men (Table 1) we have identified both in terms of transcripts and proteins the two main isoforms of estrogen receptors, not only the full-length but also some variants [22-23]. Besides the wild-type of ERα, an additional transcript related to exon1-deleted variant of ERα with a 46 kDa molecular weight has been reported in ejaculated sperm. Concerning ERα, two isoforms (in terms of transcript and protein) corresponding to the expected sizes (full-length and shorter one) were detected in germ cells whereas in spermatozoa only the PCR product was found [22]. Aquila *et al.* [2] have described the presence of ERα and ERβ in human ejaculated spermatozoa both as transcripts and proteins corresponding to the well-characterized ER forms; these discrepancies with our studies could be due to the different methodologies used. Solakidi *et al.*[45] have also demonstrated by confocal analysis the presence of the two main ERs in ejaculated sperm Recently Aschim *et al.* [3] reported the presence of several splice variants of ERβ in human testicular cells but the proteins have not been yet identified and thus their specific functions remain to be elucidated.

Conclusion

It is today obvious that 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, the existence of various estrogen sources and the presence of ERs in ejaculated sperm have been clearly demonstrated [14]. Therefore these data open new considerations about the role of estrogens all along the male genital tract and probably in the sperm mobility and the fertilizing ability.

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