**Review**

**Oestrogens and spermatogenesis**

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The role of oestrogens in male reproductive tract physiology has for a long time been a subject of debate. The testis produces significant amounts of oestrogenic hormones, via aromatase, and oestrogen receptors (ERs) are selectively expressed in cells of the testis as well as the epididymal epithelium, depending upon species. This review summarizes the current knowledge concerning the presence and activity of aromatase and ERs in testis and sperm and the potential roles that oestrogens may have in mammalian spermatogenesis. Data show that physiology of the male gonad is in part under the control of a balance of androgens and oestrogens, with aromatase serving as a modulator.

**Keywords:** testis; oestrogen; spermatogenesis; oestrogen receptor; germ cell

1. INTRODUCTION

The mammalian testis is a complex organ that serves two important functions: synthesis of steroids and production of spermatozoa that are controlled by gonadotrophins and numerous locally synthesized factors (Saez 1994) and among them oestrogens (see review, Carreau et al. 1999). Aromatase, which is the last step in the steroidogenesis pathway leading to the formation of oestrogens from androgens, is localized in the cellular endoplasmic reticulum of numerous tissues. The role of oestrogens in the physiology of male reproductive tract of mammals has for a long time been a subject of debate, even though more and more evidence suggests that oestrogens are involved via their specific receptors (see reviews of O'Donnell et al. 2001; Hess 2003; Carreau et al. 2008). Indeed, 70 years ago, Zondek (1934) discovered an oestrogenic hormone in stallion urine; but at the beginning, oestrogen production by testicular tissues was more a curiosity and it was only 30 years later that several publications provided evidence that testes are able to synthesize and secrete oestrogens. Hendry et al. (1962) were the first to demonstrate that the human testis produce oestrogens and moreover hCG stimulates that production, whereas castration exerts an opposite effect. Hendry et al. (1983) confirmed the testicular source of oestrogens and reported higher levels in the spermatic vein of men. Consequently, the presence of large quantities of oestrogens in the rete testis fluid and the spermatic vein of numerous mammals has been reported (reviewed by Hess 2003). Therefore, besides a well-known negative feedback of oestrogens in the hypothalamus–hypophysis complex, it has become more obvious that testicular oestrogens could play a role locally in the male gonad and the reproductive tract, especially after the discoveries of patients genetically deficient in aromatase (see review of Rochira et al. 2005) and the oestrogen receptor (ER) knockout mouse (review by Hess et al. 2002). Moreover, decreased sperm counts and increased male reproductive tract disorders (cryptorchidism, hypospadias and testicular cancer) in men have been described and related to a deleterious effect of endocrine disruptors (Toppari et al. 1996; Skakkebaek 2004). In this review, we will summarize the data concerning aromatase sources in the male genital tract, the presence of ERs in testis and the roles of oestrogens in mammalian spermatogenesis. There will also be a special emphasis on recent knowledge obtained with the presence of GPR30 in germ cells (Sirianni et al. 2008).

2. OESTROGEN SOURCES IN MAMMALIAN TESTICULAR CELLS

(a) Cyp19 gene

The aromatase enzyme complex comprises two proteins: a specific cytochrome P450 (P450arom) encoded by the CYP19 gene and a ubiquitous NADPH cytochrome P450 reductase. In humans, Cyp19 gene is located in the 21.2 region of the long arm of the chromosome 15 and is approximately 123 kb length (Sebastian & Bulun 2001). This gene contains nine exons (exons II–X) encoding for a unique protein with a molecular weight of 55 kDa (Nakajin et al. 1986) and upstream of exon II, a regulatory region is present (Simpson et al. 1994). The 5′ region contains 10 promoters, which are used according to the needs and the characteristics of the tissue. However, regardless of the promoter used, each untranslated first exon is spliced into a common splice junction localized 39 bp upstream of the translational start site (AUG; Simpson et al. 1997). In mouse, the Cyp19 gene is localized on chromosome 9, and Golovine et al. (2003) have shown that three promoters specifically control aromatase gene expression.

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One contribution of 17 to a Theme Issue ‘The biology and regulation of spermatogenesis’.
In rat, the CYP19 gene is situated on chromosome 8 and up to now three promoters have been described: the promoter PI.f used in brain (Yamada-Mouri et al. 1996) but also in testis (Silandre et al. 2007), the promoter PII (Young & McPhaul 1998), which is the main one directing aromatase gene expression in gonads (Lanzino et al. 2001; Pezzi et al. 2004), and the promoter PI.tr only used in testis (Silandre et al. 2007). Indeed, in figure 1, we have provided a schematic presentation of these promoters used according to age in the rat gonad, which suggests a very precise control of the expression of aromatase and the amount of oestrogens produced.

(b) Testicular oestrogens in mammals

As abundantly documented in the literature, it is difficult to find a tissue completely devoid of aromatase gene expression (Simpson et al. 1994); indeed, it is now well established that the mammalian testis is able to produce oestrogens (table 1; Hess 2003; Hess & Carnes 2004; Carreau et al. 2006). Early in the study of testicular aromatase, it was thought that its expression was in Sertoli cells during development in the rodent species, but then only in Leydig cells in the adult testis (see reviews of Hess & Carnes 2004; Carreau et al. 2006). In some species such as pig (see review by Conley & Hinshelwood 2001) and human (Inkster et al. 1995), a testicular P450arom mRNA was thought to be present only in Leydig cells. Despite the large amount of data published in different species, the precise localization of aromatase has been a subject of debate. Some of the variations observed between immunohistolocalization and enzyme activity in cell culture could result from an absence of endocrine and/or paracrine regulation (Carreau 1994), such as after in vivo germ cell depletion or lack of cell–cell contacts in culture dishes (Papadopoulos et al. 1987; Carreau et al. 1998).

The availability of antibodies allowed for immunohistolocalization of aromatase in Leydig cells of young and adult rats (Kurosumi et al. 1985). After being purified, aromatase was cloned and primers made available for checking transcripts together with measurements of aromatase activity and immunohistological studies. Thus, with these tools Nitta et al. (1993) were the first to demonstrate that mouse germ cells express aromatase. In male rat gonad, several distinct sites of aromatization have been reported and oestrogen synthesis evolves with age: in mature rat, Leydig cells are considered as the major source of oestrogens, whereas in the immature animal, this synthesis is in Sertoli cells (Rommerts et al. 1982; Papadopoulos et al. 1986). This has been confirmed in vitro using specific primers to detect aromatase transcripts (Levallet & Carreau 1997; Genissel & Carreau 2001).

Additionally, we have shown in adult rats that germ cells represent a new source of oestrogens (Janulis et al. 1996, 1998; Levallet et al. 1998a). The expression of...
The aromatase gene is higher in meiotic prophase cells (pachytene spermatocytes) than in round spermatids or testicular spermatozoa; conversely, aromatase activity (measured by the tritiated water release method) is greater in haploid germ cells than in the younger spermatocyte germ cells, a finding also confirmed by the immunolocalization of the protein that was intensively expressed in elongated spermatids (Levallet et al. 1998b) and newly released sperm (Janulis et al. 1998). The presence of aromatase mRNA has been reported in gonocytes, spermatogonia as well as in preleptotene spermatocytes (Silandre et al. 2007). However, in purified myoid cells, we were unable to detect any aromatase transcript by RT–PCR and therefore, the aromatase gene is present in all stages of germ cell development (figure 2) so far studied (Carreau et al. 2008).

In male bank vole, aromatase has also been described not only in somatic cells but also in germ cells and the protein is quantitatively more abundant during the breeding season corresponding to the full development of spermatogenesis (Bilinska et al. 2001; Kotula-Balak et al. 2003). Similar observations in terms of seasonal changes in the expression of steroidogenic enzymes have been made in the black bear by Tsubota et al. (1997). In bison (Kopera et al. in press) and the roe deer (Schon & Blottner 2008), somatic and germ cells expressed aromatase. In boars, all data available are in agreement that Leydig cells represent the only source of oestrogens (Raeside & Renaud 1983; Mutembe et al. 2003), which was confirmed in the minipig (Weng et al. 2005).

3. Oestrogen receptors in testis

It has been known for many years that oestrogens bind to proteins in male reproductive tissues, especially the epididymis, and now there is clear evidence that ERα (ESR1) and ERβ (ESR2) are localized in specific cells of the testis, efferent ductules and epididymis, with species specificity (see reviews by Hess 2000, 2003, 2004; Hess et al. 2001, 2002; Hess & Carnes 2004; Sierens et al. 2005). ERβ has a rather ubiquitous expression throughout the male reproductive system.
but ERs has greater specificity, with only two regions of the male reproductive system consistently being demonstrated to express ERs in every species, namely Leydig cells of testis and epithelium of efferent ductules, the region connecting rete testis to the head of the epididymis (Hess et al. 2001; Hess 2002). Immunohistochemical staining for ERs is more intense in efferent ductule epithelium than in any other tissue and its mRNA expression in this tissue is 3.5-fold higher than even in uterus (Hess et al. 1997b), which traditionally has been the standard for ER expression.

In testis, the reports of ERs and ERs expression are highly variable (table 2), with major differences between species, as well as between individuals within a species. Results also differ between immunohistochemical localization of the receptors and mRNA analysis of testicular tissues and cells (Saunders et al. 2001; Hess & Carnes 2004; Carreau et al. 2007a). This confusion in the literature over ER localization in the male reproductive system may require reinvestigation to more accurately determine oestrogen responsive cell types in the different tissues and among different species.

In mouse testis, ERs was found only in Leydig cells and some peritubular myoid cells (figure 4), whereas ERs was found in some Leydig cells, Sertoli cells and some germ cells, particularly spermatocytes (Zhou et al. 2002; Kutola-Balak et al. 2005). In general, ERs has been noted in the interstitial space, whereas ERs is most often located within the seminiferous epithelium. However, using frozen sections and new antibodies against the amino- or carboxy-terminal regions of ERs, very solid data revealed that ERs is also expressed in Sertoli cells and some germ cells of the rat (Lucas et al. 2008). In the same study, 17β-oestradiol treatment of cultured immature Sertoli cells resulted in a translocation of both ERs and ERs from the nucleus to the plasmalemma (Lucas et al. 2008). Based on these new data, the entire field of ER localization in the male reproductive system may require reinvestigation to more accurately determine oestrogen responsive cell types in the different tissues and among different species.

In human testis, most early reports suggested no labelling for ERs in any of the testicular cells (Pelletier & El-Alfy 2000; Saunders et al. 2001; Mäkinen et al. 2001; Siersens et al. 2005). However, an occasional paper suggested that ERs could be found in germ cells, particularly spermatocytes and spermatids (Pentikainen et al. 2000; Lambard et al. 2004). Even as early as 1981, it was suggested that human spermatozoa showed specific binding sites for 17β-oestradiol near the plasma membrane (Cheng et al. 1981a,b). Then in 1998, it was shown that the spermatozoaon contained both mRNA and protein for ERs (Durkee et al. 1998) and more recent studies have confirmed this finding of mRNA and protein for ERs as well as ERs in human ejaculated sperm (Durkee et al. 1998; Aquila et al. 2004; Lambard et al. 2004; Solakidi et al. 2005). Sperm appear to express the expected size (66 kDa) of ERs, but also a shorter isoform of 46 kDa (Lambard et al. 2004; Solakidi et al. 2005). This smaller form was only found in spermatozoa and is located on the membrane (Lambard et al. 2004), which is consistent with this protein being similar to GPR30 (G protein-coupled receptor 30), an integral membrane protein capable of mediating the rapid effects of oestrogen through a non-genomic pathway (Filardo et al. 2002, 2007; Revankar et al. 2005).

4. OESTROGENS AND THE REGULATION OF SPERMATOGENESIS

It has been known for many years that oestrogen has an essential role in regulating the hypothalamus–pituitary–testis axis and thus indirectly regulates the luteinizing hormone (LH) and testosterone (T) balance through a feedback loop (O’Donnell et al. 2001). It is also now well recognized that testicular and germ cell oestrogen has a direct role in the regulation of down-stream physiology, as the highest concentration of ERs is found in epithelial cells lining the efferent ductules (Hess et al. 2002; Hess 2003), whose primary function is to reabsorb luminal fluid and increase the concentration of sperm before they enter the epididymis (Hess 2002). However, evidence for oestrogen having a direct action on the seminiferous epithelium has been lacking. Opinions favouring a role for oestrogen in spermatogenesis have typically come from indirect evidence (Hess et al. 2002; Hess 2003; Hess & Carnes 2004; Carreau et al. 2007a,b, 2008): (i) prenatal exposure to oestrogens, (ii) expression of aromatase in the testis, (iii) expression of ERs and ERs in the testis, (iv) various knockout animal models, and (v) anti-oestrogen treatment (table 3). Others have carefully examined the important role of oestrogen in the development of testis and male reproductive tract (Sharpe 2000; O’Donnell et al. 2001; Sharpe 2001, 2006; Albrecht et al. 2009); therefore, this review will examine oestrogen’s potential function only in the adult testis and the regulation of spermatogenesis.

(a) Oestrogen receptor knockout

2The original ERs knockout mouse, the neo-ERKO (Lubahn et al. 1993; Eddy et al. 1996), generated by inserting a neo gene into exon 2, produced the first evidence that a classical ER was essential for male fertility. However, spermatogenesis was reportedly normal up to about 10 weeks and subsequent degeneration of the seminiferous epithelium and eventual tubular atrophy appeared to be related to the accumulation of fluid in the rete testis (Eddy et al. 1996). A more detailed study of the neo-ERKO mouse revealed that fluid accumulation was due to the failure of efferent ductules to reabsorb luminal fluids, which then backed up into the rete testis and seminiferous tubules (figure 5), eventually inducing degeneration of the seminiferous and testicular atrophy (Hess et al. 1997a, 2000; Zhou et al. 2001). In these early studies of the neo-ERKO mouse, the only evidence suggesting a direct effect on the seminiferous epithelium was data showing a decrease in fluid secretion, presumably by the Sertoli cell (Hess et al. 1997a).
Table 2. ESR1 (ERα) and ESR2 (ERβ) present (+) or absent (−) in the testis, examined by immunohistochemistry, in situ hybridization and RNA analysis. Peritub, peritubular myoid; spgonia, spermatogonia; spcyte, spermatocyte; sptid, spermatid; /, not determined.

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[c]Nie et al. (2002).
[d]Nie et al. (2002).
[g]Gonzalez-Moran et al. (2008).

Figure 3. Localization of aromatase in ejaculated human spermatozoa using confocal microscopy. CD 46 (red); specific marker of inner acrosomal membrane; chromatin is localized with DAPI (blue) and the aromatase is revealed by a polyclonal antibody (green). Adapted from the publisher.

Subsequent studies showed that oestrogen’s major effect was on the regulation of ion transporters and aquaporin water channels in efferent ductules (Zhou et al. 2001; Oliveira et al. 2005; Ruz et al. 2006), but effects on these molecules in testis was not found.

The neo-ERKO mouse resulted in the presence of a truncated ERα protein (Lubahn et al. 1993; Couse et al. 1995); therefore, the floxed-ERKO mouse, generated by homologous recombination and Cre recombinase-mediated excision of exon 3, resulted in complete deletion of ERα transcripts downstream of exon 2 and complete ERα and ERβ null mutants (Dupont et al. 2000). However, the male phenotype of this complete ERα knockout was essentially identical to that of the neo-ERKO, although a more recent floxed-ERαKO mouse has shown a 30 per cent decrease in testis weight at day 84 (Chen et al. 2009) compared with the neo-ERKO, which showed increased testis weight at day 81 (Hess et al. 1997a). The ERβKO, neo- or floxed-, mice have shown no significant effects on spermatogenesis (Couse et al. 1997; Dupont et al. 2000), although one study has shown Leydig cell hyperplasia and an increase in the number of spermatogonia (Gould et al. 2007). The double ERαβKO mice appear to be essentially the same as the ERαKO mice (Couse et al. 1997; Dupont et al. 2000), suggesting that ERβ, although having widespread expression in the testis and male reproductive tract, has a limited role in testicular function. Thus, the question of whether oestrogen plays a
Table 3. Experimental animal models in the study of testicular oestrogen. A, infertility or decreased fertility or delayed infertility; B, increased or decreased LH and/or testosterone; C, change in testis weight or testicular atrophy; D, seminiferous tubular disruption; E, Leydig cell effects; F, efferent ductule luminal dilatation; G, decreased efferent ductule epithelial height; H, decreased efferent ductule function +, present; -, not observed; I, decreased water and ion transport molecules; J, effects on sperm counts/motility; K, effects on prostate/seminal vesicles; /, not determined; esulfotransKO, oestrogen sulphotransferase knockout; ArKO, aromatase knockout.

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<sup>c</sup>Cousé et al. (1999) and Dupont et al. (2000).

<sup>d</sup>Fisher et al. (1998) and Robertson et al. (2001, 2002).

<sup>e</sup>McDevitt et al. (2007, 2008) and Weiss et al. (2008).

<sup>f</sup>Sinkovic et al. (2008, #159519; 2009, #17036), McDevitt et al. (2007, #15322) and Li & Rahman (2008).

<sup>g</sup>Sinkovic et al. (2009).

<sup>h</sup>Fowler et al. (2000), Gill et al. (2001), Hirotsugu et al. (1997), Luthra et al. (2003) and Simpson (2003).


<sup>k</sup>Hoyt et al. (1998) and Neubauer et al. (1993, 1995).

<sup>l</sup>Qian et al. (2001).


<sup>n</sup>Faqi et al. (2004), Morrissey & Watson (2003), Mitchell et al. (2001) and Robertson et al. (2002).

Direct role in spermatogenesis was unanswerable by the ER null mice.

(b) Aromatase knockout

The phenotype observed in the neo-ERKO mice generated considerable interest in the aromatase knockout (ArKO) mouse, in which the natural oestrogen ligand was removed. However, the ArKO mouse did not result in a neo-ERKO phenotype (Robertson et al. 1999, 2002; O’Donnell et al. 2001) and spermatogenesis appeared normal until the males began to age (table 3). Thus, it appears that oestrogen is important for the long-term maintenance of spermatogenesis, particularly the production of round spermatids and formation of the acrosomal granule, suggesting a role for oestrogen in the differentiation of spermatocytes (Robertson et al. 2002). Testis weight was not reduced in ArKO males until about 1 year, but degeneration of round spermatids began to appear as early as 12–14 weeks (Robertson et al. 1999, 2002). It was hypothesized that the standard soy-based diets may contain enough oestrogenic compounds to prevent a complete

Figure 4. (a) ERα and (b) ERβ in the adult mouse testis. Immunohistochemical localization of ERα using perfusion fixation with neutral buffered formalin and the NCL-ER-6F11 antibody (Novocastra, Newcastle upon Tyne, UK) gave strong staining in Leydig (Lc), other interstitial cells and peritubular myoid cells (Pc) of the testis. Staining for ERβ was more intense in some spermatocytes (Spc) than other germ cells, but all germ cells, except the elongated spermatids (Es) were positive, including the round spermatids (Rs). Scale bar, 50 μm.
oestrogen knockout in the ArKO mouse; therefore, the Simpson group carefully evaluated these males when fed soy+ and soy– diets (Robertson et al. 2002).

Hence, the study suggested that dietary phytoestrogens may be ‘agonistic’ in the absence of endogenous oestrogen (ArKO mice), but ‘antagonistic’ when present with endogenous oestrogens (wild type (WT)). We have recently demonstrated (Hamden et al. 2008) that phytoestrogens, probably via their antioxidant role against the reactive oxygen species, are helpful in protecting the male reproductive functions, especially during ageing.

Removing soy phytoestrogens from the diet of ArKO mice has provided the most significant data to date to demonstrate that oestrogen does indeed have a role in the maintenance of spermatogenesis, at least after 12–14 weeks of age. Total withdrawal of oestrogens (endogenous and dietary soy) did not produce spermatogenic disruptions prior to 14 weeks, but at 1 year the disruption of spermatogenesis was more severe than in the ArKO + soy males, as there was a significant decline in spermatocytes, round and elongated spermatid numbers (Robertson et al. 2002). At 14 weeks, soy-deficient diets produced decreased seminiferous tubular diameters in the ArKO mice, but increased in tubular lengths in both the ArKO and WT mice, which were explained by the separate effects of oestrogen on germ cells versus Sertoli cells. There was also an increase in the number of Sertoli cells in the ArKO + soy males.

It was surprising that the ArKO mouse did not induce a neo-ERKO phenotype (Robertson et al. 1999, 2002; O’Donnell et al. 2001). The major testicular effects observed in the neo-ERKO were caused by the back-pressure of fluid owing to the failure of efferent ductules to reabsorb luminal fluids downstream (Hess et al. 1997a, 2002; Zhou et al. 2001; Hess 2003). In ArKO males, testicular degeneration with ageing is independent of the efferent ductule abnormalities. It was hypothesized that ERα expression in the male reproductive tract, where its expression is 3.5-fold greater than uterus (Hess et al. 1997b; Robertson et al. 2002), could be independent of its endogenous ligand and thus would remain in efferent ductule tissues in the ArKO male (Hess & Carnes 2004). Toda et al. (2008) tested this hypothesis, demonstrating that ERα and other smaller bands in Western blot analysis were indeed present in the ArKO tissues. Table 4 lists several potential explanations for the differences between ArKO and neo-ERKO male testicular phenotypes, including the possibility that oestrogen function is a combination of activities through its receptors, both ligand dependent and independent, as well as rapid effects through a membrane receptor via growth factor receptors (Levin 2009a,b).

(c) Direct effects of oestrogen on seminiferous epithelium

Several studies have now presented data supporting the hypothesis that oestrogen does have a direct role in the regulation of spermatogenesis. An indirect role through the hypothalamic–pituitary–testicular feedback loop has been well established (O’Donnell et al. 2001; Balasinor et al. 2006; Ebling et al. 2006) and direct effects on Leydig cell function are well known, as ERs is typically expressed in these cells with most fixation methods and antibodies (Hess et al. 2002; Lucas et al. 2008). The anti-oestrogen ICI 182,780 inhibits T production in WT Leydig cells but not in neo-ERKO cells (Akingbemi et al. 2003). In mice expressing human aromatase, Leydig cell hypertrophy was observed without elevated LH (Strauss et al. 2009). Others have shown oestrogen-dependent regulation of Leydig cell Cyp17a1 (Tong et al. 2004) and Star (Houk et al. 2004). Although the immunolocalization of ERα has not been consistent in the seminiferous epithelium, the ArKO-soy data clearly suggested a direct effect of oestrogen on the seminiferous epithelium in the absence of efferent ductule dysfunction (Robertson et al. 2002; Toda et al. 2008).

ERKO mice lack ERα throughout the development and therefore do not provide an answer to the question of whether the receptor is essential for adult testicular function. Thus, the observed changes in the ERKO male reproductive system could be due to developmental defects and not adult dysfunction. This hypothesis
was tested by treating adult animals with the pure anti-oestrogen IC1 182,780 or Faslodex (AstraZeneca, Macclesfield, Cheshire, UK), which blocks both ERα and ERβ and also downregulates the receptor protein (Wakeling & Bowler 1991, 1992; Wakeling 1995; Sun et al 2002; Berry et al 2008). Anti-oestrogen treatment of the adult rodent simulated many of the ERα KO mouse phenotypes (table 5), but there were differences between rats and mice in their testicular responses. In rat, the anti-oestrogen exposure increased testicular weight by 45 day, with dilation of seminiferous tubules, which was due to fluid accumulation in efferent ductules and rete testis (Oliveira et al 2001, 2002). Testicular swelling was followed by total atrophy of the seminiferous tubules, with ageing.

The mouse efferent ductules and rete testis also responded to IC1 182,780, similar to those of the rat and resembled the neo-ERKO model, except the anti-oestrogen that did not induce the transient increase in testicular weight and total atrophy (Lee et al 2000; Cho et al 2003). Although effects on the efferent ductules were noted as early as 4 days after treatment (Cho et al 2003), effects on the testis were delayed, similar to those seen in the ERα KO (Eddy et al 1996; Hess et al 1997a; Weiss et al 2008), but seminiferous tubular atrophy was heterogeneous and focal, as seen in ArKO mice (Fisher et al 1998; Robertson et al 2001, 2002; Toda et al 2008), and not due to fluid back-pressure. Others have also found apparent direct effects of IC1 182,780 on seminiferous epithelium (Gancarczyk et al 2004; Anahara et al 2006), including effects within 6 days of treatment (Anahara et al 2006).

The ERKO and ArKO animal models suggested that direct effects of oestrogen may be occurring in the seminiferous epithelium, but definitive evidence for this hypothesis was not found until new animal and tissue models were designed. Lucas et al (2008) used improved immunohistochemistry and in vitro culture of isolated Sertoli cells to show specific staining for ERα in immature and adult rat Sertoli cells, which demonstrated that both ERα and ERβ migrate to the cell membrane within 10 min following treatment with 17β-oestradiol (figure 6). These data are contrary to nearly every report in the literature over

<table>
<thead>
<tr>
<th>characteristic</th>
<th>neo- or floxed-ERKO</th>
<th>ICI 182,780b,c</th>
</tr>
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<tbody>
<tr>
<td>rete testis dilation</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>seminiferous tubule dilation</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>Sertoli cell ERα migration to membrane</td>
<td>not determined</td>
<td>yes</td>
</tr>
<tr>
<td>decrease cortactin in Sertoli cells</td>
<td>not determined</td>
<td>yes</td>
</tr>
<tr>
<td>transient increase in testis weight</td>
<td>yes</td>
<td>yes^d</td>
</tr>
<tr>
<td>seminiferous epithelial degeneration testicular atrophy</td>
<td>yes</td>
<td>yes, with age</td>
</tr>
<tr>
<td>increased testosterone</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>decreased Leydig cell function</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>abnormal efferent duct epithelium normal expression of ERβ in efferent ductules</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>decrease in AQP9</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>decrease in NHE3 (SLC9A3)</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>decrease in carbonic anhydrase II (CA2)</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>increase in CFTR^f</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>decreased cauda sperm number</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
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<td>yes</td>
</tr>
<tr>
<td>decreased fertility</td>
<td>yes</td>
<td>yes, with age</td>
</tr>
</tbody>
</table>


^bFaslodex formulation (AstraZeneca, Macclesfield, Cheshire, UK); 5 mg per mouse per week for five weeks.


^dLess increase than in neo-ERKO.

^fFocal seminiferous tubular atrophy but not total testicular atrophy.

^gCystic fibrosis transmembrane conductance regulator.
the past 10 years and thus require that future studies incorporate improved methods for preserving ER for tissue localization.

Another novel animal model that has changed our understanding of oestrogen action in the testis came from the combination of ERKO mice and mice having mutations either in the DNA-binding domain (Weiss et al. 2008) or in the ligand-binding domain of ERα (Sinkvicius et al. 2008, 2009). The absence of ligand binding (ENERKI) demonstrated that both oestrogen-independent as well as oestrogen-dependent signalling pathways are working in the male reproductive tract (Sinkvicius et al. 2009).

In the ENERKI, ligand-independent ERα is capable of activating the efferent ductules and preventing fluid accumulation, possibly working through the growth factor receptor pathway (Sinkevicius et al. 2009). However, oestrogen-dependent ERα signalling was shown to be important for the progression of normal germ cell development (Sinkvicius et al. 2009), in support of the earlier ArKO findings (Robertson et al. 2001, 2002). The absence of DNA binding (NERKI) resulted in a rescue of the neo-ERKO phenotype, producing normal spermatogenesis, but persistent effects on aquaporin-1 in efferent ductules, although without inducing fluid accumulation (Weiss et al. 2008). Thus, it now appears that indeed oestrogen and its major receptor, ERα, have important roles in the regulation of spermatogenesis, particularly with ageing and that this activity occurs through both rapid non-classical membrane-associated/growth factor receptors (Levin 2009a; Santen et al. 2009), as well as classical transcriptionally mediated pathways. Future studies are required to better understand the separation of these pathways and their potential interactions with other steroid receptors that coexist in the same cell types.

**Rapid effects of oestrogen and the role of GPR30**

A large body of evidence has demonstrated that oestrogens can function not only through the classical genomic effect but also can trigger rapid responses that involve transduction pathways different from those activated by ERs (Levin 2005, 2009a). Recently, several studies showed that the GPR30 is able to mediate oestrogen action in both rodents and humans target tissues (Filardo et al. 2002; Maggiolini et al. 2004; Revankar et al. 2005; Thomas et al. 2005; Levin 2009b).

The recent demonstration that GPR30 is expressed in a spermatogonia mouse cell line highlights a role for this receptor after activation with oestradiol in the control of mouse spermatogonia cell proliferation (Sirianni et al. 2008). Our most recent study (Chimento et al. in press) demonstrated that the rat pachytene spermatocytes express ERα, ERβ and GPR30. For the first time, we showed that oestrogens, through both GPR30 and ERα, are able to activate the rapid EGFR/ERK/c-jun signalling cascade, which in turn triggers an apoptotic mitochondrial pathway involving an increase in Bax expression correlated to a reduction of cyclin A1 and B1 levels and thus, induces spermatocyte apoptosis. It is well known that pachytene cells before further differentiation can proliferate or die through apoptosis (Perrard & Durand 2009). Moreover, taking into account the presence of a biologically active aromatase in spermatocytes (Carreau et al. 2010), it will be very interesting to study the molecular mechanisms under oestradiol control that are involved in this important stage of spermatogenesis.

In mice, it has been claimed that GPR30 is not involved in oestrogenic responses of the reproductive organs (Otto et al. 2009). Indeed, these authors generated Gpr30-deficient mice and showed that mutant male and female are fertile. However, it is noteworthy that data are missing about the spermatogenetic process and a careful examination of oestrogenic response was carried out only in uterus and mammary gland. Although GPR30 is not an ER and considerable controversy remains regarding its role in oestrogen-mediated activity, others have suggested that it may collaborate with the membrane ERs to mediate signaling through the growth factor receptor pathway (Levin 2009b).

**5. TESTICULAR TUMOURS AND OESTROGENS**

The overexpression of aromatase, which leads to an oestrogen–androgen imbalance, leads to Leydig cell hyperplasia, dysmorphic seminiferous tubules and disrupted spermatogenesis (Li et al. 2001). It is clear that according to the mouse model, age is important since if the overexpression of aromatase is developed during gestation, 100 per cent of the males are infertile but conversely if the promoter is activated at puberty only 50 per cent of the males are fertile.

In human seminomas, excess oestrogens induce an impairment of spermatogenesis (Nakazumi et al. 1996), as also observed in mice overexpressing aromatase (Fowler et al. 2000; Li et al. 2001). In addition, we have reported (Roger et al. 2005) the expression of a functional aromatase complex in seminoma cells and confirmed that the JKT-1 cell (a pure human testicular seminoma cell line), similar to tumour seminoma cells and human normal testicular basal germ cells, expresses ERβ, including ERβ1 and ERβ2, but not
ERα. Seminoma cells are able to respond to oestrogens through a possible autocrine or paracrine loop and thus, these female hormones contribute to human testicular germ cell cancer proliferation by rapid activation of ERK1/2 and PKA through a membrane ER, as demonstrated recently (Bouskine et al. 2008). This non-genomic effect confirms a new basis for understanding the oestrogenic control of spermatogenesis and evaluating the role of exposure to endocrine disruptors (xenoestrogens) during malignant transformation of testicular germ stem cells. In addition, a very recent work (Rago et al. 2009) has identified ERβ1 and ERβ2 in seminomas and embroyonal carcinoma. Together with the greater presence of ERα in Leydig tumours cells (Carpino et al. 2007), and the existence of membrane ER, it is obvious that oestrogens are functioning in testicular cancers but further studies are necessary to elucidate the mechanisms of action involved.

6. CONCLUSION

It is clear from this review that the testis is equipped with the ability to produce significant amounts of oestrogenic hormones, via aromatase, and expresses ERα and ERβ in Leydig cells as well as the seminiferous epithelium. Oestrogenic activity appears to involve not only the classical genomic pathway, but also the newly discovered rapid membrane receptor pathway and possibly non-classical nuclear ER-tethering pathways (Sun et al. 2002; Harrington et al. 2003; Rodriguez et al. 2004). This activity of oestrogen on spermatogenesis appears to involve germ cell proliferation, differentiation and the final maturation of spermatids, as well as germ cell survival and apoptosis. The final steps of spermatid maturation, spermiogenesis, appear to be particularly sensitive and dependent upon oestrogen, especially after several waves of spermatogenesis have occurred. Based upon these new findings, it will be important to focus new studies on oestrogen-regulated genes associated with spermatogenesis and germ cell differentiation. There still remains a lack of knowledge concerning nuclear receptor cofactors in the testis and their combined activities when present together in cells containing both androgen and ERs. Such studies will help us to better understand the consequences of exposure to environmental endocrine disruptors, as well as provide a potential target for the development of a non-androgen male contraceptive.

Together with the existence of a functional aromatase, the intracrine role of oestrogens should be therefore considered in the immature germ cells as well as in the final steps of spermatooza maturation (Carreau et al. 2010). Even if numerous oestrogen-targeted genes are still missing, there are now evidences in favour of a positive role of oestrogens in the male reproduction via genomic and rapid membrane effects. Age-related studies are also necessary in order to fully understand the control of the aromatase expression and the role of oestrogens in male reproduction, as aromatase and oestrogens are probably involved in promoting sperm development and acquisition of fertilizing capacity.

Finally, new studies are suggesting that testicular cancers in men may have an oestrogen component that will need to be addressed from both an aromatase perspective, as well as receptor expression and activity or the ultimate balance between androgens and oestrogens in the male.

We are greatly indebted to our collaborators Drs Silandre and Delandre (Carreau). All this work has been supported by funding from the French Ministry of Education and Research, a Polonium grant and the Region Basse-Normandie (Carreau); CICC, a programme of CONRAD, Eastern Virginia Medical School and AstraZeneca (Hess).

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