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Role of estrogens in spermatogenesis

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1. ABSTRACT

Aromatase converts irreversibly androgens into estrogens and is present in the endoplasmic reticulum of various cells of mammalian testes ; at least in rodents, all testicular cells except peritubular cells express aromatase. In testis, high affinity estrogen receptors, ERα and/or ERβ, together with a membrane rapid effect recently described, mediate the effects of estrogens. From the experimental models, *in vitro* studies and data collected in patients, it is now demonstrated that estrogens play an important role in the testis of vertebrates. At least it is supported by the widespread distribution of estrogen receptors in the testicular cells and the simultaneous presence of a biologically active aromatase in all germ cells (especially in meiotic and post-meiotic stages). Thus the role of estrogens (intracrine, autocrine and / or paracrine) in spermatogenesis (proliferation, apoptosis, survival and maturation) and more generally, in male reproduction is now evidenced, and much more complex than previously predicted.

2. INTRODUCTION : "ESTROGENS IN TESTIS" A NEW CONCEPT

The mammalian testis serves two main functions: the synthesis and secretion of steroid hormones, and the production of spermatozoa, which are controlled by gonadotrophins and testosterone together with numerous factors produced locally (1), among them estrogens (reviews 2,3). More than 70 years ago, Zondek (4) discovers an estrogenic compound in the stallion urine, but it's only 30 years later that evidences have been published showing that human testes synthesize and secrete estrogens (5) which was confirmed later by Hendry *et al.*(6) demonstrating the testicular source of estrogens in men. Indeed in mammals the presence of large quantities of estrogens in the rete testis fluid and in the spermatic vein was reported (review 7).

Even though, the presence of estrogens in the testis is well documented, unlike androgens the role of estrogens in the physiology of the male reproductive tract

has not been fully understood and thus is extensively revisited (reviews 8,9). New considerations for a functional role of estrogens in male was the development of estrogen receptor alpha knockout mouse (ERαKO) by Lubahn *et al* (10) and for review Couse and Korach (11) and of mice lacking a functional aromatase by Fisher *et al* (12) and for review Jones *et al* (13). In addition the widespread presence of the two ERs (alpha and beta) in all testicular cells as well as in the male genital tract has been extensively demonstrated (reviews 3, 13-16).

Moreover the discovery of mutations in either human estrogen receptor alpha (17) or aromatase (review 18) genes has reinforced the idea that estrogens do play an important role, as a potent modulator of the male reproduction. So, a balance between androgens and estrogens regulated by aromatase seems to be essential for normal testicular physiology and reproduction. Therefore a revision of our knowledge on the testicular cell expression of aromatase and of estrogen receptors (ERs) together with their isoforms and the newborn GPR30 has been undertaken with a view to highlight their roles in male germ cell proliferation and maturation. In that review we will focus on the estrogen effects in spermatogenesis of some mammals, mainly rodents, having in mind the recent data related to the effect of these female hormones in human testicular functions.

3. AROMATASE AND ESTROGEN SOURCES IN TESTICULAR CELLS

3.1. Cyp19 gene

In testes the aromatase localized in the endoplasmic reticulum catalyzes irreversibly the transformation of androgens into estrogens. That enzymatic complex is composed of two proteins: an ubiquitous and non-specific NADPH-cytochrome P450 reductase and the regulated cytochrome P450 aromatase, which contains the heme and the steroid binding pocket specific for the estrogen biosynthesis. In humans, the aromatase is the product of a unique *Cyp19* gene of 123 kb length, located on the long arm of chromosome 15 in the q21.2 region. This gene contains a coding region of 9 exons plus 11 untranslated exons I used according to the characteristics of tissues (signaling pathway specific of the cell type). 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 localized upstream of the translation start site of the exon II (reviews 19-20). Eventhough there is generation of *Cyp19* variants with different 5'UTRs, the coding sequences are identical and a unique protein of 55 kDa is produced (21).

In mouse, the *Cyp19* gene (on chromosome 9) contains 10 putative promoters whom 5 are used in testis (22-23). In the male rat gonad, we have evidenced that at least 3 promoters (24) direct the expression of the aromatase gene (on chromosome 8) : the promoter PII which is the the main one used in testicular cells (25), the promoter PI.f, first characterized as a brain promoter (26) and the promoter PI.tr which is specific of the rat and used only in testis (24) Recently we have discovered two additional promoters used also in rat testis (unpublished data). As a matter of fact, we have reported the existence of various transcripts in rat germ cells different by the 3' region deleted of exon 9 coding for the heme-binding region and then unable to code for a functional aromatase (27) and their amounts is quite high (especially in pachytene spermatocytes). Whatever these promoters are used differentially according to age in the testis and thus control very tightly the amount of transcripts coding for aromatase and thus the amount of estrogens produced (review 3).

3.2. Source of estrogens in testicular cells

In the mammalian testis it is well known that estrogens are produced by the Leydig cells (review 28). However as reported by Simpson *et al* (29) it is difficult to find a tissue without aromatase. Indeed numerous studies have been undertaken to search for other source of estrogens in the testicular cells and it was claimed that in the rat, Sertoli cells are the major source in the immature animals although Leydig cells synthesize estrogens in the adults (30). In seminiferous epithelium when Sertoli cells stopped to express aromatase partially under the negative control of germ cells (31-32) these cells become an important source of estrogens. Nitta *et al* (33) were the first to demonstrate that the adult mouse germ cells express a functional aromatase and that the amount of estradiol produced is equivalent to that of Leydig cells. After that the testicular source (s) of estrogens in several mammals including rodents, bear, pig, horse and primates have been revisited (reviews 2, 9, 16, 34-36).

Despite the great number of data published in different species the precise localization of aromatase has been subject of debates. Some of the variations observed between immunohistolocalization and enzyme activity in cell cultures would be related to the absence of endocrine and/or paracrine regulation such as after *in vivo* germ cell depletion or lack of cell-cell contacts in culture dishes (37).

In our laboratory we have demonstrated that adult rat germ cells represent an other source of estrogens: the levels of P450arom transcripts are two-fold higher in pachytene spermatocytes than in spermatids (38), but it is reversed for the aromatase activity and confirmed by a strong positive staining of aromatase in elongated spermatids (review 3). It is noteworthy that all testicular cells express aromatase except peritubular-myoid cells and in spermatogonia, preleptotene spermatocytes *Cyp19* is present (Silandre *et al*, 2007). Taking into account that the amount of aromatase transcript is very low in the adult rat Sertoli cells (39) and that the aromatase is negatively controlled by germ cells (31-32), we have calculated that the aromatase activity in germ cells represents more than 50% of that of the whole testis. As a consequence since the germ cell source of estrogens is equivalent to that from Leydig cells a very tight control of the aromatase expression/activity should be exerted to avoid excess of estrogens which are very deleterious for spermatogenesis when produced in large quantity as in seminoma (40-41).

The report of Lambard *et al* (42) were in fitting with those of Janulis *et al* (43) who have clearly shown that the aromatase is localized in the cytoplasmic droplet of spermatozoa. Moreover the existence of aromatase transcripts in the epithelial cells of the rat epididymis has been reported (44).

In the bank vole, a seasonal breeder, aromatase has been described in somatic cells as well as in germ cells (45) and the immunoexpression of the protein is higher in the breeding season (46). In the boar a unique Leydig cell source of estrogens has been described (47-48). For the stallion, the aromatase is more expressed in Leydig cells (49), even though recent data are in favor for an additional source in germ cells (50-51) still need to be confirmed *in vitro* on purified cells. In black bear (52), bison (53) and deer (54) aromatase has been detected both in somatic and germ cells.

In the Rhesus monkey, testis and to a lesser extent, epididymis contains P450arom transcripts (55). In humans besides Leydig cells (56) Sertoli cells are able to synthesize estradiol (57) and aromatase is expressed in immature germ cells (58) as well as in ejaculated spermatozoa (36, 42, 59-60).The ability of human ejaculated spermatozoa to transform androgens into estrogens is now obvious even if the aromatase activity is weak (42), we have evidenced the presence of protein on western blots with a slightly lower molecular weight (49 kDa) than the aromatase of granulosa cells (53 kDa); in addition, the intensity of the signal is greater in the spermatozoa containing cytoplasmic droplets (59). The presence of aromatase has been shown by immunohistochemistry in the epithelial cells of the efferent ducts and of the caput epididymis in man (61).

4. ESTROGEN RECEPTORS

In order to exert a biological effect, testicular estrogens should interact with estrogen receptors which in turn can modulate the transcription of target genes. Therefore considering the presence of at least two main types of ERs in most of the testicular cells the physiological role of estrogens in male reproduction has been extensively revisited. In addition the demonstration of rapid membrane effect has provided new developments about the role of estrogens in male gamete maturation (review 3)

4.1. Estrogen receptors (ERs) alpha and beta and their isoforms

Genomic effects of estrogens are mediated by nuclear estrogen receptors (ER) α and β which are both expressed in testicular cells of several species (review 3). However, data concerning their localization in adult rat testis are not consistent. Indeed, ERα has been immunodetected only in Leydig cells by Fisher *et al.* (62) and by Saunders *et al.* (63) but was also revealed in the seminiferous compartment by Pelletier *et al* (64). About ERβ, all studies are in agreement concerning its localization in seminiferous tubules but data are in conflict regarding to its presence in germ cells. While Saunders *et al* (63) found ER beta in Sertoli cells and in different germ cell types (A spermatogonia, pachytene spermatocytes and round spermatids), van Pelt *et al* (65) and Pelletier *et al* (64) localized ERβ only in Sertoli cells. ERα mRNAs have been found in Leydig cells (66), Sertoli cells (unpublished data) and germ cells (Pelletier *et al*, 2000 ; our unpublished data). ERβ mRNA has been revealed in Sertoli cells and spermatocytes (67) while others retrieved them also in A spermatogonia, round spermatids (65) and Leydig cells (our unpublished data). It is of note that the presence of ERs either at transcript or protein level has never been observed in peritubular cells (62-68). Two in-frame deletions of the ERα (ERα-δ4 and ERα-δ5,6) with the full-length form have been reported in the testis (69). This deletion occurring in DNA and in hormone binding domains suggested a modification of receptor activity. However, using a specific antibody directed against the C-terminal region of the ERα protein, only the full-length form was detected by Chimento *et al* (70) in the whole adult rat testis and in purified germ cells as reported in immature rat Sertoli cells (71). Regarding ERβ, four different mRNAs have been retrieved in the rat testis (our unpublished data) but only one protein is detected in the male rat gonad (70-72). The presence of ERs in testicular cells of human is well documented (73; review 9). It was speculated that the cells most susceptible to modulation by estrogenic ligands are round spermatids in which levels of ERβ are high (74). In addition to the full length receptor ERβ, six variants have been identified in the testis and their different localization suggest specific functions in spermatogenesis (75). Both types of ERs (α and β) have been identified in isolated immature germ cells of men: the full length protein ERα (66 kDa) and one isoform lacking the exon 1 (46 kDa) whereas in mature spermatozoa, only the 46 kDa band was observed. For ERβ, two proteins which correspond to a long (60 kDa) and short (50 kDa) forms have been detected in germ cells whereas only the PCR product was found in spermatozoa (58). However, the presence of ERα and ERβ in the human ejaculated spermatozoa has been demonstrated (76-77).

4.2. GPR30 and rapid signalling

Estrogens can also exert effects called “non genomic” by activating signaling pathways from the membrane. The G protein-coupled receptor (GPR30), a seven transmembrane receptor (7TMR) seems to be implicated in these rapid effect of estrogens. Immunohistochemical analysis in murine tissue shows that GPR30 is found in the male reproductive tract, including testes, epididymis, vas deferens, and seminal vesicles as well as the prostate (review 78). GPR30 expression was also demonstrated in a mouse spermatogonial cell line GC-1 (79) and in rat pachytene spermatocytes (70). In GC-1 cell line, it was demonstrated that GPR30 activation by its specific agonist G1 induces the activation of the ERK cascade, an up-regulation of the cyclin D1 expression and proliferation (79). The use of the specific agonist G1, like addition of 17β-estradiol leads to phosphorylation of ERK1/2 and c-jun, after 30 minutes incubation (70), these effects are associated with a reduction of cyclin A1 expression and an increase of Bax mRNA levels, factors implicated in the meiotic progression and the control of apoptosis.

5- ROLE OF ESTROGENS IN SPERMATOGENESIS

5.1. Data from invalidation and surexpression of aromatase and estrogen receptors in mice

Several experimental models of mice have been developed and shown to be helpful to clarify the role of estrogens in testes of vertebrates (review 8). There is evidence from estrogen receptor α gene knock out (ERKOα) mouse that estrogens are necessary for the achievement of fertility (11). Animals that lack a functionnal *Esr1* gene are sterile and sperm recovered from the cauda epididymis exhibits a low percent motility, beat less vigorously and are ineffective at *in vitro* fertilization. Howewer, this infertility is not due to a primary defect in spermatozoa since the transplantation of *Esr1*KO germ cells into a wild-type reproductive tract yield normal offspring (80-81). Recent studies show that the *Esr1*KO epididymis fails to properly acidify the luminal milieu, due to defects in the expression of specific acid/base regulators. This leads to defects in sperm intracellular pH and motility that can be partially rescued by bypassing the consequences of the abnormal epididymal milieu (82). In a companion paper Joseph *et al* (83) report that ERαKO spermatozoa have two distinct morphological defects, an increased propensity for spontaneous acrosome reactions and severe flagellar coiling. These defects appear to be at least partly due to the decreased osmolarity of the ERαKO luminal environment because when ERαKO sperm are incubated in a more wild-type-like osmotic environment of high-pH and low osmolarity, the proportion of straight sperm flagella are significantly reduced. Together the results indicate that the defect in flagellar coiling of *Esr1*KO sperm is not intrinsic to the axoneme, but is rather a consequence of development within an abnormal microenvironment.

The last model of ERβKO (84) in contrary of the first models developed displays a sterility in male. In these mice, authors show the absence of alternative splicing transcripts of ERβ. The males are sterile but the initial cause is unknown because testis and epididymis histology are normal and their spermatozoa appeared normally mobile (84).

The male mice deficient in aromatase (ArKO) develop normally and the genital tract is anatomically in the control range when compared to the wild-type. The males are able to sire and to produce litters; however starting from the age of 5 months onwards some of ArKO males start to have failures of spermatogenesis and by the age of one year all males develop abnormal spermatogenesis with a blocage of germ cell maturation at the spermatid stage i.e there is a 50% decrease of both the number of round and elongated spermatid together with an increase of apoptotic features when compared to the wild-type animal (85).

Conversely the overexpression of aromatase (AROM+) in mice (86-87) induces Leydig cell hyperplasia associated with abnormal spermatogenesis following an excess of endogenous estrogens. However an age-related effect has been reported and infertility of 100% of males occurs when overexpression was performed during fetal life but only in 50% of mice when it was realized during puberty (review 88).

The estrogen role in the testis has been enlightened recently via an other model in which an association of ERαKO mice with mice having mutations either in the DNA-binding domain (89) or in the ligand-binding domain of ERα (90) have been developed. The absence of ligand binding demonstrates that both estrogen-independent and dependent signaling pathways are concerned in the male germ cell development and final maturation of sperm cells (90).

GPR30 has been identified in a variety of human and rodent estrogen target tissues (91) including the testis (92). Immunocytochemical studies have identified the intracellular localization of GPR30 in the endoplasmic reticulum, Golgi apparatus (93) and plasma membrane (94). Recent studies demonstrated that GPR30 is expressed in mouse testes and in a GC-1 spermatogonia mouse cell line leading to a control of mouse cell proliferation by estradiol (79). In our study (70) we demonstrated that GPR30 is expressed in adult rat pachytene spermatocytes and that estrogens through both GPR30 and ERα are able to activate the rapid EGFR/ERK/c-jun signaling cascade, which in turn triggers an apoptotic mitochondrial pathway involving an increase in Bax expression and a concomitant reduction of cyclin A1 and B1 levels These genes are involved in the balance between cellular proliferation and apoptosis and it is indeed well known that before diffrentiation a number of pachytene spermatocytes die (95). In an other study in round spermatids of adult rat we have shown that the rapid membrane effect of estradiol is also efficient in controlling apoptosis and maturation / differentiation of these haploid germ cells (Chimento *et al*, submitted). Our data are likely in agreement with the studies performed in ERKO mice (especially ERαβKO) which phenotype is different from ArKO and thus, a residual ER activity via other pathways should be considered.

Keeping in mind that these germ cells (PS and RS) are equipped with both a functional aromatase and ERs (rapid and genomic pathways) , the estrogen-regulated genes would be interesting to study. In GPR30 knock out mice it has been claimed that GPR30 is not concerned (96) since both male and female are fertile; however no data on spermatogensis are available and as suggested by Levin (97) a coordinated effect of estrogen via GPR30 and ERα should be taken into account.

5.2. Estrogens and germ cells development

At least in rodents, spermatogenesis is in part under estrogens control, i.e the stem germ cell number and the spermatid maturation (98); moreover estradiol enhances recovery of spermatogonia after irradiation of rat testis (99). Neonatal administration of estrogens to male rat induces an increase of the number of spermatogonia at day 16 of life (100). In the immature bank vole, exposure to low dose of estradiol leads to an acceleration of the onset of spermatogenesis which is blocked by the injection of the antiestrogen ICI 182,780 (101). An improvement of

the recrudescence of spermatogenesis in estradiol treated rodents has been reported (102-103). However, exposure of the adult male rat to a high phytoestrogen diet disrupts spermatogenesis by increasing germ cell apoptosis (104). Conversely endogenous estradiol inhibits the gonocytes development in mice (105). It is obvious that according to species, the estrogen effects are different even opposite if we consider the gonocytes proliferation (review 106). In addition D'Souza *et al* (107) have demonstrated that the spermatid elongation (steps 8 to 19) is androgen dependant whereas the differentiation of round spermatid (steps 1 to 6) is under estrogen control. That is also supported by a significant decrease of round and elongated spermatids numbers but not of spermatogonia and spermatocytes in ArKO mice which thus is in favor for a positive and / or survival role of estrogens in spermatids (85). Moreover the abnormal acrosome development in the ArKO mouse suggests that acrosome formation could be an estrogen dependent process (85) which is supported by high levels of aromatase in the Golgi of the developing spermatids (33) as well as by the presence of estrogen receptors in these germ cells (reviews 3, 8; our unpublished data).

Moreover treatment of adult monkey with an aromatase inhibitor suggest that estrogens are important for spermatid differentiation (108). In a recent work, a positive effects of estrogens on germ cell development in the fetal testis of baboon has been reported (109).

In addition we have demonstrated that estrogens or phytoestrogens delay testicular damage during aging in male rat, probably via their antioxidant role against the reactive oxygen species (110).

Aromatase and ERs have been demonstrated in most of the testicular cells in humans (review 36). Indeed Beck *et al* (111) and Idaomar *et al* (112) have described a positive role of estrogens for improving the human sperm motility. It has been reported that estradiol is a survival/antiapoptotic factor for human germ cells *in vitro* (73). Berensztein *et al* (113) have immunolocalized aromatase and ERβ in gonocytes and spermatogonia in newborn and infantile testes but the role of estrogens in that developing gonad remain to be clarified.

So the role of oestrogens in man especially in the reproductive function is becoming more obvious especially after the publication of Smith *et al* (17) concerning a man with a non-functional ERα and several reports (eight today) related to men deficient in aromatase (reviews 13, 18). What wais significant when a biopsy was performed (114) is the great variation of spermatogenesis efficiency between seminiferous tubules (some of them having a full germ cell development whereas other tubules are empty or hypospermatogenetic) has already obeserved in ArKO mice (review 115).

The excess estrogen syndrome in man has been recently reviewed (116) : only few families have been studied and all male patients exibit an early puberty and gynaecomastia associated with an hypogonadism consecutive to low gonadotropins after the overexpression of aromatase in numerous tissues leading to increased levels of estrogens.

In addition in human seminomas, excess of estrogens induces alteration of spermatogenesis (40), as observed in AROM+ mice (review 88). Seminoma cells are able to respond to estrogens via a membrane ER and thus these hormones contribute to human testicular germ cell proliferation (117) as observed in the mouse GC-1 cell line (79).

6. CONCLUSIONS AND PERSPECTIVES

Besides a well-known negative feedback of estrogens on the hypothalamus-hypohysis complex, it has become more obvious that testicular estrogens could play a role locally in the gonad, especially after the data obtained either from patients genetically deficient in aromatase (review o18) and from the ArKO or ERα KO mice (review 3). Moreover decreased sperm counts and increased male reproductive tract disorders (cryptorchidism, hypospadia, testicular cancer) in men have been published and a deleterious effect of endocrine disruptors suggested (118-119). Therefore agents able to mimic estrogens can alter the role of endogenous hormone leading to impairment of male gamete development. The non-genomic effect of estrogens confirms a new basis for understanding the estrogenic control of spermatogenesis and evaluating the role of exposure to endocrine disruptors (xenoestrogens) during malignant transformation of testicular germ cells.

The main question is related to rat or mouse which are poor models to analyze the mechanisms concerned by estrogens in human male fertility (quality of spermatogenesis) since in the null mice the direct role of estrogens is not clarified suggesting therefore that estrogen-dependent and independent as well as rapid membrane effect should be taken into account (97, 120). On male rodent germ cells the existence of both membrane and genomic effects of estrogens have been demonstrated (70, 79) with a special emphasis on proliferation, apoptotic and survival genes.

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 (review 3).

Furthermore one should kept in mind that not only rodent spermatozoa but ejaculated human spermatozoa express a functional aromatase (review 60) and together with ERs these data open new considerations about the role of estrogens all along the male genital tract as well as in sperm functions.

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8. REFERENCES

1 . J.M. Saez: Leydig cells : endocrine, paracrine and autocrine regulation. *Endocr Rev* 15, 574-626 (1994)

2. S. Carreau, C.Genissel, B.Bilinska and J. Levallet: The oestrogen sources in the testis and the reproductive tract of the male. *Inter J Androl* 22, 211- 223 (1999)

3. S. Carreau and R. Hess: Oestrogens and spermatogenesis. *Phil Trans R Soc B* 365, 1517-1535 (2010)

4. B. Zondek: Mass excretion of oestrogenic hormone in the urine of the stallion. *Nature (London)*, 193, 209-210 (1934)

5. M.F. Jayle, R. Scholler, A. Sfikalis and M. Héron : Excrétion des phénolstéroïdes et des 17 cétostéroïdes après administration de gonadotrophines chorioniques à des hommes. *Clin Chem Acta* 7, 212-20 (1962)

6. W.F. Hendry, J.M. Parslow and J. Stedronska: Exploratory scrotomy in 168 azoospermic males. *Brit. J Urol* 55, 785-791 (1983)

7. R.A. Hess: Estrogen in the adult male reproductive tract: a review. *Reprod Biol Endocrinol* 9, (1): 52 (2003)

8. L. O'Donnell, K.M. Robertson, M.E. Jones and E.R. Simpson: Estrogen and spermatogenesis. *Endocr Rev* 22, 289-318 (2001)

9. S. Carreau, S. Bourguiba, C. Delalande, D. Silandre, L. Said, I. Galeraud-Denis and S. Lambard: Estrogens and Male Reproduction. *Current Med Chem Immunol Endocrinol Metab Agents* 8, 59-65 (2008)

10. D.B. Lubahn, J.S. Moyer, T.S.Golding, J.F. Couse, K.S. Korach and O. Smithies:Alteration of reproductive function but not prenatal sexual development after insertional disruption of the mouse estrogen receptor gene. *Proc Natl Acad Sci USA* 90, 11162–11166 (1993)

11. J.F. Couse and K. Korach: Estrogen receptor null mice : what have we learned and where will they lead us? *Endocr Rev* 20, 358-417 (1999)

12. C.R. Fisher, K.H. Graves, A.F.Parlow and E.R. Simpson: Characterization of mice deficient in aromatase (ArKO) because of targeted disruption of the cyp19 gene. *Proc Natl Acad Sci USA* 95, 6965–6970 (1998)

13. M.E.E. Jones, W.C. Boon, K. McInnes, L.Maffei, C. Carani and E.R. Simpson: Recognizing rare disorders: aromatase deficiency. *Nature Clin Pract Endocrinol & Metab* 3, 414-421 (2007)

14. P.T.K. Saunders, R.M. Sharpe, K. Williams, S. Macpherson, H.Urquart, D.S. Irvine, and M.R. Millar: Differential expression of oestrogen receptor α and β proteins in the testes and male reproductive system of human and non-human primates. *Molec Human Reprod* 7, 227-336 (2001)

15. J.E. Sierens, S.F. Sneddon, F. Collins F, M. R. Millar and P. T. K. Saunders: Estrogens in Testis Biology. *Ann NY Acad Sci* 1061, 65-76 (2005)

16. R.A. Hess and K. Carnes: The role of estrogen in the testis and the male reproductive tract : a review. *Anim Reprod* 1, 5-30 (2004)

17. E.P. Smith , J. Boyd, G.R. Frank, H; Takahashi, R.M.Cohen, B. Specker, T.C.Williams, D.B.Lubahn and K.S. Korach: Estrogen resistance caused by a mutation in the estrogen-receptor gene in a man.*N Engl J Med* 331, 1056–1061(1994)

18. V. Rochira, A.R.M. Granata, B. Madeo, L. Zirilli, G. Rossi and C. Carani: Estrogens in males : what we have learned in last 10 years? *Asian J Androl* 7, 3-20 (2005)

19. S. Sebastian and S.E. Bulun : A highly complex organization of the regulatory region of the human CYP19 (aromatase) gene revealed by the Human genome project. *J Clin Endocrinol Metab* 86, 4600-4602 (2001)

20. M. Demura, S. Reierstad, J.E. Innes and S.E. Bulun. Novel promoter I.8 and promoter usage in the CYP19 (Aromatase) gene. *Reprod Sci* 15, 1044-1053. (2008)

21. E.R. Simpson,Y. Zhao, V.R.Agarwal, M.D. Michael,S.E. Bulun, M.M.Hinshelwood, S.Graham-Lorence, T. Sun, C.R. Fisher, K.Qin, and C.R. Mendelson: Aromatase expression in health and disease. *Recent Progress in Hormone Research* 52,185-212 (1997)

22. J.D. Chow, E.R. Simpson and W.C. Boon: Alternative 5'-untranslated first exons of the mouse Cyp19A1 (aromatase) gene. *J. Steroid Biochem. Mol. Biol.* 115, 115-125 (2009).

23. H. Zhao, J. Innes, D.C. Brooks, S. Reierstad, M.B. Yilmaz, Z. Lin and S.E. Bulun: A novel promoter controls Cyp19a1 gene expression in mouse adipose tissue. *Reprod. Biol. Endocrinol.* 24, 7:37. (2009).

24. D. Silandre, C. Delalande, P. Durand and S. Carreau: Three promoters PII , PI.f and PI.tr direct the expression of aromatase (cyp19) gene in male rat grem cells. *J Molec Endocrinol* 39, 169-181 (2007)

25. M. Lanzino, S. Catalano, C. Genissel, S. Ando, S. Carreau, K. Hamra and J. McPhaul : Aromatase messenger RNA is derived from the proximal promoter of the aromatase gene in Leydig, Sertoli, and germ cells of the rat testis. *Biol Reprod* 64,1439-1443 (2001)

26. N.Yamada-Mouri, S. Hirata and J. Kato: Existence and expression of the untranslated first exon of aromatase mRNA in the rat brain. *J Steroid Biochem Molec Biol* 58,163-166 (1996)

27. J. Levallet, B. Delarue, H. Mittre and S. Carreau: Alternative splicing events in the coding region of cytochrome P450 aromatase in adult male rat germ cells. *J Mol Endocrinol* 20, 305-312 (1998)

28. S. Carreau: Leydig cell aromatase : from gene to physiological role. In : *The Leydig Cell in Health and Disease*. Eds : A.H. Payne & M.P. Hardy. Human Press Inc, Totowa, NJ, USA, 189-195 (2007)

29. E.R. Simpson, M.S. Mahendroo, G.D. Means, M.W. Kilgore, M.M. Hinshelwood, S. Graham-Lorence, B. Amarneh, Y. Ito, C.R. Fisher, M.D. Michael, C.R. Mendelson and S.E. Bulun: Aromatase cytochrome P450, the enzyme responsible for estrogen biosynthesis. *Endocr Rev* 15, 342-355 (1994)

30. V. Papadopoulos, S. Carreau, E. Szerman-Joly, M.A. Drosdowsky, L. Dehennin and R. Scholler: Rat testis 17ß-estradiol : identification by gas-chromatography-mass spectrometry and age-related cellular distribution. *J Steroid Biochem* 24,1211-1216 (1986)

31. C.Boitani, E.M.Ritzen and M. Parvinen: Inhibition of rat Sertoli cell aromatase by factor(s) secreted specifically at spermatogenic stages VII and VIII. *Mol Cell Endocrinol* 23,11-22 (1981)

32. H. Bouraïma-Lelong, M Vanneste, C. Delalande, L. Zanatta, S. Woczynski and S. Carreau: Aromatase gene expression in immature rat Sertoli cells: age-related changes in the FSH signalling pathway. *Reprod Fert Dev* 22, 508-515 (2010)

33. H. Nitta, D. Bunick, R.A. Hess, L. Janulis, S.C. Newton, C.F. Milette, Y. Osawa, Y. Shizuta, K. Toda and J.M. Bahr: Germ cells of the mouse testis express P450 aromatase. *Endocrinology* 132,1396-401(1993)

34. S. Carreau, S. Lambard, C. Delalande, I. Galeraud-Denis, B. Bilinska and S. Bourguiba: Aromatase expression and role of estrogens in male gonad : a review. *Reprod Biol Endocrinol* 1, 35 (2003)

35. S. Carreau, C. Delalande, D. Silandre, S. Bourguiba and S. Lambard: Aromatase and estrogen receptors in male reproduction . *Mol Cell Endocrinol* 246, 65-68 (2006)

36. S. Carreau, C. Delalande and I. Galeraud-Denis: Mammalian sperm quality and aromatase expression. *Microsc Res Tech* 72, 552-557 (2009)

37. S. Carreau, V. Papadopoulos and M.A. Drosdowsky : Stimulation of adult rat Leydig cell aromatase activity by a Sertoli cell factor. *Endocrinology* 122,1103-1109 (1988)

38. J. Levallet, B. Bilinska, H. Mittre, C. Genissel, J. Fresnel and S. Carreau: Expression and immunolocalization of functional cytochrome P450 aromatase in mature rat testicular cells. *Biol Reprod* 58, 919-926 (1998)

39. J. Levallet and S. Carreau: Aromatase gene expression in rat testicular cells. *C R Acad Sc Paris* 320,123-129 (1997)

40. H. Nakazumi, H. Sasano, I. Maehara, M.Ozaki, F.Tezukaa and S.Orikasa: Estrogen metabolism and impaired spermatogenesis in germ cell tumors of the testis. *J Clin Endocrinol Metab* 81, 1289-1295 (1996)

41. C. Roger, S. Lambard, A. Bouskine, B. Mograbi, D. Chevallier, M. Nebout, G. Pointis, S. Carreau and P. Fenichel: Estrogen-induced growth inhibition of human seminoma cells expressing estrogen receptor beta and aromatase. *J Mol Endocrinol* 35, 191-199 (2005)

42. S. Lambard, I. Galeraud-Denis, H. Bouraima , S. Bourguiba, A. Chocat, S. Carreau: Expression of aromatase in human ejaculated spermatozoa: a putative marker of motility. *Mol. Hum. Reprod*. 9, 117-24 (2003)

43. L. Janulis, J.M.Bahr, R.A. Hess, S. Janssen, Y. Osawa and D. Bunick: Rat testicular germ cells and epididymal sperm contain active P450 aromatase.*J Androl* 19, 65-71 (1998)

44. B. Wiszniewska: Primary culture of rat epididymal epithelial cells as a source of estrogen. *Andrologia* 34, 1-8 (2002)

45. B. Bilinska, B. Schmalz-Fraczek, M. Kotula and S. Carreau: Photoperiod-dependent capability of androgen aromatization and the role of estrogens in bank vole testis visualized by means of immunohistichemistry. *Mol Cell Endocrinol* 178, 189-198 (2001)

46. M. Kotula-Balak, M. Slomczynska, B. Fraczek, S. Bourguiba, Z. Tabarowski, S. Carreau and B. Bilinska: Complementary approaches demonstrate that cellular aromatization in the bank vole testis is related to photoperiod. *Eur J Histochem* 47, 55-62 (2003)

47. J.J. Raeside and R.L. Renaud : Estrogen and andogen production by purified Leydig cells from mature boars. *Biol Reprod* 28,727-733 (1983)

48. H. Mutembei, S. Pesch, G. Schuler and B. Hoffmann: Expression of oestrogen receptors alpha and beta and of aromatase in the testis of immature and mature Boars. *Reprod Domest Anim* 40, 228-236 (2005)

49. K.M. Eisenhauer, P.M. McCue, D.K.Nayden, Y. Osawa and J.F. Roser: Localization of aromatase in equine Leydig cells. *Domest Anim Endocrino* 11, 291–298 (1994)

50. H. Sipahutar, P. Sourdaine, S. Moslemi, B. Plainfossé and G.E. Seralini : Immunolocalization of aromatase in stallion Leydig cells and seminiferous tubules. *J Histochem Cytochem* 51, 311-318 (2003)

51. A. Hejmej, M. Kotula-Balak, J. Sadowska and B. Bilinska: Expression of connexin 43 protein in testes,

epididymides and prostates of stallions. *Equine Vet J* 39, 122-127 (2007)

52. T. Tsubota, L. Howell-Skalla, H. Nitta, Y. Osawa, J.I. Mason, P.G. Meiers, R.A. Nelson, J.M. Bahr: Seasonal changes in spermatogenesis and testicular steroidogenesis in the male black bear Ursus americanus. *J. Reprod. Fertil.* 109, 21-27 (1997)

53. I. Kopera, M. Szczepanowicz, Z. Gizejewski, J. Sadowska and B. Bilinska: Immunoexpression of aromatase in immature and adult males of the European bison (Bison bonasus, Linnaeus 1758). *Reprod Domest Anim* doi: 10.1111/j.1439-0531.2008.01280.x

54. J.Schon and S.Blottner: Estrogens are involved in seasonal regulation of spermatogenesis and sperm maturation in roe deer (Capreolus capreolus). *Gen Comp Endocrinol* 159, 257-263 (2008)

55. A.C. Pereyra-Martinez, C.E. Roselli, H.L. Stadelman, and J.A. Resko: Cytochrome P450 aromatase in testis and epididymis of male *rhesus* monkeys. *Endocrine* 16**,** 15-19 (2001)

56. A.H. Payne, R.P. Kelch, S.S. Musich and M.E. Halpern: Intratesticular site of aromatization in the human. *J Clin Endocrinol Metab* 42,1081-1087 (1976)

57. P. Foucault, S. Carreau, W. Kuczynski, J.M.Guillaumin, P. Bardos and M.A. Drosdowsky: The human Sertoli cells *in vitro*: lactate, estradiol-17ß and transferrin productions. *J Androl* 13, 361–367 (1992)

58. S. Lambard, I. Galeraud-Denis, P.T. Saunders and S. Carreau: Human immature germ cells and ejaculated spermatozoa contain aromatase and oestrogen receptors. *J. Mol. Endocrinol.* 32, 279-289 (2004).

59. S. Aquila, D. Sisci, M. Gentile, E. Middea, L. Siciliano and S. Ando: Human ejaculated spermatozoa contain active P450 aromatase. *J Clin Endocrinol Metab* 87**,** 3385-3390 (2002)

60. S. Carreau, S. Wolczynski and I. Galeraud-Denis : Aromatase, oestrogens and human male reproduction. *Phil Trans R Soc B* 365, 1571-1579 (2010)

61. F. Carpino, F. Romeo and F.V. Rago: Aromatase immunolocalization in human ductuli efferentes and proximal ductus epididymis. *J Anat* 204, 217-220 (2004)

62. J.S. Fisher, M.R. Millar, G. Majdic, P.T. Saunders, H.M. Fraser and R.M. Sharpe: Immunolocalisation of estrogen receptor-alpha within the testis and excurrent ducts of the rat and marmoset monkey from perinatal life to adulthood. *J Endocrinol* 153, 485-495 (1997)

63. P.T.K. Saunders, J.S. Fisher, R.M. Sharpe and M.R. Millar: Expression of oestrogen receptor beta (ERβ) occurs in multiple cell types, including some germ cells, in the rat testis. *J Endocrinol* 156, R13-R17 (1998)

64. G. Pelletier, C. Labrie and F. Labrie: Localization of oestrogen receptor alpha, oestrogen receptor beta and androgen receptors in the rat reproductive organs. *J Endocrinol* 165, 359-370 (2000)

65. A.M. van Pelt, D.G. de Rooij, B. van der Burg, P.T. van der Saag, J.A. Gustafsson, and G.G. Kuiper: Ontogeny of estrogen receptor-beta expression in rat testis. *Endocrinology* 140, 478-483 (1999).

66. C.N. Mowa and T. Iwagana. Expression of estrogen receptor-alpha and -beta mRNAs in the male reproductive system of the rat as revealed by *in situ* hybridization. *J Mol Endocrinol* 26, 165-174 (2001)

67. P.J. Shugrue, M.V. Lane, P.J. Scrimo and I. Merchenthaler: Comparative distribution of estrogen receptor-alpha (ER-alpha) and beta (ER-beta) mRNA in the rat pituitary, gonad, and reproductive tract. *Steroids* 63, 498-504 (1998)

68. P.T.K. Saunders, S.M. Maguire, J. Gaughan, M.R. Millar: Expression of estrogen receptor beta (ERβ) in multiple rat tissues visualized by immunohistochemistry. *J Endocrinol* 154,R13-R16 (1997)

69. K E. Friend, E.M. Resnick, L.M. Ang and M.A. Shupnik: Specific modulation of estrogen receptor mRNA isoforms in rat pituitary throughout the estrous cycle and in response to steroid hormones. *Mol Cell Endocrinol* 131, 147-155 (1997)

70. A.Chimento, R. Sirianni, C. Delalande, D. Silandre, C. Bois, S. Ando, M. Maggiolini, S. Carreau and V. Pezzi : 17β-estradiol activates rapid signaling pathways involved in rat pachytene spermatocytes apoptosis through GPR30 and ERα. *Mol Cell Endocrinol* 320, 136-144 (2010)

71. T.F. Lucas, E.R. Siu, C.A. Esteves, H.P. Monteiro, C.A. Oliveira, C.S. Porto, M.F. Lazari: 17 beta-estradiol induces the translocation of the estrogen receptors ERS1 and ERS2 to the cell membrane, MAPK3/1 phosphorylation and proliferation of cultured immature rat Sertoli cells. *Biol Reprod* 78,101-114 (2008)

72. O.M. Tirado, D.M. Selva, N. Toran, C.A. Suarez-Quian, M. Jansen, D.P. McDonnell, J. Reventos and F. Munell: Increased expression of estrogen receptor beta in pachytene spermatocytes after short-term methoxyacetic acid administration. *J Androl* 25, 84-94 2004

73. V. Pentikaïnen, K. Erkkilä, L. Suomalainen, M. Otala, M.O. Pentikaïnen, M. Parvinen, and L. Dunkel: Estradiol acts as a germ cell survival factor in the human testsi *in vitro*. *J Clin Endocrinol Metab* 85, 2057-2067 (2000)

74. P.T. Saunders, M.R. Millar, S. Macpherson, D.S. Irvine, N.P. Groome, L.R. Evans R.M., Sharpe and G.A. Scobie: ERbeta1 and the ERbeta2 splice variant (ERbetacx/beta2) are expressed in distinct cell populations in the adult human testis. *J. Clin. Endocrinol. Metab*. 87, 2706-2715 (2002)

75. E.L. Aschim, T. Saether, R. Wiger, T. Grotmol and T.B. Haugen: Differential distribution of splice variants of estrogen receptor beta in human testicular cells suggests specific functions in spermatogenesis. *J. Steroid Biochem. Mol. Biol.* 92, 97-106 (2004).

76. S. Aquila, D. Sisci, M. Gentile, E. Middea, S. Catalano, A. Carpino, V. Rago and S. Ando: Estrogen receptor (ER)alpha and ER beta are both expressed in human ejaculated spermatozoa: evidence of their direct interaction with phosphatidylinositol-3-OH kinase/Akt pathway. *J. Clin. Endocrinol. Metab*. 89, 1443-1451 (2004).

77. S. Solakidi, A.M. Psarra, S. Nikolaropoulos and C.E. Sekeris: Estrogen receptors alpha and beta (ERalpha and ERbeta) and androgen receptor (AR) in human sperm: localization of ERbeta and AR in mitochondria of the midpiece. *Hum. Reprod.* 20, 3481-3487 (2005)

78. E.R. Prossnitz, J.B. Aterburn, H.O. Smith, T.I.Oprea, L.A. Sklar and H.J. Hathaway :

Estrogen signaling through the transmembrane G protein-coupled receptor GPR30. Annu Rev Physiol 70, 165-190 (2008)

79. R. Sirianni, A. Chimento, C. Ruggiero, A. De Luca, R. Lappano, S. Ando, M. Maggiolini, and V. Pezzi: The Novel Estrogen Receptor, G Protein-Coupled Receptor 30, Mediates the Proliferative Effects Induced by 17{beta}-Estradiol on Mouse Spermatogonial GC-1 Cell Line. *Endocrinology* 149, 5043-5051 (2008)

80. D. Mahato, E.H. Goulding, K.S. Korach, E.M. Eddy: Spermatogenic cells do not require estrogen receptor-α for development or function. *Endocrinology* 141, 1273-1276 (2000).

81.D. Mahato, E.H. Goulding, K.S. Korach, E.M. Eddy : Estrogen receptor-α is required by the supporting somatic cells for spermatogenesis. *Mol. Cell. Endocrinol.* 178, 57-63 (2001).

82. A. Joseph, B. D. Shur, C. Ko, P. Chambon, R. A. Hess : Epididymal hypo-osmolality induces abnormal sperm morphology and function in the estrogen receptor alpha knockout mouse. *Biol. Reprod.,* in press (2010).

83. A. Joseph, R. Hess, D.J. Schaeffer, C. Ko, S. Hudgin-Spivey, P. Chambon, B.D. Shur: Absence of estrogen receptor alpha leads to physiological alterations in the mouse epididymis and consequence defects in sperm function. *Biol. Reprod.* In press, (2010).

84. M. C. Antal, A. Krust, P. Chambon, M. Mark : Sterility and absence of histopathological defects in non reproductive organs of a mouse ERβ-null mutant. *Proc Natl Acad Sci USA* 105, 2433-2438 (2008).

85. K.M. Robertson, L. O'Donnell, M. E. E. Jones, S. J. Meachem, W.C. Boon, C.R. Fisher, K.H. Graves, R.I. McLachlan and E.R. Simpson: Impairment of spermatogenesis in mice lacking a functional aromatase (cyp 19) gene. *Proc Natl Acad Sci USA* 96,7986-7991 (1999)

86. K.A. Fowler, K.Gill, N. Kirma, D.L. Dillehay and R.R.Tekmal: Overexpression aromatase leads to development of testicular Leydig cell tumors. *Am J Pathol* 156, 347-353 (2000)

87. X. Li, E. Nokkala, W. Yan, T. Streng, N. Saarinen, A. Wärri, I. Huhtaniemi I., R. Santti, S. Mäkelä and M. Poutanen,. Altered structure and function of reproductive organs in transgenic male mice overexpressing human aromatase. *Endocrinology.* 142, 2435-42 (2001)

88. X. Li and N. Rahman: Impact of androgen/estrogen ratio: lessons learned from the aromatase over-expression mice. *Gen. Comp. Endocrinol.* 159, 1-9 (2008).

89. J. Weiss, M.L. Bernhardt, M.M. Laronda, L.A. Hurley, C. Glidewell-Kenney, S. Pillai, M. Tong, K.S. Korach and J.L. Jameson: Estrogen actions in the male reproductive system involve estrogen response element-independent pathways. *Endocrinology* 149, 6198-6206 (2008)

90. K.W. Sinkevicius, M. Laine, T.L. Lotan, K.Woloszyn, J.H. Richburg and G.L. Greene:Estrogen dependent and -independent estrogen receptor falpha signaling separately regulate male fertility. *Endocrinology* 150, 2898-28905 (2009)

91. H. Sakamoto, K. Matsuda, K. Hosokawa, M. Nishi, J.F. Morris, E.R. Prossnitz and M. Kawata: Expression of G protein-coupled receptor-30, a G protein-coupled membrane estrogen receptor, in oxytocin neurons of the rat paraventricular and supraoptic nuclei. *Endocrinology* 148, 5842-5850 (2007)

92. J. Isensee, L. Meoli, V. Zazzu, C. Nabzdyk, H. Witt, D. Soewarto, K. Effertz, H. Fuchs, V. Gailus-Durner, D. Busch, T. Adler, M.H. de Angelis, M. Irgang, C. Otto and P.R. Noppinger : Expression pattern of G protein-coupled receptor 30 in LacZ reporter mice. Endocrinology 150, 1722-1730 (2009)

93. C.M. Revankar, D.F. Cimino, L.A. Sklar, J.B. Arterburn, E.R. and Prossnitz: A transmembrane intracellular estrogen receptor mediates rapid cell signaling. *Science* 307, 1625-1630 (2005) Comment in: Science 307(5715):1572-1573 (2005), Science. 310, 51-53; author reply 51-53 (2005)

94. T. Funakoshi, A. Yanai, K. Shinoda, M.M. Kawano and Y. Mizukami: G protein-coupled receptor 30 is an estrogen receptor in the plasma membrane. *Biochem. Biophys. Res. Commun.* 346, 904-910 (2006)

95. M.H Perrard and P. Durand : Redundancy of the effcet of TGFbeta1 and beta-NGF on the second meiotic division of rat spermatocytes. *Microsc Res Tech* 72, 596-602 (2009)

96. C. Otto, I. Fuchs, G. Kauselmann, H. Kern, B. Zevnik, P. Andreasen, G. Schwarz, H. Altmann, M. Klewer, M. Schoor, R. Vonk and K.H. Fritzemeier: GPR30 does not

mediate estrogenic responses in reproductive organs in mice. *Biol Reprod.* 80, 34-41 (2009)

97. E.R. Levin : G protein-coupled receptor 30 : estrogen recptor or collaborator? *Endocrinology* 150, 1563-1565 (2009)

98. Y. Wang, R.Thuillier and M. Culty: prenatal estrogen exposure differentially affects estrogen receptor-associated proteins in rat testis gonocytes. *Biol Reprod* 71, 1652-1664 (2004)

99. K.L. Porter, G. Shetty, G.A. Shuttlesworth, C.C.Y Weng, I.Huhtaniemi, P. Pakarinen and M.L Meistrich: Estrogen enhances recovery from radiation-induced spermatogonial arrest in rat testes. *J Androl* 30, 440-451 (2009)

100. R. Walczak-Jedrzejowska, J. Slowikowska-Hilczer, K. Marchlewsk, E. Oszukowska and K. Kula: During seminiferous tubule maturation testosterone and synergistic action of FSH with estradiol support germ cell survival while estradiol alone has pro-apoptotic effect. *Folia Histochem Cytobiol* 45, S 59-64 (2007)

101. M.Gancarczyk, A. Paziewska , S. Carreau and B. Bilinska : Dose and photoperiod- dependent effect of 17β-estradiol or ICI 182,780 administration on testicular structure, acceleration of spermatogenesis and aromatase immunoexpression in immature bank voles. *Acta Histochem* 106, 269-278 (2004)

102. F.J. Ebling, A.N.Brooks, AA.S.Cronin, H. Ford and J.B. Kerr: Estrogenic induction of spermatogenesis in the hypogonadal mouse. *Endocrinology* 141, 2861-2869 (2000)

103. T.R. Pak , G.R. Lynch and P.S. Tsai : Estrogen accelerates gonadal recrudescence in photo-regressed male siberian hamsters. *Endocrinology* 143, 4131-4134 (2002)

104. S. Assinder, R. Davis, M. Fenwick and A. Glover: Adult-only exposure of male rats to a diet of high phytoestrogen content increases apoptosis of meiotic and post-meiotic germ cells. *Reproduction* 133, 11-19 (2007)

105. G. Delbes, C. Levacher, C. Pairault, C. Racine, C. Duquenne, A. Krust and R. Habert: Estrogen receptor beta-mediated inhibition of male germ cell line development in mice by endogenous estrogens during perinatal life. *Endocrinology* 145, 3395-3403 (2004)

106. M. Culty: Gonocytes, the forgotten cells of the germ cell lineage. *Birth Defects Res* 87, 1-26 (2009)

107. R. D'Souza, M.K. Gill-Sharma, S. Pathak, N. Kedia, R. Kumar and N. Balasinor : Effect of high intratesticular estrogen on the seminiferous epithelium in adult male rats. *Mol Cell Endocrinol* 241, 41-48 (2005)

108. G. Shetty, H. Krishnamurthy, H.N. Krishnamurthy, A.S. Bhatnagar and N.R. Moudgal : Effect of long-term treatment with aromatase inhibitor on testicular function of adult male bonnet monkeys (M. radiata). *Steroids* 63 : 414-420 (1998)

109. E.D. Albrecht, M.V. Lane, G.R. Marshall, I. Merchenthaler, D.R. Simorangkir, C. R Pohl, T.M. Plant and G.P. Pepe: Estrogen promotes germ cell and seminiferous tubule development in the baboon fetal testis. *Biol Reprod* 81, 406-414 (2009)

110. K. Hamden, D. Silandre,C. Delalande, A. El Feki and S. Carreau: Protective effects of estrogens and caloric restriction during aging on various rat testis parameters. *Asian J Androl* 10, 837–845 (2008)

111. K.J. Beck, S. Herscel, R. Hungershofer and E. Schwinger:The effect of steroid hormones on motility and selective migration of X-and Y-bearing human spermatozoa. *Fertil Steril* 27,407-412 (1976)

112. M. Idaomar, J.F. Guerin, J. Lornage and J.C. Czyba : Stimulation of motility and energy metabolism of spermatozoa from asthenospermic patients by 17β-estradiol. *Arch Androl* 22,197-202 (1989)

113. N Berensztein, E.B.,Baquedano,M. S.,Gonzalez,C.R., Saraco,. I., Rodriguez, J., R. Ponzio, , M. A. Rivarola, and A. Belgorosky: Expression of aromatase, estrogen receptor a and b, androgen receptor, and cytochrome P-450 scc in the human early prepubertal testis. Pediatr.Res. 60, 740–744 (2006).

114. L Maffei, V.Rochira, L.Zirilli, P. Antunez, C. Aranda, B. Fabre, M.L. Simone, E. Pignatti, E.R. Simpson, C.D. Clyne and C.Carani : A novel compound heterozygous mutation of the aromatase gene in an adult man: reinforced evidence on the relationship between congenital oestrogen deficiency, adiposity and the metabolicsyndrome. *Clin Endocrinol* 67, 218–224 (2007)

115. V. Rochira and C. Carani : Aromatase deficiency in men: a clinical perspective. *Nat Rev Endocrinol* 5, 559–568 (2009)

116. I. Czajka-Oraniec and E.R. Simpson: Aromatase research and clinical significance. Polish *J Endocrinol* 61, in press (2010)

117. A. Bouskine, M. Nebout, B.Mograbi, F. Brucker-Davis, C. Roger and P. Fenichel: Estrogens promote human testicular germ cell cancer through a membranemediated activation of extracellular regulated kinase and protein kinase A. *Endocrinology* 149, 565–573 (2008)

118. J. Toppari, J.C. Larsen, P. Christiansen, A. Giwercman, P. Grandjean, L.J. Guillette Jr, B. Jegou, T.K. Jensen, P. Jouannet, N. Keiding, H. Leffers, J.A. McLachlan, O. Meyer, J. Muller, E. Rajpert-De Meyts , T. Scheike, R.M. Sharpe , J. Sumpter and N.E.Skakkebaek: Male reproductive health and environmental xenoestrogens. *Environ Health Perspect* 104, 741-803 (1996)

119. N.E. Skakkebaek: Testicular dysgenesis syndrome: new epidemiological evidence. I*nt J. Androl* 27, 189–201 (2004)

120. M. Maggiolini and D.Picard: The unfolding stories of GPR30, a new membrane-bound estrogen receptor. *J Endocrinol* 204, 105-114 (2010)

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