Estrogen receptors and function in the male reproductive system

Receptores e função do estrógeno no sistema reprodutor masculino

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ABSTRACT

A substantial advance in our understanding on the estrogen signaling occurred in the last decade. Estrogens interact with two receptors, ESR1 and ESR2, also known as ERα and ERβ, respectively. ESR1 and ESR2 belong to the nuclear receptor family of transcription factors. In addition to the well established transcriptional effects, estrogens can mediate rapid signaling, triggered within seconds or minutes. These rapid effects can be mediated by ESRs or the G protein-coupled estrogen receptor GPER, also known as GPR30. The effects of estrogen on cell proliferation, differentiation and apoptosis are often mediated by growth factors. The understanding of the cross-talk between androgen, estrogen and growth factors signaling pathways is therefore essential to understand the physiopathological mechanisms of estrogen action. In this review we focused on recent discoveries about the nature of the estrogen receptors, and on the signaling and function of estrogen in the male reproductive system. Arq Bras Endocrinol Metab. 2009;53(8):923-33

Keywords
Estrogens; receptors, estrogen; reproduction; male

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RESUMO

Durante a última década, ocorreu um avanço substancial no conhecimento da sinalização do estrógeno. Estrógenos interagem com dois receptores, ESR1 e ESR2, também conhecidos como ERα e ERβ, respectivamente. ESR1 e ESR2 pertencem à família de receptores nucleares, que funcionam como fatores de transcrição. Além dos bem estabelecidos efeitos transcricionais, os estrógenos medeiam a sinalização rápida, desencadeada dentro de segundos ou minutos. Esses efeitos rápidos podem ser mediados por ESRs ou pelo receptor de estrógeno acoplado à proteína G, GPER, também conhecido como GPR30. Os efeitos de estrógenos sobre a proliferação celular, diferenciação e apoptose são, muitas vezes, mediados por fatores de crescimento. Portanto, a compreensão da interação entre as vias de sinalização de andrógeno, estrógeno e fatores de crescimento é essencial para entender os mecanismos fisiopatológicos envolvidos na ação estrogênica. Nesta revisão, foram abordadas descobertas recentes sobre a estrutura dos receptores, a sinalização e a função do estrógeno no sistema reprodutor masculino. Arq Bras Endocrinol Metab. 2009;53(8):923-33

Descritores
Estrogênios; receptores estrogênicos; reprodução; masculino

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Received on Nov/9/2009
Accepted on Nov/11/2009

INTRODUCTION

Estrogens play key roles in the development and maintenance of reproductive function and fertility (1-4). Estrogens also have an important role in pathological processes observed in tissues of the reproductive system (5,6). In addition, they exert a vast range of biological effects in the cardiovascular, musculoskeletal, immune, and central nervous systems (1). The most potent estrogen produced in the body is 17β-estradiol (E2). Although estrone and estriol, two E2 metabolites, bind to estrogen receptors (ESRs) with high-affinity, they are much weaker agonists compared to E2.

In this review, we have described briefly (*i*) the structure and signaling pathways of ESRs and (*ii*) the lo-

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calization and function of ESRs in organs of the male reproductive system.

Estrogens are synthesized from androgens by the aromatase complex, which contains the cytochrome P450 enzyme encoded by the *CYP19* gene (7). Aromatase expression is detected in Sertoli-Leydig cells, spermatogonia, spermatocytes, elongate spermatids and spermatozoa in adult mice and rats (7), and in Sertoli-Leydig cells, spermatocytes, spermatids and spermatozoa in man (4). Moreover, aromatase has been immunolocalized in epithelial cells of human efferent ducts and in the proximal caput of the epididymis and in rat epididymis (4,7,8), suggesting additional sources of estrogen in the male reproductive system and the possibility of paracrine/autocrine effects.

The crucial role of estrogen for the reproductive function has been demonstrated by studies with mice with targeted disruption of ESRs (*Esr1*$^{-/-}$, *Esr2*$^{-/-}$, *Esr1*$^{-/-}$/*Esr2*$^{-/-}$), aromatase enzyme (*Cyp19*$^{-/-}$), and animals treated with the antiestrogen (ICI 182,780) (9-12). The studies with knockout animals have shown that the spermatogenesis, steroidogenesis and fertility of *Esr1*$^{-/-}$, *Esr1*$^{-/-}$/*Esr2*$^{-/-}$ and *Cyp19*$^{-/-}$ animals are affected (9,10), but none of these parameters is affected in *Esr2*$^{-/-}$ animals (10). However, all these *Esr2* mutants displayed alternative splicing transcripts. A recent study showed that a new *Esr2*$^{-/-}$ mouse mutant, in which exon 3 of *Esr2* was deleted by Cre/LoxP-mediated excision, was completely devoid of any downstream transcripts, and the males were also sterile (12). The origin for the sterility of these male mice is unknown, because their gonads and internal genital organs are histological normal and the mobility of their spermatozoa appears also normal (12). It is possible that the efferent ductules, epididymis and/or prostate, which all express ESR2 in the WT mice, may not be fully functional in mouse *Esr2*-null mutant.

ESTROGEN RECEPTORS: STRUCTURE AND SIGNALING PATHWAYS

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Cellular signaling of estrogens is mediated through two ESRs, ESR1 and ESR2 (also known as ERα and ERβ, respectively), both belonging to the nuclear receptor family of transcription factors (13,14). ESRs contain conserved structure and five distinct functional domains (1,13,14). The N-terminal A/B domain is the most variable region, and the human ESR1 and ESR2 share less than 20% amino acid identity, indicating that this domain may contribute to ER subtype-specific actions on target genes. This region contains an activation function (AF-1) that is ligand-independent and shows promoter- and cell-specific activity. The C-domain is the DNA-binding domain (DBD), and features two zinc-finger motifs, which are not only responsible for DNA-binding, but also for dimerization of the receptors, allowing the formation of homo- and heterodimers. This domain is highly conserved between ESR1 and ESR2 (95% amino acid identity), and the DBD from both receptors bind to the same estrogen response element (ERE). The D-domain is a flexible hinge between the DBD and the ligand-binding domain (LDB). This domain, which is not well conserved between ESR1 and ESR2 (only 30% amino acid identity), contains a nuclear localization signal, important for nuclear translocation. ESR1 and ESR2 share approximately 55% amino acid identity in LBD. The LBD is important for ligand binding and receptor dimerization and contains a hormone-dependent activation function (AF-2). Both AFs recruit a range of coregulatory protein complexes to the DNA-bound receptor. The LBDs of ESR1 and ESR2 have very similar three-dimensional structures. However, the amino acids lining the ligand-binding cavities of ESR1 and ESR2 differ in two positions. Furthermore, ligand-binding cavity of ESR2 is approximately 20% as large as the one from ESR1, and this may have implications for the selective affinity and pharmacology of ligands. The F-domain is less conversed between the two ESRs (less than 20% amino acid identity), and the functions of this domain remain undefined.

ESR1 and ESR2 are encoded by two different genes located on different chromosomes. Several splice variants have been described for these receptors, but whether all the variants are expressed as functional proteins is not clear (13,14). Multiple RNA splicing variants for *ESR1* have been reported in humans (13,14). Full-length human *ESR1* is 595 long amino acids, and both short *ESR1* isoforms, hESR1-46 and hESR1-36, lack the N-terminal region containing AF-1. The hESR1-36 lacks both transcriptional activation domains and contains an exon coding for myristoylation sites, thus predicting an interaction with the plasma membrane. The hESR1-46 antagonizes the proliferative actions of the hESR1-66 in MCF-7 breast cancer cells. In the rat pituitary gland, an ESR1 variant named TERP-1 (truncated ER product-1) is expressed in the lactotrope cells.

The full-length human ESR2 is 530 long amino acids and the rat and mouse ESR2 is 549 amino acids

(*Esr2_v1* isoform). Four alternative *ESR2* isoforms have been described in humans, and many of these are expressed as proteins. The mouse and rat *Esr2_v2* contains an 18-amino acid insertion in the LBD that causes a significant decrease in binding affinity. Deletion of exon 3 results in rat isoforms unable to bind to DNA (13), and the deletion of exon 5 and/or 6 in mice results in isoforms lacking various parts of the LBD. *ESR2* isoforms can differentially modulate estrogen signaling and, as a consequence, impact target gene regulation (13).

The classical mechanism of ESR action involves E2 binding to receptors located in the nucleus. After binding estrogen, ESR dissociates from its chaperone proteins, phosphorylates, and dimerizes. Hormone binding also induces a conformational change within the ligand binding domain of the receptors, and this conformational change allows coactivator proteins, for example, amplified in breast cancer-1 (AIB1), nuclear-receptor-coactivator-1 (NCoA-1/SRC1) and the p300 and CBP-associated factor (PCAF), to be recruited. These activated ESR-dimer complexes bind to specific EREs located in the promoters of target genes (1,14,15).

In humans, around one third of the genes that are regulated by ESRs do not contain ERE-like sequences (15,16). This mechanism of ERE-independent ESR activation is postulated to involve a tethering of the ligand-activated ESR to other transcription factors that are directly bound to DNA via their respective response elements. Several genes are activated by E2 through the interaction of ESRs with Fos and Jun proteins at activating protein-1 (AP-1) binding sites to induce or regulate transcriptional activity. Genes that contain GC-rich promoter sequences are regulated in a similar manner through the interaction of ESRs with the Sp-1 transcriptional factor. Other transcriptional factors are also involved, such as nuclear factor kB (NF-kB) and signal transducer and activator of transcription (STAT) five binding sites. The actions of E2-ESRs-transcription factors depend on the ligand, the cell type, and the receptor subtype (15,16).

In addition to the well-established transcriptional effects of E2 mediated by the classical nuclear ESRs (13-15), estrogen also mediates rapid effects, occurring within seconds or minutes. These rapid effects include activation of different downstream signaling pathways, for example, the mitogen-activated protein kinases (MAPKs) and phosphatidylinositol 3-kinase (PI3K) pathways, which, in turn, can modulate nuclear transcriptional events and cell proliferation (15,17). Nevertheless, recent studies have revealed the contribution of a novel ESR, namely GPER (G protein-coupled ESR, or GPR30), which belongs to the family of seven-transmembrane G protein-coupled receptors (GPCRs). The activation of this receptor also induces activation of different downstream signaling pathways. Other compounds, besides estrogen, may also activate GPER: ESR antagonists, such as fulvestrant (ICI 182,780), and G-1, which is a GPER agonist (18,19). GPER has been identified in a variety of human and rodent estrogen target tissues (18,19).

We summarized the data about ESRs localization and function in some components of the male reproductive system.

Testis

In testis from immature and adult rats, splicing variants for *Esr1* were not detected, and only a single protein band for ESR1 was detected (20). On the other hand, two splicing variants were detected for *Esr2* in testis: the *Esr2_v1* isoform (20), with high similarity with the human *ESR2* (21), and the *Esr2_v2*, which contains an in-frame insert of 54 nucleotides, only described in tissues from rodents (13). *Esr2_v2* encodes a protein that differs from the isoform 1 of ESR2 only by an insertion of 18 aminoacids in the ligand binding domain. In fact, in Western blot analysis only one protein band was distinguished with an antibody that recognizes the aminoterminal region of the receptor that is common to both ESR2 isoforms (20). The physiological implication of the existence of several ESR2 isoforms has been investigated. The human ESR2 isoform 1 apparently is the only fully functional. However, the other isoforms can heterodimerize with ESR2 isoform 1 under the stimulation of estrogens and enhance the transactivation (13). Furthermore, the human ESR2 isoform 2 has been proposed to act as a dominant negative receptor, preventing estrogen action in gonocytes (22). The differential distribution of ESR2 variants in human testicular cells may indicate that these variants have specific functions in spermatogenesis (23). On the other hand, the rodent ESR2 isoform 2 seems to be functional, although much higher concentrations of estrogen are required for its activity (13). A recent study reported the presence of the ESR2 isoforms 1 and 2 in human seminoma, in embryonal carcinoma and in their adjacent intratubular germ cells (24). This study suggests that both isoforms can mediate estrogen action in early and late neoplastic testicular germ cells.

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The expression of ESR1 and ESR2 in the fetal testis occurs very early in the development, and their distribution in the different testicular cells has been extensively studied in mammals (25). Immunohistochemical data have shown that ESR1 is present in the undifferentiated gonad and in the fetal Leydig cells until birth in rodents (2). The presence of ESR1 is also detected in Sertoli cells from immature and adult rats (20). Although germ cells from immature rats are ESR1-negative, some spermatids were positive for this receptor in the adult animal (20). On the other hand, the expression of ESR1 in human testis is controversial (3). While some authors did not find significant expression of ESR1 in human testis (26), others have found ESR1 in Leydig cells (27) and in human Leydig cell tumor (28). Indeed, the presence of ESR1 in rat and human spermatozoa has been reported (4).

Our laboratory detected ESR2 specific immunostaining in multiple cell types in rat testis, including Sertoli-Leydig cells, and in some, but not all, germ cells (20), confirming previous studies. In humans, ESR2, but not ESR1 and androgen receptor (AR), is expressed in gonocytes and spermatogonia (29). It is worth to mention that ESR2 is coexpressed with ESR1 in tumoral seminoma cells, where it may counteract the tumor cell proliferation mediated by ESR1 (30).

The role of estrogens in male reproduction is complex. Estrogens have been suggested to control Leydig cell function at various stages of development. Prenatal estrogen treatment affected the differentiation of fetal Leydig cells (31), and the regeneration of Leydig cells is inhibited by estrogen in a rat model mimicking postnatal Leydig cell development (32). Several studies also indicated a role for E2 as a paracrine/autocrine factor in the regulation of steroidogenesis in the Leydig cells from rodents (33). A recent study has demonstrated that the chronic imbalance of the androgen-estrogen ratio in the aromatase transgenic animal leads to severe abnormalities in the development, structure, and function of mouse Leydig cells, and these effects seem to be mediated by ESR1 (34).

Our laboratory detected, by immunofluorescence, the presence of ESR1 and ESR2 in the nuclei of Sertoli cells obtained from 15-day old rats (20). In cultured Sertoli cells, a physiological concentration of E2 activates a Src-mediated translocation of ESRs to the plasma membrane, which results in the activation of EGFR (epidermal growth factor receptor) and Erk1/2 (extracellular-regulated kinase 1/2). Moreover, activation of ESR1 and/or ESR2 by E2 is also involved in proliferation of immature Sertoli cells (20). In fact, estrogen affects Sertoli cell proliferation (2,20) and may suppress differentiation (2), and 5α-androstane-3β, 17β-diol (3βAdiol) is a potent modulator of ESR2-mediated gene transcription in Sertoli cell lines (35). A recent study using estrogen non-responsive ESR1 knock-in (ENERK1) mice, which have a point mutation in the LBD of ESR1 that significantly reduces interaction with and response to endogenous estrogens, but does not affect activation of ESR1 by growth factors, showed that estrogen-dependent ESR1 signaling is required for germ cell viability, most likely through support of Sertoli cell function (36). No changes are observed in the number of Sertoli cells and spermatogonia in the *Esr1*$^{-/-}$ mice (9). However, spermatogenesis arrest occurs in *Esr1*$^{-/-}$ mice (9), suggesting that Sertoli cell support of germ cell development through unidentified ESR1-mediated mechanisms, may be through activation of growth factors. Studies with the mouse spermatogonial GC-1 cell line showed that E2 rapidly activates the EGFR/Erk/fos pathway through a cross-talk between GPER and ESR1, leading to cell proliferation (37). In cultured Sertoli cells obtained from 15-day old rats, GPER participates in E2 rapid actions and may regulate apoptosis (our unpublished data). ESRs and/or GPER may mediate 17β-estradiol actions important for Sertoli cell function and maintenance, consequently affecting spermatogenesis and male (in)fertility.

Estrogens also affect directly male germ cells and regulate storage and phosphorylation of Fos proteins in the cytoplasm and nucleus of germ cells, respectively, indicating that estrogen may be involved in the mechanisms inducing spermatogonial proliferation (38). Recent studies have shown that E2 and 3βAdiol, an ESR2 selective ligand, stimulate spermatogonial deoxyribonucleic acid synthesis in rat seminiferous epithelium *in vitro* (39), and low doses of bisphenol A stimulate human seminoma (JKT-1) cell proliferation by allowing a rapid membrane-initiated activation of cAMP-dependent protein kinase (PKA) and cGMP-dependent protein kinase (PKG) signaling pathways associated with phosphorylation of the transcription factor cAMP response-element binding protein (CREB) and the cell cycle regulator retinoblastoma protein (Rb) (40).

In conclusion, ESRs and GPER may mediate 17β-estradiol actions important for the function and maintenance of testicular cells, consequently affecting spermatogenesis and male (in)fertility.

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Efferent ductules

The efferent ductules present the highest levels of ESRs ESR1 and ESR2 in the male reproductive system, and represent a major target of estrogen action. In mice and rats, ESR1 and ESR2 are expressed abundantly in ciliated and non-ciliated cells of the efferent ductule epithelium (41). In rats, ESR2 is also found in peritubular and some stromal cells (41).

The efferent ductules connect rete testis to epididymis. Great part of the head of the epididymis in man and other mammals, but not in rodents, contains these ducts, and it was first thought that the efferent ductules would only transport sperm from testis to the epididymis. However, the efferent ductules are now recognized as the main place of absorption of the fluid from the rete testis, an event that is crucial to concentrate sperm prior to entering the epididymal lumen (3).

The primary mechanism involved in the movement of fluid in the ductules is the water transport and the active transport of ions (42-44). Studies in different species have demonstrated that 50 to 96% of the fluid secreted by the seminiferous tubules is reabsorbed by the epithelial cells of the efferent ductules (3). Some proteins involved in the reabsorption of the fluid have also been identified, such as the Na^+-K^+-ATPase, the aquaporins, AQP1 and AQP9, and the carbonic anidrase II (42-44).

The efferent ductules also absorb approximately 50 to 90% of the total proteins that leave the testis (45,46). This process occurs by absorption or receptor-mediated endocytosis, the main mechanism involved in the capture of large molecules from the testicular fluid (46). The androgen binding protein (ABP) and the sulphated glycoprotein 2 (SGP-2) are examples of proteins absorbed by the efferent ducts epithelium (47,48). Some of the proteins secreted by the epithelial cells of the efferent ductules have already been identified, such as the saposin 1 (SGP-1) and the pro-enkephalins (49).

Estrogen plays a crucial role regulating the ability of the efferent ductules to reabsorb the testicular fluid and to influence the seminal fluid composition. The most severe histopathological changes after disruption of ESR1 occur in this organ. The lack of ESR1 severely impairs the absorptive function of the efferent ductules, causing fluid accumulation and luminal dilation. The rete testes of the *Esr1*$^{-/-}$ were also dilated and protruded into the testis. Treatment of rats with fulvestrant (ICI 182,780), an antiestrogen that impairs estrogen action on ESR1 and ESR2, cause morphological and functional changes in the efferent ductules similar to those seen in the *Esr1*$^{-/-}$ mice (11), including luminal dilation and reduction of the epithelial height (Figure 1A and B). On the other hand, treatment of rats with the aromatase inhibitor anastrozole does not seem to alter significantly the morphology of the efferent ductules (Figure 1C and D), and aromatase knockout animals do not present a significant morphological alteration of the efferent ductules (50). Combined with the results obtained with the ESR1 knockout animals, these results indicate that the presence of a functional ESR1 is mandatory to maintain the morphology and function of the efferent ductules.

Studies with *Esr1*$^{-/-}$ and ICI 182,780-treated mice demonstrated that estrogen regulates the mRNA levels of several proteins involved in ion transport (51) and water transport (44). Using a microarray analysis of genes differentially expressed in the efferent ductules of fulvestrant-treated animals, we have been able to identify a significant impact of the antiestrogen treatment on gene expression, and besides genes related to ion and water transport, genes related to extracellular matrix organization and sperm maturation and/or capacitation, among others, were also affected, suggesting a broader role of estrogen to regulate the morphology and function of the efferent ductules (52).

Epididymis

The epididymis is a key organ for the maturation of sperm. Its function is controlled by several hormones and growth factors and testosterone is recognized as a primary stimulus for epididymal development and sperm maturation. The mammalian epididymis is a segmented organ comprised of a single, highly coiled tubule, conventionally divided into caput, corpus and cauda regions (53).

Although the main source of estrogen in the male is the testis, it has been suggested more recently that the epididymis is also capable of estrogen biosynthesis. The P450 aromatase and the 17β-hydroxysteroid dehydrogenase I mRNA are present in the caput and cauda of rat epididymis (7). The estrogen sulfotransferase, which produces sulfated estrogens that do not bind to ESRs, is highly expressed along the bovine epididymis (54). ESR1 is expressed in the epididymis of several species (55), where it seems to participate in the regulation of narrow cells of the initial segment of the epididymis and clear cells in the remaining segments. In the bonnet monkey, ESR1 and ESR2 are expressed in

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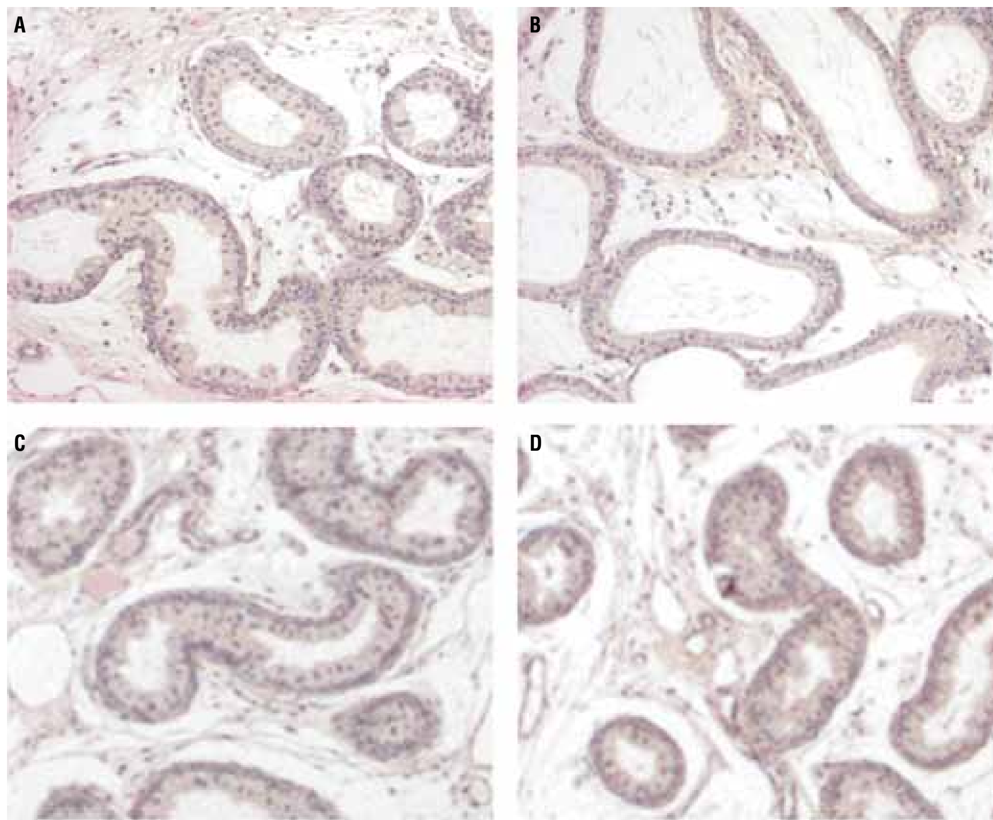

Figure 1. Morphological changes in the efferent ductules of animals treated with the antiestrogen fulvestrant (10 mg/animal, once a week, for two months, subcutaneously). Note the dilation of the lumen of the ductules and the reduced highness of the epithelial layer in animals treated with the antiestrogen (B), compared to control animals (A). No significant morphological changes were observed in the efferent ductules removed from animals treated with the aromatase inhibitor anastrozole (0.1 mg/kg, daily, during one month, orally) compared to control (C and D, respectively). Representative photomicrographies of cross sections of efferent ductules stained with hematoxylin-eosin. Scale bar 100 µm.

all three regions of the epididymis (56), in contrast to other primates, in which expression levels are very low (26). In the boar, ESR2 is localized in the principal and basal cells of all three epididymal regions, and ESR1 was localized in the principal cells of the caput, some cells of the corpus and was not present in the cauda (57). In the rat, Atanassova and cols. (58) showed that the expression of ESR1 varied with age. On day 10 and on days 25-35, the epithelial expression of ESR1 was absent, but it was detectable on day 18 in epithelial cells in the caput, corpus and proximal cauda. In contrast, ESR2 expression was immunolocalized to epithelial and some stromal cells at all ages (58).

The role of estrogen in epididymal function is still not completely understood (55). In contrast with the dramatic effect on the efferent ductules, the *Esr1*$^{-/-}$ animal does not present significant alterations on epididymis morphology (9). Nevertheless, treatment of animals with estrogens or reduction of the endogenous estrogen seem to affect the development or function of the epididymis. It has been shown that reduction of the endogenous estrogen by treatment with an aromatase inhibitor delayed epididymal development in boars (57). The gestational exposure of rats to diethylstilbestrol decreased expression of the AR mRNA and stimulated the ESR1 mRNA in the offspring (59). Exposure of adult male rats to dietary phytoestrogens reduced fecundity, and increased ESR1 and AR mRNA in the initial segment, but decreased them in the cauda of the epididymis (60). A microarray analysis of the effects of the antiestrogen fulvestrant on gene expression in the epididymis of the bonnet monkey has demonstrated that the expression of several genes involved in fluid absorption is affected by the treatment, including a reduction in aquaporin 1 and NHE3 expression (61). Treatment of mice with the antiestrogen fulvestrant (ICI 182,780) induced capping and vesiculation of narrow cells in the initial segment, accumulation of PAS-positive granules in apical cells of the caput of the epididymis, an increase in lysosomal granules in clear cells of the corpus and cauda, and a decrease in the concentration of cauda sperm, progressive sperm motility and a decre-

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ase in fertility (55). In the male rat, treatment with the nonsteroidal type I selective estrogen receptor modulator (SERM) tamoxifen induced alterations in testis and epididymis that seem to be responsible for a decrease in sperm concentration and motility (62). Estrogen may also be involved in the ejaculatory process, regulating the contractile activity of the rabbit epididymis during semen emission (63).

Vas deferens

Although the literature refers that, in most species, ESR2 but not ESR1 is present in the epithelium of the vas deferens (3), using real time PCR we have been able to detect the mRNA for both receptors, as well as for the GPER in the vas deferens of adult rats (64). Using immunohistochemistry study in vas deferens from rats at different ages, Atanassova and cols. (58) showed that the expression of ESR1 changed with age and it was confined to a band of periductal stromal cells in animals from 18-35 days, but it was absent in adult animals. The expression of ESR1 in stromal cells in the vas deferens contrasts with the predominantly epithelial immunoexpression in the epididymis of 18-day old rats. On the other hand, expression of ESR2 was detected at all ages (58). Chronic prostatitis and epithelial abnormalities in the vas deferens were observed in newborn Sprague-Dawley male rats, treated from days 1-5 with two SERMs, tamoxifen and toremifene or two estrogens, ethinylestradiol and diethylstilbestrol. These changes involved hyperplasia and development of subepithelial glandular structures of vas deferens (65). In a previous study, Atanassova and cols. (66) had already reported stromal and epithelial abnormalities in the vas deferens and cauda epididymis, in adult rats treated neonatally with diethylstilbestrol, which were related to a delay in the development of basal cells. These abnormalities included: coiling of the normally straight initial vas deferens, gross epithelial alterations, widening of the periductal non-muscle layer, infiltration of the immune cells across the epithelium to the lumen, and reduction or absence of sperm from the vas deferens lumen. All together, these observations suggest that estrogen may be important to determine the development of a normal epithelium and stroma of the vas deferens. Whether estrogen is also involved in other functions of the organ, such as regulation of absorptive function or secretion of proteins, still remains to be determined.

Prostate

Studies using different experimental approaches have revealed that estrogens play an essential role in the normal development and function of the prostate, and in the etiology of prostatic diseases (5,6). Direct effects of estrogen on the prostate are mediated through ESR1 and ESR2 (5,6).

ESR1 is primarily localized to the stromal cells of the adult prostate in men, dogs, monkeys and rodents (5,6). Studies in rodent prostate have shown a high percentage of stromal cells expressing ESR1 (mRNA and protein) throughout prenatal morphogenesis, and that this proportion significantly declines thereafter, which suggests a specific role for ESR1 in the prostate development (5,6). On the other hand, after estrogen exposure, it is possible to detect this receptor in the epithelium (67). The close proximity of ESR1-positive stromal cells to epithelial cells allows possible paracrine effects of estrogens on the prostate epithelium. In fact, studies using mice with disruption of ESRs ($Esr1^{-/-}$ and $Esr2^{-/-}$ mice) have shown that ESR1 mediates estradiol-induced squamous metaplasia in the prostate (67). Moreover, recent studies have demonstrated that ESR1 acts through a paracrine mechanism to regulate prostatic branching morphogenesis, and is involved in proliferation and differentiation of prostatic stromal compartment (68). Recently, prostatic hormonal carcinogenesis has been shown to be mediated by *in situ* estrogen production and ESR1 signaling (69).

In humans, ESR1 has also been observed in stromal cells during fetal development (5,6). However, while one study shows ESR1 protein only in stromal cells, other demonstrates the presence of ESR1 in fetal prostatic utricle and periurethral epithelium during mid-to-late gestation. Squamous metaplasia is directly associated with epithelial ESR1 in the periurethral ducts and stromal ESR1 in the peripheral prostatic acini. There is evidence that estrogens acting through stromal ESR1 may contribute at some level to the etiology of the most prevalent prostatic diseases, including chronic prostatitis, benign prostatic hyperplasia (BPH), and carcinogenesis and cancer progression (6).

In the rat and murine prostate, ESR2 (mRNA and protein) is mainly localized to differentiated luminal epithelial cells (5,6). Expression of ESR2 is low at birth, increases as epithelial cells differentiate, and reaches its maximum with the onset of secretory capacity at puberty, which suggests a role for ESR2 in the differentiated functions of the rat prostate. Furthermore, $Esr2^{-/-}$ mice show

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hyperproliferative prostatic epithelium and altered cellular differentiation, with accumulation of basal-intermediary cells. It has also been suggested that the anti-proliferative effect mediated by ESR2 could function as a brake for the androgenic stimulation of prostate growth. Nevertheless, conflicting results about the role of ESR2 in prostate proliferation have been observed among the different studies using *Esr2*$^{-/-}$ mice. While epithelial hyperplasia with increased BrdU labeling has been shown in prostate from *Esr2*$^{-/-}$ mice, other studies using this same model or a different *Esr2*$^{-/-}$ model presented different results (5).

The development pattern for ESR2 in the human prostate differs from the rodent (5). ESR2 is expressed throughout the urogenital sinus epithelium and stroma in early fetal development and this expression is maintained in most epithelial and stromal cells throughout the gestation, suggesting the involvement of ESR2 and estrogens in morphogenesis and differentiation of the prostate. While this pattern is maintained postnatally for several months, ESR2 expression decreases in adluminal cells at puberty. While some studies have shown the expression of ESR2 in basal epithelial cells, with lower stromal expression, other have shown high expression of ESR2 in both basal and luminal epithelial cells of the adult human prostate.

Prostatic epithelial ESR2 may play a role as pro-differentiation, anti-proliferative, anti-inflammatory and as an inducer of anti-oxidant genes. The loss of ESR2 may contribute to prostate cancer progression in organ confined disease, and the strong reemergence of ESR2 at metastatic sites implicates a potential role in androgen-independent progression (5,6). Thus, although ESR2 is the predominant ESR expressed in the adult prostate, its role has not yet been clearly established.

Estrogens have also been shown to act synergistically with androgens to induce BPH in dogs (70), and prostatic cancer in adult Noble rats (71,72). Although the mechanism of estrogen action in the prostate has received attention for more than two decades, it is still poorly understood. Cross-talk among the receptors of the nuclear steroid receptor family might also occur in the prostate, and the regulation of AR, ESR1 and ESR2 during prostate development, maturation and carcinogenesis remains as an important research area. In primary cultured human prostate stromal cells and prostate stromal cell line WPMY-1, recent studies showed that 17β-estradiol activates ERK pathway and cell proliferation through rapid signaling of ESR1 and that GPER is not involved in these processes (73). A recent study from our laboratory showed that the treatment of rats with the antiestrogen fulvestrant induces a decrease on ERK1/2 figure 2 phosphorylation and prostatic cell proliferation (unpublished data). Figure 2 summarizes the effects of 17β-estradiol via ESR1 and ESR2 in rat prostate.

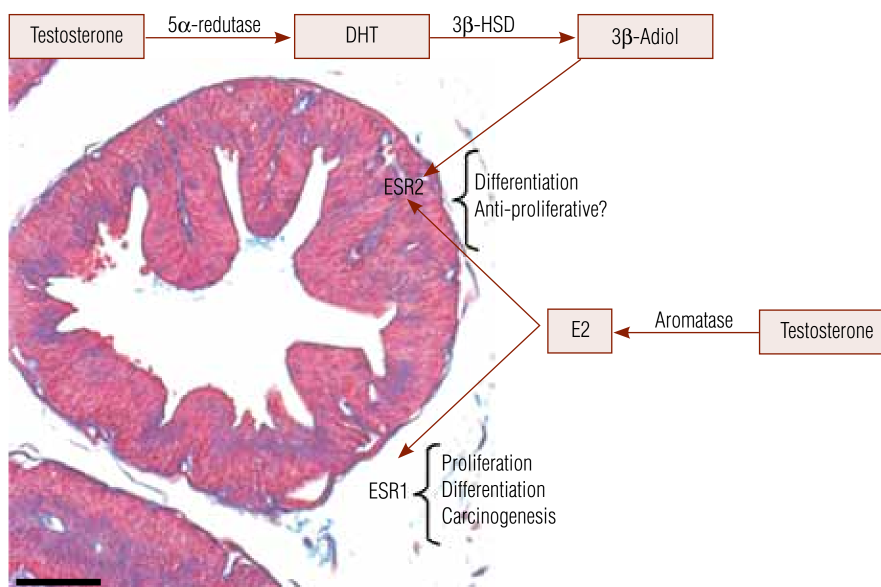

Figure 2. Cross section of rat ventral prostate stained with Masson's trichrome. Schematic representation of the local estrogen signaling mechanisms. Testosterone is locally metabolized to 17β-estradiol (E2) by aromatase, and acts via ESR1 and ESR2. Testosterone is also metabolized to DHT (5α-dihydrotestosterone) by 5α-reductase. DHT is metabolized to 3β-Adiol (5α-androstane-3β, 17β-diol) by 3β-hydroxysteroid oxidoreductase, and acts via ESR2. The effects of 17β-estradiol via ESR1 in the stroma and epithelia include proliferation, differentiation and carcinogenesis. Estrogen action via ESR2 may modulate differentiation, among other possible actions. The antiproliferative effect of ESR2 remains to be better determined. Scale bar 15 μm.

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These findings provide a fundamental basis for future studies to determine the physiological roles of estrogen in the ventral prostate and for new approaches in prostatic diseases, such as benign prostate hyperplasia and prostatic cancer. It is possible that ESR-specific agonists and antagonists may provide the most beneficial therapeutic strategies in future clinical trials.

CONCLUDING REMARKS

Cellular and physiological roles of ESRs and GPER are becoming better defined for tissues of the male reproductive system. In this review, we have discussed the advances in knowledge about the localization, molecular signaling and function of these receptors. The results obtained in our laboratory and those from the literature support the concept that estrogens play an important role in modulating the function of the male reproductive tract throughout the species. Furthermore, estrogens play a role in (in)fertility and carcinogenesis. The use of selective ligands, siRNA approaches and knockout animals will certainly enhance our understanding on the role of each receptor in the male reproductive function.

Acknowledgements: we thank Espedita M.J. Silva and Sabrina A. Souza e Silva for technical assistance. This study was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (Fapesp, grant nos. 2006/60406-7; 2007/52471-6; 2008/56564-1 to MFML and CSP).

Disclosure: no potential conflict of interest relevant to this article was reported.

REFERENCES

1. Nilsson S, Mäkelä S, Treuter E, Tujague M, Thomsen J, Andersson G, et al. Mechanisms of estrogen action. Physiol Rev. 2001;81(4):1535-65.
2. O'Donnell L, Robertson KM, Jones ME, Simpson ER. Estrogen and spermatogenesis. Endocr Rev. 2001;22(3):289-318.
3. Hess RA, Carnes K. The role of estrogen in testis and the male reproductive tract: a review and species comparison. Anim Reprod. 2004;1(1):5-30.
4. Carreau S, de Vienne C, Galeraud-Denis I. Aromatase and estrogens in man reproduction: a review and latest advances. Adv Med Sci. 2008;53(5):139-44.
5. Prins GS, Korach KS. The role of estrogens and estrogen receptors in normal prostate growth and disease. Steroids. 2008;73(3):233-44.
6. Ellem SJ, Risbridger GP. The dual, opposing roles of estrogen in the prostate. Steroid Enzymes and Cancer. 2009;1155:174-86.
7. Carreau S, Bourguiba S, Lambard S, Galeraud-Denis L, Genissel C, Bilinska B, et al. Reproductive system: aromatase and estrogens. Mol Cell Endocrinol. 2002;193(1-5):137-43.
8. Carpino A, Romeo F, Rago V. Aromatase immunolocalization in human ductuli efferentes and proximal ductus epididymis. J Anat. 2004;204(Pt 3):217-20.
9. Eddy EM, Washburn TF, Bunch DO, Goulding EH, Gladen BC, Lubahn DB, et al. Targeted disruption of the estrogen receptor gene in male mice causes alteration of spermatogenesis and infertility. Endocrinology. 1996;137(11):4796-805.
10. Dupont S, Krust A, Gansmuller A, Dierich A, Chambon P, Mark M. Effect of single and compound knockouts of estrogen receptors alpha (ERalpha) and beta (ERbeta) on mouse reproductive phenotypes. Development. 2000;127(19):4277-91.
11. Oliveira CA, Carnes K, França LR, Hess RA. Infertility and testicular atrophy in the antiestrogen-treated adult male rat. Biol Reprod. 2001;65(3):913-20.
12. Antal MC, Krust A, Chambon P, Mark M. Sterility and absence of histopathological defects in nonreproductive organs of a mouse ERbeta-null mutant. Proc Natl Acad Sci U S A. 2008;105(7):2433-8.
13. Heldring N, Pike A, Andersson S, Matthews J, Cheng G, Hartman J, et al. Estrogen receptors: how do they signal and what are their targets. Physiol Rev. 2007;87(3):905-31.
14. Ellmann S, Sticht H, Thiel F, Beckmann MW, Strick R, Strissel PL. Estrogen and progesterone receptors: from molecular structures to clinical targets. Cell Mol Life Sci. 2009;66(15):2405-26.
15. Björnström L, Sjöberg M. Mechanisms of estrogen receptor signaling: convergence of genomic and nongenomic actions on target genes. Mol Endocrinol. 2005;19(4):833-42.
16. O'Lone R, Frith MC, Karlsson EK, Hansen U. Genomic targets of nuclear estrogen receptors. Mol Endocrinol. 2004;18(8):1859-75.
17. Song RX, Santen RJ. Membrane initiated estrogen signaling in breast cancer. Biol Reprod. 2006;75(1):9-16.
18. Filardo EJ. Epidermal growth factor receptor (EGFR) transactivation by estrogen via the G-protein-coupled receptor, GPR30: a novel signaling pathway with potential significance for breast cancer. J Steroid Biochem Mol Biol. 2002;80(2):231-8.
19. Prossnitz ER, Sklar LA, Oprea TI, Arterburn JB. GPR30: a novel therapeutic target in estrogen-related disease. Trends Pharmacol Sci. 2008;29(3):116-23.
20. Lucas TF, Siu ER, Esteves CA, Monteiro HP, Oliveira CA, Porto CS, et al. 17beta-estradiol induces the translocation of the estrogen receptors ESR1 and ESR2 to the cell membrane, MAPK3/1 phosphorylation and proliferation of cultured immature rat Sertoli cells. Biol Reprod. 2008;78(1):101-14.
21. Saunders PT, Millar MR, Macpherson S, Irvine DS, Groome NP, Evans LR, et al. ERβ1 e ERβ2 splice variant (ERβcx/β2) are expressed in distinct cell populations in the adult human testis. J Clin Endocrinol. 2002;87(6):2706-15.
22. Gaskell TL, Robinson LL, Groome NP, Anderson RA, Saunders PT. Differential expression of two estrogen receptor beta isoforms in the human fetal testis during the second trimester of pregnancy. J Clin Endocrinol Metab. 2003;88(1):424-32.
23. Aschim EL, Saether T, Wiger R, Grotmol T, Haugen TB. Differential distribution of splice variants of estrogen receptor beta in human testicular cells suggests specific functions in spermatogenesis. J Steroid Biochem Mol Biol. 2004;92(1-2):97-106.
24. Rago V, Romeo F, Giordano F, Ferraro A, Andò S, Carpino A. Identification of ERbeta1 and ERbeta2 in human seminoma, in embryonal carcinoma and in their adjacent intratubular germ cell neoplasia. Reprod Biol Endocrinol. 2009;7:56.
25. Delbes G, Levacher C, Habert R. Estrogen effects on fetal and neonatal testicular development. Reproduction. 2006;132(4):527-38.
26. Saunders PT, Sharpe RM, Williams K, Macpherson S, Urquart H, Irvine DS, et al. Differential expression of oestrogen receptor alpha and beta proteins in the testes and male reproductive system of human and non-human primates. Mol Hum Reprod. 2001;7(3):227-36.
27. Pelletier G, El-Alfy M. Immunocytochemical localization of estrogen receptors alpha and beta in the human reproductive organs. J Clin Endocrinol Metab. 2000;85(12):4835-40.

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28. Carpino A, Rago V, Pezzi V, Carani C, Andò S. Detection of aromatase and estrogen receptors (ERalpha, ERbeta1, ERbeta2) in human Leydig cell tumor. Eur J Endocrinol. 2007;157(2):239-44.
29. Berensztein EB, Baquedano MS, Gonzalez CR, Saraco NI, Rodriguez J, Ponzio R, et al. Expression of aromatase, estrogen receptor alpha and beta, androgen receptor, and cytochrome P-450scc in the human early prepubertal testis. Pediatr Res. 2006;60(6):740-4.
30. Roger C, Lambard S, Bouskine A, Mograbi B, Chevallier D, Nebout M, et al. Estrogen-induced growth inhibition of human seminoma cells expressing estrogen receptor beta and aromatase. J Mol Endocrinol. 2005;35(1):191-9.
31. McKinnell C, Atanassova N, Williams K, Fisher JS, Walker M, Turner KJ, et al. Suppression of androgen action and the induction of gross abnormalities of the reproductive tract in male rats treated neonatally with diethylstilbestrol. J Androl. 2001;22(2):323-38.
32. Abney TO, Myers RB. 17 beta-estradiol inhibition of Leydig cell regeneration in the ethane dimethylsulfonate-treated mature rat. J Androl. 1991;12(5):295-304.
33. Tong MH, Christenson LK, Song WC. Aberrant cholesterol transport and impaired steroidogenesis in Leydig cells lacking estrogen sulfotransferase. Endocrinology. 2004;145(5):2487-97.
34. Strauss L, Kallio J, Desai N, Pakarinen P, Miettinen T, Gylling H, et al. Increased exposure to estrogens disturbs maturation, steroidogenesis, and cholesterol homeostasis via estrogen receptor alpha in adult mouse Leydig cells. Endocrinology. 2009;150(6):2865-72.
35. Sneddon SF, Walther N, Saunders PT. Expression of androgen and estrogen receptors in Sertoli cells: studies using the mouse SK11 cell line. Endocrinology. 2005;146(12):5304-12.
36. Sinkevicius KW, Woloszyn K, Laine M, Jackson KS, Greene GL, Woodruff TK, et al. Characterization of the ovarian and reproductive abnormalities in prepubertal and adult estrogen non-responsive estrogen receptor alpha knock-in (ENERKI) mice. Steroids. 2009;74(12):913-9.
37. Sirianni R, Chimento A, Ruggiero C, De Luca A, Lappano R, Ando S, et al. The novel estrogen receptor, G protein-coupled receptor 30, mediates the proliferative effects induced by 17beta-estradiol on mouse spermatogonial GC-1 cell line. Endocrinology. 2008;149(10):5043-51.
38. Cobellis L, Reis FM, Driul L, Vultaggio G, Ferretti I, Villa E, et al. Estrogen receptor alpha mRNA variant lacking exon 5 is co-expressed with the wild-type in endometrial adenocarcinoma. Eur J Obstet Gynecol Reprod Biol. 2002;102(1):92-5.
39. Wahlgren A, Svechnikov K, Strand ML, Jahnukainen K, Parvinen M, Gustafsson JA, et al. Estrogen receptor beta selective ligand 5alpha-Androstane-3beta, 17beta-diol stimulates spermatogonial deoxyribonucleic acid synthesis in rat seminiferous epithelium in vitro. Endocrinology. 2008;149(6):2917-22.
40. Bouskine A, Nebout M, Brücker-Davis F, Benahmed M, Fenichel P. Low doses of bisphenol A promote human seminoma cell proliferation by activating PKA and PKG via a membrane G-protein-coupled estrogen receptor. Environ Health Perspect. 2009;117(7):1053-8.
41. Oliveira CA, Mahecha GA, Carnes K, Prins GS, Saunders PT, França LR, et al. Differential hormonal regulation of estrogen receptors ERalpha and ERbeta and androgen receptor expression in rat efferent ductules. Reproduction. 2004;128(1):73-86.
42. Ilio KY, Hess RA. Localization and activity of Na+,K(+)-ATPase in the ductuli efferentes of the rat. Anat Rec. 1992;234(2):190-200.
43. Hansen LA, Clulow J, Jones RC. The role of Na+-H+ exchange in fluid and solute transport in the rat efferent ducts. Exp Physiol. 1999;84(3):521-7.
44. Oliveira CA, Carnes K, França LR, Hermo L, Hess RA. Aquaporin-1 and -9 are differentially regulated by oestrogen in the efferent ductule epithelium and initial segment of the epididymis. Biol Cell. 2005;97(6):385-95.
45. Veeramachaneni DN, Amann RP. Endocytosis of androgen-binding protein, clusterin, and transferrin in the efferent ducts and epididymis of the ram. J Androl 1991;12(5):288-94.
46. Hermo L, Morales C. Endocytosis in nonciliated epithelial cells of the ductuli efferentes in the rat. Am J Anat. 1984;171(1):59-74.
47. Pelliniemi LJ, Dym M, Gunsalus GL, Musto NA, Bardin CW, Fawcett DW. Immunocytochemical localization of androgen-binding protein in the male rat reproductive tract. Endocrinology. 1981;108(3):925-31.
48. Hermo L, Wright J, Oko R, Morales CR. Role of epithelial cells of the male excurrent duct system of the rat in the endocytosis or secretion of sulfated glycoprotein-2 (clusterin). Biol Reprod. 1991;44(6):1113-31.
49. Garrett SH, Garrett JE, Douglass J. In situ histochemical analysis of region-specific gene expression in the adult rat epididymis. Mol Reprod Dev. 1991;30(1):1-17.
50. Toda K, Okada T, Hayashi Y, Saibara T. Preserved tissue structure of efferent ductules in aromatase-deficient mice. J Endocrinol. 2008;199(1):137-46.
51. Lee KH, Finnigan-Bunick C, Bahr J, Bunick D. Estrogen regulation of ion transporter messenger RNA levels in mouse efferent ductules are mediated differentially through estrogen receptor (ER) alpha and ER beta. Biol Reprod. 2001;65(5):1534-41.
52. Yasuhara F, Gomes GR, Siu ER, Suenaga CI, Maróstica E, Porto CS, et al. Effects of the antiestrogen fulvestrant (ICI 182,780) on gene expression of the rat efferent ductules. Biol Reprod. 2008;79(3):432-41.
53. Johnston DS, Jelinsky SA, Bang HJ, DiCandeloro P, Wilson E, Kopf GS, et al. The mouse epididymal transcriptome: transcriptional profiling of segmental gene expression in the epididymis. Biol Reprod. 2005;73(3):404-13.
54. Frenette G, Leclerc P, D'amours O, Sullivan R. Estrogen sulfotransferase is highly expressed along the bovine epididymis and is secreted into the intraluminal environment. J Androl. 2009;30(5):580-9.
55. Hess RA, Zhou Q, Nie R, Oliveira C, Cho H, Nakaia M, et al. Estrogens and epididymal function. Reprod Fertil Dev. 2001;13(4):273-83.
56. Shayu D, Kesava CC, Soundarajan R, Rao AJ. Effects of ICI 182780 on estrogen receptor expression, fluid absorption and sperm motility in the epididymis of the bonnet monkey. Reprod Biol Endocrinol. 2005;3:10.
57. Pearl CA, At-Taras E, Berger T, Roser JF. Reduced endogenous estrogen delays epididymal development but has no effect on efferent duct morphology in boars. Reproduction. 2007;134(4):593-604.
58. Atanassova N, McKinnell C, Williams K, Turner KJ, Fisher JS, Saunders PT, et al. Age-, cell- and region-specific immunoexpression of estrogen receptor alpha (but not estrogen receptor beta) during postnatal development of the epididymis and vas deferens of the rat and disruption of this pattern by neonatal treatment with diethylstilbestrol. Endocrinology. 2001;142(2):874-86.
59. Yamamoto M, Kohara S, Kobayashi T, Shirai M, Nisikawa O, Arishima K. Effects of maternal exposure to diethylstilbestrol on epididymal development in rat offspring. J Vet Med Sci. 2009;71(3):375-8.
60. Glover A, Assinder SJ. Acute exposure of adult male rats to dietary phytoestrogens reduces fecundity and alters epididymal steroid hormone receptor expression. J Endocrinol. 2006;189(3):565-73.
61. Shayu D, Hardy MP, Rao AJ. Delineating the role of estrogen in regulating epididymal gene expression. Soc Reprod Fertil Suppl. 2007; 63:31-43.
62. Motrich RD, Ponce AA, Rivero VE. Effect of tamoxifen treatment on the semen quality and fertility of the male rat. Fertil Steril. 2007;88(2):452-61.
63. Vignozzi L, Filippi S, Morelli A, Luconi M, Jannini E, Forti G, et al. Regulation of epididymal contractility during semen emission, the first part of the ejaculatory process: a role for estrogen. J Sex Med. 2008;5(9):2010-6.

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64. Cardoso L, Nascimento A, Royer C, Porto C, Lazari M. Locally produced relaxin may affect testis and vas deferens function in rats. Reproduction 2009 [Epub ahead of print] DOI: 10.1530/REP-09-0146.
65. Karlsson S. Histopathology and histomorphometry of the urogenital tract in 15-month old male and female rats treated neonatally with SERMs and estrogens. Exp Toxicol Pathol. 2006;58(1):1-12.
66. Atanassova N, McKinnell C, Fisher J, Sharpe RM. Neonatal treatment of rats with diethylstilboestrol (DES) induces stromal-epithelial abnormalities of the vas deferens and cauda epididymis in adulthood following delayed basal cell development. Reproduction. 2005;129(5):589-601.
67. Risbridger G, Wang H, Young P, Kurita T, Wang YZ, Lubahn D, et al. Evidence that epithelial and mesenchymal estrogen receptor-alpha mediates effects of estrogen on prostatic epithelium. Dev Biol. 2001;229(2):432-42.
68. Chen M, Hsu I, Wolfe A, Radovick S, Huang K, Yu S, et al. Defects of prostate development and reproductive system in the estrogen receptor-alpha null male mice. Endocrinology. 2009;150(1):251-9.
69. Ricke WA, McPherson SJ, Bianco JJ, Cunha GR, Wang Y, et al. Prostatic hormonal carcinogenesis is mediated by in situ estrogen production and estrogen receptor alpha signaling. FASEB Journal. 2008;22(5):1512-20.
70. Walsh PC, Wilson JD. The induction of prostatic hypertrophy in the dog with androstanediol. J Clin Invest. 1976;57(4):1093-7.
71. Leav I, Ho SM, Ofner P, Merk FB, Kwan PW, Damassa D. Biochemical alterations insex hormone-induced hyperplasia and dysplasia of the dorsolateral prostate of Noble rats. J Natl Cancer Inst. 1988;80(13):1045-53.
72. Yu M, Leav BA, Leav I, Merk FB, Wolfe HJ, Ho SM. Early alterations inras protooncogene mRNA expression in testosterone and estradiol-17 beta induced prostatic dysplasia of noble rats. Lab Invest. 1993;68(1):33-44.
73. Zhang Z, Duan L, Du X, Ma H, Park I, Lee C, et al. The proliferative effect of estradiol on human prostate stromal cells is mediated through activation of ERK. Prostate. 2008;68(5):508-16.

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