The Penis:
A New Target And Source Of Estrogen In Male Reproduction

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Abstract
In the past decade, interest and knowledge in the role of estrogen in male reproduction and fertility has gained significant momentum. More recently, the cellular distribution and activity of estrogen receptors (a and ß)(ER) and aromatase (estrogen synthesis) has been reported in the penis, making the penis the latest "frontier" in the study of estrogen in male reproduction. ER and aromatase are broadly and abundantly expressed in various penile compartments and cell types (erectile tissues, urethral epithelia, vascular and neuronal cells), suggesting the complexity and significance of the estrogen-ER system in penile events. Unraveling this complexity is important and will require utilization of the various resources that are now at our disposal including, animal models and human lacking or deficient in ER and aromatase and the use of advanced and sensitive techniques. Some of the obvious areas that require our attention include: 1) a comprehensive mapping of ER-a and -ß cellular expression in the different penile compartments and subpopulations of cells, 2) delineation of the specific roles of estrogen in the different subpopulations of cells, 3) establishing the relationship of the estrogen-ER system with the androgen-and rogen receptor system, if any, and 4) characterizing the specific penile phenotypes in human and animals lacking or deficient in estrogen and ER. Some data generated thus far, although preliminary, appear to challenge the long held dogma that, overall, androgens have a regulatory monopoly of penile development and function.

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Introduction

Over half a century ago, the first report documenting synthesis of estrogen by male gonads was published (Zondek 1934; Goldzieher and Roberts 1952; Leach et al., 1956; Morse et al., 1962; Attal, 1969; Carreau et al., 1999). However, interest in estrogen's role in male reproductive events only began to gain momentum in the past decade, largely catapulted by the unexpected data generated by studies from: 1) male transgenic mice lacking functional estrogen receptors (ERs), aromatase or over-expressing aromatase, 2) newly discovered mutations in ER-α and aromatase in human, and 3) from reports that exposure to environmental estrogens may have detrimental effects on male reproductive development and health (Greene et al., 1939; Stillman 1982; Sharpe and Skakkebaek, 1993; Korach, 1994; Smith et al., 1994; Eddy et al., 1996; Krege et al., 1998; Carreau et al. 1999; Couse and Korach, 1999; McKinnell et al., 2001; Simpson, 2003, 2004; Hewitt et al., 2004). Clearly, based on these emerging evidences, estrogen exerts important influence on male reproductive events.

Estrogen, which in the male is mainly produced by the testis and, to a lesser extent, by the brain and adrenal gland, exerts its effects on male reproduction via its receptors, estrogen receptors (ERs) that have been localized in the gonads, reproductive tract, accessory glands (Hess et al., 1997a,b, 2000; Mowa and Iwanaga, 2001) and, more recently, in the male external genitalia, the penis (Jesmin et al., 2002; Crescoli et al., 2003; Dietrich et al., 2003; Goyal et al., 2004). The present review provides a general update on the emerging data of the latest 'frontier' in the study of estrogen's action on male reproduction, the penis, including, 1) the distribution pattern of ER and aromatase, 2) the effects of ER and estrogen deficiency and excess on penile development and structure, and 3) the likely role of estrogen on penile events.

An overview of penis development and structure

Development

The development of the external genitalia can be divided into two stages: 1) initial appearance of the genital tubercle, which is common to both sexes, and 2) the differentiation and growth of the genital tubercle into the penis (male) and clitoris (female) (Jirasek, 1971; Guthrie et al., 1973). Although the exact molecular

mechanisms that underlie the development of external genitalia are unclear, the later phase of development, i.e, differentiation and growth, appear to be regulated by a delicate and complex balance between estrogen and androgen (Jirasek, 1971). In the human, prior to the 9th week of gestation, the external genitalia is indifferent (Jirasek, 1971), and the primordial fetal penis and clitoris are of equal size up until the 14th week of pregnancy (Jirasek, 1971). The 12-14th weeks of fetal life constitute a critical window of development because a shift in equilibrium between androgens and estrogens may lead to the development of hypospadia, failure of scrotal folds and pseudohermaphroditisia (see review Murray et al., 2001). However, the mechanisms or factors that underlie the development of the genital tubercle, which consists of formations and initial outgrowth, are unclear. This initial phase of external genital development is not sex steroid hormone-dependent, but is regulated by distal urethral epithelia, which expresses several genes, including Fgf8, Fgf10, Msx1, Hoxd13 and Bmp4 (Murakami and Mizuno, 1986; Kondo et al., 1997; Mortlock and Innis, 1997; Haraguchi et al., 2000).

The onset of masculanization of the male external genitalia coincides with Leydig cell-derived androgen production and absence of androgen in female fetus leads to passive development of the external genitalia (Jost, 1953; see review Hiort and Holterhus, 2000, 2003). The role of estrogen in this process is unclear.

Structure

General structure

The penis is a pendulous organ hinged to the pubic arch and is composed of two major components: 1) three columns of erectile tissues are divided into a paired corpora cavernosa and a corpus spongiosum, which are both covered by the tunica albiginea, and 2) the glans penis. The paired corpora cavernosa are laterally located along the dorsal body and the corpus spongiosus is located medially on the lower portion and has a urethra that passes through it. Regionally, the penis can be divided into 3 parts, namely: a) the root, anchored to the pubic arch, b) the body, forming the central and largest portion and, c) the glans, which is the most distal and expanded extremity.

General vasculature

The vasculature of the penis plays a very important role in erection. *Arterial:* The complex terminal arterial network of the common penile artery is supplied by the internal pudendal artery, a branch of the hypogastric artery. The common penile artery divides into the dorsal, cavernosus and bulbourethral arteries that either directly supply the cavernosus space or branch into the convulated helicine ateries, which ultimately open into the cavernosus spaces. The walls of these spaces are

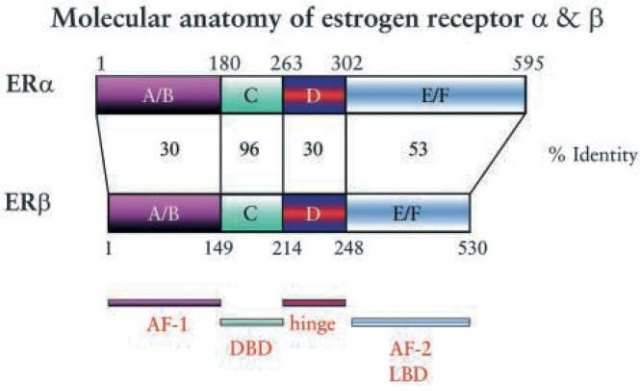

Fig. 1. Model illustrating the molecular anatomy of estrogen receptor (ER) subtypes α and ß, divided into four structurally distinct functional domains: the N-terminal A/B region, containing activation function 1 (AF-1); the C- or DNA-binding domain (DBD); the hinge region or D domain; and the C terminus– or ligand-binding domain (LBD), which contains a ligand-binding pocket and sites for cofactor binding, transactivation (AF-2), nuclear localization, and interactions with heat shock protein complex. Numbers in the upper and lower panels represent the number of amino acids in ER-α and ER-ß subtypes, respectively. The numbers in the middle panel represent percentage of homology between the specific domains of each ER subtype. [Reprinted from Mowa and Papka, 2004 The Role of Sensory Neurons in Cervical Ripening: Effects of Estrogen and Neuropeptides. J. Histochem. Cytochem. Volume 52(10): 1249-1258, 2004, Histochemical Society].

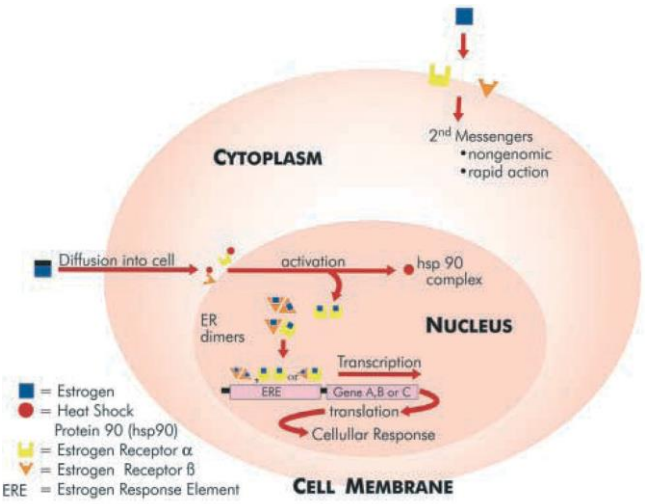

Fig. 2. Schematic diagram of the ER and its mechanism of action in a model cell such as a sensory neuron. Estrogen diffuses into the cell and binds to the ER to initiate ER activation and dimerization (homo- and heterodimers). The ligand receptor complex in turn binds to the estrogen response elements on the promoter of the DNA to initiate transcription, translation, and, ultimately, cellular response. Rapid response to estrogen may be mediated by the recently described membrane ER (ER-X). [Reprinted from Mowa and Papka, 2004 The Role of Sensory Neurons in Cervical Ripening: Effects of Estrogen and Neuropeptides. J Histochem Cytochem, Volume 52(10): 1249–1258, 2004 by Histochemical Society].

formed by trabeculae, made of thick bundles of smooth muscles and collagen material and, peripherally, they are lined by vascular endothelial cells. *Venous:* The blood from cavernous spaces is drained by a series of subtunical veinous plexus that "pierce" through the tunica albiginea as emissary veins to form the circumflex veins, which in turn converge dorsally into the deep dorsal veins. Other draining veins include periurethral, crural and cavernosal. Collectively, these veins drain into the urethral vein that supplies the internal pudendal vein (Fernandez et al., 1991).

General innervation

The penis is richly innervated by the sympathetic, parasympathetic (autonomic), sensory and motor (somatic) nerves. The sympathetic and parasympathetic converge to form the cavernosus nerves that innervate the columnal erectile tissues (cavernosus and spongiosus) and largely modulate neurovascular events associated with erection and detumescence. On the other hand, the somatic nerves are largely involved in regulating sensation and contraction of the crural muscles (Mowa and Papka, 2003; El-Sakka and Lue, 2004).

Biology of estrogen receptor and synthesis

Estrogen receptors

Estrogen's effects are principally mediated by two estrogen receptor (ER) subtypes, ER-α, the "classical" subtype cloned in 1986 (Koike et al., 1987) and ER-ß, a more recently cloned subtype (Kuiper et al., 1996) (Fig. 1). Different isoforms of ER-ß have since been identified, including ER-ß2, ER-ßcx, and ER-ß variants that are altered by a deletion within the DNA binding domain (ER-ß1δ3 and ER-ß2δ3) (Moore et al., 1998; Ogawa et al., 1998).

ERs belong to the steroid/retinoid receptor superfamily that has structural and functional similarities (Mangelsdorf et al., 1995). They (ERs) are divided into four structurally distinct, functional domains: 1) the N-terminal A/B region, which has the activation function 1 (AF1) that regulates transcriptional activation, 2) the C region (mid region) or DNA binding domain (DBD) that mediates specific DNA binding, 3) the less known D domain or hinge region, 4) the C terminus or ligand binding domain (LBD) containing the ligand binding pocket and sites for cofactor binding, transactivation (AF2), nuclear localization and interactions with heat

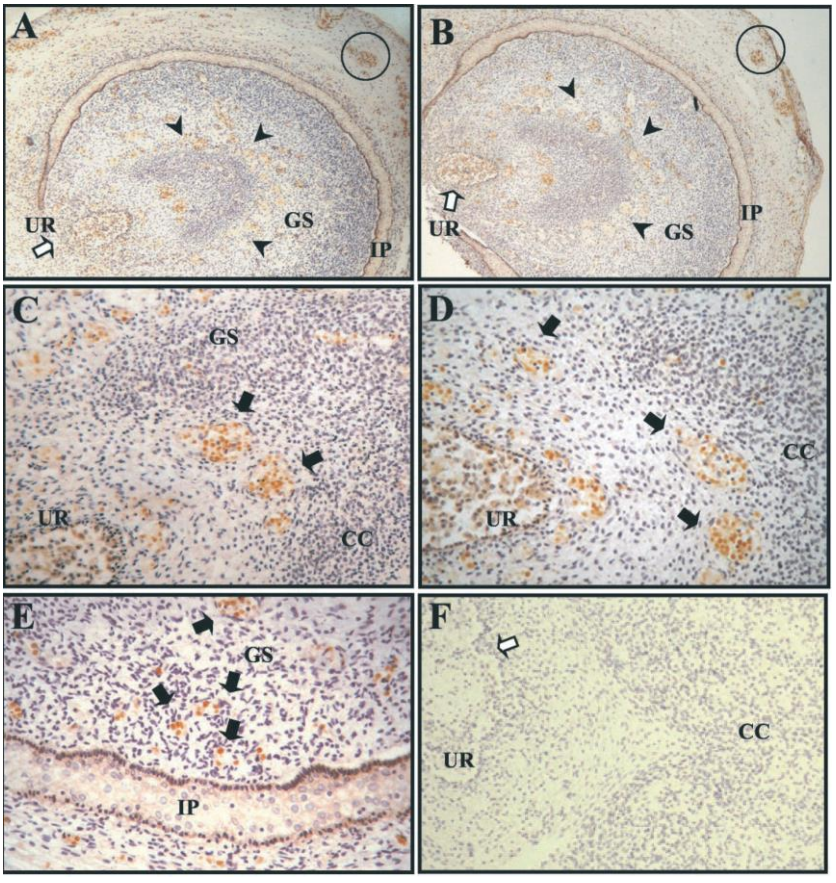

Fig. 3. Immunohistochemistry for ER and AR in human fetal penile tissue. Transverse sections of human male external genitalia (MEG) of a fetus at the 12th week of gestation. White arrows indicate the forming male urethra (UR); black arrowheads in the glans stroma (GS) indicate the condensing connective tissue of the forthcoming CC. Note the inner prepuce (IP) surrounding the forming CC. **A and B.** Low magnification of the MEG. Immunostaining for ER-positive **(A)** and AR-positive **(B)** cells was present in several portions of the MEG, including the skin (circle), the UR, and the GS. Positivity for both sex steroid receptors occurred apparently in the same structures. **C and D.** Panels correspond to A and B, respectively. Note that blood vessels (black arrows) surrounding and invading the CC are positive for ER and AR. Stromal cells of the UR were also stained. **E.** ER immunostaining at the level of the prepuce showing positive endothelial and smooth muscle cells. **F.** Negative control avoiding primary antibodies. A and B, x 80; C-F, x 150. [Reprinted from Crescioli et al., Expression of functional estrogen receptors in human fetal male external genitalia. J Clin Endocrinol Metab, 88 (4):1815-1824, with permission from The Endocrine Society].

shock proteins (see review; Weihua et al., 2003) (Fig. 1). Upon binding to its receptors, the ERs dimerize and control transcription by binding, in most cases, to the classical consensus estrogen response elements (ERE) in the promoter region of the target genes, leading to a cellular response (Fig. 2) (see review; Weihua et al., 2003). Alternatively, ER can induce transcription by binding to AP-1 and Sp-1, where ER-α and ER-ß have different, and in some cases, opposing effects (Phillips et al., 1993; Umayahara et al., 1994; Webb et al., 1995). Rapid effects of estrogen on neurons are exerted via the recently identified, but less characterized, membrane estrogen receptor (McEwen, 1991), which may be identified as ER-X (Toran-Allerand et al., 2002).

Estrogen synthesis

Estrogens, 17ß-estradiol and estrone, are produced from C19 androgens, testosterone and androstanedione, respectively, by cytochrome P450 arom (P450arom), a product of CYP19 gene. P450arom is a mono-oxygenase microsomal enzyme complex present in endoplasmic reticulum that irreversibly converts the aromatizable C19 androgens to estrogens through 3 consecutive hydroxylation reactions at the A-ring (Lephart 1996; Pereyra-Martinez et al. 2001; Carreau et al. 2002; Wiszniewska 2002). Aromatase is expressed in a tissue-specific manner and is composed of two proteins, namely, 1) a ubiquitous NADPH-cytochrome P450

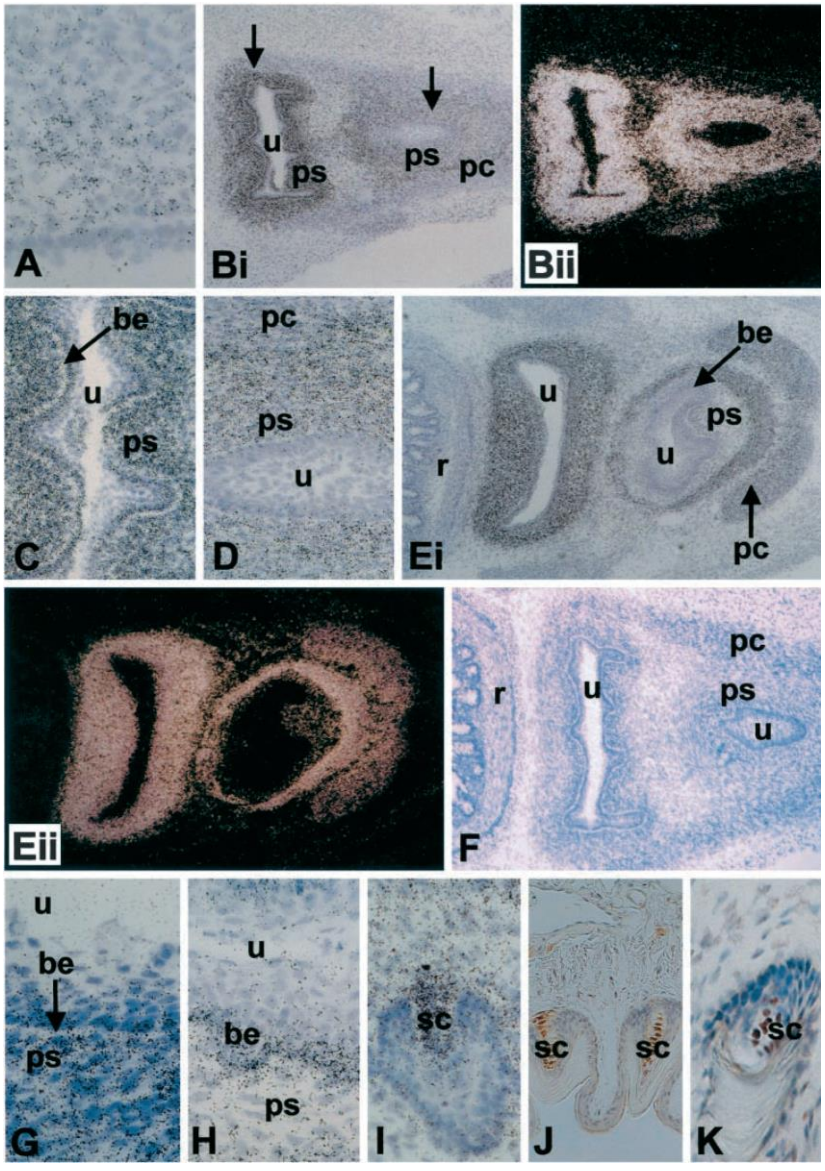

Fig. 4. *In situ* hybridization analysis showing gene expressions of ERa in the rat penis. **A.** Distinct ERα mRNA signals were seen in the mesenchyme of the developing penis at fetal d 17. **B–E.** The transverse sections of the primordial penis exhibited more pronounced signals of ERα mRNA in the proximal portion (left arrow in panel Bi) than the distal region (right arrow in panel **Bi**) at postnatal d 3. Urethral epithelium, especially the basal epithelium (be), and penile spongiosus (ps) of the proximal penis were more strongly labeled than those of the distal penis **(D)** (pc, penile cavernosus; u, urethral lumen). **C and D** are higher magnifications (x 400) of **B**, whereas **Bi** and **Bii** are bright-field and dark-field images, respectively. **Ei** and **Eii** are also bright- and dark-field images of the transverse section of the penis at postnatal d 7 taken at a more rostral or cranial position. **F.** No grain (ERα mRNA signal) was observed in the transverse section of the penis at postnatal d 3 when excessive cold probe was added to a little amount of the corresponding hot probe during the hybridization process. **G-H.** The stratified epithelia of the middistal penile urethra at postnatal d 2 (**G**) and 14 (H) showed significant signals of ERa mRNA in the basal epithelial layer (be). Strong ERa mRNA signals in the spongiosus (ps) were seen at postnatal d 2 **(G)**, which declined at postnatal d 14 **(H). I.** Distinct and intense signals of ERα mRNA were localized to the lamellated sensory corpuscles (sc) of the glans penis at postnatal d 24. **J.** Immunohistochemical detection for S100 protein. Positive staining (brown) was found in the lamellated sensory corpuscles (sc). **K.** Immunohistochemical detection for active caspase 3, a member of caspase superfamily, which initiates apoptotic events. Positive staining (brown) was found in the lamellated sensory corpuscles (sc). x 200 (x 100 for B, E, and F). [Reprinted from Jesmin et al. Evidence for a potential role of estrogen in the penis: Detection of estrogen receptor-α and -ß messenger ribonucleic acid and protein. Endocrinology, December 2002, 143(12):4764–4774, with permission from The Endocrine Society].

reductase and 2) a cytochrome P450 aromatase, which contain the heme and the steroid pocket (Lephart 1996; Carreau et al. 1999, 2002).

Distribution of ER and aromatase in the penis

Estrogen receptors

The earliest evidence implicating estrogen in penile events emerged from human and, later, laboratory and wildlife studies several decades ago (see review Toppari et al., 1996). It was shown that exposure of male offspring to estrogen-like endocrine disruptors *in utero* induce micropenis and hypospadia (Toppari et al., 1996). However, in order to understand the effects of estrogen and the specific mechanisms underlying its action on penile events, it was essential to demonstrate presence of ER, the principal mediator of estrogen action. Indeed, the earlier data that implicated estrogen-like endocrine disruptors in penile development, served as the impetus to the subsequent ER distribution studies. However, the failure of the initial study to localize ER in fetal human corpus cavernosus by Kalloo et al. (1993) using immunohistochemistry, may have dampened interest in this field, until very recently in the early 2000s, following the first report by Jesmin and others (2002), which was shortly confirmed by others (Crescioli et al., 2003; Dietrich et al., 2004; Goyal et al., 2004). The recent success in demonstrating ER in penile tissues could, in part, be attributed to new advancement in technology with increased sensitivity.

Prenatal

ER is expressed in the developing penis, as early as the prenatal stage in both the rat and human fetuses (Jesmin et al., 2002; Crescioli et al., 2003) (Figs. 3, 4a). In the human, by the 12th week of gestation, the earliest age investigated, ER was expressed throughout the entire developing organ, including the skin, urethra, glans, stromal cells of corpus cavernosus, stromal cells under urethra, smooth muscle cells under the level of inner prepuce and the endothelial and smooth muscle cells of the blood vessels in the corpus cavernosus (Crescioli et al., 2003). More importantly, ER was co-localized with the androgen receptor (AR) (Crescioli et al., 2003). Subsequent cultures of fetal human penile smooth muscle cells when analyzed by RT-PCR, Western blot and immunohistochemistry demonstrated both ER subtypes, ER-α and ER-ß mRNAs and proteins (Crescioli et al., 2003). In the rat, ER-α mRNA was demonstrated by *in situ* hybridization in the mesenchyme of the undifferentiated penis by day 17 of gestation, the earliest age examined. There were no

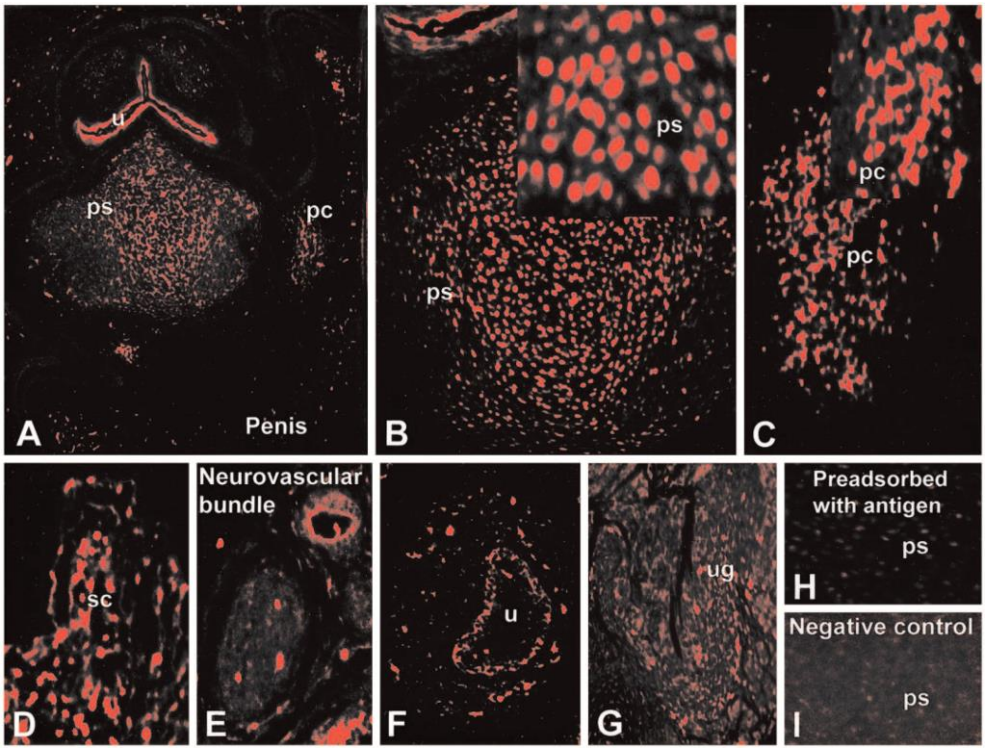

Fig. 5. Immunofluorescent findings for ERa in the rat penis. **A.** Penile ERa distribution in postnatal d 1 rat (low magnification, x 100). Positive staining (red) was found in the penis spongiosus (ps), urethra (u), and penis carvenosus (pc). **B and C.** Immunoreactivity was found in the penis spongiosus (ps) and penis carvenosus (pc) (high magnifications x 200, [inset, x 400]). **D.** Positive staining was also found in the sensory corpuscle (sc) of glans penis (x 400). **E.** Immunoreactivity was less in the neurovascular bundle of body penis (x 200). **F and G.** Positive staining was found in the urethra (u) and urethral gland (ug) (magnification, x100). **H**. No staining was observed in the penile spongiosus (ps) when peptide-adsorbed antibody was used (x 200). **I.** Primary antibody (data not shown) or secondary antibody (data presented) showed no immunoreactivity with the penile spongiosus (ps), indicating the specificity of the antibodies (x 400). [Reprinted from Jesmin et al. Evidence for a potential role of estrogen in the penis: Detection of estrogen receptor-α and -ß messenger ribonucleic acid and protein. Endocrinology, December 2002, 143(12):4764–4774, with permission from The Endocrine Society].

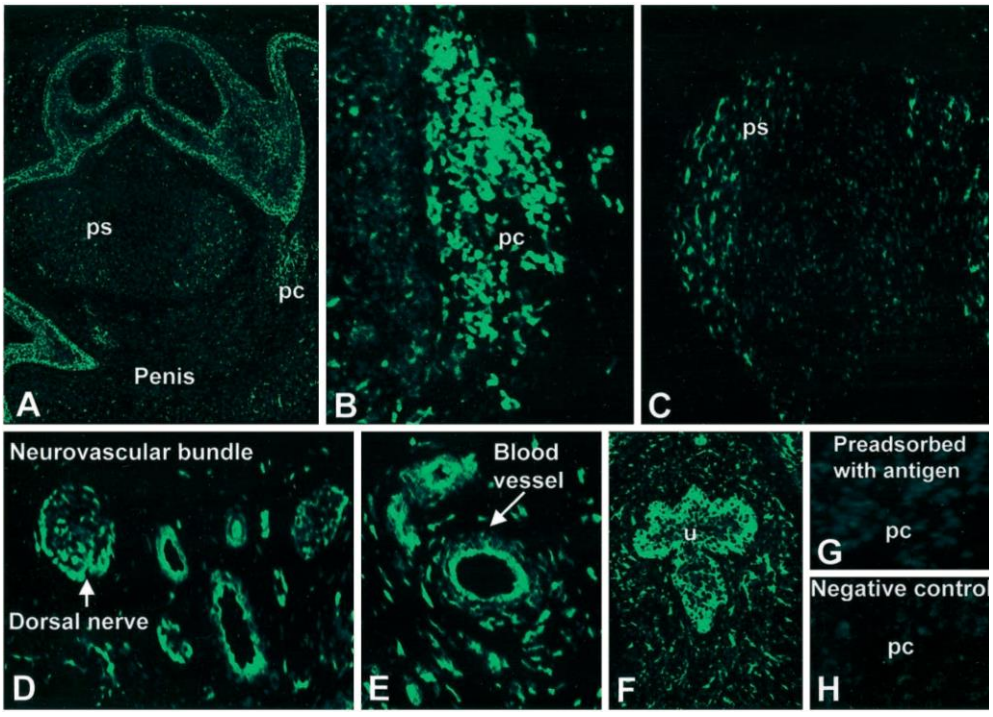

Fig. 6. Immunofluorescenct findings for ERb in the rat penis. **A.** Penile ERß distribution in postnatal d 1 rat (low magnification, x 100). **B and C.** Immunoreactivity found in carvenosus (pc) of body penis much more strongly than in the penis spongiosus (ps) (high magnification, x 400). **D.** Positive staining was seen in the neurovascular bundle of body penis (x 200). E, Positive staining found in the penile artery of root penis (x 400). **F.** Positive staining found in urethra (u) (x 100). **G.** No staining was observed in the penile cavernosus (pc) when peptide-adsorbed antibody was used (x 400). **H.** Primary antibody (data not shown) or secondary antibody (data presented) showed no immunoreactivity with the penile cavernosus (pc), indicating the specificity of the antibodies (x 400). [Reprinted from Jesmin et al. Evidence for a potential role of estrogen in the penis: Detection of estrogen receptor-α and -ß messenger ribonucleic acid and protein. Endocrinology, December 2002, 143(12):4764–4774, with permission from The Endocrine Society].

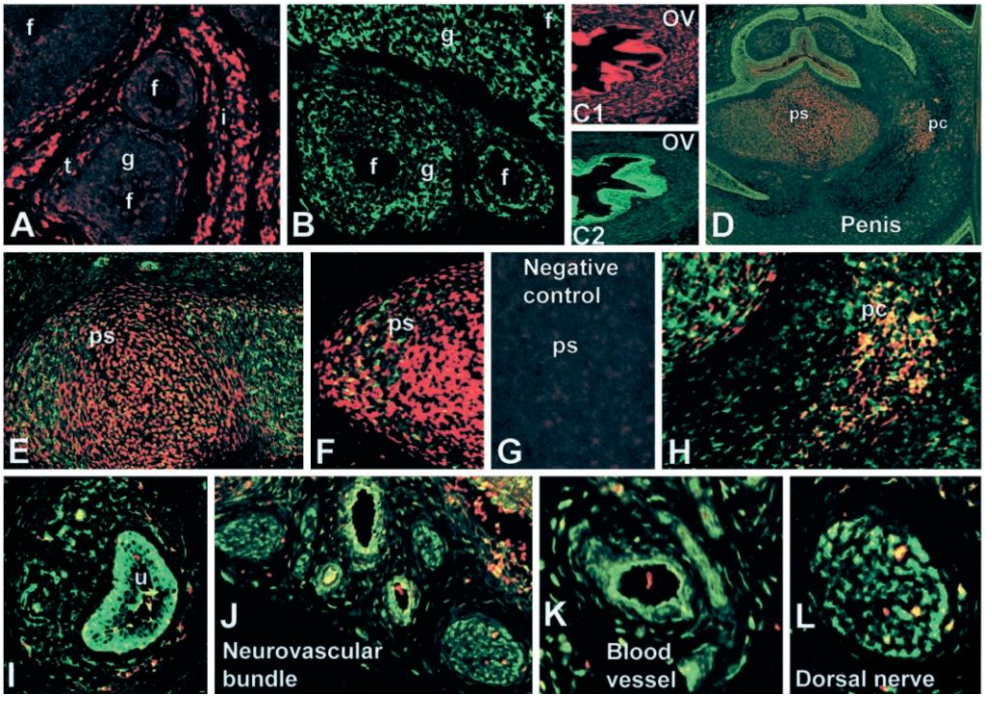

Fig. 7. Immunohistological localization of ERα (red) and ERß (green) in the rat ovary (**A–C**) and penis **(D–L**). **A.** In the adult female rat ovary, ERa was immunolocalized to thecal cells (t) and interstitial gland cells (i) but not to granulosa cells (g) of follicles (f). **B.** In the same ovary, ERb was predominantly detected in granulosa cells (g) of follicles (f). **C.** Intense ERß staining (C1) was detected in luminal epithelium and muscle cells of the oviduct (ov), whereas ERα (C2) was expressed mainly in epithelium of the oviduct (ov). **D.** Immunofluorescence double labeling for ERα and ERß in 1-d-old rat penis (low magnification, x 100). **E and F.** ERß was expressed more abundantly than ERα in the penis spongiosus (ps) (high magnification, x 200 and x 400). **G.** Primary antibodies (data not shown) or secondary antibodies used for double labeling (data presented) showed no reactivity with the penile spongiosus (ps), indicating the specificity of the antibodies (x 400). **H.** Both of ERα and ERß were present in the penis cavernosus (pc) (high magnification, x 400). **I.** ERα was more dominant in the urethra than ERß (x 100). **J–L.** ERß immunoreactivity was main in the neurovascular bundle, dorsal artery, and dorsal nerve (x 200 for J, x 400 for K and L). [Reprinted from Jesmin et al. Evidence for a potential role of estrogen in the penis: Detection of estrogen receptor-α and -ß messenger ribonucleic acid and protein. Endocrinology, December 2002, 143(12):4764–4774, with permission from The Endocrine Society].

ER–α mRNA signals in the epithelium and for ER-ß mRNA (Jesmin et al., 2002) (Fig. 4a).

Postnatal

By as early as neonatal period, the distribution pattern of ER in the rat was almost as widespread in a subpopulation of cell types as in human fetal penis: during the first week of birth, the corpus spongiosus, basal epithelia, stromal cells adjacent to the urethral epithelium and blood vessels expressed signals of both ER-α and -ß mRNAs and proteins, as demonstrated by both in situ hybridization and immunohistochemistry (Jesmin et al., 2002) (Figs. 3-7). Signals for ER-α in the rat penis were more discrete and intense compared to ER-ß, which were more diffuse and moderate (Jesmin et al., 2002). Additionally, ER-α mRNA and protein were also revealed in the sensory corpuscles of the glans and, together with ER-ß, in neurovascular bundle and dorsal nerve (Jesmin et al., 2002). Double immunofluorescence of ER-α and -ß in the penis of 1-d-old rat showed a predominant distribution of ERα in the penis spongiosus, and a predominant distribution of ERß in the urethra and neurovascular bundle (including dorsal blood vessels and dorsal nerve) (Jesmin et al., 2002) (Fig. 7). The penis cavernosus appeared to express ERα and ERß equally. In another study by Goyal et al. (2004) that exclusively used immunohistochemistry, ER-α was largely negative in most rat corpus cavernosus cells of both immature and mature, and only weak to moderate and very weak in some cells of the tunica albuginea and fibroblasts of the intercrural stroma, respectively. This is in contrast to earlier observations reported in rats by Jesmin et al. (2002) and in human by Cresoli (2003), which showed a more widespread distribution of ER (Figs. 3-7). However, the expression pattern of ER in the corpus spongiosus as described by Goyal et al. (2004) were overall consistent with those described by Jesmin et al. (2002) and Crescioli et al. (2003) (Fig. 8).

Adulthood

The only report on ER expression in the penile tissue

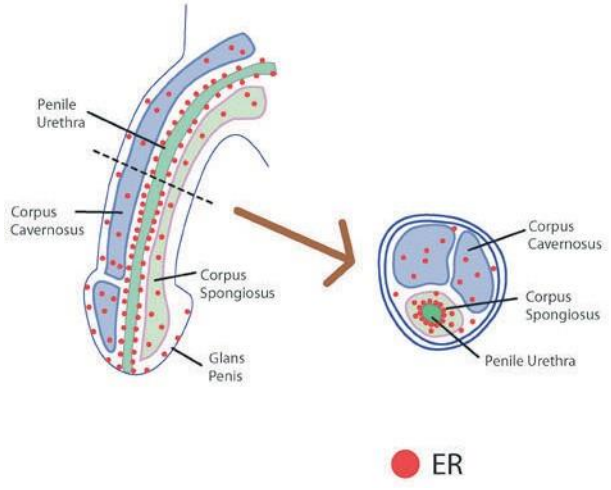

Fig. 8. Schematic diagram shows an overview distribution of ER in the penile tissues (glans penis, penis spongiosus, penis cavernosus, penile urethra). Bottom right, cross section of penis.

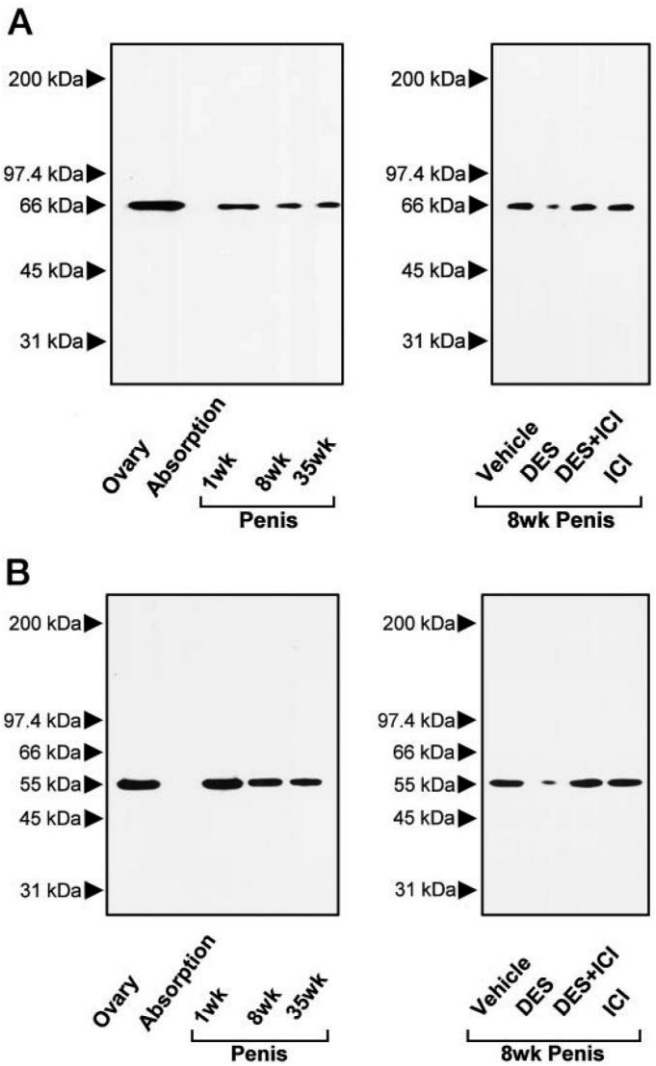

Fig. 9. Immunoblot analysis for ERα (A1) and ERß (B1) in the penile tissues of 1-, 8-, 35-wk-old rats (lanes 3-5). The ovary from the adult female rat (lane 1) was used as a positive control. No band was seen when the antibody had been pre-adsorbed with antigen. Lower panels (A2, B2) show changes in the blots for ERα and ERß obtained from the penile tissues of 8-wk-old rats after vehicle treatment (lane 1), DES treatment (lane 2), DES+ICI treatment (lane 3), and ICI treatment (lane 4) for 3 wk. [Reprinted from Jesmin et al. Evidence for a potential role of estrogen in the penis: Detection of estrogen receptor-α and -ß messenger ribonucleic acid and protein. Endocrinology, December 2002, 143(12):4764–4774, with permission from The Endocrine Society].

of men was recently described by Dietrich and others (2004) using immunohistochemistry and is principally consistent with the pattern observed in adult rat, as well as the human fetal penis described earlier (Jesmin et al., 2002; Crescioli et al., 2004) (Figs. 3-8). In men ER-α and ER-ß are largely co-expressed in the smooth muscle cells and endothelia (sporadically) of corpora cavernosus and spongiosus, although ER-ß is predominant (Dietrich et al., 2004). Both ER subtypes are also expressed in the periurethral and urethra. In the rat, ER mRNA is localized in the basal region of the urethra and lamellated sensory corpuscles, but with slightly decreased intensity in the adult. ERß mRNA was more diffused and was expressed in penis cavernosus, urethral glands, blood vessels, and dorsal nerve of penile spongiosus (Jesmin et al., 2002).

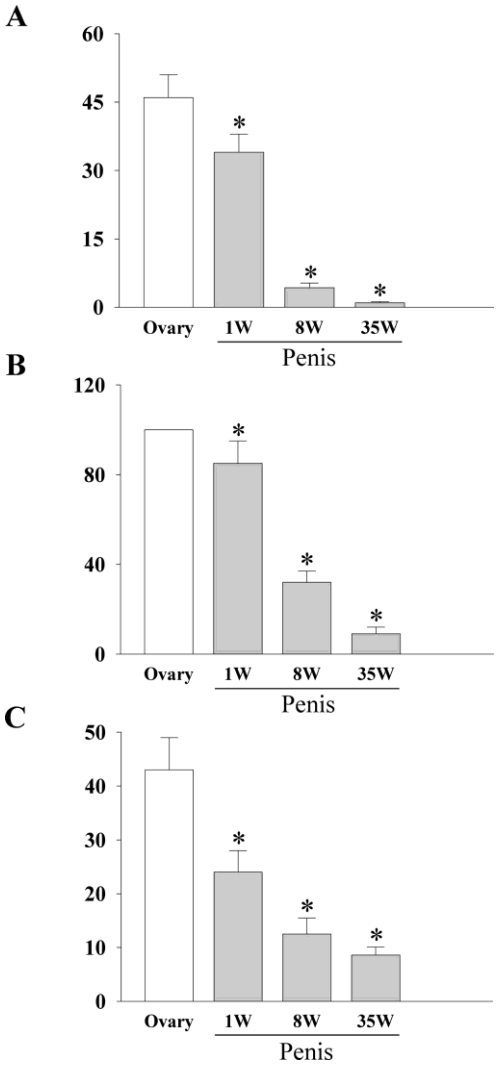

Fig. 10. A. Levels of aromatase in rat adult ovary and penises at 1, 8, 35 weeks (1W, 8W, 35W) as determined by ELISA. **B.** Gene expression level of aromatase in the penile tissues of 1-, 8-, and 35-week-old rats and in the adult ovary which was used as a positive control tissue. Expression of aromatase mRNA was quantitatively evaluated by real-time PCR. **C.** Tissue levels of 17ß estradiol in rat adult ovary and rat penile tissues at 1, 8, and 35 weeks. The 17ß-estradiol level was determined by enzyme immunoassay. Data are means ± S.D. (n=6–7). *P=0·01 compared with the corresponding values obtained in the ovary. [Reprinted from Jesmin et al. Aromatase is abundantly expressed by neonatal rat penis but downregulated in adulthood. J. Mol. Endocrinol., 2004, 33, 343-359, with permission from The Society of Endocrinology].

Aromatase

Evidence of the ability of male gonads to produce estrogen was reported more than half a century ago. Estrogen in the male is also known to be produced by the brain and adrenal gland, although to a lesser extent (see review Carreau et al., 1999, 2002; Simpson, 2003). However, it is only very recently that evidence of aromatase presence, activity and cellular distribution in penile tissues have been reported (Jesmin et al., 2004; Vignozzi et al., 2004). The pattern of aromatase distribution during development and adulthood are overall comparable to that of ER, based on *in situ* hybridization and immunolabeling: aromatase is localized to the penis spongiosus, penis cavernosus, urethra, sensory corpuscle of glans penis, blood vessel,

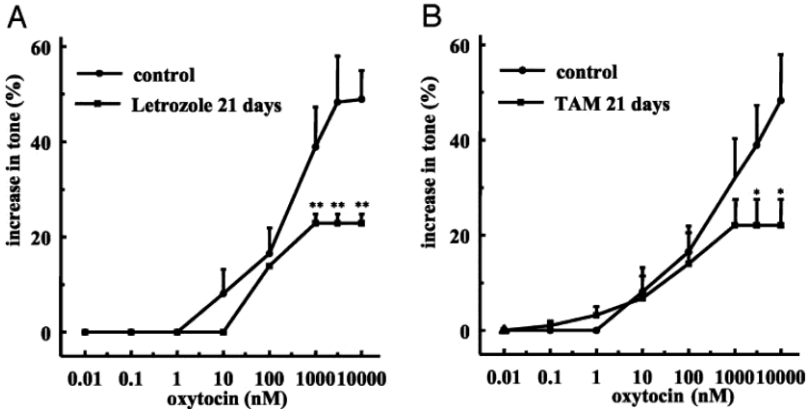

Fig. 11. Effect of estrogen ablation in sexually mature, intact rabbits. Responsiveness of CC to OT was tested in untreated rabbits (**A** and **B**; F; n=6 in three separate animals) or in animals treated daily for 3 wk with letrozole (2.5 mg/kg; A; f; n =6 in three separate animals) or TAM (0.250 mg/kg; B; f; n =6 in three separate animals). Ordinate, Contractile activity, expressed as a percentage of the maximal response obtained with KCl (80 mM); abscissa, concentrations of oxytocin (nanomolar). Data were expressed as the mean ± SEM. *: P<0.05; **: P<0.01 (vs. control). [Reprinted from Vignozzi et al. Oxytocin receptor is expressed in the penis and mediates an estrogen-dependent smooth muscle contractility. Endocrinology, April 2004, 145 (4): 1823-1834, with permission from The Endocrine Society].

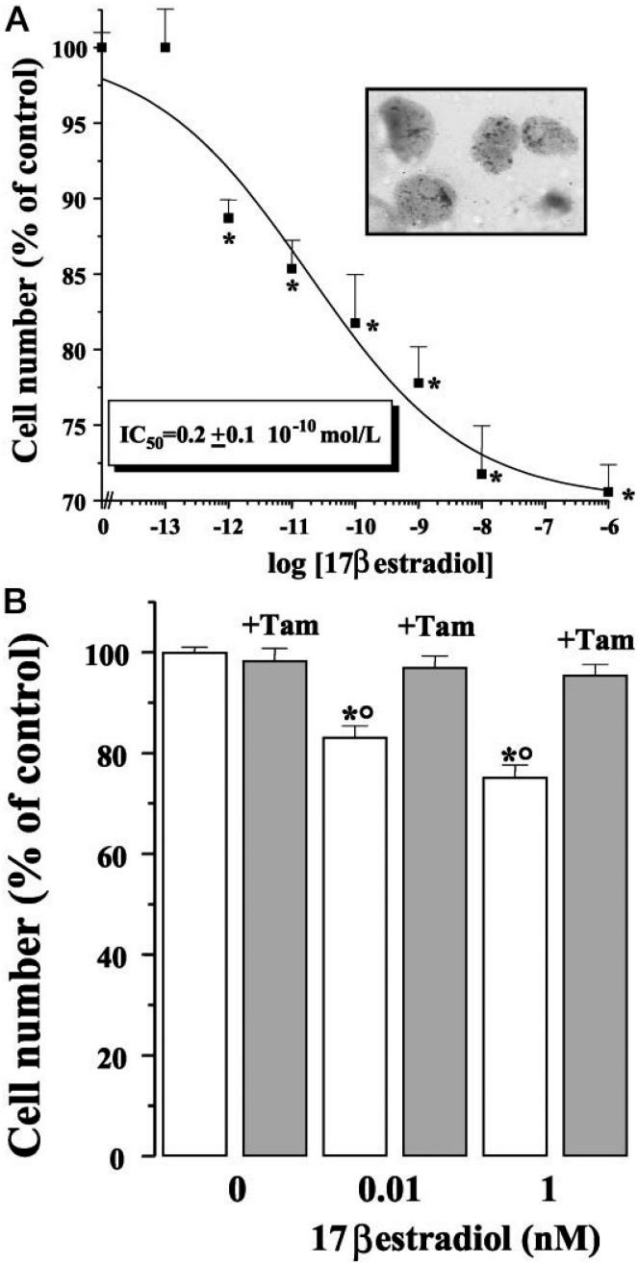

Fig. 12. Estrogens inhibit proliferation in hfPSMC. **A.** Twenty-four hour incubation with increasing concentrations of 17ß-estradiol [10^{-13} to 10^{-16} mol/liter] resulted in a statistically significant decrease in hfPSMC proliferation rate with IC50 = 0.2±0.1 x 10^{-10} mol/liter. Ordinate: Cell number expressed as percentage of the control; abscissa: logarithmic scale of 17ß-estradiol molar concentrations. Data were expressed as the means ± SEM values obtained in six different experiments. *: P<0.001 vs. control (0 nM), Student's t test for paired data. Inset: Immunocytochemistry in hfPSMC shows positivity for ER in cell nuclei (x 300). **B.** The inhibitory effect of 17ß- estradiol on hfPSMC was counteracted by a fixed dose of the antiestrogen tamoxifen (+Tam, 100 nmol/liter). Data were expressed as the means ± SEM values obtained in three different experiments. *: P<0.01 vs. control (0 nM); °: P<0.005 vs. Tam-treated. Student's t test for unpaired data. [Reprinted from Crescioli et al., Expression of functional estrogen receptors in human fetal male external genitalia. J. Clin. Endocrinol. Metab. 88 (4):1815-1824, with permission from The Endocrine Society].

dorsal nerve and primordial os penis at postnatal day 1 (Jesmin et al., 2004).

Levels of ER and aromatase change with age: Jesmin and others (2002, 2004) have reported an age-dependent expression of ER and aromatase levels using a variety of techniques, including RT-PCR, Western blot, ELISA, RIA and in situ hybridization. ER was detected in 1-wk-old rat penis, but was barely detectable at 8 and 35 wk of age (Jesmin et al., 2002) (Fig. 9). Although ERß was detected in all ages examined, it also tended to decrease like ER-α with age (Jesmin et al., 2002) (Figs. 9,10). However, this observation has not yet been confirmed by others. It is essential that a more thorough and comprehensive data on ER and aromatase distribution patterns during different ages and species be determined.

Role of estrogen in penile events

Although our knowledge on the physiological role of estrogen in penile development and function is beginning to surface, it is for the most part limited, unclear and in some cases is yet to be reproduced. Much of what we know about the effects of estrogen on penile events in human, wildlife and laboratory animals is largely based on in utero exposure to environmental estrogens or mega pharmacological doses of estradiol (McLachlan et al., 1975; Sharpe and Skakkebaek, 1993). The fact that estrogen receptors and aromatase are localized in a diverse subpopulation of penile cells, including neurons, epithelia, stroma, glandular cells, suggests that its function in the penis is likely to be cell-specific and complex (Jesmin et al., 2002, 2004; Crescioli et al., 2003; Dietrich et al., 2004; Goyal et al., 2004; Vignozzi et al., 2004).

Penile postorgasm flaccidity

Oxytocin is released during male orgasm and is believed to participate in the ejaculatory process (Ogawa et al., 1980). In the human and rabbit, oxytocin receptors have been localized in the corpus cavernosus and in vitro studies in human and rabbit model of hypogonadotropic hypogonadism. These studies have shown that the oxytocin receptor mediates corpus cavernosus contractility under the influence of steroid hormone mileau (Vignozzi et al., 2004). Estrogen, but not testosterone, restores oxytocin responsiveness, an effect attenuated by aromatase and ER blockers, suggesting that estrogen, acting via ER and oxytocin, may be involved in penile postorgasm flaccidity (Vignozzi et al., 2004) (Fig. 11).

Effect of estrogen on penile growth

When varying doses of estrogen were administered to smooth muscle cells of the human fetal corpus cavernosus in vitro, they exerted an anti-proliferative effects in a dose-dependent manner, an effect that was

opposite to testosterone (Crescioli et al., 2003) (Fig. 12). This observation in human cells is consistent with the elegant in vivo data from rats by Goyal et al. (2004) and Yucel and others (2003) demonstrating an inhibitory effect of DES on rat penile growth and differentiation, and urethral closure, respectively (Figs. 13, 14). Taken together, these data, together with data from xenoestrogens, suggest that the balance between androgens and estrogen is critical in penile growth and differentiation. Exactly how estrogens and androgens and their respective receptors interact is unclear. However, it may be that some of the penile effects currently attributed to androgens, such as androgen receptor down-regulation (Takane et al., 1990), may be mediated by estrogens via aromatization of testosterone (Lin et al., 1993).

Other potential roles of estrogen in the penis

Based on the localization of ER in diverse penile compartments and cell subpopulations, estrogen likely plays a broad range of functions in the penis, which at this point are purely speculative.

Blood vessel

The presence of ER and aromatase in the blood vessel lining and walls in rat and human penile tissues suggests that estrogen may influence the penile vasculature, possibly, as is the case in other body tissues, by influencing synthesis of vascular chemical mediators such as NO and prostacyclin (Calles-Escandoon and Cipolla, 2001). Of interest to erection, estrogen may influence the erectile tissue in a rapid non-genomic and NO-independent mechanism, as reported recently by Kim and others (2004), by stimulating the Maxi-K channel of the cavernosus myocyte.

Nerves

ER and aromatase in the rat and human are localized to the penile nerves (Jesmin et al., 2002, 2004; Crescioli et al., 2003). The presence of ER-α and aromatase in the rat sensory corpuscle of glans penis, containing the highest concentration of sensory nerve fibers vital for sexual sensation (Kandel et al., 2001), is interesting and intriguing, particularly because both ERα and ERß have

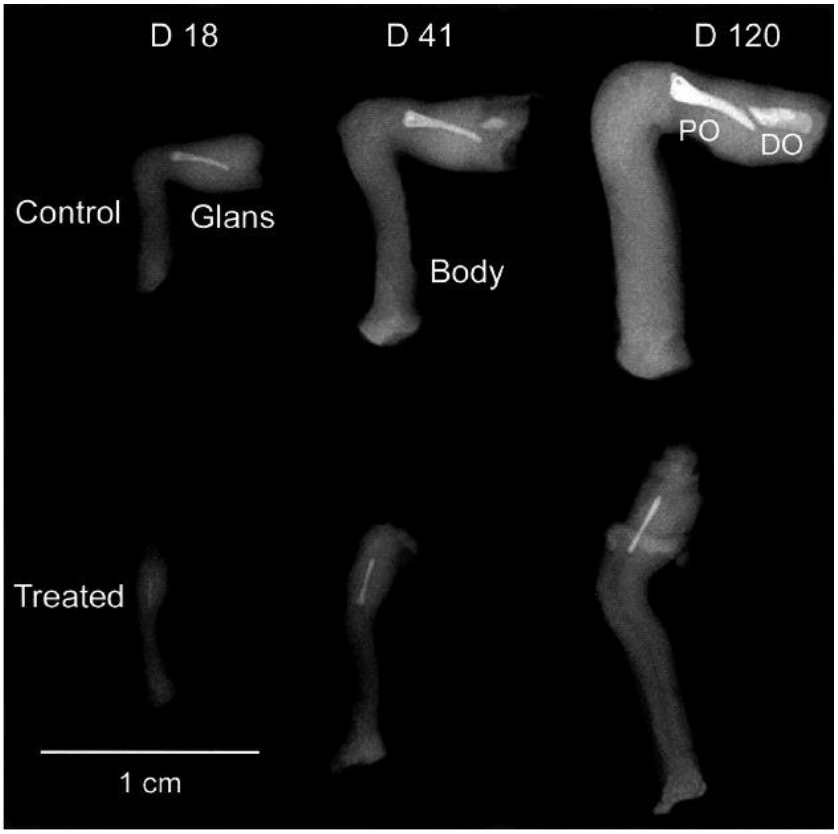

Fig. 13. Radiographs of the penis at 18, 41, and 120 days of age in rats treated with oil (control) or DES neonatally. Note reductions in length and diameter of the body and glans of the penis, a reduction in thickness of the proximal part of the os penis (PO), and the lack of development of the distal part of the os penis (DO), as a result of treatment. Scale bar 5 1 cm. [Reprinted from Goyal et al. Abnormal morphology of the penis in male rats exposed neonatally to diethylstilbestrol is associated with altered profile of estrogen receptor-a protein, but not of androgen receptor protein: a developmental and immunocytochemical study. Biology of Reproduction, with permission from The Society for the Study of Reproduction].

been identified in neural circuits involving central neurons, autonomic and sensory ganglionic neurons, and spinal cord neurons in areas that have connections with the male reproductive systems (Taleghany et al., 1999; Burke et al., 2000; Murphy and Hoffman, 2001). Estrogen may likely be important in the survival and vitality of sensory corpuscles and, consequently, on sexual sensation. It is also important to note that the main efferent parasympathetic pathway supplies vasodilating innervation to the cavernosus bodies, whereas the main sympathetic pathway supplies mostly the vasoconstriction innervation, thus chiefly mediates

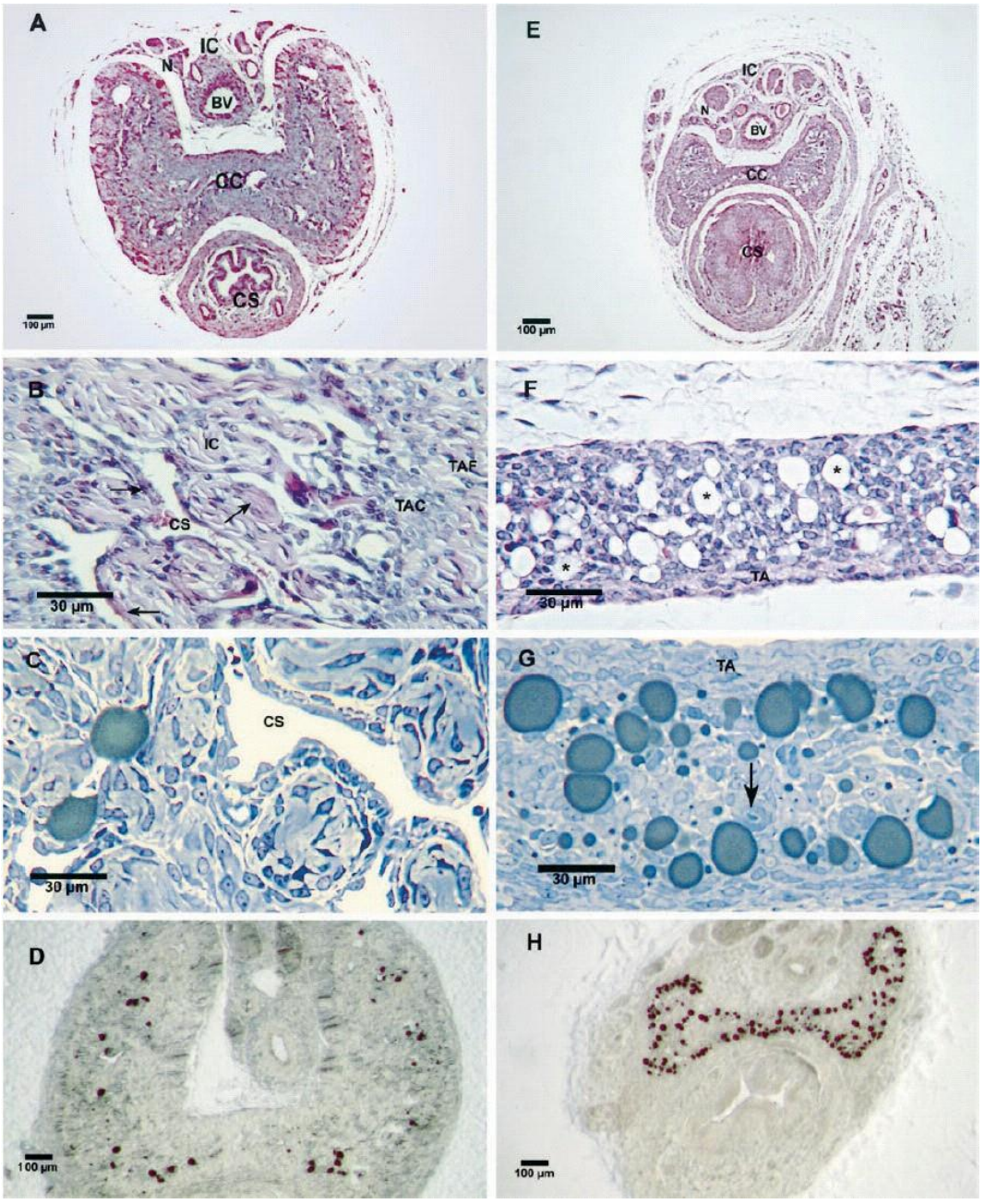

Fig. 14. Micrographs from the body of the penis in control rats **(A–D)** and in rats treated with DES neonatally **(E–H)** at 18 days of age. **A, E.** Note similarity in spatial arrangement of different parts of the body of the penis between control and treated rats: paired corpora cavernosa (CC), corpus spongiosus (CS), and intercrural septum (IC) containing blood vessels (BV) and nerves (N). **B.** Corpus cavernosus penis from the control rat showing tunica albuginea fibrous (TAF), tunica albuginea cellular (TAC), cavernous spaces (CS), and intercavernous septa (IC) containing fibers, stromal fibroblasts, and smooth muscle cells (arrows) under the endothelium of cavernous spaces. **F.** Conversely, in the corpus cavernosum penis of the treated rat, note absence of cavernous spaces, decreased thickness of tunica albuginea (TA), and increased deposition of empty-appearing fat cells (*). **C and G.** Epoxy sections of the corpus cavernosum from the control and treated rats. Note increased deposition of fat cells in the treated rat; cavernous spaces (CS), tunica albuginea (TA), small arteriole (arrow). **D and H.** In these low-magnification, unparaffinized sections of the body of the penis, note much higher accumulation of fat cells in the corpora cavernosa penis of the treated rat. **A, B, E, and F.** Hematoxylin and eosin, (C and G) toluidine blue, (**D and H**) fat stain. Scale bars: A, D, E, and H, 100 mm; B, C, F, and G, 30 mm. [Reprinted from Goyal et al. Abnormal morphology of the penis in male rats exposed neonatally to diethylstilbestrol is associated with altered profile of estrogen receptor-a protein, but not of androgen receptor protein: a developmental and immunocytochemical study. Biology of Reproduction, with permission from The Society for the Study of Reproduction].

detumescence (Kandel et al., 2001).

Finally, it is interesting to note that levels of ER, together with AR and progesterone receptor (PR) are down-regulated in the penile crura of aging rats and are associated with aging-related erectile dysfunction (Shirai et al., 2003).

Effect of xenoestrogens, knockout and mutation of aromatase and ER on the penis

Xenoestrogens

As stated above, interest in the role of estrogen in penile events was, to a large measure initiated by reports that *in utero* exposure of male offspring (human, laboratory and wildlife) to estrogen-like endocrine disruptors (EED) induce micropenis (McLachlan et al., 1975; Raman-Wilms et al., 1995; Visser et al., 1998). Subsequent studies reported that mothers with significant intake of foods rich in estrogens (phytoestrogen) were more likely to give birth to boys with hypospadia (see North and Golding, 2000). Other EED, such as the estrogen antagonist tamoxifen, when given to neonatal rats permanently disrupts differentiation of os penis and completely erases epidermal projections and keratinization of the glans penis (Iguchi et al., 1990; Deveci et al., 1997). Clearly, taken together, these data strongly point to the presence of an estrogen system in the penis. However, the specific mechanisms underlying the effects of EED on penile development are unclear.

Human mutations and Knockout animal models of aromatase and ER

Data obtained from studies of animal models lacking ER and aromatase and men with mutations of these genes, have greatly enhanced our understanding of the functional significance of estrogen and its receptors in male fertility (Korach, 1994; Smith et al., 1994; Morishima et al., 1995; Eddy et al., 1996; Carani et al., 1997, 1999; Bilezikian et al., 1998; Fisher et al., 1998; Krege et al., 1998; Faustini-Fustini et al., 1999; Li et al., 2001; Rochira et al., 2001). However, the effects of these mutations and gene deletions or over-expressions on the penile events have not been adequately addressed. Based on the distribution and abundance of ER and aromatase in the developing penis and the inhibitory effects of EED on penile growth, one would expect that a lack or deficiency of aromatase and or ER in animal models and human, respectively, would lead to enhanced penile growth or size. To the contrary, and surprisingly, the preliminary data, thus far, report normal development of the male external genitalia in men, suggesting that estrogen and ER are not important regulators of penile development and growth (Morishima et al., 1995; Carani et al., 1997, 1999; Bilezikian et al., 1998; Faustini-Fustini et al., 1999). There is need to undertake detailed and systematic investigations using the available animal models in order to address these discrepancies. Additionally, and of equal importance, it is essential to investigate whether deficiency or deletion of the estrogen-ER system influence the androgen-AR system in the penis, as is the case in some areas of the brain (McAbee and DonCarlos, 1999). These studies should shed light on the extent or degree to which the two hormonal systems interact in regulating penile development and function.

Conclusion

The field of estrogen and ER in penile events is the latest 'frontier' in the study of estrogen in male reproduction. Although important findings are beginning to emerge, most of these findings need to be reproduced by other workers to arrive at a consensus. Secondly, there remains a lot of unanswered questions. The most notable questions include: 1) the interaction of the estrogen-ER system with the principal regulator of penile function, the androgen-AR system, 2) a more detailed cellular expression of ER-α and -ß in the different penile compartments and subpopulations of cells in development, adulthood and in different species, 3) delineation of the specific functional roles of estrogen in the different subpopulations of cells, 4) a more systematic and comprehensive study of the impact of lack or deficiency of estrogen and ER on penile events and on the androgen-AR system. Thus far, data emerging from various studies appear to suggest a role for estrogen in penile development and growth. These data may challenge the long held dogma that androgens are the sole regulators of penile development and function.

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