Estrogen receptor-α mediates estrogen-inducible abnormalities in the developing penis

H O Goyal1, T D Braden3, P S Cooke4, M A Szewczykowski4, C S Williams1, P Dalvi1 and J W Williams2

Departments of 1Biomedical Sciences and 2Biology and CBR/RCMI, Tuskegee University, Tuskegee, Alabama 36088, USA, 3Department of Anatomy, Physiology and Pharmacology, Auburn University, Auburn, Alabama, USA and 4Department of Veterinary Biosciences, University of Illinois, Urbana, Illinois, USA

Correspondence should be addressed to H O Goyal who is now at Department of Biomedical Science, College of Veterinary Medicine, Nursing and Allied Health, Tuskegee University, Tuskegee, Alabama 36088, USA; Email: goyalho@tuskegee.edu

Abstract

Previously, we reported an association between estrogen receptor-α (ERα) upregulation and detrimental effects of neonatal diethylstilbestrol (DES) exposure in the rat penis. The objective of this study was to employ the ERα knockout (ERαKO) mouse model to test the hypothesis that ERα mediates DES effects in the developing penis. ERαKO and wild-type C57BL/6 mice received oil or DES at a dose of 0.2 μg/pup per day (0.1 mg/kg) on alternate days from postnatal days 2 to 12. Fertility was tested at 80–240 days of age and tissues were examined at 96–255 days of age. DES caused malformation of the os penis, significant reductions in penile length, diameter, and weight, accumulation of fat cells in the corpora cavernosa penis, and significant reductions in weight of the bulbospongiosus and levator ani muscles in wild-type mice. Conversely, ERαKO mice treated with DES developed none of the above abnormalities. While nine out of ten male mice sired pups in the wild-type/control group, none did in the wild-type/DES group. ERαKO mice, despite normal penile development, are inherently infertile. Both plasma and intratesticular testosterone levels were unaltered in the DES-treated wild-type or DES-treated ERαKO mice when compared with controls, although testosterone concentration was much higher in the ERαKO mice. Hence, the resistance of ERαKO mice to developing penile abnormalities provides unequivocal evidence of an obligatory role for ERα in mediating the harmful effects of neonatal DES exposure in the developing penis.


Introduction

It is well known that androgens are critical in development of male reproductive organs in all mammals studied to date (George & Wilson 1994). It is also known that dihydrotestosterone, a 5α-reductase reduced testosterone metabolite, is more critical than testosterone in development of those organs which are derived from the urogenital sinus, genital tubercle, and genital swelling in males, including the penis (Anderson & Clark 1990, George & Wilson 1994). Notably, the early differentiation and morphogenesis of male reproductive organs corresponds to the testosterone surge by fetal Leydig cells that occurs at about 12 weeks of gestation in humans (Williams-Ashman & Reddi 1991, George & Wilson 1994, Klonisch et al. 2004) and at late gestation and early neonatal period in rodents (Ward & Weisz 1984, El-Gehani et al. 1998). Alterations in androgenic activity during the critical period of differentiation, resulting from abnormalities in testosterone, 5α-reductase, or androgen receptor, cause maldevelopment of internal and external male genitalia, including hypospadias and shorter penis (Gray et al. 2001, Sultan et al. 2001, Kim et al. 2002, Foster & Harris 2005). In a situation where androgen receptors are inherently lacking, such as in androgen insensitivity syndrome, external genitalia develop as those of females (Wiener et al. 1997, Regadera et al. 1999, Hiort 2000).

Unlike the case with androgens, the role of estrogen in development of male reproductive organs, especially in the penis, remains largely unknown; although both estrogen receptors (ERs) and/or aromatase enzyme have been localized in the developing penis of a number of species, including humans (Crescioli et al. 2003, Schultheiss et al. 2003, Dietrich et al. 2004) and rodents (Jesmin et al. 2002, 2004), and rabbits (Srilatha & Adaikan 2004). Additionally, epidemiological studies have suggested links between inappropriate estrogen exposure and higher frequency of reproductive
abnormalities in men and wild animals (Toppari et al. 1996, McLachlan et al. 2001, Safe et al. 2001, Mosconi et al. 2002, Fisher 2004, Vidaeff & Sever 2005, Storgaard et al. 2006, reviews). In this context, it is noteworthy that male offspring of women exposed to diethylstilbestrol (DES) during pregnancy have higher incidence of epididymal cysts, cryptorchidism, hypospadias, and smaller penis (Gill et al. 1979, Swan 2000, Klip et al. 2002), as well as higher incidence of hypospadias in sons of women exposed to DES in fetus, implying a transgenerational effect (Klip et al. 2002, Brouwers et al. 2006); pregnant mothers with high intake of phytoestrogens as a result of vegetarian diet are more likely to give birth to boys with hypospadias (North & Golden 2000); laboratory animals exposed neonatally to estrogen develop hypospadias (McLachlan et al. 1975, Kim et al. 2004, Newbold 2004); neonatal exposure to estrogen at low doses enlarges the adult prostate gland, while higher doses have the opposite effect (vom Saal et al. 1997, Gupta 2000, Putz et al. 2001, vom Saal & Hughes 2005); and perinatal estrogen exposure predisposes the prostate gland to a precancerous growth at adulthood by an epigenetic mechanism (Ho et al. 2006). Hence, prenatal and/or neonatal exposure to estrogens can have permanent, and even transgenerational, deleterious effects on the development of male reproductive organs; however, the mechanism underlying estrogen-inducible abnormal phenotypes in the male reproductive tract, as well as in the penis, remains unknown.

Recently, we reported permanent dysmorphogenesis of the penis and loss of fertility in adult rats-treated neonatally with DES or estradiol valerate (Goyal et al. 2004a,b, 2005a,b). Specifically, these studies showed significant reductions in penile length and weight, maldevelopment of the os penis, and accumulation of fat cells and loss of cavernous spaces and smooth muscle cells in the body of the penis. Furthermore induction of these novel phenotypes is dose-dependent, requires a critical window of exposure, and is associated with decreased plasma testosterone and upregulation of ERx expression in the body of the penis. These observations lead us to hypothesize that a functional ERx-mediated pathway is required in induction of aberrant penile development.

Hence, the objective of this study is to test the above hypothesis by treating ERx knockout (ERxKO) mice neonatally with DES because they inherently lack ERx and, therefore, should be resistant to DES-inducible penile abnormalities, which should develop in the wild-type mice treated similarly.

Materials and Methods

Animals and treatments

Heterozygous breeding pairs for the ERx null allele were crossed to generate ERxKO and wild-type C57BL/6 mice in a continuous breeding scheme at the University of Illinois at Urbana-Champaign. Pups were treated with DES at a dose of 200 ng (0.1 mg/kg) in 25 μl olive oil, per pup, every other day, from postnatal days 2 to 12 (note, the same dose regimen was used in our previous studies in the rat, Goyal et al. 2004a,b). Controls received olive oil only. DNA was isolated between 8 and 12 days of age, from tail tissues using DirectPCR Lysis Reagent (Viagen Biotech Inc., Los Angeles, CA, USA) and used to determine genotype. Animals were shipped to Tuskegee University at adulthood for further study. Animals at both universities were maintained at 22–23 °C ambient temperature, 55–60% relative humidity, and 12 h light:12 h darkness cycle, and had free access to food (Rodent Chow 5001; Purina Mills, St.Louis, MO, USA) and water for 24 h. Animals were handled in accordance with the guidelines for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, National Research Council, National Academy Press, Washington, DC, 1996). All animal procedures were approved by the Institutional Animal Care and Use Committees of the University of Illinois at Urbana-Champaign and Tuskegee University.

Body and organ weights

All animals were weighed and terminated at 96–255 days of age. The seminal vesicles were weighed as a marker for DES effects on the accessory gland because estrogens are known to have an inhibitory effect on this gland (Goyal et al. 2003, 2004a,b). The testes and epididymides were also weighed, but the data are not included in the study because the weight of both organs in the wild-type/DES mice and that of the testes in the ERxKO/DES KO mice varied widely among animals. These variations resulted from retention of the testis and epididymis of one or both sides in the inguinal canal in more than 70% of the wild-type/DES mice and from accumulation of the testicular fluid in the testis of one or both sides of the ERxKO mice, which worsened with age.

Penis and penile skeletal muscles

The penis was measured for length, diameter, and weight and was processed as described previously (Goyal et al. 2005a,b). Briefly, the penis was exposed up to the ischial arch, and its stretched length was measured from the tip of the glans penis to the midpoint of the ischial arch, and the diameter was measured from the middle of the body of the penis. After removing the free loose connective tissue, the entire penis was weighed. Two to three millimeter long sections from the middle of the body were processed for histopathology and histochemistry (n≥5 per group). For histopathology, tissues were fixed in formaldehyde, and 5 μm thick paraffin sections were
stained with hematoxylin and eosin (H&E) for general morphology and with Masson’s Trichrome stain for smooth muscle. For histochemical demonstration of fat, formaldehyde-fixed tissues were en bloc stained for 8 h with 1% osmium tetroxide dissolved in 2.5% potassium dichromate solution, and then processed for paraffin embedding. In addition, epoxy sections (1 μm thick) of glutaraldehyde-fixed tissues were stained with toluidine blue. For evaluating the development of os penis, 1 penis from each group was radiographed with a cabinet radiograph system (Faxitron series, Hewlett-Packard) as described previously (Goyal et al. 2004b).

The rodent penis is surrounded by three pairs of skeletal muscles: ischiocavernosus extends from the ischial arch to the middle of the dorsal surface of the body of the penis; bulbospongiosus surrounds the ventro-lateral surface of the bulb of the penis; and levator ani forms a sling-like band around the anus and is connected dorsally to the bulbospongiosus. The latter two muscles were isolated, freed from connective tissue and fat, and weighed separately.

Digital images of paraffin and epoxy sections, as well as of the radiograph, were captured with a Leitz Orthoplan microscope (Vashaw Scientific Inc., Norcross, GA, USA) and the Kodak Microscopy Documentation System 290 (Eastman Kodak Company), and were assembled with the use of Adobe Photoshop 7.0.

**Plasma and intratesticular testosterone**

For plasma testosterone, one blood sample was collected by cardiac puncture from each animal just prior to necropsy; and for intratesticular testosterone, a part of the right testis was collected from each animal at the time of necropsy. Both plasma and testicular parenchyma were frozen at −20 °C until assayed. Testes were processed in accordance with the protocol described by Park et al. (2002). Briefly, ~50 mg testicular tissue was homogenized in PBS. Eight volumes of ether were added to the homogenate and vortexed vigorously. The aqueous phase was snap-frozen, and the organic supernatant was transferred to a secondary tube and air dried. Just prior to assay, samples were resuspended in PBS. Testosterone in plasma and testicular tissue was measured using a COAT-A-COUNT testosterone RIA (Diagnostic Products Corporation, Los Angeles, CA, USA) according to manufacturer’s protocol. The sensitivity of the assay was 0.2 ng/ml. The intra- and interassay coefficients of variations were 6 and 12% respectively.

**Fertility**

Eight to ten adult male mice from each group were transferred to mating cages and cohabited with untreated, 70- to 80-day-old females (1:2) for 12 days. At the end of cohabitation, females were separated and allowed to deliver at term. The number of pups was recorded for each litter.

**Statistical analysis**

Statistical analyses were performed using Sigma Stat statistical software (Jandel Scientific, Chicago, IL, USA). Two-way ANOVA was performed on all parameters. Treatment groups with means significantly different (P<0.05) from controls were identified using the Student–Newman–Keuls (SNK) test. When data were not distributed normally, or heterogeneity of variance was identified, analyses were performed on transformed data or ranked data.

**Results**

**Body and organ weights**

The mean body weight in the adult wild-type and ERαKO control mice was 25.88 and 26.53 g respectively, and was not altered as a result of DES treatment (25.68 vs 26.82 g). Seminal vesicles were weighed as a marker for DES effects in the male accessory sex organs. Their weight was reduced by almost 70% in the wild-type/DES mice when compared with that of wild-type controls, but was not significantly (P>0.05) different between the ERαKO/DES and ERαKO/control mice (Fig. 1). Generally, seminal vesicles were one, one and half or two times heavier in the ERαKO mice than in the wild-type/control mice.

![Figure 1](https://www.reproduction-online.org)

*Figure 1* The paired weight of the seminal vesicles (SV) in wild-type and ERαKO adult mice treated neonatally with oil (control) or DES. An asterisk indicates a significant (P<0.05) reduction in weight in the wild-type mice, as a result of DES treatment. Bars with different superscripts indicate significantly higher weight in the ERαKO/control mice than in the wild-type/control mice (P<0.05). Data are expressed as mean ± s.e.m.
**Penile measurements**

The mean measurements of the penis, including weight, length, and diameter, were significantly ($P<0.05$) lower in wild-type/DES mice when compared with those of wild-type/control mice (Fig. 2); and the decrease was more pronounced for weight than length (56% vs 25%). Conversely, DES exposure failed to exhibit any of the above detrimental effects in the penis of ERαKO mice. All penile measurements in the ERαKO/DES or ERαKO/control mice were similar to those in the wild-type/control mice.

**Penile skeletal muscles**

The weight of both bulbospongiosus and levator ani muscles was significantly ($P<0.05$) lower in the wild-type/DES mice when compared with that of the wild-type/control mice (Fig. 3), although the percent reduction was much higher for the former muscle (68% vs 35%). In contrast, DES failed to affect the weight of either muscle in the ERαKO mice. The weight of both muscles was almost 10% higher in both the ERαKO/DES and ERαKO/control mice than in the wild-type/control mice; however, the increase was significant ($P<0.05$) only in the case of the bulbospongiosus muscle.

**Penile gross morphology, histopathology, and histochemistry**

**Gross morphology**

The mouse penis, similar to the rat penis (Goyal et al. 2004a), consists of a root, a cylindrical body, and a bulbous glans penis. An os penis extends from the distal end of the body to the tip of the glans penis and is responsible for the right angle bend between the body and the glans penis in a non-erect penis (Fig. 4). Based on gross and radiographic examinations, striking effects of neonatal DES exposure in the wild-type mice included a reduction in the right angle and malformations of the os penis, including its underdevelopment and less calcification. Conversely, these effects were completely lacking in the penis of DES-treated ERαKO mice, which was morphologically similar to that of the ERαKO/control and wild-type/control mice (Fig. 4).
Histopathology and histochemistry

The body of the penis in both wild-type and ERα KO mice consists of three erectile bodies: two corpora cavernosa that are located dorso-lateral to the urethra and are partly separated by an intercrural septum that brings blood vessels and nerves to the crura and a corpus spongiosus that is located ventrally and surrounds the urethra (Fig. 5A–D). The corpora cavernosa contain endothelial-lined cavernous spaces (also called sinusoids), thin strands of smooth muscle cells surrounding the endothelium, and connective tissue trabeculae between cavernous spaces. In addition, a thick tunica albuginea capsule, consisting of an outer fibrous layer and an inner cellular layer, surrounds the corpora cavernosa.

An examination of paraffin sections stained with hematoxylin and eosin or Masson’s Trichrome (distinguishes smooth muscle cells from collagen fibers) revealed that, among various histological components of the penis, the tunica albuginea capsule and connective tissue trabeculae were less developed in the DES-treated wild-type mice when compared with those of the wild-type/control mice (Fig. 6A and B). In addition, an examination of epoxy sections stained with toluidine blue (Fig. 6C and D) and of undeparaffinized sections stained with osmium tetroxide (Fig. 7A–D) showed an abnormal accumulation of fat cells in the DES-treated wild-type mice. Again, none of the above histopathological abnormalities resulted in the penis of DES-treated ERα KO mice, which was structurally similar to that of the wild-type/control or the ERα KO/control mice.

Plasma and intratesticular testosterone

Neonatal DES exposure did not significantly (P<0.05) alter the mean concentration of plasma or intratesticular testosterone in the wild-type or the ERα KO mice at adulthood (Fig. 8); however, both concentrations were almost three- to four-fold higher in the DES- or oil-treated ERα KO mice than in the wild-type/control mice.

Fertility

While nine out of ten males in the wild-type/control group sired pups, with a mean litter size of 6.5 pups (ranging from 4 to 11 pups/l), no male sired a pup in the DES-treated wild-type group or ERα KO groups treated with oil or DES (n=8/group).

Discussion

The objective of this study was to test the hypothesis that ERα is essential in mediating detrimental effects of
neonatal estrogen exposure in the developing penis. To test this hypothesis, we employed the ERα KO mouse model because it lacks ERα genetically. We reasoned that if ERα expression is obligatory then ERαKO mice should be resistant to the harmful effects of neonatal DES exposure. Results of the present study showed for the first time that ERαKO males were completely resistant to the harmful effects of neonatal DES exposure, which were consistently observed in the penis of wild-type C57BL/6 mice, providing unequivocal evidence of an obligatory role for ERα in mediating estrogen-inducible abnormalities in the developing penis.

The penis is not the only reproductive organ in which the presence of ERα is crucial for estrogen-inducible developmental deformities. The prostate gland in the ERαKO males treated neonatally with DES on postnatal days 1, 3, and 5 and examined at different ages up to 18 months of age were normal in all age groups, while that of the wild-type mice treated similarly developed alterations at the cellular and molecular levels, including epithelial hyperplasia and dysplasia, which worsened with age (Prins et al. 2001). Adult ERαKO female mice treated with DES neonatally failed to develop pathological abnormalities that were observed in the uterus, oviduct, and vagina of similarly treated wild-type mice (Couse et al. 2001). On the other hand, mice lacking ERβ (ERβKO) and treated with DES neonatally developed abnormalities in the prostate (Prins et al. 2001) and female genital organs (Couse & Korach 2004), similar to those observed in the DES-treated wild-type mice. Thus, the above studies, as well as our present study, underscore an essential role of ERα in mediating detrimental effects of neonatal estrogen exposure not only in the penis but also in other reproductive organs of both sexes.
Similarities in penile abnormalities presently observed in DES-treated wild-type mice with those previously reported from our laboratory in DES-treated rats (Goyal et al. 2005a, b), including malformations of the os penis; reductions in weight, length, and diameter of the penis; reductions in thickness of the tunica albuginea capsule and connective tissue trabeculae in the corpora cavernosa penis; and abnormal accumulation of fat cells in the corpora cavernosa penis; clearly identify the penis as a target organ for harmful effects of neonatal estrogen exposure. Similarly, other studies also reported a smaller penis in laboratory animals exposed neonatally to estrogen (McLachlan et al. 1975, Zadina et al. 1979, Newbold 2004); and the rabbit penis treated with bisphenol A (Moon et al. 2001) or tetrachlorodibenzo-dioxin (Moon et al. 2004) had subtunical deposition of fat in the corpora cavernosa penis. Additionally, alligators from Lake Apopka, FL, contaminated with environmental pollutants had smaller phalluses (Guillette et al. 1994, 1996, Milnes et al. 2005); and male offspring of women exposed to DES during pregnancy had smaller penises (Gill et al. 1979, Swan 2000).

However, unlike in the DES-treated rat penis, which also showed loss of cavernous spaces (sinusoids) and smooth muscle cells (Goyal et al. 2004a, b, 2005a, b), both structures were present in the corpora cavernosa penis of wild-type mice treated neonatally with DES. Reasons for this difference may be attributed to differences in the species, or to the developmental stage of the penis at the time of estrogen exposure, because, even within the rat, the severity of loss of smooth muscle cells and cavernous spaces and the degree of accumulation of fat cells was higher in the penis exposed to estrogen from postnatal days 1 to 6 than that exposed from postnatal days 7 to 12.
we previously found an association between ERα upregulation and penile abnormalities in the developing rat penis treated neonatally with DES (Goyal et al. 2004b, 2005b). Similarly, ERα upregulation is associated with abnormal development of rodent female reproductive tract (Yamashita et al. 1990, Markey et al. 2005), mammary gland (Tekmal et al. 2005), male reproductive tract (Sato et al. 1994), prostate gland (Prins & Birch 1997, Prins et al. 2001), and seminal vesicles (Williams et al. 2001). In addition, ERα overexpression is shown to inhibit growth and angiogenic factors in the endometrial carcinoma cell line Ishikawa (Ali et al. 2004); and ERα is the main regulator of estrogenic effect on adipose tissue since an alteration in estrogen/ER signaling during development results in dramatic changes in adipocyte number (Cooke & Naaz 2004).

Another mechanism of estrogen-inducible, developmental penile abnormalities may involve alterations in androgen action because androgen receptors are ubiquitously present in the rat penis (Goyal et al. 2004b), and their concentration is maximal prior to puberty (Rajfer et al. 1980, Takane et al. 1990). Additionally, neonatal estrogen exposure downregulates androgen receptor level in male reproductive organs (Prins & Birch 1995, McKinnell et al. 2001, Williams et al. 2001, Woodham et al. 2003) and lowers plasma testosterone (Sharpe et al. 1998, Atanassova et al. 2000). Testosterone coadministration with estrogen mitigates developmental abnormalities affecting the male reproductive tract (Rivas et al. 2003). In vitro treatment with androgens promotes smooth muscle differentiation and inhibits adipocyte differentiation in C3H 10T1/2 pluripotent mesenchymal cells (Bhasin et al. 2003, Singh et al. 2003), and castration induces subcutaneous fat deposition and loss of smooth muscle cells in the corpora cavernosa of the rabbit penis (Traish & Kim 2005, Traish et al. 2005). Taken together, these observations raise the possibility that lower androgen action via estrogen-induced decrease in testosterone or androgen receptor or both as potential mechanisms contributing to penile abnormalities.

Although neonatal DES exposure in the present study did not significantly alter the adult level of plasma or intratesticular testosterone in wild-type mice, the neonatal level of intratesticular testosterone in rat pups treated with DES for 1–3 postnatal days was reduced by 90% at postnatal days 5–8 (Goyal et al. 2005b), the developmental period when stromal cells start differentiation in the rat penis (Murakami 1986, 1987), thus suggesting a role for estrogen-induced lower androgen action in inducing penile abnormalities by reprogramming stromal cell differentiation. In this context, observations that endogenous estrogens or DES exposure to fetal or neonatal Leydig cells decreased testosterone secretion in wild-type mice, but not in ERαKO mice, implied an ERα-mediated inhibitory role of estrogen in testosterone secretion (Delbes et al. 2005). Supporting...
this role and, in agreement with the previous studies (Eddy et al. 1996, Akingbemi et al. 2003), both plasma and intratesticular testosterone levels are much higher in ERαKO mice than in wild-type mice. Hence, it is hypothesized that the perinatal testosterone surge, typical for rodents from late gestation to first week of life, may be a natural mechanism of downregulating ERα expression in penile stromal cells and thereby safeguarding their normal differentiation at a critical time of penile development. In other words, estrogen-induced downregulation of the perinatal testosterone surge results in ERα upregulation in penile stromal cells and, consequently, abnormal development of the penis in the wild-type mice. Alternatively, the lack of effect in the ERαKO mice could be due to the increased testosterone, which provided protection against DES effects in the same way that testosterone administration protected against DES effects in the neonatal rat (Rivas et al. 2003).

In agreement with our previous observations in the rat (Goyal et al. 2005b), the weight of both penile skeletal muscles, bulbospongious and levator ani was not only significantly decreased but also was differentially decreased, with the former muscle undergoing a much higher decrease, as a result of neonatal estrogen exposure to wild-type mice. The mechanism of this differential decrease may lie in differences in androgen receptor concentration because both muscles are androgen dependent (Breedlove & Arnold 1983, Hadi Mansouri et al. 2003) and, interestingly, both muscles are more developed in ERαKO mice than in wild-type/ control mice. Likewise, a dramatic weight reduction observed in the seminal vesicle in the DES-treated wild-type mice is consistent with our previous data in the rat (Goyal et al. 2005a,b), as well as from other laboratories (Atanassova et al. 2000, Putz et al. 2001, Hendry et al. 2006); and a significantly higher weight of the seminal vesicles in ERαKO mice when compared with wild-type/ control mice is also in agreement with previous observations (Eddy et al. 1996, Couse & Korach 1999), and is probably attributed to higher testosterone levels in these animals.

In conclusion, ERαKO mice are resistant to estrogen-inducible penile abnormalities present in wild-type mice, implying an unequivocal role for ERα in mediating maldevelopment of the penis.

Acknowledgements

Authors acknowledge the technical help of Ms Salimata Kone-Coulibaly in tissue collection, and Dr John R Kammermann (Auburn University) in radiographs. This research was supported by NIH grants MBSR-5-506-GM-08091 (to HG) and RCM1-5-G12RR03059 and by USDA grant CSR-EES-ALX-TU-CTIF. The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

References


Anderson CA & Clark RL 1990 External genitalia of the rat: normal development and the histogenesis of 5α-reductase inhibitor-induced abnormalities. Teratology 42 483–496.


Couse JF & Korach KS 1999 Estrogen receptor null mice: what have we learned and where will they lead us? Endocrine Review 20 358–417.

Couse JF & Korach KS 2004 Estrogen receptor-α mediates the developmental effects of neonatal diethylstilbestrol (DES) exposure in the murine reproductive tract. Toxicology 205 55-62.


Foster PMD & Harris MW 2005 Changes in androgen-mediated reproductive development in male rat offspring exposure to a single oral dose of flutamide at different gestational ages. Toxicological Sciences 85 1024–1032.
H O Goyal and others


Park TJ, Song KY, Sohn SH & Lim IK 2002 Marked inhibition of testosterone biosynthesis by hepatotoxin nodularin due to apoptosis of Leydig cells. Molecular Carcinogenesis 34 151–163.


Srilatha B & Adaiak PG 2004 Estrogen and phytoestrogen predispose to erectile dysfunction: Do ER-α and ER-β in the cavernous play a role? Urology 63:382–386.


Received 14 November 2006
First decision 21 December 2006
Revised manuscript received 30 January 2007
Accepted 12 February 2007