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ESTROGEN-INDUCED ABNORMAL MORPHOLOGY OF THE PENIS, ALTERED SEXUAL BEHAVIOR AND LOSS OF FERTILITY IN MALE RATS

GOYAL HO[1], BRADEN TD[3], WILLIAMS CS[1], WILLIAMS JW[2], DALVI P[1] and MANSOUR M[1]

Departments of Biomedical Sciences[1] and Biology/CBR/RCMI[2], Tuskegee University, Tuskegee, AL 36088, USA, and Department of Anatomy, Physiology and Pharmacology[3], Auburn University, Auburn, AL 36849, USA.

SUMMARY

Penile abnormalities, including hypospadias and/or smaller phallus, have been reported in laboratory animals and alligators exposed to estrogenic chemicals, as well as in offspring of women treated with diethylstilbestrol (DES) during pregnancy. In addition, estrogen receptors have been identified in the penis; however, the mechanism of estrogen action in inducing penile abnormalities is not clear. In this study we determined whether reduced fertility and altered sexual behavior in adult rats treated with (DES) neonatally or at adulthood are associated with structural changes in the penis and whether testosterone (T) supplementation can reverse the effects of DES. Plasma T was significantly decreased *($P < 0.05$)*, whether DES was given neonatally or at adulthood. The penile morphology was adversely affected in rats that received DES neonatally, including reductions in length, diameter and weight of the penis, and complete replacement of cavernous spaces with fat cells in the body of the penis. None of the neonatal DES-treated males (0/6) sired a pup or produced a copulatory plug, in contrast to 6/7 in controls. Supplementation with T restored penile morphology and sexual behavior (as indicated by the presence of copulatory plugs, 6/6) to an almost normal level, but fecundity (ability to sire pups, 3/6) only partially. Conversely, penile morphology was not altered in rats that received DES at adulthood, although adverse effects on sexual behavior and fecundity, and their prevention by T supplementation, were similar to those of the neonatal group. Hence, neonatal estrogen exposure, but not adult estrogen exposure, leads to abnormal morphology of the penis; however, both exposures result in reduced fertility and altered sexual behavior. Supplementation with T prevents many estrogen-induced effects.

Key words: Estrogen; Fertility; Penis; Tesosterone.

INTRODUCTION

The role of estrogen in male reproduction remains unclear, although it is known that an exposure of fetal, neonatal, immature, and/or mature animals, including humans, to estrogens causes a range of reproductive disorders, including retained testis, atrophic testis, epididymal abnormalities, delayed puberty, and/or infertility (1). Additionally, recent reports of infertility in male mice lacking estrogen receptor α (2) or aromatase enzyme (3, 4) have underscored the significance of estrogen and its receptors in reproductive physiology.

*Correspondence Address: H. O. Goyal, Department of Biomedical Sciences, School of Veterinary Medicine, Tuskegee University, Tuskegee, AL 36088, goyalho@tuskegee.edu, 334-727-8465 (Tel), 334-727-8177 (Fax)

Our long-range goal is to understand how estrogens mediate reproductive disorders. Previously, we reported that adult rats treated neonatally (5) or at adulthood (6) with diethylstilbestrol (DES) were infertile, and the loss of fertility was associated with decreased plasma testosterone (T) and altered sexual behavior (as indicated by the absence of copulatory plugs). Objectives of this study are to determine whether DES-induced reduced fertility and altered sexual behavior are associated with penile abnormalities and whether T supplementation can reverse the effects of DES.

MATERIALS AND METHODS

Animals and treatments

Neonatal and/or adult Sprague-Dawley male and female rats (Harlan Sprague Dawley, Indianapolis, IN) were maintained under controlled conditions as described previously from our laboratory (5). All animal procedures were approved by the Institutional Animal Care and Use Committee at Tuskegee University.

(a) Neonatal treatment: Neonatal pups (5-8 males/group) received sc injections of 25 ml of olive oil containing DES (Sigma Chemical, St. Louis, MO) at a dose of 5 mg/rat/day or DES and T (100 mg/rat/day) (Sigma Cemical, St. Louis) or T alone on alternate days from postnatal day 2-12. Controls received oil only. Adult males were tested for fertility at 160 days of age and killed for evaluation of the penis and reproductive hormones at 180 days of age.

(b) Adult treatment: Adult male rats (9-10/group) received DES, or DES and T implant, or oil and empty implant (control). DES was sc injected daily at a dose of 10 mg/rat/day in 0.2 ml of olive oil for 12 days (note, similar dose induced 100% infertility in our previous experiment, 6). The T implants (2.5 cm long) were made from Silastic tubing (3.18 x 1.98 mm, Dow Corning #508-009) and placed subdermally in the scapular area. Fertility was tested on the thirteenth day of the implant, and rats were killed for examination of the penis and reproductive hormones on the fourteenth day of the implant.

Fertility: Male rats treated neonatally (Experiment A) were cohabited with untreated, adult females (1:1) for 12 days, and male rats treated at adulthood (Experiment B) were cohabited with LHRH-synchronized females (1:1) for 24 h. The cohabitation with synchronized females allowed us to visually observe the sexual behavior for the first few hours and to shorten the period of DES treatment during cohabitation since all females were expected to show estrous at a pre-determined time. The procedure for synchronization was the same as described previously from our laboratory (7). Rats were checked twice daily for the presence of copulatory plugs. Females with positive plugs were evaluated for the presence of sperm in vaginal washings, separated, and killed on the fifteenth day of pregnancy for the examination of pups, implantation sites and corpora lutea. Females without plugs were similarly examined on the fifteenth day after the end of cohabitation.

Examination of penis: The penis was grossly examined for its length and weight, and the body of the penis was processed for histopathology and histochemistry as described previously from our laboratory (7). Briefly, the penis was exposed up to the ischial arch, stretched, and measured for the length from the tip of the glans penis to the mid point of the ischial arch with a caliper. For histopathology, three to five mm-thick tissues for paraffin sections and one mm-thick tissues for epoxy sections from the middle of the body of the penis were processed and examined with a light

microscope. For histochemical demonstration of fat, tissues from the body of the penis were processed for *en bloc* staining of fat using the osmium tetroxide method (8).

Reproductive hormones: One blood sample was collected from the heart of each animal prior to necropsy, and plasma was frozen at -20°C until used for T and LH asssays as described previously (7). T was measured using a COAT-A-COUNT testosterone radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA) and LH using materials obtained through NHPP, NIDDK, and Dr. A. F. Parlow.

Statistics: Statistical analyses were performed using Sigma Stat Statistical Software (Jandel Scientific, Chicago, IL). Treatment groups with means significantly different *($P < 0.05$)* from controls were identified using the Dunnett test.

RESULTS

Body weight: The mean body weights (± SEM) at 180 days of age in male rats treated neonatally with DES or DES and T or T alone were similar and not significantly *($p < 0.05$)* different from that of controls (489 ± 27.9). Conversely, the body weights of rats treated with DES or DES and T adulthood were significantly lower *($P < 0.05$)* and were almost 85% and 92% of controls, respectively.

Plasma testosterone: The mean concentration of plasma T was decreased to 16-35% of controls, whether rats received DES neonatally or at adulthood (Table 1). Conversely, co-administration of T with DES at adulthood, but not neonatally, increased plasma T concentration to the control level (1.73 ng/ml *vs* 1.92 ng/ml in controls). The mean plasma LH level ranged from 0.4 to 0.7 ng/ml among groups, but did not differ from that of controls *($P < 0.05$)*, whether DES was given neonatally or at adulthood.

Fertility in male rats treated neonatally with DES: While all females mated with control males (n = 7) had copulatory plugs, and six of them had sperm in vaginal washings and delivered pups (fertility index, 86%), none of the females mated with DES-treated males (n = 6) had plugs or sperm or pups (Table 1). Conversely, while all females mated with the DES plus T group (n = 6) had plugs, only three had sperm in vaginal washings and delivered pups, and the mean numbers of pups/litter was only 3.3, in contrast to 13.6 in controls. All fertility parameters in male rats treated with T alone were similar to those of controls.

Fertility in male rats treated at adulthood with DES: While 78% of females (7/9) mated with males in the control group had plugs, sperm in vaginal washings, and pups, only 20% (2/10) had sperm in vaginal washings and pups and none (0/10) had a plug in the DES group. Conversely, 50-60% of females mated with the DES plus T group had plugs, sperm in vaginal washings, and pups (Table 1). The number of pups ranged from 10-19 in the control and from 13-15 in the DES plus T group, in contrast to two pups in one female and nine pups in the second female of the DES group.

Gross observations of the penis: The penis of the adult, control rats had a cylindrical body, a bulbous glans penis, and a right angle between the two. The unique feature of the rat penis is a cylindrical bone, the OS penis that has proximal (closer to the body) and distal parts and extends from the distal end of the body to the tip of the right angle. The penis was adversely affected in adult rats treated neonatally with DES, but T co-administration with DES restored all features, except weight (Table 1; Fig. 1A). Conversely, DES treatment, with or without T, at adulthood had minimal to no effect on gross parameters of the penis (Table 1).

Table 1. Data on fertility, penis and plasma hormones of neonatal and adult rats. C, control; DES, DES-treated; DES+T, DES and T co-administered; T, T - treated.

	Neonatal[a]				Adulthood[b]		
Parameter	**C**	**DES**	**DES+T**	**T**	**C**	**DES**	**DES+T**
Fertility							
Pregnant/mated	6/7	0/6	3/6	5/7	7/9	2/10	5/10
Copulatory plug/mated	7/7	0/6	6/6	5/7	7/9	0/6	6/10
Sperm/vaginal washings	6/7	0/6	3/6	5/7	7/9	2/10	6/10
Pups/litter	13.6±2.1	NA	3.3±0.8	16.8±0.8	15.5±1.2	5.5±3.5	14.4±0.4
Implantations	13.6±2.1	NA	3.3±0.8	17.0±1.1	15.8±1.1	5.5±3.5	14.4±0.4
Corpora Lutea	16.6±1.0	NA	16.3±1.6	18.2±0.8	16.7±0.7	14.5±0.5	16.6±0.8
Penis[c]							
Length (mm)	42±0.5	31±0.7*	39±0.4	42±0.2	41±0.3	40 ±0.4	40±0.6
Weight (mg)	341±13	136±8*	254±10*	329±9	354±6	344±5	357±7
Plasma Hormones[c]							
Testosterone (ng/ml)	1.5±0.2	0.55±0.3*	0.4±0.1*	1.0±0.3	1.9±0.3	0.3±0.1*	1.7±0.1
LH (ng/ml)	0.7±0.06	0.5±0.1	0.7±0.1	0.7±0.1	0.6±0.06	0.5±0.03	0.4±0.01

Data are presented as means ± SE. [a] DES (5 μg/rat/day), T (100 μg/rat/day) on alternate days from postnatal day 2-12. [b] DES (10 μg/rat/day) daily for 12 days, T (implant). [c] Statistically analyzed for significance. Significantly different from controls *($P < 0.05$)*.

Microscopic observations of the penis: The body of the adult rat penis consists of paired corpora cavernosa penis that are located dorsolaterally and a corpus spongiosus penis that is located ventrally and surrounds the urethra. The corpora cavernosa penis in adult, control rats were surrounded by thick tunica albuginea and consisted of a network of endothelial-lined cavernous spaces that were underlain by smooth muscle cells and separated by dense collagen fibers (Figs. 1B, C). On the other hand, the corpora cavernosa penis in adult animals treated neonatally with DES had much thinner tunica albuginea, lacked cavernous spaces and smooth muscle cells, and were filled with fat cells, as revealed by epoxy sections stained with toluidine blue and by paraffin sections stained *en block* with osmium tetroxide (Figs. 1D, E).

There appeared to be virtually complete replacement of cavernous spaces by fat cells as a result of the neonatal DES treatment, although a few arterioles were invariably observed. The co-administration of T with DES prevented almost all DES-induced structural defects; however, fat cells were still relatively more frequent in the DES plus T group (Figs. 1F, G) than in controls, where only isolated fat cells were encountered (compare Fig. 1C with 1G). The penile morphology in adult rats treated neonatally with T alone was essentially similar to that of controls (Figs. 1H, I).

Unlike in rats treated neonatally with DES, the morphology of the corpora cavernonsa penis in rats treated with DES or DES plus T at adulthood was not different from that of controls. There was no evidence of increased accumulation of fat cells, nor was there any indication of loss of cavernous spaces, smooth muscle cells, or collagen fibers.

Unlike the corpora cavernosa penis, the morphology of the corpus spongiosus was unaltered, whether rats received DES noenatally or at adulthood.

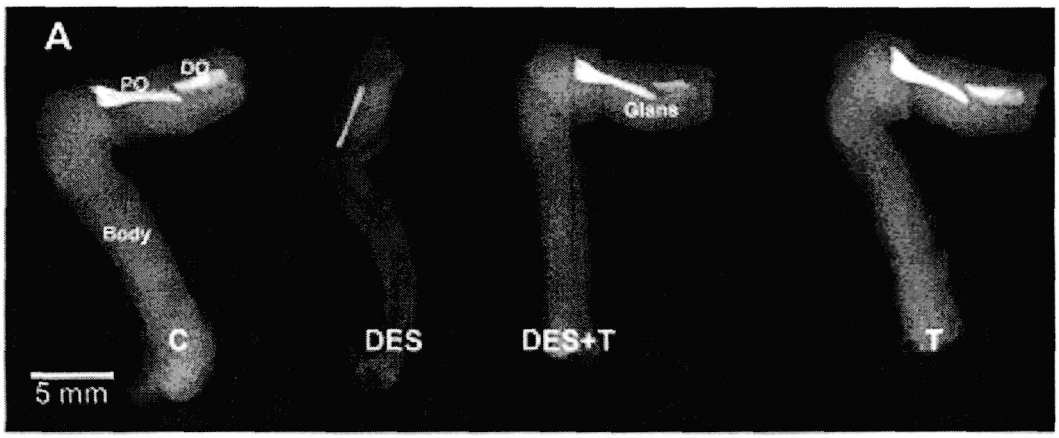

Fig. 1A : Radiographs of the adult penis in control rats (C) and in rats treated neonatally with DES, or DES and testosterone (T), or T. Note reductions in the length and thickness of the penis, the angle between the body and glans of the penis, and the thickness of the proximal part of the os penis (PO), and the lack of development of the distal part of the os penis (DO) in rats treated neonatally with DES and their restoration to almost normal levels in rats co-administered with T and DES.

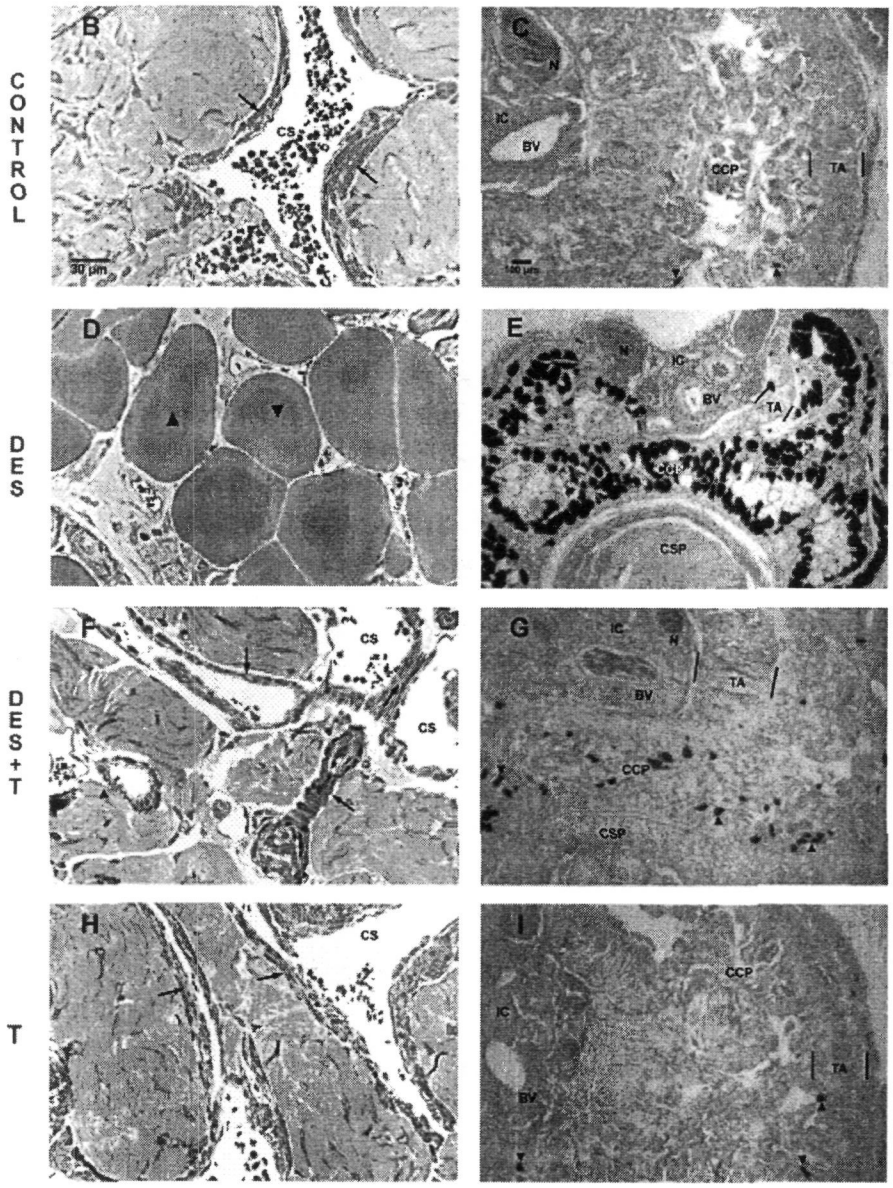

Fig. 1B-H : Micrographs from the body of the adult penis in control rats (**B, C**) and in rats treated neonatally with DES (**D, E**), DES and T (**F, G**) and T alone (**H, I**) retain (**D, F**). **B,D,F,H.** In these micrographs of epoxy sections, note the presence of cavernous spaces (CS) and smooth muscle cells (arrow) in the corpus cavernosus penis of the control and T-treated rats, in contrast to replacement of these structures by fat cells (arrowhead) in the DES-treated rats. Note that co-administration of T and DES reversed many DES-induced effects. **C, E, G, H.** In these low magnification, un-paraffinized sections of the body of the penis, note the presence of isolated fat cells (arrowhead) in the control and T-treated rats, accumulation of fat cells in the corpus cavernosum penis (CCP) of the DES-treated rats, and, in comparison to the latter, a dramatic reduction in fat cells in the DES and T-treated rats. Other structures shown are intercrural septum (IC) containing blood vessel (BV) and nerves (N), tunica albuginea (TA) surrounding the corpus cavernosus penis, and corpus spongiosus penis (CSP). B, D, F and H, toluidine blue, bar equals 30 mm; C, E, G and I, fat stain, bar equals 100 mm.

DISCUSSION

This study evaluated the effects of neonatal *versus* adult exposure to DES on the penis, sexual behavior and fertility in male rats and determined whether co-administration of T with DES can reverse DES-induced effects. Results demonstrated that DES exposure in neonatal rats, but not in adult rats, resulted in abnormalities of the penis including, reductions in length and weight, underdevelopment of the os penis, and virtually complete replacement of cavernous spaces and associated smooth muscle cells by fat cells in the corpora cavernosa penis. Similar histopathological changes in the penis have not been reported in the rat or any other species, except in rabbits that were treated with Bisphenol A at 8-12 weeks of age for 12 days and examined at four or eight weeks after the treatment (9). However, reductions in the length of the penis in rats treated neonatally with estrogen (10) and abnormally small phalluses in alligators exposed to an excessive spill of estrogenic compounds in Lake Apopka in Florida (11, 12) have been documented.

It is well known that the erection of the penis in species with a vascular penis, such as man and rats (13), depends upon the relaxation of smooth muscle cells and the engorgement of cavernous spaces with blood. The replacement of both of these structures by fat cells in the corpora cavernosa penis, but not in the corpus spongiosus, of rats treated with DES neonatally, but not at adulthood, is suggestive of a selective effect of estrogen within the body of the penis and of a critical period of exposure during the development of the penis.

The selective effect appears to be at the level of the helicine artery since both cavernous spaces and smooth muscle cells in the corpora cavernosa penis are direct descendents of this artery (13). It is hypothesized that the helicine artery, rather than breaking into cavernous spaces, formed arterioles and capillaries (in other words, behaved like other body arteries), which provided the needed nutrients to the corpora cavernosa. Estrogens have been shown to inhibit the proliferation of smooth muscle cells of injured blood vessels (14, 15), the endogenous estrogen metabolite, 2-methoxyestradiol, inhibits angiogenesis and thus, suppresses tumor growth (16), and estrogen receptors have been identified in blood vessels of the corpus cavernous penis at postnatal day 1 in rats (17).

In addition to and/or instead of estrogens, the decreased T during the critical period of the penile development may be responsible for the observed abnormalities in the penis since T and its metabolite, dihydrotestosterone, are essential for the development of external genitalia, including the penis (18), and androgen receptors have been identified in penile smooth muscle cells (19). The present observations that the co-administration of T with DES to neonatal rats reversed many DES-induced effects provide credence to the above concept. Also, supporting the concept are data from previous studies including, that the penile abnormalities resulting from castration at birth were partially restored upon androgen substitution at the time of castration in rats (20) and that the co-administration of T with DES prevented most of the morphological abnormalities affecting the male reproductive tract of rats treated neonatally with DES (21). It is noteworthy that we found plasma T concentration at an undetectable level at 18 and 41 days of age in rats that received DES neonatally (personal observations). Similarly, previous studies also reported a marked reduction in plasma T in rats treated neonatally with estrogens (22, 23). In this context, it will be interesting to find out whether similar penile abnormalities could be induced by low T resulting from GnRH antagonist and/or by competitive inhibition of androgen receptor by flutamide treatment.

The present results confirmed earlier reports of reduced fertility and/or altered sexual behavior in male rats that received estrogen neonatally (5, 22, 24) or at adulthood (6, 25, 26). Interestingly, both of these fertility-related disorders in the latter group of animals were not associated with structural

changes in the penis and were almost prevented by co-administration of T with DES, implying that they probably resulted from low T-induced altered sexual behavior. Low T in both men (27) and animals (28) is associated with depressed sexual desire and performance. The decreased sexual activity in castrated rats can be reversed by T substitution (29-31). Androgen insensitive testicular feminization mice have reduced masculine sexual behavior (32). The absence of a structural change in the penis of rats treated with DES at adulthood and of its presence in rats treated with DES neonatally should not be surprising considering that both androgen (33, 34) and estrogen (17) receptors in the rat penis are present at a peak level before puberty and are reduced to a barely detectable level in adulthood.

Another interesting finding of the study was that, despite restoration of normal sexual behavior, the mean number of pups per litter was less than four in rats treated neonatally with DES and T, in contrast to more than 13 in rats that received DES and T at adulthood, or T alone neonatally, or in controls. Based upon an analysis of implantation sites and corpora lutea, the decreased litter size probably resulted from higher pre-implantation loss. The reason(s) for the higher pre-implantation loss are difficult to explain with the available data but could be attributed, in part, to low mean plasma T concentration (0.40 ng/ml in the neonatal DES and T group, in contrast to 1.7 ng/ml in the adult DES and T group and 1.5 ng/ml in controls). The low plasma concentration of T has been shown to compromise sperm functions, including forward motility (5, 6). In addition, the low T may led to weaker erection and/or premature ejaculation, which then deposited seminal plasma with fewer sperm or no sperm (as indicated by the presence of sperm in vaginal washings in 3/6 females, in contrast to copualtroy plugs in 6/6 females mated with males of the neonatal DES and T group). In this context, it is noteworthy that the mean weight of the penis in the neonatal DES and T group, although significantly *($P < 0.05$)* higher than in the neonatal DES group (254 mg *vs* 136 mg), was still significantly lower *($P < 0.05$)* than in controls (341 mg). In addition, despite apparently normal histology of the corpora cavernosa penis, fat cells were still more frequent in the neonatal DES and T group than in controls. It will be interesting to find out whether a higher dose of T supplementation (for example, 200 mg/rat/day, instead of 100 mg/rat/day, the dose currently used) will bring all fertility-related parameters to control levels.

In conclusion, our results provide evidence that exposure of neonatal, but not adult, male rats to DES induces abnormal morphology of the penis, although both exposures lead to lower fertility and altered sexual behavior. The co-administration of T with DES restores almost normal fertility in rats treated with DES at adulthood, and restores nearly normal penile morphology, but only partially restores fertility, in rats treated with DES neonatally. Hence, these data suggest that the loss of fertility and/or abnormal morphology of the penis probably resulted from DES-induced decrease in T.

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REFERENCES

1 Toppari J, Larsen JC, Christiansen P, Giwercman A, Grandjean P, Guillette, LJ, Jegou B, Jensen TK, Jouannet P, Keiding N, Leffers H, McLachlan JA, Meyer OM, Muller J, Rajpert-De Myets E, Scheike T, Sharpe R, Sumpter J and Skakkebaek NE (1996). Male reproductive health and environmental xenoestrogens. *Environ Health Perspect* **104:** 741-803.

2 Eddy EM, Washburn TF, Bunch DO, Goulding EH, Gladen BC, Lubahn DB and Korach KS (1999). Targeted disruption of the estrogen receptor gene in male mice causes alteration of

spermatogenesis and infertility. *Endocrinology* **137:** 4796-4805.

3 Fisher CR, Graves KH, Parlow AF and Simpson ER (1998). Characterization of mice deficient in aromatase (ArKO) because of targeted disruption of the cyp 19 gene. *Proc Natl Acad Sci USA* **95:** 6965-6970.

4 Honda S, Harada N, Ito S, Takagi Y and Maeda S (1998). Disruption of sexual behavior in male aromatase-deficient mice lacking exons 1 and 2 of the cyp 19 gene. *Biochem Biophys Res Commun* **252:** 445-449.

5 Goyal HO, Robateau A, Braden TD, Williams CS, Srivastava KK and Ali K (2003). Neonatal estrogen exposure of male rats alters reproductive functions at adulthood. *Biol Reprod* **68:** 2081-2091.

6 Goyal HO, Braden TD, Mansour M, Williams CS, Kamaleldin A and Srivastava KK (2001). Diethylstilbestrol-treated adult rats with altered epididymal sperm numbers and sperm motility parameters, but without alterations in sperm production and sperm morphology. *Biol Reprod* **64:** 927-934.

7 Goyal HO, Braden TD, Williams CS, Dalvi P, Williams JW and Srivastava KK (2004). Exposure of neonatal male rats induces abnormal morphology of the penis and loss of fertility. *Reprod Toxicol* **18:** 265-274.

8 Luna LG (1968). *Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology*, edn 3, McGraw-Hill Book Company, New York, pp. 143-145.

9 Moon DG, Sung DJ, Kim YS, Cheon J and Kim JJ (2001). Bisphenol A inhibits penile erection via alteration of histology in the rabbit. *Int J Imp Res* **13:** 309-316.

10 Zadina JE, Dunlap JL and Gerall AA (1979). Modifications induced by neonatal steroids in reproductive organs and behavior of male rats. *J Comp Physiol Psychol* **93:** 314-322.

11 Guillette LJ, Gross TS, Masson GR, Matter JM, Percival HF and Woodward AR (1994). Developmental abnormalities of the gonad and abnormal sex hormone concentrations in juvenile alligators from contaminated and control lakes in Florida. *Environ Health Perspect* **102:** 680-688.

12 Guillette LJ, Pickford DB, Crain DA, Rooney AA and Percival HF (1996). Reduction in penis size and plasma testosterone concentration in juvenile alligators living in a contaminated environment. *Gen Comp Endocrinol* **101:** 32-42.

13 Benson GS (1994). Male sexual function : erection, emission, and ejaculation. In : Knobil E and Neil JD (eds.), *The Physiology of Reproduction,* 2 nd edn, Vol. I, Raven Press, New York pp. 1489-1506.

14 Vargas R, Wroblewska B, Rego A, Hatch J and Ramwell PW (1993). Oestradiol inhibits smooth muscle cell proliferation of pig coronary artery. *Br J Pharmacol* **109:** 612-617.

15 Goyal MK and Oparil S (2001). Direct estrogen effects on the cardiovascular system. In: Manni and Verderame MF (eds.), *Contemporary Endocrinology: Selective Estrogen Receptor Modulators: Research and Clinical Applications*, Humana Press. Totowa, NJ, pp. 99-119.

16 Fotsis T, Zhang Y, Pepper MS, Adlercreutz H, Montesano R, Nawroth PP and Schweigerer L (1994). The endogenous oestrogen metabolite 2- methoxyoestradiol inhibits angiogenesis and suppresses tumor growth. *Nature* **368:** 237-239.

17 Jesmin S, Mowa CN, Matsuda N, Salah-Eldin A-E, Togashi H, Sakuma I, Hattori Y and Kitabatake A (2002). Evidence for a potential role of estrogen in the penis: detection of estrogen receptor-a and –ß messenger ribonucleic acid and protein. *Endocrinology* **143:** 4764-4774.

18 George FW and Wilson JD (1994). Sex determination and differentiation. In: Knobil E and Neil JD (eds.), *The Physiology of Reproduction*, 2 nd edn, Vol. I, Raven Press, New York, pp. 3-28.

19 Takane KK, Husmann DA, McPhaul MJ and Wilson JD (1991). Androgen receptor levels in the rat penis are controlled differently in distinctive cell types. *Endocrinology* **28:** 2234-2238.

20 Beach FA, Noble RG and Orndoff RK (1969). Effects of perinatal androgen treatment on responses of male rats to gonadal hormones in adulthood. *J Comparat Physiol Psych* **68:** 490-497.

21 Rivas A, McKinnell C, Fisher JS, Atanassova N, Williams K and Sharpe RM (2003). Neonatal coadministration of testosterone with diethylstilbestrol prevents induction of most reproductive tract abnormalities in male rats. *J Androl* **24:** 557-567.

22 Brown-Grant K, Fink G, Greig F and Murray MA (1975). Altered sexual development in male rats after oestrogen administration during the neonatal period. *J Reprod Fertil* **44:** 25-42.

23 Atanassova N, McKinnell C, Walker M, Turner KJ, Fisher JS, Morley M, Millar MR, Groome NP and Sharpe RM (1999). Permanent effects of neonatal estrogen exposure on reproductive hormone levels, Sertoli cell number, and the efficiency of spermatogenesis at adulthood. *Endocrinology* **140:** 5364-5373.

24 Atanassova N, McKinnell C, Turner KJ, Walker M, Fisher JS, Morley M, Millar MR, Groome NP and Sharpe RM (2000). Comparative effects of neonatal exposure of male rats to potent and weak (environmental) estrogens on spermatogenesis at puberty and the relationships to adult testis size and fertility: evidence for the stimulatory effects of low estrogen levels. *Endocrinology* **141:** 3898-3907.

25 Robaire B, Duron J and Hales BF (1987). Effect of estradiol-filled polydimethylsiloxane subdermal implants in adult male rats on the reproductive system, fertility, and progeny outcome. *Biol Reprod* **37:** 327-334.

26 Kaneto M, Kanamori S, Hishikawa A and Kishi K (1999). Epididymal sperm motion as a parameter of male reproductive toxicity: sperm motion, fertility, and histopathology in ethinylestradiol-treated rats. *Reprod Toxicol* **13:** 279-289.

27 Robbins A (1996). Androgens and male sexual behavior. *Trends Endocrinol Metabol* **7:** 345-350.

28 Meisel RL and Sachs BD (1994). The physiology of male sexual behavior. In: Knobil E and Neil JD (eds.), *The Physiology of Reproduction*, 2 nd edn, Vol. II, Raven Press, New York, pp. 3-106.

29 Baum ML and Vreeburg JTM (1973) Copulation in castrated male rats following combined treatment with estradiol and dihydrotestosterone. *Science* **182:** 283-285.

30 Wee BE, Weaver DR and Clemens LG (1988). Hormonal restoration of masculine sexual behavior in long-term castrated B6D2F1 mice. *Physiol Behav* **42:** 77-82.

31 Heaton JPW and Varrin SJ (1994). Effects of castration and exogenous testosterone supplementation in an animal model of penile erection. *J Urol* **151:** 797-800.

32 Olsen KL (1992). Genetic influences on sexual behavior differentiation. In: Gerall AA, Moltz H and Ward IL (eds.), *Handbook of Behavioral Neurobiol ogy: Sexual Differentiation*, Vol. II, Plenum Press, New York, pp. 1-40

33 Rajfer J, Namkung PC and Petra PH (1980). Identification, partial characterization and age-related changes of a cytoplasmic androgen receptor in the rat penis. *J Steroid Biochem* **13:** 1489-1492.

34 Takane KK, George FW and Wilson JD (1990). Androgen receptor of rat penis is downregulated by androgen. *Am J Physiol* **21:** E46-50.