Endocrine, *vol. 9, no. 3, 253–261, December 1998* 0969–711X/98/9:253–261/$10.25

Androgen Receptor Expression in the Testes and Epididymides of Prenatal and Postnatal Sprague-Dawley Rats

Li You and Madhabananda Sar

Endocrine, Reproductive, and Developmental Toxicology Program, Chemical Industry Institute of Toxicology, Research Triangle Park, NC

The androgen receptor (AR) plays a critical role in sexual differentiation and in the virilization of the male reproductive system. A clear understanding of AR expression at the early stages of sexual development will help elucidate the sensitivity of perinatal animals to endocrine modulation by external agents, such as some environmental chemicals. Immunohistochemistry was used in this study to localize the AR in the differentiating testis and epididymis of Sprague-Dawley rats starting from gestation day 15 until postnatal day 21. Positive AR staining was found on gestation day 15 in the mesenchymal as well as in the epithelial cells in the mesonephros. Weak staining was also observed in a small number of interstitial cells in the primordial testis at this age. The fetal interstitial and peritubular myoid cells showed positive AR immunoreactivity early in development, but the Sertoli cells did not overtly express the receptors until postnatal day 5. The intensity of staining and number of AR-positive cells in the testis and epididymis increased over time. The epithelium in the mesonephros-derived tissues, including rete testis and epididymis, appeared to exhibit a higher capacity to express AR than the rest of the testicular tissue. The results demonstrate that AR expression in the primordial male reproductive system is highly specific to time and cell type and modify previous understanding on the timing of AR expression in the testicular tissue. Since AR-positive cells at various developmental stages may be potential sites of interaction with chemicals that adversely affect sexual differentiation, improved understanding of AR ontogeny will help in investigating the effects of AR-reactive agents, such as environmental antiandrogens, with respect to specific windows of sensitivity.

Received June 29, 1998; Revised September 15, 1998; Accepted September 29, 1998.

Author to whom all correspondence and reprint requests should be addressed: Dr. Li You, Chemical Industry Institute of Toxicology, 6 Davis Drive, P. O. Box 12137, Research Triangle Park, NC 27709-2137. E-mail: you@ciit.org

Key Words: Androgen receptor; testis; epididymis; sexual differentiation; immunohistochemistry; ontogeny; development; rat.

Introduction

Testosterone (T) is the main testicular steroid hormone and the major circulating androgen. In some cells, T is metabolized by the converting enzyme 5α-reductase to dihydrotestosterone (DHT) *(1)*. During fetal life, T is responsible for the virilization of the wolffian duct and its derivatives, whereas DHT mainly acts to differentiate the urogenital sinus and external genitalia *(2,3)*. Both T and DHT bind to the same androgen receptor (AR), which is encoded by a gene on the X chromosome and belongs to a superfamily of nuclear receptors *(4,5)*.

AR has been demonstrated at different developmental stages in a number of tissues and cell types that are involved in reproduction and sexual behavior *(6–11)*. The level of AR expression in the fetal as well as the adult reproductive tract is believed to be regulated by androgens or androgenic agents *(9,12–14)*. AR is expected to be in place at the time that T starts to be synthesized in the fetal testis, since it represents the only known mechanism for mediating the action of androgens *(9)*. The fetal rat testis starts to produce testosterone by gestation day (gd) 15 *(15,16)*. AR is detectable in the motoneurons of the lumbar spinal cord *(11)* and in the area containing the differentiating wolffian duct *(10)* on gd 15, when gd 1 is defined as the day after overnight mating. However, the time of initial AR expression in the fetal testis is not clear, and the time profile of AR expression during sexual differentiation in organs derived from the wolffian duct has not been determined.

Androgens play a critical role in the virilization of the male urogenital system. The temporal and spatial characteristics of AR expression are among the major factors defining androgenic function. Fetal and early postnatal sexual development has been regarded as highly sensitive to external endocrine modulations. The sexual and reproductive functions of animals can be permanently affected by events occurring in this perinatal period of time *(17)*,

which may extend for about 4 wk from gd 15 to postnatal day (pnd) 21 *(18)*. We conducted an immunohistochemical study to investigate AR expression in testicular and epididymal tissues in prenatal and postnatal male rats. This information will help evaluate possible effects of exogenous agents on AR expression during this critical developmental period.

Results

Nuclear AR immunostaining was detected in the male reproductive tract at all examined time-points, ranging from gd 15 to pnd 21. The method used in this study for AR protein detection is sensitive: optimal immunostaining was consistently obtained with low concentrations (1–2 μg/mL) of the primary antibody. The AR immunostaining was specific: preadsorption of the antibody with fourfold excess of the synthetic peptide that corresponded to the 21 amino acid sequence of the immunogen reduced the staining to background level. The tissue slides from animals of different ages were processed immunohistochemically at the same time with the same treatment so that AR staining intensities among different ages and tissue types could be compared. Unless otherwise stated, all the results reported in this article have been observed in at least three rats at each time-point, with a minimum of three independent slides from each rat processed at different times.

AR in the Wolffian Duct-Associated Structures and Testis During the Prenatal Period

AR was clearly observed in the mesonephros-derived structures at all times examined. On gd 15, the structures of mesonephros and the testis can be clearly identified (Fig. 1A), with the former containing mesonephric tubules (Fig. 1A) and the latter containing seminiferous cords (Fig. 1A,B). AR immunostaining staining was evident in the mesonephros, mainly in the mensenchymal cells surrounding the mesonephric tubules, but also in the epithelial cells of the tubules themselves (Fig. 1A). In one sample, prominent AR staining was found in the cells surrounding the wolffian duct (not shown). In the fetal testis at that stage, germ cells were observed in the testicular cords (Fig. 1B). In the interstitial space outside the arches of the testicular cords, a few cells were found with faint staining (Fig. 1B). This staining, although very weak and appearing in only a small number of cells, was eliminated when sections were incubated with the preadsorbed AR antibody. The appearance of weak AR immunostaining in the interstitial tissue of the gd 15 fetal testis was consistent among different samples from fetuses harvested from different pregnant dams.

The wolffian duct became prominent on gd 17, whereas the AR staining surrounding it increased in intensity and spread more widely among the mesenchymal cells in the differentiating epididymis (Fig. 1C,D). The testis showed a marked increase in AR staining, mostly in the interstitium. AR staining also began to appear on gd 17 in the elongated nuclei of the peritubular cells that line the outer boundary of seminiferous cords, but the staining was limited to just a few of the peritubular cells (Fig. 1D). On gd 19, the testis exhibited a highly convoluted seminiferous tubule system, but the fetal epididymis developed from the wolffian duct, and showed its anatomical characteristics as the caput and cauda epididymis became morphologically distinguishable (Fig. 1E). At this time, the number of AR-positive cells in the interstitial tissue was increased compared with gd 17, and the staining in the peritubular cells became more uniform (Fig. 1F). On both gd 17 and 19, seminiferous tubules were filled with prespermatogonia and the presumptive Sertoli cells; however, none of these showed AR immunoreactivity.

AR in the Testis and Epididymis in the Early Postnatal Period

The number of seminiferous tubules and germ cell layers in the testis continually increased throughout the period examined (data not shown). Similarly, the development of the epididymis during this period was also represented by a continual increase in the convolution of the epididymal ducts (data not shown). The presumptive Sertoli cells were visible in the testicular cords beginning at gd 15 (Fig. 1B), but they did not show AR immunoreactivity throughout the prenatal and the early neonatal stages. Positive AR staining could not be detected in the Sertoli cells until pnd 5, and it was considerably weaker than that in the adjacent peritubular and interstitial cells (Fig. 2A,B). Afterward, the number of AR-positive Sertoli cells and their immunostaining intensity increased over time, and the appearance of AR staining in the Sertoli cells became less variable among the seminiferous tubules on pnd 21 (Fig. 2D). Compared with pnd 10 (Fig. 2C), the nuclear staining of the Sertoli cells on pnd 21 appeared to be more condensed, suggesting continual differentiation of these cells. In addition, as the number of germ cells in the seminiferous tubules increased from pnd 5 (Fig. 2B) to 21 (Fig. 2D), an alignment of the Sertoli cells in circles of a single cell layer seemed to occur within the seminiferous tubules (Fig. 2D). Changes in Sertoli cell AR staining can also be observed in Figs. 3 and 4, in which positive AR staining for the Sertoli cells could be identified on pnd 5 (Fig. 4A) and 10 (Fig. 3B), but not on pnd 1 (Fig. 3A,C).

Shortly after birth, the rete testis exhibited a markedly higher level of AR staining than the nearby testicular tissue occupied by seminiferous tubules, and most of this increase can be attributed to the higher AR staining intensity in the epithelial cells that are abundant in rete testis (Fig. 2E). In the epididymis, an apparent shift in the AR staining pattern occurred as the organ continued to differentiate. During fetal life, AR staining in the epididymis was mostly found in the mesenchymal cells and seemed to distribute evenly in the tissue, whereas staining in the epithelial cells was less prominent and nonuniform (Fig. 1D,F). Following birth, AR expression in the epididymis shifted to

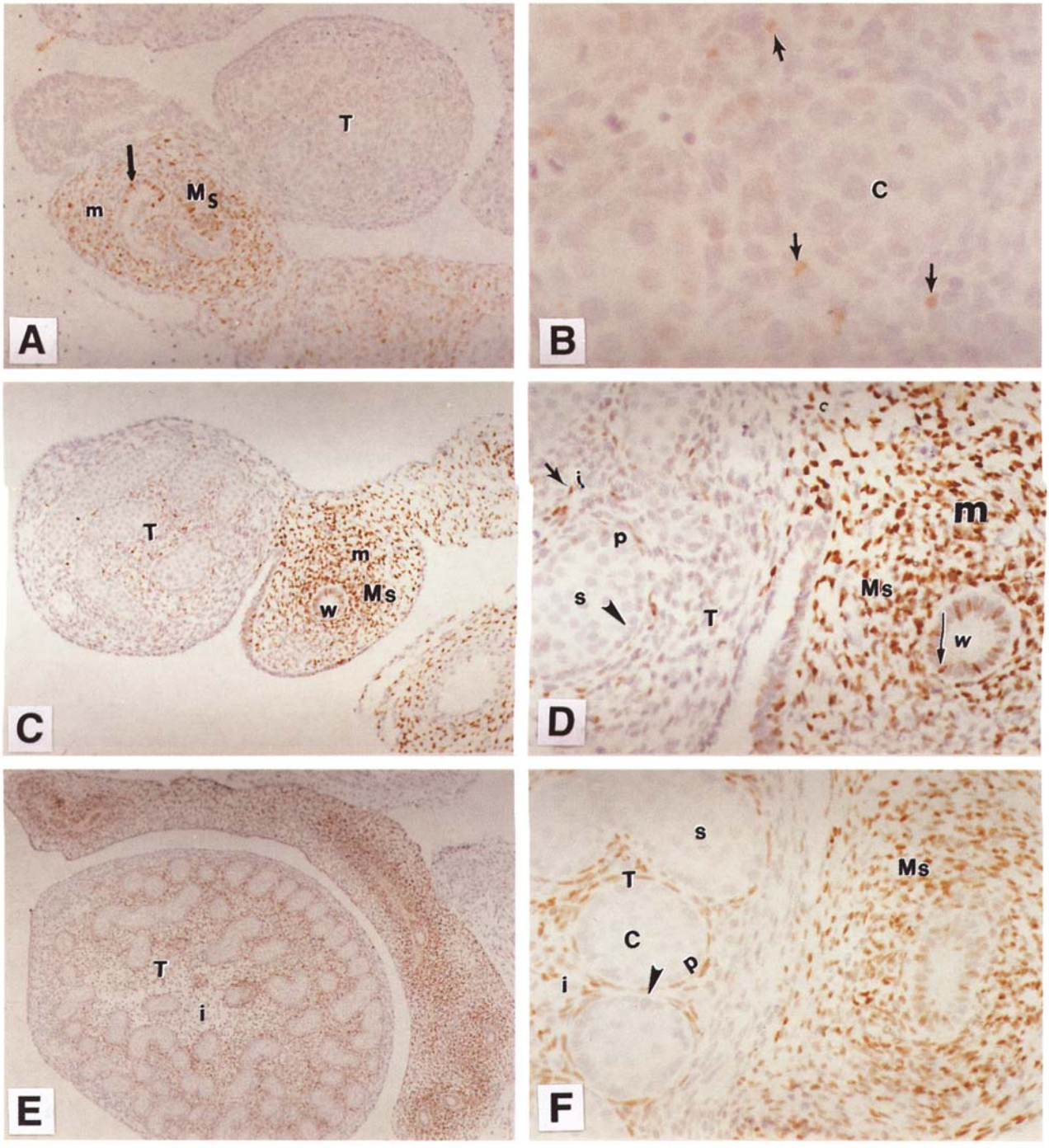

Fig. 1. AR localization in the differentiating fetal testis and associated ducts on gd 15 **(A,B)**, 17 **(C,D)**, and 19 **(E,F)**. On gd 15, in the tissue sections of the primordial testis (T) and mesonephros (Ms), AR was detected in the nuclei of the mesenchymal cells (m) and epithelial cells (→) around the mesonephric tubules (A) and in the interstitial cells (→) of the testis (B). The staining increases around the wolffian duct (w) and in the testicular interstitium (i) on gd 17 (D) and 19 (F). The AR staining in the peritubular cells (p) can first be seen on gd 17 (D). On both gd 17 and 19, seminiferous tubules were filled with prespermatogonia (s) and the presumptive Sertoli cells (arrowhead) (D,F). Magnifications: ×290 (A), ×650 (B), ×200 (C), ×560 (D,F), and ×108 (E).

a pattern in which the most intensive AR staining was seen in the epithelial cells of the epididymal duct on pnd 21 (2F). Earlier stages of this shifting from predominately mesenchymal AR staining to predominately epithelial AR staining can be seen on pnd 1 (Fig. 3A,B) and pnd 10 (Fig. 4C). Preadsorbing the primary antibody with the synthetic peptide that had the same amino acid sequence as the immunogen eliminated the AR staining (Figs. 3D and 4D).

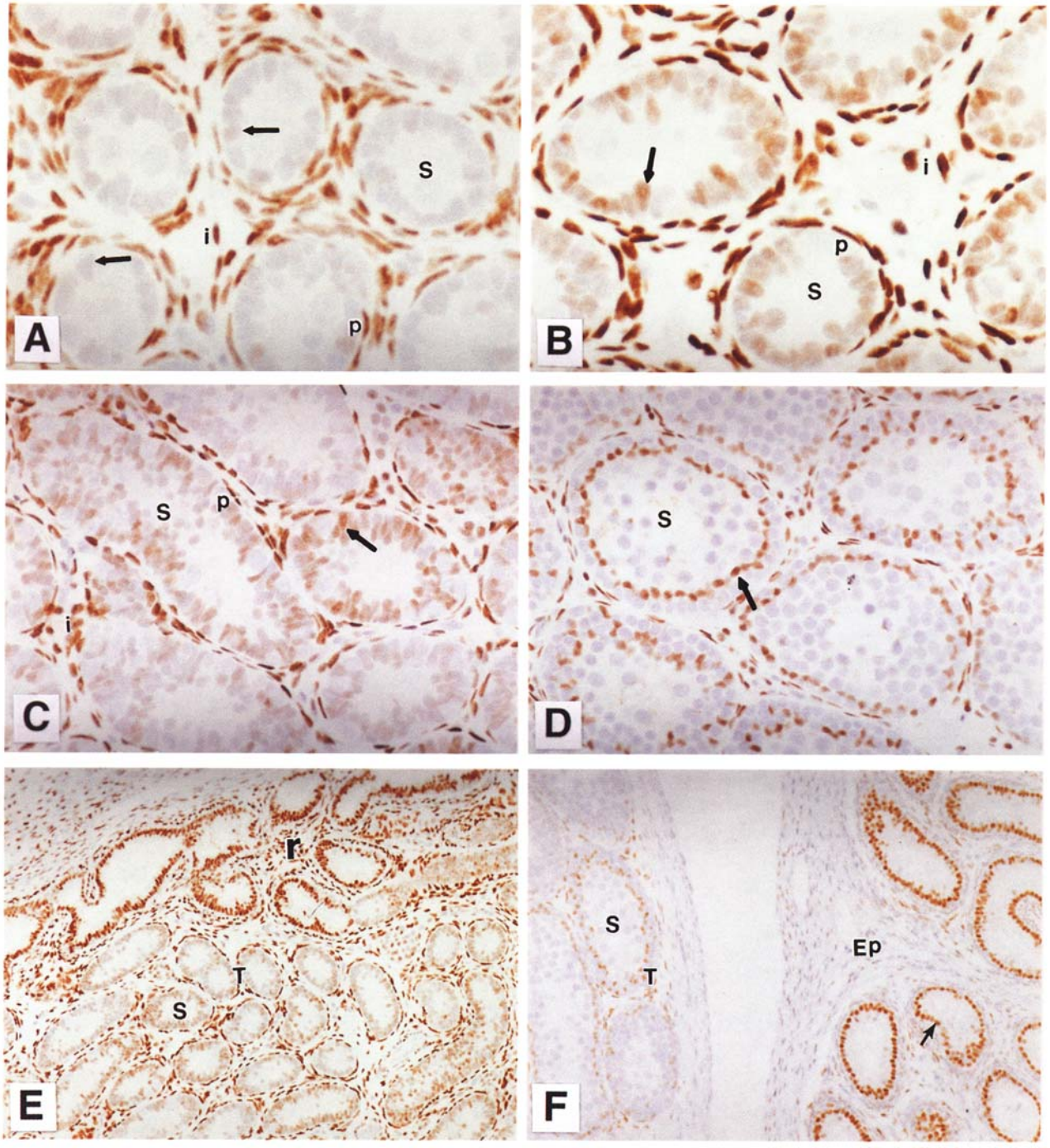

Fig. 2. Testicular tissue **(A–F)**, rete testis (r in E), and epididymal tissues (Ep in F) immunostaining for AR during postnatal development. The intensity of nuclear AR immunostaining increases in general from pnd 2 (A) to pnd 5 (B). Although all the postnatal sections had AR-positive staining for the peritubular (p) and interstitial cells (i), the Sertoli cells (Æ) in the seminiferous tubules (S) do not show overt AR staining until pnd 5 (B). The staining is unevenly distributed among the seminiferous tubules on pnd 5 and 10 (B,C), but becomes virtually uniform on pnd 20 (D). Nuclear AR staining in the Sertoli cells is condensed from pnd 10 to 20, and a movement of the these cells toward the centers of the tubules can be seen on pnd 20 as compared with pnd 10. AR is expressed at a higher level in the rete testis (r) than in the adjacent testicular tissue (T) on pnd 5 (E). On pnd 21, the AR staining is mostly concentrated in the epithelial cells (→) of the duct in the epididymis (F). Magnifications: ×1080 (A,B,C,D) and ×540 (E,F).

Discussion

This study describes the time-course for AR expression in developing rat testes and epididymides during the fetal and early postnatal stages. The presence of AR in the mesonephros of the male rat fetus was clearly demonstrated to occur as early as gd 15. Although most of the immunostaining was localized in the nuclei of mesenchymal cells

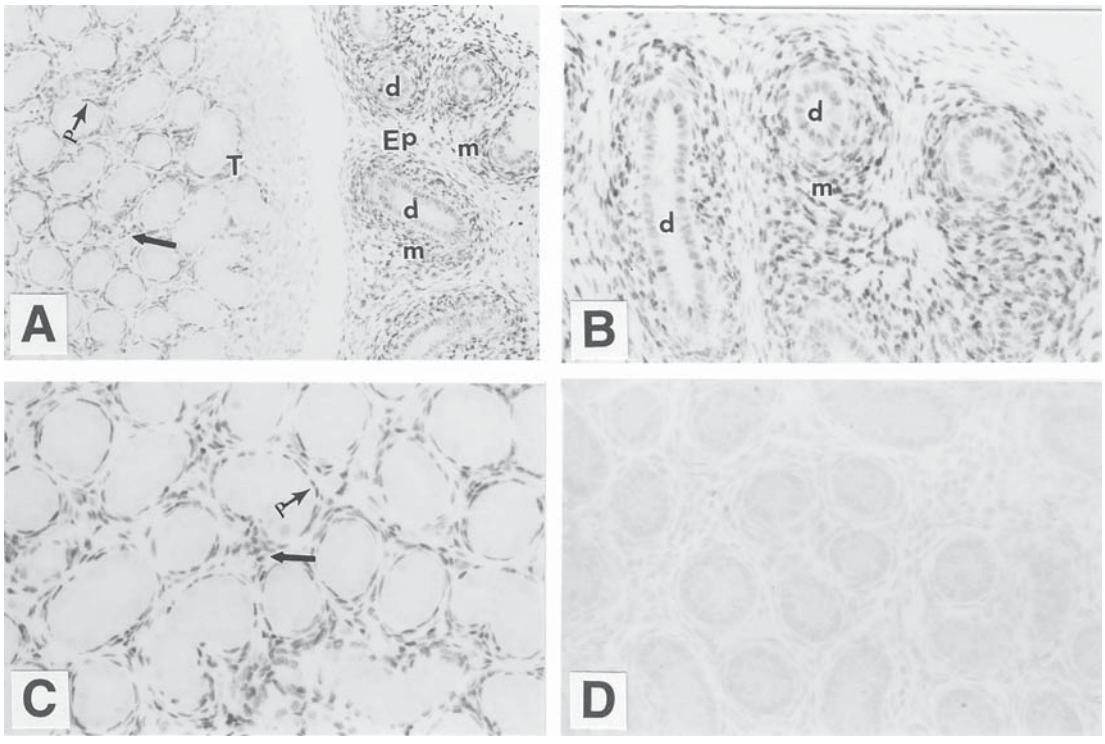

Fig. 3. AR immunostaining in the testis **(A,C)** and epididymis (A,**B**) on pnd 1. The staining begins to concentrate in the mesenchymal cells (m) around the ducts (d) (A,B) of the epididymis (Ep). In the testis (T in A,C), the interstitial (→) and peritubular (p) cells are AR-positive. The epididymal tissue is more intensely stained for the AR than the testicular tissue (A). Preadsorbing the primary antibody with the 21 amino acid synthetic peptide that corresponds to the sequence of the immunogen eliminates the AR staining **(D)**. Magnifications: ×160 (A), ×325 (B,C,D).

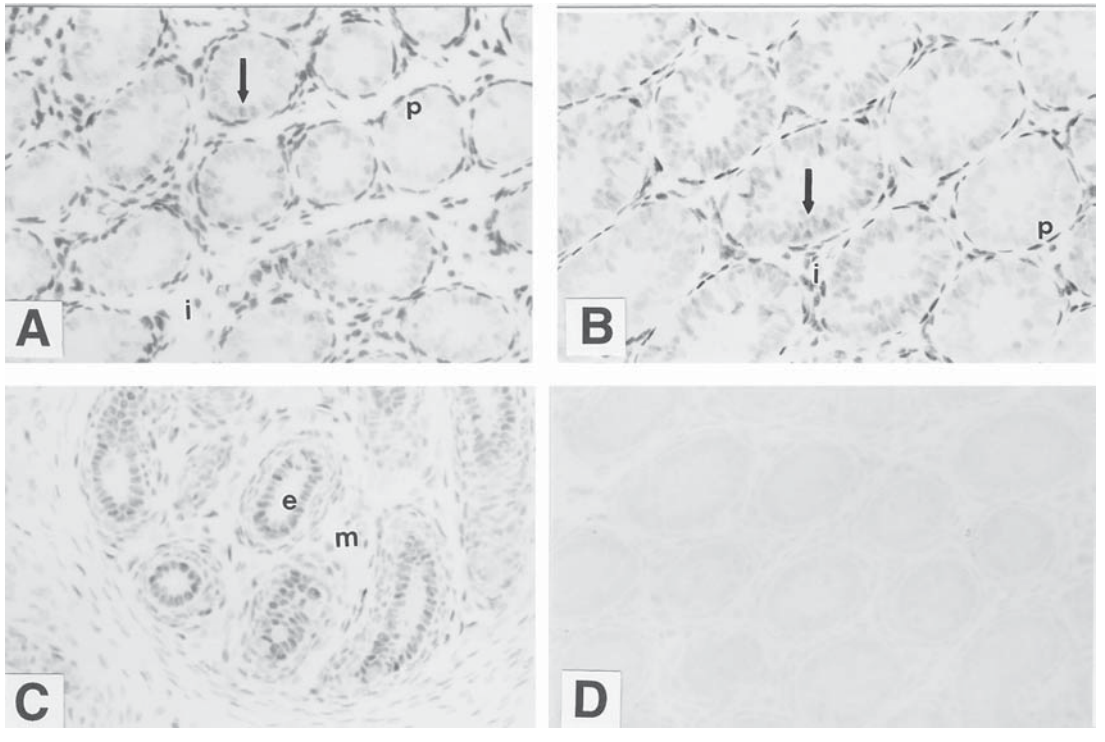

Fig. 4. AR immunostaining on gd 5 and 10. AR staining in nuclei of the Sertoli cells (→) in the testis increases from pnd 5 **(A)** to pnd 10 **(B)** as the seminiferous tubules grow in size. The peritubular (p) and interstitial (i) cells are also positively stained. The prominence of AR staining in the epididymis has shifted to the ductal epithelial cells (e) on pnd 10 from previously mesenchymal cells (m) **(C)**. Preadsorption of the primary antibody eliminates any AR staining in the pnd 5 testis **(D)**. Magnifications: ×325 (A,B,C,D).

surrounding the mesonephric tubules and the differentiating wolffian duct, AR protein was also detected in the nuclei of some epithelial cells. In addition, the fetal testis appeared to express AR on gd 15, although at a much lower level than that found in the mesonephros. From the faintness of the AR staining signal in the testicular tissue on gd 15 (Fig. 1B), we believe this age is very close to the starting point of testicular AR expression. However, we do not know exactly how much earlier the fetal rats would have started to express AR in the testicular as well as the mesonephric tissues, since gd 15 is the earliest time-point we examined. In any event, our results indicate that the fetal testis starts to express AR much earlier than previously believed *(7)*. The expression of AR in the testis throughout the prenatal period was confined to the peritubular cells and the interstitial cells, with the number of AR-positive cells and their immunostaining intensity steadily increasing over time. Immunoreactive AR was not overtly expressed in the Sertoli cells until pnd 5, although a few Sertoli cells could be detected with minimal AR staining on pnd 3 (not shown). On pnd 5, the Sertoli cell AR staining was relatively weak compared with the interstitial and peritubular myoid cells. AR staining became less varied among the Sertoli cells in different seminiferous tubules around pnd 21.

In male rat fetuses, blood testosterone was detectable on gd 15.5 *(15,16)*. Somewhat expectedly, we observed the beginning of AR expression around the same time as the first fetal testosterone surge. In a study with Wistar rats, Majdic et al. *(7)* demonstrated AR immunoreactivity in the mesenchymal cells surrounding the differentiating wolffian duct on gd 16.5, but not on gd 15.5. In the present study, AR immunostaining was clearly demonstrated in the mesenchymal as well as in the epithelial cells in the wolffian duct on gd 15. Owing to different definitions of fetal age, 16.5 d in the Majdic et al. study *(7)* were comparable to 17 d in our study in which the day of vaginal sperm positivity was counted as gd 1 and the animals were routinely killed in the morning. This difference is important because our finding of AR expression on pnd 15 indicates that the machinery to mediate androgen action is available *in situ* at about the time that testosterone begins to be synthesized. The AR in the mesonephros at that stage provides the means for testosterone to facilitate wolffian duct differentiation, which is already in progress during the time span of pnd 15–17. This early differentiation process can be seen in Fig. 1A and 1C: the mesonephric tubules on pnd 15 were replaced by a prominent wolffian duct on pnd 17. The different findings in the timing of early AR expression between the Majdic et al. study *(7)* and the present one can be attributed either to the sensitivity of the primary antibody or to the use of different rat strains.

AR expression has also been reported on gd 15 in the genitofemoral nucleus of the lumbar spinal cord, which is believed to be involved in testicular descent *(11)*. In addition, the onset of luteinizing hormone (LH) receptor expression in the fetal testis was reported to begin on gd 15 *(19)*. These findings, together with the results of the current study, again suggest that androgens start to exert their virilization effects on the male rat reproductive organs around gd 15.

Throughout the prenatal period, AR was expressed earlier and more prominently in the mesonephros tissues and (later) in the epididymis, in comparison with the testicular tissue. Shortly after birth, AR staining was apparently stronger in the rete testis than in other parts of the organ. Since both the rete testis and epididymis are derived from the mesonephros of the embryo, tissues of mesonephric origin may have a higher capacity to express AR. This apparent greater intensity of AR staining in the rete testis and epididymis seems to be dependent on cell type. The epithelial cells lining the anastomosing passages in the rete testis and epididymal ducts exhibited very high levels of AR staining, whereas the seminiferous epithelium, consisting of the germ cells and the Sertoli cells, only showed relatively weaker staining in the Sertoli cells.

The presence of AR in the adult Leydig cell has long been known *(20)*. In addition to mediating the negative feedback inhibition of testosterone biosynthesis by androgens in mature animals, the function of AR in the Leydig cells has been primarily linked to postnatal development of Leydig cell progenitors and immature Leydig cells *(13,21)*. Adult Leydig cells are believed to come from different cell lineage than fetal Leydig cells *(22)*. In the fetal testicular interstitium, the steroidogenic enzyme 3β-hydroxysteriod dehydrogenase was identified in a cell population different than that of the cells expressing AR *(7)*. The identity of those AR-positive fetal interstitial cells, which showed detectable AR expression on gd 15 in the present study, is currently not clear. However, the presence of AR has been demonstrated in the progenitors of adult Leydig cell in pnd 21 rats *(22)*. This suggests the possibility that those same progenitor cells at fetal stage, although steroidogenically inactive, may nonetheless express AR and therefore represent the AR-positive fetal interstitial cells in question. In contrast to the interstitium, the present study did not detect any positive AR staining in the germ cells during the period examined, although AR can be identified in the nuclei of spermatids in adult rats *(23)*.

Our finding of neonatal expression of AR in the Sertoli cells agrees with some reports *(24,25)*, but differs from others. Suarez-Quian et al. *(26)* reported that AR in the Sertoli cells was not detectable until pnd 15. The present study clearly demonstrates that the expression of immunoreactive AR in Sertoli cells starts as early as pnd 5. However, the nuclear staining was comparatively weak and appeared in some of the cells in certain seminiferous tubules, but not in others (Fig. 2C). This finding of uneven distribution of AR staining in different seminiferous tubules at both pnd 5 and 10 differs from a previously report in which AR staining in the Sertoli cells was uniform among

the tubules at pnd 5 and 14 *(25)*. Our observation of nuclear AR staining in the Sertoli cells is also in clear contrast to the observation made by Majdic et al. *(7)*, in which a perinuclear, instead of nuclear staining was ascribed to the Sertoli cell on pnd 5. The lack of AR in the fetal and early neonatal Sertoli cells suggests that androgens are not likely to be directly involved with Sertoli cell functions during that period of time; rather, any effects that androgens may have on Sertoli cell development may be exerted through indirect means. An example of this is the stimulating effect of androgens on the peritubular cells to secrete a paracrine factor (P-Mod-S), which modulates Sertoli cell function *(27)*. Additionally, testosterone may have effects on the Sertoli cells by its conversion to estrogen, since the aromatase, which catalyzes this conversion, has been located in these cells during the perinatal period *(28)*. However, Sertoli cells may still play a role in androgen production by the Leydig cells, since paracrine secretion by the Sertoli cells has been shown to facilitate differentiation of the Leydig cells *(29)*.

Changes in the appearance of AR staining in Sertoli cells were evident from pnd 5 to 21. In addition to a general increase in the number of positively stained Sertoli cells during this time span, apparent condensation of the nuclear staining and an alignment of the Sertoli cells also occurred. This alignment resulted in a pattern of Sertoli cell staining similar to that of adult rat *(24)*. This particular morphological feature is actually observable, although not discussed, in previously published microphotographic data *(25,26)*. These morphological changes precede the onset of spermatogenesis and suggest differentiation of Sertoli cells in preparation for the support of sperm production.

The Sertoli cells are known to proliferate rapidly during the late fetal and early postnatal stages. The total number of Sertoli cells reaches its plateau around pnd 21, and then a constant Sertoli cell number is maintained afterward throughout puberty and adulthood *(30)*. The main factor affecting Sertoli cell proliferation is the follicle-stimulating hormone (FSH), and FSH receptors can be found in Sertoli cells on gd 17 *(22)*. A progressive increase in FSH receptors is well correlated with an increase in Sertoli cell number *(29)*. The increase in AR expression in the Sertoli cells appears to be inversely related to the number of FSH receptors, since the responsiveness of the testis to FSH showed a decline by pnd 5 *(31)*. It is therefore interesting to note that the onset of AR expression in Sertoli cells coincides with the beginning of a decline in Sertoli cell responsiveness to FSH.

Properly functioning AR is essential for normal male sexual development. In humans or animals with testicular feminization syndrome (Tfm), which is caused by mutations in the AR, the affected males produce normal levels of testosterone, but exhibit a female phenotype *(1)*. Animal studies with flutamide, a nonsteroidal antiandrogen that competitively blocks androgen binding to the AR, showed that it produced a variety of developmental defects in the reproductive system of male offspring when administered to pregnant dams. Depending on the time and dose level of the exposure, these effects may include cryptorchidism, hypospadias, and reduced growth of the prostate *(32,33)*. Experimental evidence also indicates that depriving androgens perinatally predisposes adult male rats to feminine sexual behavior *(34)*.

In recent years, some environmental chemicals have been reported to affect the male reproductive system by interacting with the AR *(14,35)*. These compounds have been shown to block the AR in vitro, and they are capable of producing a number of antiandrogenic effects in male animals when given in large bolus doses. Although data regarding the effects of low-level exposures to those chemicals are lacking, it has been postulated that exposures to small amounts of hormonally active agents during developmentally critical periods could result in permanent alterations in sexual and reproductive functions *(36)*.

The high sensitivity of developing animals to AR-mediated endocrine modulations is probably related to the temporal and spatial characteristics of AR expression during the early phases of sexual differentiation. In addition, the relatively small number of receptor sites and low concentrations of endogenous ligand may themselves contribute to this sensitivity. Whether levels of environmental chemicals to which humans may be exposed are sufficient to affect sexual differentiation adversely remains to be determined, however.

Materials and Methods

Animal Husbandry

Timed-pregnant Sprague-Dawley rats were purchased from Charles River Laboratories (Raleigh, NC). Young adult female rats were cohabited with male rats overnight, and copulation was verified in the morning by the presence of sperm in the vagina. The day following the overnight mating was designated as gd 1. On arrival at the animal facility from the breeder on gd 3, the animals were housed individually in polycarbonate cages and were provided *ad libitum* with NIH-07 rodent chow (Zeigler Bros., Gardners, PA) and deionized tap water. The animal room was controlled at a temperature range of 22–25°C and humidity of 50 ± 10%, with 12-h (7:00–19:00) light cycles. This study was conducted in accordance with principles and procedures under National Institute of Health guidelines and approved by the Institutional Animal Care and Use Committee, Chemical Industry Institute of Toxicology (CIIT).

Tissue Preparation

On gd 15, 17, and 19, pregnant rats were asphyxiated in a CO_2 chamber, and the fetuses were quickly removed from the uterine horns. With the reproductive structures exposed by opening the abdominal cavity, the fetal bodies were

placed into 10% buffered formalin for 48 h. Additional pregnant rats were allowed to give birth, and the pups were kept with their dams until pnd 21. The male neonatal pups were decapitated on pnd 1, 2, 3, 5, 10, or 21, with the 24 h following birth defined as pnd 1. The testes and epididymides of the pups were separated from the rest of the body and fixed in the same way as the fetal tissues. All the tissues were placed in 70% ethanol after the 48-h fixation until they were embedded into paraffin blocks, which were then cut into 5-µm sections and mounted on glass slides.

Immunohistochemistry

The tissue slides were deparaffinized by immersing sequentially in xylene (3 min × 4), 100% ethanol (3 min × 2), and 95% ethanol (3 min × 2). After a 5-min wash in 0.01 *M* sodium phosphate-buffered saline (PBS), pH 7.6, sections were treated with 3% hydrogen peroxide in PBS for 8 min. The slides were washed in PBS, transferred to a citrate buffer (0.01 *M*), pH 5.5–5.7 (HIER, Ventana Medical System, Inc., Santa Barbara, CA), and heated in a microwave oven (4 min × 3) for antigen retrieval. After cooling to room temperature, the slides were washed in PBS (5 min × 2), followed by incubation with 2% normal goat serum (NGS) in PBS for 20 min. The sections were incubated with a polyclonal anti-AR antibody (PA1-111, Affinity BioReagents, Inc., Golden, CO) at a concentration of 1–2 µg/mL in 2% NGS overnight at 4°C. The AR antibody was raised against a synthetic peptide of 21 amino acids corresponding to the N-terminus of rat and human AR.

After a wash in PBS for 5 min, the sections were incubated with a secondary antibody, a biotinylated goat antirabbit IgG (I:200 or 1:400), for 30 min. Following another PBS wash, the slides were incubated with avidin–biotin peroxidase complex (Elite ABC kit, VECTASTAIN, Burlingame, CA) at 1:200 dilution for 30 min. After a PBS wash, the sections were treated with freshly prepared liquid diaminobenzidine (BioGenex, San Ramon, CA) for 5–10 min and washed in PBS for 10 min before they were counterstained with hematoxylin and mounted with Permount. All treatments, unless otherwise indicated, were carried out at room temperature.

The specificity of the AR immunoreaction was confirmed by incubating sections with the preadsorbed AR antibody, which prevented specific immunostaining. Preadsorbed AR antibody was prepared by incubating AR antibody with the 21 amino acid synthetic peptide that corresponds to the sequence of immunogen (1 µg of AR antibody plus 4 µg of peptide in 1 mL of 2% NGS for 24–48 h at 4°C). The peptide was synthesized by the Protein Synthesis Laboratory, Department of Microbiology and Immunology, University of North Carolina, Chapel Hill, NC. The avidin–biotin peroxidase technique has been described previously for localization of steroid receptors *(37)*.

Acknowledgments

We are grateful for the support and encouragement provided by Henry Heck during the study. We also wish to thank Henry Heck, Frank Welsch, and Paul Foster for critical review of the manuscript, Delorise Williams for skilled preparation of the histochemical tissues, and Barbara Kuyper for editorial assistance. This study was supported in part by funds from the Chlorine Chemistry Council.

References

1. George, F. W. and Wilson, J. D. (1994). In: *The Physiology of Reproduction*, vol. 1, 2nd ed., Knobil, E. and Neill, J. D. (eds.). Raven: New York, pp. 3–28.
2. Wilson, J. D., George, F. W., and Griffin, J. E. (1981). *Science* **2111,** 1278–1284.
3. Anderson, C. A. and Clark, R. L. (1990). *Teratology* **42,** 483–496.
4. Evans, R. M. (1988). *Science* **240,** 889–895.
5. Zhou, Z. X., Wong, C. I., Sar, M., and Wilson, E. M. (1994). *Recent Prog. Horm. Res.* **49,** 249–274.
6. Sar, M., Lubahn, D. B., French, F. S., and Wilson, E. M. (1990). *Endocrinology* **127,** 3180–3186.
7. Majdic, G., Millar, M. R., and Saunders, P. T. K. (1995). *J. Endocrinol.* **147,** 285–293.
8. George, F. W. and Nobel, J. F. (1984). *Endocrinology* **115,** 1451–1458.
9. Bentvelsen, F. M., McPhaul, M. J., and Wilson, J. D. (1994). *Mol. Cell. Endocrinol.* **105,** 21–26.
10. Bentvelsen, F. M., Brinkmann, A. O., van der Schoot, P., van der Linder, J. E. T. M., van der Kwast, T. H., Boersma, W. J. A., et al. (1995). *Mol. Cell. Endocrinol.* **113,** 245–253.
11. Cain, M. P., Kramer, S. A., Tindall, D. J., and Husmann, D. A. (1994). *J. Urol.* **152,** 766–769.
12. Blok, L. J., Bartlett, J. M. S., Bolt-De Vries, J., Themmen, A. P. N., Brinkmann, A.O., Weinbauer, G. F., et al. (1991). *J. Steroid Biochem. Mol. Biol.* **40,** 343–347.
13. Shan, L. X., Bardin, C. W., and Hardy, M. P. (1997). *Endocrinology* **138,** 1259–1266.
14. Kelce, W. R., Lambright, C. R., Gray, L. E., Jr, and Roberts, K. P. (1997). *Toxicol. Appl. Pharmacol.* **142,** 192–200.
15. Warren, D. W., Haltmeyer, G. C., and Eik-Nes, K. B. (1973). *Biol. Reprod.* **8,** 560–565.
16. Feldman, S. C. and Bloch, E. (1978). *Endocrinology* **102,** 999–1007.
17. Sharpe, R. M. and Skakkebaek, N. E. (1993). *The Lancet* **341,** 1392–1395.
18. Ojeda, S. R. and Urbanski, H. F. (1994). In: *The Physiology of Reproduction*, vol. 2, 2nd ed., Knobil, E. and Neill, J. D. (eds.). Raven: New York, pp. 363–409.
19. Warren, D. W., Huhtaniemi, I. T., Tapanainen, J., Dufau, M. L., and Catt, K. J. (1984). *Endocrinology* **114,** 470–476.
20. Sar, M., Stumpf, W. E., McLean, W. S., Smith, A. A., Hansson, V., Nayfeh, S. N., et al. (1975). In: *Hormonal Regulation of Spermatogenesis*, French, G. S., Hansson, V., Ritzen, E. M., and Nayfeh, S. N. (eds.). Plenum: New York, pp. 311–319.
21. Saez, J. M. and Lejeune, H. (1996). In: *The Leydig Cell*, Payne A. H., Hardy M. P., and Russell, L. D. (eds.). Cache River Press: Vienna, IL, pp. 383–406**.**
22. Hardy, M. P, Gelber, S. J., Zhou, Z., Penning, T. M., Ricigliano, J. W., Ganjam, V. K., et al. (1991). *Ann. NY Acad. Sci.* **637,** 152–163.
23. Vornberger, W., Prins, G., Musto, N. A., and Suarez-Quian, C. A. (1994). *Endocrinology* **134,** 2307–2316.

24. Sar, M., Hall, S. H., Wilson, E. M., and French, F. S. (1993) *The Sertoli Cell*, Russell, L. D. and Griswold, M. D (eds.). Cache River Press: Cleawater, FL, pp. 509–516.
25. Bremner, W. J., Millar, M. R., Sharpe, R. M., and Saunders, P. T. K. (1994). *Endocrinology* **135,** 1227–1234.
26. Suarez-Quian, C. A., Oke, B. O., Vornberger ,W., Prins, G., Xiao, S., and Musto, N. A. (1996). In: *Cellular and Molecular Regulation of Testicular Cells*, Desjardins, C. (ed.). Springer-Verlag: New York, pp. 189–212.
27. Norton, J. N. and Skinner, M. K. (1989). *Endocrinology* **124,** 2711–2719.
28. Rommerts, F. F., de Jong, F. H., Brinkmann, A. O., and van der Molen, H. J. (1982). *J. Reprod. Fertil*. **65,** 281–288.
29. Pelliniemi, L. J., Frojdman, K., and Paranko, J. (1993). In: *The Sertoli Cell*, Russell, L. D. and Griswold, M. D. (eds.). Cache River Press: Clearwater, FL, pp. 87–113.
30. Bardin, C. W., Cheng, C. Y., Mustow, N. A., and Gunsalus, G. L. (1994). In: *The Physiology of Reproduction*, vol. 1, 2nd ed., Knobil, E. and Neill, J. D. (eds.). Raven: New York, pp. 1291–1933.
31. Buzek, S. W. and Sanborn, B. M. (1988). *Biol. Reprod*. **39,** 39–49.
32. Silversides, D. W., Price, C. A., and Cooke, G. M. (1995). *Can. J. Physiol. Pharmacol*. **73,** 1582–1588.
33. Cain, M. P., Kramer, S. A., Tindall, D. J., and Husmann, D. A. (1995). *Urology* **46,** 553–558.
34. Gladue, B. A. and Clemens, L. G. (1982). *Physiol. Behav*. **29,** 263–267.
35. Kelce, W. R., Christy, R. S., Laws, S. C., Gray, L. E., Kemppainen, J. A., and Wilson, E. M. (1995). *Nature* **375,** 581–585.
36. Kavlock, R. J., Daston, G. P., DeRosa ,C., Fenner-Crisp, P., Gray, L. E., Kaattari, S., et al. (1996). *Environ. Health Perspectives* **104(Suppl. 4),** 715–740.
37. Sar, M. (1985). In: *Techniques in Immunocytochemistry*, vol. 3., Bullock, G. R. and Petrusz, P. (eds.). Academic: New York, pp. 43–54.