Physiological Androgen Insensitivity of the Fetal, Neonatal, and Early Infantile Testis Is Explained by the Ontogeny of the Androgen Receptor Expression in Sertoli Cells

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Context: Although gonadotropins and testosterone are high in the fetal/early postnatal periods, Sertoli cells remain immature and spermatogenesis does not progress. We hypothesized that Sertoli cells do not respond to testosterone because they do not express the androgen receptor.

Objective: The objective of the study was to describe the precise ontogeny of androgen receptor expression in the human testis from fetal life through adulthood.

Design: This was an immunohistochemical study on testicular biopsies from fetal, neonatal, prepubertal, pubertal, and adult human testes.

Main Outcome Measures: Quantification of androgen receptor expression in Sertoli cells was measured. Evaluation of androgen receptor expression in peritubular and interstitial cells as well as anti-Müllerian hormone and inhibin-α was also performed.

Results: Androgen receptor expression was first observed in the nuclei of few Sertoli cells at the age of 5 months. Labeling was weak in 2–15% of Sertoli cells until 4 yr of age and progressively increased thereafter. High levels of androgen receptor expression were observed in more than 90% from the age of 8 yr through adulthood. Androgen receptor was positive in peritubular cells and variable in interstitial cells. Anti-Müllerian hormone immunolabeling was strong in all Sertoli cells from fetal life throughout prepuberty and weakened progressively as spermatogenesis developed. Inhibin-α expression was detected in all Sertoli cells from fetal life through adulthood.

Conclusions: A lack of androgen receptor expression could explain a physiological Sertoli cell androgen insensitivity during fetal and early postnatal life, which may serve to protect the testis from precocious Sertoli cell maturation, resulting in proliferation arrest and spermatogenic development. **(*J Clin Endocrinol Metab* 93: 4408–4412, 2008)**

Testicular maturation is a complex process in which germ and somatic cells evolve through different phases. In the fetal testis, Sertoli cells and germ cells are enclosed in the seminiferous tubules, surrounded by peritubular cells, whereas Leydig cells differentiate in the interstitial tissue. Sertoli and Leydig cells have endocrine functions, respectively secreting anti-Müllerian hor-

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Abbreviations: AMH, Anti-Müllerian hormone; AR, androgen receptor.

TABLE 1. Source of tissues used in this study

	Necropsy[a]	Paratesticular lesions[b]	Testicular lesions[c]	Cryptorchidism[d]	Leukemia[e]
Fetal	19				
0–6 months	20	2	2		
Older than 6 months (prepubertal)	4	2	2	4	5
Pubertal					5
Adult					3

[a] Testes were collected at necropsy from patients who died of disorders not related to endocrine or metabolic diseases (fetuses: acute chorioamnionitis, 22%; abruptio placentae, 11%; uterine rupture, 6%; central nervous system hemorrhage, 22%; congenital heart defects, 11%; intestinal malformation, 6%; autosomal aneuploidies, 22%; 0–6 months: congenital heart defects, 32%; respiratory distress syndrome, 20%; severe diaphragmatic hernia, 16%; sudden infant death syndrome, 16%; gut volvulus, 16%; prepubertal: pneumonia, 75%; meningitis, 25%). Necropsies were done within the following 12 h.

[b] Normal testicular tissue was present as part of the surgical piece sample obtained for various paratesticular lesions (epididymal cysts, mesothelial cyst, paratesticular adenomatoid tumor).

[c] Normal testicular tissue was present adjacent to benign testicular tumors (teratoma).

[d] Only histologically normal testicular tissue of biopsies obtained during orchiopexy was included in this study. None of the samples had dysgenetic features.

[e] Only histologically normal testicular tissue of biopsies obtained for the staging of patients with leukemia was included in this study.

mone (AMH) and testosterone. Gonadotropins are the main regulators of testicular function. After birth, the gonadotropin axis remains active for approximately 3–6 months and subsequently enters a quiescent state until the onset of puberty. During the quiescent period of the gonadotropin axis, Leydig cell testosterone secretion also declines to almost undetectable levels, but Sertoli cells remain active, as revealed by intense cell proliferation and AMH and inhibin B secretion (reviewed in Ref. 1). Germ cells, mainly represented by spermatogonia, also proliferate but do not go through meiosis before puberty.

The dramatic changes occurring in testicular volume and endocrine and spermatogenic functions during pubertal development are driven by the reactivation of pituitary gonadotropin secretion. LH induces Leydig cell steroidogenesis resulting in an elevation of intratesticular androgen concentration. Testosterone provokes Sertoli cell maturation, both morphologically (2) and functionally, *e.g.* reflected in down-regulation of AMH expression (3), and germ cells undergo meiosis, the hallmark of adult spermatogenesis driving to sperm production.

An intriguing feature of testicular development is that, although the gonadotropin axis and testosterone production are as active in the fetal and early postnatal periods as in puberty, Sertoli cell morphology remains immature and germ cells do not go through meiosis. Likewise, down-regulation of AMH expression, a conspicuous feature of androgen action on Sertoli cells (4, 5), does not occur in the fetal and early postnatal gonad despite elevated intratesticular testosterone levels. Androgens act through binding to the androgen receptor (AR), a transcription factor widely expressed in target cells. Androgen-sensitive organs, like Wolffian ducts and the primordia of external genitalia, express the AR from early fetal life, but the precise ontogeny of AR expression in the testis has not been described in humans. We hypothesized that Sertoli cells do not respond to the high intratesticular androgen concentration existing in fetal and early postnatal life because they do not express the AR. The aim of our study was to describe the precise ontogeny of AR expression in the human testis from fetal life through adulthood to determine whether lack of AR expression could explain a physiological Sertoli cell androgen insensitivity during fetal and early postnatal life.

Materials and Methods

Tissue samples

Paraffin-embedded tissue sections were obtained from the archives of the pathology laboratories of the Centro de Investigaciones Endocrinológicas, Hospital de Niños R. Gutiérrez (Buenos Aires), and the Hospital La Paz (Madrid) and from the Cooperative Human Tissue Network (http://chtn.nci.nih.gov/). The study protocol was approved by the respective institutional review boards and ethics committees. Samples were available from 19 fetal testes (16–39 gestational wk), 20 neonatal testes (1–30 d), four testes in the early postnatal hypothalamic-pituitary-gonadal activation period (1.5–6 months), 17 testes in the infantile period and childhood (7 months to 9 yr), five pubertal testes (11–14 yr), and three adult testes (18–56 yr). Details are given in Table 1.

Immunohistochemistry

Immunohistochemical localization of the AR, AMH, and inhibin α-subunit was performed as follows: sections mounted on slides coated with 3-aminopropyltriethoxy-silane (Sigma Chemical Co., St. Louis, MO) were submitted twice to microwave pretreatment (for AR, AMH, and inhibin) for 2.5 min at 800 W in sodium citrate buffer 0.01 M (pH 6) and subsequently blocked with Tris-buffered saline containing 1% BSA. The primary antibodies used were a mouse monoclonal antibody raised against a synthetic peptide sequence comprising amino acids 301–320 of the human AR (BioGenex Laboratories, Inc., Dublin, Ireland), a rabbit polyclonal antibody raised against recombinant human AMH (6), or a mouse monoclonal antibody raised against inhibin α-subunit (7). All sections were incubated overnight at 4 C. After incubations with the primary antibody, the sections were carefully washed and the supersensitive peroxidase or alkaline phosphatase ready-to-use detection systems (BioGenex, San Ramón, CA) were used. These reactions were revealed by incubation with 3, 3′-diaminobenzidine/H_2O_2 or diluted in phosphate buffer for peroxidase kits or with fast red diluted in naphthol phosphate for alkaline phosphatase systems. Negative controls were treated with nonimmune rabbit or mouse serum as adequate.

The proportion of cells exhibiting positive immunostaining for the AR was calculated by counting 200 cells per gonad. This was based on an estimation of the sample size needed to estimate a proportion with absolute precision (8). We fixed desired absolute precision level at 6% (*i.e.* an accepted maximum error of ± 3%) for cell populations anticipated to be at least 60% positive and at 2% (*i.e.* ± 1%) for cell populations anticipated to be less than 10% positive. Confidence level was fixed at 95%. Sampling was performed by counting all cells found in at least five blindly selected microscopic fields, using a ×100 objective, and a ×10 ocular. Field selection was performed as follows: starting from the upper left microscope field of the section, the stage was moved manually by 1 point of the stage rule to the right edge and then 1 point downward.

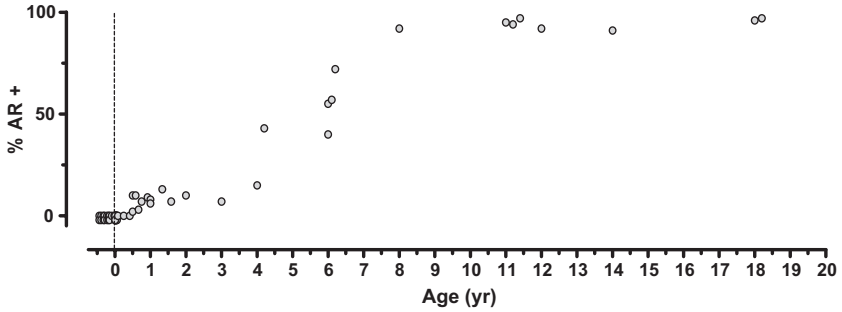

FIG. 1. Percentage of Sertoli cells with positive immunostaining for the AR in human testes. Dotted line, birth. Note that in the fetal and neonatal periods, there is superposition of points.

Results

AR labeling was negative in fetal Sertoli cells (Figs. 1 and 2) and was first observed in the nuclei of a few Sertoli cells at the ages of 5 months. A weak immunostaining was present in only 2–15% of Sertoli cells until 4 yr of age. Thereafter AR expression progressively increased in Sertoli cells: staining was more intense and a higher proportion of nuclei were positive. More than 90% of Sertoli cell nuclei expressed high levels of the AR from the age of 8 yr through adulthood. AR labeling was positive in peritubular cell nuclei from fetal life through adulthood. The proportion of interstitial cell nuclei positive for AR showed changes from fetal life through adulthood: 66–81% in fetal testes, 39–71% in the first yr of life, 29–55% in ages 2–6 yr, 61–82% in prepubertal testes older than 8 yr, and 87–95% in pubertal and adult gonads.

As expected, AMH immunolabeling was strong in the cytoplasm of all Sertoli cells from fetal life throughout prepuberty (Fig. 2). As already described (9), AMH expression was weaker in pubertal testes than in prepubertal testes. Furthermore, AMH was extremely faint or undetectable in seminiferous tubules with meiotic cells. Premeiotic AMH-positive tubules coexisted with meiotic AMH-negative tubules in the same sample (Fig. 2). Inhibin-α expression was detected in the cytoplasm of all Sertoli cells from fetal life through adulthood (not shown). Until the 35th fetal week, inhibin-α immunoexpression was stronger in Leydig cells than Sertoli cells. Thereafter no signal was observed in the interstitial tissue until pubertal onset.

Discussion

Our results depict the precise ontogeny of the expression of the AR in Sertoli cells, which gives further insight into the understanding of the physiology and pathophysiology of male gonadal development. The ontogeny of the AR expression in the testis had previously been described in rodents (5, 10). However, because there is a close continuity between neonatal period and pubertal development, these laboratory animals are inadequate experimental models to study certain aspects of human testicular development, characterized by a long period elapsing from birth to the beginning of puberty. We were the first to communicate preliminary results on the ontogeny of the testicular AR in human testicular tissue from the fetal period to adulthood (11), and we expand our work in this report. Previous studies had analyzed AR expression in a limited number of gonads from boys younger than 5 yr (12), adolescents (13), or adults (14). Here we provide a more exhaustive study of the ontogeny of the AR, and its correlation with other markers of testicular development, in a large number of samples encompassing testicular development from fetal life through adulthood. We show that AR expression is not detected by immunohistochemistry in the fetal Sertoli cell; it first appears as a weak signal in few Sertoli cell nuclei approximately 5 months after birth and increases significantly between ages 4 and 8 yr. Thereafter an intense signal is present in almost all Sertoli cells.

A limitation of immunohistochemistry is its sensitivity. We are aware that very low expression of AR might not be detected in fetal, neonatal, and early infantile Sertoli cells. However, a constant positive labeling was present in either the epididymis of the same sample or peritubular and interstitial cells, which served as an internal control of the technique. Furthermore, our results are in line with the known physiological actions of androgens within the testis. Whereas immature Sertoli cell proliferation is dependent on FSH (15), Sertoli cell maturation is mainly driven by androgens (2, 16). When Sertoli cells are completely mature, they cease to proliferate. Strong AMH expression is a typical feature of immature (fetal and prepubertal) Sertoli cells. In normal or precocious puberty, androgens down-regulate AMH secretion (4), with the onset of meiosis resulting in a further inhibition of AMH expression (5), whereas in the androgen insensitivity syndrome, AMH levels remain high at puberty despite the increase of testosterone levels (17).

Spermatogenesis is regulated by a complex endocrine and paracrine regulatory network involving Sertoli, peritubular, and Leydig cells (18). However, Sertoli cell action of the AR is an absolute requirement for androgen maintenance of complete spermatogenesis, as shown in cell-specific AR knockout models (19, 20). It is intriguing that the high levels of testosterone secreted by the testes during the fetal and early postnatal periods, similar to those observed in puberty and adulthood, are not capable of inducing morphological changes in Sertoli cells, inhibiting AMH expression, or triggering spermatogenesis. This suggested that the fetal and early postnatal Sertoli cells are physiologically insensitive to androgens. Our observations, showing the absence of AR expression in fetal and early infantile Sertoli cells, support this hypothesis.

The relevance of our novel findings concerning the significant increase in Sertoli cell AR expression between ages 4 and 8 yr is related to the fact that this may explain why in patients with testotoxicosis, the first signs of the disorder (*e.g.* testicular enlargement) are usually observed after the age of 3–4 yr (21). It therefore seems that, although the underlying activating mutation of the LH receptor is present from conception, the resulting sustained Leydig cell androgen secretion does not affect Sertoli cells and spermatogenesis in the first years of life. Interestingly, in a recently reported patient in whom central precocious puberty was diagnosed at the age of 11 months because of increase

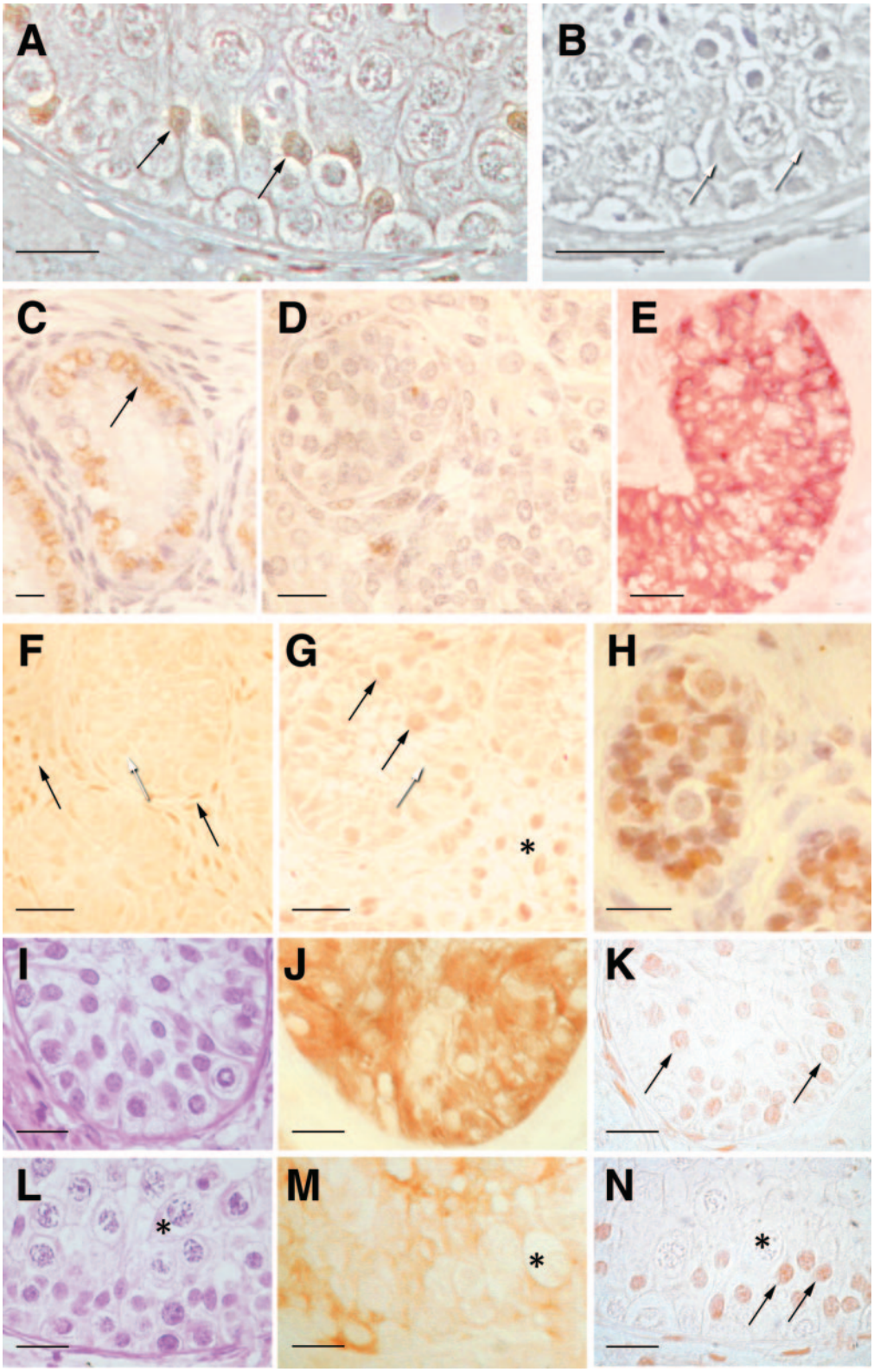

FIG. 2. Immunostaining for the AR and AMH in human testes and epididymis. A and B, Phase-contrast microscopy of adult testes with complete spermatogenesis. A, Strong positivity of AR in all Sertoli cell nuclei (*arrows*). Peritubular cell nuclei are also positive. B, A negative control of the same sample shows nonreactive Sertoli cell nuclei (open *arrows*). C–E, Age of 28 fetal wk; AR is positive in the nuclei of most epididymal epithelial cells (C, *arrow*) but not in testicular Sertoli cells (D, nuclear contrast with hematoxylin). AMH is positive in the cytoplasm of all Sertoli cells (E). F–N, Postnatal life. F, Three months. AR staining is negative in all Sertoli cell nuclei (*empty arrow*) but positive in peritubular and Leydig cell nuclei (*filled arrows*). G, Two years. Few Sertoli cell nuclei are AR positive (*filled arrows*), whereas most remain negative (*empty arrow*). Note AR-positive cells in interstitial cells (*asterisk*, *lower right corner*). H, Six years. More than half of Sertoli cell nuclei are positive. I–N, Eleven years. The six panels correspond to a pubertal testis that shows a mixture of premeiotic tubules (I, J, and K) and others with meiotic development. (L, M, and N). Premeiotic seminiferous tubules, as seen by hematoxylin-eosin staining (I), show positive AMH staining in Sertoli cell cytoplasm (J) and AR reactivity in all Sertoli cell nuclei (K, *arrows*). Seminiferous tubules with meiotic spermatocytes (*asterisks*) observed in hematoxylin-eosin slides (L) show mostly negative AMH staining in the cytoplasm (M) and Sertoli cells with positive AR staining in their nuclei (N, *arrows*). *Bars*, 20 μm.

in the size of the penis, AMH levels were not down-regulated despite high testosterone levels (22). This clearly indicates that androgen secretion, abnormally high for age, triggered peripheral signs of pubertal development but was unable to induce Sertoli cell maturation.

In conclusion, we propose that the absent or low expression of the AR in fetal, neonatal, and early infantile Sertoli cells represents a transient, physiological state of cell-specific androgen insensitivity, which may serve to protect the testis from precocious Sertoli cell maturation, resulting in proliferation arrest and spermatogenic development. The progressive increase of the AR expression in Sertoli cells during childhood does not represent a risk in normal conditions owing to the quiescent state of the gonadotropin axis, but it becomes unveiled in disorders characterized by an early activation of Leydig cell testosterone production.

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