Ontogeny of the Androgen Receptor Expression in the Fetal and Postnatal Testis: Its Relevance on Sertoli Cell Maturation and the Onset of Adult Spermatogenesis

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KEY WORDS testosterone; LH receptor; Leydig cell; testicular maturation

ABSTRACT From fetal life to adulthood, the testis evolves through maturational phases showing specific morphologic and functional features in its different compartments. The seminiferous cords contain Sertoli and germ cells, surrounded by peritubular cells, and the interstitial tissue contains Leydig cells and connective tissue. Sertoli cells secrete anti-Müllerian hormone (AMH), whereas Leydig cells secrete androgens. In the fetal and early postnatal testis, Leydig cells actively secrete androgens. Sertoli cells are morphologically and functionally immature—e.g., they secrete high levels of AMH—and germ cells proliferate by mitosis but do not enter meiosis. During infancy and childhood, Leydig cells regress and testosterone secretion declines dramatically. Sertoli cells remain immature and spermatogenesis is arrested at the premeiotic stage. At puberty, Leydig cells differentiate again, and testosterone concentration increases and provokes Sertoli cell maturation—e.g., down-regulation of AMH expression—and germ cells undergo meiosis, the hallmark of adult spermatogenesis driving to sperm production. An intriguing feature of testicular development is that, although testosterone production is as active in the fetal and early postnatal periods as in puberty, Sertoli cells and spermatogenesis remain immature until pubertal onset. Here, we review the ontogeny of the androgen receptor expression in the testis and its impact on Sertoli cell maturation and the onset of pubertal spermatogenesis. We show that the absence of androgen receptor expression in Sertoli cells underlies a physiological stage of androgen insensitivity within the male gonad in the fetal and early postnatal periods. *Microsc. Res. Tech. 72:787–795, 2009.*

INTRODUCTION

During its ontogeny, the testis evolves through maturational phases showing specific morphologic and functional features in the different testicular compartments. When the fetal testis becomes differentiated from the gonadal ridge, two typical compartments can be distinguished: the seminiferous cords, containing Sertoli and germ cells, surrounded by peritubular cells, and the interstitial tissue, containing Leydig cells and connective tissue. Sertoli and Leydig cells have endocrine functions, which are essential for fetal sex differentiation. Sertoli cells secrete anti-Müllerian hormone (AMH), whereas Leydig cells secrete androgens. Germ cells, mainly represented by gonocytes or prespermatogonia, proliferate by mitosis. Gonadotropins are the chief regulators of testicular function. In humans, gonadotrophin secretion remains high for ~3–6 months after birth and subsequently decreases. During the rest of childhood, the gonadotrope is quiescent until the onset of puberty. Androgen production follows the same pattern. During infancy and childhood, Sertoli cells remain immature both morphologically and functionally—e.g., they secrete high levels of AMH. Spermatogenesis is also immature: massive entry into meiosis only takes place at puberty. The significant increase in testicular volume, the changes in hormone secretion, and the activation of full spermatogenesis occurring during pubertal development are due to the reactivation of pituitary gonadotropin secretion. LH drives Leydig cell maturation resulting in active steroidogenesis and the elevation of intratesticular androgen concentration. Testosterone induces morphological and functional Sertoli cell maturation, resulting in down-regulation of AMH expression, and germ cells undergo meiosis and attain sperm production, the hallmark of adult spermatogenesis.

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 cells and spermatogenesis remain immature until pubertal onset. Here, we will review the ontogeny of testicular development, especially focusing on the androgen receptor (AR)

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expression in the testis and its impact on Sertoli cell maturation and the onset of pubertal spermatogenesis.

The Developing Testis: From Fetal Life to Adulthood

The Initial Stages. The cell populations of the fetal testis have three distinct origins, as demonstrated in rodents: Sertoli cells derive from the coelomic epithelium covering the gonadal ridge; peritubular cells, Leydig cells, and the diverse elements of the connective tissue originate mainly in the mesonephric mesoderm of the gonadal ridge; and germ cells are initially extragonadal. Stemming from the epiblast, primordial germ cells migrate through the extraembryonic mesoderm of the yolk sac and the dorsal mesentery of the hind gut to reach the gonadal ridges in the fifth week. During migration, primordial germ cells proliferate actively but do not differentiate any further. Upon arriving in the undifferentiated genital ridge, germ cells continue to proliferate by mitosis and maintain bipotentiality (i.e., to undergo oogenesis or spermatogenesis) for ~1 week. Then, germ cells in the male gonad become enclosed in the seminiferous cords and differentiate into the spermatogonial lineage, which does not enter meiosis until the onset of puberty. Prevention of entry into meiosis is a specific effect of male somatic cells since germ cells entering a prospective ovary or those which have failed to enter gonads of either sex enter meiosis at approximately the same time and develop into oocytes, irrespective of their chromosomal pattern (McLaren and Southee, 1997). Recent studies have shown that retinoic acid produced by the mesonephros acts as a meiosis inducer (Bowles et al., 2006; Koubova et al., 2006). Germ cells embedded in the seminiferous cords do not enter meiosis, because they are protected from retinoic acid action: Sertoli cells express CYP26B1, an enzyme that catabolizes retinoic acid, thus behaving as a meiosis-preventing factor (Bowles et al., 2006; Koubova et al., 2006).

The Interstitial Tissue. In the differentiating human fetal testis, Leydig cells develop, proliferate, and are capable of androgen synthesis and secretion in response to placental chorionic gonadotropin (in the human, hCG). The most highly differentiated Leydig cells exist between the 8th and 18th weeks; then Leydig cells regress slowly until birth (O'Shaughnessy et al., 2006; Pelliniemi and Niemi, 1969). In the second half of gestation, fetal LH commands Leydig cell function. After birth, Leydig cell number increases again, with a peak at about 3 months of age, with a subsequent progressive decrease (Codesal et al., 1990). Leydig cell testosterone secretion increases in the first 3 weeks following birth (Bergadá et al., 2006; Forest et al., 1973) and persists during 3–6 months in response to the active LH secretion by the pituitary (Forest et al., 1973). Then, gonadotropin secretion declines dramatically and Leydig cells dedifferentiate (Chemes, 1996; Nistal et al., 1986). This axis remains quiescent until pubertal onset, when pituitary LH secretion increases again. No typical Leydig cells are observed in the testis during childhood. At pubertal onset, proliferation of fibroblast-like cells containing lipid droplets occurs in the interstitial tissue and peritubular wall. A similar picture is observed in prepubertal testes of boys treated with hCG (Chemes et al., 1985). This is followed by various steps of cell maturation leading successively to immature and mature Leydig cells.

Although devoid of typical Leydig cells, the testis of the prepubertal boy is competent for testosterone production in vivo or in vitro. Testosterone secretion comes from mesenchymal Leydig cell precursors present in the interstitial tissue and the tubular wall, which are capable of binding hCG, and express steroidogenic enzymes (Fig. 1). This capacity is increased after hCG stimulation treatment (Chemes et al., 1985) and during pubertal development, when immature and mature Leydig cells progressively differentiate from elongated progenitor cells (Fig. 1). During differentiation, precursor cells enlarge, their cytoplasm becomes abundant and eosinophilic, and their nuclei round-up with prominent nucleoli and heterochromatic granules forming a peripheral layer underlying the nuclear envelope. Smooth endoplasmic reticulum and typical steroidogenic mitochondria with tubular cristae become prominent. As Leydig cell maturation proceeds, hCG binding and the expression of steroidogenic enzymes become stronger (Shan and Hardy, 1992).

The presence of truncated forms of the LH receptor has been described in the testes of adult rats treated with the Leydig-cell cytotoxic agent ethylene dimethane sulfonate (EDS) (Tena-Sempere et al., 1994). Using in situ hybridization, we studied the cellular expression pattern of three mRNAs forms of the LH receptor in Leydig cells and their precursors after EDS-mediated Leydig cell depletion in rats. Adult rats (60–70 days old), housed under controlled conditions of light (14-h light/10-h darkness) were injected i.p. with a single dose of EDS (75 mg/kg of body weight) dissolved in dimethylsufoxide-water (1:3, vol:vol) or with vehicle only as control, following a validated protocol (Tena-Sempere et al., 1994). After 7, 14, or 30 days, rats were sacrificed; testes were fixed in paraformaldehyde 4% in phosphate-buffered saline, embedded in paraffin wax, and sectioned at 5–7 μm for in situ hybridization experiments as previously described (Carré-Eusèbe et al., 1996). Three oligonucleotide primer pairs were synthesized following the rat cDNA LH receptor sequence for PCR amplification from rat DNA and subsequent hybridization probe synthesis. LHR-740-S 5′-ATTTCTTCCACCAAATTGCA-3′ and LHR-902-AS 3′-TCCTTAAACGGCTTCTTTCT-5′ were used to obtain a probe detecting sequences of the extracellular region of the LH receptor. LHR-1921-S 5′-GTATGCGATCTTCACGAAGG-3′ and LHR-2101-AS 3′-TCACGTGACAGTTGTCGGGT-5′ were used to obtain a probe detecting sequences of the intracellular region of the LH receptor. Finally, LHR-3912-S 5′-GTGTATAGACTGTATGTGCC-3′ and LHR-4323-AS 3′-GGGAAGAGATTACCTAATGTG-5′ were used to synthesize a probe hybridizing of the 3′-UTR of the LH receptor (Hu et al., 1994). PCR products were cloned into pGEM-T Easy® (Promega); the constructs were subsequently linearized and transcribed with T7 or T3 RNA polymerase to obtain the sense or the antisense probes, using the RNA digoxigenin labeling kit® (Boehringer) as already described (Carré-Eusèbe et al., 1996). Digoxigenin-labeled RNA was detected with antidigoxigenin antibody coupled to alkaline phsophatase (Boehringer) and revealed by reaction with nitro blue tetrazolium salt, 5-bromo-4-chloro-3-in-

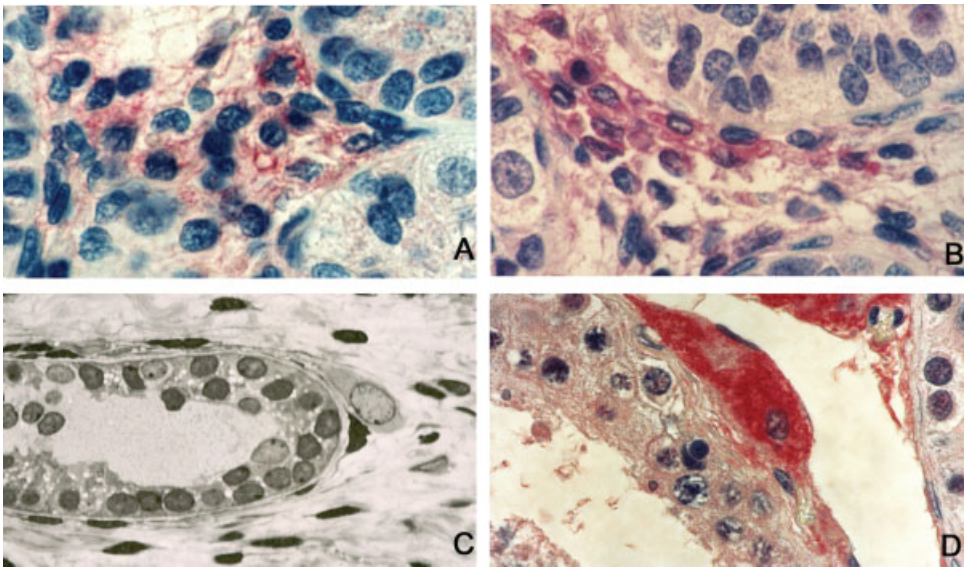

Fig. 1. Leydig cell development in the prepubertal (**A–C**) and adult (**D**) human testis. A: 3β-Hydroxysteroid dehydrogenase (3β-HSD) immunolabeling; (B) testosterone immunolabeling; (C) hCG treatment 1,500 IU/day for 3 days; hematoxylin-eosin staining showing a differentiating peritubular Leydig cell precursor; (D) testosterone immunolabeling. Magnification: ×1,000.

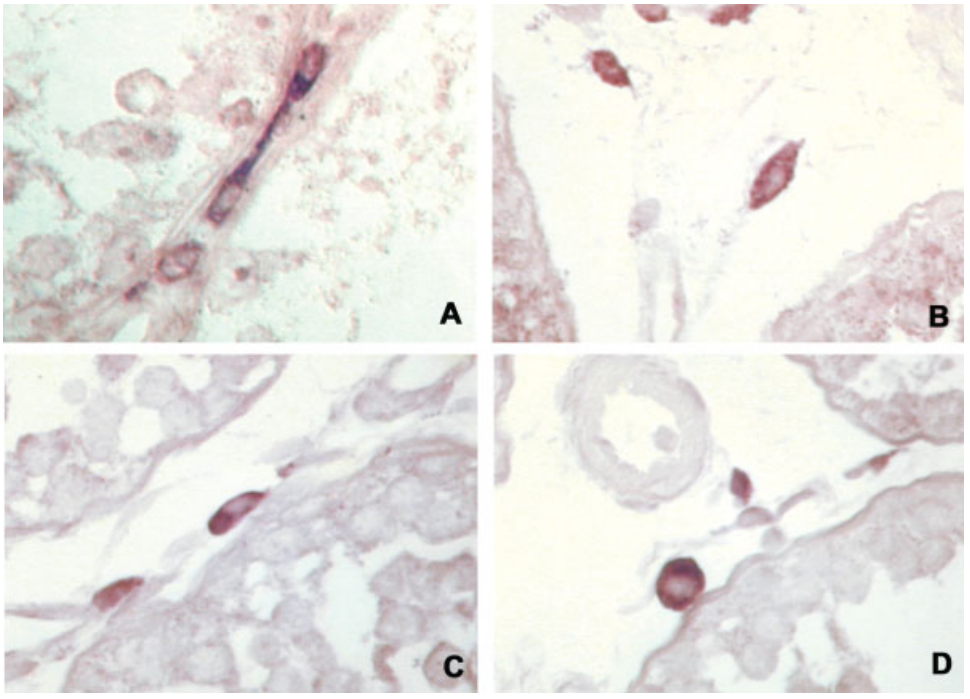

Fig. 2. In situ hybridization studies showing LH receptor mRNA expression in Leydig cells and their precursors in the testes of rats after EDS treatment. **A:** Seven days after EDS, an elongated Leydig cell progenitor expresses the LH receptor extracellular domain mRNA (0.74 kb). **B–D:** Fourteen days after EDS, immature Leydig cells express the intermediate-size (1.92 kb, B) and full-length (3.91 kb, C) LH receptor mRNA. Mature Leydig cells (D) express the full-length LH receptor mRNA. Magnification: ×1,000.

dolyl phosphate (Boehringer), and levamisole (Sigma) in the dark.

EDS destroys mature Leydig cells after administration. Seven days after a single EDS injection, elongated Leydig cell progenitors and immature Leydig cells are already present, and progress to mature Leydig cells after 14 days (Sharpe et al., 1988; Tena-Sempere et al., 1994). We observed that the shorter mRNA (0.74 kb), corresponding to the extracellular domain of the LH receptor was present in elongated Leydig cell progenitors 7 days after EDS treatment (Fig. 2). These elongated cells are morphologically similar to mesenchymal cells

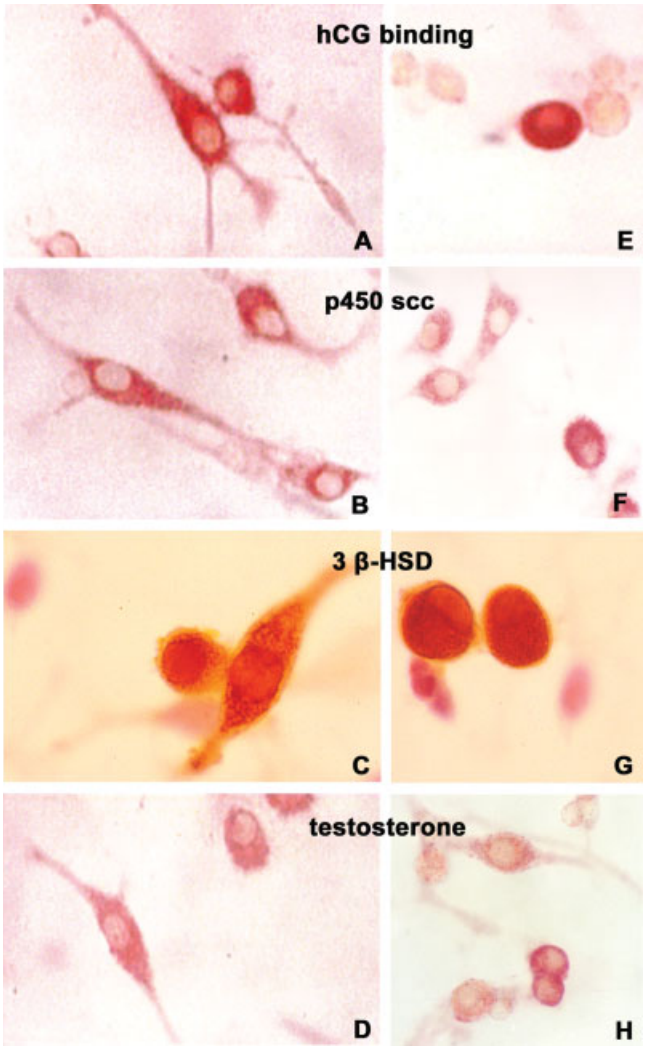

Fig. 3. Immunolabeling for hCG binding, cytochrome P450scc, 3β-hydroxysteroid dehydrogenase (3β-HSD) and testosterone in cultured Leydig cell mesenchymal precursors (**A–D**) and mature Leydig cells (**E–H**) from rats 21 days after EDS treatment. Magnification: hCG binding, P450scc, and testosterone: ×1,000; 3β-HSD: ×2,000.

and can only be identified as Leydig cell progenitors by expression analyses. The intermediate size mRNA (1.92 kb) was also observed at 7 days, but in immature Leydig cells. The full-length LH receptor mRNA (3.91 kb) was found in immature and mature Leydig cells from day 14 after EDS administration. Interestingly, elongated Leydig cell progenitors are capable of binding hCG and express steroidogenic enzymes and synthesize testosterone (Fig. 3).

The Seminiferous Tubules. The seminiferous tubules formed by Sertoli cells, germ cells, and peritubular cells do not show major changes during fetal life, infancy, and childhood in humans. Tubular diameter remains stable (∼50–60 μm), and Sertoli cells show typically immature features: they have oval, elongated nuclei with a regular shape; inter-Sertoli cell tight junctions are absent, cell proliferation is active, and AMH expression level is high. The germ cell population, represented first by gonocytes and subsequently

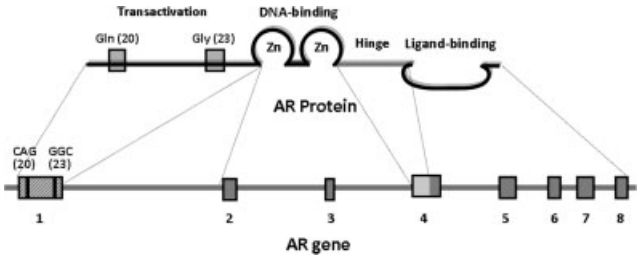

Fig. 4. Androgen receptor (AR) gene and protein.

by spermatogonia, proliferate by mitosis. Meiotic entry is not the rule (Nistal et al., 1982). During these periods, the seminiferous tubules moderately grow in length owing mainly to Sertoli cell proliferation (Rey et al., 1993b), driving a slight change in testicular volume, which reaches 2–3 mL before the initiation of puberty. Sertoli cell proliferation and AMH expression are regulated by FSH (Lukas-Croisier et al., 2003).

At pubertal onset, intratesticular levels of testosterone increase earlier than serum levels (Pasqualini et al., 1981). Sertoli cells undergo maturation, and stop dividing (Steinberger and Steinberger, 1971), show enlarged cytoplasm and nuclei with irregular shape, develop tight intercellular junctions (Chemes et al., 1979a), and significantly decrease their AMH expression (Rey et al., 1993a, 1996). After Sertoli cell maturation, pubertal spermatogenesis is triggered: spermatogonia divide and differentiate into primary spermatocytes, which go through the two successive meiotic divisions, resulting in the formation of spermatids. As a consequence of these processes, tubular diameter increases four- to fivefold (∼200–250 μm), and testicular volume reaches 20–25 mL at the end of puberty.

The Androgen Receptor. The androgen receptor (AR) is a transcription factor, member of the steroid hormone receptor superfamily, nearly ubiquitously expressed in mammalian tissues. The AR protein has a variable NH_2-terminal domain, a highly conserved DNA-binding domain (DBD), a hinge domain, and a ligand-binding domain (LBD) [reviewed by Lee and Chang (2003)]. The molecular mass of AR is 110 kDa, with ∼918 amino acids (Fig. 4). The NH_2-domain is characterized by the existence of polyglutamine and polyglycine repeats that result in variations in the length of the AR. The AR gene, containing eight exons, maps to the long arm of the X chromosome at Xq11-12.

When the AR binds to testosterone or, with higher affinity, to dihydrotestosterone, it undergoes conformational changes allowing the DBD of the AR to interact with androgen response elements in androgen target genes. The DBD is a 68-amino acid region folding into two zinc-finger structures (Fig. 4). The 295-amino acid region behind the DBD, including the hinge region and the LBD, is responsible for dimerization and androgen binding. A nuclear localization signal is located within the second zinc finger and the hinge region. This nuclear localization signal is responsible for nuclear trafficking of AR upon binding to testosterone or dihydrotestosterone. The most variable region is the N-terminal domain (NTD), composed of 555 amino acids, and containing one region involved in transcriptional acti-

vation of androgen responsive genes referred to as AF-1, and ranging from amino acids 141–338. Two other trans-activation domains, called AF-2 and AF-5, exist in the LBD and NTD, respectively. Phosphorylation may influence AR trans-activation. The polyglutamine repeat of the NH_2-terminal domain has an average length of 20, ranging from 8 to 31. There is an inverse correlation between the length of the polyglutamine repeat and the AR transcriptional activity, that is, long polyglutamine repeats reduce AR trans-activation. This might result from the influence of the repeat on interaction with co-regulators. Alternatively, short polyglutamine repeats may interfere with the AR NH_2-terminal domain phosphorylation or interdomain interaction of AR [reviewed by Lee and Chang (2003)]. The length of polyglycine repeats may also influence AR activity.

AR upregulates the expression of responsive genes upon binding to androgen response elements, which are mainly located in the target gene promoter region [reviewed by Lee and Chang (2003) and Heemers and Tindall (2007)]. More rarely, AR represses the expression of target genes, with or without DNA interaction (Eacker et al., 2007; Heckert et al., 1997; Palvimo et al., 1996). Furthermore, several AR coregulators, including coactivators or corepressors, have been identified. Coregulators interact with receptor proteins via protein–protein interactions, and play important roles in transcriptional activation. They might be involved in the pathogeny of certain cases of androgen insensitivity (Adachi et al., 2000).

Androgen-Dependent Function of the Seminiferous Tubule Cell Populations

The local effect of androgens within the testis has been acknowledged for more than 30 years in rodents (Chemes et al., 1982; Steinberger, 1971). The expression of the AR in the seminiferous tubules further supported the hypothesis (Hansson et al., 1975). Recent mouse knockout models conditionally impairing AR expression in the different cell populations of the testis have added relevant information (De Gendt et al., 2004; Tan et al., 2005; Tsai et al., 2006; Wang et al., 2006; Xu et al., 2007; Zhang et al., 2006).

Testosterone is the main stimulus for Sertoli cell maturation and for the attainment of full spermatogenesis during puberty in rodents (Chemes et al., 1979a,b). In humans with precocious puberty owing to an early activation of the hypothalamic-pituitary-gonadal axis, testicular volume increases as a result of an early onset of pubertal spermatogenesis. Interestingly, the same occurs in patients with gonadotropin-independent precocious puberty (or testotoxicosis), where an activating mutation of the LH/CG receptor induces an early increase of Leydig cell testosterone secretion despite a completely inactive state of the gonadotrope (Gondos et al., 1985; Misrahi et al., 1998; Rey et al., 1993a; Rosenthal et al., 1983). On the other hand, lack of AR expression in Sertoli cells in patients with cryptorchidism (Regadera et al., 2001) or other testicular disorders (Brehm et al., 2006; Steger et al., 1996, 1999) correlates with an undifferentiated histological phenotype and spermatogenic failure. Altogether, these clinical observations point to androgen action as the most important factor for the achievement of qualitatively full spermatogenesis.

On the other hand, the lack of androgen action within the gonad results in an immature aspect of Sertoli cells, with persistent AMH expression (Rey et al., 1994, 1999; Young et al., 2003) and incomplete spermatogenesis, in patients with congenital hypogonadotropic hypogonadism (Iovane et al., 2004), inactivating mutations of the LHβ subunit (Lofrano-Porto et al., 2007) or the LH/CG receptor (Kremer et al., 1995), or with androgen insensitivity owing to AR mutations (Quigley et al., 1995).

Similarly, experimental mouse models with knockouts of the gonadotropin-releasing hormone or its receptor, the gonadotropin common-α subunit, the LHβ subunit, the LH/CG receptor [reviewed by Huhtaniemi et al. (2006)] or the AR are azoospermic, with spermatogenesis arrested at the pachytene stage of the first meiotic division (De Gendt et al., 2004; Yeh et al., 2002) and maintain AMH expression (Al-Attar et al., 1997; Chang et al., 2004). Interestingly, conditional AR knockout models in which AR expression has been specifically ablated in germ (Tsai et al., 2006), peritubular (Zhang et al., 2006) or Sertoli (Chang et al., 2004; De Gendt et al., 2004; Wang et al., 2006) cells have demonstrated that only Sertoli cell AR expression is essential for spermatogenesis.

The Ontogeny of AR Expression in the Testis and Its Functional Relevance

As already mentioned, Leydig cells are very active as regards testosterone secretion in humans from early fetal life through 3–6 months after birth and then from the onset of puberty. However, while the high-intratesticular testosterone levels are capable of inducing Sertoli cell maturation and trigger full spermatogenesis at puberty, no such changes are observed during the fetal and early postnatal periods. In fact, fetal and neonatal spermatogenesis is characterized by the existence of gonocytes and prespermatogonia, with no meiotic progression. Furthermore, Sertoli cells respond to androgen action during puberty by changing their cytological and functional aspects: they develop tight junctions (Chemes et al., 1979a), stop dividing, and decrease AMH expression (Franke et al., 2004; Fröjdman et al., 1999; Rey et al., 1996). Yet, during fetal life and the first months after birth, the high levels of testosterone are unable to induce the aforementioned cytological changes and AMH expression remains at its highest levels (Rey et al., 1996). Similar observations have been made in the monkey (Sharpe et al., 2003) and rodents (Al-Attar et al., 1997).

To get insights into the reasons underlying this physiological state of androgen insensitivity of the Sertoli cell in the fetus and neonate, we and others assessed the ontogeny of the AR expression within the testis. A similar pattern has been observed in rodents (Al-Attar et al., 1997; Bremner et al., 1994), monkeys (Sharpe et al., 2003), and humans (Berensztein et al., 2006; Boukari et al., 2009; Chemes et al., 2008). AR expression is observed from early fetal life in the nuclei of most peritubular and Leydig cells. During childhood, typical Leydig cells are no longer observed in the interstitial tissue, yet AR expression is present in ~30–50% of interstitial mesenchymal cells. After pubertal onset,

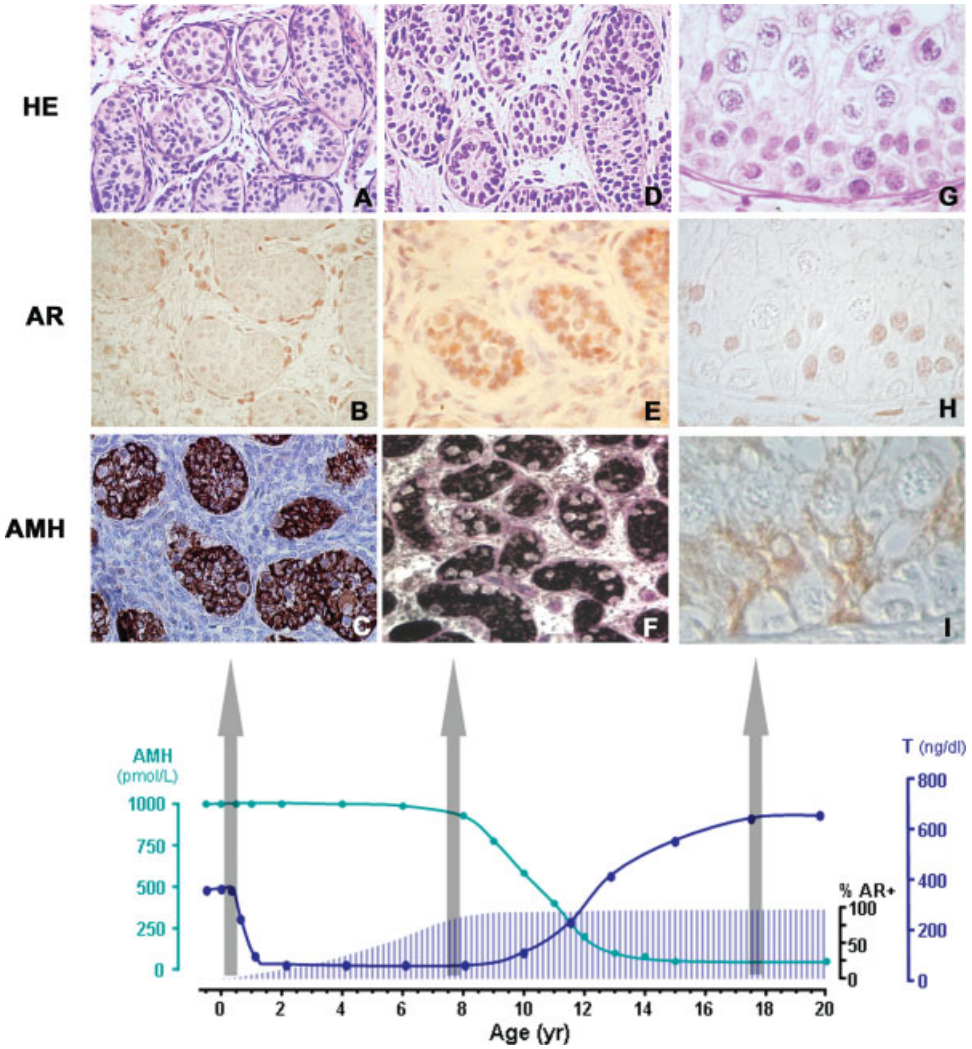

Fig. 5. Relationship between intratesticular androgen levels, androgen receptor (AR) expression and AMH in the human testis from fetal life to puberty. At 3 months (**A–C**), there are Leydig cells in the interstitial tissue that secrete testosterone; because the AR is expressed only in peritubular and interstitial cells but not in Sertoli cells (% of AR positive cells is null), AMH cannot be inhibited by testosterone and is therefore highly expressed. At 8 years (**D–F**), most Sertoli cells express the AR, but the interstitial tissue does not have mature Leydig cells and testosterone is low; therefore, Sertoli cells remain immature, AMH is high, and no meiosis occurs. At late puberty and adulthood (**G–I**), the increase in intratesticular concentration of testosterone acts on Sertoli cells to provoke their maturation, reflected in an inhibition of AMH expression and also in the development of adult spermatogenesis. Magnification: A–F: ×200; G–I: ×500. Modified from Chemes et al. 2008. J Clin Endocrinol Metab 93:4408–4412. Copyright 2008, The Endocrine Society.

Leydig cell nuclei show positive for AR again. Peritubular cells are always intensely positive, and germ cells are negative or mildly positive. In mice, the significance of AR in Leydig cells is related mainly to steroidogenic function (Xu et al., 2007), whereas in peritubular cells AR is important for quantitative—yet not qualitative—spermatogenesis (Zhang et al., 2006). Sertoli cells are AR-negative during the whole fetal life and the early postnatal period in rodents, monkeys, and humans (Fig. 5). In the mouse, intratesticular androgens are elevated from birth; when the AR expression increases in Sertoli cells (i.e., between postnatal days 4–9), AMH production decreases and meiosis is initiated (Fig. 6). In the monkey, there is also a negative correlation between AR and AMH expression in Sertoli cells (McKinnell et al., 2001). In the human, there is a much longer prepubertal period (infancy plus childhood) during which intratesticular testosterone is extremely low. AR expression appears faintly in a low proportion of Sertoli cell nuclei in the second half of the first year of life. A progressive increase is observed between ages 4–8 years (Fig. 5). By the age of pubertal onset (9–14 years), all Sertoli cell nuclei are AR-positive and respond to the increase of local androgen concentration with cytological signs of maturation, AMH downregulation and massive entry of germ cells into meiosis (Fig. 6) (Chemes et al., 2008). The factors that regulate AR expression in Sertoli cells remain unknown.

In line with the previous observations, clinical and experimental data support the existence of a period of physiological Sertoli cell insensitivity to androgens.

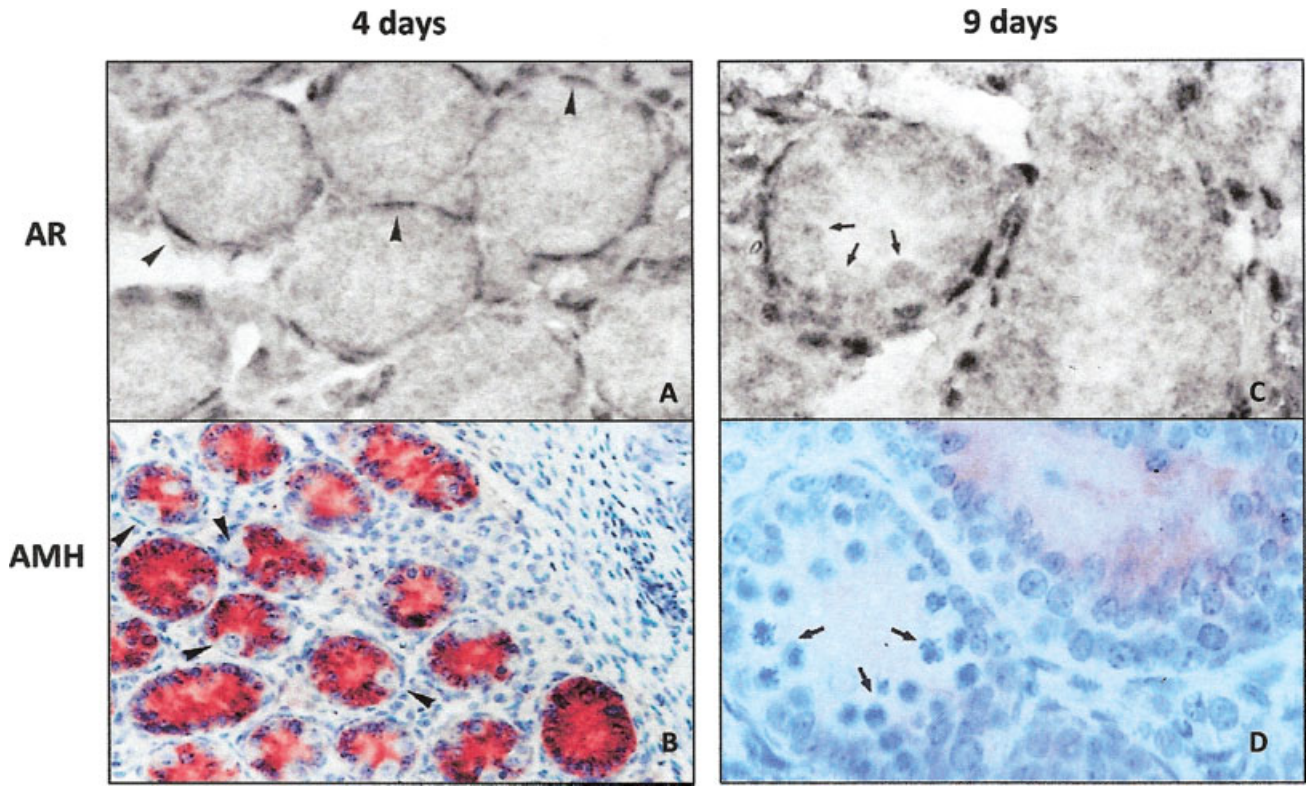

Fig. 6. Relationship between androgen receptor (AR) and AMH expression in the postnatal mouse testis. Until day 4, the AR is expressed in the peritubular, but not Sertoli, cells. Although testosterone is elevated in the mouse at this age, AMH expression remains high. By day 9, some Sertoli cell nuclei express the AR, and AMH expression is inhibited. Magnification: **A**, **B**, and **D:** ×300; **C:** ×150. Modified from: Al-Attar et al. 1997. J Clin Invest 100:1335–1343. Copyright 1997, American Society for Clinical Investigation.

Male patients with precocious puberty are rare before the age of 3–4 years and have not been described in the first year of life. In a recently reported patient in whom central precocious puberty was suspected at the age of 11 months because of an increase in penile size, AMH levels were not downregulated despite high-testosterone levels (Bergadá et al., 2007). This clearly indicated that androgen secretion, abnormally high for age, triggered peripheral signs of pubertal development (increase in penile size) but was unable to induce Sertoli cell maturation, which would have resulted in AMH downregulation. Similarly, in two infants with hypogonadotropic hypogonadism, prolonged hCG treatment induced Leydig cell androgen secretion to high levels, but AMH was not inhibited (Bougnères et al., 2008). In in vitro studies, a prepubertal mouse Sertoli cell line devoid of the AR expression has shown high levels of AMH expression despite high local androgen concentration in the culture medium. Yet, AMH expression was significantly downregulated when the Sertoli cell line was transfected with an AR expression vector and incubated with androgen-containing medium (Boukari et al., 2009).

Postpubertal spermatogenesis is adversely affected by fetal exposure to endocrine disrupters (Bay et al., 2006). These effects cannot be mediated by a direct antiandrogenic effect on germ cells, because the latter do not express the AR. Sertoli cell-mediated effect does not seem plausible either, because the AR expression in Sertoli cells attains significant levels only after birth. The mechanism for the fetal basis of adult onset disease is still poorly understood. The recent demonstration of the ability of environmental compounds to promote transgenerational transmission of adult onset disease—e.g., the exposure of a pregnant female rat to environmental endocrine disruptors, vinclozolin, or methoxychlor, resulting in male offspring of several generations with reduced spermatogenetic capacity—points to epigenetic reprogramming of the male germ line, due, for example, to DNA methylation (Anway et al., 2006, 2008). Antiandrogens may also act via paracrine mechanisms involving Leydig and/or peritubular cells, which express the AR from early fetal life. Alternatively, some of the antiandrogenic effects may be explained by the estrogenic action of certain endocrine disrupters, acting directly on fetal Sertoli cells (Sharpe, 2001), which express the estrogen receptor β (Boukari et al., 2007).

CONCLUDING REMARKS

The absent or low expression of the AR in fetal, neonatal, and early infantile Sertoli cells underlies a physiological state of cell-specific androgen insensitivity. This transient state may serve to protect the testis from precocious Sertoli cell maturation, which would result in the arrest of their proliferation and the precocious onset of pubertal 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 gonadotrope, but it becomes unveiled in disorders characterized by an early activation of Leydig cell testosterone production. At the time of puberty, when an adequate number of Sertoli cells has been achieved, intratesticular testosterone increases and acts on Sertoli cells with high AR expression, resulting in Sertoli cell maturation, downregulation of AMH expression and the onset of full spermatogenesis.

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