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Mini-Review

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The Role of Androgen Signaling in Male Sexual Development at Puberty

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Abbreviations: AMH, anti-Müllerian hormone; AR, androgen receptor; ARE, androgen response element; CREB, cAMP response element binding; DHT, dihydrotestosterone; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; FSH, follicle-stimulating hormone; G, genital; GnRH, gonadotropin-releasing hormone; LBD, ligand-binding domain; LH, luteinizing hormone; PH, pubic hair; PSA, prostate-specific antigen

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Abstract

Puberty is characterized by major changes in the anatomy and function of reproductive organs. Androgen activity is low before puberty, but during pubertal development, the testes resume the production of androgens. Major physiological changes occur in the testicular cell compartments in response to the increase in intratesticular testosterone concentrations and androgen receptor expression. Androgen activity also impacts on the internal and external genitalia. In target cells, androgens signal through a classical and a nonclassical pathway. This review addresses the most recent advances in the knowledge of the role of androgen signaling in postnatal male sexual development, with a special emphasis on human puberty.

Key Words: androgen receptor, AMH, Leydig, testis, Sertoli, transcriptional regulation

Puberty is a unique stage during postnatal development, of variable duration according to species, characterized by substantial anatomical and physiological changes leading to the mature state, typical of adulthood, of most organs. Throughout history, most of the attention has been directed to the physiology and pathology of the organs in their adult stage (1). The accelerated progress of technological tools during the last decades has nurtured the advancements in developmental biology, encompassing both prenatal and postnatal stages, until the achievement of the mature state. Androgen action is key for the virilization of the fetus but after birth, particularly in humans and other long-lived mammals, the prepubertal stage is characterized by a lack of evident activity in gonadal steroid secretion. During pubertal development, the testes resume the production of androgens, whose actions become patent in the development of male secondary sexual characteristics.

The onset and progression of puberty are controlled are controlled by the hypothalamic-pituitary-gonadal axis. The hypothalamus synthesizes gonadotropin-releasing

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hormone (GnRH) and releases it in a pulsatile manner to the portal system that drives it to the anterior pituitary where they reach the gonadotrophs expressing the GnRH receptor (2). Gonadotrophs secrete both luteinizing hormone (LH), responsible for androgen synthesis in Leydig cells, and follicle-stimulating hormone (FSH), acting on the seminiferous tubule (3). The hypothalamic-pituitary-gonadal axis is active during fetal development and for 3 to 6 months after birth in the human male. Thereafter, an active inhibition of GnRH secretion ensues throughout childhood, probably due to the effect of neurotransmitters such as catecholamines, GABA, and glutamate, and to the most recently described makorin ring-finger protein 3 (MKRN3) (2). A progressive increase in pulsatile GnRH secretion is responsible for the onset and progression of puberty. The mechanisms leading to the reinstatement of pulsatile GnRH secretion involve a complex interaction between genetic and environmental factors. Specific microRNAs (miRNAs) have recently been shown to lift the inhibitory actions of prepubertal blockers (4, 5), thus leading to the activation of kisspeptin and tachykinin systems that control GnRH neuron activity (2).

The testes are not only a source but also a target of androgen action, and major physiological changes occur in the various cell populations of the male gonads in response to variations in intratesticular testosterone concentrations. Testosterone is the most abundant circulating androgen produced by the testes. Dihydrotestosterone (DHT) is a more potent androgen (6), produced essentially in peripheral tissues by the classical pathway involving 5α-reduction from testosterone, and also by a "backdoor" pathway in the absence of testosterone as a precursor (7). In target cells, androgens act essentially through 2 different mechanisms, 1 classical and 1 nonclassical, both involving the same receptor (8). There is a differential impact of androgen action on the various target organs according to the stage of development. This review will address the most recent advances in the knowledge of the role of androgens and their signaling mechanisms in the different postnatal stages of male sexual development, with a special focus on human puberty.

Androgen Action in Target Cells

Testosterone and DHT

Testosterone and DHT are the main androgens in primates. The testis is the principle source of testosterone, whereas DHT is essentially produced in target tissues through the action of 5α-reductases. There are 2 physiologically relevant isoenzymes with 5α-reductase activity: type 1, encoded by *SRD5A1*, and type 2, encoded by *SRD5A2* (9). Expression is tissue- and age-dependent. In humans, 5α-reductase type 1 is not expressed in the fetus but can be detected in nongenital skin and liver at birth. While hepatic expression persists throughout life, expression in nongenital skin is transient during infancy and reappears at puberty in nongenital skin, including scalp where it is found in the sebaceous gland. Type 2 isoform of 5α-reductase is expressed at high levels in the derivatives of the Wolffian duct (epididymis, vas deferens, and seminal vesicles) and of the urogenital sinus (prostate and urethra) as well as in genital skin and scalp, and to a lesser extent in liver during fetal life. It can also be transiently detected in nongenital skin during infancy. Expression in liver, male reproductive tissues, and genital skin is high throughout life (10). A type 3 isoform has more recently been identified in prostate cancer tissue (11) but appears not to be involved in normal reproductive physiology (12).

A second pathway of DHT synthesis—less abundant in the adult but physiologically important in the fetus (13)—bypasses testosterone, and it is thus called the "backdoor pathway" (14). First described in the tammar wallaby (15), this route for DHT production involves 17OH-progesterone reduction by 5α-reductase type 1, followed by 3α-reduction by AKR1C2 or AKR1C4 to 17OH-allopregnanolone. The latter is subjected to 17,20 lyase activity of P450c17, yielding androsterone that is transformed to androstanediol by the hydroxysteroid dehydrogenase 17β-HSD3 in the gonads, or 17β-HSD5 (AKR1C3) in the adrenals. Androstanediol is finally 3α-oxidized by 17β-HSD6 (also known as retinol dehydrogenase, RoDH) in target tissues to yield DHT (Fig. 1).

Androgen signaling

The direct effects of androgen in target cells is mediated by the androgen receptor (AR), a member of the nuclear receptor subfamily 3, group C, member 4 (NR3C4). The AR is a 110-kDa protein, encoded by a gene mapping to Xq12, initially described as a ligand-activated transcription factor consisting of 4 main domains: an N-terminal domain, a 2-zinc-finger DNA-binding domain, a hinge region holding the nuclear localization signal, and a ligand-binding domain (LBD). In its unliganded form, the AR resides in the cytoplasm due to the association of its LBD with multiprotein complexes of chaperones and co-chaperones (Fig. 2), such as the heat shock proteins HSP23, HSP40, HSP56, HSP70, and HSP90 (16), or proteins of the FKBP family (17).

Classical pathway of androgen signaling

Androgens passively diffuse through the cell membrane and bind to the AR LBD. At the low hormone levels

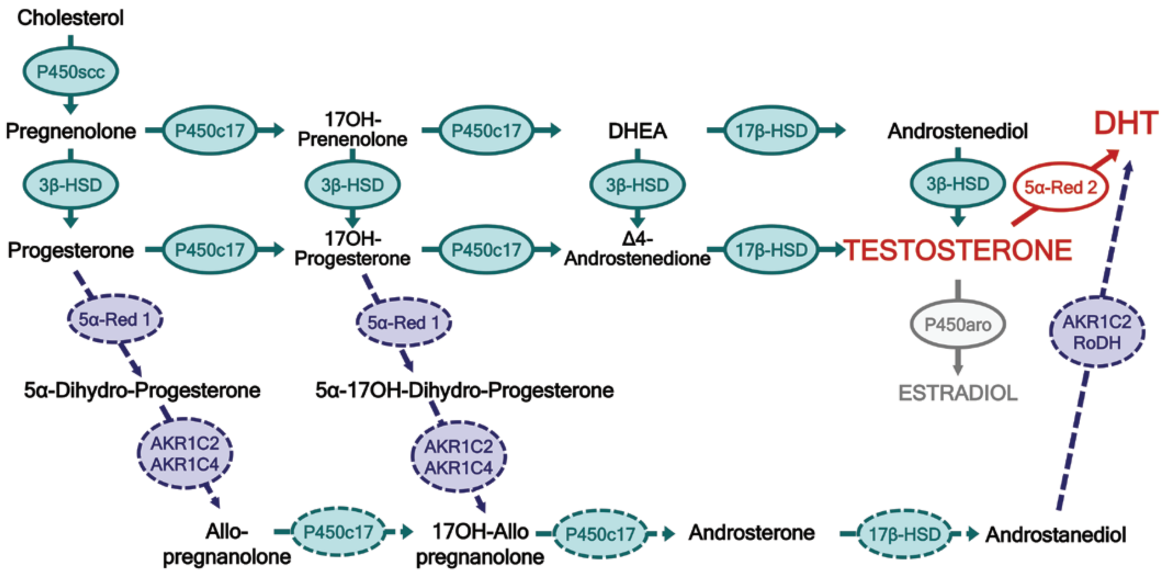

Figure 1. Sex steroid synthesis in the male: the classical pathway is shown in green, and the "backdoor" pathway of DHT synthesis is shown in blue.

observed in target tissues, DHT is more potent than testosterone because it has a 4-fold higher binding affinity for the AR and a 3-fold slower rate of dissociation than testosterone. However, there are no such differences at higher testosterone concentrations as those observed within the testes (18). Androgen binding to the LBD induces a conformational change in the AR that results in the exposure of its nuclear localization signal, which promotes the translocation of the AR to the nucleus mediated by interactions with importins that facilitate the transit through the nuclear pore complex (19-21).

In the nucleus, 2 AR molecules homodimerize and bind through their DNA-binding domains to androgen response elements (ARE) present in the promoters of target genes (Fig. 2). Classical AREs are 15-mer sequences formed by 2 palindromic repeats of 6 base pairs (5′-AGAACA-3′) separated by a 3-nondefined-base spacer, thus resulting in 5′-AGAACAnnnTGTTCT-3′, which can be recognized by all class I receptors, including the glucocorticoid, mineralocorticoid, and progesterone receptors. A second type of ARE, resembling more direct repeats of 5′-AGAACA-3′-like motifs, are only recognized by the AR and thus called selective AREs (22, 23). Classical and selective ARE sequences have been described for a large number of androgen-regulated genes (22). The AR dimers, acting through their N-terminal domain with a strong activation function domain (AF-1) and their LBD with a weaker AF-2, recruit a variety of co-activators or co-repressors that promote or inhibit transcriptional activity of target genes (16). These coregulators include modifiers of DNA structure, such as BRG1 and SNF, histone modifiers such as CBP/p300 and NCoR, and coordinators of transcription such as ARC and TRAP (24). Alternatively, the androgen-bound AR can interact with other transcription factors that have their own response elements in target gene promoters, eg, *NGFR* (formerly known as the p75 neurotrophin receptor) (25), *CGA* coding for the glycoprotein hormones α-chain (26), *LHB* encoding the LHβ chain (27), and *AMH* coding for anti-Müllerian hormone (28). In these cases, ligand-bound AR action does not require the existence of classical ARE sequences (Fig. 2). Whichever the underlying mechanism is, these "classical" or "genomic" pathways of androgen action are relatively slow mechanisms that require between 30 and 45 minutes after androgen stimulation for transcriptional activity to be modified, and even additional time is needed to be reflected in modifications of target protein levels.

Nonclassical pathways of androgen signaling

The nonclassical or "nongenomic" pathways induce responses within seconds of DHT stimulation that cannot be explained by the typical genomic mechanisms. Through its proline-rich region (aa 352 to 359), the AR associates with the SH3 domain of SRC (29, 30) triggering its tyrosine kinase activity, which results in phosphorylation of the epidermal growth factor (EGF) receptor (EGFR) (30). Activation of MAP kinase signaling ensues, including RAF, MEK, and ERK, followed by p90RSK kinase and final phosphorylation of transcription factors (Fig. 2), such as the cAMP response element binding (CREB) protein within 1 minute (31). The AR has also been shown to traffic and localize near the cell membrane (32-34), a process mediated by MEK1/2, AKT, and ERK1/2 signaling, leading to SRC phosphorylation (35). Recently, ZIP9, a member of a zinc transporter family unrelated to the classic AR, has been described as a membrane-bound AR, involved in Sertoli cell

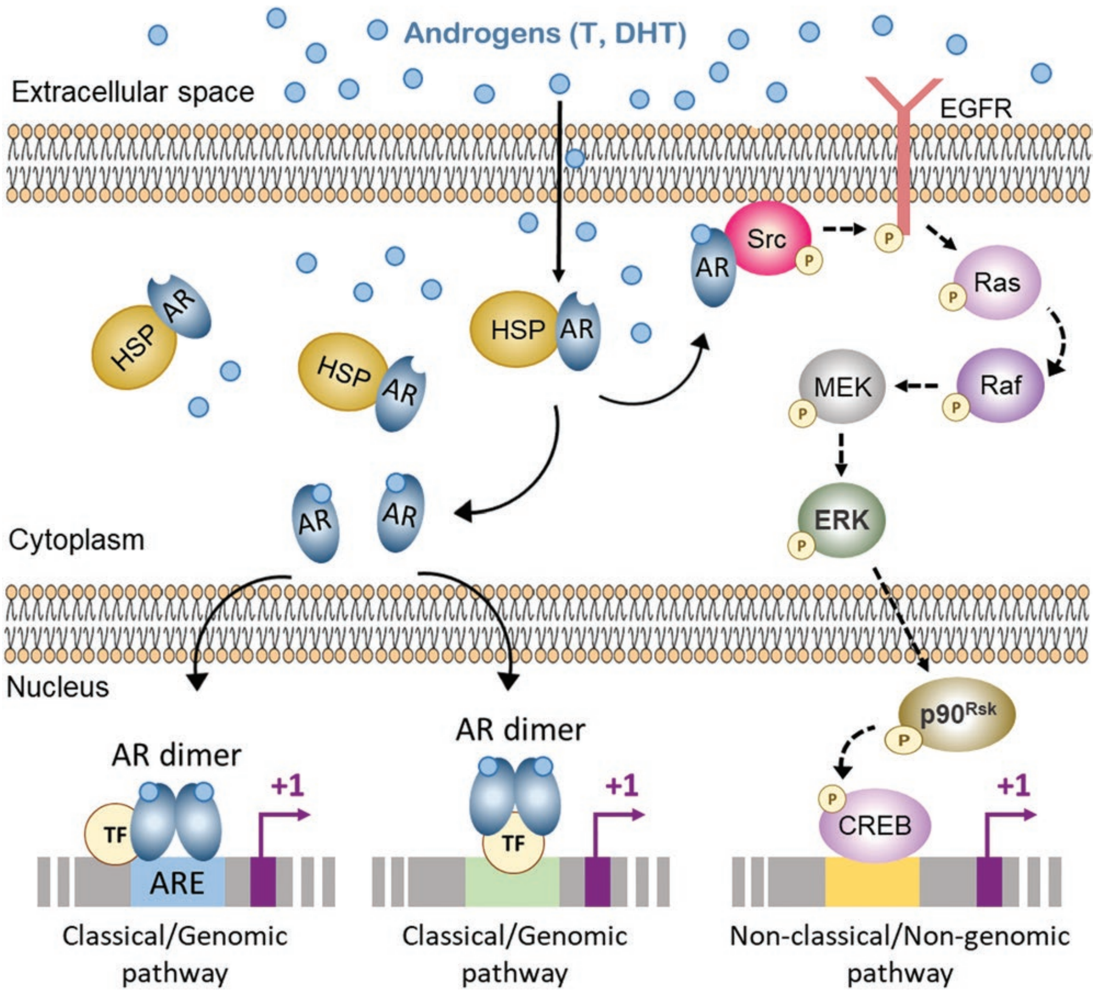

Figure 2. Pathways of androgen signaling. Androgens, such as testosterone (T) or dihydrotestosterone (DHT) represented as blue circles, cross the cell membrane and bind to the androgen receptor (AR) in target cells, displacing chaperones as the heat shock proteins (HSP), In the "classical" or "genomic" pathway, the ligand-bound AR translocates to the nucleus and forms homodimers that interact with androgen response elements (ARE) in target gene promoters or with other transcription factors (TF), finally regulating gene expression. In the "nonclassical" or "nongenomic" pathway, the ligand-bound AR migrates to the inner side of the cell membrane and interact with the Steroid receptor coactivator (Src) and activates the epidermal growth factor receptor (EGFR) signaling cascade involving eg, the mitogen-activated protein kinase (MEK), the extracellular signal-regulated kinase (ERK), and the cAMP response element binding protein (CREB). Modifed from: Edelsztein NY, Rey RA. Importance of the androgen receptor signaling in gene transactivation and transrepression for pubertal maturation of the testis. *Cells.* 2019;8:1-17, with permission from the authors © 2019, licensee MDPI, Basel, Switzerland (open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license).

physiology through ERK1/2-mediated phosphorylation of transcription factors CREB and ATF1 (36).

AR-independent pathways of androgen action

Androgens are converted to estrogens in the gonads and many other organs by the enzyme aromatase, a member of the cytochrome P450 superfamily. Estrogens signal by binding to classical intracellular estrogen receptors ERα and ERβ or to the G-protein coupled membrane receptor GPER (37). Many effects observed in association with male-range circulating androgen levels do not involve AR signaling, but are predominantly mediated by aromatization and estrogen signaling (38-40) or the nonspecific interaction and modulation of the activities of other nuclear receptors by androgens (23).

Androgens During Postnatal Development of the Male Reproductive Tract

The period elapsing between birth and the onset of puberty shows significant differences between mammalian species. Humans and other primate species are characterized by a long prepubertal stage, contrasting with rodents, in which pubertal changes start a few days after birth. Therefore, caution is essential when extrapolating experimental results obtained in rodents to primate reproductive developmental physiology. In humans, the prepubertal stage is usually divided into 3 sequential periods: the neonatal period includes the first 4 weeks of life, infancy comprises the first 2 years, and childhood is of variable length, until pubertal development begins at a variable age between 9 and 14 years in the male (Fig. 3). Similar stages are less clearly defined in other primates and cannot be distinguished in rodents.

Rather than a point in postnatal development, puberty is an extended maturational stage—of variable duration according to species—that shows spectacular changes in most reproductive organs leading them to the adult mature state. Anatomical changes of the genitalia, described by Marshall and Tanner (41), are classified into 5 stages from the prepubertal stage 1 to full development at stage 5 (Fig. 3). Completion of pubertal development of the genitalia takes

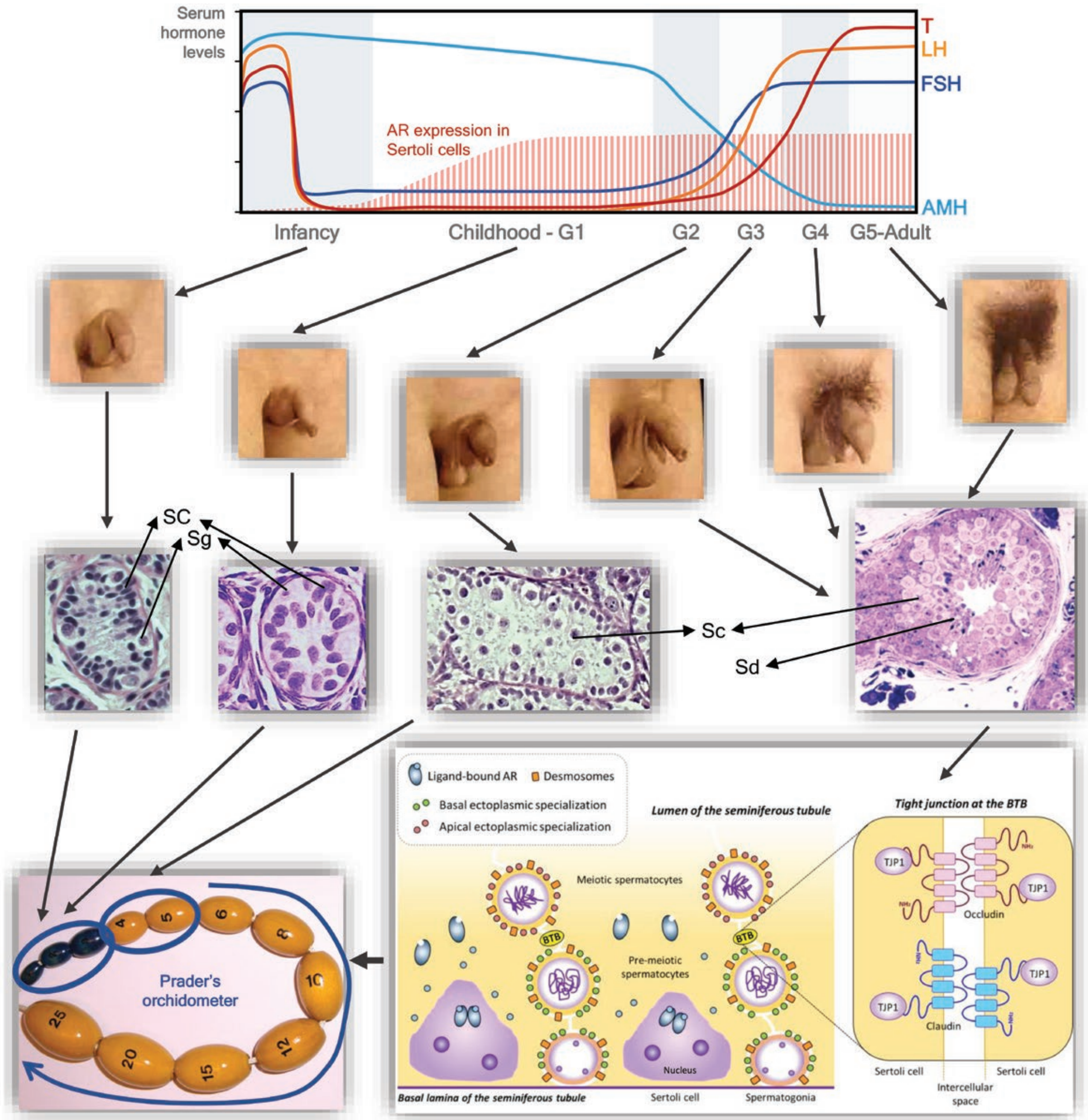

Figure 3. Schematics of changes in serum hormone levels, anatomy of the external genitalia, histology of the testis, illustrative components of the blood-testis barrier (BTB, reproduced with permission from ref. (8)), and testicular volume (in mL, as compared to Prader's orchidometer) throughout postnatal life in humans. Abbreviations: AMH, anti-Müllerian hormone; AR, androgen receptor; G1-G5, genital stages according to Marshall and Tanner (41); SC, Sertoli cells; Sc, spermatocytes; Sd, spermatids; Sg, spermtaogonia; T, testosterone. Prader's orchidometer: numbers represent testicular volume in mL.

approximately 3 years. In rodents, the onset of puberty is less well defined; the first changes in testicular physiology resembling those observed in humans, eg, the entry of testicular germ cells into meiosis, occur approximately at day 7 in mice. Achievement of the adult status, as defined by the acquisition of fertility, occurs rapidly at 6 to 8 weeks of age.

Androgen effects within the testis

Testicular changes during the prepubertal period

The testis is structured into 2 compartments, the seminiferous tubules and the interstitial tissue. Very few changes occur from birth until the onset of puberty (Fig. 3). The seminiferous tubules are solid with no lumen, formed by Sertoli cells and germ cells. Sertoli cells represent the largest testicular component until the onset of puberty. Immature Sertoli cells are small and oval-shaped, with elongated nuclei arranged in a palisade-like disposition. Functional characteristics of immature Sertoli cells include their expression of genes involved in cell division, growth, and metabolism (42). Archetypal features of the prepubertal Sertoli cell include its high expression of AMH (43), as well as its proliferative capacity, in response to FSH and other local factors (44). Germ cells are represented almost exclusively by spermatogonia, which divide by mitosis but do not enter meiosis until puberty. The germinal epithelium is surrounded by a basement membrane and peritubular myoid cells.

Between the seminiferous tubules lies the interstitial tissue, containing Leydig cells or their precursors and components of the connective tissue. Leydig cells are the source of androgens, showing substantial changes throughout development. Differences exist between rodent and primate Leydig cell differentiation and function, as reviewed in detail elsewhere (45-47). Primate Leydig cells are highly dependent on placental human chorionic gonadotropin (hCG) or pituitary LH (48). Neonatal activation occurring in humans (49) persists during infancy for 3 to 6 months (50); this period is often referred to as "mini-puberty," although clear physiological differences from true puberty exist (51). Subsequently, a prolonged period of inactivity exists during the rest of infancy and childhood in humans (Fig. 3). In other primates, this stage is usually called the "juvenile phase." Infantile or immature Leydig cells and their precursors do not show spontaneous steroidogenic capacity (52), but they have the capacity to respond to exogenous stimulation with hCG (53).

Role of androgen signaling in the prepubertal testis

The neonatal Leydig cells produce high amounts of testosterone, in approximately the same range as in puberty, both in rodents (54) and humans (55, 56). The high circulating level of testosterone is reflected in penile growth during the first months after birth in humans (57). The intratesticular concentrations of testosterone are high enough to saturate AR binding sites independently of transformation to DHT (18). The AR is expressed in peritubular myoid cells and Leydig cells but not in germ or Sertoli cells in neonates (54, 58-61). Therefore, androgens exert limited effects on the seminiferous tubules at this stage. One of the rare androgen actions within the testis during early postnatal life in humans involves germ cells, inducing the development and transformation of gonocytes into Ad spermatogonia. This process is impaired in boys with congenital central hypogonadism resulting in an impaired androgen surge (62) or with androgen insensitivity syndrome due to *AR* gene mutations (63). Androgen signaling is probably mediated through peritubular myoid cells. Other subtle modifications observed in Sertoli cell biology, such as testosterone-induced membrane potential depolarization and increased calcium uptake, have been explained by a noncanonical pathway independent of the AR in neonatal rats (64).

Interestingly, the prevailing physiological state of androgen resistance of Sertoli cells, derived from their lack of AR expression during fetal and early postnatal life (Fig. 3), seems critical for normal testicular development. Despite being exposed to adult-range intratesticular androgen concentrations during almost a year in humans (6 to 7 months *in utero* plus 3 to 6 months after birth), Sertoli cells do not show the morphologic maturation changes observed at puberty (8): they continue to produce high amounts of the immature marker AMH (60, 61) and to proliferate in response to FSH (44). In fact, the total number of Sertoli cells generated in this stage will have a direct influence on sperm output in adulthood, since each Sertoli cell is capable of sustaining a limited number of germ cells (65). When premature AR overexpression was experimentally induced in mouse Sertoli cells, their final population was significantly reduced and, although progression to meiosis and adult spermatogenesis was prematurely achieved, absolute spermatogenic output was visibly impaired (66). The physiological state of androgen insensitivity is maintained for approximately one year after birth in humans. Thereafter, Sertoli cells start expressing the AR (Fig. 3), and exposure to intratesticular androgen elevation, eg, in central precocious puberty, triggers Sertoli cell maturation and adult spermatogenic development in boys as young as 2 years of age. Both processes are reversible after androgen withdrawal (67, 68). Interestingly, spermatogenic development occurs with testicular volume that is smaller than that observed during normal puberty, suggestive of a precocious arrest of Sertoli cell proliferation, as observed in transgenic mice precociously overexpressing the AR in the testis (66).

Physiological changes and the role of androgen signaling in the pubertal testis

In humans, the first clinical sign associated with the onset of puberty is the increase in testicular volume, passing from 2-3 mL to 4 mL when compared to Prader's orchidometer (Fig. 3), or from 1.8 mL to 2.7 mL when more precisely measured by ultrasonography (69, 70). As already mentioned, the main difference within the testis between "mini-puberty" and true puberty stems from Sertoli cell responsiveness to androgens (51). At the moment of gonadotroph pubertal reactivation—which occurs between 9 and 14 years in humans, between 2 and 4 years in other primates, and by the end of the first postnatal week in mice—all Sertoli cells express the AR (54, 58-61, 71) but still have an immature phenotype (Fig. 3). Indeed, their expression of AMH is typically high and they are unable to support adult spermatogenesis (54, 60, 72, 73). Sertoli cells proliferate in response to the FSH surge (44, 65), which initiates before that of LH (56, 65, 74). This provokes the initial enlargement in testicular volume in humans (Tanner stage G2).

Subsequently, the progressive increase in pituitary LH pulses during pubertal stages G2 and G3 promotes a new wave of Leydig cell differentiation (52, 53, 75-77) and a gradual increase in intratesticular testosterone concentration (78-80). When it reaches the threshold to saturate AR binding sites, with no need to transformation to the more potent androgen DHT (13), testosterone leads to Sertoli cell maturation, increased peritubular myoid cell activity (81) and final Leydig cell development (82). Sertoli cell maturation is reflected in the upregulation of a large number of genes and the downregulation of others (8, 42, 83-87). The secretion of the immaturity marker AMH wanes during puberty (Fig. 3), especially between stages G2 and G3 in humans (56) and similarly in monkeys (88), bovines (89), swine (90), and rodents (54). This is explained by a direct effect of androgens on Sertoli cells, resulting in downregulation of AMH expression (28). However, the *AMH* gene promoter does not have a classical ARE, and experimental findings in the peripubertal Sertoli cell line SMAT1 (91) indicate that the ligand-bound AR could potentially interact with the transcription factor SF1 or its response element on the *AMH* promoter to hamper SF1-dependent induction of *AMH* transcription (Fig. 4) (28). The relevance of SF1 in AMH transactivation in the fetal testis had already been shown in rodents (92) and humans (93). In the absence of AR expression in Sertoli cells at the age of puberty, eg, in patients with androgen insensitivity

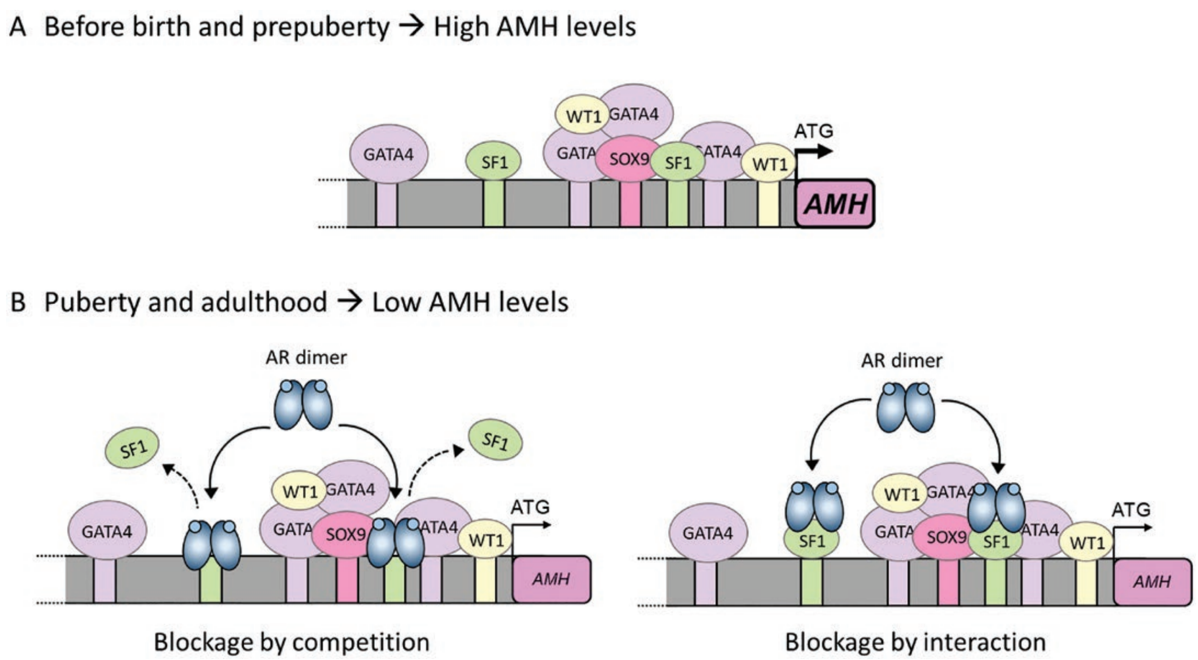

Figure 4. Molecular mechanism explaining the androgen-induced downregulation of anti-Müllerian hormone (AMH) expression in pubertal Sertoli cells. (A) Before puberty, in the absence of androgen action, AMH is highly expressed in response to transcription factors SF1, GATA4 and WT1. (B) During puberty and adulthood, the ligand-bound androgen receptor (AR) inhibits *AMH* transcription through either a direct interaction with SF1 sites on the *AMH* promoter (blockage by competition, which impedes SF1 binding to its specific response elements) or a protein-protein interaction with SF1 (blockage by interaction, resulting in the inactivation of SF1 transcriptional activity). In both cases, the AR prevents SF1 from upregulating *AMH* expression. Reproduced from: Edelsztein NY, Rey RA. Importance of the androgen receptor signaling in gene transactivation and transrepression for pubertal maturation of the testis. *Cells.* 2019;8:1-17, with permission from the authors © 2019, licensee MDPI, Basel, Switzerland (open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license).

syndrome (94), *Tfm* mice (54) or mice with Sertoli cell-specific AR knockout (95), AMH expression persists at prepubertal levels or even higher.

Among Sertoli cell-expressed genes that are upregulated by androgens during puberty, of particular importance are those involved in the establishment of the blood-testis barrier (8, 42, 83, 85). The blood-testis barrier creates a protected microenvironment for meiotic (spermatocytes) and postmeiotic germ cells (spermatids) in the adluminal compartment of the seminiferous tubules (Fig. 3), separated from the basal compartment containing premeiotic germ cells (spermatogonia). The adluminal compartment is inaccessible to the immune system, thus avoiding autoimmune reactions to spermatocytes and spermatids, which do not exist in early life when the immune system develops. The mature blood-testis barrier consists of Sertoli cell membrane specializations, especially tight junctions and gap junctions. Claudin-11 and connexin-43 are the main components of tight and gap junctions, respectively. Their expression increases with Tanner stages in humans, as AMH wanes (96). In mice, claudin-3 and claudin-11, and components of the cytoskeleton, such as TJP1 (also known as ZO1), also show a significant increase by postnatal day 10, in coincidence with the first testicular signs of pubertal onset (97). The androgen dependence of the blood-testis barrier components became evident in studies showing a decreased expression in the hypogonadal *hpg* mouse, with a positive response to DHT (98), as well as in *Tfm* mice (99) and *AR* knockout models (84, 87, 100). Further support was provided by results of ChIP experiments showing the existence of functional ARE sequences in the promoters of mouse genes *Cldn13* and *Tjp2iso3* (101), suggesting that the classical AR-mediated pathway is involved. Androgens can also upregulate *Cldn3* and *Cldn5* through the nongenomic pathway involving ERK1/2, CREB and ATF1 (102). In all these cases, the disruption in the formation of the blood-testis barrier is associated with an incomplete progression through meiosis. On the other hand, experimentally induced premature overexpression of the AR in mouse Sertoli cells drives precocious upregulation of *Cldn11* and *Tjp1*, and early development of the blood-testis barrier and of meiotic onset (103).

Once Sertoli cells have acquired a mature phenotype, the onset of adult spermatogenesis occurs, characterized by increased proliferation of germ cells and their entry into meiosis (51). Diploid spermatogonia give rise to primary spermatocytes that undergo the 2 successive meiotic divisions to produce haploid spermatids (Fig. 3). The latter further mature to form spermatozoa that are released to reach the epididymis. The duration of the full spermatogenic process from spermatogonial differentiation to sperm release is approximately 74 days in humans (104); however, the process is rather inefficient during the first stages of puberty, and spermarche only occurs about 1 to 2 years after pubertal onset, when boys are in Tanner stage G3 and have a mean testicular volume of 10 mL (105, 106). Interestingly, intratesticular testosterone concentration is already high by this stage (78), but not serum testosterone (56), which underscores the importance of the paracrine action of androgens on the seminiferous tubules. Indeed, spermatogenic development and consequent testicular enlargement are indicative of local testosterone production, as also observed in boys with Leydig cell tumors (107) or testotoxicosis, a condition due to an activating mutation in the LH receptor (108), and in patients with central hypogonadism treated with gonadotropins (109). Conversely, high circulating androgen levels due to excessive adrenal production, eg, congenital adrenal hyperplasia, or to exogenous testosterone administration, are unable to achieve sufficient intratesticular androgen concentration to induce spermatogenesis. The achievement of full adult spermatogenesis results in a further increase in gonadal size attaining >15 mL (orchidometer) or >10.2 mL (ultrasonography) (70). At this stage, the histology of the gonads is characterized by seminiferous tubules with open lumina. Sertoli cells have a typical columnar feature, and germ cells are the largely predominant population (Fig. 3).

Extensive evidence exists on the physiological importance of androgens on spermatogenic development at 3 stages: (a) spermatogonial proliferation and differentiation, (b) progression through meiosis, and (c) spermatid development and spermiation (110). Surprisingly, the mechanisms that connect androgen-induced Sertoli cell maturation and germ cell progression through meiosis have not been elucidated. In mice, retinoic acid is critical for meiotic entry (111, 112), although in humans other factors also seem to be involved (113). The enzyme CYP26B1 degrades retinoic acid, thus preventing meiotic entry in the fetal and prepubertal testis (114). Interestingly, CYP26B1 expression in Sertoli cells wanes at puberty (115), suggesting a potential downregulation by androgens like that observed for AMH (28). However, experimental results in the peripubertal Sertoli cell SMAT1 line ruled out a direct action of androgens on CYP26B1 expression (115). One possibility is that the androgen-driven changes in Sertoli cell cytoskeleton provokes changes in the germ cell cytoskeleton resulting in passage from the basal to the adluminal compartment of the seminiferous tubule. This immune-privileged microenvironment would be influential for germ progression through meiosis (116).

Impaired androgen signaling results in defective spermatogenesis. The role of ligand-bound AR action in the progression of spermatogenesis through meiosis after pubertal onset has been clearly demonstrated in conditions such as central hypogonadism, impaired Leydig cell steroidogenesis and androgen insensitivity, either naturally occurring in humans or experimentally induced in animal models (117). Once again, Sertoli cells are the main mediators, since germ cells do not express the AR during puberty and adulthood (118, 119). In patients with androgen insensitivity syndrome, a moderately increased risk for germ cell neoplasia in situ has been described. Particularly in partial forms of androgen insensitivity, residual androgen signaling has been suggested to promote neoplastic germ cell proliferation from puberty onwards (120).

Androgen effects on the internal reproductive tract

The main androgen-dependent organs of the internal male reproductive tract include the epididyimides, the vasa deferentia, and the seminal vesicles, all derivatives of the mesonephric Wolffian ducts, and the prostate, which originates in the urogenital sinus.

Epididymis

In the neonatal period and early infancy, the epididymal duct is formed by a single epithelial layer lying on a basement membrane and surrounded by myoid cells (121). The AR is expressed mainly in the epithelial cells of the epididymis (122), in which they induce maturation features during pubertal development (123-125). Conversion to DHT seems to be important in spite of the high local androgen levels (126). Maturational changes include cell proliferation and coiling, such that in the human 6 meters of tubule become packed into the small organ lying above the testis in the scrotum (127). Three topographical portions can now be clearly identified: caput, corpus, and cauda. The caput and corpus show a predominant secretory function, mainly involved in sperm maturation, while the cauda primarily serves as a storage site for mature spermatozoa. The androgen signaling pathways in postnatal development are poorly known. Recent studies using genome-wide protocols including DNase-seq, RNA-seq and ChIP-seq have characterized the transcriptome and occupancy of specific transcription factors in the different segments of the epididymis. Expression of the AR seems to play a major functional role essentially in the caput epididymis (128). AR ChIP-seq experiments have identified new cofactors, such as CCAAT/Enhancer binding protein-β (CEBPβ) and Runt-related transcription factor-1 (RUNX1), required for AR binding at a subset of sites in human epididymis epithelial cells (129). Regional expression of AR coregulators may play a role for the differential androgen actions observed along the epididymis (130).

Vas deferens

During infancy and childhood, the vas deferens is lined by a columnar epithelium with short stereocilia resting on a basement membrane and a basal lamina of connective tissue, surrounded by 3 layers of muscular tissue with ill-defined limits (131). During pubertal development, the wall and the lumen of the vas deferens increase in diameter. The epithelium becomes pseudostratified, with columnar and basal cells, and the 3 muscular layers can be clearly distinguished (131). Expression of the AR is induced by the PI3K/AKT pathway (132) in the epithelial cells (122), where they mediate androgen action, eg, inducing the expression of *Itm2b*, a member of the type II integral membrane protein, during pubertal maturation (133). EGF-mediated signaling interferes with AR-dependent maturation in the epithelial cells, thus allowing cell proliferation; conversely, when androgen signaling prevails, DHT exerts an inhibitory effect on the EGFR-induced ERK activity and favors the maintenance of mature state (134).

Seminal vesicle

During childhood, the epithelium of the seminal vesicles consists of basal and mucus-producing glandular cells with relatively scarce activity. The size of the seminal vesicles grows slowly during childhood (135). During pubertal development, the columnar epithelium of the seminal vesicles becomes highly convoluted and pseudostratified with active protein secretory machinery in response to DHT (136). The AR is expressed in all cell types (stromal, smooth muscle, and epithelial cells), and a vital role for AR signaling via the smooth muscle cells has been demonstrated for normal seminal vesicle structure and function (137).

Prostate

The prostate, the largest accessory male sex organ, is formed by glands communicating with the urethra through excretory ducts. The glands are surrounded by a stroma, containing connective tissue and smooth muscle. Three concentric zones can be distinguished surrounding the urethra: the innermost zone formed by mucosal glands, surrounded by the internal zone consisting of submucosal glands, and externally the peripheral zone containing the by tubule-alveolar glands. The epithelial cells of the glands are formed by 3 distinct lineages: basal, luminal, and neuroendocrine cells (138). At birth in humans, the glandular aspect is evident, with most acini showing a lumen. During the following weeks, the epithelial cells of the glands become taller, as a sign of androgen-dependent activity, and some of the acini show the typical features of the adult prostate. After the sixth month, there is an involution of the glandular aspect, and little change is seen in childhood (139). During puberty, prostate size increases from 0.5 to

2 g to reach 12 to 20 g in the young adult. This is due to the development of the acini into glandular structures lined by a secretory, columnar epithelium and, to a lesser extent, of the stroma (140).

Normal androgen levels (141) and expression of the AR (142) and the enzyme 5α-reductase 2 (143) are essential for prostate development in fetal and postnatal life. AR is present in both the epithelial and the stromal cells, and the androgenic effects on prostate development is mediated through mesenchymal-epithelial interactions. Selective cell disruption of the AR has clearly shown that fetal and postnatal prostate development and epithelial proliferation depend mainly on androgen-dependent paracrine signals originating in stromal cells (144), whereas AR signaling in epithelial cells maintains their functionally differentiated phenotype and restrains their proliferation specially in the anterior lobe (145, 146). Androgen signaling through the classical AR pathway has a critical role in mediating WNT action on mouse prostate development (147). The subcellular androgen-dependent mechanisms involved in pubertal development of the prostate have been poorly studied. The AR co-chaperone FKBP52 has a specific role in prostate androgen-regulated maturation (17). The prostate-specific antigen (PSA; also known as kallikrein-related peptidase 3, encoded by *KLK3*) is a functional marker of androgen action produced by prostate gland epithelial cells. PSA concentration is extremely low or undetectable in prepubertal boys and during Tanner stage G2, reflecting the low circulating levels of testosterone; PSA levels increase progressively from stages G3 to G5 of normal pubertal development, in correlation with serum testosterone (148), are elevated in boys with precocious puberty and decrease when androgen production is curtailed by GnRH analog treatment (149), and are low in patients with delayed puberty or other conditions characterized by androgen deficiency (150). PSA levels reflect the direct transcriptional activation exerted by DHT-bound AR on classical ARE sequences present in the *KLK3* gene promoter (151).

The external genitalia

Changes during childhood and pubertal development

The penis consists of a root, the body or shaft, and the glans. The body is enveloped in skin and contains the erectile tissues: the 2 corpora cavernosa and the corpus spongiosum. Penile size shows little variations among human ethnic groups, with a mean length between 3 and 4 cm at birth (152). Penile length shows a very modest increase during infancy and childhood (Fig. 3), approximately 1 mm per month during the first 6 months after birth (57) and 2 to 3 mm per year during childhood (153).

The scrotum is the cutaneous sac that holds the testes outside of the abdominal cavity. Covered by skin and essentially formed by smooth muscle, is the dartos muscle, or the dartos fascia. The skin of the scrotum and the pubic area is hair-bearing, with sebaceous and sweat glands. The hair is scarce, fine, and lacking the medulla layer, ie, it is vellus hair, until the onset of puberty.

Genital (G) and pubic hair (PH) development during human puberty has been characterized in detail by Marshall and Tanner (41). Together with testicular size increase in stage G2, the scrotum enlarges and its skin texture changes and reddens (Fig. 3). Subsequently in stage G3, the penis grows first in length and then in breadth, together with a further enlargement of the testes and scrotum. Before the genitalia progress to stage G4, sparse growth of long, slightly pigmented pubic hair can be seen at the base of the penis; this stage of pubic hair development is known as PH2. Subsequently, the penis further enlarges in length and breadth, and the glans develops, the testes and scrotum also enlarge, with darkening of the scrotal skin (stage G4), and pubic hair becomes curled, darker, and coarser, spreading sparsely (PH3). This coincides with peak height velocity in adolescents. In the following months, the external genitalia and pubic hair reach the adult stages (G5 and PH5). This usual sequence of events may be altered in certain conditions, such as early adrenarche or other situations of excess androgen production by the adrenals, where pubic hair may appear before stage G2.

Role of androgen signaling in the pubertal changes of external genitalia

The normal development and trophism of the external genitalia are fully dependent on continuous androgen stimulation from fetal life until the completion of puberty. The AR is expressed in stromal and endothelial cells of the erectile tissue of the corpus cavernosum, corpus spongiosum and glans penis (154, 155) and in the fibroblasts and hair follicles of the genital skin (156-158). Circulating testosterone levels reaching these organs are insufficient to produce an appropriate effect, thus 5α-reductase activity for transformation into DHT is critical (159). *SRD5A2* expression is high in genital skin. A deficiency in DHT synthesis or action in early fetal life results in genital ambiguity, whereas a later production deficiency leads to micropenis and hypotrophic scrotum. Interestingly, when the problem relies on 5α-reductase activity, the development and function of organs exposed to high testosterone levels is not affected, eg, in patients with mutations in *SRD5A2* Wolffian duct derivatives adjacent to the testis (epididymis and vas deferens) differentiate in utero and Sertoli cells mature and support spermatogenesis at puberty (159). The expression of the AR and 5α-reductase

2 does not seem to show major changes from fetal life to puberty, whereas that of 5α-reductase 1 increases after birth (10, 159).

Changes in penile size, and scrotal and pubic hair trophism, follow the increase of circulating testosterone levels during human pubertal development: changes are very subtle or absent in Tanner stage 2 (41) when serum androgens concentrations are roughly similar to those observed before pubertal onset (56); from stage G3 onwards, there is a progressive increase in serum testosterone associated with enlargement of the penis and scrotum and development of genital skin hair (Fig. 3). Surprisingly few studies exist on the molecular signaling pathways underlying androgen action in the external genitalia. In the rat, penile growth is in part explained by testosterone regulation of keratin 33B expression through AR binding to an ARE sequence present in the *Krt33b* promoter (160).

Conclusions

Androgens play a major role during male pubertal development. The testis is the major source of testosterone, which acts in a paracrine way mainly through Sertoli and peritubular myoid cells to induce and maintain adult spermatogenesis. Rapid responses are mediated by nongenomic pathways whereas the best characterized long-term actions involving upregulation and downregulation of androgen-dependent genes are mediated by genomic pathways. In the internal and external genitalia, testosterone needs to be the more potent androgen DHT to be efficacious. While the effects of androgens and of their withdrawal have been extensively characterized at the level of the internal and external genitalia, remarkably little information exits on the molecular mechanisms involved.

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