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Leydig Cell Function in Man

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INTRODUCTION

In humans, as in all mammalian males, Leydig cells are the main source of the androgenic hormone, testosterone, which is essential for male sexual differentiation, gamete production and maturation, and development of secondary sexual characteristics. In this chapter, the development, steroidogenic function, and regulation of human Leydig cells are summarized. Clinical aspects of androgen secretion and pathology related to Leydig cells are also reviewed. The information presented is based, in part, on studies conducted in laboratory animals because of the larger database available for these species.

LEYDIG CELL DEVELOPMENT

Leydig cells were first described by the German histologist Franz Leydig in 1850 (1). In all mammalian species, Leydig cells are located in the interstitial compartment of the testis, between and surrounding the seminiferous tubules (2). Human Leydig cells are epithelioid and ovoid or polygonal. Other cytological features include eosinophilic cytoplasm, euchromatic round eccentric nuclei with a peripheral distribution of heterochromatin, and conspicuous nucleolus. The predominant cytoplasmic organelle is the smooth endoplasmic reticulum (SER), which is characteristically more abundant in steroidogenic cells when compared to other cell types. Mitochondria and lipid droplets are also numerous in Leydig cells and play a role in steroidogenesis that is discussed later in this

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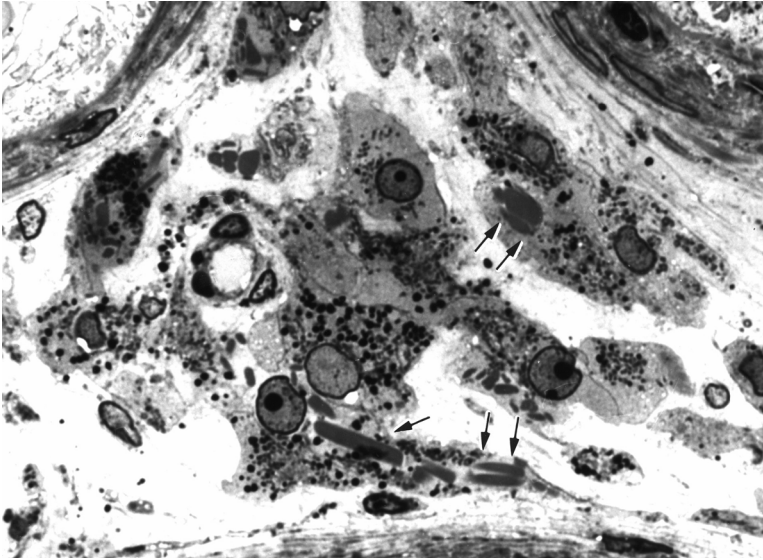


Fig. 1. Testicular interstitial space of an adult man (original image magnification $\times 7,000$). This semithin plastic section shows mature Leydig cells surrounding a capillary. In humans, Leydig cells are embedded in a loose connective tissue. The arrows point to Reinke crystals in the cytoplasm of Leydig cells. (Photo supplied by Dr. Hector Chemes, from ref. 13, with the publisher's permission.)

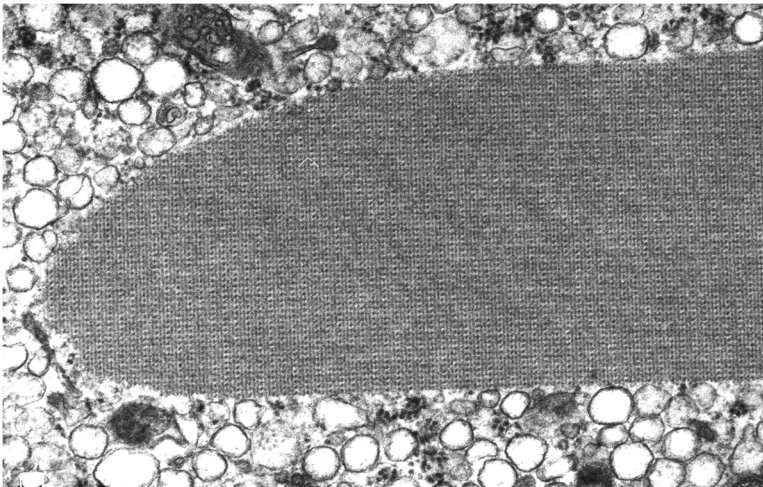


Fig. 2. A Reinke crystal. This electron micrograph (original image magnification $\times 50,000$) shows the highly ordered lattice of filaments within these crystals. (Photo supplied by Dr. Hector Chemes, from ref. 13, with permission from the publisher.)

chapter (3,4). Reinke crystals are observed exclusively in human Leydig cells and are believed to be indicative of diminished steroidogenic capacity during aging (5–7). Human Leydig cell structure and Reinke crystals are shown in Figs. 1 and 2.

In humans, blood levels of testosterone peak three times during development (8). The first peak occurs at 12–14 wk of gestation, during the fetal differentiation of Leydig cells (9). Testosterone levels then decline and are low for the remainder of gestation

and the early neonatal period. A second peak at 2 mo postpartum is associated with renewed Leydig cell proliferation. Leydig cells then atrophy a second time, and, for the next decade, the interstitium is populated by steroidogenically inactive precursor cells. The adult generation of Leydig cells differentiates pubertally and is complete by 12 to 13 yr of age. Serum levels of testosterone average 6 ng/mL during adulthood (10). Finally, there is a decline in testosterone secretion with aging, which varies in its age of onset. The age-related testosterone decrease is caused primarily by gradual atrophy and loss of adult Leydig cells and also by declines in steroidogenic capacity (11). The fetal, neonatal, and adult epochs of testosterone secretion are associated with three separate waves of increases and declines in Leydig cell numbers (12).

Fetal Leydig Cells

Until recently, the precursors of Leydig cells were believed to be of mesenchymal origin and derived from cells in the mesonephros (primitive kidney) (14–16). However, recent tissue recombination studies do not support the hypothesis that precursor cells migrate into the interstitium from the mesonephros, and ontogeny from the embryonic neural crest has been suggested (17). By the sixth week of gestation, seminiferous cord formation within the gonadal blastema simultaneously creates the outer interstitial compartment (18). Fetal Leydig cells become identifiable in this compartment among the undifferentiated mesenchymal cells at 8 wk of gestation (19). Then, Leydig cell numbers increase continuously and reach a maximum by 14 to 15 wk, when they fill the space between the cords and comprise more than half the volume of the fetal testis. Although testosterone is first detectable in the testis as early as 6 to 7 wk of gestation (20), the sharp increment in the numbers of fetal Leydig cells is accompanied by further rises in androgen concentrations in testicular tissue, blood, and amniotic fluid, which reach a maximum at week 15 (21). After the 16th wk of gestation, the numbers of fetal Leydig cells, serum testosterone concentrations, and testicular mRNA levels for at least two of the testosterone biosynthetic enzymes, P450scc and P45017 α , decline (21–23). At birth, the total number of Leydig cells per testis is 60% lower compared to the prenatal peak, and the remaining Leydig cells are half the size (21).

Neonatal Leydig Cells

Just after birth, the number of Leydig cells again increases to a peak at 2 to 3 mo of age, contributing to a second surge in plasma testosterone levels. At this stage, Leydig cells contain abundant SER membranes and mitochondria, as well as varying amounts of lipid droplets (4,24–27). In the neonatal testis, fetal Leydig cells persist through at least 3 mo after birth. Postnatal increase in the number of these cells most likely results from recruitment of interstitial precursor cells. After this increase, neonatal Leydig cell numbers regress rapidly to a nadir by the end of the first year. The neonatal period is relatively brief, extending only through the first year of life (28,29).

After the first year and continuing for a decade, Leydig cells are in a state of prepubertal quiescence. During this phase, well-differentiated Leydig cells are absent from the interstitial space. In their place are partially differentiated Leydig cells and primitive fibroblastic cells. At this stage, Leydig cells are dispersed in a loose connective tissue matrix and contain elongated nuclei with scarcely visible cytoplasm. It has been proposed that these partially differentiated Leydig cells and primitive fibroblasts are precursors of adult Leydig cells (13,25,30–32).

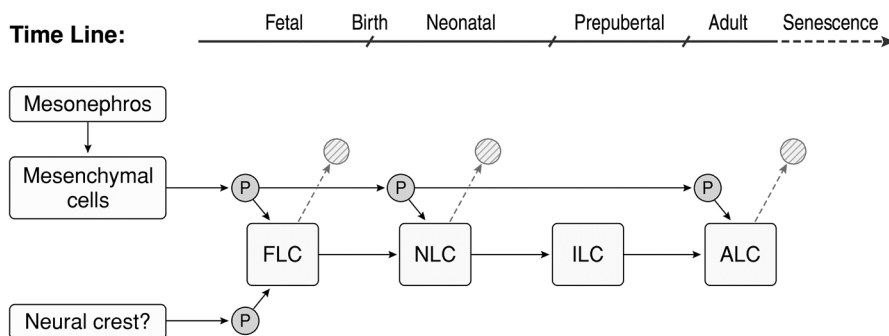


Fig. 3. Human Leydig cell development. FLC, fetal Leydig cells; NLC, neonatal Leydig cells; ILC, immature Leydig cells; ALC, adult Leydig cells, P, fibroblastic precursors of Leydig cells. The green cross-hatched circles indicate degeneration of FLC, NLC, and ALC. The yellow compartment represents stem cells, including mesonephros-derived mesenchyme and/or neural crest. The time line shows the developmental period: fetal (from 8 wk gestation to birth), neonatal (first year of life), prepubertal (from second year to approx 10 yr of life), and adult (from approx 13 yr old onward). According to the hypothesized ontogeny, mesonephros-derived mesenchymal cells, or possibly neural crest, supply precursors for FLC. After birth, NLC arise from redifferentiation of FLC and new differentiation from mesenchymal cell precursors. After the first year of life, NLC regress to the partially differentiated ILC. ILC are then present through childhood. Starting at approx 10 yr of age, ILC and mesenchymal precursors again differentiate, maturing into ALC. With senescence, Leydig cells begin to lose full steroidogenic function and, as indicated, atrophy.

Adult Leydig Cells

The precursor cells for adult Leydig cells begin their transformation at approx 10 yr of age, and differentiation is complete by 13 yr of age (33). During puberty, the number of adult Leydig cells increases and reaches a maximum of 5×10^8 per testis in the early 20s (25). At this time, a third peak in testosterone concentrations occurs. Thereafter, Leydig cell numbers gradually decrease by 50% in men aged 60 yr and older (34). Between ages 20 and 60 yr, there is an equilibrium in the numbers of Leydig cells, which comprise approx 4% of the volume of the mature testis (14). The overall scheme of human Leydig cell development is summarized in Fig. 3.

STEROIDOGENIC FUNCTION OF LEYDIG CELLS

The primary function of Leydig cells is to synthesize and secrete androgenic steroids. The process of androgen biosynthesis requires the activities of four enzymes: cytochrome P450 cholesterol side chain cleavage enzyme (P450scc), 3β -hydroxysteroid dehydrogenase/ Δ^{5-4} isomerase (3β -HSD), cytochrome P450 17α -hydroxylase (P450 17α), and 17β -hydroxysteroid dehydrogenase (17β -HSD) (35). Leydig cells are the only cells in the testis containing P450scc and 3β -HSD. Thus, Leydig cells are the sole testicular location for the first two steps in steroidogenesis converting cholesterol, the substrate for all steroid hormones, to pregnenolone and pregnenolone to progesterone (36–38). Thereafter, with the catalytic activities of P450 17α and 17β -HSD, steroidogenesis proceeds to the ultimate product, testosterone (39). The synthetic process involves three hydroxylations (at carbons 17, 20, and 22), two cleavages (at carbons

20–22 and 17–20), two dehydrogenations ($3\beta,17\beta$), and one Δ^{5-4} isomerization (the steroidogenic pathway is shown in Fig. 4).

Conversion of Cholesterol to Pregnenolone

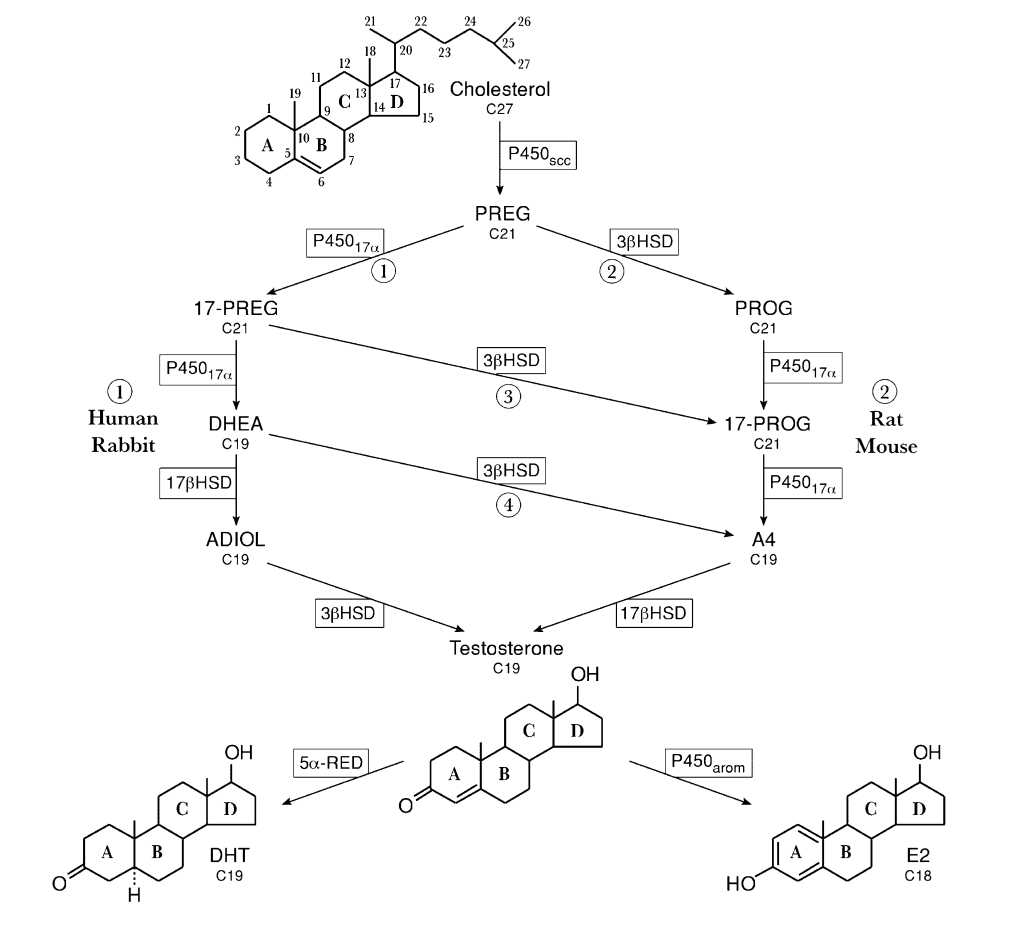
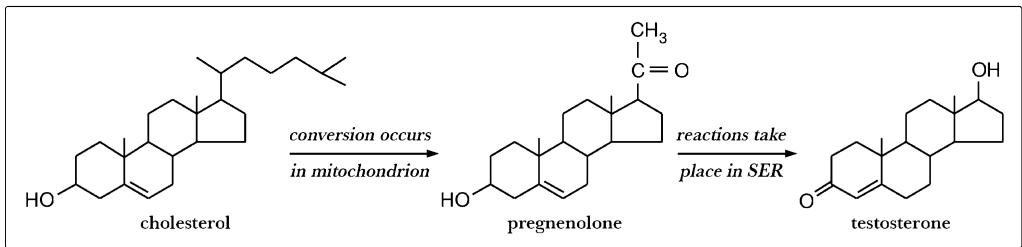
In all species, the first step in androgen biosynthesis is the conversion of cholesterol to pregnenolone, which is catalyzed by P450scc located in the inner mitochondrial membrane. This reaction also requires a mitochondrial electron transfer system, consisting of adrenodoxin and adrenodoxin reductase, to convey electrons from NADPH to P450scc (40). The P450scc enzyme catalyzes three sequential oxidation reactions of cholesterol, with each reaction requiring 1 molecule of O_2 and one molecule of nicotinamide adenine dinucleotide phosphate (NADPH). The first reaction is hydroxylation at C_{22} , followed by hydroxylation at C_{20} to yield (20,22) R-hydroxycholesterol, which is cleaved between C_{20} and C_{22} to yield the C_{21} steroid pregnenolone and isocaproaldehyde (41,42). Isocaproaldehyde is unstable and quickly oxidized to isocaproic acid (43).

The rate-limiting step in the synthesis of steroid hormones is not the first enzymatic reaction catalyzed by P450scc but rather the transport of precursor cholesterol from intracellular sources to the inner mitochondrial membrane and subsequent loading of cholesterol into the catalytic site of P450scc (44,45). Hydrophobic cholesterol cannot traverse the aqueous intermembrane space of mitochondria and reach the P450scc rapidly enough by simple diffusion to support acute steroid synthesis (46). Thus, cholesterol is mobilized by carrier proteins. Both steroidogenic acute response (StAR) protein and peripheral benzodiazepine receptor (PBR) are believed to participate in cholesterol delivery to the mitochondria, with StAR being primarily involved in gonadotropin-stimulated transfer.

Cholesterol, the raw material for steroidogenesis, is obtained from (1) lipoprotein in circulation, with low-density lipoprotein (LDL) being the primary source in humans; (2) *de novo* synthesis from acetate in the SER; and (3) free cholesterol liberated by cholesterol esterase from less directly available esters in lipid droplets (47).

Conversion of Δ^5 -3 β -Hydroxysteroids to Δ^4 -3-Ketosteroids

After cholesterol side-chain cleavage, two different pathways (Δ^5 and Δ^4) have been identified, which are defined by the position of one of the double bonds in the steroid molecule. In the Δ^4 pathway, pregnenolone, which has a double bond between carbons 5 and 6 of the steroid backbone, is converted to progesterone, which has a double bond between carbons 4 and 5. An isomerization reaction shifts the position of the double bond from between carbons 5 and 6 to between carbons 4 and 5. In the Δ^5 pathway, Δ^5 to Δ^4 isomerization does not occur until the last step, in which androstenediol is converted to testosterone. The intermediates of the two pathways are Δ^5 -3 β -hydroxysteroids and Δ^4 -3-ketosteroids, respectively. The conversion of Δ^5 -3 β -hydroxysteroids to Δ^4 -3-ketosteroids is accomplished by 3 β -HSD enzyme, which uses NAD^+ as a cofactor, sequentially catalyzing a 3 β dehydrogenation and isomerization (48). The 3 β -HSD enzymes can act on three Δ^5 intermediates, pregnenolone, 17 α -hydroxypregnenolone, and dehydroepiandrosterone, converting them, respectively, to the Δ^4 steroids progesterone, 17 α -hydroxyprogesterone, and androstenedione. The predominant Δ^5 to Δ^4 conversion occurs most commonly with either pregnenolone or dehydroepiandrosterone as substrates rather than 17 α -hydroxypregnenolone. Whether the Δ^5 - or Δ^4 -pathway is followed from pregnenolone to



Steroidogenic Enzymes	Abbreviation	Cofactors	Location
Cholesterol side-chain cleavage enzyme	P450 _{scc}	NADPH	Mitochondrial inner membrane
17 α -hydroxylase/ C17-20 lyase	P450 _{17α}	NADPH	Smooth endoplasmic reticulum
3 β -hydroxysteroid dehydrogenase/ Δ^5 -4 isomerase	3 β HSD	NAD+	Smooth endoplasmic reticulum
17 β -hydroxysteroid dehydrogenase	17 β HSD	NADP+	Smooth endoplasmic reticulum
Aromatase	P450 _{arom}	NADPH	Smooth endoplasmic reticulum
5 α -reductase	5 α -RED	NADPH	Endoplasmic reticulum/nuclear envelope

Fig. 4. Pathway of testosterone biosynthesis. Of the four possible biosynthetic pathways, the first or Δ^5 predominates in the rabbit and human, whereas the second or Δ^4 predominates in rodents. CHOL, cholesterol; PREG, pregnenolone; 17-PREG, 17-hydroxypregnenolone; DHEA, dehydroepiandrosterone; ADIOL, androstenediol; PROG, progesterone; 17-PROG, 17-hydroxyprogesterone; A4, androstenedione; T, testosterone; DHT, dehydrotestosterone; E₂ 17 β -estradiol.

testosterone depends on the species and developmental status of the Leydig cell. In humans, the Δ^5 pathway is predominant (49–52). Mammalian 3β -HSDs comprise a large family of enzymes (53,54). In the human branch of this family, two genes encode two corresponding, type I and II, 3β -HSD enzymes. Both types dehydrogenate 3β -hydroxysteroids and isomerize C_{21} and C_{19} steroids. Type I 3β -HSD is exclusively present in placenta and skin, whereas type II is the predominant form expressed in the adrenal, ovary, and testis. In the testis, type II 3β -HSD activity is localized exclusively in Leydig cells (37,55).

Conversion of C_{21} Steroids into C_{19} Steroids

Conversion of C_{21} to C_{19} requires two steps, 17α -hydroxylation and cleavage of the C_{17-20} bond, both of which are catalyzed by a single enzyme, the cytochrome P450 17α -hydroxylase/ C_{17-20} lyase (P45017 α). This enzyme catalyzes the bioconversion of pregnenolone and progesterone to the C_{19} steroids, dehydroepiandrosterone and androstenedione, respectively (56,57). In this two-step reaction, 17α -hydroxypregnenolone or 17α -hydroxyprogesterone exist as transient intermediates that are rapidly converted to dehydroepiandrosterone or androstenedione. The substrate preference and reaction velocities of P45017 α differ, depending on the species and tissue source. In humans, P45017 α has a higher affinity for 17α -hydroxypregnenolone, with dehydroepiandrosterone as the end product, and fails to show detectable C_{17-20} lyase activity with 17α -hydroxyprogesterone as substrate to generate androstenedione (58,59). In contrast, rat P45017 α readily cleaves the Δ^4 - C_{21} 17α -hydroxypregnenolone and Δ^5 - C_{21} 17α -hydroxyprogesterone to dehydroepiandrosterone and androstenedione, respectively. Guinea pig P45017 α also has a high affinity for Δ^4 - C_{21} steroids, (54), and both species contrast with the human, where the Δ^5 pathway predominates

Conversion of Dehydroepiandrosterone to Testosterone

The microsomal enzyme 17β -hydroxysteroid dehydrogenase (17β -HSD) catalyzes the interconversion of dehydroepiandrosterone and androstenediol, or androstenedione and testosterone. The 17β -dehydrogenase reaction is reversible, in contrast to the previous reactions in testosterone biosynthesis (60). Following the Δ^5 pathway in humans, the C_{19} product, dehydroepiandrosterone, is converted to androstenediol by 17β -HSD, and then androstenediol is converted to testosterone by 3β -HSD. In the Δ^4 pathway occurring in rodents, 17β -HSD uses androstenedione as substrate to produce testosterone (61). Testosterone is the principle steroid end product secreted by adult Leydig cells, but it can be further metabolized to 17β -estradiol (E_2) and dehydrotestosterone (DHT) before secretion.

Conversion of Testosterone to E_2

Aromatization of testosterone is catalyzed by the microsomal enzyme, cytochrome P450 aromatase (P450arom). Once substrate binding occurs, there is a sequential hydroxylation, oxidation, and removal of the C_{19} carbon, followed by aromatization of the A ring of the steroid. The entire aromatase reaction uses three molecules of oxygen and three electrons donated by NADPH (62–64). In the testis, aromatase activity is detectable in Leydig cells, Sertoli cells, and germ cells (65–69). The relative contributions of each of these testicular cell types to testicular aromatase activity varies with age and between species (70). In the rat and mouse, germ cells are a significant site of activity during adulthood (68). Sertoli cells contribute to testicular aromatase activity

only in immature animals (71). However, in humans, Leydig cells are the only source of testicular estrogens at all ages (39). Sertoli cell cultures from juvenile monkeys expressed follicle-stimulating hormone (FSH) driven aromatase (72).

Conversion of Testosterone to DHT

DHT, a more potent androgen than testosterone, is produced in a reaction catalyzed by the 5α -reductase enzyme. This conversion generally occurs in androgenic target tissues, such as the prostate gland, but 5α -reductase activity is high in Leydig cells prepubertally. Two isoforms of 5α -reductase, type I and type II, have been identified. The type II isoform is found in human prostate tissue and in Leydig cells (73,74).

Testosterone Secretion

The first step of androgen biosynthesis, catalyzed by P450scc, occurs in Leydig cell mitochondria, and subsequent steps occur in the SER. After synthesis, testosterone, which is lipophilic, moves out of the Leydig cell by passive diffusion, down a concentration gradient. There is no evidence of packaging testosterone into secretory granules (8). In the testis, testosterone diffuses freely into the interstitial space and associates in rodents with androgen-binding protein (ABP) produced by Sertoli cells (75,76) as a transport vehicle to the seminiferous tubules and epididymis.

Testosterone enters the testicular blood capillaries that are immediately adjacent to Leydig cells. Once a part of the systemic circulation, secreted testosterone binds to plasma proteins and is present in both bound and unbound forms. In humans, more than 95% of testosterone is complexed with proteins, both the high-affinity ($K_D = 1$ nM) sex hormone-binding globulin (SHBG) and the low-affinity ($K_D = 1000$ nM) albumin (Alb). The proportion of testosterone that is unbound or loosely bound represents the biologically active fraction, which freely diffuses from capillaries into cells. In contrast, the SHBG-bound fraction is believed to act as a reservoir for the steroid.

SHBG is a plasma protein synthesized and secreted by the liver. As its name suggests, SHBG has the ability to bind androgens and estrogens and the capacity to regulate the free concentrations of the steroids that bind to it. SHBG also participates in signal transduction for sex steroids at the cell membrane. SHBG binds with high affinity to a specific membrane receptor (R_{SHBG}) in prostate stromal and epithelial cells, wherein the SHBG / R_{SHBG} complex forms. Once an appropriate steroid, e.g., 3α -androstenediol or estradiol, binds to this complex, an increase of intracellular cyclic adenosine monophosphate (cAMP) occurs and intracellular signal transduction is initiated. Moreover, SHBG not only is a plasma protein secreted by the liver but also is expressed in the prostate tissue itself, specifically by prostate stromal and epithelial cells (77). (See Chapter 17.) The process of testosterone secretion and regulation of Leydig cell function are illustrated in Fig. 5.

REGULATION OF LEYDIG CELL FUNCTION

Steroidogenesis in adult Leydig cells is controlled by the pituitary gonadotropic hormones. Of the two gonadotropins, luteinizing hormone (LH) is the main stimulus for androgen biosynthesis. LH signal transduction is initiated on binding of the hormone to specific receptors on the Leydig cell surface. The receptor for LH (LH-R) belongs to a superfamily of G protein-coupled receptor (GPCR) (78).

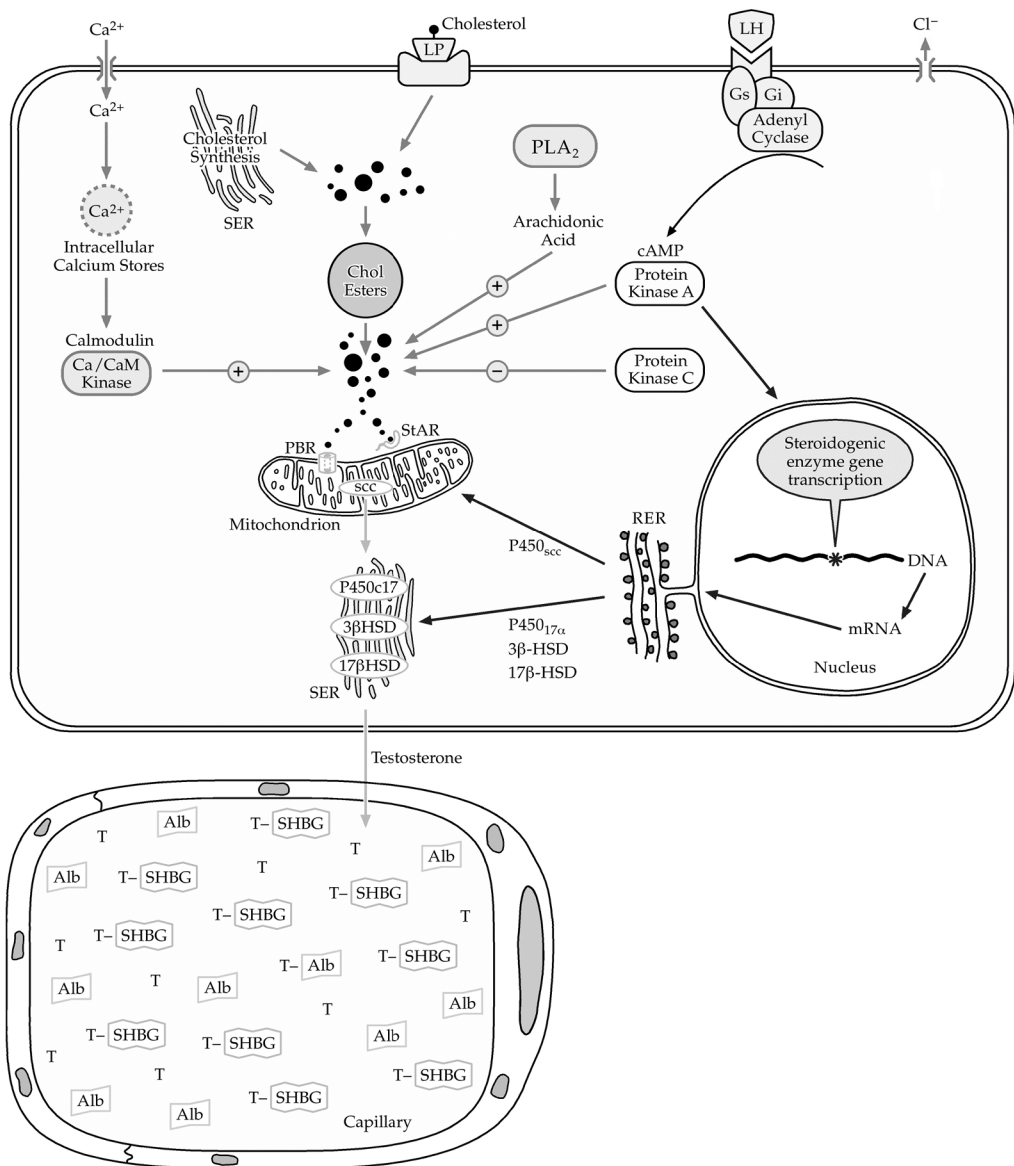


Fig. 5. Acute regulation of Leydig cell steroidogenesis by luteinizing hormone (LH). The complex of LH and LHRs triggers adenylate cyclase-mediated increases cytoplasmic cyclic adenosine monophosphate (cAMP) levels. Activation of protein kinase A and cholesterol esterase results in cholesterol mobilization from extracellular lipoprotein (low-density lipoprotein) and intercellular sources (including *de novo* synthesis) in the smooth endoplasmic reticulum (SER) and released from lipid droplet stores. Cholesterol moves across the outer to the inner membrane of the mitochondrion via shuttle proteins steroidogenic acute response (StAR) and peripheral benzodiazepine receptor (PBR). Cholesterol side-chain cleavage enzyme catalyzes the initial step in steroid biosynthesis. The subsequent steps are catalyzed by P450_{17α}, 3βHSD, and 17βHSD in the SER. The ultimate product, testosterone, moves out of the Leydig cell down a concentration gradient and is carried to androgen-responsive tissues by androgen-binding protein (rodents) and sex hormone-binding globulin (SHBG) (humans). The response to LH also involves an increased cytoplasmic calcium, synthesis of arachidonic acid, and efflux of chloride ions. The mechanism of Ca²⁺ action involves a calcium/calmodulin (Ca/CaM) protein kinase. Activation of phospholipase A₂(PLA₂) produces arachidonic acid (AA), which has a stimulatory effect on cholesterol mobilization to mitochondria. (Courtesy of P.N. Schlegel and M.P. Hardy (2002) [79] with the publisher's permission.)

Acute Effects of LH

Two types of responses to LH are seen in Leydig cells. The acute response triggers a rapid production of steroid within minutes (46). In the acute LH signal transduction process, LH-Rs interact with intracytoplasmic adenylate cyclase to form the second messenger adenosine 3',5'-cAMP. A sharp increase in cytoplasmic cAMP levels elicits a cascade of events leading to testosterone synthesis, including increased translocation of cholesterol from the cytosol to the inner mitochondrial membrane, conversion of cholesterol to pregnenolone, and, ultimately, transformation of steroid intermediates into testosterone. In addition to stimulating cAMP formation, LH acts on other intracellular signaling systems, including release of calcium from internal stores, synthesis of arachidonic acid (AA) and its metabolites from membrane phospholipid, and efflux of chloride ions, which have all been linked to the steroidogenic process (80).

The acute response of Leydig cells to LH does not require new transcription of mRNA (81). However, carrier proteins are required in the cholesterol translocation process for the rapid steroid production in the acute response. StAR is a 30-kDa molecular weight cholesterol transporter, and blockade of its synthesis by cycloheximide prevents the LH-induced increase in testosterone biosynthesis (82). Transient transfection of COS-1 cells with the cDNA for StAR increases the conversion of cholesterol to pregnenolone. StAR gene mutations result in the pathological condition of congenital lipoid adrenal hyperplasia (lipoid CAH), in which patients are unable to convert cholesterol to pregnenolone. This confirms a cholesterol-shuttling role for StAR protein in steroidogenesis (46,83,84). StAR protein biochemistry, as it is currently understood, may not completely account for transfer of cholesterol across mitochondrial membranes (85). Another candidate protein for cholesterol trafficking is the mitochondrial PBR, which is an 18-kDa integral outer mitochondrial membrane phosphoprotein that has a high affinity for cholesterol binding (86). PBR expression decreases are correlated with the decreased steroid synthesis (87). PBR plays a role in the maintenance of the basal pool of mitochondrial cholesterol (88). It has been postulated that PBR functions in cholesterol transport in steroidogenic tissues by mediating the entry, distribution, and/or availability of cholesterol within mitochondria (87,89). Recently, it was proposed by West et al. that PBR associates with StAR and that the two proteins may work in tandem at the outer mitochondrial membrane (90).

Chronic Effects of LH

LH also has long-term trophic effects on Leydig cells, requiring both transcription and increased translation of proteins. Chronic stimulation by LH is required for maintenance of Leydig cell steroidogenic enzyme levels and to support the steroidogenic organelle apparatus, including mitochondrial membrane potential and SER volume.

Inhibition of LH action can be achieved by hypophysectomy (91–93), suppression of gonadotropins through steroid administration (94–96), and neutralization of LH or LH-releasing hormone (GnRH) by specific antibodies (97). LH blockade eliminates the chronic effects of this hormone, causing Leydig cell atrophy and loss of cellular volume, SER, steroidogenic enzyme activities (particularly P-45017 α and P-450scc), LH receptor numbers, and the ability to secrete testosterone in response to LH (91,95). In LH-deprived rats, Leydig cell structure and function are restored by LH replacement

(93,94,96). Similarly, daily injections of LH to the hypogonadal mouse markedly increase steroidogenic enzyme activities (98).

In addition to LH, FSH and local cell-cell interactions participate in the regulation to Leydig cells. FSH regulatory control of Leydig cells is based on the correlation between serum FSH levels and the steroidogenic response of Leydig cells to LH during sexual maturation in humans. Because FSH-R are present only in the Sertoli cells, FSH acts on Leydig cells indirectly through Sertoli cell-secreted factors (99,100). Huhtaniemi and colleagues (*see* Chapter 6), in a study of FSH β And FSH-R knockout mice, recently demonstrated FSH signaling involvement in Leydig cell development. LH alone is sufficient for normal postnatal development of Leydig cells only if FSH-R are present. In the absence of LH, FSH stimulates Leydig cell steroidogenesis (101). Sertoli cells may also modulate Leydig cell numbers via paracrine interactions (14,102,103). Several factors produced by Sertoli cells, including insulin-like growth factor (IGF) 1, epidermal growth factor (EGF)- α , transforming growth factor (TGF)- α , and TGF- β , inhibin, and activin, influence Leydig cells. TGF- β is believed to be a strong inhibitor of Leydig cell steroidogenesis, whereas IGF-1 is a stimulator. EGF- α and TGF- α stimulate steroidogenesis during adulthood but may inhibit differentiation of immature Leydig cells. Germ cells, through their interactions with Sertoli cells, are believed to affect Leydig cells indirectly (81).

CLINICAL ASPECTS

Aging of Leydig Cells

Reproductive function declines as men grow older. An age-associated decline in plasma testosterone concentration occurs even in healthy men (104,105), although there is considerable variation in the age of onset (106). The level of testosterone in the blood stream declines on average by 1.2% each year for men over 40 yr of age. Because SHBG rises, the level of free (bioavailable) testosterone in the blood decreases more with age compared to total testosterone (106,107). Because clearance of testosterone does not rise with age, it is reasonable to deduce that the age-related decreases in androgen concentrations result from decreased Leydig cell androgen production (108,109).

Age-related declines in testosterone could be caused by decreased Leydig cell numbers and atrophy of their structure and/or reduced steroidogenic ability. Leydig cell numbers are inversely correlated with age, decreasing 44% by age 58 compared to 32-yr-old men (34). Leydig cell numbers decline because of degeneration rather than dedifferentiation (110). In addition, aged Leydig cells contain cytoplasmic or intranuclear crystalline inclusions, lipofuscin granules, diminished SER, and smaller and fewer mitochondria compared to young men (7,111–113). Older men with higher serum LH and low serum testosterone levels also have a large number of abnormal Leydig cells, suggesting that Leydig cell structural changes are related to changes in steroidogenic function (112). Age-related declines in steroidogenesis are caused by a global reduction in steroidogenic enzyme gene expression and by decreases in the rate of cholesterol transfer to mitochondria (114–117). In fact, the senescence of Leydig cells is involved at all aspects of the steroidogenic process, from LH binding to the steroidogenic reactions in the SER. Zirkin and colleagues report that reactive oxygen, produced as a by-product of steroidogenesis itself, may be responsible for age-related reductions testosterone production (118).

Therefore, unless Leydig stem cells can be recruited to restore the Leydig cell numbers, age-related declines in testosterone levels are unavoidable. The endocrine changes with aging in men are reviewed more thoroughly in Chapter 14.

Leydig Cell Tumors

Testicular tumors occur at a rate of 2 cases per 100,000 men and constitute 1% of all tumors in men. Leydig cell tumors (LCTs) account for 1 to 3% of testicular tumors (119). LCTs were first identified by Sacchi in 1895 (120). Although they are rare gonadal stromal tumors, LCTs may occur at any age and represent approx 40% of all non-germ cell testicular tumors (121). Most LCTs are unilateral; only 3% are bilateral (121).

LCTs are found at all ages from 2 to 90 yr, with a peak occurrence in the fifth decade of life. In boys, these tumors typically occur between the ages of 4 and 10 yr and account for approx 10% of cases of precocious puberty (122). They are uniformly benign hormonally active tumors that present with macrogenitosomia (a syndrome that is characterized by precocious enlargement of the genitals), including an enlarged phallus and/or prostate, and premature growth of pubic hair (120). Adult men with LCTs often present with a painless testicular mass, usually associated with gynecomastia, infertility, decreased libido, and other feminizing features. Gynecomastia is bilateral in 90% of the cases (123,124).

LCTs secrete both androgenically and estrogenically active steroids. Unlike normal Leydig cells, androgen secretion by the tumor is independent of pituitary control. LCTs have an abnormally high aromatase activity and secrete E_2 (125). The resulting elevated E_2 and decreased, or low-normal T values cause gynecomastia and infertility (126). This alteration in the T/ E_2 ratio, together with suppressed LH secretion, may be useful for clinical diagnosis.

Macroscopically, the lesions are generally small, yellow to brown, well circumscribed, and rarely hemorrhagic or necrotic. Microscopically, they consist of uniformly polyhedral packed cells with round and slightly eccentric nuclei, and eosinophilic granular cytoplasm with lipoid vacuoles, lipofuscin granules, and occasionally, Reinke's crystals (127).

Most LCTs are benign, but approx 10% are malignant (128). Large size, extensive necrosis, gross or microscopic evidence of infiltration, invasion of blood vessels, and excessive mitotic activity are all features that suggest malignancy. However, the presence of metastases, most often to pelvic lymph nodes and bone, is the only reliable criterion of malignancy. The appearance of metastases may be delayed for as many as 9 yr (129).

Inguinal orchidectomy is the treatment of choice for benign tumors. Recent reports have cited testis-sparing enucleation as an alternative treatment for benign lesions in children, especially those with bilateral tumors (130,131). Testis-sparing surgery is also an option for children with the clinical and biochemical findings typical of LCTs and an ultrasonographically defined encapsulated intratesticular mass. In cases managed by enucleation, local relapse remains a possibility, even when specimen margins are free of tumor cells and uniformly benign (120). Malignant LCTs are radioresistant and chemoresistant and have a poor prognosis. The mean survival time after surgery for patients with malignant LCTs is approx 3 yr (127).

Leydig cell hyperplasia (LCH) shares the same clinical presentation as LCTs, including painful gynecomastia and decreased libido in adults, precocious puberty in children,

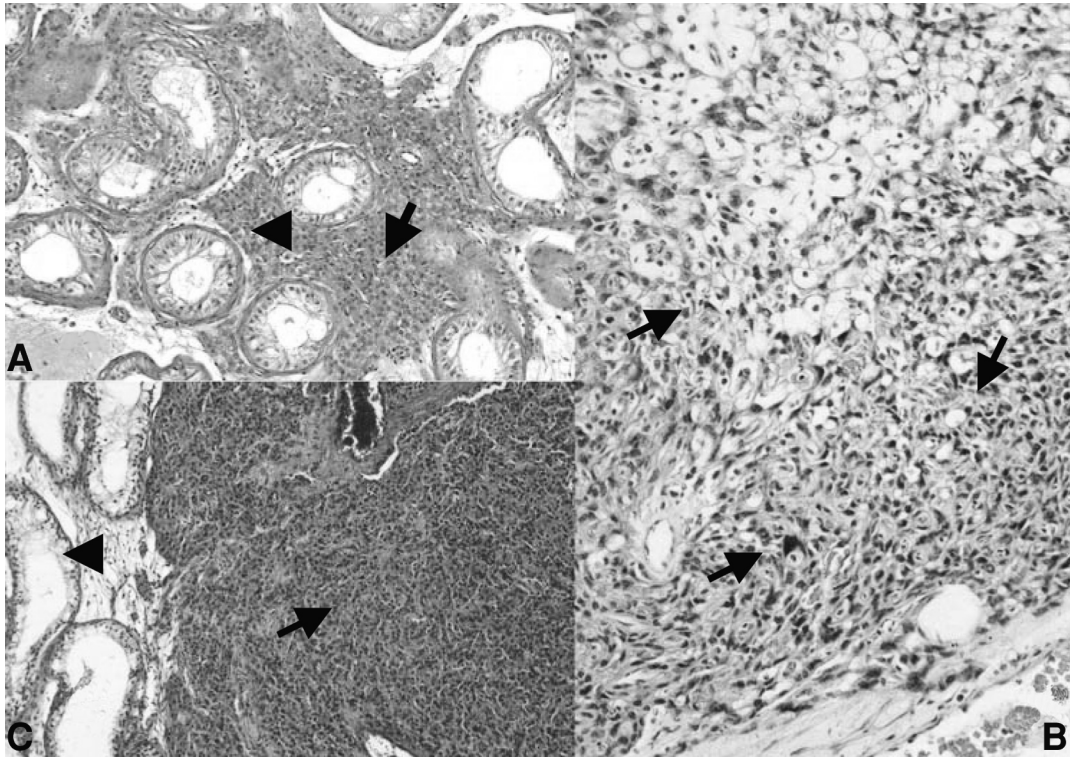


Fig. 6. Benign and malignant Leydig cell tumors (LCTs). The upper left panel, (A) an example of Leydig cell hyperplasia (LCH), is seen with an increased number of Leydig cells and an intertubular growth pattern (original image magnification $\times 200$). The lower left panel, (C) is a benign LCT with a loss of normal tubules and peripheral compression of adjacent seminiferous tubules (original image magnification $\times 100$). In the right panel, (B) a malignant LCT is shown with significant nuclear atypia and an invasive border (original image magnification $\times 300$). (Photos supplied by Drs. Cathy Naughton and Peter Humphrey of Washington University School of Medicine.)

and complaints of infertility or even palpable testicular masses. However, histological features help to distinguish LCH from normal Leydig cells and benign LCTs. Hyperplastic Leydig cells are arranged in diffuse, multifocal, small nodules and lack cytological atypia, frequent mitoses, necrosis, and vascular invasion. Hyperplastic Leydig cells usually infiltrate between seminiferous tubules, whereas benign LCTs form nodules that compress surrounding tubules. LCTs grow as nodules, efface normal testicular architecture, with loss of seminiferous tubules, and compress the adjacent tissue. Most LCH cases are multifocal and bilateral (132). Figure 6 shows the histological appearance of benign and malignant LCTs, as well as LCH.

Leydig Cells as a Target for Male Contraception

Current approaches to fertility control are predominantly targeted to women. However, men have traditionally and historically played an important role in contraception. Thus, until the second half of the 20th century, periodic abstinence, coitus interruptus, condoms, and vasectomy, all of which are male-directed or male-oriented methods, were

the only means for couples to limit family size. However, all of these methods are associated with limited effectiveness, lower acceptability, and partial irreversibility. Therefore, protracted efforts have been made to develop an endocrine male contraceptive regimen. Although our limited understanding of the complex regulatory mechanisms underlying normal spermatogenesis makes it difficult to identify specific testicular targets for pharmacological disruption, both T and FSH are required for complete spermatogenesis in humans. Therefore, to suppress spermatogenesis, the pituitary gonadotropic stimulus is inhibited, thereby abrogating Leydig cell steroidogenesis and nullifying FSH simultaneously. The consequent depletion of intratesticular testosterone and loss of FSH action result in a collapse of spermatogenesis without affecting stem cells. Maintenance of the spermatogonial stem cell population ensures that hormonal suppression of spermatogenesis is reversible. Because testosterone is necessary for spermatogenesis (133), Leydig cells represent an obvious target for hormonal contraception.

Hormonal contraception targets Leydig cells, suppressing their androgenic function. How much testosterone is needed for normal spermatogenesis in humans remains to be determined. In rats, intratesticular testosterone concentrations as low as 5% of normal support the complete spermatogenic process, whereas in the nonhuman primate, testicular androgen levels of 30% of baseline do not prevent complete suppression of germ cell development (134,135). Therefore, testosterone levels must be reduced below a threshold for successful interruption of spermatogenesis. In primates, however, the selective suppression of Leydig cell testosterone production is not sufficient to accomplish the goal of fertility regulation, and the additional inhibition of FSH secretion is necessary (136). One effective approach may be to suppress both LH and FSH secretion and, simultaneously, supply androgen to avoid peripheral androgen deficiency (137).

Leydig Cell Toxicology

Several agents have been identified as Leydig cell toxicants, including ethanol, ethane 1,2 dimethanesulphonate (EDS), 2,3,7,8 tetrachlorodibenzo-p-dioxin (TCDD), and steroid hormone receptor antagonists. These toxicants can damage Leydig cells in three ways: overstimulation or inhibition of steroidogenesis, induction of tumor formation, and promotion of cell death. Leydig cells are vulnerable to several toxins through direct actions and/or by disruption of the hypothalamic–pituitary axis.

Toxicants, such as ethanol, interfere with Leydig cell steroidogenesis by interfering with LH secretion, LH receptor binding, intracellular signal transduction pathways, and steroidogenic enzyme activities. Ethanol, for example, decreases LH secretion and reduces LH receptor binding and intracellular cyclic guanosine 5'-monophosphate (GMP) levels. Hence, chronic alcohol abuse causes declines in testosterone levels (138–140). Tumor formation and cell death are also observed after toxicant exposures. Carcinogenesis is considered to be a consequence of multiple insults to the genome. Necrosis and apoptosis have both been implicated in the process of toxicant-related Leydig cell death, with ethylene dimethanesulfonate exposure as the experimental paradigm (141).

SUMMARY

Leydig cells represent the endocrine proportion of the testis. In all mammalian males, Leydig cells are the main site of testosterone synthesis and secretion and are,

thus, essential for male reproductive function. Developmentally, human Leydig cells appear in three separate stages: fetal, neonatal, and pubertal. Each of these stages underlies a corresponding epoch of testosterone secretion in the male lifespan.

Testosterone is synthesized from cholesterol in a series of reactions catalyzed by four enzymes. The first enzyme, P450_{scc}, converts cholesterol to pregnenolone and is located in the inner mitochondrial membrane. Therefore, movement of cholesterol from the Leydig cell cytosol into the mitochondria is the rate-limiting step and is performed by the carrier proteins StAR and PBR. The three other biosynthetic enzymes, 3 β -HSD, P45017 α , and 17 β -HSD, are situated in the SER. Testosterone can be metabolized into other steroids, DHT and E₂, primarily by two enzymes: 5 α -reductase and P450_{arom}. The Δ^5 and Δ^4 steroidogenic pathways are followed in Leydig cells, with one preferred over the other depending on the species and developmental status. In humans, the Δ^5 pathway from 17 α -hydroxypregnenolone to dehydroepiandrosterone is predominant.

Development of steroidogenesis in Leydig cells is regulated by the pituitary gonadotropic hormone LH. FSH and cell–cell interactions in the testis also participate in this regulatory process. Decreased numbers and atrophy of cytological structure, as well as reduced steroidogenic ability, occur during Leydig cell aging, lowering testosterone secretion in older men. LCTs have a low incidence in humans, constituting only 1–3% of testicular neoplasms, with the highest incidence being for men in their 50s. Because testosterone is necessary for spermatogenesis, Leydig cells are a target for male hormonal contraception. Control of male fertility through suppression of Leydig cells will require identifying a level of intratesticular androgen concentration that is sufficient for spermatogenesis while maintaining libido and skeletal muscle mass in the periphery. Studies of reproductive toxicants have shown that disruption of Leydig cell steroidogenic function cannot be ignored as a causative factor.

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REFERENCES

1. Glees P. Leydig, Franz von. In: Gillispe CC, ed. Dictionary of Scientific Biology. Charles Scribner's Sons, New York, 1973, pp. 301–303.
2. Schulze C. Sertoli cells and Leydig cells in man. *Adv Anat Embryol Cell Biol* 1984;88:1–104.
3. Christensen AK. Leydig cells. In: Greep RO, Astwood EB, eds. Handbook of Physiology. Vol. V. American Physiological Society, Washington DC, 1975, pp. 57–94.
4. Huhtaniemi I, Pelliniemi LJ. Fetal Leydig cells: cellular origin, morphology, life span, and special functional features. *Proc Soc Exp Biol Med* 1992;201:125–140.
5. Irby DC, Kerr JB, Risbridger GP, de Kretser D. Seasonally and experimentally induced changes in testicular function of the Australian bush rat (*Rattus fuscipes*). *J Reprod Fertil* 1984;70:657–666.
6. Kerr JB, Abbenhuis DC, Irby DC. Crystalloid formation in rat Leydig cells. An ultrastructural and hormonal study. *Cell Tiss Res* 1986;245:91–100.
7. Mori H, Fukunishi R, Fujii M, Hataji K, Shiraishi T, Matsumoto K. Steroidogenical analysis of Reinke crystalloids in human Leydig cells. *Pathol Anat* 1978;380:1–10.

8. Bardin CW, Hardy MP, Catterall JF. Androgens. In: Adashi E, ed. Reproductive Endocrinology, Surgery, and Technology. Lippincott-Raven Publishers, Philadelphia, 1995, pp. 505–525.
9. Muller J, Skakkebaek NE. The prenatal and postnatal development of the testis. *Baillieres Clin Endocrinol Metab* 1992;6:251–271.
10. Carlstrom K, Eriksson A, Stege R, Rannevik G. Relationship between serum testosterone and sex hormone-binding globulin in adult men with intact or absent gonadal function. *Int J Androl* 1990;13:67–73.
11. Paniagua R, Nistal M, Saez FJ, Fraile B. Ultrastructure of the aging human testis. *J Electron Microsc Tech* 1991;19:241–260.
12. Prince FP. Ultrastructural evidence of mature Leydig cells and Leydig cell regression in the neonatal human testis. *Anat Rec* 1990;228:405–417.
13. Chemes HE. Leydig cell development in humans. In: Payne AH, Hardy MP, Russell L, eds. *The Leydig Cell*. Cache River Press, Viena IL, 1996, pp. 175–201.
14. de Kretser DM, Kerr JB. The cytology of the testis. In: Ernst K, Neil JD, eds. *The Physiology of Reproduction*. Vol. 1. Raven, New York, 1994, pp. 1177–1290.
15. Wartenberg H. Human testicular development and the role of the mesonephros in the origin of a dual Sertoli cell system. *Andrologia* 1978;10:1–21.
16. Wartenberg H. Differentiation and development of the testes. In: Burger H, de Kretser D, eds. *Comprehensive Endocrinology, the Testis*. Raven, New York, 1981, pp. 39–80.
17. Schulze W, Davidoff MS, Holstein AF. Are Leydig cells of neural origin? Substance P-like immunoreactivity in human testicular tissue. *Acta Endocrinol (Copenh)* 1987;115:373–377.
18. Gondos B. Development and differentiation of the testis and male reproductive tract. In: Steinberger A, Steinberger E, eds. *Testicular Development, Structure, and Function*. Vol. 3. Raven, New York, 1980, p. 20.
19. Voutilainen R. Differentiation of the fetal gonad. *Horm Res* 1992;38:66–71.
20. Tapanainen J, Kellokumpu-Lehtinen P, Pelliniemi L, Huhtaniemi I. Age-related changes in endogenous steroids of human fetal testis during early and midpregnancy. *J Clin Endocrinol Metab* 1981;52:98–102.
21. Reyes FI, Winter JSD, Faiman C. Endocrinology of the fetal testis. In: Bergur H, de Kretser DM, eds. *The Testis*. Raven, New York, 1989, pp. 119–142.
22. Zondek LH, Zondek T. Fetal hilar cells and Leydig cells in early pregnancy. *Biol Neonate* 1975;30:193–199.
23. Voutilainen R, Miller WL. Developmental expression of genes for the steroidogenic enzymes P450scc (20,22-desmolase), P450c17 (17 α -hydroxylase/17,20-lyase), and P450c21 (21-hydroxylase) in the human fetus. *J Clin Endocrinol Metab* 1986;63:1145–1150.
24. Nistal M, Paniagua R. *Testicular and Epididymal Pathology*. Thieme-Stratton Inc., New York, 1984.
25. Nistal M, Paniagua R, Regadera J, Santamaria L, Amat P. A quantitative morphological study of human Leydig cells from birth to adulthood. *Cell Tissue Res* 1986;246:229–236.
26. Pelliniemi LJ, Niei M. Fine structure of the human foetal testis. I. The interstitial tissue. *Z Zellforsch Mikrosk Anat* 1969;99:507–522.
27. Mancini RE, Vilar O, Lavieri JC, Abbenhuis DC, Heinrich JJ. Development of Leydig cells in normal human testis: a cytological, cytochemical and quantitative study. *Am J Anat* 1963;112:203–214.
28. Forest MG, Cathiard AM, Bertrand JA. Evidence of testicular activity in early infancy. *J Clin Endocrinol Metab* 1973;37:148–151.
29. Fouquet JP, Meusy-Dessolle N, Dang DC. Relationships between Leydig cell morphometry and plasma testosterone during postnatal development of the monkey, *Macaca fascicularis*. *Reprod Nutr Dev* 1984;24:281–296.
30. Prince FP. Ultrastructure of immature Leydig cells in the human prepubertal testis. *Anat Rec* 1984;209:165–176.
31. Chemes H, Cigorraga S, Bergada C, Schteingart H, Rey R, Pellizzari E. Isolation of human Leydig cell mesenchymal precursors from patients with the androgen insensitivity syndrome: testosterone production and response to human chorionic gonadotropin stimulation in culture. *Biol Reprod* 1992;46:793–801.
32. Chemes HE, Gottlieb SE, Pasqualini T, Domenichini E, Rivarola MA, Bergada C. Response to acute hCG stimulation and steroidogenic potential of Leydig cell fibroblastic precursors in humans. *J Androl* 1985;6:102–112.
33. Hardy MP, Zirkin BR. Leydig cell function. In: Lipshultz LI, Howards SS, eds. *Infertility in the Male*. Mosby-Year Book, St. Louis, MO, 1997, pp. 59–70.

34. Neaves WB, Johnson L, Porter JC, Parker CR, Jr., Petty CS. Leydig cell numbers, daily sperm production, and serum gonadotropin levels in aging men. *J Clin Endocrinol Metab* 1984;59:756–763.
35. Hall PF. Testicular steroid synthesis. In: Knobil E, Neill JD, eds. *The Physiology of Reproduction*. Raven, New York, 1994, pp. 1335–1362.
36. Rouiller V, Gangnerau MN, Vayssiere JL, Picon R. Cholesterol side-chain cleavage activity in rat fetal gonads: a limiting step for ovarian steroidogenesis. *Mol Cell Endocrinol* 1990;72:111–120.
37. Dupont E, Zhao HF, Rheaume E, et al. Localization of 3β -hydroxysteroid dehydrogenase/ Δ^5 - Δ^4 isomerase in rat gonads and adrenal glands by immunocytochemistry and in situ hybridization. *Endocrinology* 1990;127:1394–1403.
38. Pelletier G, Dupont E, Simard J, Luu-The V, Belanger A, Labrie F. Ontogeny and subcellular localization of 3β -hydroxysteroid dehydrogenase (3β -HSD) in the human and rat adrenal, ovary and testis. *J Steroid Biochem Mol Biol* 1992;43:451–467.
39. Payne AH, Kelch RP, Musich SS, Halpern ME. Intratesticular site of aromatization in the human. *J Clin Endocrinol Metab* 1976;42:1081–1087.
40. Simpson ER. Cholesterol side-chain cleavage, cytochrome P450, and the control of steroidogenesis. *Mol Cell Endocrinol* 1979;13:213–227.
41. Boyd GS, Simpson ER. Studies on the conversion of cholesterol to pregnenolone in bovine adrenal mitochondria. In: Mckern KW, ed. *Functions of the Adrenal cortex*. Vol. 1. Appleton-Century-Crofts, New York, 1968, pp. 49–76.
42. Burstein S, Gut M. Intermediates in the conversion of cholesterol to pregnenolone: kinetics and mechanism. *Steroids* 1976;28:115–131.
43. Schulster D, Burstein S, Cooke BA. Biosynthesis of steroid hormone. In: Schulze C, Burstein S, Cooke BA, eds. *Molecular Endocrinology of the Steroid Hormones*. John Wiley & Sons, London, 1976, pp. 44–74.
44. Black SM, Harikrishna JA, Szklarz GD, Miller WL. The mitochondrial environment is required for activity of the cholesterol side-chain cleavage enzyme, cytochrome P450_{sc}. *Proc Natl Acad Sci USA* 1994;91:7247–7251.
45. Farkash Y, Timberg R, Orly J. Preparation of antiserum to rat cytochrome P-450 cholesterol side chain cleavage, and its use for ultrastructural localization of the immunoreactive enzyme by protein A-gold technique. *Endocrinology* 1986;118:1353–1365.
46. Stocco DM, Clark BJ. Regulation of the acute production of steroids in steroidogenic cells. *Endocr Rev* 1996;17:221–244.
47. Freeman DA, Rommerts FFG. Regulation of Leydig Cell cholesterol transport. In: Payne AH, Hardy MP, Russell LD, eds. *The Leydig Cell*. Cache River, New York, 1996, pp. 232–236.
48. de Launoit Y, Simard J, Durocher F, Labrie F. Androgenic 17β -hydroxysteroid dehydrogenase activity of expressed rat type I 3β -hydroxysteroid dehydrogenase/ Δ^5 - Δ^4 isomerase. *Endocrinology* 1992;130:553–555.
49. Rajfer J, Sikka SC, Swerdloff RS. Lack of a direct effect of gonadotropin hormone-releasing hormone agonist on human testicular steroidogenesis. *J Clin Endocrinol Metab* 1987;64:62–67.
50. Rey R, Campo S, Ayuso S, Nagle C, Chemes H. Testicular steroidogenesis in the Cebus monkey throughout postnatal development. *Biol Reprod* 1995;52:997–1002.
51. Preslock JP. In vitro steroidogenesis in subhuman primates. In: Steinberger E, Steinberger A, eds. *Testicular Development, Structure, and Function*. Raven, New York, 1980, pp. 249–268.
52. Preslock JP, Steinberger E. Testicular steroidogenesis in the common marmoset, *Callithrix jacchus*. *Biol Reprod* 1977;17:289–293.
53. Labrie F, Simard J, Luu-The V, et al. Structure and tissue-specific expression of 3β -hydroxysteroid dehydrogenase/5-ene-4-ene isomerase genes in human and rat classical and peripheral steroidogenic tissues. *J Steroid Biochem Mol Biol* 1992;41:421–435.
54. Tremblay Y, Fleury A, Beaudoin C, Vallee M, Belanger A. Molecular cloning and expression of guinea pig cytochrome P450_{c17} cDNA (steroid 17α -hydroxylase/17,20 lyase): tissue distribution, regulation, and substrate specificity of the expressed enzyme. *DNA Cell Biol* 1994;13:1199–1212.
55. Dupont E, Labrie F, Luu-The V, Pelletier G. Ontogeny of 3β -hydroxysteroid dehydrogenase/ Δ^5 - Δ^4 isomerase (3β -HSD) in rat testis as studied by immunocytochemistry. *Anat Embryol (Berl)* 1993;187:583–589.
56. Hauffa BP, Miller WL, Grumbach MM, Conte FA, Kaplan SL. Congenital adrenal hyperplasia due to deficient cholesterol side-chain cleavage activity (20, 22-desmolase) in a patient treated for 18 years. *Clin Endocrinol (Oxf)* 1985;23:481–493.

57. Hall PF. Cytochrome P-450 C21sc: one enzyme with two actions: hydroxylase and lyase. *J Steroid Biochem Mol Biol* 1991;40:527–532.
58. Pang S, Yang X, Wang M, et al. Inherited congenital adrenal hyperplasia in the rabbit: absent cholesterol side-chain cleavage cytochrome P450 gene expression. *Endocrinology* 1992;131:181–186.
59. Fevold HR, Lorence MC, McCarthy JL, et al. Rat P450(17 α) from testis: characterization of a full-length cDNA encoding a unique steroid hydroxylase capable of catalyzing both Δ^4 - and Δ^5 -steroid-17,20-lyase reactions. *Mol Endocrinol* 1989;3:968–975.
60. Payne AH, O' Shaughnessy PJ. Structure, function and regulation of steroidogenic enzymes in the Leydig cell. In: Payne AH, Hardy MP, Zirkin BR, eds. *The Leydig Cell*. Cahun River, New York, 1996, p. 260.
61. Hardy MP, Gelber SJ, Zhou ZF, et al. Hormonal control of Leydig cell differentiation. *Ann NY Acad Sci* 1991;637:152–163.
62. Fishman J, Goto J. Mechanism of estrogen biosynthesis. Participation of multiple enzyme sites in placental aromatase hydroxylations. *J Biol Chem* 1981;256:4466–4471.
63. Thompson EA, Jr., Siiteri PK. Utilization of oxygen and reduced nicotinamide adenine dinucleotide phosphate by human placental microsomes during aromatization of androstenedione. *J Biol Chem* 1974;249:5364–5372.
64. Thompson EA, Jr., Siiteri PK. The involvement of human placental microsomal cytochrome P-450 in aromatization. *J Biol Chem* 1974;249:5373–5378.
65. Raeside JI, Lobb DK. Metabolism of androstenedione by Sertoli cell enriched preparations and purified Leydig cells from boar testes in relation to estrogen formation. *J Steroid Biochem* 1984;20:1267–1272.
66. Raeside JI, Renaud RL. Estrogen and androgen production by purified Leydig cells of mature boars. *Biol Reprod* 1983;28:727–733.
67. Canick JA, Makris A, Gonsalus GL, Ryan KJ. Testicular aromatization in immature rats: localization and stimulation after gonadotropin administration in vivo. *Endocrinology* 1979;104:285–288.
68. Dorrington JH, Fritz IB, Armstrong DT. Control of testicular estrogen synthesis. *Biol Reprod* 1978;18:55–64.
69. Nitta H, Bunick D, Hess RA, et al. Germ cells of the mouse testis express P450 aromatase. *Endocrinology* 1993;132:1396–1401.
70. Rommerts FF, de Jong FH, Brinkmann AO, van der Molen HJ. Development and cellular localization of rat testicular aromatase activity. *J Reprod Fertil* 1982;65:281–288.
71. Dorrington JH, Khan SA. Steroid production, metabolism, and release by Sertoli cells. In: Russell L, Griswold M, eds. *The Sertoli Cell*. Cahun River, Clearwater, FL, 1993, pp. 537–549.
72. Majumdar SS, Winters SJ, Plant TM. Procedures for the isolation and culture of Sertoli cells from the testes of infant, juvenile, and adult rhesus monkeys (*Macaca mulatta*). *Biol Reprod* 1998;58:633–640.
73. Andersson S, Russell DW. Structural and biochemical properties of cloned and expressed human and rat steroid 5 α -reductases. *Proc Natl Acad Sci USA* 1990;87:3640–3644.
74. Andersson S, Bishop RW, Russell DW. Expression cloning and regulation of steroid 5 α -reductase, an enzyme essential for male sexual differentiation. *J Biol Chem* 1989;264:16249–16255.
75. Gonsalus GL, Bardin CW. Sertoli-germ cell interactions as determinants of bidirectional secretion of androgen-binding protein. *Ann NY Acad Sci* 1991;637:322–326.
76. Gonsalus GL, Larrea F, Musto NA, Becker RR, Mather JP, Bardin CW. Androgen binding protein as a marker for Sertoli cell function. *J Steroid Biochem* 1981;15:99–106.
77. Hryb DJ, Nakhla AM, Kahn SM, et al. Sex hormone-binding globulin in the human prostate is locally synthesized and may act as an autocrine/paracrine effector. *J Biol Chem* 2002;277:26618–26622.
78. McFarland KC, Sprengel R, Phillips HS, et al. Lutropin-choriogonadotropin receptor: an unusual member of the G protein-coupled receptor family. *Science* 1989;245:494–499.
79. Schlegel PN, Hardy MP. Male Reproductive Physiology. In: Partin K, Peters N, eds. *Campbell's Urology*, Vol. 2. Saunders, Philadelphia, 2002, pp. 1437–1474.
80. Cooke BA, Choi MC, Dirami G, Lopez-Ruiz MP, West AP. Control of steroidogenesis in Leydig cells. *J Steroid Biochem Mol Biol* 1992;43:445–449.
81. Saez JM. Leydig cells: endocrine, paracrine, and autocrine regulation. *Endocr Rev* 1994;15:574–626.
82. Luo L, Chen H, Stocco DM, Zirkin BR. Leydig cell protein synthesis and steroidogenesis in response to acute stimulation by luteinizing hormone in rats. *Biol Reprod* 1998;59:263–270.
83. Lin D, Sugawara T, Strauss JF, 3rd, et al. Role of steroidogenic acute regulatory protein in adrenal and gonadal steroidogenesis. *Science* 1995;267:1828–1831.

84. Sugawara T, Lin D, Holt JA, et al. Structure of the human steroidogenic acute regulatory protein (StAR) gene: StAR stimulates mitochondrial cholesterol 27-hydroxylase activity. *Biochemistry* 1995;34:12506–12512.
85. Christenson LK, Strauss JF. Steroidogenic acute regulatory protein (StAR) and the intramitochondrial translocation of cholesterol. *Biochim Biophys Acta* 2000;1529:175–187.
86. Li H, Papadopoulos V. Peripheral-type benzodiazepine receptor function in cholesterol transport. Identification of a putative cholesterol recognition/interaction amino acid sequence and consensus pattern. *Endocrinology* 1998;139:4991–4997.
87. Papadopoulos V, Amri H, Boujrad N, Vidic B, Garnier M. Targeted disruption of the peripheral-type benzodiazepine receptor gene inhibits steroidogenesis in the R2C Leydig tumor cell line. *J Biol Chem* 1997;272:32129–32135.
88. Krueger KE, Papadopoulos V. Peripheral-type benzodiazepine receptors mediate translocation of cholesterol from outer to inner mitochondrial membranes in adrenocortical cells. *J Biol Chem* 1990;265:15015–15022.
89. Papadopoulos V, Amri H, Boujrad N, et al. Peripheral benzodiazepine receptor in cholesterol transport and steroidogenesis. *Steroids* 1997;62:21–28.
90. West LA, Horvat RD, Roess DA, Barisas BG, Juengel JL, Niswender GD. Steroidogenic acute regulatory protein and peripheral-type benzodiazepine receptor associate at the mitochondrial membrane. *Endocrinology* 2001;142:502–505.
91. Swerdloff RS, Heber D. Endocrine control of testicular function from birth to puberty. In: HB, D Kretser, eds. *The Testis*. Raven, New York, 1981, pp. 108–126.
92. Mori H, Christensen AK. Morphometric analysis of Leydig cells in the normal rat testis. *J Cell Biol* 1980;84:340–354.
93. Teerds KJ, Closset J, Rommerts FF, et al. Effects of pure FSH and LH preparations on the number and function of Leydig cells in immature hypophysectomized rats. *J Endocrinol* 1989;120:97–106.
94. Russell LD, Corbin TJ, Ren HP, Amador A, Bartke A, Ghosh S. Structural changes in rat Leydig cells posthypophysectomy: a morphometric and endocrine study. *Endocrinology* 1992;131:498–508.
95. Keeney DS, Mendis-Handagama SM, Zirkin BR, Ewing LL. Effect of long-term deprivation of luteinizing hormone on Leydig cell volume, Leydig cell number, and steroidogenic capacity of the rat testis. *Endocrinology* 1988;123:2906–2915.
96. Wing TY, Ewing LL, Zegeye B, Zirkin BR. Restoration effects of exogenous luteinizing hormone on the testicular steroidogenesis and Leydig cell ultrastructure. *Endocrinology* 1985;117:1779–1787.
97. Awoniyi CA, Santulli R, Chandrashekar V, Schanbacher BD, Zirkin BR. Quantitative restoration of advanced spermatogenic cells in adult male rats made azoospermic by active immunization against luteinizing hormone or gonadotropin-releasing hormone. *Endocrinology* 1989;125:1303–1309.
98. O'Shaughnessy PJ. Steroidogenic enzyme activity in the hypogonadal (hpg) mouse testis and effect of treatment with luteinizing hormone. *J Steroid Biochem Mol Biol* 1991;39:921–928.
99. Orth J, Christensen AK. Localization of 125I-labeled FSH in the testes of hypophysectomized rats by autoradiography at the light and electron microscope levels. *Endocrinology* 1977;101:262–278.
100. Bardin CW. The Sertoli cell. In: Knobil E, ed. *The physiology of reproduction*. Vol. 2. Raven, New York, 1988.
101. Baker PJ, Abel MH, Charlton HM, Huhtaniemi IT, O'Shaughnessy PJ. Failure of normal Leydig cell development in FSH receptor deficient mice but not in FSH β -deficient mice. *Endocrinology*, 2003;144:138–145.
102. Feig LA, Bellve AR, Erickson NH, Klagsbrun M. Sertoli cells contain a mitogenic polypeptide. *Proc Natl Acad Sci USA* 1980;77:4774–4778.
103. Ewing L, Keeney D. Leydig cells: structure and function. In: Desjardins C, Ewing LL, eds. *Cell and Molecular Biology of the Testis*. Oxford University Press, New York, 1993, pp. 137–165.
104. Belanger A, Candas B, Dupont A, et al. Changes in serum concentrations of conjugated and unconjugated steroids in 40- to 80-year-old men. *J Clin Endocrinol Metab* 1994;79:1086–1090.
105. Dabbs JM, Jr. Age and seasonal variation in serum testosterone concentration among men. *Chronobiol Int* 1990;7:245–249.
106. Gray A, Feldman HA, McKinlay JB, Longcope C. Age, disease, and changing sex hormone levels in middle-aged men: results of the Massachusetts Male Aging Study. *J Clin Endocrinol Metab* 1991;73:1016–1025.
107. Harman SM, Tsitouras PD. Reproductive hormones in aging men. I. Measurement of sex steroids, basal luteinizing hormone, and Leydig cell response to human chorionic gonadotropin. *J Clin Endocrinol Metab* 1980;51:35–40.

108. Vermeulen A, Rubens R, Verdonck L. Testosterone secretion and metabolism in male senescence. *J Clin Endocrinol Metab* 1972;34:730–735.
109. Baker HW, Burger HG, de Kretser DM, et al. Changes in the pituitary-testicular system with age. *Clin Endocrinol (Oxf)* 1976;5:349–372.
110. Neaves WB, Johnson L, Petty CS. Age-related change in numbers of other interstitial cells in testes of adult men: evidence bearing on the fate of Leydig cells lost with increasing age. *Biol Reprod* 1985;33:259–269.
111. Mori H, Hiromoto N, Nakahara M, Shiraishi T. Stereological analysis of Leydig cell ultrastructure in aged humans. *J Clin Endocrinol Metab* 1982;55:634–641.
112. Paniagua R, Amat P, Nistal M, Martin A. Ultrastructure of Leydig cells in human ageing testes. *J Anat* 1986;146:173–183.
113. Nistal M, Codesal J, Paniagua R. Multinucleate spermatids in aging human testes. *Arch Androl* 1986;16:125–129.
114. Giusti G, Gonnelli P, Borrelli D, et al. Age-related secretion of androstenedione, testosterone and dihydrotestosterone by the human testis. *Exp Gerontol* 1975;10:241–245.
115. Piotti LE, Ghiringhelli F, Magrini U. Apropos of the testicular function of the aged: histochemical and biological observations. *Rev Fr Endocrinol Clin* 1967;8:479–491.
116. Purifoy FE, Koopmans LH, Mayes DM. Age differences in serum androgen levels in normal adult males. *Hum Biol* 1981;53:499–511.
117. Serio M, Gonnelli P, Borrelli D, et al. Human testicular secretion with increasing age. *J Steroid Biochem* 1979;11:893–897.
118. Zirkin BR, Chen H. Regulation of Leydig cell steroidogenic function during aging. *Biol Reprod* 2000;63:977–981.
119. Mottola A, di Cello V, Saltutti C, Bianchi S. Leydig cell tumor of the testis. Therapeutic and anatomicopathologic clinical study of 2 new cases. *Arch Esp Urol* 1989;42:433–435.
120. Rich MA, Keating MA. Leydig cell tumors and tumors associated with congenital adrenal hyperplasia. *Urol Clin North Am* 2000;27:519–528.
121. Cortez JC, Kaplan GW. Gonadal stromal tumors, gonadoblastomas, epidermoid cysts, and secondary tumors of the testis in children. *Urol Clin North Am* 1993;20:15–26.
122. Ducharme JR, Collu R. Pubertal development: normal, precocious and delayed. *Clin Endocrinol Metab* 1982;11:57–87.
123. Fallick ML, Lin WW, Lipshultz LI. Leydig cell tumors presenting as azoospermia. *J Urol* 1999;161:1571–1572.
124. Kondoh N, Koh E, Nakamura M, et al. Bilateral Leydig cell tumors and male infertility: case report. *Urol Int* 1991;46:104–106.
125. Sasano H, Nakashima N, Matsuzaki O, et al. Testicular sex cord-stromal lesions: immunohistochemical analysis of cytokeratin, vimentin and steroidogenic enzymes. *Virchows Arch A Pathol Anat Histopathol* 1992;421:163–169.
126. Kuhn JM, Duranteau L, Rieu MA, Lahlou N, Roger M, Luton JP. Evidence of oestradiol-induced changes in gonadotrophin secretion in men with feminizing Leydig cell tumours. *Eur J Endocrinol* 1994;131:160–166.
127. Ritchie JP. Neoplasms of the testis. In: Walsh P, Reitik A, Vaughan E, Wein A, eds. *Campbell's Urology*. WB Saunders, Philadelphia, 1992, pp. 1222–1263.
128. Grem JL, Robins HI, Wilson KS, Gilchrist K, Trump DL. Metastatic Leydig cell tumor of the testis. Report of three cases and review of the literature. *Cancer* 1986;58:2116–2119.
129. Mellor SG, McCutchan JD. Gynaecomastia and occult Leydig cell tumour of the testis. *Br J Urol* 1989;63:420–422.
130. Konrad D, Schoenle EJ. Ten-year follow-up in a boy with Leydig cell tumor after selective surgery. *Horm Res* 1999;51:96–100.
131. Rushton HG, Belman AB. Testis-sparing surgery for benign lesions of the prepubertal testis. *Urol Clin North Am* 1993;20:27–37.
132. Naughton CK, Nadler RB, Basler JW, Humphrey PA. Leydig cell hyperplasia. *Br J Urol* 1998;81:282–289.
133. Marshall GR, Jockenhovel F, Ludecke D, Nieschlag E. Maintenance of complete but quantitatively reduced spermatogenesis in hypophysectomized monkeys by testosterone alone. *Acta Endocrinol (Copenh)* 1986;113:424–431.
134. Bartlett JM, Weinbauer GF, Nieschlag E. Differential effects of FSH and testosterone on the maintenance of spermatogenesis in the adult hypophysectomized rat. *J Endocrinol* 1989;121:49–58.

135. Weinbauer GF, Gockeler E, Nieschlag E. Testosterone prevents complete suppression of spermatogenesis in the gonadotropin-releasing hormone antagonist-treated nonhuman primate (*Macaca fascicularis*). *J Clin Endocrinol Metab* 1988;67:284–290.
136. Wickings EJ, Nieschlag E. The effects of active immunization with testosterone on pituitary-gonadal feedback in the male rhesus monkey (*Macaca mulatta*). *Biol Reprod* 1978;18:602–607.
137. Weinbauer GF, Nieschlag E. The Leydig Cell as a Target for Male Contraception. *The Leydig Cell*. Cache River, New York, 1996, pp. 640–649.
138. Adams ML, Forman JB, Kalicki JM, Meyer ER, Sewing B, Cicero TJ. Antagonism of alcohol-induced suppression of rat testosterone secretion by an inhibitor of nitric oxide synthase. *Alcohol Clin Exp Res* 1993;17:660–664.
139. Pajarinen JT, Karhunen PJ. Spermatogenic arrest and Sertoli cell-only syndrome—common alcohol-induced disorders of the human testis. *Int J Androl* 1994;17:292–299.
140. Van Thiel DH, Gavalier JS. Hypothalamic-pituitary-gonadal function in liver disease with particular attention to the endocrine effects of chronic alcohol abuse. *Prog Liver Dis* 1986;8:273–282.
141. Morris ID. Leydig cell toxicology. In: Payne AH, Hardy MP, Russell LD, eds. *The Leydig Cell*. Cache River, New York, 1996, pp. 575–585.



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