Leydig Cell and Spermatogenesis

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Abbreviation

AKR1C14	3α-hydroxysteroid dehydrogenase
cAMP	Adenosine 3′5′-cAMP
CYP11A1	Cytochrome P450 cholesterol side chain cleavage enzyme
CYP17A1	Cytochrome 17α-hydroxylase/17,20-lyase
hCG	Human chorionic gonadotrophin
HSD17B3	17β-Hydroxysteroid dehydrogenase isoform 3
HSD3B	3β-Hydroxysteroid dehydrogenase
LHCGR	Luteinizing hormone/chorionic gonadotrophin receptor
NAD^+	Nicotinamide adenine dinucleotide
NADPH	Reduced nicotinamide adenine dinucleotide phosphate
SCARKO	Conditional knockout androgen receptor in Sertoli cell
SRD5A1	Steroid 5α-reductase 1
SRY	Sex-determining region Y protein

Introduction

Testosterone is the main androgen secreted by Leydig cells in mammalians. This steroid is necessary for spermatogenesis. Many data were available in rat, mouse and human Leydig cells. In this chapter, Leydig cell development, regulation, and its function for spermatogenesis are discussed.

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The Development of Leydig Cells

The German scientist Franz Leydig first described a cell type with steroid-producing potential in the interstitial compartment of mammalian testes in 1850 [1]. This interstitial cell was named as the Leydig cell later. In mammalian males, Leydig cells are present in the interstitial area of the testis, surrounded by the seminiferous tubules (Fig. 1) [2]. These cells are void and multigonal in shape. The Leydig cell of mammalians has smooth endoplasmic reticulum, which is abundant like other steroidogenic cells such as adrenal cells. These cells also contain numerous mitochondria. The Leydig cell of mammalians also contains lipid droplets. Unlike rats and mice, another typical cytological feature of human Leydig cells is Reinke crystal, which is an indicator of reduced steroidogenic capacity during cell aging [3].

The Fetal Leydig Cell

There are two generations of Leydig cells in both rats and mice: fetal Leydig cell and adult Leydig cell [2, 4, 5]. Fetal Leydig cells are differentiated from stem Leydig cells. Although the exact origin of fetal Leydig cells are still under debate, they were believed to be originated from mesenchymal cells and cells in the mesonephros [6]. The genetic X and Y chromosomes determine sex of an embryo at fertilization [7]. The Y chromosome is required for fetal testis differentiation [8]. The sexual

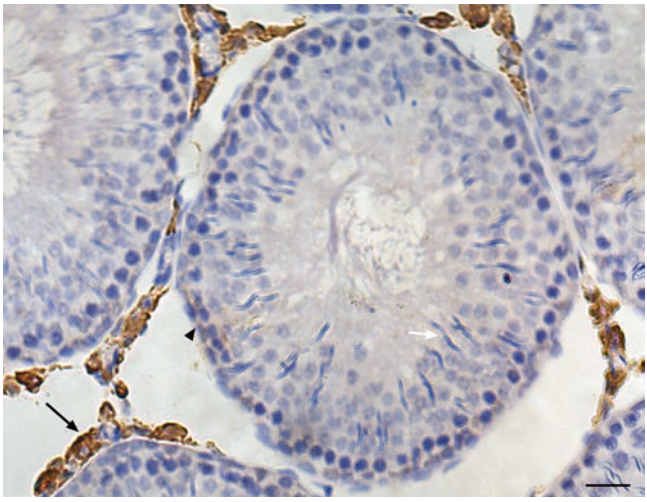

Fig. 1 Immunohistochemical staining of adult rat testis section. Immunohistochemical staining of cytochrome P450 cholesterol side chain cleavage enzyme, a biomarker of Leydig cells. Black arrow designates the Leydig cell. Black arrowhead designates the Sertoli cell, which support spermatogenesis. White arrowhead designates a sperm. Bar designates 20 μm

differentiation of the fetus starts when the gonads differentiate [9], and in the case of males, male sexual differentiation of the fetus begins when the fetal testis differentiates. However, initially, gonads are identical in XY and XX embryos and the gonads are referred as the indifferent gonads [10].

Around gestational Day 12 in mice or 14 in rats and gestational Week 6 in humans [9, 11], the bipotential gonad appears and it develops into the fetal testis under the action of sex-determining region Y protein (SRY) produced from its gene on Y chromosome [12]. SRY is a transcription factor and binds to specific regions of DNA to regulate testis-specific gene expression [8, 13, 14]. The first appearance in the Sertoli cells, and then the stem cells of fetal Leydig cells begin to migrate and start to differentiate in the interstitium of the testis. The differentiation of fetal Leydig cells from stem cells is believed to be regulated by Sertoli cell-secreted factors, such as desert hedgehog [15] and platelet derived growth factors [16], and aristaless-related homeobox [17]. During the later gestation, the number of fetal Leydig cells gradually increases and they form clusters and express steroidogenic enzymes and reach a maximum secretion of androgens during the late gestation [18]. Fetal Leydig cells involute gradually after birth [19]. There is still controversy about the fate of fetal Leydig cells in the postnatal testis [18]. A few fetal Leydig cells are believed to persist in adult mouse testis [20]. However, the contribution to testosterone secretion in the adult testis by fetal Leydig cells is minimal [19].

In humans, seminiferous tubules are formed within the gonadal blastema and create interstitial parts by gestational Week 6 [21]. Fetal Leydig cells are differentiated from undifferentiated mesenchymal cells (potential stem Leydig cells) within these interstitial compartments on gestational Week 8 [10]. The number of fetal Leydig cells increases gradually, reaching a maximum by gestational Week 14–15 [21]. Due to the formation of fetal Leydig cells, the androgen concentrations in the fetal testis change in parallel with the increase of fetal Leydig cell number. Unlike rats and mice, the numbers of fetal Leydig cells, serum testosterone levels, some steroidogenic enzyme expression begin to decline [22, 23]. The fetal Leydig cell number is approximately 60% lower than the prenatal peak at the birth [22, 23]. The primary function of fetal Leydig cells is the secretion of testosterone, which stimulates the development of both the internal and external genitalia of the male fetus [2].

The Neonatal Leydig Cell

Unlike rats and mice, humans have additional generation of Leydig cells during the neonatal period, referred as the neonatal Leydig cell. The number of neonatal Leydig cells again increases and reaches a peak at 2–3 months after birth, leading to a peak in serum testosterone concentrations. This type of Leydig cells are typical, containing abundant smooth endoplasmic reticulum, mitochondria and lipid-droplets [24, 25]. Although the exact origin of neonatal Leydig cells is still unclear, it is believed that neonatal Leydig cells differentiate from stem Leydig cells under the brief surge of pituitary activities. Then, neonatal Leydig cell number rapidly

regresses by the end of the first year of age [26]. Since then to the first decade, The Leydig cells in human testis are in quiescence with absence of well-developed Leydig cells and the interstitial area of the postnatal human testis contains stem Leydig cells or progenitor Leydig cells, which are spindle-shaped. These cells are believed to be source of adult Leydig cells because they are able to increase steroidogenic activity under the stimulation of human chorionic gonadotrophin, which also binds to the surface of luteinizing hormone/chorionic gonadotrophin receptor (LHCGR) [27–29].

The Adult Leydig Cell

Adult Leydig cells are differentiated from stem Leydig cells during the second week of age in mice and rats after they commit into spindle-shaped progenitor Leydig cells. Progenitor Leydig cells have a few smooth endoplasmic reticulum and mitochondria but have some lipid-droplets and they are abundant around postnatal Day 21 in rodents [2, 30, 31]. They express some androgen synthetic enzymes such as cytochrome P450 cholesterol side chain cleavage enzyme (CYP11A1), 3β-hydroxysteroid dehydrogenase/Δ5-4 isomerase (HSD3B), and cytochrome P450 17α-hydroxylase/17,20-lyase (CYP17A1) but lack last-step testosterone synthetic enzyme 17β-hydroxysteroid dehydrogenase isoform 3 (HSD17B3) [32, 33]. Thus, progenitor Leydig cells are capable of only making androstenedione, the precursor of testosterone [32]. Progenitor Leydig cells also contain a fair amount of androgen metabolic enzymes, steroid 5α-reductase 1 (SRD5A1) and 3α-hydroxysteroid dehydrogenase (AKR1C14) [32, 33]. Therefore, androstenedione formed is metabolized into androstanedione by SRD5A1 and further into androsterone by AKR1C14 [32]. Progenitor Leydig cells are almost irresponsive to luteinizing hormone stimulation because they almost have truncated LHCGR [30, 32]. Around postnatal Day 28–35, progenitor Leydig cells differentiate into ovoid immature Leydig cells [2]. Immature Leydig cells have increased amount of smooth endoplasmic reticulum and mitochondria and numerous lipid-droplets [31]. However, the smooth endoplasmic reticulum in immature Leydig cells are still under developmental stage [31]. Immature Leydig cells have all four androgen synthetic enzymes (CYP11A1, HSD3B, CYP17A1, and HSD17B3), thus they can make testosterone [32, 34]. However, immature Leydig cells still contain high levels of SRD5A1 and AKR1C14, thus SRD5A1 converting testosterone into dihydrotestosterone, which is further converted into 5α-androstane-3α,17β-diol, as the major secreted androgen [32, 34]. Around postnatal Day 49 and after, immature Leydig cells mature into adult Leydig cells, which are large and void and they have well developed smooth endoplasmic reticulum and many mitochondria and have almost no lipid droplets [30, 31]. Adult Leydig cells have all four androgen synthetic enzymes (CYP11A1, HSD3B, CYP17A1, and HSD17B3) and are able to make testosterone. However, the SRD5A1 is silenced in adult Leydig cells, thus testosterone cannot be metabolized further [32]. Interestingly, rat adult Leydig cells contain a small amount of

cytochrome P450 2A1, thus they can metabolize testosterone into 7α-hydroxytestosterone [35] and however, testosterone is still the major end androgen in adult Leydig cells [32]. In rat testis, stem and progenitor stem Leydig cells have high proliferative capacity and they have higher expression of cyclin A2 [36, 37]. Although immature Leydig cells have decreased proliferative capacity and decreased expression of cyclin A2 [36, 37], they still can divide once and make a maximum of about 23×10^6 cells per testis after postnatal Day 56 [38].

Adult Leydig cells in humans are developed from stem Leydig cells from about 10 years of age and development is complete by 13 years of age [39]. Adult Leydig cells increases and reaches a maximum of about 5×10^8 cells per testis in the early 20s [24] and they mainly secrete testosterone with the plasma levels of average 6 ng/mL during adulthood. The primary function of adult Leydig cells is the synthesis of androgen, which promotes the development of the second sexual characteristics of males, stimulates spermatogenesis and maintains protein synthesis at adulthood.

Steroidogenesis in Leydig Cells

The major function of adult Leydig cells is to secrete testosterone. The steps of testosterone synthesis include the enzymatic activities of four enzymes: CYP11A1, HSD3B, CYP17A1, and HSD17B3 [32]. In some precursor cell types such as progenitor and immature Leydig cells, SRD5A1 and AKR1C14 are expressed [32].

The Sources of Cholesterol in Leydig Cells

Cholesterol is the starting material for making testosterone in rat, mouse and human Leydig cells. In rats, mice and humans, cholesterol is absorbed primarily via lipoprotein in the circulation via high-density lipoprotein, which binds to the membrane receptor, scavenger receptor class B member 1, for uptake [40–42]. Cholesterol can also be taken in via lipoprotein in the circulation, after binding to the low-density lipoprotein receptor for uptake [43]. Cholesterol can be also de novo synthesized from acetyl CoA in the smooth endoplasmic reticulum via a series of enzymatic reactions: (1) acetyl CoA units are linked to form 3-hydroxy-3-methylglutaryl coenzyme A; (2) 3-hydroxy-3-methylglutaryl coenzyme A is catalyzed into mevalonate; (3) mevalonate is converted to isopentenyl pyrophosphate; (4) isopentenyl pyrophosphate is lined to 30-carbon squalene; and (5) squalene cyclizes to lanosterol and further metabolized to form cholesterol [44]. Cholesterol is also capable of being obtained from the liberation of esters in lipid droplets by cholesterol esterase [45].

Cholesterol Transportation Within Leydig Cells

The first enzyme to use cholesterol is CYP11A1, which is located in the inner membrane of the mitochondrion. Cholesterol cannot pass through the aqueous mitochondrial lumen to reach the CYP11A1 in the inner membrane of mitochondrion. It is believed that cholesterol is transported by some carrier proteins. One of the most important carrier proteins is steroidogenic acute regulatory protein [46, 47], which transports cholesterol to together with peripheral benzodiazepine receptor [48]. However, the role of peripheral benzodiazepine receptor in steroidogenesis is still controversial. CRISPR/Cas9-mediated deletion of peripheral benzodiazepine receptor in mouse MA-10 Leydig cells does not alter steroidogenesis [49] and but alters mitochondrial fatty acid oxidation without altering mitochondrial membrane potential [50]. Another study shows that the peripheral benzodiazepine receptor disruption causes reduction of both steroidogenesis and mitochondrial membrane potential [51]. However, global deletion of peripheral benzodiazepine receptor in mice does affect Leydig cell steroidogenesis [52–54].

Androgen Synthetic Pathways

In Leydig cells from rats, mice, and humans, all steroids need CYP11A1 for the first catalysis from substrate cholesterol to generate pregnenolone. After that, there is a clear species difference in the steroidogenic pathways between rodents and humans. In rodents, the Δ^4 pathway (pregnenolone → progesterone → 17α-hydroxyprogesterone → androstenedione → testosterone) is the preferable pathway (Fig. 2). In the Δ^4 pathway, pregnenolone is preferably bounded by HSD3B, catalyzing the formation of progesterone. In human Leydig cells, the Δ^5 pathway (pregnenolone → 17α-hydroxypregnenolone → dehydroepiandrosterone → androstenedione → testosterone) is the preferable pathway (Fig. 2).

CYP11A1 Catalysis

Cholesterol is the substrate of CYP11A1, which converts it into pregnenolone. CYP11A1 is present in the inner membrane of mitochondrion [55]. A single gene (*Cyp11a1* in rodents and *CYP11A1* in humans) encodes CYP11A1 [56–58]. The reaction of CYP11A1 requires a mitochondrial electron transfer system, which consists of adrenodoxin and adrenodoxin reductase [55]. CYP11A1 catalyzes three sequential oxidative reactions of cholesterol, and each oxidative reaction needs one molecule of oxygen and one molecule of nicotinamide adenine dinucleotide phosphate (NADPH) [55, 59]. The first oxidative reaction happens at C22, then the second oxidative reaction happens at C20 to produce [20, 22] R-hydroxycholesterol,

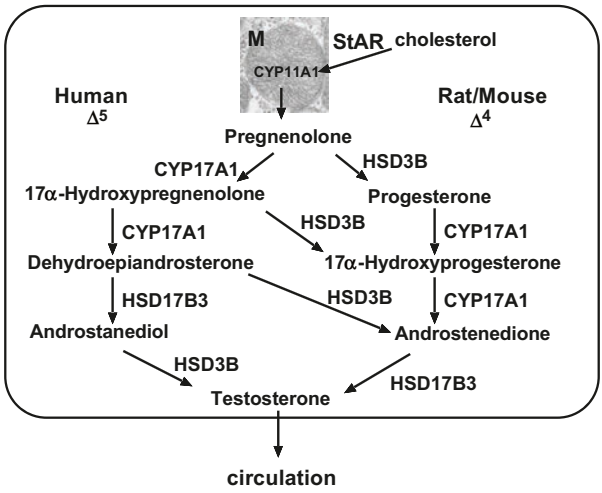

Fig. 2 The Δ^4 and Δ^5 steroidogenic pathways in rodent and human testis. Rat or mouse Leydig cell takes Δ^4 steroidogenic pathway while human Leydig cell takes Δ^5 steroidogenic one. *M* mitochondrion, *StAR* steroidogenic acute regulatory protein, *CYP11A1* cytochrome P450 cholesterol side chain cleavage enzyme, *CYP17A1* cytochrome 17α-hydroxylase/17,20-lyase, *HSD3B* 3β-hydroxysteroid dehydrogenase, *HSD17B3* 17β-hydroxysteroid dehydrogenase isoform 3

which is unstable and is cleaved between C20 and C22 to produce pregnenolone and isocaproaldehyde [60, 61].

HSD3B Catalysis

Pregnenolone is believed to diffuse from mitochondria into smooth endoplasmic reticulum, where HSD3B is located. In rats, two genes (*Hsd3b1* and *Hsd3b2*) encode respective HSD3B isoforms [62]. In rat Leydig cells, type I HSD3B is predominant isoform [62]. In mice, the corresponding counterpart is *Hsd3b6* gene, which encodes HSD3B6 [63]. Two human *HSD3B* genes with 81.9% identity were cloned: *HSD3B1* mainly exists in placenta and *HSD3B2* predominantly occurs in human Leydig cells [64]. HSD3B has two steps of catalysis: dehydrogenation and isomerization of a double bond in the steroid molecule and it requires nicotinamide adenine dinucleotide (NAD^+) as the coenzyme [62]. Rodent and human HSD3B take different pathway for catalysis. Rodent HSD3B uses pregnenolone as the substrate to dehydrogenize it at 3β-hydroxyl group of this steroid. Pregnenolone has a double bond between carbons 5 and 6 and the isomerase activity of HSD3B converts the double bond between carbons 4 and 5 in progesterone (Fig. 2). In human Leydig cells, HSD3B catalyzes the CYP17A1 products, 17α-hydroxypregnenolone and dehydroepiandrosterone, into 17α-hydroxyprogesterone and androstenedione (Fig. 2), respectively [65].

CYP17A1 Catalysis

A single gene (*Cyp17a1* in rodents and *CYP17A1* in humans) encodes CYP17A1 [66–68]. This enzyme has two activities: 17α-hydroxylase and C17,20-lysase activities. CYP17A1 is located in the smooth endoplasmic reticulum. CYP17A1 catalysis depends on the Δ^4 (rodent) or Δ^5 (human) pathway. In the Δ^4 pathway, CYP17A1 catalyzes progesterone to 17α-hydroxyprogesterone by 17α-hydroxylase activity and the later further into androstenedione by C17,20-lyase (Fig. 2) [69]. Each reaction requires coenzyme, NADPH [55], which transfers electrons via cytochrome P450 oxidoreductase [55]. In the Δ^5 pathway, CYP17A1 catalyzes pregnenolone into 17α-hydroxyprogesterone and the later further into dehydroepiandrosterone (Fig. 2). CYP17A1 takes either Δ^4 (rodent) or Δ^5 (human) pathway, depending on the species and tissue location. Human CYP17A1 has a higher affinity for 17α-hydroxypregnenolone and has almost no C17,20-lyase activity with 17α-hydroprogesterone [68]. However, rodent CYP17A1 can catalyze both 17α-hydroxypregnenolone and 17α-hydroxyprogesterone [68].

HSD17B3 Catalysis

Although there many 17β-hydroxysteroid dehydrogenase isoforms [70], the rat [32] and mouse *Hsd17b3* [71] and human *HSD17B3* [72] encode HSD17B3, which is the isoform in Leydig cells for the last-step of testosterone synthesis. HSD17B3 is located in the smooth endoplasmic reticulum. HSD17B3 catalyzes androstenedione into testosterone. HSD17B3 catalysis requires NADPH as its coenzyme. The production of testosterone is considered an end-product in adult Leydig cells.

SRD5A1 Catalysis

In the rodent precursor cells, mainly progenitor and immature Leydig cells, SRD5A1 is highly expressed [32, 34, 73]. Rat [74] and mouse [75] *Srd5a1* as well as human SRD5A1 [76] encode SRD5A1. SRD5A1 is located in the smooth endoplasmic reticulum of Leydig cells [77]. SRD5A1 catalyzes the conversion of testosterone into the more potent androgen, dihydrotestosterone under the assistance of coenzyme, NADPH. SRD5A1 in progenitor and immature Leydig cells also catalyzes androstenedione into androstanedione [32].

AKR1C14 Catalysis

In the rodent Leydig cells, AKR1C14 is expressed and down-regulated with the development of rat Leydig cells during puberty [31, 32, 78]. Mouse Leydig cells also express AKR1C14 [34]. Rat [79] and mouse [80] *Akr1c14* encode AKR1C14. AKR1C4 is located in the cytoplasmic part of Leydig cells [81]. AKR1C14 catalyzes the conversion of dihydrotestosterone into weak androgen 5α-androstane-3α, 17β-diol [32]. It also catalyzes the conversion of androstanedione into weak androgen, androsterone [32].

Testosterone Secretion

When testosterone is synthesized after HSD17B3 catalysis in the smooth endoplasmic reticulum, it passively diffuses out of Leydig cells via concentration gradient. In the interstitial fluid after diffusion, testosterone is bound to androgen binding protein, a protein secreted by Sertoli cells [82]. Bound androgen binding protein-testosterone is transported into the rodent seminiferous tubules and epididymis [82]. When entering the circulation, testosterone in the blood is bound to some plasma proteins. In humans, two types of plasma proteins bind to testosterone: sex hormone binding globulin and albumin. Sex hormone binding globulin is secreted by human liver, and is a high-affinity testosterone binding protein with a KD of 1 nM and albumin is a low-affinity testosterone binding protein with a KD of 1000 nM. The biological activity of testosterone is free testosterone levels in the serum, which depends on sex hormone binding globulin and albumin levels.

Regulation of Leydig Cell Development and Function

Adult Leydig cells were differentiated from stem Leydig cells. Stem Leydig cells have been identified in rats [36], mice [83], and humans [84]. In the rat model, many growth factors such as platelet-derived growth factor-AA [36, 85], platelet-derived growth factor-BB [85], leukemia inhibitory factor [36], epidermal growth factor [36], fibroblast growth factor 1 [86], fibroblast growth factor 2 [85, 87], fibroblast growth factor 16 [88], nerve growth factor [89], insulin-like growth factor 1 [85], desert hedgehog [85], activin A [85, 90], and kit ligand [36, 85, 91] stimulate the proliferation of stem Leydig cells, while other factors, including platelet-derived growth factor −AA [92], nerve growth factor [89], desert hedgehog [85], insulin-like growth factor 1 [93], androgen [85], fibroblast growth factor 12 [94], and parathyroid hormone-related protein [95] stimulate the differentiation of these cells (Fig. 3). In mouse stem Leydig cells, platelet-derived growth factor-AA and platelet-derived growth factor-BB, and desert hedgehog seem also to regulate its

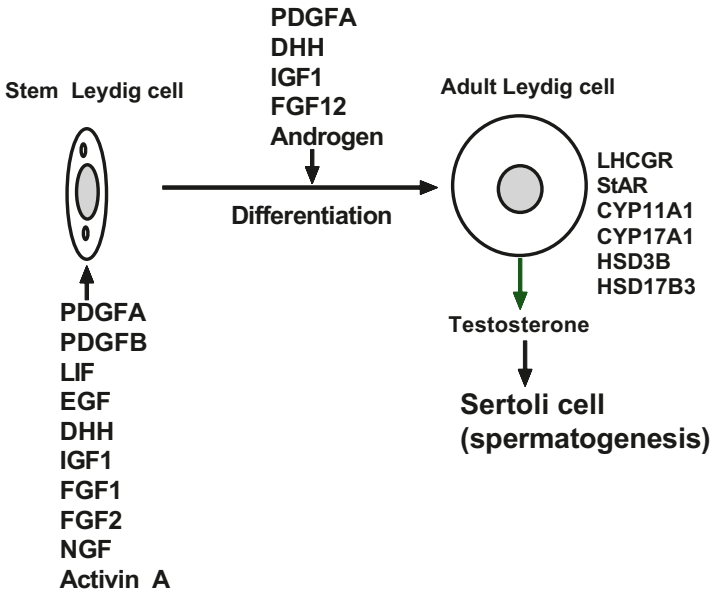

Fig. 3 Illustration of hormones and growth factors to regulate Leydig cell development. PDGFA, PDGFB, LIF, EGF, DHH, IGF1, FGF1, FGF2, NGF, and activin A stimulate stem Leydig cell proliferation. PDGFA, DHH, IGF1, FGF12, androgen stimulate stem Leydig cell differentiation in the Leydig cell lineage. *PDGFA* platelet-derived growth factor-A, *PDGFB* platelet-derive growth factor B, *LIF* leukemia inhibitory factor, *EGF* epidermal growth factor, *DHH* desert hedgehog, *IGF1* insulin growth factor-like 1, *FGF1* fibroblast growth factor 1, *FGF2* fibroblast growth factor 2, *NGF* nerve growth factor

development, as the knockout of platelet-derived growth factor A [96], platelet-derived growth factor receptor α [97] and platelet-derived growth factor β [97] and desert hedgehog [98] causes involution and absence of adult Leydig cells (Fig. 3).

When stem Leydig cells enter the Leydig cell lineage, luteinizing hormone and other growth factors seem to positively regulate its differentiation in rat and mouse models. LHCGR is expressed in progenitor, immature and adult Leydig cells [30]. Luteinizing hormone binds to the LHCGR in progenitor and immature Leydig cells, inducing their proliferation [37]. The action of luteinizing hormone to induce proliferation of these precursor cells of Leydig cells, possibly via interacting epidermal growth factor receptor/ERK1/2 pathways [99, 100].

In adult Leydig cells, luteinizing hormone is the major regulator of steroidogenesis. There are acute and chronic effects for luteinizing hormone. The acute effects take place within minutes [47]. This process acts through bound luteinizing hormone-LHCGR, triggering intracytoplasmic adenylate cyclase to increase adenosine 3′5′-cAMP (cAMP) to mobilize steroidogenic acute regulatory protein for cholesterol transportation [47]. Besides cAMP signaling, other signaling pathways including release of calcium, efflux of chloride ions, and production of arachidonic acid [101]. Luteinizing hormone also has chronic trophic actions on immature and

adult Leydig cells, up-regulating the expression of many steroidogenesis-related genes, including *Lhcgr*, *Scarb1*, *Cyp11a1*, and *Cyp17a1* [32, 102]. The chronic action of luteinizing hormone possibly exerts via cAMP/PKA/cAMP responsive element binding protein [2].

Onset and Maintenance of Spermatogenesis by Testosterone

Spermatogenesis takes place in the seminiferous tubules to eventually release of spermatozoa in the testis. The detailed process of spermatogenesis is reviewed in the other chapters. The effects of Leydig cell on spermatogenesis mostly act via secreting hormones, mainly androgen. The importance of androgen for the regulation of spermatogenesis is proven by pharmacological treatment of androgens and the conditional knockout androgen receptor.

Pharmacological Treatment of Androgens

Spermatogenesis depends on action of androgens. Luteinizing-immunization to deplete luteinizing action in Leydig cells induces the reduction of testis weight due to blocked spermatogenesis, indicating the importance of androgens for spermatogenesis [103]. Hypophysectomized rats develop testicular involution due to disrupted spermatogenesis and the androgen administration before hypophysectomy is capable of preventing these effects [104]. Using a drug ethane dimethane sulfonate to delete Leydig cells in adult rats and administration of high doses of androgen, Sharpe et al. showed that Leydig cell factors other than testosterone are not essential for maintenance of spermatogenesis in rats [105]. Testosterone is able to maintain the spermatogenesis in intact rats [106, 107], in estradiol-inhibited rats [108], and in gonadotropin-releasing hormone vaccine rats [109].

Clinical study in hypogonadotropic hypogonadal patients demonstrates that testosterone can partially maintain spermatogenesis and even fertility in some cases [110]. Testosterone and hCG have been demonstrated to initiate spermatogenesis in hypogonadotropic hypogonadal patients although the sperm production was much lower in many patients [111–113].

Androgen Action on Sertoli Cells

Germ cells themselves do not express androgen receptor [114, 115]. Indeed, germ cell conditional androgen receptor knockout mice have normal spermatogenesis [116]. Therefore, androgen action is most likely via indirect somatic cell-mediated mechanism. These somatic cells include Sertoli cells, myoid cells, and Leydig cells.

Androgen receptor is expressed in Sertoli cells [117, 118], myoid cells [114, 119, 120], and Leydig cells [30, 121]. The effects of androgen on spermatogenesis via androgen receptor have been demonstrated in Sertoli cell conditional androgen receptor knockout mice. Using Sertoli cell specific anti-Müllerian hormone promoter (only expressed in Sertoli cells) to drive Cre recombinase to create two Sertoli cell androgen receptor conditional knockout models: androgen receptor exon 2 deletion in Sertoli cell (SCARKO) [122] and S-AR$^{-/Y}$ mice [123]. Both knockout models have a normal phenotype of external male reproductive tract phenotype but blocked spermatogenesis at the level of meiosis [122, 123]. The defects of spermatogenesis in SCARKO and S-AR$^{-/Y}$ mice are as severe as those in androgen depletion in wild-type mice, indicating that androgen acts mostly via genomic androgen-dependent pathway. This is further confirmed by the severe spermatogenesis arrest in Sertoli cell conditional knockout of androgen receptor deleting exon 3, which encodes the DNA-binding domain [124], like SCARKO and S-AR$^{-/Y}$. This indicates that non-genomic action of androgen receptor plays a minor role in the regulation of spermatogenesis by Sertoli cells. A mouse model with decreased androgen receptor (AR$^{flox(ex1\text{-}neo)/Y}$) shows germ cells can complete meiosis but fails to complete spermiogenesis [125]. This finding supports the contention that androgen is also required for spermatogenesis beyond meiosis.

Conclusion

Leydig cells are critical cell types in the testis and they differentiate from stem Leydig cells. They control sperm cell meiosis and spermiogenesis beyond meiosis via secreting androgen, which acts on androgen receptor in Sertoli cells in the regulation of spermatogenesis.

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Conflict of Interest The authors declared that no competing interests exist.

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