Chapter 1

Morphological Bases of Human Leydig Cell Dysfunction

Maria P. De Miguel, Pilar Gonzalez-Peramato and Manuel Nistal

Additional information is available at the end of the chapter

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Abstract

In this chapter, we describe the histophysiology of human Leydig cells, their cytological characteristics, their differentiation processes, and the physiopathological processes occurring at various times throughout life. We first focus on the normal development of fetal Leydig cells as well as the pathologies of fetal Leydig cells that can affect numbers or hyperplasic processes (e.g., hypogonadotropic hypogonadism, cryptorchidism, congenital Leydig cell hyperplasia secondary to diabetes, and isoimmunization). Next, we explain the changes occurring at puberty with the onset and differentiation of adult Leydig cells and the pathophysiology of delayed puberty. We then describe the histophysiology of adult Leydig cells and the most frequent pathologies (e.g., hypogonadotropic hypogonadism, testicular dysgenesia, mild androgen insensitivity syndrome, 5-α-reductase defect, and Klinefelter syndrome). Finally, we discuss the morphological changes of these cells in the elderly.

Keywords: fetal Leydig cells, adult Leydig cells, testosterone, puberty, elderly, dysgenesia, pathology

1. Introduction

Leydig cells are the primary producers of testosterone. They perform important functions during fetal life, in the first months of life, and in adults, ranging from male sexual differentiation and testicular descent to the acquisition of a normal number of spermatogonial stem cells, the development of spermatogenesis and thus fertility, and the maintenance of typical male characteristics in adulthood.

2. Fetal stage

2.1. Origin and development of fetal Leydig cells

During development, two different types of Leydig cells appear sequentially, fetal Leydig cells and adult Leydig cells, which display different functional characteristics, reflecting their morphology, steroid capacity, and regulatory mechanisms. Fetal Leydig cells are detected immediately after gonadal sex differentiation [1]. Adult Leydig cells begin to develop at the beginning of puberty and acquire their maximum numbers at the end of puberty. The origin of both Leydig cell types is debated: some suggest a parent-common progenitor pool in the fetal testis [2, 3] and others have suggested that Leydig cells in adults might originate in precursors to fetal Leydig cells [4]. In addition, a three-phase model of the development of Leydig cells has been proposed: fetal, neonatal, and adult Leydig cells [5].

2.1.1. Development of fetal Leydig cells

The mesonephros and the coelomic epithelium are considered the two most plausible origins of fetal Leydig cells. The progenitor cells expressing SF1 actively proliferate and ultimately lose this marker when cell differentiation begins in week 8 of human gestation. The first sign of differentiation is the histochemical detection of 3-beta hydroxysteroid dehydrogenase (3β-HSD) in the cytoplasm. About 83% of 3β-HSD-positive cells show a high intensity of androgen receptors (ARs) immunoreaction.

Fetal Leydig cells express various steroidogenic enzymes required for androgen synthesis, such as acute regulatory protein, CYP11A1 (P450 side chain cleavage), CYP17A1 (P450C17), and 3β-HSD. The most important androgenic product of fetal Leydig cells is androstenedione. Fetal Sertoli cells make testosterone from androstenedione through the enzyme 17-β hydroxysteroid dehydrogenase (HSD17β3) at this stage of fetal testis development. Differentiation of fetal Leydig cells has been a subject of debate in recent decades and appears to be an androgen-independent process [6]. Their proliferation and subsequent development do so under the stimulus of human chorionic gonadotropin (hCG) in the first half of the gestation period and with the help of luteinizing hormone (LH) secreted by the pituitary gland in the last half. Both hormones are glycoproteic in nature and act through the same receptor (LHCGR).

2.1.2. Evolution throughout fetal life

Fetal Leydig cells are identified by positivity for the 3β-HSD enzyme. The maximum peak of Leydig cell numbers is reached at week 19 of gestation (**Figure 1**). Remarkably, this peak occurs in parallel with the disappearance of one of the two layers of peritubular cells of the seminiferous epithelium, the outermost layer of peritubular cells, between weeks 17 and 19 of gestation. These two occurrences also match the maximum production of testosterone between the 14th and 19th weeks of fetal life. After a few weeks of Leydig cell quiescence, from week 22 of gestation, a progressive decrease in the number of Leydig cells is observed.

Most fetal Leydig cells disappear after birth; however, a subpopulation persists in the postnatal testis that is estimated to represent 10% of all Leydig cells in adulthood [7–9]. Given that

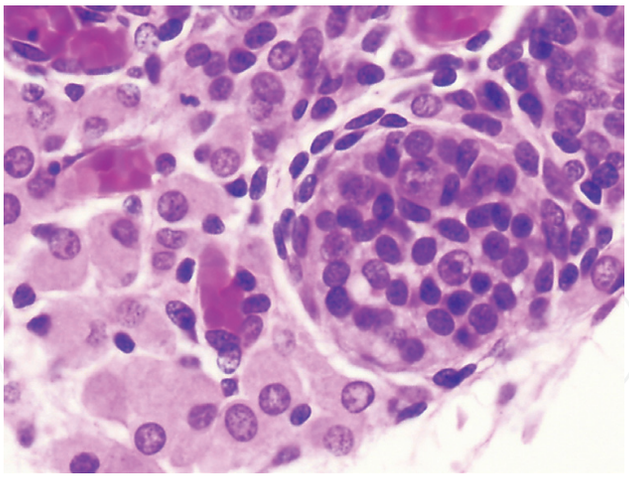

Figure 1. Fetus at 19 weeks. Seminiferous tube showing two gonocytes in the center, with the remaining cells, the Sertoli cells. In the interstitium, Leydig cells with peripheral nuclei and broad eosinophilic cytoplasm are abundantly found.

fetal Leydig cells do not express HSD17β3, they cannot directly synthesize testosterone; thus, their functional importance in adults is unknown.

2.2. Functions of fetal Leydig cells

2.2.1. Differentiation of the genitals

Under the action of testosterone, the differentiation of the Wolff tubes takes place. This differentiation is ipsilateral and gives rise to the development of the epididymis, vas deferens, and seminal vesicle. When testosterone is converted into dihydrotestosterone by the enzyme 5-α-reductase, differentiation of the prostate and development of the external genitalia occur: male urethra, penis, and scrotum.

2.2.2. Testicular descent

The two stages of testicular descent, transabdominal and inguinoscrotal, are controlled directly or indirectly by fetal Leydig cells. Transabdominal descent depends on an androgen-independent peptide, insulin-like growth factor 3 (INLS3), whereas inguinoscrotal descent is mediated by androgens through the masculinization they produce in the genitofemoral nerve neurons. Those neurons produce calcitonin gene-related peptide (CGRP), which is responsible for the development that the gubernaculum acquires, allowing testicle descent [10].

2.3. Pathology of fetal Leydig cells

2.3.1. Defective androgen synthesis

Defects in the synthesis of androgens due to various autosomal hereditary syndromes result in absent or incomplete virilization. They include lipoid congenital adrenal hyperplasia (lipoid CAH), deficits in 3-βHSD/17,20-lyase, α-hydroxylase 17/17,20-lyase deficiency, deficit in 17,20 desmolase, and deficit in 17-β hydroxysteroid dehydrogenase.

2.3.1.1. Lipoid CAH

It is the most severe form of congenital adrenal hyperplasia. It affects the synthesis of adrenal and gonadal steroid hormones. Genetically 46,XY patients are phenotypically women with severe salt-losing syndrome. There is no uniformity in the histological descriptions of the Leydig cells; in some cases, they are described as having large accumulations of lipids [11], whereas in others, such lipid inclusions are absent [12].

2.3.1.2. 3-βHSD/17,20-lyase deficit

Patients with a mild deficiency have normal external genitalia; some consult for hypospadias or micropenis [13], and the testicles are smaller than normal.

2.3.1.3. 17-α Hydroxylase/17,20-lyase deficiency

Patients have a significant degree of undermasculinization; they can even mature as girls and consult at puberty for amenorrhea. The testes show a delay in pubertal development and hyperplasia of Leydig cells.

2.3.1.4. 17,20 Desmolase deficit

It presents with a high variability in external genital development, from a female phenotype to males with micropenis that is hardly virilized, bifid scrotum, perineal hypospadias, and cryptorchidism, due to the insufficient production of testosterone during fetal life.

2.3.1.5. 17-β-Hydroxysteroid dehydrogenase deficit

Patients show a female phenotype at birth, grow up as girls, and suffer significant virilization at puberty. The cryptorchid testis or testis housed in the labia majora has low spermatogenesis development, testicular mixed atrophy, Sertoli cell-only testis histology, and in all cases, Leydig cell hyperplasia [14].

2.3.2. Hypoplasia of Leydig cells

Inactivating mutations in the LHCGR gene, primarily those involving amino acid sequences, result in a rare 46,XY disorder in sexual development (DSD) [15]. A spectrum of phenotypes can occur, ranging from a severe form with a female phenotype (Leydig cell hypoplasia (LCH) type I) when various mutations, deletions, or insertions produce a complete inactivation of LHCGR to a milder form of LCH (type II) when some receptor activity is maintained with male undervirilization (delayed puberty, primary hypogonadism, micropenis, or hypospadias) [16]. The testes in patients with LCH type I remain in the adult as a pattern of infant development with an absence of germ cells and Leydig cells. The epididymides and vas deferens are absent or hypoplastic. In patients with LCH type II, a pattern similar to the previous one has been reported, with the presence of small groups of Leydig cells and focal spermatogenesis [17].

2.3.3. Transient androgenic insufficiency

Testicular descent is a complex process in which multiple factors are involved sequentially and synergistically, highlighting the role of the hypothalamic-pituitary-testicular axis. Congenital cryptorchidism has been linked to a hormonal dysfunction, emphasizing the role of testosterone. Androgenic insufficiency during the second and third trimesters of pregnancy secondary to poor stimulation of the Leydig cells either by the pituitary hormones or by HCG has been suggested. This insufficiency might be low and transient given that no other genital anomalies except epididymal anomalies are present [18].

2.3.4. Congenital hyperplasia of Leydig cells

The presence in the newborn of a nodular or a diffuse Leydig hyperplasia is most often due to hyperstimulation by the mother's hCG, which, under certain conditions, can pass to the fetus in large quantities (**Figure 2**).

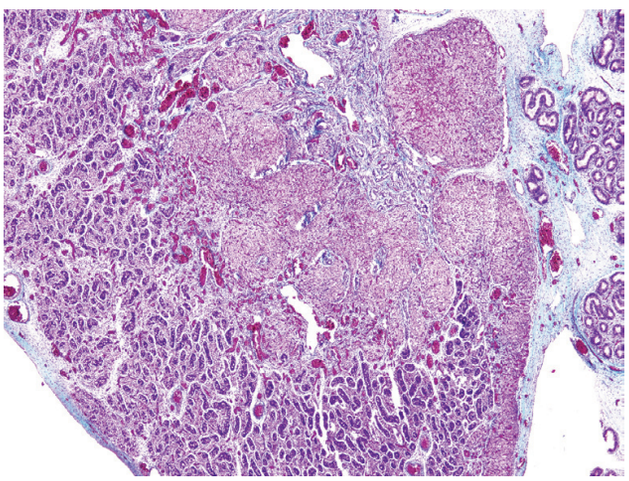

Figure 2. Congenital Leydig cell hyperplasia in a son of a mother with diabetes. Several nodules of Leydig cells are observed located in the testicular mediastinum and subalbuginea region.

Children of mothers with diabetes who have very edematous placentas could suffer this phenomenon. It has also been observed in triploid fetuses, in newborns with Beckwitz-Wiedemann syndrome, nonimmune hydrops fetalis, Rh isoimmunization or leprechaunism, and in complicated pregnancies [19].

3. Neonatal stage

3.1. Mini-puberty

In the first few months of postnatal life, a second wave of Leydig cell proliferation occurs (**Figure 3**), which depends on a transient reactivation of the hypothalamic-pituitary-testicular axis that begins immediately after birth. This stage is known as mini-puberty and begins with

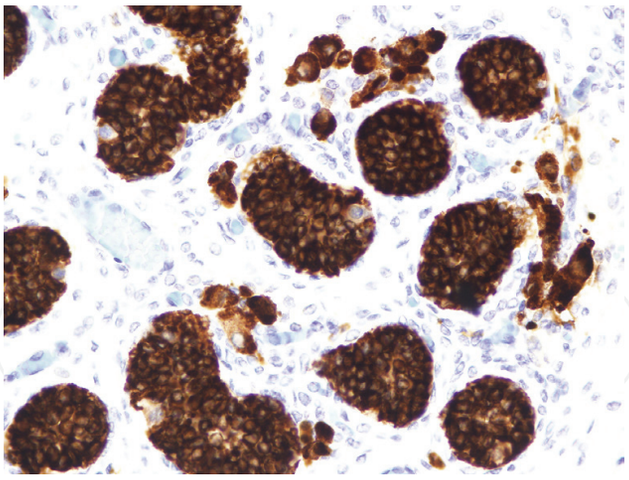

Figure 3. Infant of 3.5 months of age. The testicular parenchyma shows small clusters of Leydig cells between the seminiferous tubules. Immunostaining for inhibin in brown.

an elevation in LH and follicle-stimulating hormone (FSH). Then, an increase in the number of Leydig cells reaches a peak in the third month after birth, and secondarily, an increase in the production of testosterone and estradiol [20]. This transient stimulation determines important changes in the various cells of the testicle. An increase in the number of Sertoli cells ensuring normal spermatogenesis in adult life [21] and a transformation of gonocytes into adult spermatogonia reaches a peak by postnatal day 100. Six months after birth, coinciding with the decrease in GnRH pulses, a loss of germ cells then begins. Outside the genital tract, mini-puberty is key to the masculinization of the brain.

3.2. Pathology of mini-puberty

The most frequent pathology at this stage of development is hypogonadotropic hypogonadism, which is transient in most patients with undescended testes and permanent in other conditions such as Kallmann syndrome, multiple pituitary hormone deficiency, and DAX-1 mutations [22]. The lack of gonocyte differentiation into adult spermatogonia can be the cause of many infertility disorders that have been considered idiopathic until now.

4. Childhood

After mini-puberty, the testicle enters a resting phase that lasts until the end of the third year of life. During this period, most fetal Leydig cells involute and disappear [8]. At the beginning of the fourth year, coinciding with the activation of the androgenic receptors in the Sertoli cells, a wave of proliferation and differentiation of the germ cells begins in the seminiferous tubules. This wave is observed at the appearance of various types of spermatogonia and type I spermatocytes. The purpose of this process is not well understood, but it leads to a renewal of the germ cells. After a short time, the most differentiated cells (the spermatocytes) undergo apoptosis and are phagocytosed by Sertoli cells. Until the beginning of puberty, no Leydig cells are observed in the interstitium but are represented by fibroblastic-like cells with some lipid vacuoles.

The most frequent pathology of Leydig cells during this period is a persistence of abundant fetal Leydig cells and Leydig cell hyperplasia.

4.1. Persistence of fetal Leydig cells

The persistence of isolated fetal Leydig cells throughout childhood and even in adult testes is a normal occurrence. In any case, it is an androgen-independent subpopulation [9]. What is pathological is the presence of abundant Leydig cell clusters that also show a large cytoplasmic vacuolization due to the presence of abundant lipid inclusions. Most patients with these alterations are DSD carriers. The persistence of fetal Leydig cells is characteristic of the testis of patients with DSD with NR5A1 gene mutations (**Figure 4**), congenital lipoid adrenal hyperplasia (due to StAR mutations) [23], or a deficit of 5α-reductase and androgen insensitivity. These alterations are remarkable in the first years of life.

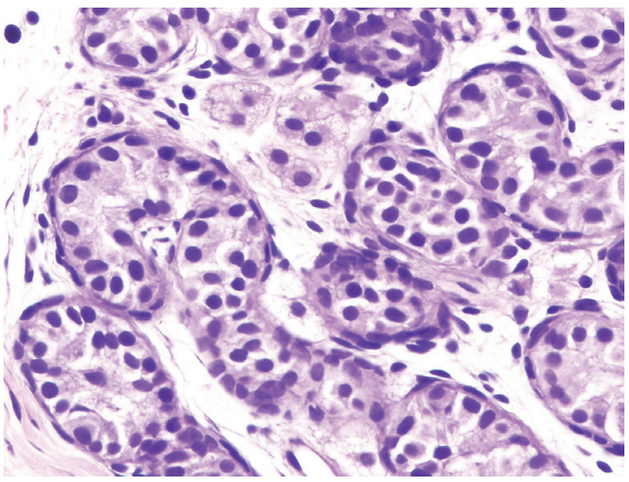

Figure 4. Patient with 46,XY DSD by mutation in the NR5A1 gene, showing Leydig cells in clusters and isolated between seminiferous tubules with a few germ cells showing highly vacuolated cytoplasms due to the high lipid content.

4.2. Hyperplasia of Leydig cells

The presence of abundant Leydig cells in childhood is a rare occurrence and is clinically manifested by symptoms of precocious puberty. It is observed in familial testotoxicosis, in some cases of McCune-Albright syndrome (MAS), Leydig cell hyperplasia/Leydig cell tumor, and extratesticular hCG-secreting tumors.

4.2.1. Familial testotoxicosis

It is also known as gonadotropin-independent precocious puberty (GIPP) or familial male-limited precocious puberty (FMPP) and is an autosomal-dominant disorder caused by a constitutive activating mutation of the LH/CGR gene [24]. It is a form of precocious male puberty characterized by the early maturation of Leydig cells and spermatogenesis in the absence of pituitary gonadotropic stimulation [25] (**Figure 5**).

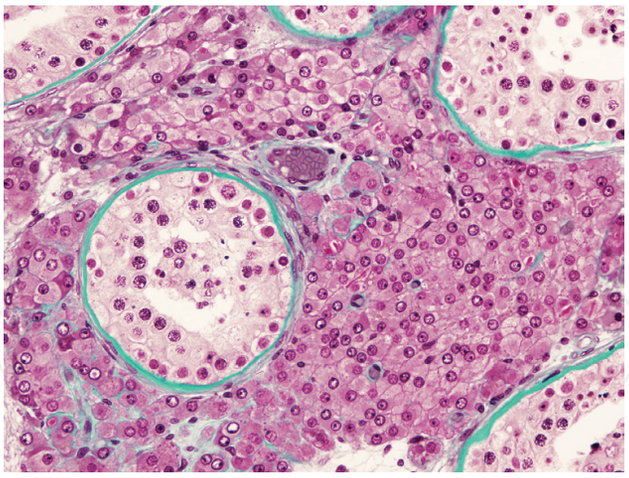

Figure 5. Hyperplasia of Leydig cells surrounded by seminiferous tubules with spermatogenesis in a 3-year-old patient with testotoxicosis.

4.2.2. McCune-Albright syndrome

It is considered an example of mixed peripheral and early precocious puberty. This syndrome is characterized by polyostotic fibrous dysplasia, "*café au lait*" skin pigmentation, and autonomous endocrine hyperfunction. At the testicular level, it is manifested by a secondary macroorchidism, in some cases associated with isolated hyperfunction of the Sertoli cells, and in other cases to a hyperfunction of the Leydig cells without the activation of the Sertoli cells [26].

4.2.3. Leydig cell hyperplasia/Leydig cell tumor

More frequently than the above situations, hyperplasia of Leydig cells in childhood is observed, which manifests clinically with the same symptoms as functioning Leydig cell tumors. Histologically, there are several nodules of hypertrophic Leydig cells located between or surrounded by seminiferous tubules with complete spermatogenesis. Whether Leydig cell hyperplasia with focal spermatogenesis in childhood is a precursor lesion of some tumors of Leydig cells is a matter of debate.

4.2.4. Leydig cell hyperplasia secondary to extratesticular hCG-secreting tumors

Numerous extratesticular tumors have been described (mediastinal, retroperitoneal, basal ganglia, pineal, or suprasellar region) that manifest with precocious pseudopuberty. All are hCG secretors. Leydig cell hyperplasia is mostly moderate but could be responsible for complete spermatogenesis [27].

5. Puberty

5.1. Differentiation of adult Leydig cells

The onset of puberty depends on both genetic and environmental factors. Under the action of gonadotropin-releasing hypothalamic hormone (GnRH), gonadotropic cell receptors of

the anterior lobe of the pituitary gland are stimulated, and the synthesis of FSH and LH begins. The onset of puberty occurs around the age of 9 years. Adult Leydig cells originate at the onset of puberty after an active proliferation of undifferentiated stem cells. Under the stimulus of LH, these cells first become stellate or spindle-shaped, from either the tubular or perivascular wall, and begin to show steroidogenic activity. Initially, as immature cells, they produce 5-α-reduced androgens more than testosterone, and later when they have acquired a polyhedral shape, the mature cells produce testosterone. Leydig cells are arranged isolated or in small groups between the seminiferous tubules, which in turn are increasingly developing the germ line, in a proportion estimated at 1.2 groups (regardless of the number of cells) per cross-tubular section. The change in testicular size is rapid and detectable at an average age of 13.5 years and is followed 1 or 2 years later by an increase in body height and the development of male secondary sexual characteristics.

5.2. Delay in the differentiation of adult Leydig cells

The most frequent pathology observed during puberty is a delay in the differentiation of Leydig cells. This delay causes an absence of or low testosterone levels, and as a consequence, a serious defect in spermatogenesis in the testicle occurs that is clinically expressed as delayed puberty. Delayed puberty is suspected when at the age of 14 years, the testis has not reached 3 ml of volume and its major axis is less than 2.5 cm. If the defect persists beyond 18 years, the patient probably carries a form of hypogonadotropic hypogonadism.

5.2.1. Constitutional delay of growth and puberty (CDGP)

Delayed puberty is a symptom for which adolescents are frequently sent to the endocrinologist, considered a minor GnRH deficit [28]. Many cases are inherited as an autosomal-dominant, -recessive, or X-linked trait. It is twice as frequent in boys as in girls. Although delayed puberty has multiple possible causes, the conjunction of elevated serum ghrelin and low concentrations of leptin is worth noting. Histologically, the testes remain prepubertal; no tubular development and adult Leydig cells are observed.

5.2.2. Delayed puberty associated with chronic illness

A number of situations can lead to delayed puberty, from malnutrition to chronic diseases (e.g., gastrointestinal diseases, chronic anemia, recurrent infections, immunodeficiency, respiratory diseases, and endocrine diseases). Even excessive exercise at this age could be a cause. The testis in most cases shows isolated small-sized Leydig cells with poor immunostaining for testosterone.

6. Adulthood

6.1. Histophysiology of adult Leydig cells

Leydig cells represent 3.8% of the testicular volume [29]. As polyhedral cells arranged in isolation or in small clusters (**Figure 6**), they are frequently observed in the tunica propria of the

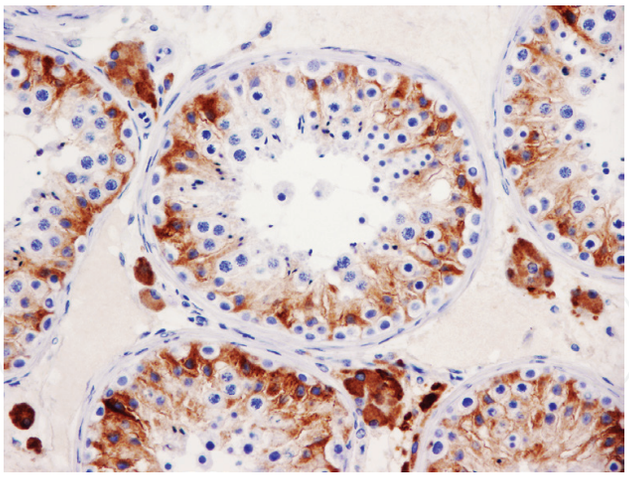

Figure 6. Transverse sections of seminiferous tubules with complete spermatogenesis in an adult patient. Immunostaining for inhibin highlights both Sertoli cell and Leydig cell clusters in the interstitium.

seminiferous tubules (peritubular Leydig cells) and as ectopic cells (testicular mediastinum, interlobular septa, albuginea, epididymis, and spermatic cord).

Cellular characteristics include an eosinophilic cytoplasm and an eccentric nucleus with one or two nucleoli. In the cytoplasm, the machinery is at the service of the transformation of cholesterol into testosterone, which includes the presence of abundant lipid droplets, significant development of the smooth endoplasmic reticulum, mitochondria with tubular crests, peroxisomes, and lipofuscins. Many cells contain Reinke crystals as well. Leydig cell clusters are joined by gap junctions.

The proper function of Leydig cells depends not only on the presence of LH receptors but a complex paracrine network in which Sertoli cells, germ cells, peritubular cells, macrophages, telocytes, and even vascular endothelial cells are involved [30]. Leydig cells show morphological changes in relation to the six stages of the cycle of the human seminiferous epithelium [29]. In adulthood, they rarely show mitosis [31]. Their numbers begin to decrease slowly after puberty, and at 60 years, there are approximately half as many as at age 20. However, the production of testosterone is maintained well until the end of the fifth decade due to the high number of cells.

6.2. Pathology of adult Leydig cells

6.2.1. Alterations in number

6.2.1.1. Absence or incomplete maturation of Leydig cells

It is the characteristic histological picture of most hypogonadotropic hypogonadisms (HHs). They include normosmic idiopathic HH, HH with anosmia or Kallmann syndrome, and HH due to LH deficiency. Another situation in which a small number of Leydig cells are observed occurs when LH is biologically inactive.

The most frequent genetic anomalies identified as causes of normosmic idiopathic HH are those in genes related to the synthesis and secretion of GnRH, such as GNRHR/GNRH1, TAC3/TACR3, KISS1R and KISS1, FGFR1 and CHD7. In the HH associated with anosmia described by [32] and

many years later known as Kallmann syndrome, KAL1 gene mutations are predominant. In these two types of hypogonadism, serum determinations of FSH and LH are very low or undetectable. The testicles show a childhood or a pubertal development pattern depending on the complete or partial absence of GnRH. In the first case, a testicular interstitium in which Leydig cells and their precursors are absent or are undetectable (**Figure 7**) is worth noting. In the second case, in which patients have a low GnRH, they present pulsatile secretions of FSH and LH that ensure maturation of isolated Leydig cells and then some secretion of testosterone allowing for testicular development that is closer to that of a pubertal testis [33, 34].

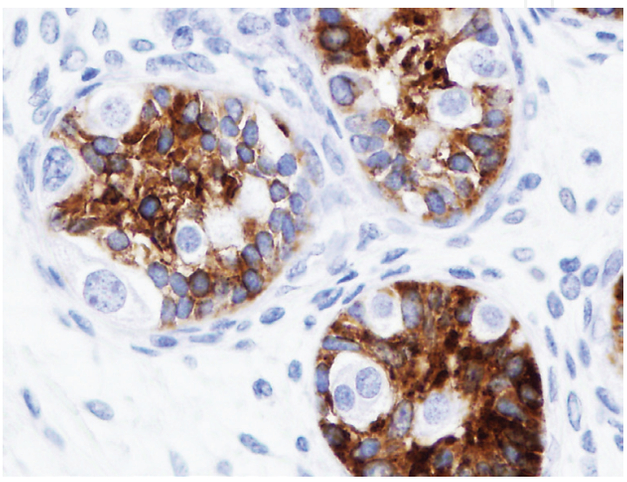

Figure 7. Adult patient of 26 years with hypogonadotropic hypogonadism with anosmia (Kallmann's syndrome). The interstitium shows a complete absence of Leydig cells. At the tubular level, binucleated spermatogonia and intense immunostaining for inhibin are observed in the cytoplasm of Sertoli cells.

In the isolated LH deficit (Pasqualini-Bur-McCullagh syndrome, fertile eunuch syndrome), serum levels of FSH are normal, whereas those of LH and testosterone are very low [35]. In some cases, the cause is a mutation in both the LH β subunit gene and in the gonadotropin-releasing hormone receptor. The testicles present complete but quantitatively abnormal spermatogenesis at the tubular level with a depopulated interstitium or very few Leydig cells. A minimum production of testosterone and a proper functioning of Sertoli cells needed for spermatogenesis are achieved. Among the many complex syndromes in which this deficit in the development of Leydig cells can be seen are Prader-Willi syndrome, Bardet-Biedl syndrome, Biemond syndrome, Frasser syndrome, and hypogonadism associated with dermatological diseases, ataxia, or central demyelination.

6.2.1.2. Focal hyperplasia of Leydig cells

It is a very common condition in ex-cryptorchid testicles, in infertile patients, and in peritumoral parenchyma that has been conserved in germ cell tumors. The focal hyperplasia is defined by the presence of clusters of at least 14 Leydig cells and by the fact that they do not distort the testicular architecture, conserving the seminiferous tubules without englobing them. Focal hyperplasia is typically associated with poor spermatogenesis (**Figure 8**). Patients frequently suffer a decrease in testosterone/LH ratio and testosterone/estradiol ratio. The presence of a focal hyperplasia of the Leydig cells represents a loss of the normal paracrine

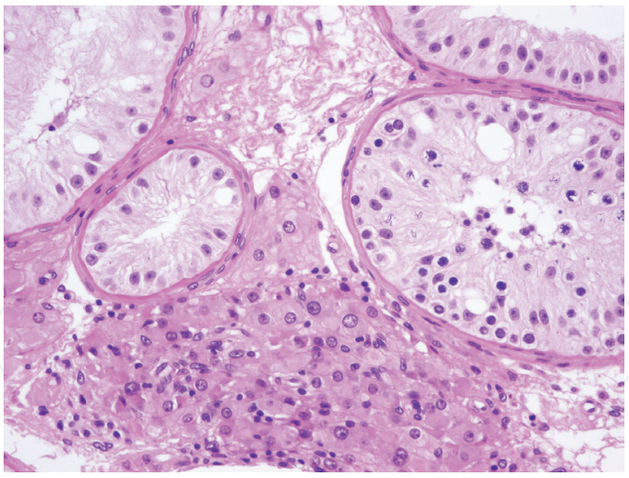

Figure 8. Focal nodular hyperplasia of Leydig cells in an adult male aged 30 years with cryptorchidism. Testicular parenchyma with mixed atrophy shows tubules with spermatogenesis and tubules with only Sertoli cells.

interactions between Sertoli cells, peritubular myoid cells, and Leydig cells. Some believe it to be the result of a defect in fetal life that probably depends on Sertoli cells, given that Sertoli cells are responsible for the quantity of Leydig stem cells [36]. Focal hyperplasia of Leydig cells is included among the most frequent lesions of patients with testicular dysgenesis [37].

6.2.1.3. Diffuse hyperplasia of Leydig cells

With this condition, Leydig cells are not only increased in number (hyperplasia) but often show hypertrophy. This hyperplasia is observed in patients with androgen insensitivity, a defect in 5-α reductase, DSD with dysgenetic testis, in many infertile patients of uncertain etiology and between the conserved seminiferous tubules of a testicle carrying a germ-cell tumor secreting β-hCG or even in the contralateral testis of such tumor.

The androgen resistance syndromes are a group of DSDs characterized by a 46,XY karyotype and phenotypes ranging from female (testicular feminization) to normal males who consult for infertility. The cause must be found in one of the almost 1000 mutations described in the AR gene. The three main forms, CAIS (complete testicular feminization), PAIS (partial testicular feminization), and MAIS (slight or minimal androgen insensitivity) [38], have been described according to the phenotype. The complete form is rarely diagnosed in childhood; patients consult for amenorrhea, or the testicles are an incidental finding during a herniorrhaphy. PAIS is diagnosed more often in childhood, associated with surgical interventions for the reconstruction of the external genitalia. Patients with CAIS and PAIS have a pathognomonic histological image: a testicular interstitium populated by numerous Leydig cells next to the seminiferous tubules, which, due to the absence of androgenic receptors in Sertoli cells, remain at infantile development (**Figure 9**). The Leydig cells usually do not show hyperplasia; many have hyperchromatic nuclei and/or abundant cytoplasmic vacuoles. Two-thirds of the testicles have Sertoli/Leydig hamartomas, which are nonencapsulated nodular formations constituted by parenchyma similar to the rest of the testicle.

Patients with MAIS frequently consult for infertility. They are phenotypically males with minimal malformations of the external genitalia, simple coronal hypospadias, or a prominent midline

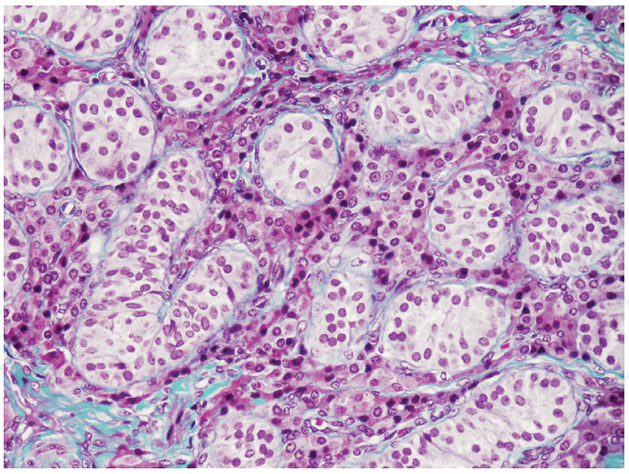

Figure 9. Patient of 20 years with 46,XY DSD with complete androgen insensitivity. The diffuse Leydig cells hyperplasia contrasts with the prepubertal development of the seminiferous tubules.

raphe of the scrotum. On the biopsy, a diffuse hyperplasia of Leydig cells surrounding each of the seminiferous tubules is observed. The presence of spermatogenesis varies from case to case [39].

Patients with a defect in 5-α reductase rarely show spermatogenesis, and the seminiferous epithelium is reduced to only Sertoli cells with incomplete maturation. The interstitium contains an elevated number of Leydig cells whose morphology is apparently normal.

In adult patients with dysgenetic testis secondary to an abnormal secretion or action of the anti-Müllerian hormone (AMH) (Sohval syndrome, male dysgenetic pseudohermaphroditism, and male with uterus), the function of Leydig cells is poor and many patients have hypergonadotropic hypogonadism. Leydig cells are not only increased in number in the interstitium but it is worth noting that a high number of Leydig cells are peritubular, forming rings in between the tubular wall (**Figure 10**).

In the testicular tumors secreting β-hCG, a marked diffuse hyperplasia of Leydig cells is generally observed. In some cases, and coinciding with burned-out tumors, Leydig cells are not only increased

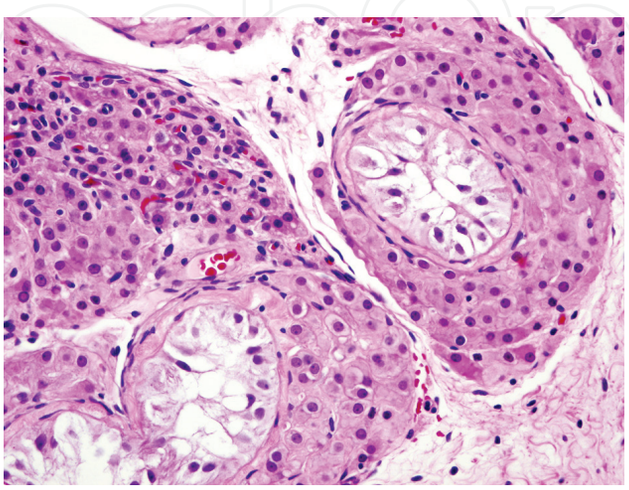

Figure 10. Male pseudohermaphroditism patient with dysgenetic testes. Note the presence of concentric rings of Leydig cells in the thickness of the tubular wall.

in number but their cytoplasm is balonized and does not show its characteristic eosinophilia. The appearance of a high number of macrophage clusters between the Leydig cells is common.

6.2.1.4. Nodular hyperplasia of Leydig cells

The presence of Leydig cell nodules has classically been considered as one of the more common histologic features in patients with 47,XXY Klinefelter syndrome. The pathology is associated with a diffuse tubular hyalinization with or without the presence of isolated tubules with only Sertoli cells or with spermatogenesis (**Figure 11**).

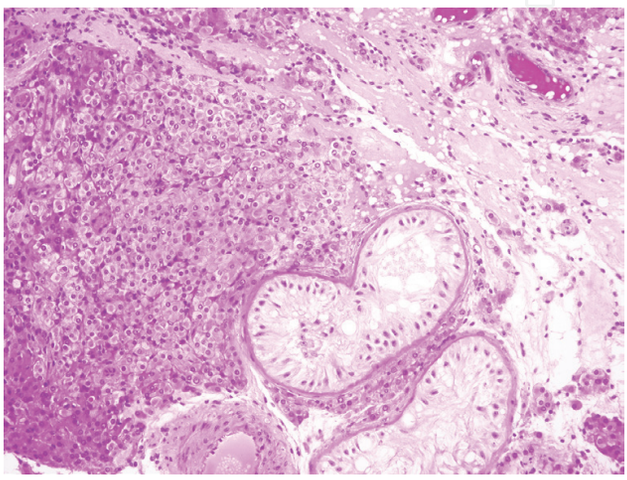

Figure 11. Klinefelter syndrome in a 32-year-old patient who consulted for infertility. Next to a nodular Leydig cells, hyperplasia seminiferous tubules with only Sertoli cells and sclerotic tubules are observed.

This form of hyperplasia is more about histological appearance than actual hyperplasia, if we consider the small size of the testicles and the disappearance of the seminiferous tubules. Both quantitative and qualitative studies have concluded that the number of Leydig cells is not increased [40]. The population of Leydig cells present is also not uniform. There are hypertrophic cells, involuted cells, and immature cells [41]. In most variants of Klinefelter syndrome, such as those with karyotypes 46,XX / 47,XYY, 48,XXYY, 48,XXXY, and 49,XXXY, Leydig cell hyperplasia is less important and can be both nodular and diffuse [42]. The 46,XX males with a normal phenotype and normal external genitalia and most patients with microdeletions of the AZF region of the Y chromosome also present Leydig cell hyperplasia that is frequently diffuse [43].

6.2.2. Alterations in Leydig cell location

There are two different situations in testicular pathology in which Leydig cells do not have a direct relationship with Sertoli cells: when they are located in the perilobular area and when they are inside the seminiferous tubules. Perilobular distribution refers to the presence of Leydig cells surrounding each testis lobule (**Figure 12**), characteristic of patients with androgen insensitivity, although it is probably a diffuse hyperplasia that isolates the seminiferous tubules.

The other location is inside seminiferous tubules, which have frequently lost the seminiferous epithelium (**Figure 13**). This ectopic location is associated with tubular dysgenesia, such as

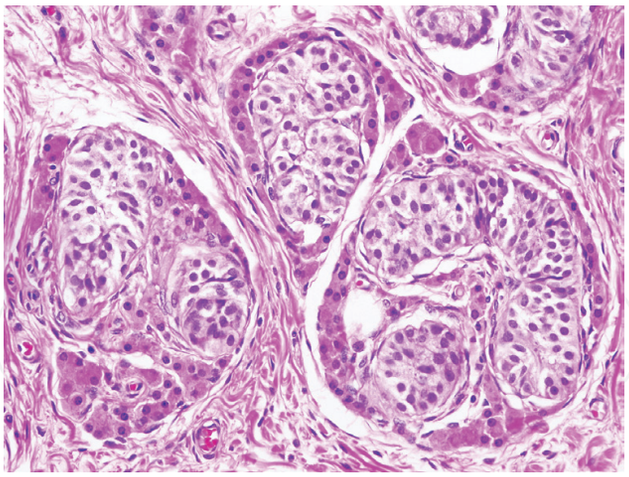

Figure 12. Preferential perilobular distribution of Leydig cells in a patient with complete androgen insensitivity. Abundant fibrosis in the interstitium stands out.

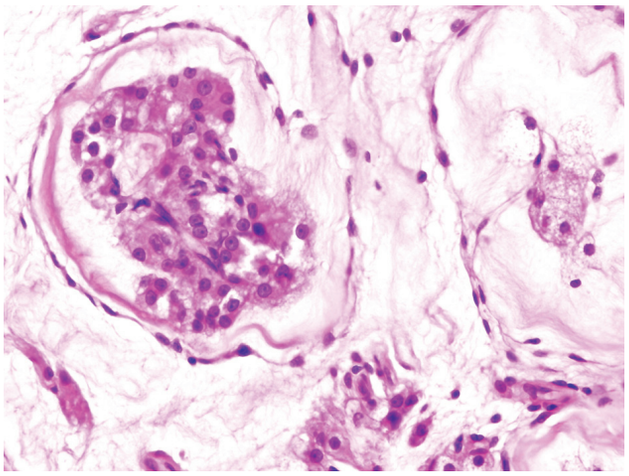

Figure 13. Leydig cells inside the sections of two seminiferous tubules in a 42-year-old patient with cryptorchidism. In contrast with atrophic characteristics of interstitial Leydig cells, normal-looking or xantomized Leydig cells in intratubular location can be observed.

Klinefelter syndrome, cryptorchid testicles that do not descend until adult age, and in some infertile patients. In many cases, the intratubular Leydig cells are accompanied by blood vessels, probably representing a migration of both Leydig cells and blood vessels normally present in the tubular wall to the interior once the cells of the seminiferous epithelium disappear.

6.2.3. Qualitative alterations

Among these alterations are Leydig cells with signs of hypertrophy, xantomized cytoplasm, and the presence of lamellar inclusions in the cytoplasm.

6.2.3.1. Cellular hypertrophy

It is common in most cases of hyperplasia; however, various degrees of cell hypertrophy with normal Leydig cell groups can coexist in the same testis. *Cytoplasmic xantomization* is always associated with cellular hypertrophy (**Figure 14**). The appearance of a pale cytoplasm with multiple

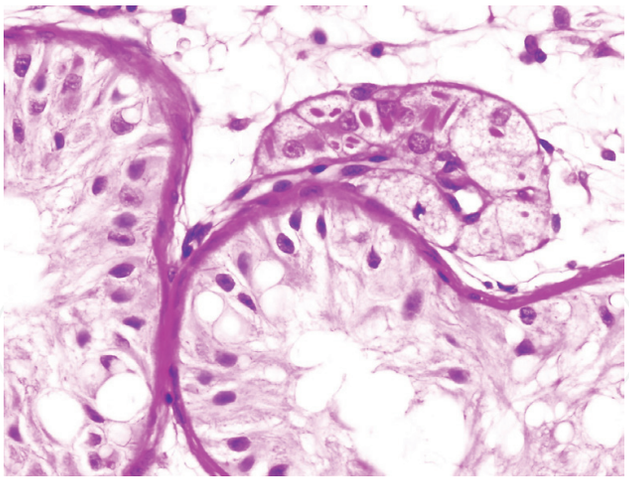

Figure 14. Testicular biopsy of an azoospermic patient. A cluster of Leydig cells with markedly xantomized cytoplasm with persistence of some Reinke crystals is shown. The nuclei of some cells are pyknotic and retracted. The neighboring seminiferous tubules lack a germ line.

small vacuoles expresses the unusually high accumulation of lipids. The cells are arranged singly or in clusters and are frequently found in patients with cryptorchidism, in DSD with dysgenetic testes, in some infertile patients, and in some elderly patients. The fact that these Leydig cells very often present with retracted, hyperchromatic nuclei in which it is difficult to recognize the nucleolus suggests that these cells have completed their biological cycle and degenerates in this way. The relationship with the various pathologies in which they are more frequently found makes it possible that many of them are dysgenetic. The presence of lamellar inclusions in the cytoplasm of Leydig cells is a characteristic feature of patients with adrenoleukodystrophy. In the variety of adrenoleukodystrophy termed adrenomyeloneuropathy, symptoms of gonadal dysfunction (loss of libido, erectile dysfunction, scant pubic hair, gynecomastia, and testicular atrophy) are observed. The patients also show a decreased seminal volume, oligozoospermia, and elevated FSH and LH. In the testis, there is severe and progressive damage of the seminiferous epithelium and alterations in Leydig cells, such as the appearance of lamellar cytoplasmic inclusions similar to the ones in adrenal cortex cells and cerebral cells [44].

7. Elderly patients

7.1. Late-onset hypogonadism

Age negatively affects all functions of the male genital tract, especially after age 65, with large inter-individual differences. Various terms have been proposed to describe this situation as "andropause," "male climacteric," "aging male syndrome," or "symptomatic" androgen deficiency in aging men. "Late-onset hypogonadism" (LOH) has been considered the most appropriate term [45, 46]. LOH is a clinical and biochemical syndrome secondary to a decrease in the serum levels of free testosterone (total testosterone will still be preserved in serum two or three decades longer). This late hypogonadism is already present in 3.1–7.0% of men between 30 and 69 years of age, but it is found in 18.4% of those over 70 years. The minimum criteria for diagnosis have been established when three clinical symptoms are present: decreased libido,

morning erection, and erectile dysfunction associated with a total testosterone level of less than 11 nml/L and a free testosterone level of less than 220 pmol/L [47, 48]. Hypogonadism in some cases has been described as primary and in others as secondary.

7.2. Interpretation of elder testis histology

Assessment of Leydig cells in the elderly is complicated, and it is often difficult to distinguish between what might be considered a consequence of normal aging of the Leydig cells and what is pathological. Sometimes, the cause is local (varicocele, obstruction of the spermatic tract), and other times, it is systemic (the impact of ischemia in a complicated arteriosclerosis, hypertension or in chronic diseases and their treatments).

7.2.1. Primary alterations

Primary alterations of Leydig cells consubstantial with physiological aging are as follows: a decrease in number, the tendency to form small clusters, cellular atrophy, abundant intranuclear inclusions, multinucleation [49] (**Figure 15**), an increase in lipofuscins, an increase in lipids, immunoexpression of keratins (**Figure 16**), and a decreased immunohistochemical expression of testosterone. The number of Leydig cells at the age of 70 is less than half than at 20 years. The tendency to form clusters becomes more important in the peripheral zones of the parenchyma, under the albuginea and in the proximities of the testicular mediastinum. The cellular atrophy is revealed by pyknotic nuclei, irregular nuclear contours, and reductions in the size of the cytoplasm [50, 51]. Multinucleation appears around the age of 60 but is isolated; it is very frequent toward the age of 70, and from this age, multinucleated cells can be observed in more than half of the microscopic fields at high magnification [52]. Not all multinucleated cells are Leydig cells; an important percentage of them are telocytes (CD34-positive fibroblasts). Paracrystalline inclusions become more abundant and are frequently observed inside the nucleus [53]. The increase in lipofuscins is progressive and appears in both single-nucleated and multinucleated cells [54]. The increase in lipid vacuoles and the scarce immunostaining for testosterone is related to involutive changes in the structures

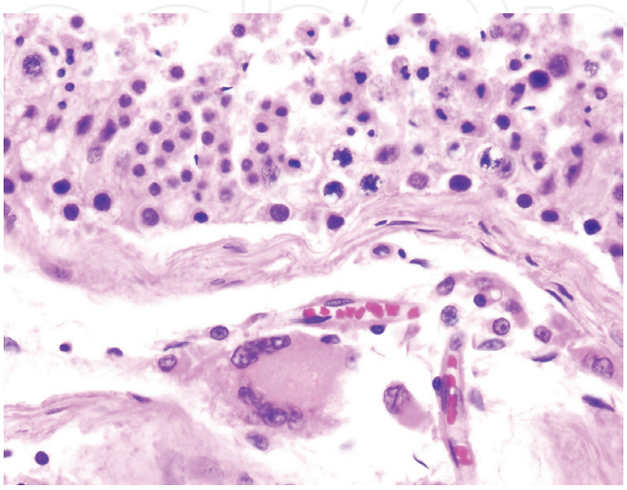

Figure 15. Multinucleated Leydig cell with typical horseshoe nuclei. Autopsy finding of a 65-year-old patient who died of myocardial infarction.

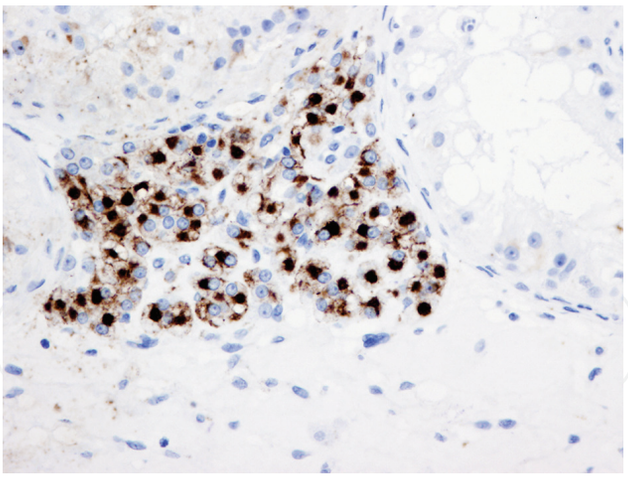

Figure 16. Small-sized Leydig cell cluster showing intense cytoplasmic cytokeratin AE1/AE3 immunostaining in a 75-year-old patient. Autopsy finding.

related to steroidogenesis (e.g., smooth endoplasmic reticulum, mitochondria) [55]. Most of the alterations correspond to those of a primary hypogonadism [56, 57].

7.2.2. Secondary alterations

The most well-known secondary changes are secondary to an obstruction, ischemia, multiple transfusions, acquired hypogonadotropic hypogonadism, and estrogen treatment. When the blockage occurs near the testicle, the seminiferous tubules of several lobules first dilate and later lose the seminiferous epithelium and become sclerosed. Leydig cells acquire a pseudohyperplastic appearance. When a branch of the testicular artery develops arteritis or even partial obstruction by arteriosclerosis, the seminiferous tubules atrophy and the Leydig cells disappear [58]. This image contrasts with the conservation of the surrounding parenchyma. In patients with chronic anemia who undergo multiple transfusions, iron accumulates in the cytoplasm of Leydig cells. In acquired hypogonadotropic hypogonadism, an involution of both the seminiferous tubules and the Leydig cells occurs. The seminiferous epithelium is reduced to only dedifferentiated Sertoli cells and isolated spermatogonia. The thickened tubular wall conserves the elastic fibers, and the Leydig cells involute until they disappear. Estrogen treatment was widely used in the past in prostate cancer, although currently it is only used before proceeding to surgical gender change. Parallel with the dedifferentiation of the seminiferous epithelium is a rapid involution of the Leydig cells, which are reduced to small pyknotic cells with retracted nuclei with some cytoplasmic vacuoles [59].

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Conflict of interest

The authors declare no conflict of interest.

Author details

Maria P. De Miguel[1]*, Pilar Gonzalez-Peramato[2] and Manuel Nistal[3]

*Address all correspondence to: mariapdemiguel@gmail.com

1 Cell Engineering Laboratory, La Paz University Hospital Research Institute, IdiPAZ, Madrid, Spain

2 Department of Pathology, La Paz University Hospital, Madrid, Autonomous University of Madrid, Madrid, Spain

3 Department of Anatomy, Histology and Neuroscience, Autonomous University of Madrid, Madrid, Spain

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