https://doi.org/10.52973/rcfcv-e35521 Revista Científica, FCV-LUZ / Vol. XXXV

Histological analysis of epididymis and testis in the dynamics of postnatal ontogenesis from one month to puberty of Ouled Djellal lambs

Análisis histológico del epidídimo y los testículos en la dinámica de la ontogénesis postnatal desde un mes hasta la pubertad de los corderos Ouled Djellal

***Yamina Belkhiri*[1,2]*, *Farida Bouzebda–Afri*[3], *Zoubir Bouzebda*[3], *Souheyla Benbia*[1,2], *Ramzi Lamraoui*[1,3]**

[1]*University of Batna 2, Faculty of Natural and Life Sciences, Biology of Organisms Department. Batna, Algeria.*
[2]*University of Batna 2, Biotechnology's Laboratory of the Bioactive Molecules and the Cellular Physiopathology. Batna, Algeria.*
[3]*Souk–Ahras University, Biotechnologies and Health. Institute Agronomic and Veterinary Sciences, Laboratory of Animal Productions. Souk Ahras, Algeria.*
**Corresponding author: y.belkhiri@univ–batna2.dz*

ABSTRACT

The aim of the study was to analyze the ontogeny of the epididymal and seminiferous epithelium in the testis of Ouled Djellal lambs throughout postnatal development until puberty. A total of 24 Ouled Djellal lambs, aged between 1 and 8 months (mos), were used in this study, with three lambs selected at each mo of age. The lambs were surgically castrated monthly, and routine histology along with histomorphometry was performed on the processed epididymal and testis tissues using AxioVision Rel 4.6 software. Statistical analysis revealed that testis weight augmented at an accelerated rate between 4 and 8 mos postnatal, coinciding with significant testicular histology modifications. These changes included notable increases in the seminiferous tubules' diameter and a rise in testosterone levels. The height of epithelium, as well as luminal and ductal diameters were found to be statistically different (P<0.05) among the epididymis's caput, corpus, and cauda segments, with the maximum levels recorded during puberty. At 1 and 2 mos of age, there were two cell kinds present inside the epithelium of seminiferous: support cells and gonocytes. By 3 mos of age, the first spermatogonia were formed from gonocytes, coinciding with the testicles reaching an average weight of 4 g. Spermatogenesis was initiated as gonocytes underwent mitosis, giving rise to progenitors that further differentiated into spermatogonia. Around 4 and 5 mos of age, the seminiferous tubules began to exhibit a lumen and primary spermatocytes (spermatocytes I). Around the age of 6 mos, secondary spermatocytes and round spermatids formed a single row within the seminiferous tubules. At ages 7 and 8 mos, all generations of germ stem cells were present in the seminiferous tubules. At 8 mos of age, spermatozoa were became apparent in several segments of the epididymis in the Ouled Djellal breed, signaling the start of puberty.

Key words: Histomorphometry; Ouled Djellal lamb; puberty; spermatogenesis; testis; epididymis

RESUMEN

El objetivo del estudio fue analizar la ontogenia del epitelio epididimario y seminífero en el testículo de los corderos Ouled Djellal a lo largo del desarrollo postnatal hasta la pubertad. Se utilizaron un total de 24 corderos Ouled Djellal, con edades comprendidas entre 1 y 8 meses, seleccionando tres corderos en cada mes de edad. Los corderos fueron castrados quirúrgicamente mensualmente, y se realizó histología de rutina junto con histomorfometría en los tejidos epididimarios y testiculares procesados utilizando el software AxioVision Rel 4.6. El análisis estadístico reveló que el peso testicular aumentó a un ritmo acelerado entre los 4 y los 8 meses postnatales, coincidiendo con modificaciones significativas en la histología testicular. Estas modificaciones abarcaron incrementos significativos en la medida de los túbulos seminíferos y un incremento en los niveles de testosterona. Se encontró que el diámetro ductal, el diámetro luminal y la altura del epitelio eran estadísticamente diferentes (P<0.05) entre las regiones caput, corpus y cauda del epidídimo, con los niveles máximos registrados durante la pubertad. A los 1 y 2 meses de edad, había dos tipos de células presentes en el epitelio seminífero: células de soporte y gonocitos. A los 3 meses de edad, se formaron los primeros espermatogonios a partir de gonocitos, coincidiendo con el peso promedio de los testículos alcanzando 4 g. La espermatogénesis se inició cuando los gonocitos sufrieron mitosis, dando lugar a progenitores que se diferenciaron en espermatogonios. A los 4 y 5 meses de edad, se observó inicialmente un lumen y espermatocitos primarios (espermatocitos I) en los túbulos seminíferos. A los 6 meses de edad, espermatocitos secundarios y espermatides redondas formaron una única fila dentro de los túbulos seminíferos. A los 7 y 8 meses de edad, todas las generaciones de células madre germinales se encontraban en los seminiferous tubules. A los 8 meses de edad, se observaron espermatozoides en varios segmentos del epidídimo en la especie Ouled Djellal, dando inicio a la etapa de pubertad.

Palabras clave: Histomorfometría; corderos Ouled Djellal; pubertad, espermatogénesis; testículo; epidídimo

Received: 23/09/2024 Accepted: 26/12/2024 Published: 21/02/2025

INTRODUCTION

For domestic animals to have successful reproductive management, it is crucial to comprehend when puberty and sexual development begin. The timing of sexual maturity can significantly influence an individual's reproductive efficiency. Characterizing puberty and the initial stages of sexual maturation is crucial for selecting males within a specific breed, Early sexual maturity shortens the generation interval and permits early progeny testing, thereby enhancing the selection process. In this regard, it is noteworthy that most sheep breeds are seasonal producers, and that the age at which males reach sexual puberty varies according to a number of factors, including season, breed, age, body weight, development of the testes, and the enhanced efficacy of endocrine gland secretions [1].

In lambs (*Ovis aries*), gonocytes move centrifugally from their central position toward the base membrane during the early postnatal stage, where they transform into spermatogonial stem cells and produce differentiated spermatogonia [2]. Postnatal testicular development and function are governed by a complex interplay of growth factors, cytokines, and gonadotropins, alongside the establishment of the hypothalamo–hypophyseal–gonadal axis. The onset of the pre–pubertal period is marked by the initiation of spermatogenesis, leading to sperm formation due to the progressive increase in LH pulses. Spermatogenesis is a systematic process that is ongoing in males, but it doesn't happen in every seminiferous tubule at the same time [3].

Instead, it develops in wave–like maturation sequences called seminiferous epithelium cycles. The first signs of puberty are the completion of spermatogenesis and the sperm cells found in the epididymis or seminiferous tubules [4].

In sheep (*Ovis aries*), measuring the most fundamental morphometric traits of the reproductive system is a useful tool for assessing the breeding soundness and potential fertility of breeding males. Testicular biometrics, in particular, are crucial for interpreting spermatogenesis, selecting superior rams, and assessing sexual maturity throughout postnatal development. Also, Ibrahim *et al.* [5] noted that qualitative changes in testicular components and spermatogenic activities must be assessed and estimated using testicular morphological examination in any breed or species. In addition to histological analysis, the study of the epididymis provides reliable information for determining the stage of maturity. Anatomically, the epididymis of ovine species is separated into cauda (tail), corpus (body) and caput (head). It contains the final segments of the efferent ducts and features a densely coiled epididymal duct along its entire length [4]. The epididymis plays crucial roles in sperm storage, maturation, and absorption [6].

In male sheep, sex hormones are vital for completing the sexual development process [1]. Testosterone is crucial in initiating sexual desire in ram lambs, as demonstrated by the first emergence of spermatozoa in the ejaculate [7]. Fluctuations in testosterone levels throughout the postnatal period can have a substantial impact on the spermatogenesis process as well as the size and weight of the testicles and accessory reproductive organs [8].

The timing of puberty and sexual maturity, as well as sexual development have been studied in Blackbelly sheep [2], Corriedale singleton lambs [4], Ghezel breed rams [9] and Najdi and Naemi ram lambs [10]. However, similar studies have not yet been conducted on Ouled Djellal rams, also known as the White Arab breed or Arbia, which are native to the arid and semi–arid regions of Algeria. Given the likely significant variation both between and among different breeds, a comprehensive study on this aspect of reproductive function in Ouled Djellal rams is required. Therefore, it was essential to describe the morphometric characteristics of the epididymis, as well as the different seminiferous epithelial cells, both in terms of quality and quantity during prepubertal development in the Ouled Djellal ram breed. This study focused on the timing of the development of certain kinds of germ cells, the commencement of spermatogenesis, and the onset of puberty.

MATERIALS AND METHODS

Animals and location

The investigation was conducted at the Khebbaba farm in Mezloug (Setif), Algeria. Mezloug is located around 10 km South–East of Setif in latitude 36°6'28" North and longitude 5°20'13" East, with a mean altitude of 933 m. The climate in Setif is semi–arid, characterized by four distinct seasons. The average temperature ranges from 4.6°C in January to 35°C in July, with an annual average rainfall of 456 mm.

A total of 24 Ouled Djellal ram lambs were used, with three lambs at each month of age between 1 and 8 months (mos). Animals were born in autumn. Throughout the trial the rams were fed a typical growth ration and had unfettered access to both mineral block and water. Each lamb's body weight (BW) was measured at the beginning of the experiment utilizing a livestock scale tailored for small ruminants (Marechalle Weighing, France).

Tissue collection and histological procedure for light microscopy

The testes were removed following anesthetic induction and lateral scrotal incision. Then, for histomorphometric analysis, the left testes and epididymides were subjected to fixation in a 10% formalin solution, while the right testes were used for biometric analysis.

The formalin–fixed tissues were processed as follows: removal of the fixative with an ascending ethanol series (sequential immersion in 70%, 95%, and 100% ethanol for 2 h, clearing with xylene 2 h, embedding in paraffin, sectioning with a microtome (Leica RM2125 RT, Germany), mounting 5 µm thick sections on glass slides, and staining with hematoxylin–eosin. A light microscope (ZEISS, Axioplan, Germany) with a digital camera (MICROCAM MA88-500, Germany) was used to take digital images of the testes and the three parts of epididyma (cauda, corpus, and caput) for morphometric analysis. The images were examined using the AxioVision Rel 4.6 software (Carl Zeiss, Thornwood, USA).

The tissue area filled by tubular and interstitial tissue in each selected field was quantified using a 40× objective. Seminiferous tubule diameter was measured at 40× magnification. Thirty spherical (or almost spherical) tubule profiles were selected and evaluated for each testis in each lamb. The seminiferous epithelium's height was also measured using these sections.

The weight of both testes (in g) was directly converted into their volume (VT), as the testicular volume density in mammals is known to be approximately one, according to Belkhiri *et al.* [11]. The relative volume (Vr) of the seminiferous tubules, calculated as the area occupied by the tubules divided by the total area of the field. The total seminiferous tubule volume per testis (VTS) was then obtained by multiplying Vr by VT. To determine the seminiferous tubules' total length (LST) (m), the tubules were modeled as a single cylinder with radius r and a volume of seminiferous tubules (VTS). Equation $LST=VTS/\pi r^2$ is obtained by using the formula $VTS=\pi r^2 LST$; Where: $\pi = 3.14$.

In the epididymis, each region's ductal diameter (caput, corpus, and cauda) was measured by randomly selecting and analyzing 20 ductal cross–sections per animal, ensuring the sections were as round as feasible. Additionally, the epithelial height and luminal diameter were measured in these sections. The horizontal distance, excluding the stereocilia, between the epithelium's base (basal lamina) and its apical border was used to describe the epithelium's height. The greatest distance between two apical margins across the lumen was measured as the luminal diameter, and the maximum distance across basal laminas was measured as the tubular diameter [12].

Each lamb's testis at each age was used to investigate 30 cross–sections of seminiferous cords and tubules in order to study the development of postnatal germ cell populations.

As described by Bahaodini *et al.* [13] various spermatogenic cells, such as support cells, Sertoli cells, gonocytes, spermatogonia, primary spermatocytes (leptotene, zygotene, pachytene, and diacinese stages), secondary spermatocytes, and spermatids (round and elongated), were categorized based on particular morphological. This evaluation was performed at 100×.

Blood concentration of testosterone

From one month old until puberty, blood samples were taken every month from the external jugular vein to measure plasma testosterone levels. Following a 10 min centrifugation (Thermo Scientific Heraeus® Labofuge® 200, Germany) at 1500 × g, the samples were decanted and stored at -20°C (Thermo Scientific™ Forma, Germany) until analysis. Testosterone levels were measured using electrochemiluminescence immunoassay (ECLIA).

Statistical analysis

Values are expressed as the mean with the standard error of the mean (SEM). Differences in weight measurements, testosterone levels, and histomorphometric values across diverse postnatal ages were assessed using one–way variance analysis (ANOVA), followed by a post–hoc analysis using Tukey's HSD test, with statistical analysis performed using SPSS (Ver. 2023). Significant differences were considered as *P*–values of 0.05 or below.

RESULTS AND DISCUSSION

This study is the first to describe the development of testicular parenchyma and epididymal epithelium in Ouled Djellal ram lambs during postnatal growth. In each animal, sexual maturity was marked by the presence of spermatozoa in the epididymal lumen [5, 6]. Understanding and mastering male reproduction is a cornerstone in breeding and management programs.

Age–related changes in weight measurements

Table I shows variations in total weight, testicular weight, and epididymis weight with age. The findings of the current study showed notable monthly variations in body weight (BW) ($P<0.05$) of Ouled Djellal ram lambs from the first (9.60 ± 0.36 kg) to the eighth month (43.80 ± 0.48 kg) (TABLE I). These findings are largely consistent with data from other studies [9, 10], with minor differences likely attributable to breed and environmental factors in which the rams were raised. Body weight variations can likely be attributed to differences in overall body development, which reflect anabolic processes and growth rates. Additionally, hormonal influences, particularly from growth hormone and other hormones linked to body mass development, as well as differences in genotype, may also contribute significantly to these variations [14].

TABLE I
Weight measurements and testosterone levels in Ouled Djellal lambs at different ages

Age (months)	Parameters			
	Epididymis weight (g)	Testicular weight (g)	Body weight (kg)	Testosterone (ng·ml^{-1})
1	0.51 ± 0.02^{a}	1.30 ± 0.11^{a}	9.60 ± 0.36^{a}	0.07 ± 0.01^{a}
2	1.02 ± 0.05^{ab}	2.40 ± 0.19^{ab}	16.80 ± 0.73^{bc}	0.09 ± 0.00^{a}
3	2.18 ± 0.32^{b}	4.00 ± 0.15^{b}	22.80 ± 0.48^{c}	0.11 ± 0.01^{a}
4	5.16 ± 0.17^{c}	15.80 ± 1.95^{c}	29.80 ± 0.73^{d}	0.12 ± 0.07^{a}
5	10.03 ± 0.43^{d}	20.60 ± 2.20^{d}	33.00 ± 0.00^{de}	0.20 ± 0.00^{b}
6	21.50 ± 0.15^{e}	58.40 ± 0.97^{e}	35.60 ± 0.24^{ef}	0.31 ± 0.03^{c}
7	29.32 ± 0.47^{f}	77.00 ± 0.01^{f}	38.40 ± 0.24^{fg}	0.50 ± 0.02^{d}
8	38.98 ± 0.13^{g}	99.40 ± 0.24^{g}	43.80 ± 0.48^{h}	0.58 ± 0.03^{e}

The differences between ages within columns are indicated by different superscripts $^{a, b, c, d, e, f, g}$ ($P<0.05$)

These results showed that testicular and epididymal development in Ouled Djellal rams was slower ($P<0.05$) in the initial postnatal period and accelerated from 4 to 8 mos of age ($P<0.001$), approximately the time when spermatogenesis begins. Therefore, the 4 mos of age marks a crucial period in the pubertal development of Ouled Djellal ram lambs. In selecting males of this breed, testis weight is significantly more relevant than body weight.

This finding supports previous observations documented in various ram breeds, including Arrabi and Awassi sheep males [14]. The first sluggish period of testicular growth is marked by low blood testosterone levels, but the ensuing fast growth phase is marked by much greater testosterone concentrations. This hormonal shift plays a critical role in accelerating testicular growth and the onset of spermatogenesis [15]. Wańkowska [16] identified another factor in her research on sheep, noting that increased body weight correlates with heightened secretion of metabolic hormones like growth hormone, which directly affects the growth of body organs, including the testes. Other studies have demonstrated that

a quick increase in testicular weight coincided with elevated serum testosterone concentrations, as well as enhanced gonadotropin receptor concentrations and affinity. Li *et al.* [17] demonstrated that high levels of postnatal serum gonadotropins and testicular gonadotropin receptors are believed to have triggered testicular development. This growth was sustained by the increased responsiveness of Leydig and Sertoli cells to reduced blood FSH and LH concentrations, as observed in male sheep.

Age–related changes in testosterone levels

TABLE I shows the variations in testosterone levels with age. According to these findings, there were no significant changes in testosterone levels between one and four months ($P>0.05$). Low testosterone levels throughout the neonatal phase do not inhibit spermatogonia formation, during the early stages of spermatogenesis is unaffected by androgens and gonadotropins. Indeed, Sertoli cells do not have receptors for androgenic hormones during the majority of the early neonatal period [18]. Zirkin and Papadopoulos [19] demonstrated in rats that the early infantile period is characterized by relatively stable yet reduced testosterone levels. This is a result of the progressive decline of fetal Leydig cells and the ongoing development of mitotically active progenitor Leydig cells, which predominantly synthesize dihydrotestosterone (DHT) and 3α–androstanediol, rather than testosterone [20]. These results demonstrated a sudden and rapid increase ($P<0.05$) in testosterone levels among Ouled Djellal lambs between 4 mos (0.18 ± 0.01 ng·ml^{-1}) and 8 mos of age (0.58 ± 0.01 ng·ml^{-1}). These results are comparable to those of Al–kawmani *et al.* [10], who observed an increase in testosterone concentration between 5 and 9 mos of age in Najdi and Naemi sheep. Zornitzki *et al.* [21] showed that the increase in testosterone levels can be attributed to various factors, including species, breed, age, environmental conditions, and seasonal variations.

Age–related changes in testicular histology

At 1 and 2 mos of age, the testicular parenchyma was consisted of several seminiferous cords. Every single sex cord was encircled by a unique basement membrane and many rows of elongated peritubular cells called myoid cells. Many stromal or interstitial cells, including Leydig cells, were seen in the stroma in the areas between the sex cords (FIG. 1). This result agrees with Elzoghby *et al.* [22] in lamb. Only two types of cells were observed in the seminiferous cords: the majority were supporting cells, which were arranged peripherally and perpendicular to the base membrane. The other type of cells was the gonocytes, which had larger nuclei compared to the supporting cells. Gonocytes were arranged near or even within the wall of the supporting cells' nuclei.

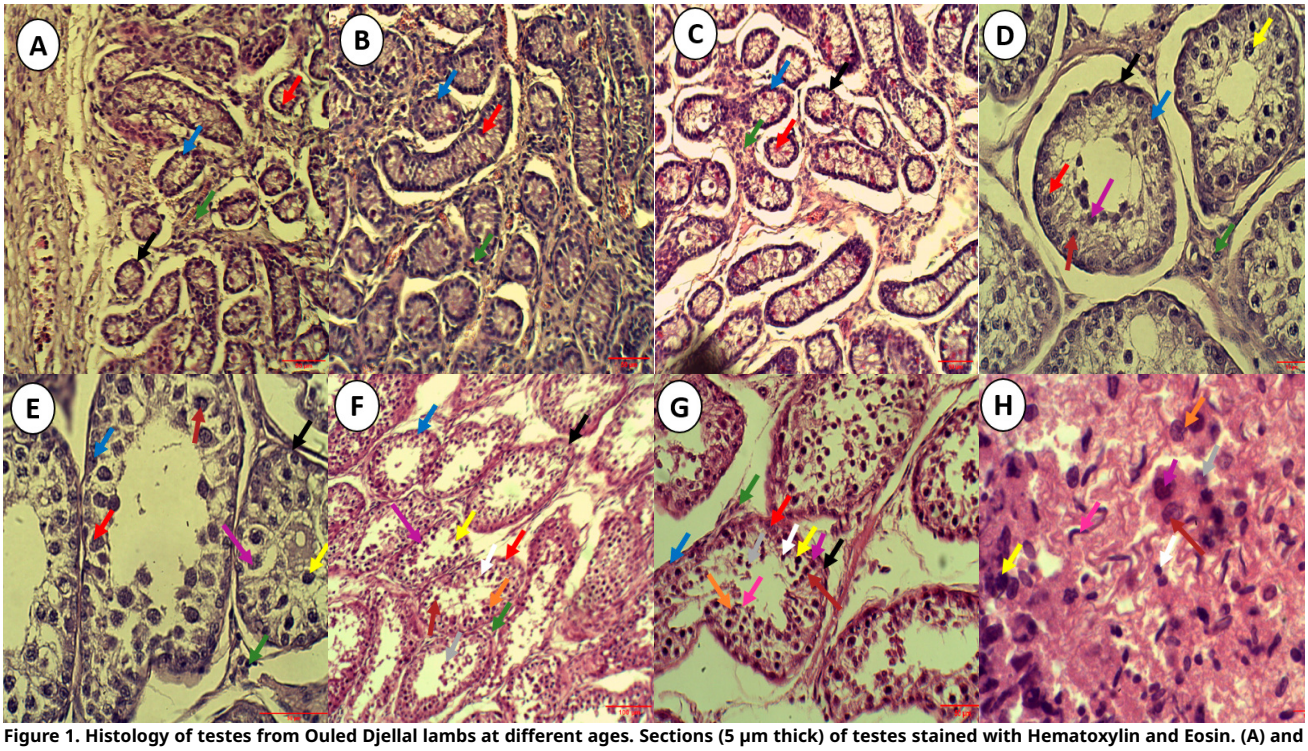

Figure 1. Histology of testes from Ouled Djellal lambs at different ages. Sections (5 µm thick) of testes stained with Hematoxylin and Eosin. (A) and (B) At 1 and 2 months of age, the seminiferous tubules were composed of supporting cells (blue arrow) and gonocytes (red arrow). Each sex cord was surrounded by a distinct basement membrane (black arrow), and The seminiferous tubules were divided by interstitial tissue rich in Leydig cells (green arrow) (100× magnification). (C) At 3 months of age, the seminiferous tubules consisted of spermatogonia (red arrow) and Sertoli cells (blue arrow) (100× magnification). (D) and (E) At 4 and 5 months of age, the seminiferous tubules contained spermatogonia (red arrow), Sertoli cells (blue arrow), leptotene spermatocytes (brown arrow), zygotene spermatocytes (yellow arrow), pachytene spermatocytes (violet arrow), and diplotene spermatocytes (orange arrow) (40× magnification). (F) At 6 months of age, the seminiferous tubules included all of the previously mentioned cells, with the addition of secondary spermatocytes (white arrow) and round spermatids (grey arrow) (40× magnification). (G) At 7 months of age, the seminiferous tubules contained all the configurations marking the different stages of spermatogenesis, though in very small numbers(40× magnification). (H) At 8 months of age, the seminiferous tubules showed completely mature spermatogenesis (100× magnification)

At 3 mos of age, spermatogonia are the offspring cells of gonocytes that enter mitosis, marking the beginning of spermatogenesis when the testicle reaches an average weight of about 4 g. This founding was similar to that in the Blackbelly breed (9-12 week) [2], however, considerably later than in the Nadji breed lamb (2 mos) [23] and in Ghazel breed lamb (1 month) [9]. The beginning of spermatogenesis may differ across lamb breeds. Increased serum FSH concentrations, mediating effects via FSH receptors located on Sertoli cells, enhance the transformation from from gonocytes into spermatogonia [24]**.** Sertoli cell differentiation in Ouled Djellal lambs began around the time of spermatogenesis and lasted around 2 mos. These early differentiated Sertoli cells supported the earliest steps of spermatogenesis, which included the differentiation of early spermatogonia and the subsequent development of germ cells during meiosis during the first wave of the cycle. In bull calves, testosterone has been proposed to induce the differentiation of indifferent supporting cells (immature Sertoli cells) [20, 25]**.**

At four months of age, primary spermatocytes begin to emerge, corresponding to a testicular weight of 15.8 g, but they were few and present in only a few tubules. One month later (at 5 mos), with a testicular weight of 20.6 g, primary spermatocytes were present in all tubules. In contrast, pachytene spermatocytes were found in the majority of tubules at 12 weeks earlier than the age at which the quantity of sertoli cells were constant (15 weeks) [2]. Santi *et al.* [26] demonstrated that mean serum FSH concentrations and FSH receptor (FSH–R) levels were elevated when the immature germ cells, occupied the majority of the seminiferous tubules. This most likely helped these immature germ cells proliferate and differentiate into primary spermatocytes in response to FSH. The lumen of the seminiferous tubules appears when the central cells disintegrate and the intercellular material of the sexual cords liquefies. This occurred at 4 mos in the Ouled Djellal lambs, which was similar to that of the Ghezel breed rams [9]. At this postnatal age, we detected a sharp and significant rise in the tube diameter of Ouled Djellal rams. The lumen formation, occurring mainly after the placing of the blood–testis barrier, in parallel to the production of fluid from the sertoli cell, as well as the transport of fluid in interstitial tissue by Sertoli cells [27].

In the present study, secondary spermatocytes first appeared at 6 mos of age. According to Oduwole *et al.* [28], increased LH receptor activity may have stimulated the LH–dependent secretion of testosterone, which is necessary for the development of primary spermatocytes into secondary spermatocytes. The age at which spermatids were first observed was 6 mos, which was similar to that of the Black Bengal goats [29], and was earlier than the Nadji lambs (7 mos) [23] and later than the Blackbelly lambs (5 mos) [2]. Rajak *et al.* [20] found that insufficient androgen levels result in an immediate halt in the meiotic transition of primary spermatocytes to spermatids, thereby inhibiting sperm production.

At 7 mos of age, the histological appearance was indicative of sexually mature sheep. The seminiferous tubules exhibited configurations marking different stages of spermatogenesis, but in very small numbers (FIG. 1). All the lumina of the tubules were empty.

These results indicated that spermatozoa were present in the epididymal lumen, as revealed by histological studies, suggests that Ouled Djellal rams reach puberty at around 8 mos of age. This finding is inconsistent with other studies on the same breed that estimated the onset of puberty based on the first detection of sperm in ejaculate, which occurred at approximately 228 days [30]. In contrast, Blackbelly sheep had spermatozoa in the various segments of the epididymis at 18-21 weeks of age, indicated the beginning of puberty [2]. Environmental factors affect sexual development in lambs, with the season of birth influencing the time between puberty and sexual maturity. Lambs born in the autumn exhibit faster testicular growth compared to those born in the spring. Nutrition is another key environmental factor impacting the onset of puberty [31].

Age–related changes in the numbers of germ cells, Sertoli cells, and Leydig cells

The results of different germ cells number at different age of lambs are presented in TABLE II. The mean count of indifferent supporting cells (immature Sertoli cells) per testis increased between 1 and 3 mos of age ($P<0.05$). As these cells differentiated into mature Sertoli cells, the average Sertoli cell count per testis increased until 4 mos of age, then gradually declined until 8 mos ($P<0.05$). Our results revealed that the reduced proliferation of Sertoli cells after 4 mos of age supports the hypothesis that these cells cease dividing to promote the development of the seminiferous epithelium during the initial wave of spermatogenesis. This cessation of mitotic activity coincides with several key events: an increase in Sertoli cell nuclear volume, the initiation of tubular fluid secretion, lumen formation, the emergence and widespread proliferation of primary spermatocytes, and the development of the Sertoli cell barrier and cytoskeleton [32]. During the foetal and neonatal periods, thyroid hormone receptors are extensively expressed on Sertoli cells, with T3 playing a vital role in controlling Sertoli cell proliferation and, more crucially, maturation [33]. Since each Sertoli cell has a limited capacity to support germ cells, the number of Sertoli cells in the adult testis determines both testicular size and daily sperm production.

These results showed that the number of gonocytes/spermatogonia per testis remained low from 1 to 3 mos of age ($P>0.05$). A significant increase in the germ cell population occurred after 4 mos of age ($P<0.05$). After 7 mos of age, the number of spermatogonia showed a propensity to stabilize ($P>0.05$). This process has also been reported in other breeds, such as Ghezel, at 4 mos of age [9]. The number of primary and secondary spermatocytes, round and elongated spermatids progressively increased with age once they first appeared.

The results showed that from the 1st to the 4th month of age, the pattern of Leydig cell numbers and serum testosterone levels was similar (TABLES I and II). After 4 mos, the amount of Leydig cells in each testis increased, reaching 3.20×10^9 by 8 mos of age. This phase of development seems to be reflected in the elevated testosterone production observed from 4 to 8 mos of age. Bagu *et al.*, [25] suggested that higher testosterone production in bull calves is either a result of increased cellular synthesis or a rise in the number of Leydig cells. Zirkin and Papadopoulos [19] showed that mature adult Leydig cells have a much higher capability for testosterone release as they acquire more organelle components required for steroid synthesis and exhibit greater responsiveness to circulating LH.

TABLE II
Number (Mean ± SE) of different cell types per testis in Ouled Djellal lambs at different ages

Age (months)	Total number per testis (× 10^9)										
	Supporting cells / Sertoli cells	Gonocytes / Spermatogonia	Primary Spermatocytes	Primary Spermatocytes L	Primary Spermatocytes P	Primary Spermatocytes Z	Primary Spermatocytes D	Secondary Spermatocytes	Round Spermatids	Elongated Spermatids	Leydig cells
1	1.58 ± 0.12^{a}	0.06 ± 0.01^{a}	–	–	–	–	–	–	–	–	0.49 ± 0.02^{a}
2	4.01 ± 0.68^{b}	0.10 ± 0.02^{a}	–	–	–	–	–	–	–	–	0.51 ± 0.01^{a}
3	7.76 ± 1.22^{c}	0.13 ± 0.03^{a}	–	–	–	–	–	–	–	–	0.52 ± 0.00^{a}
4	24.92 ± 6.58^{d}	0.49 ± 0.18^{b}	2.62 ± 1.04^{a}	1.23 ± 0.56^{a}	0.47 ± 0.17^{a}	0.90 ± 0.31^{a}	–	–	–	–	0.58 ± 0.00^{a}
5	23.37 ± 9.47^{de}	0.93 ± 0.38^{c}	6.75 ± 2.65^{b}	2.59 ± 1.09^{b}	0.81 ± 0.23^{b}	2.45 ± 0.99^{b}	0.87 ± 0.35^{a}	–	–	–	0.79 ± 0.01^{c}
6	19.38 ± 4.68^{f}	1.62 ± 0.39^{d}	14.85 ± 3.98^{c}	3.80 ± 0.85^{c}	2.34 ± 0.71^{c}	4.65 ± 0.51^{c}	2.40 ± 0.66^{b}	4.73 ± 1.29^{a}	19.42 ± 4.74^{a}	–	0.89 ± 0.10^{d}
7	12.05 ± 1.79^{g}	1.33 ± 0.23^{be}	15.86 ± 2.79^{cd}	4.16 ± 0.53^{d}	4.90 ± 1.00^{d}	4.61 ± 0.55^{c}	2.55 ± 0.42^{bc}	5.98 ± 1.05^{b}	22.86 ± 3.83^{b}	8.33 ± 2.04^{a}	2.14 ± 0.05^{e}
8	6.43 ± 0.91^{ce}	1.32 ± 0.11^{be}	18.52 ± 1.73^{e}	4.60 ± 1.13^{de}	5.72 ± 0.77^{e}	5.49 ± 1.50^{d}	3.91 ± 0.44^{d}	10.34 ± 1.63^{c}	27.93 ± 3.26^{c}	63.77 ± 6.69^{b}	3.20 ± 0.17^{f}

The differences between ages within columns are indicated by different superscripts $^{a, b, c, d, e, f, g}$ (P<0.05).

Age–related changes in testicular morphometric parameters

TABLE III presents data on the total volume of seminiferous tubules, their total length and diameter, and the volume of the interstitium during testicular development from 1 to 8 mos of age.

The volume percent filled by the seminiferous tubule varied considerably across the eight groups (P<0.05). The volume of seminiferous tubules varied from 38.23 ± 3.45 to 86.70 ± 1.81 %. A similar finding was observed in Assam goats [34]. The increase in the area occupied by seminiferous tubules within the testis is associated with testicular growth, suggesting that the parenchyma expands more rapidly to produce sufficient spermatozoa for breeding rams at puberty. We also observed a steady decrease in interstitial volume, which contrasted with a gradual increase in seminiferous tubule occupancy within the parenchyma. A similar decrease in intertubular space was recorded in Black Bengal goat [29].

TABLE III
Morphometric values (Mean ± SE) of the testicular seminiferous tubules in Ouled Djellal lambs

Age (month)	Parameters			
	Total seminiferous tubule volume per testis (%)	Seminiferous tubules diameter (μm)	Length of seminiferous tubules (m)/testis	Interstitium volume (%)
1	38.23 ± 3.45^{a}	42.04 ± 3.06^{a}	495.19 ± 83.30^{a}	61.76 ± 3.45^{h}
2	43.86 ± 2.53^{b}	45.55 ± 1.85^{a}	696.28 ± 90.17^{b}	56.13 ± 2.53^{fg}
3	46.47 ± 2.16^{bc}	54.615 ± 3.71^{b}	986.88 ± 91.57^{c}	53.52 ± 2.16^{f}
4	55.57 ± 2.06^{d}	78.11 ± 13.86^{c}	3293.80 ± 269.84^{d}	44.42 ± 2.06^{e}
5	74.15 ± 1.55^{e}	91.78 ± 9.31^{d}	3304.71 ± 356.42^{e}	25.84 ± 1.55^{cd}
6	77.63 ± 1.89^{ef}	112.94 ± 15.20^{de}	3431.75 ± 183.76^{ef}	22.36 ± 1.89^{c}
7	80.90 ± 1.56^{g}	150.39 ± 4.69^{f}	3680.63 ± 333.59^{g}	19.09 ± 1.56^{b}
8	86.70 ± 1.81^{h}	176.96 ± 10.03^{g}	3730.11 ± 597.83^{h}	13.29 ± 11.81^{a}

The differences between ages within columns are indicated by different superscripts $^{a, b, c, d, e, f, g}$ (P<0.05)

The diameter of seminiferous cords/tubules is an excellent parameter for evaluating the progression of spermatogenesis during postnatal testis development, as well as the maturation of Sertoli cells and their fluid secretion, which leads to lumen formation [35]. These results demonstrated that throughout postnatal development until puberty, both the diameter and length of the seminiferous cords/tubules steadily increased (TABLE III). A similar finding was reported in Blackbelly sheep [2] and Black Bengal goat [29]. In the early weeks after birth, the only germ cells connected with Sertoli cells were gonocytes and spermatogonia [32]. Since spermatogonia were located near the basal membrane and more mature germ cells were absent, the elongation of the tubules during this period was primarily caused by spermatogonia and Sertoli cells mitosis. However, as spermatogenesis initiated, additional germ cell types emerged and proliferated, leading to a significant increase in tubule diameter. This enlarged parenchyma is due to a significant increase in the diameter of the seminiferous tubules around puberty. After 4 mos of age, our study found a substantial rise in tubular diameter in Ouled Djellal rams, indicating rapid tubule development at the onset of spermatogenesis and prior to puberty.

Age–related changes in the histmorphometry of the epididymis

This study's microscopic analysis of the epididymis showed that the ductus epididymis at 1, 2, and 3 mos of age was lined by simple epithelial cells, which might be cuboidal or columnar in shape. These cells' nuclei ranged from circular to oval and were characterized by the presence of minute microvilli (stereocilia) (FIG. 2). At 4 and 5 mos of age, the ductus epididymis was covered by pseudostratified epithelium that included microvilli. This epithelial layer was made up of tall principal cells with elongated, narrow nuclei, as well as smaller basal cells with round to oval nuclei. At 6 mos of age, pseudostratified columnar epithelium with more prominent microvilli surrounded the ductus epididymis. By 7 mos of age, the ductus epididymis was characterized by tall columnar epithelium with elongated microvilli and an appearance of near maturity. At 8 mos of age, numerous spermatozo a were appeared accumulating in the cauda, corpus, and caput epididymis lumens, with no significant age–related histological modifications noted in the epididymis. These results align with those found by

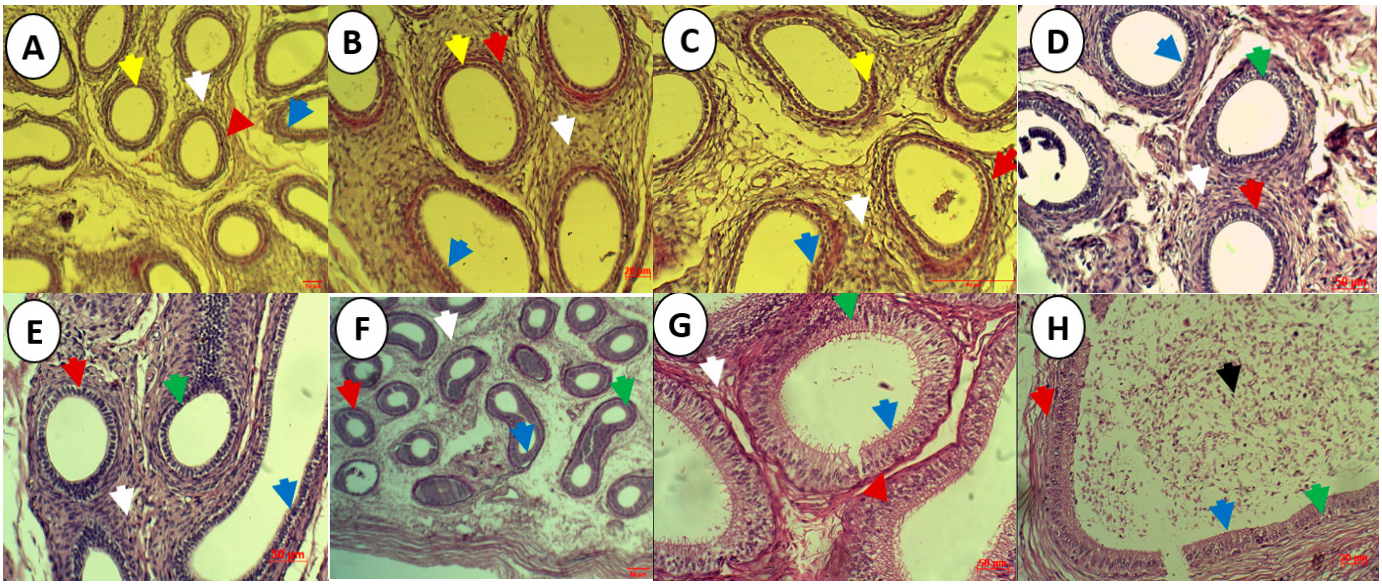

Figure 2. Histological Development of Epididymis in Ouled Djellal Lambs at Different Ages. Sections (5 µm thick) of testes stained with Hematoxylin and Eosin. (A) (B) (C) At 1, 2, and 3 months of age: simple cuboidal to columnar epithelial cells with minute microvilli (stereocilia) were the constituents of the ductus epithelium (blue arrow head). A muscular layer of smooth muscle (red arrow head) surrounded the duct. The tube itself was encased by connective tissue (white arrow head) (10× magnification). (D) (E) At 4 and 5 months of age, there were some microvilli and pseudostratified columnar epithelium (green arrow head) lining the ductus epididymis (10× magnification). (F) The pseudostratified columnar epithelium with prominent microvilli lined the ductus epididymis at six (6) months of age (4× magnification). (A) At 7 months of age, the ductus epididymis had almost mature look due to its highly columnar epithelial composition (20× magnification). (H) At 8 months of age, the completely formed epithelium included a ductus epididymis with with abundant mature sperms in the lumen (40× magnification)

Bielli *et al*. [4] in Corriedale singleton lambs. At puberty, mature spermatozoa predominantly appeared in the cauda epididymis, with fewer instances observed in the corpus and caput epididymis. This finding aligns with previous studies indicating that the cauda epididymis of animals has a higher concentration of sperm [35].

Table IV presents the average measurements of the duct diameter, lumen diameter, and epithelial height across various segments of the epididymis in the examined Ouled Djellal ram lambs. Throughout all three regions, a gradual increase in tubular diameter, luminal diameter, and epithelial height was observed with age (P<0.05), becoming more pronounced during the pubertal stages. These morphometric changes were especially significant in the corpus and cauda regions, with the most substantial alterations occurring in the cauda (P<0.05). Given that the epididymal ducts play a crucial part in sperm maturation, which favorably affects sperm morphology, their parietal growth may have improved their functioning.

CONCLUSION

The postnatal developmental investigation revealed that the testis in rams grows smoothly and progressively, as shown by weight measurements and histomorphometric characteristics.

TABLE IV
Morphometric parameters of the epididymis in Ouled Djellal lambs at different ages

Age (month)	Parameters								
	Tubular diameter (µm)			Luminal diameter (µm)			Epithelial height (µm)		
	Caput	Corpus	Cauda	Caput	Corpus	Cauda	Caput	Corpus	Cauda
1	$52.32 \pm 9.45^{a.1}$	$70.58 \pm 8.43^{a.2}$	$82.51 \pm 7.87^{a.3}$	$39.25 \pm 11.98^{a.1}$	$40.25 \pm 10.25^{a.1}$	$44.91 \pm 11.23^{a.3}$	$7.11 \pm 2.34^{a.1}$	$9.42 \pm 1.92^{a.2}$	$10.11 \pm 2.34^{a.2}$
2	$66.48 \pm 10.98^{b.1}$	$89.25 \pm 9.34^{b.2}$	$98.65 \pm 10.98^{b.3}$	$40.66 \pm 12.02^{a.1}$	$46.43 \pm 11.36^{ab.2}$	$54.18 \pm 10.45^{b.3}$	$8.28 \pm 3.25^{a.1}$	$10.65 \pm 1.98^{a.2}$	$13.08 \pm 2.24^{b.3}$
3	$70.32 \pm 10.76^{bc.1}$	$90.98 \pm 10.67^{b.2}$	$100.25 \pm 12.34^{b.3}$	$50.36 \pm 11.36^{b.1}$	$58.32 \pm 12.25^{c.2}$	$64.75 \pm 12.98^{c.3}$	$11.12 \pm 1.94^{b.1}$	$13.93 \pm 2.87^{b.2}$	$15.90 \pm 2.98^{c.3}$
4	$77.74 \pm 11.76^{c.1}$	$96.43 \pm 12.43^{c.2}$	$118.32 \pm 12.98^{c.3}$	$59.39 \pm 12.08^{c.1}$	$67.25 \pm 12.98^{d.2}$	$74.60 \pm 13.76^{d.3}$	$15.58 \pm 2.45^{c.1}$	$16.34 \pm 2.34^{c.1}$	$17.98 \pm 2.67^{cd.3}$
5	$87.45 \pm 12.67^{cd.1}$	$103.43 \pm 12.09^{d.2}$	$124.98 \pm 14.65^{cd.3}$	$75.98 \pm 14.25^{d.1}$	$78.36 \pm 14.62^{e.2}$	$88.74 \pm 13.45^{e.3}$	$21.57 \pm 2.98^{d.1}$	$18.32 \pm 1.98^{ce.2}$	$22.57 \pm 1.98^{e.3}$
6	$90.85 \pm 13.96^{d.1}$	$120.78 \pm 13.76^{e.2}$	$198.36 \pm 14.23^{e.3}$	$89.96 \pm 15.21^{e.1}$	$90.27 \pm 14.79^{f.1}$	$94.28 \pm 14.34^{f.3}$	$24.73 \pm 1.00^{de.1}$	$26.39 \pm 2.78^{f.2}$	$31.73 \pm 3.02^{f.3}$
7	$127.14 \pm 15.67^{e.1}$	$134.98 \pm 15.32^{f.2}$	$234.54 \pm 15.17^{f.3}$	$105.98 \pm 15.85^{f.1}$	$115.74 \pm 26^{g.2}$	$122.07 \pm 15.98^{g.3}$	$31.28 \pm 3.01^{f.1}$	$29.09 \pm 2.98^{g.2}$	$37.28 \pm 2.87^{g.3}$
8	$166.70 \pm 14.45^{f.1}$	$187.34 \pm 13.57^{g.2}$	$266.70 \pm 15.43^{g.3}$	$169.25 \pm 16.25^{g.1}$	$172.36 \pm 14.25^{h.2}$	$228.03 \pm 18.45^{gh.3}$	$30.94 \pm 2.90^{f.1}$	$43.52 \pm 3.23^{h.2}$	$46.14 \pm 3.03^{h.3}$

The differences between ages within columns are indicated by different superscripts $^{a, b, c, d, e, f, g, h}$ (P<0.05). Differences between regions of the epididymis within a row are indicated by different superscript numbers $^{1, 2, 3}$ (P<0.05)

At 4 months of age, there was a large rise in testicular and epididymal weight, as well as layering of the epithelium of the seminiferous tubules, a noticeable elevate in seminiferous tubule diameter, and higher testosterone levels. This represents the seminiferous tubule lumen's development. By 8 months of age, the seminiferous epithelium had completely matured, with all varieties of spermatogenic cells present, including spermatozoa in the lumen of the seminiferous tubules and epididymis, indicating the onset of puberty.

Conflict of interest

No potential conflict of interest was reported by the authors.

BIBLIOGRAPHIC REFERENCES

[1] Jumaa AJ, Kassim WY. The influence of age, gender and weight on sex hormone levels and histological development of reproductive organs in Arabi sheep. Al–Mustansiriyah J. Sci. [Internet]. 2023; 33(5):27–32. doi: https://doi.org/n4wp

[2] Herrera–Alarcón J, Villagómez–Amezcua E, González–Padilla E, Jiménez–Severiano H. Stereological study of postnatal testicular development in Blackbelly sheep. Theriogenology [Internet]. 2007; 68(4):582–591. doi: https://doi.org/dj38vd

[3] Sarlós P, Egerszegi I, Balogh O, Molnár A, Cseh S, Rátky J. Seasonal changes of scrotal circumference, blood plasma testosterone concentration and semen characteristics in Racka rams. Small Rumin. Res. [Internet]. 2013; 111(1–3):90–95. doi: https://doi.org/f4sf98

[4] Bielli A, Genovese P, Ungerfeld R, Katz H. Histology of lamb epididymal development. Anat. Histol. Embryol. [Internet]. 2007; 36(6):437–441. doi: https://doi.org/bbj2k8

[5] Ibrahim AA, Aliyu J, Ashiru RM, Jamilu M. Biometric study of the reproductive organs of three breeds of sheep in Nigeria. Int. J. Morphol. [Internet]. 2012 [cited 24 Jul. 2024]; 30(4):1597–1603. Available in: https://n9.cl/v0nj5

[6] Kangawa A, Otake M, Enya S, Yoshida T, Shibata M. Histological development of male reproductive organs in microminipigs. Toxicol. Pathol. [Internet]. 2016; 44(8):1105–1122. doi: https://doi.org/n4wq

[7] Salhab SA, Zarkawi M, Wardeh MF, Al–Masri MR, Kassem R. Development of testicular dimensions and size, and their relationship to age, body weight and parental size in growing Awassi ram lambs. Small Rumin. Res. [Internet]. 2001; 40(2):187–191. doi: https://doi.org/chs2kz

[8] Gofur MR, Khatun A, Sadi MS, Aktar S, Alam ME. Exogenous Testosterone Causes Delayed Postnatal Testicular Development and Disrupts Spermatogenesis. J. Sci. Technol. Res. [Internet]. 2023; 4(1):25–36. doi: https://doi.org/n4wr

[9] Nazari–Zenouz F, Moghaddam G, Hamidian G, Ashrafi J, Rafat SA, Qasemi–panahi B. Postnatal testicular development and testosterone changes in Ghezel ram lambs. Small Rumin. Res. [Internet]. 2016; 141:70–76. doi: https://doi.org/f82drn

[10] Al–Kawmani AA, Alfuraiji MM, Kandeal SA, Abul Farah M, Alanazi KM. Pubertal changes in testicular parameters and secretion of testosterone in Najdi and Naemi ram lambs under desert conditions. Indian J. Anim. Res. [Internet]. 2018 [cited 20 Jul. 2024]; 52(2):212–219. doi: https://n9.cl/dfmmqv

[11] Belkhiri Y, Benbia S, Djaout A. Influence of season on stereological and histomorphometric characteristics of testes of Ouled Djellal rams in Algeria. Thai J. Vet. Med. [Internet]. 2020; 50(3):297–304. doi: https://doi.org/n4wt

[12] Rashad D, Kandiel M, Agag M, El–Khawagah A, Mahmoud K, Ahmed Y., Abou El–Roos MEA, Sosa GAM. Histomorphometry of dromedary camel epididymis and its correlation with spermatozoa characteristics during their epididymal transport. Benha Vet. Med. J. [Internet]. 2018; 35(1):1–11. doi: https://doi.org/n4wv

[13] Bahaodini A, Owjfard M, Tamadon A, Jafari SM. Low frequency electromagnetic fields long–term exposure effects on testicular histology, sperm quality and testosterone levels of male rats. Asian Pacific J. Reprod. [Internet]. 2015; 4(3):195–200. doi: https://doi.org/n4ww

[14] Al–Sadoon AA, Al–Yasery AJ, Al–Khagani IY. Comparative morphological and anatomical study to development of testes and epididymis in males of Arrabi and Awassi sheep. Plant Arch. [Internet]. 2019 [cited 20 Jul. 2024]; 19(1):181–190. Available in: https://n9.cl/i82t7f

[15] Kuiri–Hänninen T, Koskenniemi J, Dunkel L, Toppari J, Sankilampi U. Postnatal testicular activity in healthy boys and boys with cryptorchidism. Front Endocrinol. [Internet]. 2019;10:1–12. doi: https://doi.org/n4wx

[16] Wańkowska M. Influence of testicular hormones on the somatostatin–GH system during the growth promoted transition to puberty in sheep. Theriogenology [Internet]. 2012;77(3):615–627. doi: https://doi.org/d7qrjc

[17] Li L, Lin W, Wang Z, Huang R, Xia H, Li Z, Deng J, Ye T, Huang Y, Yang Y. Hormone regulation in testicular development and function. Int. J. Mol. Sci. [Internet]. 2024; 25(11):5805. https://doi.org/n4wz

[18] Picut CA, Remick AK, de Rijk EP, Simons ML, Stump DG, Parker GA. Postnatal development of the testis in the rat:morphologic study and correlation of morphology to neuroendocrine parameters. Toxicol. Pathol. [Internet]. 2015; 43(3):326–342. doi: https://doi.org/n4w2

[19] Zirkin BR, Papadopoulos V. Leydig cells: formation, function, and regulation. Biology of reproduction. [Internet]. 2018; 99(1), 101–111. https://doi.org/gc76zm

[20] Rajak SK, Kumaresan A, Gaurav MK, Layek SS, Mohanty TK, Muhammad–Aslam MK, Tripathi UK, Prasad S, De S. Testicular cell indices and peripheral blood testosterone concentrations in relation to age and semen quality in crossbred (Holstein Friesian × Tharparkar) bulls. Asian–Australasian J. Anim. Sci. [Internet]. 2014; 27(11):1554–1561. doi: https://doi.org/f6nvbr

[21] Zornitzki T, Tshori S, Shefer G, Mingelgrin S, Levy C, Knobler H. Seasonal variation of testosterone levels in a large cohort of men. Int. J. Endocrinol. [Internet]. 2022; 2022:6093092. doi: https://doi.org/n7m3

[22] Elzoghby E, Sosa G, Nah M. Postnatal development of the sheep testis. Benha Vet. Med. J. [Internet]. 2014[cited 19 August 2024]; 26(2):186-190. Available in: https://n9.cl/bgp2a

[23] Al–Kawmani AA, Alfuraiji MM, Abou–Tarboush FM, Alodan MA, Farah MA. Developmental changes in testicular interstitium in the Najdi ram lambs. Saudi J. Biol. Sci. [Internet]. 2014; 21(2):133–137. doi: https://doi.org/g4mj5t

[24] Rossi P, Dolci S. Paracrine mechanisms involved in the control of early stages of mammalian spermatogenesis. Front Endocrinol. [Internet]. 2013; 4:1-8. doi: https://doi.org/n4w3

[25] Bagu ET, Cook S, Gratton CL, Rawlings NC. Postnatal changes in testicular gonadotropin receptors, serum gonadotropin, and testosterone concentrations and functional development of the testes in bulls. Reproduction [Internet]. 2006; 132(3):403–411. doi: https://doi.org/bvjbqq

[26] Santi D, Crépieux P, Reiter E, Spaggiari G, Brigante G, Casarini L, Rochira V, Simoni M. Follicle–Stimulating Hormone (FSH) action on spermatogenesis: a focus on physiological and therapeutic roles. J. Clin. Med. [Internet]. 2020; 9(4):1014. doi: https://doi.org/g7ktbm

[27] Auharek SA, de França LR. Postnatal testis development, Sertoli cell proliferation and number of different spermatogonial types in C57BL/6J mice made transiently hypo – and hyperthyroidic during the neonatal period. J. Anat. [Internet]. 2010; 216(5):577–88. doi: https://doi.org/c9dztd

[28] Oduwole OO, Huhtaniemi I T, Misrahi M. (2021). The roles of luteinizing hormone, follicle–stimulating hormone and testosterone in spermatogenesis and folliculogenesis revisited. Int. J. Mol. Sci. [Internet]. 2021; 22(23):12735. doi: https://doi.org/n4w4

[29] Gofur MR, Sadi MS, Aktar S, Khatun A, Awal MA, Alam ME, Rauf SMA, Matsuo K. Biometrical and histomorphometrical changes of testis in the dynamics of postnatal ontogenesis from birth to puberty of Black Bengal goat. J. Adv. Vet. Anim. Res. [Internet]. 2023; 10(2):237-243. doi: https://doi.org/n4w5

[30] Boussena S, Bouaziz O, Hireche S, Derqaoui L, Dib AL, Moula N. Apparition de la puberté chez les agneaux mâles de race Ouled Djellal. Revue Med. Vet. [Internet]. 2016 [cited 22 Jul. 2024]; 167(9-10):274–282. Available in: https://n9.cl/m8fnb

[31] Ledezma–Torres R A, Sánchez–Dávila F, Rodríguez–Miranda DA, Luna–Palomera C, Grizelj J, Vázquez–Armijo JF, López–Villalobos N. Sexual performance and semen quality of pubertal lambs treated with different weaning methods. Arch. Anim. Breed. [Internet]. 2022; 65(3):259–265. doi: https://doi.org/n4w6

[32] Meroni SB, Galardo MN, Rindone G, Gorga A, Riera MF, Cigorraga SB. Molecular mechanisms and signaling pathways involved in sertoli cell proliferation. Front. Endocrinol. [Internet]. 2019; 10(224):22. doi: https://doi.org/gmcp2f

[33] Gao Y, Lee, W M, Cheng, C Y. Thyroid hormone function in the rat testis. Front. Endocrinol. [Internet]. 2014; 5(188):1-7. doi: https://doi.org/n4w7

[34] Sarma K, Devi J. Morphometrical changes of the seminiferous tubules and leydig cells in Assam goats (*Capra hircus*) from birth to 10 months. J. Appl. Anim. Res. [Internet]. 2017; 45(1):268–274. doi: https://doi.org/n4w8

[35] Olukole SG, Obayemi TE. Histomorphometry of the Testes and Epididymis in the Domesticated Adult African Great Cane Rat (*Thryonomys swinderianus*). Int. J. Morphol. [Internet]. 2010; 28(4):1251–1254. doi: https://doi.org/n4w9