Correlation of Reproductive Histological Characteristics with Age, 5α-Reductase and Androgens in Arabi Rams (*Ovis aries*)

Research Article

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ABSTRACT

The aim of this study was to evaluate the correlation between histological features of the reproductive system and age, 5α-reductase-2 (5αR2), and androgenic hormone levels in Arabi rams. Thirty Arabi rams were used in three age groups: lambs, rams with 9-12 months of age in the early puberty period, and adult rams with 3-4 years of age. Blood samples were collected to evaluate the serum 5αR2, testosterone, and dihydrotestosterone (DHT) concentrations. Subsequently, testes, epididymis, and prostate samples were collected after slaughter of the animals for histologic evaluations. Serum concentrations of 5αR2, DHT, testosterone, and the number of testicular spermatocytes, spermatids, and Leydig cells in adult rams were higher (P<0.05) than those in lambs and early puberty rams. The highest (P<0.05) population of spermatogonia cells was observed in early pubertal rams. There was a significant (P<0.05) increase in the diameter of the seminiferous tubule and its lumen, the germinal epithelium's thickness, and the epididymis's epithelial thickness in relation to age. The epithelial thickness and the secretory vesicle diameter of the prostate increased (P<0.05) with age. There were significant (P<0.05) positive correlations between germinal epithelium thickness and testosterone (r=0.81) as well as DHT (r=0.81) concentrations; however, it was not significant (P>0.05) for 5αR2. In conclusion, at the beginning of sexual maturity, the tissue structure of the reproductive system in rams does not reach its full development, and it is only after physical maturity that these structures reach their full development.

KEY WORDS dihydrotestosterone, maturity, *Ovis aries*, SRD5A2, testis tissue, testosterone.

INTRODUCTION

The reproductive performance of the animals is one of the most important traits, due to its impact on the overall profitability of the flock. Management of the males is necessary to ensure the success of the flock productively and economically. The age of onset of puberty in ram lambs varies across breeds and environmental conditions Nevertheless, it is characterized by a series of endocrine events that activate the reproductive axis, which allows the Sertoli and Leydig cells to become responsive to the gonadotropin action, resulting in the initiation of spermatogenesis (Maquivar *et al.* 2021).

Iranian sheep breeds differ in several factors, including their genetic potential for meat, milk, and wool production. These breeds are traditionally named according to the keepers' tribe or geographic origin, but they have also been classified according to morphological features and productive performance (Ruiz-Larrañaga *et al.* 2020). Arabi sheep is one of the most important dual-purpose (meat and wool) sheep native breeds of Iran, which are characterized as white, cream, black, and dark/bright brown color, horned

rams and polled ewes, fat-tailed, medium-sized. Most of these sheep are raised in Khuzestan province in the south-west of Iran. They are well adapted to humid tropical environmental conditions (Roshanfekr *et al.* 2015).

5α-reductase is a system of NADPH-dependent enzymes that catalyzes the irreversible conversion of testosterone to its corresponding 5α-reduced metabolite, i.e., dihydrotestosterone (DHT) with more androgenic effect than testosterone (Zhu and Imperato-McGinley, 2008). The actions of androgens such as testosterone and dihydrotestosterone are mediated via the androgen receptor, a ligand dependent nuclear transcription factor and member of the steroid hormone nuclear receptor family (Davey and Grossmann, 2016). Although testosterone and DHT interact with the same androgen receptor, both hormones produce distinct biological responses (Luetjens and Weinbauer, 2012). Deficiency of 5α-reductase enzyme results in intrauterine abnormal male sexual development and also during puberty. Biochemical findings that support the diagnosis of 5- Alpha reductase deficiency are that of a normal serum testosterone value and an increased ratio of serum testosterone to DHT (Noroozi Asl *et al.* 2023). Animal studies showed that male genitalia virilization resulted from the conversion of testosterone into dihydrotestosterone, a reaction catalyzed by the 5α-reductase enzyme (Batista and Mendonca, 2020).

It has been reported that mutations in the 5α-reductase-2 gene (SRD5A2) are responsible for a disorder in male sexual differentiation. The specific short-chain dehydrogenase reductase enzymes, known as 5α-reductases, are best known for their role in the masculinization of the male reproductive tract (Wilson *et al.* 1995). These isozymes are expressed in several male reproductive tissues, including the epididymis (Robaire *et al.* 1977). It is well recognized that there is a decline in serum testosterone concentration with age, associated with a decrease in the amplitude of diurnal variation (Imperato-McGinley *et al.* 1980). DHT plays a critical role in male sexual development, and a deficiency of this hormone disrupts the formation of the external sex organs before birth. During puberty, an increase in male sex hormone levels leads to the development of some secondary sex characteristics. Using pharmacological inhibitors of 5α-reductase in humans leads to increased sexual dysfunction, ejaculatory dysfunction, erectile dysfunction, testicular atrophy, and hypogonadism (Baas *et al.* 2018). 5α-reductase has different activities in the epididymal microsomal and nuclear fractions, whereas 5α-reductase inhibitors may decrease fertilization success and increase implantation loss (Robitaille and Langlois, 2020). The ratio of testosterone to DHT decreases in the epididymal cavity compared to the testes (Falvo *et al.* 2015). Therefore, DHT may play an important role in the epididymis.

Histologic quantification of the testicular parenchyma, such as tubule diameter and germinal epithelium thickness, is important in studying of male reproductive function. The volumetric proportion of seminiferous tubules and inter-tubular tissues such as the tubule diameter and thickness of seminiferous tubules are directly related to sexual activity (Paula *et al.* 1999; Shukla *et al.* 2013; Aissanou and Ayad, 2022). The volumetric proportion of Leydig cells in the intertubular tissues and quantitative parameters directly related to the seminiferous tubule, such as the tubule diameter, the thickness of the seminiferous epithelium, and the length of the tubule, are positively correlated with spermatogenic activity, provide information on the level of spermatogenic activity and are indicators of this activity in studies of testicular function (Andreussi *et al.* 2014).

The postnatal development of the testes is a period during which the somatic and germ cells proliferate and differentiate, resulting in the first round of spermatogenesis and setting the framework for continuous sperm production in the future (Nazari-Zenouz *et al.* 2016). The analysis of testicular development through biometric measurements is highly important, as it shows a significant correlation with reproductive activity (Salhaba *et al.* 2001). Testicular histomorphological evaluation is known to elucidate physiological processes from the perspective of form and function. The quantification of testicular histology is a valuable tool for evaluating the sperm capacity of animals (Rocha *et al.* 2022). Therefore, the aim of this study was to evaluate the variation in histological characteristics of the reproductive system as an indicator of reproductive potential in relation to age and its association with concentrations of blood serum 5α-reductase-2 (5αR2), testosterone, and DHT concentrations in Arabi rams (*Ovis aries*).

MATERIALS AND METHODS

Location and Animals
The present study was conducted from the summer of 2021 to the fall of 2022 at the research farm of Agricultural Sciences and Natural Resources University of Khuzestan in the Khuzestan province of Iran. A total of 30 Arabi rams (*Ovis aries*) were used and divided into three age categories: 1) lambs less than one month old (mean BW of 9±2 kg, n=10), 2) rams in the early puberty period with 9-12 months old (mean body weight (BW) of 40±2 kg, n=10), and 3) adult rams with 3-4 years old (mean BW of 62±3 kg, n=10).

Ethical approval
All study procedures were approved by the Department of Animal Science, Agricultural Sciences and Natural Resources University of Khuzestan (Approval no: 4.07.2021/4).

Blood serum parameters

Blood samples of jugular vein (5 mL) were collected from lambs, early puberty, and adult rams using venoject tubes and immediately transferred to centrifuge tubes cooled on ice. Then, samples were centrifuged for 15 min and the serum was recovered and stored at −20 °C until assayed. The concentrations of isoenzyme 5α-reductase-2, testosterone, and dihydrotestosterone (DHT) in blood serum were determined using sheep kits from ZellBio GmbH, Germany (Cat No. ZB-10093C-Sh9648), and the ELISA method.

Histologic assessment

Testes with epididymis and prostate samples were collected immediately after the animals were slaughtered, fixed in buffered 10% formalin (pH 7), and transported to the laboratory as soon as possible. In the laboratory, physiological saline containing antibiotics was used to wash the samples and surrounding tissues were carefully removed with a scalpel blade (Hasani Al-Ameri, 2022). Fixed samples were dehydrated with different concentrations of ethanol (70-90%), cleared and embedded in melted paraffin wax. After preparation of the samples, step serial sections 5 μm thick were obtained using a microtome and then stained with hematoxylin-eosin and used for histologic evaluation by a light microscope and Dino capture software. Three slides were prepared for each replicate. The investigated factors in testicular tissue samples included seminiferous tubule diameter, seminiferous tubule lumen diameter, epithelial thickness, and counting of testicular cell types, whilst those in prostate tissue samples included epithelial thickness, lumen diameter, and secretory unit diameter. In order to measure and count the histometric indices of this research, 5 slides from each sample and 5 microscopic fields in each slide (100 μm diameter) were evaluated (Gholami *et al.* 2015).

Statistical analysis

Data were analyzed using SPSS software (SPSS, 2011) by completely random design. Variations in serum and histologic parameters among different age groups were evaluated using one-way ANOVA and Duncan's multiple range test to detect differences among groups. Pearson's correlation coefficient test was used to evaluate correlations between subjective parameters (Petrie and Watson, 2013).

RESULTS AND DISCUSSION

The serum concentrations of 5αR2, DHT and testosterone among different ages of rams are shown in Table 1. Age variations were observed in the serum parameters so that 5αR2, DHT, and testosterone levels in adult rams (3-4 years old) were significantly ($P<0.05$) higher than those in lambs

(less than one month) and in early pubertal rams (9-12 months old). As shown in Table 2, the number of testicular spermatocytes, spermatids, and Leydig cells was significantly higher in adult rams than in lambs and early pubertal rams. The highest ($P<0.05$) population of spermatogonia cells was observed in early pubertal rams. In contrast, the highest ($P<0.05$) number of Sertoli cells was observed in lambs. The morphometric evaluation of the seminiferous tubules and epididymis showed a significant increase in the diameter of the seminiferous tubules and their lumen, the thickness of the germinal epithelium as well as the epithelial thickness of the epididymis in relation to age (Tables 3 and 4).

Histologic examination of the prostate (Table 5) showed an increasing trend in epithelial thickness and secretory vesicle diameter of this gland with increasing age. The diameter of the prostate lumen was not affected ($P>0.05$) by the age of the rams.

The results showed a lower density of seminiferous tubules and a higher density of intertubular tissue in lamb testis. In early pubertal rams, the population of seminiferous tubules as well as their diameter and germinal epithelium thickness increased and reached a maximum in adult rams (Figures 1 and 2). The epithelial thickness of the epididymis was lower in lambs and with increasing age and reaching the stage of early pubertal rams and then mature rams, it had an increasing trend (Figure 3). The structure of the prostate gland in lambs, early pubertal rams and adult rams is shown in Figures 4 and 5. The density of secretory units was low in lambs and then increased in early pubertal rams. A relatively higher distribution of secretory units with a significant increase in prostate diameter was observed in adult rams.

The analysis of the correlation between the histological parameters of the testis and prostate with the assessed blood parameters in Arabi rams showed significantly positive correlations between the thickness of the germinal epithelium and the concentrations of testosterone ($P<0.05$, $r=0.81$) and DHT ($P<0.05$, $r=0.81$). Other correlations between the parameters examined were however not significant (Tables 6 and 7).

In this study, the highest 5α-reductase activity and androgenic hormone levels were observed in adult rams compared to lambs and rams in early puberty. Puberty corresponds to the transition to adulthood and can be defined in rams as the time when fertile spermatozoa are present in the ejaculate (Kridli *et al.* 2007). Some authors define puberty as the time when rams show interest in females in estrus by successive mounting with ejaculation (Belibasaki and Kouimtzis, 2000). It is also described as the stage of sexual maturation when a ram is able to display full sexual behavior, producing and releasing gametes (Price *et al.* 1991).

Table 1 Serum parameters at different ages of Arabi rams

Groups	Age	Serum parameters		
		5αR2 (pg/mL)	DHT (pg/mL)	Testosterone (pg/mL)
Lambs	(<1 month)	3283.30[b]	37.48[b]	128.17[b]
Early puberty rams	(9-12 months)	3300.00[b]	81.60[b]	210.00[b]
Adult rams	(3-4 years)	3950.02[a]	815.05[a]	3080.02[a]
SEM	-	90.74	118.73	408.94
P-value	-	0.022	0.001	0.026

5αR2: 5α-reductase type 2 enzyme and DHT: dihydrotestosterone.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 2 Testicular cell counts at different ages in Arabi rams

Groups	Age	Average number of cells					
		Sertoli	Spermatogonia	Spermatocyte	Early spermatid	Late spermatid	Leydig
Lambs	(<1 month)	11.00[a]	13.14[c]	0.00[c]	0.00[c]	0.00[c]	3.50[c]
Early puberty rams	(9-12 months)	9.19[b]	20.36[a]	16.81[b]	13.85[b]	1.16[b]	6.27[b]
Adult rams	(3-4 years)	8.52[b]	17.14[b]	37.11[a]	29.39[a]	33.39[a]	9.08[a]
SEM	-	0.49	1.34	6.98	5.37	6.93	1.05
P-value	-	0.024	0.03	0.013	0.001	0.0001	0.011

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 3 Morphometric approach to the seminiferous tubules at different ages in Arabi rams

Groups	Age	Average diameter (μm)		
		Seminiferous tubule	Seminiferous tubule lumen	Germinal epithelium thickness
Lambs	(<1 month)	64.34[c]	26.93[c]	32.42[c]
Early puberty rams	(9-12 months)	136.38[b]	67.80[b]	57.08[b]
Adult rams	(3-4 years)	195.12[a]	89.84[a]	98.76[a]
SEM	-	23.95	11.68	12.32
P-value	-	0.0001	0.0001	0.001

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 4 Epithelial thickness (μm) of the epididymis at different ages in Arabi rams[1]

Epididyme	Lambs	Early puberty rams	Adult rams	SEM	P-value
Epithelium thickness	28.21[c]	47.36[b]	57.18[a]	5.41	0.011

[1] Lamb (<1 month of age); early pubertal rams (9-12 months of age); adult rams (3-4 years of age).
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

**Table 5** Prostate gland characteristics (μm) at different ages in Arabi rams

Groups	Age	Epithelium thickness	Lumen diameter	Secretory vesicle diameter
Lambs	(<1 month)	7.89[c]	14.02	22.52[c]
Early puberty rams	(9-12 months)	11.30[b]	14.71	26.78[b]
Adult rams	(3-4 years)	12.89[a]	15.36	30.94[a]
SEM	-	0.94	0.31	1.55
P-value	-	0.0001	0.25	0.002

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Early pubertal development associated with increased body weight is desirable for improved reproductive performance (Emsen, 2005; Elmaz *et al.* 2008). Alternatively, puberty is determined by testicular growth, separation of the penis from the prepuce, and androgenic hormone concentrations (Moulla *et al.* 2018). Threshold concentrations of testosterone are required for the acquisition and expression of adult sexual behavior. Sex differences in adult reproductive behavior and hormonal responsiveness are the result of permanent organizing effects of testosterone and its metabolites on brain development (Perkins and Roselli, 2007). The results of the present study show that although the rams reached sexual maturity, the levels of 5α-reductase, testosterone, and DHT increased to some extent.
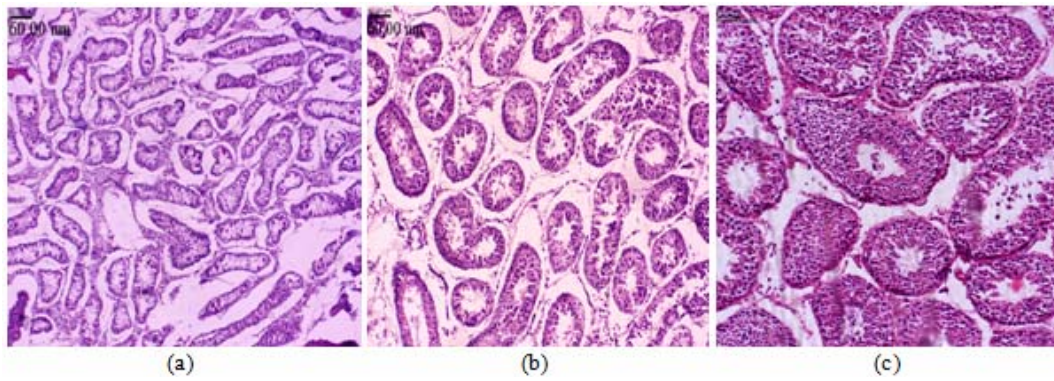
Figure 1 (a) Lower density of seminiferous tubules and high ratio of interstitial connective tissue to seminiferous tubules in the testes of lambs; (b) increase in density and diameter of testicular seminiferous tubules in early pubertal rams compared to lambs; (c) increase in density, diameter and germinal epithelial thickness of seminiferous tubules and decrease in amount of interstitial connective tissue in adult rams compared to lambs and early pubertal rams (H & E staining, X 100)
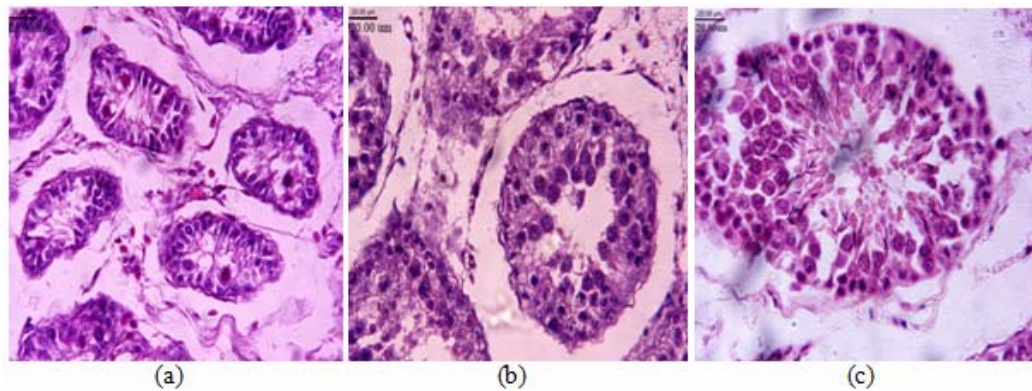

Figure 2 Testis structure of lambs (A), early pubertal rams (B) and adult rams (C); Diameter of the seminiferous tubules, the thickness of the germinal epithelium and the wave of spermatogenesis in rams are greater and more complete than in early pubertal rams and lambs (H & E staining, X 100)
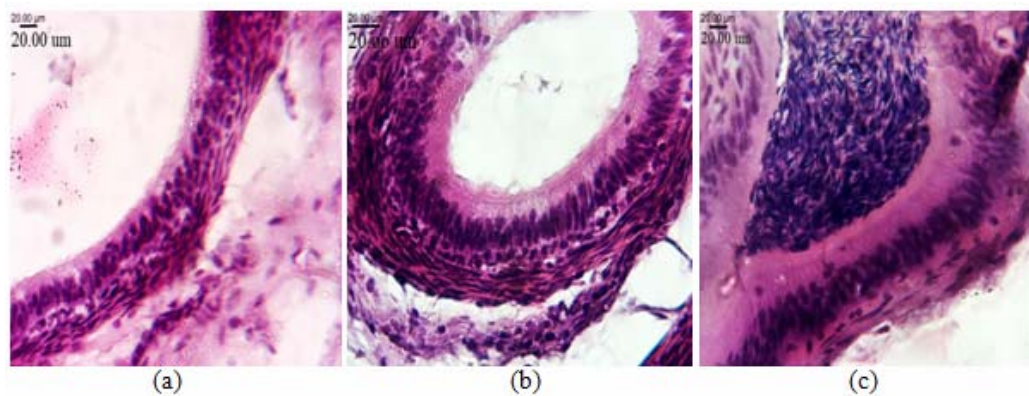

Figure 3 Epididymal tissue in lambs (A), early pubertal rams (B) and adult rams (C). Note the increase in epithelial thickness as the animal ages (H & E staining, X 400)

The concentration of these enzymes and hormones reaches its maximum value only after the animal has reached its full sexual and physical maturity. The findings show that the DHT deficiency caused by finasteride, which altered the expression of 5α-reductase in the epididymis, may have destabilized the function of this organ (Kolasa, 2006). In one study, the mean diameter of the seminiferous tubules and the number of Leydig cells in one year old (early puberty) native Iraqi rams were 264 μm and 3.5, respectively (Al-Kelaby *et al.* 2017).
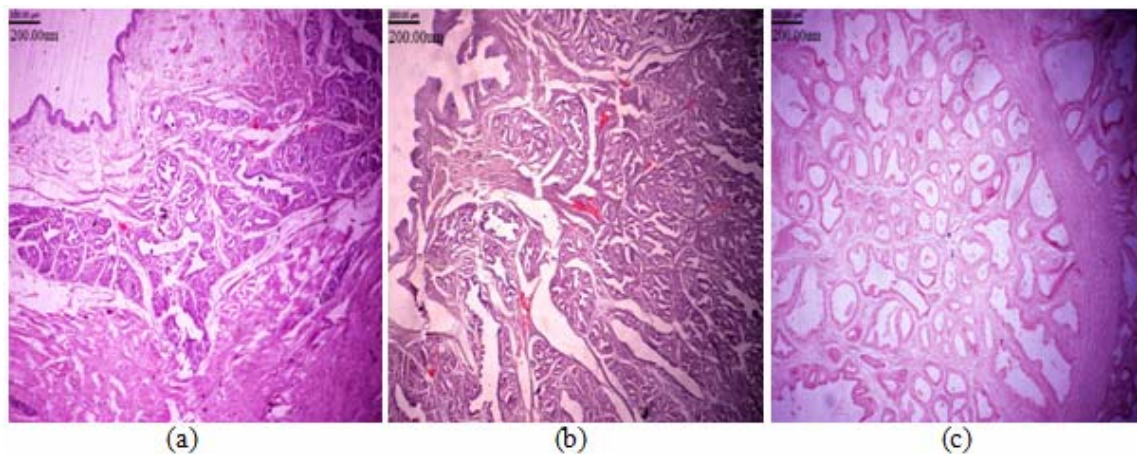
Figure 4 Prostate structure; low density of secretory units in lambs (A), increasing density of secretory units in early pubertal rams (B), high distribution of secretory units with increase in diameter in adult rams (C) (H & E staining, X 40)
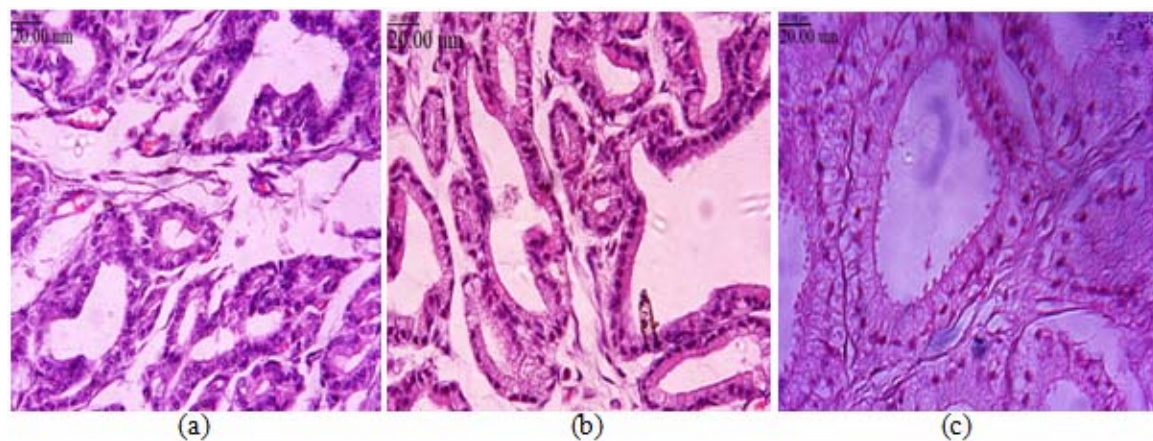
Figure 5 Prostate tissue in lambs (A), rams in early puberty (B) and adult rams (C). The diameter of secretory units and epithelium height of secretory units, which indicates active secretory units, is evident in rams compared to early pubertal rams and lambs (H & E staining, X 100)

Table 6 The Pearson correlation co-efficient (r) for serum 5αR2 and androgenic hormones with testicular histologic parameters in Arabi rams

Parameter	5αR2	Testosterone	DHT
Seminiferous tubule diameter	0.35	0.68	0.67
Seminiferous lumen diameter	0.30	0.62	0.63
Leydig cell No.	0.38	0.77	0.78
Germinal epithelium thickness	0.54	0.81*	0.80*

5αR2: 5α-reductase type 2 enzyme and DHT: dihydrotestosterone.
* (P<0.05).

Table 7 The Pearson correlation co-efficient (r) for serum 5αR2[1] and androgenic hormones with prostate histologic parameters in Arabi rams

Parameter	5αR2	Testosterone	DHT
Epithelium thickness	0.27	0.59	0.60
Secretory vesicle diameter	0.43	0.72	0.71

5αR2: 5α-reductase type 2 enzyme and DHT: dihydrotestosterone.

In the present study, seminiferous tubule diameter and Leydig cell count in Arabi rams were 136μm and 6.27, respectively. In another report, the mean Sertoli cell population in 4-month-old crossbred Santa Inês lambs was 5.27 (Rocha *et al.* 2022), which was lower than that observed in the Arabi lambs (11) of the present study. Some studies in men have reported normal testicular histology in patients with 5α-reductase deficiency during infancy and prepubertal ages; however, almost all patients suffer from impaired spermatogenesis during the adult period (Wada *et al.* 2022). In our study, the higher concentration of 5αR2 in adult rams than in lambs was associated with an increase in the testicular cell population and therefore higher spermatogenesis. Lack of 5 alpha-reductase activity may inhibit the development of spermatogonia into spermatocytes regardless of the location of the testis (Hadziselimovic and Dessouky, 2008). In one research, the interstitial tissue was more predominant than the seminiferous tubules in the testis of sheep of the newborn period, whereas the seminiferous tubules grow in the subsequent age periods which leads to a decrease in the content of interstitial tissue. Leydig cells were found in three age groups studied. These findings are similar to our results (Khasaev *et al.* 2018). In the 5α-R2 deficiency, histological evaluation revealed heterogeneous seminiferous tubules and a thickened basement membrane. The majority of these seminiferous tubules displayed central lumina and normal diameters. They contained mature Sertoli cells, with limited borders and visible nucleoli, or involuting Sertoli cells, with lobulated shapes, irregular borders, and inconspicuous nucleoli (Vija *et al.* 2014).

The microscopic studies revealed the sequential changes in the diameter of the seminiferous tubules and also in the seminiferous epithelium at different ages during the postnatal development of the indigenous sheep testis. The mean diameter of seminiferous tubules of an early pubertal ram was 176.22 μm (Sadi and Gofur, 2022), which was higher than that observed in Arabi rams (136.38 μm).

Low serum testosterone concentrations and high androstenedione concentrations are responsible for the initial slow phase of testicular growth and vice versa for the later rapid phase of testicular growth in animals (Chacur *et al.* 2018). Our results showed a significant positive correlation between testosterone and dihydrotestosterone concentrations with germinal epithelium thickness in the testes. Androgens play an important role in the organization, development, and function of many reproductive tissues and other biological processes. Testosterone is directly involved in the development and differentiation of Wolffian duct-derived structures such as epididymides. The impact of testosterone and DHT differed for some measures. These differences may relate to the degree of androgen exposure (DHT is a more potent androgen) or the contribution from estradiol derived from the aromatization of testosterone (Bormann *et al.* 2011). In Ghezel rams, plasma testosterone levels were significantly positively correlated with the number of Leydig cells, spermatocytes, spermatogonia, and seminiferous tubule and lumen diameters (Nazari-Zenouz *et al.* 2016). Histological analysis of testes from rats treated with testosterone showed the non-intact arrangements of seminiferous tubules and the development of a wide space between the tubules, beside the shape of the tubules changed to become an oval-like shape. These changes in the tubular shape result in an elongation of the tubules thus increasing the mean diameter of seminiferous tubules in comparison with those of the control group (Mutalip *et al.* 2013). In guinea fowls, a significant positive correlation was observed between plasma testosterone concentrations and Sertoli and germ cell populations, seminiferous tubular diameter, and seminiferous tubular length (Abdul-Rahman *et al.* 2017).

In the present study, the Sertoli cell population of Arabi lambs was maximum at one month of age, whereas in the Ghezel rams, Sertoli cell proliferation occurred up to 3 months of age, and a proliferative phase was found in the number of Sertoli cells during the 3rd to 5th month. After 5 months of age, no significant changes in the number of Sertoli cells were observed (Nazari-Zenouz *et al.* 2016). Postnatal testicular growth results from increases in both seminiferous tubule length and intertubular tissue (Hochereau-de Reviers *et al.* 1995), which somewhat is related to the effects of the environmental conditions such as nutrition and breed effect (Belkhiri *et al.* 2017).

At 2 to 4 months of age, spermatogonia and Sertoli cells were present in the testes of Najdi lambs, but there was no cell division within the seminiferous tubules. At five months of age, only a few spermatocytes and no spermatids or spermatozoa were present. In the sixth month of age, no spermatozoa or spermatids were found, but several spermatocytes were found. In the seventh month of age, no spermatozoa but many spermatids were present. In the eighth month of age, many spermatozoa were present, but the germinal epithelium was disorganized with marked sloughing or obliteration of the lumen. In the ninth month of age, complete spermatogenesis was observed with the presence of many spermatozoa (Al-Kawmani *et al.* 2014). In our results, there were no spermatocytes and spermatid cells in the seminiferous tubules of lambs. In early pubertal rams (9-12 months of age), spermatocytes and spermatid cells increased and reached a maximum in physically mature rams (3-4 years of age).

The appearance of the seminiferous tubule lumen is a consequence of the secretion and accumulation of tubular fluid produced by Sertoli cells. The presence of this lumen indicates that the process of development and differentiation of the seminiferous epithelium is functional

(Boukenaoui *et al.* 2012). In our study, the lumen diameter of the seminiferous tubules increased with increasing age of the animal and reached the maximum in fully mature rams at 3-4 years of age compared to lambs and early pubertal rams. Therefore, rams that have recently reached sexual maturity have not reached their maximum reproductive capacity, and only after physical maturity, is the highest reproductive efficiency achieved.

In Awassi ram lambs, there was a gradual and linear increase in testicular volume from 3 to 17 months of age, with the highest increase in testicular morphometric parameters occurring between 7 and 10 months of age (Salhaba *et al.* 2001). Based on the histological findings in the present study, seminiferous tubules of Arabi rams are not completely mature during the early puberty period in 9-12 years old. With increasing age and body weight of rams in 3-4 years old, the highest seminiferous tubule diameter and germinal epithelium thickness of testis and epithelium thickness of epididymis, as well as highest epithelium thickness and secretory vesicle diameter of prostate gland were observed. Overall, the present study in Arabi rams showed that the blood serum concentrations of testosterone and DHT had a positive correlation with the thickness of germinal epithelium in the testis. In the early sexual maturity of rams, the histological characteristics of the testis, epididymis, and prostate do not reach their full development and function. After increasing the age and size of the ram and reaching physical maturity, the structures of the reproductive system reach their maximum efficiency. Therefore, histological evaluation of testes, epididymis, and prostate may be a more accurate indicator to determine the reproductive potential of rams.

CONCLUSION

In general, according to the hormonal, enzymatic, and histological results of the present study, when the ram reaches early puberty and becomes sexually active, its reproductive capacity is still incomplete and it can probably fertilize a limited number of ewes during mating. It is only after reaching physical maturity and completing the growth of the animal that reproductive performance reaches its maximum capacity and is expected to fertilize more ewes.

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