Macroanatomy, Histomorphometry, and Androgen Receptor Expression in the Epididymis of Kacang Goats Aged 4, 8, and 12 Months

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Abstract

The epididymis is a crucial component of the goat's reproductive structure. The epididymis is responsible for the transportation, concentration, and maturation of sperm. This study aimed to examine the differences in the structure of macro and microanatomy, tissue composition, and the existence of androgen receptors in the epididymis of Kacang goats aged 4, 8, and 12 months. The assessment of macroscopic organ growth was done immediately after sampling, whereas microscopic measurements were carried out following histological preparations using hematoxylin-eosin (HE) and immunohistochemical (IHC) procedures. The results on the macroscopic anatomy of the epididymis indicated a significant association between age with the width of the caput dexter and sinister and the circumference of the right cauda. However, no significant relationship was found between age with the corpus length and the left cauda's circumference. Significant variations were observed in the diameter and concentration of the agglutinated spermatozoa in the lumen during histomorphometry of the epididymis in three age groups of Kacang goats. There were no statistically significant variations in the expression of androgen receptors among the three age groups. This study showed that the correlation coefficient test reveals a positive relationship between age and the caput width and corpus length dimensions, indicating that these measurements tend to grow as age increases. On the other hand, the diameter of the agglutinated spermatozoa in the epididymal lumen exhibits significant variations between the ages of 4 months, with the ages of 8 months and 12 months, suggesting that the sperm becomes fully matured by the age of 8 months.

Keywords: androgen receptor, *Capra aegragus hircus*, epididymis, histomorphometry, Kacang goat

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INTRODUCTION

Kacang goat or *Capra aegagrus hircus* is an indigenous Indonesian goat breed known for its compact physique, low maintenance requirements, and vital capacity to thrive in the native environment. Furthermore, this breed has exceptional reproductive capabilities. These goats are highly sought after for breeding due to their moderate body size, low maintenance requirements, rapid breeding and high fertility rates, short birth intervals, and fast development (Tmaneak *et al*., 2016).

The development of the reproductive organs varies among individual goats. The growth is determined by genetic and non-genetic factors, often known as environmental factors (Bahari *et al*., 2023). Genetic factors in goats impact the growth and development of organs in both males and females. An intriguing area of study involves the maturation of the reproductive organs in male Kacang goats. This study was particularly significant as it can shed light on the development of male goat reproductive organs across different age groups. Ultimately, this knowledge can enhance goat population growth and facilitate strategic livestock breeding. Male Kacang goats may be categorized into several age groups. The first group consists of goats aged 4–6 months, during which they reach sexual maturity. The second group includes goats aged 6–12 months when they experience their first sexual desire. The third group comprises goats aged 10–18 months, who are suitable for mating and contributing to

the growth of the goat population (Kurniasih *et al*., 2013).

Male goats are typically favored for their higher reproductive output than female goats, prioritizing their production features. Understanding the reproductive process in goats, particularly male *C. aegagrus hircus*, is crucial for breeders since it directly impacts the advancement of animals. The male goat's reproductive system comprises the testes, retetestis, vas efferens, epididymis, vas deferens, and urethra (Lestari and Ismudiono, 2014).

The epididymis is a crucial reproductive organ in goats. The epididymal tract comprises elongated, coiled tubules on the testicles related to the efferent duct and ductus deferens. Anatomically, the epididymis is split into three parts: the caput (head), corpus (body), and cauda (tail) (Hanifah *et al*., 2020). The epididymis is an organ responsible for the transportation, concentration, and maturation of sperm. This organ plays a crucial function in absorbing fluid from the seminiferous tubules of the testicles and directing spermatozoa to the ductus deferens (Purnama *et al*., 2019). It then combines with seminal plasma and is ejaculated into the female reproductive canal (Dalimunthe *et al*., 2017). The androgen receptor (AR) plays a crucial role in the epididymis of goats. The AR regulates the expression of genes involved in sperm maturation and the development of the epididymal epithelium. In epididymis, the AR helps maintain the integrity of the epithelial cells and regulates the expression of genes involved in the maturation of spermatozoa (Yendraliza *et al*., 2023). The AR also helps to regulate the synthesis of androgens, such as testosterone, which are essential for the final stages of spermatogenesis and the development of reproductive organs. The AR is a key factor in developing and maintaining the epididymis, which is critical for the proper functioning of the male reproductive system (Goyal *et al*., 1997; Hejmej *et al*., 2006; Mansour *et al*., 2021).

This study aimed to investigate the development of the epididymis, part of the Kacang goat's reproductive organs, in three different age groups (4, 8, and 12 months). The study provides a nuanced and age-specific examination of macroanatomy, histomorphometry, and androgen receptor expression in the epididymis. This temporal study enhances our comprehension of how these characteristics fluctuate at crucial developmental phases in Kacang goats. The findings from this study can inform breeding strategies by offering insights into the ideal age at which male goats attain reproductive maturity. This can have practical implications for breeding efforts to enhance overall herd fertility.

MATERIALS AND METHODS

Ethical Approval

The authors verify that the study protocol has received ethical approval from the "Animal Care and Use Committee" of Brawijaya University, with the ethical file clearance number 002-KEP-UB-2022.

Study Period and Location

The investigation was conducted from October 2021 to February 2022. The study was conducted at the Sumbersekar Field Laboratory, a Faculty of Animal Husbandry, Brawijaya University. Histology staining was performed using hematoxylin-eosin (HE) at the Pathology Anatomy Laboratory, Faculty of Veterinary Medicine, Brawijaya University.

Experimental Design

This study used a sample of 15 male Kacang goats divided into three groups, i.e., a group of 4-month goats, 8-month goats, and 12-month goats. Each group has five goats. The goats were bought from civil farms in Tangerang.

Histopathological Evaluation of Epididymis

The goats used have adapted a week under procedure before being slaughtered. The Kacang goat’s specimens were slaughtered by severing the neck at the jugular vein, esophagus, and trachea (Nielsen *et al*., 2020). Following the slaughter, the organs were harvested and subjected to macroscopic measurements, including the caput width, corpus length, and

cauda circumferences of the epididymis. The process involves utilizing a thread to measure the width, length, and circumference, which are subsequently recorded using a ruler (v-tech®) to provide precise measurements. The method used to analyze the correlation between age groups with epididymal macroscopy measurements, which consist of caput width, corpus length, and cauda circumferences in Kacang goats, is the Pearson correlation coefficient (Tella *et al*., 2023).

The epididymis samples were preserved in 10% paraformaldehyde and then dehydrated using various alcohol concentrations. They were then clarified using three rounds of xylene, infiltrated with paraffin wax, sectioned, and then de-waxed and stained with Haematoxylin and Eosin (Raji *et al*., 2012). Histomorphometry from HE staining measured the diameter of agglutinated spermatozoa (Fitria *et al*., 2018) and the concentration of sperm cells (Noviana *et al*., 2000) in the lumen of the epididymis. The histology images were seen using a trinocular Nicon Eclipse CiL Plus light microscope with a camera set DS-Fi3 type (Nikon Corp, Tokyo, Japan) at a magnification of 100×. The observations and measurements were conducted using Image J software. The measurement was conducted in five fields of view, and the average was analyzed.

An immunohistochemical examination was conducted on tissue slices that were fixed in 10% formalin, embedded in paraffin, and had a thickness of 5 µm. Immunohistochemical staining techniques targeting AR (polyclonal antibody, BIOSS®, bs-0118R, with concentration 1 µg/µL) were carried out manually utilizing a horseradish peroxidase-labeled streptavidin-biotin detection system (Thermo Scientific, TP-060-HL). The Ultraview Universal DAB Detection and the Rabbit Monoclonal Antibody were used (Kuyucu *et al*., 2021). A brown cellular appearance distinguishes a positive reaction for the androgen receptor, whereas a lighter color indicates a negative outcome. The immunoreactivity levels were classified using a scoring method known as the Immunoreactive Score (IRS). The score range is from 0 to 12. It describes the reaction's color intensity from negative to positive reaction. A score of 0–1 indicates negative reactions, 2–3 indicates faint positive reactions, 4–8 indicates moderate positive reactions, and 9–12 indicates strong positive reactions (Fedchenko and Reifenrath, 2014). The slices were examined using a Nikon Eclipse Ci-L Plus trinocular light microscope with a camera set type DS-Fi3 (Nikon Corp, Tokyo, Japan) and captured in photographs. Direct observations did the scoring.

Statistical Analysis

The SPSS for Windows tool was used to evaluate data obtained from measurements of the epididymis. The Spearman correlation test was used for this analysis. The histomorphometry data from HE staining was analyzed using One-way ANOVA, followed by the Tukey HSD test. The nonparametric Kruskal-Wallis test was used to evaluate the expression of the androgen receptor.

RESULTS AND DISCUSSION

Gross Morphometry of Epididymis

The study found that the gross anatomical epididymis of Kacang goats at 4, 8, and 12 months consists of a convoluted, long, and finely structured tube with a lobulated shape. This tube is attached to the testis at the cranial end through the rete testis, while the caudal end is connected to the vas deferens, which is divided into the head, body, and tail. The body is positioned adjacent to the lateral aspect of the connected border of the testicle, and it is in a regular and restricted state. The tail was elongated and directly connected to the lower end of the testicle. This was in line with Al-Sadoon *et al*., (2019) on Arrabi and Awassi Sheep, Khan *et al*., (2019) on Teddy Goats, and Abdullahi Mahmud *et al*., (2015) on ruminants.

Table 1 presents the macroscopic anatomy of the epididymis of Kacang goats. The correlation of morphometry of caput width, corpus length, and cauda circumferences of the epididymis was then analyzed with age. It is evident from the data that the correlation coefficient indicates a positive number, indicating that the two variables have a link in the same direction. These findings are consistent with Al-Sadoon *et al*., (2019), who

showed that anatomical measurements of epididymis significantly increased every month as the goats aged. However, from the table, the significance value is obtained in several variables. The significance value is less than 0.05, meaning the null hypothesis (H0) is rejected, and the alternative hypothesis (H1) is accepted. Depending on the separation in prepubertal and pubertal stages, 4 months is in the prepubertal stage, and 8 and 12 months are in the pubertal stage. Khan *et al*., (2019) found in male Teddy goats that on the overall structure of the epididymis revealed a noteworthy correlation between age and the width of the caput (right and left) and the circumference of the right cauda, the average weight of the right and left epididymis and the length and width of the right and left corpus-epididymis were substantially higher during the pubertal stage compared to the pre-pubertal period. Puberty and age considerably impact the gross anatomical characteristics of the epididymis in male Teddy goats. The result showed that in the circumference of the left cauda, there were no significant differences. Pathak *et al*. (2014) found that the length, width, thickness, and diameter of the cauda epididymis in three age groups of Gaddi goats were slightly different between the left and right but statistically were not significant. Nevertheless, Table 1 showed no correlation between age and the length of the corpus and the circumference of the left cauda. It is the same as the result from Al-Sadoon *et al*., (2019), in which no significant differences were observed in all parts of the epididymis on Arrabi and Awassi Sheep.

Table 1. The macroscopic correlation analysis of the epididymis of Kacang goats

Correlation	Sig.	Correlation coefficient
Age and dexter caput width	0.010	0.640
Age and dexter corpus length	0.251	0.316
Age and dexter cauda circumferences	0.014	0.618
Age and sinister caput width	0.030	0.559
Age and sinister caput length	0.392	0.239
Age and sinister cauda circumferences	0.075	0.472

Table 2. Histomorphometry measurements on the right cauda epididymis

Parameters	4 months	8 months	12 months
The agglutinated spermatozoa diameter $\bar{x} \pm SD$ (μm)	25.92 ± 10.33^{a}	49.61 ± 16.90^{b}	61.87 ± 13.89^{b}
The agglutinated spermatozoa concentration	-/+	+/++	++

Different superscripts in the same row showed significant differences ($p < 0.05$). (-) no agglutinated spermatozoa, (+) few agglutinated spermatozoa, (++) many agglutinated spermatozoa.

Table 3. The scoring results with immunoreactive score (IRS) from immunohistochemical staining on the cauda epididymis

IRS results	Age of Kacang Goats		
	4 months	8 months	12 months
$\bar{x} \pm SD$	10 ± 0.57	11 ± 1.00	11 ± 1.15

Histomorphometry of Epididymis

The anatomy of the epididymis illustrated in this study was a convoluted epithelial tube and well-structured entity consisting of head, body, and tail segments. During the pre-pubertal stage in Teddy goats, the epididymal duct was covered by non-ciliated epithelium, but it developed cilia during the pubertal stage (Khan *et al*., 2019). This contrasts with what was found in this study in Kacang goats, which were in the prepubertal phase and had a non-ciliated epithelium (Figure 1). The epithelium lining the epididymal duct comprises pseudostratified columnar cells illustrated in Figure 2. The height of the epithelium diminishes towards the distal end, and the epithelium is enveloped by smooth muscle, which exhibits a noticeable increase in thickness towards the tail. The duct wall is joined by loose

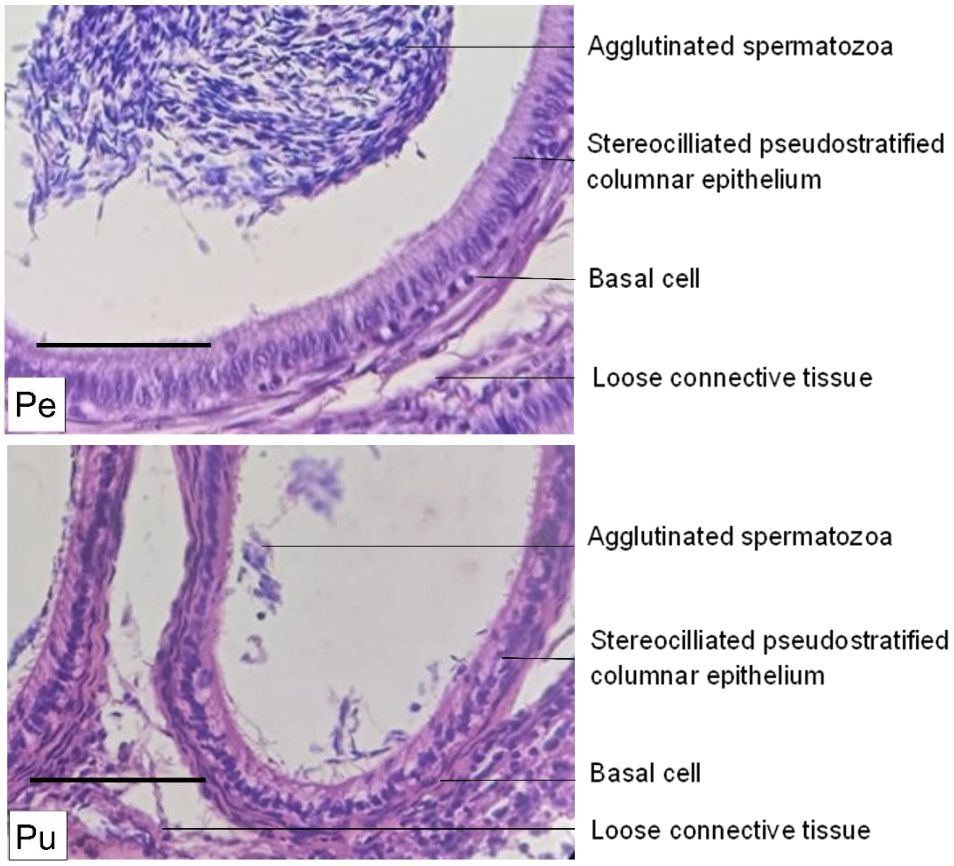

Figure 1. Histhological right ductus of the cauda epididymis in Kacang goat. Cauda epididymis in prepubertal phase (Pe) and corpus epididymis in pubertal phase (Pu). H&E 400×. — bar 100 µm.

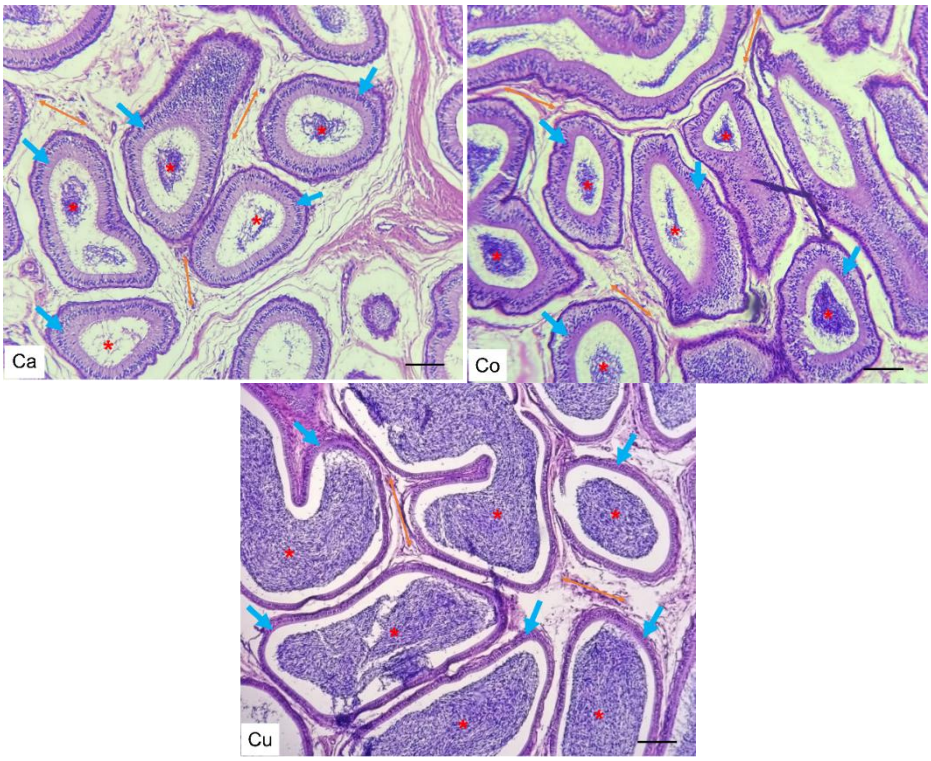

Figure 2. Histological of the caput (Ca), corpus (Co), and cauda (Cu) epididymis in Kacang goats. (*) lumen epididymis with agglutinated spermatozoa, (➔) ephitelium, (↔) loose connective tissue. H&E 100×. — bar 100 µm.

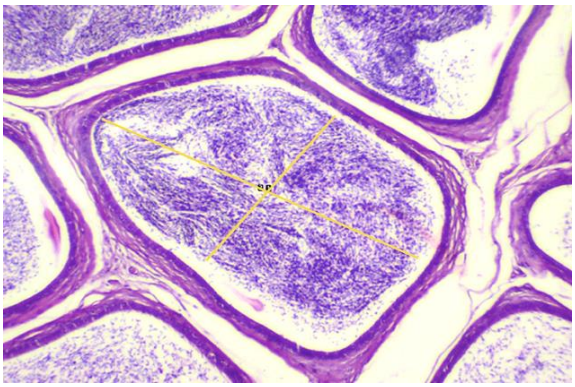

Figure 3. Histomorphometry measurements of the epididymis and diameter of the agglutinated spermatozoa using ImageJ.

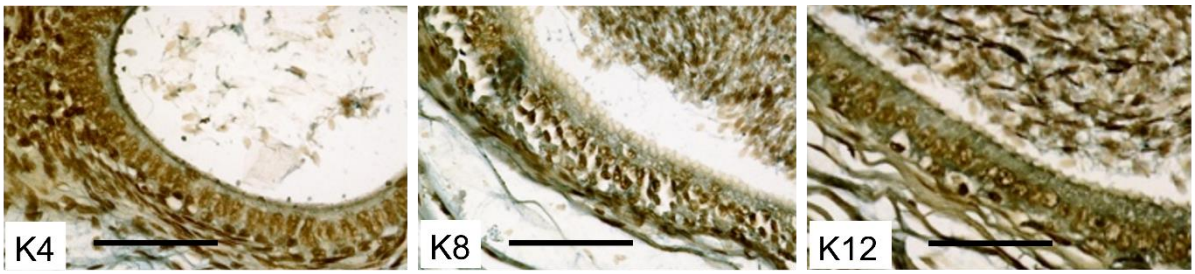

Figure 4. Immunohistochemical staining results in the epididymis of Kacang goats. (K4) 4 months old, (K8) 8 months old, and (K12) 12 months old. — bar 100 µm.

connective tissue, which contains macrophages, leukocytes, and many blood vessels and neurons. The density of capillary bundles significantly rises in the tail. From this study, we descriptively found that the epididymal lumen increased from 4 months to 12 months (Figure 2). It was in line with Khan *et al*. (2019) found in Teddy goats, in which the diameter of the epididymal tubules progressively grew from the start of the head to the end of the tail in both groups, prepubertal and pubertal.

This study used male Kacang goats from three different age groups: 4, 8, and 12 months. The wider diameter of the lumen and agglutinated spermatozoa were found in the cauda epididymis in three age groups, as shown in Figure 3. Noviana *et al*. (2000) found that the epididymis cauda had the largest diameter among the collected spermatozoa. Due to its larger storage capacity, the cauda region is the primary reservoir for spermatozoa within the epididymis. Around 75% of epididymis spermatozoa are stored in the cauda epididymis region (Andaruisworo *et al*., 2023). The expansive lumen of the cauda epididymis is characterized by a much larger diameter of the spermatozoa collection compared to the head and corpus regions (Wijayanti *et al*., 2023). The circumference of the right cauda is substantially higher during the pubertal stage compared to the pre-pubertal period. It is the background of why the histomorphometric measurements were conducted on histological preparations of the right cauda epididymis organ of the goats. The histomorphometry criteria assessed the agglutinated spermatozoa diameter and concentration inside the epididymal lumen.

Table 2 shows the average diameter of agglutinated spermatozoa in the epididymal lumens of three age groups of Kacang goats. The average diameter of agglutinated spermatozoa in the 12-month age group is 61.87 µm; in the 8-month age group, it is 49.61 µm; and in the 4-month age group, it is 25.92 µm. The one-way analysis of variance (ANOVA) indicated a statistically significant disparity ($p < 0.05$) in the diameter of the agglutinated spermatozoa. Subsequently, the Tukey HSD test confirmed a significant difference between the average diameter of the agglutinated spermatozoa in the 4-month age group compared to the eight and 12-month age groups. The diameter of agglutinated spermatozoa can be affected by different factors.

The variations in these parameters observed in different age groups (4, 8, and 12 months) can be attributed to the epididymis' natural developmental processes and spermatozoa's maturation. The epididymis is responsible for sperm maturation, storage, and transportation (Rodriguez-Martinez *et al*., 2022; Prayogo *et al*., 2022). As goats progress through different age groups, the epididymis undergoes functional changes that can affect the diameter of the ductus epididymis. The dimensions of the epithelium, including its tubular diameter and height, increased from birth to late prepuberty (Archana *et al*., 2011). In a previous study, the prepubertal period (4 months) and pubertal period (8 and 12 months) have significantly different luminal diameters of the epididymis (Khan *et al*., 2019).

The difference found in agglutinated spermatozoa between prepubertal (4 months) and pubertal (8 and 12 months) appears in Table 2. The sperm contents were observed on the periphery and increased throughout the lumen of tubules of the body region of epididymis (Khan *et al*., 2019). This situation happened because the prepubertal stage (4 months) still has an incomplete phase to produce spermatozoa, resulting in some of the epididymal lumens lacking or containing just a tiny number of spermatozoa. In the early pubertal stage (8 months), spermatogenesis does not happen in the optimum process, resulting in some spermatozoa in the lumen of the epididymis (Saputro *et al*., 2022). In Bengal goat kids, immature sperm cells were observed in individuals as young as 4.5 months old, while viable sperm cells were found in the cauda epididymis of castrated kids. However, semen ejaculates with adequate numbers of mature and motile sperm cells could only be collected from individuals aged six months and older (Wajdi *et al*., 2021). In addition, the best age for semen ejaculate characteristics was shown to be between 11 and 12 months (Rava *et al*., 2023).

Androgen Receptors Expression of Epididymis

Distinct variations in androgen receptor expression were detected across three different age groups labeled with immunohistochemical stains. Picture from immunohistochemical stains were analyzed, and variations were assessed using an IRS scoring method (Fedchenko and Reifenrath, 2014).

The average scoring of AR using the Immunoreactive Score (IRS) in the cauda epididymis of three age groups of Kacang goats is presented in Figure 4 and Table 3. The data showed that there are no significant differences between groups of age. Perobelli *et al*. (2013) also discovered that androgen deprivation from pre-puberty up to peripuberty did not affect the histological structure of the epididymis either at puberty or at maturity. The critical factor controlling epididymal activity is androgens; however, the processes by which these hormones work are not entirely known (Zaya *et al*., 2012; Fiqih *et al*., 2021). The activities of androgens such as testosterone and dihydrotestosterone are mediated by the androgen receptor (AR), a ligand-dependent nuclear transcription factor and member of the steroid hormone nuclear receptor family (Davey and Grossmann, 2016). In in vitro studies, positive immunostaining for AR was detected in cultured cells derived from the initial segment/caput, corpus, and cauda from both prepubertal and adult rats (Zaya *et al*., 2012; Sangen *et al*., 2021). The expression of AR in both prepubertal and adult animals shows that androgens are crucial for epididymal development prior to the arrival of sperm and for normal sperm maturation; however, the relative relevance of the hormone to these processes remains unclear. This study also suggests a slight difference in the expression of AR at the epididymis because androgens are essential for both the development of the epididymis and maturation function.

CONCLUSION

Considering that these results might be attributed to the typical physiological fluctuations linked to growth and maturation is crucial. Additional investigation involving comprehensive molecular and cellular examinations may be required to reveal the precise processes responsible for these alterations

and their functional importance in the reproductive process of *C. aegagrus hircus*.

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AUTHORS' CONTRIBUTIONS

HP: conceptualization and drafted the manuscript. HP and DPP: performed statistical analysis and visualized figures and tables. DPP, WAS, and AF: treated the animal laboratory and project administration; SS: supervision and conceptualization. All authors have read, reviewed, and approved the final paper.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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