Age-Related Morphometric and Hormonal Changes in the Testes and Epididymis of Hamdani Sheep

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Abstract

This study aimed to evaluate age-related changes in testicular and epididymal morphometry alongside key reproductive and metabolic hormones in Hamdani sheep during the first year of life. Sixteen clinically healthy male lambs were allocated into four age groups (6, 8, 10, and 12 months). Testicular and epididymal measurements were recorded post-slaughter, and serum concentrations of testosterone, thyroid hormones (T3 and T4), calcium, and albumin were analyzed. Results revealed a significant ($P \leq 0.05$) progressive increase in testicular size, weight, and volume with advancing age, with peak values observed at 12 months. Epididymal development, including segmental weights and total length, followed a similar pattern, indicating functional maturation of sperm storage and transport systems. Testosterone concentrations increased significantly in older groups, coinciding with enhanced morphometric development. Thyroid hormones peaked at 10 months, suggesting increased metabolic activity during the peri-pubertal stage. Additionally, calcium and albumin levels showed age-related variations linked to metabolic and reproductive functions. In conclusion, reproductive development in Hamdani sheep is strongly age-dependent and closely associated with endocrine and metabolic changes. The period between 10 and 12 months represents a critical window for sexual maturation, highlighting its importance for breeding management strategies.

Keywords: morphometry, sexual maturity, testes, Iraqi sheep

Introduction:

The testis represents the primary male reproductive organ responsible for spermatogenesis and androgen synthesis, thus playing a pivotal role in determining reproductive efficiency in sheep. The structural development and functional maturation of the testes are governed by complex interactions between endocrine regulation and environmental influences, particularly during the critical transition to puberty [1,2].

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Testicular growth and epididymal development are influenced by a combination of intrinsic and extrinsic factors, including age, genetic background, nutritional status, seasonality, and management practices. Among these, age is considered the most decisive factor affecting reproductive maturation, as it directly regulates both morphometric development and endocrine activity [3]. Furthermore, environmental cues such as photoperiod and nutrition play a crucial role in modulating the hypothalamic–pituitary–gonadal (HPG) axis, thereby affecting testosterone secretion and spermatogenic processes [4].

Several studies have demonstrated that testicular dimensions and circulating hormone concentrations increase progressively with age, particularly during the pre-pubertal and pubertal stages [5]. In addition, significant breed-related variations in morphometric and hormonal characteristics have been reported, reflecting underlying genetic differences in reproductive potential [6]. However, most available studies have focused on non-local breeds or broad age intervals, which limits their applicability to indigenous sheep populations.

In Iraq, sheep production represents a vital component of the livestock sector, contributing significantly to meat production and rural livelihoods. Despite its importance, there is a noticeable lack of recent, region-specific data addressing the relationship between testicular morphometry and endocrine changes during early sexual development. This knowledge gap is particularly evident within the critical growth period between 6 and 12 months of age, which corresponds to the transition toward sexual maturity.

Therefore, the present study was designed to provide an integrated morphometric and hormonal evaluation of testicular and epididymal development in Hamdani sheep under the environmental conditions of Diyala Governorate. The outcomes of this study are expected to enhance the understanding of reproductive physiology and support the development of scientifically based breeding and management strategies.

Materials and Methods

Study Area and Experimental Design

This study was conducted between November 2024 and March 2025 at the Animal Physiology Laboratory, College of Agriculture, University of Diyala, in collaboration

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with a certified private diagnostic laboratory. The experiment followed a descriptive and analytical design to evaluate age-related changes in morphometric and hormonal characteristics of the testes and epididymis in Hamdani sheep.

A total of sixteen clinically healthy male Hamdani sheep were included in the study. Animals were categorized into four age groups representing key stages of sexual development: 6, 8, 10, and 12 months (n = 4 per group). Sample size was determined based on animal availability and the need to maintain homogeneity within groups.

Sample Collection

Testicular samples (right and left) were collected immediately post-slaughter from a local abattoir. After removal, samples were carefully cleaned with running water to remove residual blood and connective tissue. The epididymis was then separated from the testis using sterile surgical instruments for independent measurements.

Blood samples (4 mL) were collected from the jugular vein prior to slaughter using sterile syringes. Samples were transferred into plain tubes and centrifuged at 3000 rpm for 15 minutes to obtain serum. The serum was stored at −20°C until biochemical analysis.

Morphometric Measurements

Morphometric parameters of the testes and epididymis were recorded using standardized procedures. Testicular measurements included:

Testis weight (with and without epididymis), Testis length and width, Testis size (measured by water displacement method)

Epididymal measurements included:

Total epididymis weight and length. Weight and length of epididymal head, body, and tail

Digital calipers were used for linear measurements, while a precision balance was used for weight determination. Testicular size was estimated using the water displacement method as described by Attar and Feed [7].

Biochemical Analysis

Serum samples were analyzed to determine concentrations of key reproductive and metabolic indicators, including:

Testosterone Triiodothyronine (T3) Thyroxine (T4) Calcium Albumin

Analyses were conducted using automated biochemical analyzers with commercially available diagnostic kits (Spinreact, Spain), following the manufacturer’s protocols.

Statistical Analysis

Data were analyzed using a Completely Randomized Design

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(CRD). One-way analysis of variance (ANOVA) was performed to evaluate the effect of age on the studied parameters. Differences among means were assessed using Duncan's Multiple Range Test at a significance level of $P \leq 0.05$.

Yijk = M + Bi + eij

YiJk = the viewing value k due to the influence of factors i and J.

M = overall average.

Bj = the effect of age since j =4.

Eij = the effect of a random error that is normally distributed with a mean equal to zero and a variance of se.

Results and Discussion

Age exerted a significant effect ($P \leq 0.05$) on all reproductive, anatomical, and hormonal parameters measured in local male sheep. Testicular dimensions (length, width, and size) increased progressively with age table (1), reaching peak values in T4 (12 months), followed by T3 (10 months), while T1 (6 months) showed the lowest values. This trend reflects the normal physiological progression of testicular development associated with puberty and sexual maturation.

Table 1. Age-related changes during the first year of life in testicular morpho metric traits (length, width, and size) of Hamdani sheep (Mean ± SE).

Group	Right testis			Left testis		
	testis length (g)	testis width (mm)	testis size (cm^3)	testis length (g)	testis width (mm)	testis size (cm^3)
T1	67.68 d 3.38±	33.49 d 1.20±	58.75 d 2.39±	64.94 b 3.02±	32.85 c 1.11±	56.75 d 2.25±
T2	91.65 c 2.20±	44.19 c 1.86±	93.00 c 3.13±	89.19 ab 1.45±	42.10 b 2.01±	89.00 c 2.27±
T3	102.9 b 2.80±	57.72 b 1.13±	133.75 b 4.26±	75.07 b 22.19±	54.29 a 1.05±	116.00 b 5.33±
T4	118.13 1.22±	72.48 a 1.15±	190.0 a 8.41±	113.9 a 2.13±	61.36 a 4.22±	117.50 a 5.20±

- T1: Animal at 6 months of age, T2: Animal at 8 months of age, T3: Animal at 8 months of age, T4: Animal at 12 months of age.
- Means within the same column bearing different superscript letters are signific antly different according to Duncan's Multiple Range Test at the 0.05 level of s ignificance.

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Similarly, testicular weight and epididymal weight increased significantly table (2) in advanced age groups (T3 and T4), indicating enhanced spermatogenic activity and tissue differentiation. The observed increase in epididymal segment weights (head, body, and tail) table (3) and total epididymal length table (4) further supports the functional maturation of sperm storage and transport systems. These findings are consistent with recent studies highlighting that reproductive organ growth in rams is strongly age-dependent and closely linked to endocrine regulation [8,9].

Table 2. Age-related variation during the first year of life in testicular and epi didymal weights (testis weight, testis weight excluding epididymis, and total ep ididymal weight) in Hamdani sheep (Mean ± SE).

	Right testis			Left testis		
Group	TWwE (g)	NTW (g)	NEW (g)	TWwE (g)	NTW (g)	NEW (g)
T1	52.13 d 4.23±	43.34 d 3.41±	8.78 d 0.82±	49.80 d 4.12±	41.66 d 3.10±	7.99 c 1.10±
T2	90.07 c 4.73±	75.84 c 3.56±	14.23 c 1.27±	87.21 c 3.75±	73..28 c 2.68±	13.93 b 1.18±
T3	131.84 a 9.33±	112.2 a 9.16±	19.74 a 1.04±	118.14 a 3.57±	96.07 a 4.17±	11.07 a 2.58±
T4	118.13 b 1.22±	72.48 b 1.15±	19.00 a 8.41±	113.94 b 2.13±	61.36 b 4.22±	17.50 a 5.20±

- T1: Animal at 6 months of age, T2: Animal at 8 months of age, T3: Animal at 8 months of age, T4: Animal at 12 months of age.
- TWwE: Testis Weight with Epididymis. NTW: Net Testis Weight. NEW: Net Epididymis Weight.
- Means within the same column bearing different superscript letters are signific antly different according to Duncan's Multiple Range Test at the 0.05 level of s ignificance.

The significant increase in testosterone concentration with advancing age, particularly in T3 and T4, aligns with the observed morphological development of the testes and epididymis. Testosterone plays a central role in stimulating spermatogenesis and the growth of accessory reproductive organs, which explains the parallel improvement in

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anatomical traits. Similar relationships between testosterone levels and testicular development have been reported by [10,11].

Table 3. Age-related variation during the first year of life in epididymal regional weights (head, body, and tail) in Hamdani sheep (Mean ± SE).

Group	Right testes			Left testes		
	Head Weight (g)	Body Weight (g)	Tail Weight (g)	Head Weight (g)	Body Weight (g)	Tail Weight (g)
T1	3.05 c 0.41±	1.54 b 0.23±	4.18 c 0.49±	3.35 b 0.49±	1.52 b 0.24±	3.34 c 0.77±
T2	6.08 b 0.92±	3.20 a 0.45±	4.95 c 0.62±	5.62 b 0.70±	3.58 ab 0.47±	4.72 bc 0.43±
T3	7.63 ab 0.82±	2.54 ab 0.14±	9.56 b 0.71±	10.54 a 2.00±	4.73 a 1.58±	6.72 b 1.08±
T4	9.79 a 0.64±	3.27 a 0.67±	13.25 a 0.82±	9.43 a 0.75±	3.38 ab 0.45±	12.21 a 1.18±

- T1: The animal at 6 months' old T2: The animal at 8 months' old T3: The ani mal at 8months' old T4: The animal at 12 months' old.
- Means within the same column bearing different superscript letters are signific antly different according to Duncan's Multiple Range Test at the 0.05 level of s ignificance.

Thyroid hormones (T3 and T4) showed peak levels in T3 table (5), suggesting elevated metabolic activity during the critical phase of sexual maturation. Thyroid hormones are known to influence testicular growth indirectly by regulating metabolism and energy balance, which are essential for reproductive efficiency [12,13].

Table 4. Age-related variation during the first year of life in epididymal length parameters (total length, head, body, and tail) in Hamdani sheep (Mean ± SE).

Group	Right testis				Left testis			
	Epi Length	Head Length	Body Length	Tail Length	Epi Length	Caput Length	Corpus	Cauda Length
T1	72.41 b 4.28±	30.81 d 2.01±	11.04 c 1.16±	30.55 c 1.56±	70.42 d 4.47±	29.65 d 1.74±	10.96 c 1.28±	26.80 c 2.68±
T2	75.02 b 24.87±	42.00 c 1.24±	16.24 bc 1.18±	36.07 c 7.19±	95.92 c 2.07±	40.16 c 0.66±	15.65 bc 1.41±	40.10b 2.71±
T3	129.25 a 6.98±	50.06 b 1.13±	34.59 ab 7.69±	44.60 b 2.08±	116.52 b 11.60±	49.33 b 1.52±	30.63 b 10.44±	36.56 bc 4.86±
T4	171.78 a 9.35±	58.36 a 1.66±	56.55 a 6.62±	56.86 a 2.43±	163.66 a 5.19±	55.71 a 0.83±	52.50 a 4.17±	54.79 a 1.89±

- T1: The animal at 6 months' old T2: The animal at 8 months' old T3: The ani mal at 8months' old T4: The animal at 12 months' old.
- Epi length: Length of the epididymis.
- Means within the same column bearing different superscript letters are signific antly different according to Duncan's Multiple Range Test at the 0.05 level of s ignificance.

The increase in calcium levels in older age groups may be associated with its physiological role in cellular signaling and sperm motility, while albumin variation likely reflects changes in metabolic and nutritional status. These findings agree with recent evidence emphasizing the importance of mineral balance and protein metabolism in reproductive performance [14,15].

Overall, the superiority of T3 and T4 groups across most traits indicates that reproductive maturity in local male sheep is achieved progressively with age and is strongly influenced by hormonal activity, metabolic status, and physiological development. Minor variations between studies may be attributed to differences in breed, fenvironmental conditions, feeding systems, and management practices.

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Table 5. Age-related variation during the first year of life in reproductive and biochemical parameters concentrations associated with sexual maturation in Hamdani sheep (Mean ± SE).

Group	Calcium	Albumin	T3	T4	Testosterone
T1	6.15 b 0.06±	2.45 b 0.06±	2.33 b 0.02±	71.63 c 0.56±	2.55 d 0.29±
T2	5.22 b 0.04±	2.70 a 0.04±	2.39 b 0.01±	72.23 c 0.12±	4.42 c 0.94±
T3	10.35 a 0.89±	2.47 b 0.10±	2.77 a 0.15±	163.20 a 4.39±	6.94 b 0.05±
T4	10.55 a 0.89±	2.77 a 0.04±	2.39 b 0.05±	119.89 b 1.34±	7.37 a 0.08±

- T1: The animal at 6 months' old T2: The animal at 8 months' old T3: The ani mal at 10 months' old T4: The animal at 12 months' old.
- Means within the same column bearing different superscript letters are signific antly different according to Duncan's Multiple Range Test at the 0.05 level of s ignificance.

Conclusion

The present study demonstrated that testicular and epididymal development in Hamdani sheep is strongly age-dependent during the first year of life. A progressive increase in morphometric traits was observed, with peak development at 12 months of age. This structural maturation was closely associated with elevated testosterone concentrations in older animals. Thyroid hormones and metabolic indicators also showed age-related variations, reflecting increased physiological activity during puberty.

The synchronization between endocrine changes and reproductive organ growth highlights the integrated nature of sexual development. Overall, the period between 10 and 12 months represents a critical stage for achieving sexual maturity in local Iraqi rams.

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