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Original Article

Evaluation of Testicular Biometry and Spermatozoa Recovered after Slaughter from Cauda Epididymal of Awassi Ram

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Abstract

Rams play an important role in reproductive efficiency because each ram or semen contains half of the genetic material of its descendants. In Iraq, the Awassi sheep are the most common indigenous breed, highly adaptable to tough environmental conditions. The present study was carried out to evaluate testicular biometry and spermatozoa recovered after the slaughter at different ages in Awassi rams. A total of thirty-three pairs (n=66 testes) of Awassi rams testicles were collected after the slaughter at the abattoir in Baghdad and divided into three groups according to age. Rams less than a year old were grouped as G1, rams aged one to two years were grouped as group G2, and rams older than two years as group G3. There were significant differences (P<0.05) in testicular weight, length, width, and diameter in both right and left increased steadily in group G1 to reach a maximum in group G3. The epididymal weight and length (right and left) were significant (P<0.05) in group G3. The spermatozoa concentration obtained from the left testicle increased significantly (P<0.05) in groups G2 and G3 compared to group G1, while the right spermatozoa concentration increased significantly (P<0.05) in group G3 when compared to group G1. In conclusion, it can be concluded that the biometry of testicular and epididymal (right and left) in this study was influenced by progressing age, and the spermatozoa concentration obtained from the left testicle was higher active than the right one in adult Awassi rams.

Keywords: Awassi Rams, Biometry, Testicular, Epididymal, Spermatozoa Characteristics

1. Introduction

Rams play an important role in reproductive efficiency because each ram or semen contains half of the genetic material of its descendants (1). In Iraq, the Awassi sheep are the most common indigenous breed (2) and are highly adaptable to tough environmental conditions (3-5). It is well established that the Awassi sheep are able to produce milk and lambs under harsh environmental conditions (6, 7). The puberty in Awassi ram lambs is determined by age and the testosterone hormone level produced by the testicles (8); in fact, the Awassi ram lambs reached sexual maturity at 7 months and a body weight of 34.6 kg (9, 10). As a result, mature rams mostly had distinct semen properties from younger ones (11).

To boost reproductive performance in ram breeding programs, testicular and epididymal morphometric traits were used as selection criteria (12, 13). Moreover, a male's reproductive ability could be assessed by analyzing the testicular morph-biometric characteristics, which could contribute to the study of testicular sperm production (14). Researchers studied testicular biometry and sperm production, and it was reported that body weight and/or age are directly associated with testicular biometry.

The role of body weight points that heavier weight, a longer length, and a larger chest provide more surface area for muscle development (15). Furthermore, enhanced body size will improve reproductive organ features (16, 17). Consequently, the testicular measures associated with body weight could be vital for selecting high-value slaughtered animals for *in vitro* embryo production programs (IVEP) (18, 19).

Therefore, the present study was designed to investigate the association between testicular biometry and spermatozoa quality produced in right and left testicles on the basis of age in Awassi rams.

2. Materials and Methods

2.1. Study Location

The study was conducted in the laboratory of the Biotechnology and Environmental Center, University of Fallujah, during the period from October 2020 to January 2021.

2.2. Experimental Design

Thirty-three pairs (n=66 testes) of Awassi rams testicles were collected after the slaughter at the abattoir in Baghdad and randomly divided into three groups according to age achieved by their dentition. Rams less than a year old were group G1 (n=22 testes), rams aged one to two years were grouped as group G2 (n=22 testes), and rams older than two years as group G3 (n=22 testes). Each animal's testicle was removed and placed in a plastic bag that was then placed in an ice box of 4- 8 ℃ and transported to the laboratory as soon as possible after slaughter (20). In the laboratory, physiological saline solution (0.9 % NaCl) containing antibiotics was used to wash the testes at room temperature, and surrounding tissues were removed by scalpel blade carefully.

2.3. Testicular-Epididymal Measurements

The epididymis from each testis was carefully removed using a scalpel blade as previously described (21). Testicular weighted on a sensitive balance scale, the length, width, and diameter testicular were measured with a flexible measuring tape. The Epididymal is weighed on a sensitive balance scale, and the epididymal length is measured by flexible measuring tape.

2.4. Spermatozoa Recovery

The spermatozoa were collected by the flushing method. Briefly, a cut near the corpus and the proximal side separated each cauda epididymis. Then, the cauda epididymis was injected with 3-5 ml of normal saline and a blunted 23G needle. After that, several longitudinal incisions were made and kept in a 35-mm Petri dish for 15 min in a water bath at 37°C (22). Finally, the spermatozoa were harvested in a glass tube in Petri dishes. pH measured directly using indicator papers ranges from 5.6 - 8.0 (Madaus GmbH, Koeln, Germany). Using a microscope at a magnification of 400, determine the percentage of individual motility spermatozoa at 37ºC (23). The percentage of live and abnormal spermatozoa was assessed by means of the eosin-nigrosin staining; abnormalities spermatozoa were recorded (including head, midpiece, and tail) by a microscope at X100; the sperm concentration $X10^9$ was determined by means of a haemocytometer (24).

2.5. Statistical Analysis

The data is presented as the mean ± SE. Comparisons were conducted by one-way analysis of variance (ANOVA) followed by the Duncan Multiple Range Test. The significance level was set at $P<0.05$and data were analyzed using the SPSS Statistics 24.0 (2016).

3. Results

The results of the biometry of testicular and epididymal (right and left) obtained from different ages group of Awassi rams are present in table 1. The results revealed the effect of age of Awassi rams significant differences ($P<0.05$) on testicular weight, length, width, and diameter both right and left increased steadily in group G1 to reach a maximum in group G3. As shown in table 1, the epididymal weight and length (right and left) significantly ($P<0.05$) influenced by the advancing age Awassi rams.

The parameters of spermatozoa like pH, individual motility, live, dead, and abnormality spermatozoa (right

and left) indicate no significant difference groups (Table 2). However, the spermatozoa concentration obtained from the left increased significantly (P<0.05) in groups G2 and G3 compared to group G1, while the right spermatozoa concentration increased significantly (P<0.05) in group G3 when compared to group G1. However, the results of group G2 were similar to groups G3 and G1.

Table 1. Testicular and epididymal biometry in Awassi rams of different ages (mean±SE)

Parameters	Testicle side	Age		
		G1	G2	G3
Testicular weight(gm)	Left	89.47±3.20^{c}	129.96±7.22^{b}	195.00±13.70^{a}
	Right	80.47±2.03^{c}	121.03±5.98^{b}	196.03±9.97^{a}
Testicular length(cm)	Left	11.00±0.16^{c}	12.15±0.28^{b}	14.12±0.32^{a}
	Right	10.44±0.15^{c}	11.83±0.33^{b}	13.74±0.27^{a}
Testicular width(cm)	Left	8.63±0.20^{c}	9.72±0.14^{b}	11.40±0.36^{a}
	Right	8.18±0.21^{c}	9.27±0.20^{b}	11.45±0.34^{a}
Testicular diameter (cm)	Left	14.81±0.26^{c}	17.17±0.29^{b}	19.31±0.30^{a}
	Right	14.18±0.26^{c}	16.45±0.30^{b}	19.13±0.34^{a}
Epididymal weight(gm)	Left	15.07±0.65^{c}	21.79±0.79^{b}	30.60±2.20^{a}
	Right	13.63±0.43^{c}	20.93±0.80^{b}	28.68±1.32^{a}
Epididymal length(cm)	Left	15.14±0.30^{c}	17.70±0.51^{b}	20.40±0.29^{a}
	Right	14.50±0.55^{c}	17.41±0.46^{b}	19.59±0.36^{a}

Within the same row, different superscripts (a, b, c) show a significant (P<0.05) difference

Table 2. Evaluation of cauda epididymal spermatozoa in left and right in Awassi rams of different ages (mean±SE)

Parameters	Testicle side	Age		
		G1	G2	G3
pH	Left	6.89±0.02^{a}	6.92±0.02^{a}	6.90±0.02^{a}
	Right	6.86±0.03^{a}	6.89±0.02^{a}	6.90±0.02^{a}
Individual motility %	Left	83.18±2.26^{a}	86.81±1.54^{a}	84.54±1.05^{a}
	Right	78.18±4.00^{a}	84.54±1.96^{a}	81.36±1.79^{a}
Concentration X10^{9}	Left	5.37±0.81^{b}	7.60±0.49^{a}	7.72±0.47^{a}
	Right	5.05±0.75^{b}	6.69±0.66^{ab}	7.25±0.40^{a}
Live spermatozoa %	Left	79.16±1.98^{a}	80.48±2.15^{a}	78.80±1.64^{a}
	Right	76.40±1.75^{a}	78.87±2.07^{a}	75.16±1.86^{a}
Dead spermatozoa %	Left	20.83±1.98^{a}	19.51±2.15^{a}	21.19±1.64^{a}
	Right	23.59±1.75^{a}	21.12±2.07^{a}	24.83±1.86^{a}
Abnormal spermatozoa %	Left	8.71±0.69^{a}	8.69±1.86^{a}	8.53±1.07^{a}
	Right	9.74±0.95^{a}	9.22±2.27^{a}	7.49±1.26^{a}

Within the same row, different superscripts (a, b, c) show a significant (P< 0.05) difference

4. Discussion

The testicular biometry, weight, length, width, and diameter in both right and left Awassi rams testicles in various age groups showed that these parameters grew with age. Furthermore, age substantially impacted both right and left testicular biometry measures. According to previous research, testicular biometry evolves through time, with increases in testicular biometry related to body weight and age (14, 17, 25). However, the results of this study showed that testicular weight increased with ageing in both the right and left testicles. This is similar to the report's findings of Kabiraj, Hoque (26). The results, on the other hand, revealed that the left testicular weight was greater than the right

in different age groups. Khan, Rind (27) and Saleh (28) reported that the left testicular weight was heavier than the right.

The substantial influence of age on testicular length and width in both the right and left at various ages was higher than that observed in the previous study (29). However, Akpa, Ambali (30) reported that age and body condition scores were significant for testicular width except for the testicular length. Furthermore, Salhab, Zarkawi (9) found that the length and width of the testicles increased in tandem with the weight gain.

The current study's finding on the testicular diameter was significant in both right and left at different ages. The previous authors' findings contradict the current findings. Mahmud, Onu (31), in adult rams, it was observed that there was no substantial difference between the right and left testicles. Oyeyemi and Olusoji (32) pointed out that the diameter of the right testicle was greater than that of the left. However, Baldaniya, Patel (33) proved the existence of a positive relationship between testicular diameter and testicular weight. Furthermore, this evidence agrees with existing findings because of a syncretized development in testicular diameter and weight with increasing age and body weight in group G3.

The findings of this study revealed that rams' age caused a significant increase in epididymal weight and length in both the right and left sides in different age groups. Both testicles, on the other hand, develop at different rates. These results agreed with the findings of Ahmmad, Parvez (34) pointed out that epididymal weight and length difference between the right and left sides, with the left side having a greater tendency than the right. In addition, Turri, Madeddu (35) reported a favourable relationship between increased body weight, testicular weight, and epididymal weight. Moreover, the influence of breed of the epididymal weight of high value in mature rams was found (36).

Based on the current findings of this study, spermatozoa concentrations were more significant in groups G3 and G2 than in group G1, attributable to spermatozoa concentrations being higher in adult rams than in yearling rams (11). These results agreed with Abba and Igbokwe (37) findings that the testes and cauda epididymis sperm concentrations were higher at 30 months of age than at 18 and 24 months. Therefore, age has a substantial impact on sperm concentration (30). Moreover, According to histological evidence, the left testes of adult rams were significantly more active than the right testes (38).

In conclusion, it can be concluded that the biometry of testicular and epididymal (right and left) in this study was influenced by progressing age, and the spermatozoa concentration obtained from the left testicle was higher more active than the right one in adult Awassi rams.

Authors' Contribution

Study concept and design: M. H. A.
Acquisition of data: M. H. A.
Analysis and interpretation of data: M. H. A.
Drafting of the manuscript: M. H. A.
Critical revision of the manuscript for important intellectual content: M. H. A.
Statistical analysis: M. H. A.
Administrative, technical, and material support: M. H. A.

Ethics

The study was conducted in accordance with ethical committee of the University of Fallujah, Fallujah, Iraq

Conflict of Interest

The authors declare that they have no conflict of interest.

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