AGRICULTURAL BIOLOGY,
2019, V. 54, № 2, pp. 239-245
(SEL'SKOKHOZYAISTVENNAYA BIOLOGIYA)

ISSN 2412-0324 (English ed. Online)
ISSN 0131-6397 (Russian ed. Print)
ISSN 2313-4836 (Russian ed. Online)

UDC 636.38:636.082:591.463:591.8

doi: 10.15389/agrobiology.2019.2.239eng
doi: 10.15389/agrobiology.2019.2.239rus

TESTICULAR HISTOMORPHOMETRY AND SPERM CHARACTERISTICS IN Lori RAMS (*Ovis aries* L.)

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The authors declare no conflict of interests
Received December 21, 2018

Abstract

Lori sheep breed is one of the important genetic resources in the Zagros region of Iran. Accurate knowledge of reproduction of Lori sheep is an essential step for optimal utilization of its potential. The present research aimed to study the testis histology and identify the sperm parameters in Lori rams. Ten healthy rams 2-3 years, were selected. After slaughtering, the testes were removed and epididymis minced to release sperm. The averages of weight, volume, length and diameter of testis were 237.00±6.56 g, 235.30±3.77 cm^3, 9.81±0.32 cm and 7.63±0.42 cm, respectively. Study of testicular sections was shown that the mean diameter, lumen and height of epithelium of seminiferous tubules were 220.04±8.73 μm, 125.10±13.99 μm and 65.68±13.54 μm, respectively. The mean number of Sertoli cells, spermatogonia, primary spermatocytes, secondary spermatocytes and spermatids per each section were 1.90±0.87, 6.02±1.44, 15.30±5.90, 9.65±3.82 and 7.60±2.63, respectively. Rate of motility, viability and normal morphology of released spermatozoa from epididymis were 64.42 %, 83.81 % and 90.5 %, respectively. The knowledge generated in the present study can be used in reproduction for comparative reproductive biology of mammalian species.

Keywords: testis, Sertoli cells, Lori sheep, morphometry

Sheep production is considered as a main economic activity for maximizing income and providing better income to the rural poor of the tropical countries. More than 1000 distinct sheep breeds are found in different parts of the world accounting 1172 million sheep population [1]. Lori sheep (*Ovis aries*) deemed, as dual-purpose sheep is suitable sources of wool and meat, Therefore, is of desirable economic efficiency in nomadic and rural systems [2].

Irrespective of animal species, understanding of reproductive parameters is an important factor in herd expansion [3]. Applied studies on spermatogenesis can be effective in preserving and improving genetics and hence increasing the reproductive capacity of livestock animals [4]. Estimation of reproductive parameters in male animals vary between different area such as the caliper method [5], orchidometry and ultrasonography in human, and goat [6, 7]. Seminiferous tubular length in goat occupied about 85% of parenchyma testis [8]. Testis weight in feral pig was estimated as 23.7±1.8 g [9]. In Arabian ram, seminiferous tubule diameter estimated in early winter is 220.97±12.15 μm [10].

There has been little research reported on reproductive parameters of Iranian breed sheep [11]. The results presented by us in this report supplement this data.

The present research aimed to study histomorphometry of testicular tissues and some sperm characteristics in Lori rams.

Techniques. Sperm parameters. The experiment was conducted at the Agriculture and Natural Resources collage of Lorestan University on ten Lori rams with 2-3 years. The weight of rams was 70.0±5.0 kg. After weighting,

slaughtering (Approved by Institutional Animal Ethics Committee, Lorestan University, Khorram abad 02.2018) and removing the testis from the scrotum, epididymis was incised in multiple locations with a scalpel to release the sperm. The part of epididymis agitated to encourage release of the sperm from the tissue into the surrounding culture media. Sperm collected from the epididymis, diluted with sodium citrate 2.9% at 37 °C. Sperm motility was evaluated by computer assisted semen analysis (CASA, Pro, Way Bulb Type Microscope), Straight movement, zigzag movement (Wavy movement), Non-progressive motile (vibrate movement) and Non-motile were registered by CASA (Computer-aided sperm analysis). The Eosin-Nigrosin staining method, for the differentiation of live and dead spermatozoa was applied on Sperm samples for determination of viability and abnormality rate. Nigrosin stain increases the contrast between the background and head of sperm, making sperm easier to visualize. Eosin stains only the dead sperm, turning them a dark pink, whereas live sperm appears white. The 5 μl of semen was mixed with 20 μl of eosin/nigrosin solution on glass microscope slide [12]. The prepared sperm samples were smeared on microscope slides and fixed by air-drying at room temperature for 10 min before observation. Viable sperm remained unstained and dead cells were totally or partially pink to red/brown. Viable sperms were further classified as morphologically normal or abnormal, depending on the head, midpiece, and tail morphology. At least 200 sperm cells were examined using a light microscope at ×400 magnification [13].

The hypo-osmotic swelling test (HOS) estimates the functional integrity of the sperm's plasma membrane. The HOS test estimates membrane integrity by determining the ability of the sperm membrane to maintain equilibrium between the sperm cell and its environment. Entry of the fluid due to hypo-osmotic stress causes the sperm tail to coil and balloon or "swell." A higher percentage of swollen sperm indicates the presence of sperm having a functional and intact plasma membrane. The 100rL incubated semen was added to the 1ml HOST solution, mixed gently with the pipette and kept at 37 °C for at least 30 minutes. Evaluated 200 spermatozoa by microscopy at ×400 magnification. All forms of swollen tails calculated as live spermatozoa [14, 15].

Immediately after slaughtering, the testes were removed from the scrotum. The diameter and length of testes were measured using a digital caliper (10-rm precision). The weight of testes was determined by digital electrical balance. Volume of testis with mass measurement were estimated using water displacement method (Archimedes' Principle) [10, 16]. To determine the testicular volume, formula (1), was used where m and v show testis weight (grams) and testicular volume (cm^3) respectively:

$$\rho = \frac{m}{V}. \quad [1]$$

Histological sections. The preparation of tissue sections were modified Culling 1974 [17], Fixation (buffer formalin 10%), dehydration (ethylic alcohol with increasing concentrations of 50, 70, 90, and 100%), clearing (xylene), saturation and embedding in paraffin. The embedded samples in paraffin cut using a microtome, such that the sample thickness was 6 μm. Histological sections were stained with hematoxylin-Eosin. Seminiferous epithelium was measured at ×400 magnification using a calibrated ocular micrometer (IS capture-Tucsen). The diameter of seminiferous tubules was taken in 5-10 randomly selected cross sections of apparently round seminiferous tubules per section [18]. Epithelial height was measured in the same tubules. The Germ cells with round nuclei were characterized spermatogium, whereas irregular nuclei were characterized Sertoli cells [19]. The number of Sertoli cells, and the germ cells (spermatogonia, the primary and secondary spermatocytes, spermatids) were deter-

mined using an Olympus microscope (Olympus Corporation, Japan) ×400 magnification and test grid (15×15 cm). The number of tubules in each tissue sections was calculated under a microscope with ×100 magnification and formula (2):

$$V_v = \frac{P(y) \times 100}{P(ref)}, \quad (2)$$

where $P(y)$ stands for sum of points following on the tissue section, and $P(ref)$ denotes total number of points in the test grid [20].

The number of seminiferous tubules per each grid, the number of primary spermatogonia, number of secondary spermatogonia, number of Sertoli cells and the number of spermatid were counted in 10 round or nearly round cross sections of seminiferous tubules chosen randomly.

For the obtained indicators, mean values (M) and standard deviations (±SD) were calculated.

Results. The results related to semen characteristics and testicular morphometry are shown in Table 1 and 2. The seminiferous tubules consist of a multilayered germinal epithelium containing spermatogonia and Sertoli cells (Fig. 1). Spermatogenesis starts with the production of primary spermatocyte on the basement membrane and continues to the production of spermatozoid in the center of the tubules. The spermatogonia in tissue sections were observed as spherical and large, around the seminiferous tubules. Moreover, the Leydig and myoid cells were found normally with blood vessels in the interstitial tissue of the testes (Fig. 2). Number of Sertoli cells in tissue section was low near to spermatogonia (see Fig. 2).

1. Different parameters of testis morphometry and semen in Lori rams (*Ovis aries* L.) (n = 10, Agriculture and Natural Resources College of Lorestan University, Iran)

Sperm classes, %	M±SD	Sperm parameters	M±SD	Parameter of motility	M±SD	Testis parameter	M±SD
Straight movement	64.42±4.22	Cell integrity, %	61.70±11.88	VAP, мкм/с	92.67±1.40	Weight, g	237.0±6.56
Zigzag movement	29.45±5.85	Normality, %	5.90±0.96	VCL, мкм/с	126.76±8.50	Testis weight/body weight, %	0.33±0.10
Vibrate movement	24.88±1.12	Viability, %	83.81±4.61	VSL, мкм/с	74.72±5.13	Volume, cm^3	235.3±3.77
Non-motile	14.24±0.84	Motility, %	86.30±4.73	BCF, Hz	14.46±60	ρ, g/cm^3	1.01±0.02
		Concentration, $\times 10^9$	2.68±0.17	STR, %	80.63±4.60	Diameter, cm	7.63±0.42
				LIN, %	58,94±1,11	Length, cm	9,81±0,32

N o t e. VCL stands for velocity of curved line, VAP for velocity of average path, VSL for velocity of straight line, STR for straightness: STR = (VSL/VAP) × 100; LIN for linearity: LIN = (VSL/VCL) × 100, and BCF for beat frequency.

2. Functional parameters of tissue section of testis in Lori rams (*Ovis aries* L.) (n = 10, Agriculture and Natural Resources College of Lorestan University, Iran)

Parameter	M±SD	Parameter	M±SD
Diameter of seminiferous tubules, μm	220.04±8.73	Density of seminiferous tubules	87.66±1.99
Epithelium length, μm	65.68±13.54	Number of vessels/mm^2	1.37±0.65
Lumen diameter, μm	125.11±13.99	Length of seminiferous tubules in mm^3	10.09±2.18
Number of tubule	4.95±1.07	Number of Sertoli cell/mm^2	1.90±0.87
Number of primary spermatocyte/mm^2	15.30±5.90	Number of Spermatogonium/mm^2	6.02±1.44
Number of secondary spermatocyte/mm^2	9.65±3.82	Number of Spermatid/mm^2	7.60±2.63

The diameter of the seminiferous tubules and epithelial height of seminiferous tubules in Lori ram was recorded 220.04±8.73rm and 65.68±13.54rm, respectively, which was similar to that reported in some domestic ram. The average diameters of seminiferous tubules and epithelial height of seminiferous tubules in goat were 197.20, and 39.2 μm, respectively [5]. Akosman et al. [21] found that the mean diameters of seminiferous tubules in Holstein-Friesian and Simmental breeds of cattle were 226.68 μm and 223.44 μm, respectively. Arrighi et al. [13] reported that the mean diameter of seminiferous tubules in buffaloes

was 243.19 μm. In general, the diameter of seminiferous tubules in most mammals varies from 180 to 350 μm [18, 22].

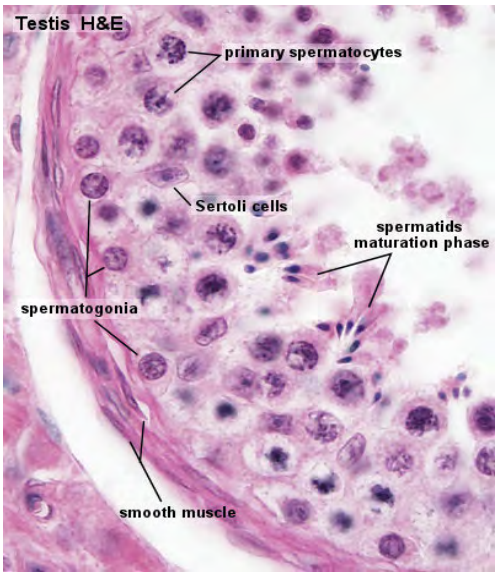

Fig. 1. Different cells of seminiferous tubules in testis of Lori rams (*Ovis aries* L.). Primary spermatocytes, Sertoli cells, spermatogonia, spermatids (maturation stage) and smooth muscles are visible. Histological sections, hematoxylin and eosin staining, light microscopy (Olympus Corporation, Japan), magnification ×400.

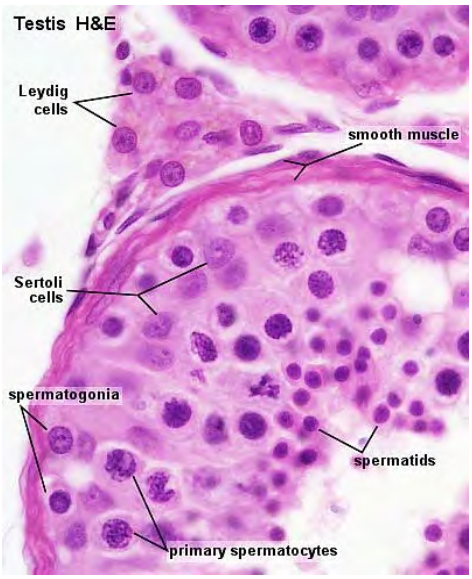

Fig. 2. Interstitial tissue, germ cells and sertoil cell in testis of Lori rams (*Ovis aries* L.). Leydig and Sertolli cells, spermatogonia, primary spermatocytes, spermatids and smooth muscles are visible. Histological sections, hematoxylin and eosin staining, light microscopy (Olympus Corporation, Japan), magnification ×400.

The determination of testis volume and characteristics of seminiferous tubules is an important index in identifying maturity and evaluating spermatogenesis [23, 24]. In this experiment, the testis parameters in Lori rams were similar to the results found by Martins et al. [25] and Mohammadzadeh et al. [5]. These findings were reported for Santa Ines rams [26] and European sheep breeds such as Merino, Corriedale, Suffolk, and Lori rams [27].

The Sertoli cell was the major controller of testis development and efficiency of spermatogenesis [28].). Sperm production capacity in testes is adjusted with the help of events related to proliferation of Sertoli cells that happens before the beginning of sexual maturity. Since Sertoli cells are referred as the nourishing for sperm production, number of Sertoli cells is closely correlated with spermatogenesis process [29]. In the present research, the Sertoli cells with triangular nuclei and expanded cytoplasm were observed at ×400 magnification.

Testis size in the mature males is correlated with reproductive capacity and total number of Sertoli cells in various species of mammals [30]. The mean number of Sertoli (Sustentacular) cells recorded in the Lori ram was 1.90±0.87 (see Fig. 2).

The seminiferous density comprised the main compartment of the testis which changed from 70 to 90% of the testis parenchyma in most mammals [30]. In this study, the seminiferous density obtained was 87.66±1.99% in Lori ram at 2-3 year of ages. In domestic cat, the volume density of the seminiferous tubule was reported to be 90% [18]. Result of this experiment were shown that, large part of the testis was occupied by seminiferous tubules. The percentage of seminiferous tubules in Lori goats was re 80-85 percent [5]. Franca and Godinho [18] reported that the percentages of seminiferous tubules in mammals were not substantially different: 87, 83, 80-87, 73, 88, and 81 percent in rabbits, pigs,

rams, stallions, cats, and bulls respectively. In the present research, the mean numbers of Sertoli cells, spermatogonia, initial spermatocytes, and secondary spermatocytes per mm^2 were estimated as 1.90±0.87, 6.02±1.44, 15.30±5.90, 9.65±3.82, and 7.60±2.60, respectively. Relevant information pertaining to this parameter was found to be very scant in the available literature.

Thus, quantitative study of the number of cells, tubule diameter, testis volume and its other parameters can provide effective help in better understanding of the spermatogenesis process in native breed. Since there is a high correlation between seminiferous density and spermatogenesis, several parameters such as leydig cell, size of nucleus of spermatogonia are need to determined.

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