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COMPARISON OF SEMEN CHARACTERISTICS IN ROMANOV AND Lori Bakhtiari RAMS

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Abstract

One of the important aims in animal breeding is using the advantages of bio diversity. Romanov sheep is Russian native breed and has prominent features, such as high percent of twinning. Lori-Bakhtiari breed is dual-purpose (meat and wool) in Iran. Regarding the governmental policies, confluence between these two breeds is on the agenda. The aim of this research is to study comparatively some of the sperm features of rams in both breeds in terms of concentration, motility, morphometry. Semen samples of Lori and Romanov rams were collected in husbandry research station known as Shuli in Chaharmahal Bakhtiari Province in Iran. In this experiment, sperm parameters in Romanov ram including large diameter, small diameter, perimeter and area of head, tail length, mid piece and end piece length were estimated as 9.16±0.37 μ, 4.77±0.41 μ, 24.47±0.97 μ, 24.89±1.94 μ^2, 57.59±1.50 μ, 15.40±1.20 μ and 42.19±2.10 μ, respectively. A significant difference was observed between the large diameter and head area of sperm, while the small diameter and perimeter of sperm were similar. Motility of type A in Romanov rams was significantly higher. The tail and mid piece of Romanov sperms was significantly taller than these parts in Lori's rams. It seems that taller length of the mid piece and more mitochondria concentration are the causes of higher motility in Romanov spermatozoa.

Keywords: ram, Romanov breed, Lori Bakhtiari breed, sperm, motility, morphology

Iran also has numerous comparative advantages in terms of animal husbandry and products in such a way that every domestic animal nearly can be reared and maintained. This advantage has led to place Iran as one of the most lucrative and important countries. Of the goals of the animal breeding institutions is to apply cross breeding in order to use the genetic potentials and increase livestock resistance. Advancements could be seen considerably in the first generation. Different goals are determined due to the environmental conditions for sheep management. Those areas in where wool production is most important, crossbreeding program tends to select wool breeds. In areas where meat is more important economically, those breeds are used which have high growth rate, feed efficiency and meat quality [1]. Exploiting the most production potential of sheep is the only way which is profitably suitable and explainable [2].

Romanov sheep is Russian native breed and imported to France for research purposes by IRNA (French National Institute for Agricultural Research) research center in 1963 for the first time. This breed is premature and has outstanding characteristics such as a high percentage of twinning, high maternal instinct and high physical ability of the newborn lambs. This breed has rather tall legs. The other important aspect of Romanov is high resistant of ewes and lambs and their vigor. Lambing is easy. The mean weight of Romanov ram and

ewe are 55-80 kg and 40-50 kg, respectively. Rams have aggressive behavior during breeding, and they have sexual activity in all of seasons [3].

Lori Bakhtiari sheep is a heavy and outstanding breed in terms of the production features in Iran. They are reared in the south-west of Iran, especially in Chaharmahal Bakhtiari province. The main place of its rearing is Chaharmaahal Bakhtiari. For its top features, it is reared in the neighboring provinces such as Isfahan, Lorestan, Kohgiloye va Boyerahamd and Khuzistan. It's ewe known as Haftlang. Its fat tail is big and deep. Fat tail is a groove cause to divide and suspense. It's divided into two completely separate sections (Fig. 1). Sometimes the end of the fat tail is placed in the lower hocks [3].

Fig. 1. Rams of the local Iranian breed Lori Bakhtiari (left) **and a crossbred animal F_1** (right). A very long fat tail characteristic of the breed is considerably shortened in the offspring from crossing.

Exposing Romanov ram to the sun decreased sperm volume, total motility, individual motility, concentration, viability and normal sperms. Testis length and its diameter in Romanov ram were higher than the native Finns [4]. The color of semen is dependent upon the sperm concentration and varied from cream to white. If the sperm concentration is high in the semen, the semen color tends to be cream and if the sperm concentration in semen is low, its color tends to be white and watery [5]. Studies show that there is a direct relationship between the number of live sperms and pregnancy [6].

It is planned in order to produce the hybrid of Romanov breed and reduce some features of Lori Bakhtiari breed such as the large size of fat tail and low twining [7]. There is a project for the possibility of crossbreeding between Romanov ram and Lori Bakhtiari ewes (see Fig. 1). There is not any study about morphologic parameters in Lori Bakhtiari semen and Romanov ram in Iran.

The aim of this research was the comparison of the semen parameters of both breeds in terms of volume, concentration, motility and sperm morphometry.

Techniques. Rams were managed in the research station of Jahad Keshavarzi Organization in Chaharmahal Bakhtiari province — Iran at the same feeding and maintenance conditions. A total of 24 rams aged 2-3 years, were kept in the husbandry research station known as Shuli station which 12 of them were Lori Bakhtiari and the other 12 were Romanov rams. Shuli belongs to Jahad Keshavarzi Organization in Chaharmahal Bakhtiari province with longitude 50°, latitude 32° and the height of 2017 meters above the sea level. With the help of artificial vagina semen samples were collected. Semen was stored at 36 °C to prevent the cold shock. Samples were transferred in the laboratory for semen analysis.

Fresh semen was diluted with Hepes TCM 199 (Tissue Culture Medium, Sigma-Aldrich, Inc., USA) in proportion 1:250. From diluted sample 5μl was placed on Makler Counting Chamber (New York Microscope Company, Inc., USA) and evaluated under the microscope using CASA (Computer-aided sperm

analysis) software. To study the sperm morphology, smear was prepared. For smears staining, Rapid Sperm Staining (RSS) Differentiation quick kit was used. This kit contains three solutions of A, B and C. At first, solution A was poured on the smear in such a way that the smear surface is completely covered. After 75 seconds, it is evacuated and the surface of smear was covered with B solution for 60 seconds and evacuated. Finally, C solution was poured on the smear for 15 sec and it was rinsed with a very slow water flow. Smears were dried completely at room temperature. After staining stage, about 100 sperms in the field of microscope equipped with Isc capture program were examined in ×40 lenses. Different parameters, including area, perimeter, large and small diameter of sperm head, different parts of the sperm body and tail were determined.

Mean (M), standard errors of mean (±SE) and statistical significance (P) of the differences between the studied parameters in the two rocks were calculated.

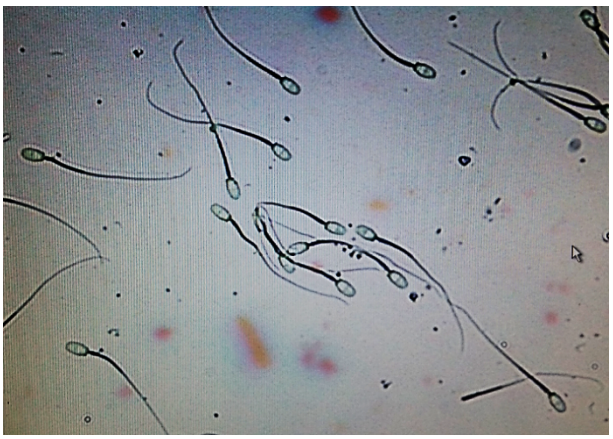

Fig. 2. Sperm of Lori Bakhtiari ram (magnification lenses ×20).

Results. Figure 2 shows the sperm samples under the microscope with ×20 magnifications in Lori Bakhtiari ram.

Different characteristics of semen samples of Lori Bakhtiari and Romanov rams are shown in Table 1. Morphological parameters and motility features of sperm between two breeds of Romanov and Lori Bakhtiari were compared.

Morphological parameters of experiment, including large diameter, small diameter, perimeter (μ) and area (μ^2) related to the sperm head are shown in Table 2. The length of tail (μ), length of the mid piece and length of end piece are related in the body of sperm. The results of comparing of sperm morphology in Lori and Romanov rams showed that there was a significant difference between large diameter and the head area of sperm ($P < 0.01$). There was not any significant difference between the small diameter and the perimeter of the head sperm between these two breeds. There was a significant difference between the sperm tail and the length of mid piece while there was not any significant difference between two breeds in the end piece (see Table 2).

Sperm motility class A, B, C, D, actual sperm velocity, straight movement, mean of the straight movement (μ/sec), mean of rotation angle, maximum range of lateral motility (μ), frequency of lateral motility (Hz), percent of linear movement, percent of rotation angle and standard percentage of straight motility are among sperm motility. The results are shown in Table 3.

Comparing the mean of sperm parameters in Lori Bakhtiari and Romanov rams indicated that sperm concentration, percent of sperm motility, percent of proceeding sperm, percent of sperm motility class A, sperm motility class D, real velocity of sperm, sperm velocity in the straight line, mean of velocity in the straight line, mean rotation angle and frequency of lateral motility had a significant difference in both breeds ($P < 0.05$). There is a significant relationship between the percent of sperm motility Class B, percent of sperm motility Class D, percent of linear of sperm motility, mean rotation angle and percent standard

straight sperm motility in both breeds (see Table 3).

1. Some parameters of ram and semen analysis in Romanov and Lori Bakhtiari rams (Shuli, Chaharmahal Bakhtiari, Iran)

Breed	Age, years	Ram color	Ram No	Replication of semen sample	Semen color	Sperm concentration, $\times 10^{12}$/ml
Lori Bakhtiari	3.0	White	885954	1	Beige	2.2
Lori Bakhtiari	2.5	White	917163	1	Beige	1.6
Lori Bakhtiari	2.5	White	865821	1	Beige	1.0
Lori Bakhtiari	2.5	White	917163	2	Beige	1.5
Lori Bakhtiari	2.5	White	865821	2	Beige	1.8
Lori Bakhtiari	3.0	White	885954	2	Beige	2.0
Lori Bakhtiari	3.0	White	885954	3	Beige	1.9
Lori Bakhtiari	2.5	White	917163	3	Beige	1.6
Lori Bakhtiari	2.5	White	865821	3	Beige	1.6
Lori Bakhtiari	2.5	White	865586	1	Beige	1.6
Lori Bakhtiari	2.5	White	865586	2	Beige	1.5
Lori Bakhtiari	2.5	White	865586	3	Beige	1.6
Romanov	2.0	Black	50202	1	Beige to white	1.8
Romanov	2.0	Black	50202	2	Beige to white	1.5
Romanov	2.0	Black	50202	3	Beige to white	1.6
Romanov	2.0	Black	52028	1	Beige to white	1.7
Romanov	2.0	Black	52028	2	Beige to white	1.6.
Romanov	2.0	Black	52028	3	Beige to white	1.7
Romanov	2.0	Black	52017	1	Beige to white	1.6
Romanov	2.0	Black	52017	2	Beige to white	1.4
Romanov	2.0	Black	52017	3	Beige to white	1.6
Romanov	2.0	Black	50141	1	Beige to white	1.8
Romanov	2.0	Black	50141	2	Beige to white	1.5
Romanov	2.0	Black	50141	3	Beige to white	1.6

2. Comparison of morphological parameters in semen sample of Lori Bakhtiari and Romanov rams (M±SE, Shuli, Chaharmahal Bakhtiari, Iran)

Parameter	Lori Bakhtiari	Romanov	P
Large diameter, μ	8.10±0.37	9.16±0.37	0.000
Small diameter, μ	4.63±0.33	4.77±0.41	0.130
Perimeter, μ	24.29±1.50	24.47±0.97	0.570
Area, μ^2	26.97±1.18	24.89±1.94	0.000
Tail length, мк	56.11±1.90	57.59±1.50	0.007
Middle piece, μ	14.11±1.10	15.40±1.20	0.000
End piece, μ	42.01±2.60	42.19±2.10	0.750

3. Comparison of sperm characteristics in Lori Bakhtiari and Romanov rams (M±SE, Shuli, Chaharmahal Bakhtiari, Iran)

Parameter	Lori Bakhtiari	Romanov	P
Concentration, $\times 10^6$/мл	5308.8±0.15	6764.4±1.2	0.028
Sperm motility, %	39.24±0.80	59.47±1.60	0.048
Percent of proceeding sperm	33.55±0.42	52.37±2.10	0.042
Sperm motility class A, %	21.47±1.10	38.26±1.20	0.320
Sperm motility class B, %	12.07±1.90	14.11±0.60	0.490
Sperm motility class C, %	5.68±1.90	7.09±1.60	0.290
Sperm motility class D, %	60.76±0.34	40.53±0.72	0.048
Actual sperm velocity, μ/sec	38.87±0.42	58.95±1.70	0.019
Straight movement, μ/sec	24.12±1.40	41.08±0.30	0.027
Mean of the straight movement, μ/sec	27.87±1.60	45.82±0.36	0.025
Mean of rotation angle, °	9.27±1.60	15.17±1.50	0.047
Maximum range of lateral motility, μ	1.72±1.40	2.23±1.90	0.030
Frequency of lateral motility, Hz	0.41±0.22	0.74±0.36	0.022
Linear movement, %	36.63±0.65	48.26±2.00	0.086
Rotation angle, %	50.15±0.72	60.49±1.50	0.089
Standard of straight motility, %	52.79±1.20	63.29±1.60	0.069

According to Table 3, the highest number of sperm was obtained in Romanov ram (6764.4 million/ml vs. 5308.8 million/ml). The highest percentage of motile sperm was in Romanov (59.47 % vs. 39.24 %). The highest percentage of proceeding of sperm was related to Romanov (52.37 % vs. 33.55 %). The highest percentage of sperm motility in Class A was seen in Romanov ram (38.26 % vs.

21.47 %). The highest real velocity of sperm was in Romanov (58.95 μ/sec vs. 38.87 μ/sec). Sperm velocity in straight line (μ/sec) was higher in Romanov (45.82 μ/sec vs. 27.87 μ/sec). The highest rotation angle of sperm was observed in Romanov (15.13° vs. 9.27°). Sperm lateral motility was higher in Romanov ram (2.23 μ vs. 1.73 μ) (see Table 3).

Morphology of sperm has a great influence on sperm motility and ovum fertilization. Normally, at least 30 % of sperms should have a normal shape [8]. The abnormality of sperm could have different forms. For example twin heads, two tail and microcephlic sperm. Abnormal sperm is not able to move in a balanced way and these sperms cannot fertile the ovum [9]. In this experiment, some abnormal forms such as sperm bent tail were seen in the semen sample of Romanov rams.

Semen color is one of the important indicators determining reproduction activity. Sperm color of rams and cows changes from light yellow to cream. If the concentration of sperm increases in semen, the semen color will tend to be cream and if the sperm concentration is low in semen, its color will change into watery [5]. In this experiment, the color of semen sample in Lori Bakhtiari was cream, and in Romanov was whitish. Higher concentration of sperm is in Romanov rams (6.7×1012/ml vs. 5.3×1012/ml).

Motility is the most important factor in fertility. Motility of sperm is important for its movement through the reproductive system. For this reason, there is a correlation between motility and fertility. Increasing the percent of straight motility and fertility of sperm will go up. In this experiment, there was no significant relationship between the rate of sperm motility in both breeds of Lori-Bakhtiari and Romanov in Class B and C ($P < 0.05$), while the motility in classes A and D in both breeds was significant. Sperm of Romanov breed was higher than Lori Bakhtiari breed in terms of motility A ($P > 0.05$). Using some component and time variation could change the sperm motility. In a study [10], melatonin in non-reproductive season had no significant effect on motility in Lori Bakhtiar ram. In another research [11], changes of luteinizing hormone (LH), follicle-stimulating hormone (FSH) and testosterone hormones in Romanov and Al de France rams were studied. Results showed that FSH secretion in blood plasma had no significant difference in both breeds, while LH secretion and testosterone in Romanov were higher and its secretion started earlier. It seems that the cause of better reproduction activity performance in Romanov is the specific secretion of gonadotropins and testosterone [12]. Results from comparison of sperm motility and fresh semen of ram and the sperm motility after 24 hours in vitro showed that the motility of fresh sperms was significantly higher than the semen sample kept after 24 hours in vitro. Decreasing motility to less than 20 % will reduce fertility. The percent of motile sperms in bulls is 50-80 %. The results showed that the percent of sperm motility is high at the beginning of sample evaluation, while sperm motility reduced after a short time [13]. Some male infertile bull had high motile sperms. In fertile rams, the motility percent was 60-70 and sperms having abnormality in body and tail had poor motility [14]. In this experiment, the percent of sperm motility in Romanov ram was higher than in Lori Bakhtiari (59.47 % vs. 39.24 %). Considering a minimum motility of 60 to 70 percent for sperm, it seems that Lori Bakhtiari rams would have a low fertility rate. In this experiment, other measured factors using CASA indicate the priority of Romanov ram than Lori Bakhtiari ram. The real rate of sperm, percent of sperm proceeding, actual velocity of sperm, sperm velocity in straight line (μ/sec), mean velocity in straight line, mean rotation angle (degree), maximum range of lateral motility (μ), frequency of lateral motility (Hz), percent of

linear of sperm motility, mean percent of rotation angle and standard percent of straight line sperm motility in Romanov ram were higher than in Lori Bakhtiari ($P > 0.05$).

Morphometric study of germ cells especially sperm is very important in breed characteristics. Different methods are applied for morphometric study. J. Yániz et al. [15] investigated the morphometric features of acrosome and the nucleus of ram by staining the semen samples using florescence microscope. In the first group, some appropriate digital pictures were taken from sperm, head and nucleus using Potassium Iodide stain. The pictures were studied by a computer program of CASA. In the other group, Hoechst stain was used sperm nucleolus of ram and was investigated by CASA software. There was no significant relationship between the data from the first and second groups. Different dimensions of the sperm head were analyzed by CASA. Head length, head width, proportion of width to length, area and perimeter were estimated 8.08 r, 4.80 r, 0.59, 29.13 r^2 and 23.93 r, respectively [16]. Potassium iodine was used to compare sperm nucleuses of bull, boar and ram. Morphometric studies in ram sperm showed that acrosome of ram sperm is long and wide, while it is small in bull [17]. In this experiment, sperm parameters in Romanov ram were estimated including large diameter, small diameter, perimeter and area of head, tail length and mid piece length as 9.16±0.37, 4.77±0.41, 24.47±0.97, 24.89±±1.94, 57.59±1.5, 15.40±1.2 and 42.19±2.1 μ, respectively. Comparing data from Table 2 related to sperm morphometry of Romanov sperm and Lori Bakhtiari ram showed that head area of Romanov sperm is significantly greater than sperm head in Lori Bakhtiari ($P > 0.01$). The length of middle piece of Romanov sperm was significantly greater than Lori Bakhtiari sperm. Referring to Table 2, sperm motility of Romanov ram was higher than Lori Bakhtiari sperm. It seems more mitochondria accumulate in the mid piece of Romanov sperm (because of the longer middle part of Romanov sperm than Lori Bakhtiar). This structure causes to provide more energy for sperm motility.

Therefore, due to the comparison of motility classes of sperm in Romanov and Lori rams, it seems that increasing of sperm motility in Class A in Romanov breed is because of the longer mid piece and high concentration of mitochondria. Other features of semen analysis indicate the priority of this breed's sperm to Lori Bakhtiari breed.

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