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Improving the Technology for Assessing the Semen of Beige Karakul Sheep

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Abstract: The article presents the research results on the effective use of seed products from breeding rams in reproduction for the conservation and targeted propagation of genetic resources of Karakul sheep of rare beige color, which are on the verge of extinction. Selected freshly obtained ejaculates from 6 beige rams of the Karakul breed in the amount of 32 samples, meeting the requirements of the sperm production standard for sheep, were studied by the speed of sperm movement and the frequency of occurrence of the acrosomal area of sperm. It was established that the seed production of producers in terms of the speed of sperm cell movement ranged from 80-169 μm/s and the area of the acrosome in the head of the sperm ranged from 40-86%. Various parameters for their integration have been determined and various gradations of parameters have been developed, which are divided into 9 groups with the functional characteristics of sperm products. Testing samples of freshly obtained sperm in artificial insemination of sheep showed that among all groups, the most effective was the semen produced by rams that have a VAP movement speed of at least 110 μm/sec and a sperm acrosomal area of at least 60%, ensuring the fertility of ewes up to 100%, which is higher in compared to the traditional assessment method by 22%. During cryopreservation of freshly obtained semen, in all 9 groups of frozen-thawed semen, the speed of sperm cell movement decreases by 30.7-97.4 μm/sec and the area of the acrosome in the head of the sperm decreases by 9.2-36.7% and is, respectively, within 49, 62-68.67 μm/sec and 17.4-65.0% and significantly affects the fertility of ewes. The developed method for selecting ram sperm with new morphological parameters and the speed of sperm cell movement in the ejaculate increases the genetic progress of beige color reproduction in Karakul sheep populations from 12.0-22.0%. The data presented in the article on the morphological structure of sperm, their motility, and their fertilizing ability indicate their great importance in the reproduction of especially valuable beige-colored genotypes in astrakhan sheep breeding.

Keywords: Karakul Breed, Ram, Beige Color, Ejaculate, Quality and Motility of Sperm, Acrosome, Sperm, Fertility

Introduction

The Karakul breed of sheep belongs to the astrakhan direction of productivity and differs from other breeds of sheep in its wide variety of original colors, shades, and colors on the Anushka (Degen, 2013).

The huge variation in the colors of Karakul sheep is the result of natural and targeted artificial selection to meet the exquisite requirements of Karakul consumers (Mashner *et al*., 2016).

Beige color is one of the most original and beautiful color variations of hair pigmentation on the fleece of Astrakhan sheep and as Astrakhan raw material has gained universal recognition in business fashion and is in high demand in the fur market (Buzu, 2018).

Today, in the populations of astrakhan sheep, there is a numerical reduction in individuals of beige color, that is, they have become very few and are in danger of extinction (Ibrahim, 1998).

This circumstance urgently requires the preservation of beige-colored individuals and their intensive reproduction. However, breeding beige sheep by selection is very difficult and requires a long period of time due to their small number in the selection aspect and the recessiveness of beige color in genetic terms. Moreover, the Law of the Republic of Kazakhstan "On the protection, reproduction, and use of fauna" provides the basic concept that any fauna is a collection of animals living on the territory of the country and refers to natural resources (Law of the Republic of Kazakhstan, 2004; 2024).

Karakul breeding in Kazakhstan is well combined with camel breeding (Baimukanov *et al*., 2022; Bekenov *et al*., 2023; Kargaeva *et.al*., 2022; Yuldashbayev *et al*., 2023) and herd horse breeding (Iskhan *et al*., 2019; Baimukanov *et al*., 2023; Kargaeyeva *et al*., 2023).

Regarding the legal status of any state for the use, improvement, and conservation of genetic resources of endangered animals, they are provided for in the provisions of the (FAO) of the United Nations (FAOLEX Database, 2018; FAO, 2008; Radionov, 2015).

Improving the genetic resources of local livestock breeds is a priority in Kazakhstan, particularly in matters of reproduction and livestock increase (Baimukanov *et al*., 2021; 2024).

In this regard, the development of progressive reproductive technologies that allow the maximum and effective use of rams with particularly rare colors contributes to the accelerated reproduction and preservation of multi-breed populations of beige sheep (El Amiri and Rahim, 2024).

The reproductive potential of breeding rams is characterized by qualitative and quantitative indicators of seed production (Abebe *et al*., 2023; Yuldashbayev *et al*., 2022).

Currently, the interstate standard GOST 32200-2013 Means of reproduction is in force. Ram sperm. Specifications (Product for reproduction. Sperm of rams. Specifications) for freshly obtained diluted, freshly obtained undiluted, and frozen ram sperm intended for artificial insemination of sheep, where minimum standards are provided (GOST 32200-2013, 2018).

Existing traditional visual microscopic methods for assessing sperm do not always reliably predict the quality and fertilizing abilities of native and thawed sperm, due to the subjectivity of determining the qualitative and quantitative indicators of sperm.

In recent years, to analyze ejaculate, manufacturers have begun to use a hardware-software complex (CASA), which is based on modern technologies in electro-optics, computer algorithms, and video microscopy (Iolchiev *et al*., 2011).

Computer analysis of semen production CASA has enormous potential as a tool for increasing reproduction in animal husbandry and not just for describing measurements of sperm parameters as such and the integration of automated sperm analysis parameters for the effective use of the functional characteristics of sperm in the reproduction of populations is becoming relevant (Tadzhieva and Sulima, 2015).

In this aspect, a special role in the fertilization of eggs is played by the acrosome - a secretory vesicle, which is formed from the vesicles of the Golgi apparatus, starting from the early stages of spermiogenesis, located in the form of a "cap" on the anterior pole of the sperm nucleus. Proteolytic enzymes are localized in the acrosome matrix, which is involved in the interaction of sperm and egg and ensures penetration through the zona pellucida (ZP) (Ramalho-Santos *et al*., 2002; Sutovsky *et al*., 2003).

Acrosome biogenesis is an important aspect of spermogenesis and it has been established that at present the molecular mechanism regulating this event remains unknown (Berruti and Paiardi, 2011).

The sperm acrosome contains two groups of enzymes (hyaluronidase and acrosin), which play an important role in the fertilization process. The detection in the ejaculate of increased content of sperm with an abnormal acrosome is one of the reasons for idiopathic infertility in agricultural producers, even with standard spermogram parameters (Efimovna and Bocharova, 2014).

A sperm without an acrosome is not able to fertilize an egg on its own. In addition, one of the most important functions of sperm is its motility, i.e. the speed of their movement to deliver the sperm head with an acrosome to the egg (Ashotovich *et al*., 2013; Bailey *et al*., 2003).

In this regard, conducting research work to assess the seed production of beige-colored Karakul rams in the relationship between acrosomy and the speed of sperm cell movement in the fertilizing ability and stability of sperm freezing is of great scientific and practical interest

when propagating their livestock by artificial insemination and creating a gene pool of semen of the most valuable male genotypes.

Purpose of the Study

Development of an effective method for selecting the seed of Karakul sheep of rare, disappearing beige color for preservation and use in accelerated reproduction of their genetic resources.

Research Objectives

1. Quantitative and qualitative analysis of sperm production
2. Assessment of morphological parameters of sperm production
3. Development of effective criteria for the selection of sperm products to increase their fertilizing ability
4. Study of the influence of cryogenic factors on the fertilizing ability of sperm

Materials and Methods

Research was carried out in the period 2021-2022 in the farm "Tore" of the Saryagash district of the Turkestan region and laboratory studies were carried out at the Research Laboratory "Agricultural Biotechnology" of the South Kazakhstan University named after. M. Auezov.

The object of the research was Karakul sheep of beige color, including a light beige shade - 2 animals, a medium beige shade - 2 animals, and a dark beige shade - 2 animals.

The material for the research was freshly obtained and frozen-thawed sperm products of 6 rams with different breeds of beige color.

The selection of breeding rams, feeding, care, maintenance, and use of them as a seed producer was carried out in accordance with the requirements of the "Instructions for conducting breeding work in karakul breeding" (Degen, 2013).

A total of 32 ejaculates were studied, of which 8 ejaculates were from rams with a light beige color, 10 with a medium beige color, and 14 ejaculates with a dark beige color.

Receiving sperm from stud rams into an artificial vagina was performed with a sperm receptacle twice daily. Each ejaculate collected was assessed for volume, color, and smell. For the study, those ejaculates from rams were selected that, upon visual assessment after their receipt, had a viscous structure, without impurities and a milky white color.

For the effective use of beige-colored Karakul rams in the breeding season during artificial insemination of sheep, 32 ejaculate samples were examined and selected that meet the requirements of GOST No. 32222-2013 “Means of reproduction. Sperm " (Table 1).

Table 1: Quality indicators of native sperm (n = 32 ejaculates)

Index	Unit	M ± m	Cv,%	Min	Max
Volume	ml	1.49±0.046	17.4	1.1	2.3
Mobility	points	9.34±0.136	8.2	8	10
Concentration	billion/ml	2.49±0.062	14.2	1.9	3.0
Number of sperm in ejaculate	billion	3.73±0.155	23.6	2.31	6.6

Note to the table: M ± m – arithmetic mean ± error of the arithmetic mean; Cv,% - coefficient of variability, in %; Min – the minimum indicator; Max – the maximum indicator

When assessing the quality of the sperm of each ejaculate from experimental rams, we used the hardware and software complex for sperm analysis “VideoTest-Sperm 2.1”, intended for the sperm analysis of animals of different species.

The speed of movement of fresh and thawed sperm cells in the samples was determined simultaneously under phase contrast conditions using the automatic “Motility” module. At the same time, the speed of sperm movement is established by tracking the trajectory and speed of movement VAP - the average speed of movement of the head along the average trajectory (μm/sec). The obtained variation data are divided into 3 categories: Slow up to 109 μm/sec (from 80-109 μm/sec), medium-from 110-139 μm/sec, and fast -over 140 μm/sec (140-169 μm/sec).

Morphological parameters and acrosomy of sperm in the studied samples were applied to ready-made glass slides with a pre-painted layer of paint using the Morphology module.

The percentage of acrosome to sperm head area was determined by fluorescence microscopy in samples stained with the Diff-Quik kit.

After each test of sperm samples, a protocol is drawn up based on the test results, which include the ram number, sample number, and the obtained sperm quality parameters.

Glucose-citrate-yolk medium was used to dilute, freeze, and store the sperm of stud rams. The composition of this medium includes (per 100 mL of distilled water): Medical anhydrous glucose -0.8 g, sodium citrate trisubstituted pentahydrate -2.8 g, polygen -0.03 g, chicken egg yolk -20.0 cm^3. The sperm of the ram was diluted 2-4 times depending on the concentration of sperm in the ejaculate. The diluted sperm was cooled for 3 h in a refrigerator at a temperature of 2-4°C. After keeping the diluted sperm in the refrigerator, the sperm was placed in the well of a fluoroplastic plate and immersed in liquid nitrogen for 1 min to further cool the granules, then the plate was removed from the liquid nitrogen and the granules were collected in containers and transferred to Dewar flasks for storing sperm. The container was marked with the ejaculate number, date of collection, ram number, and number of pellets.

To determine the concentration and speed of sperm movement, a frozen sperm pellet was thawed in a water bath at 42°C. Thawed sperm was diluted 10 times with a 2.9% sodium citrate solution.

Then, using an automatic analyzer, we calculated the exact number and set the parameters for the speed of sperm cell movement.

Semen production of rams assessed by the speed of sperm movement and acrosomy was used according to the following research scheme (Fig. 1).

For artificial insemination of beige-colored Karakul ewes during the breeding season, the volume of freshly obtained sperm introduced into the vagina was 0.1-0.2 mL and the volume of frozen sperm was 0.4 mL.

Insemination of ewes was carried out with freshly obtained sperm from 141 animals and frozen-thawed sperm from 86 animals as they came into heat, at 7-8 o'clock in the morning and again at 16-18 o'clock in the evening. The experimental results were processed using biometric algorithms. To determine the share of influence of the studied factors on the fertility of ewes, the method of analysis of variance of one-factor complexes was used. The reliability of the results obtained was determined using the Student's test.

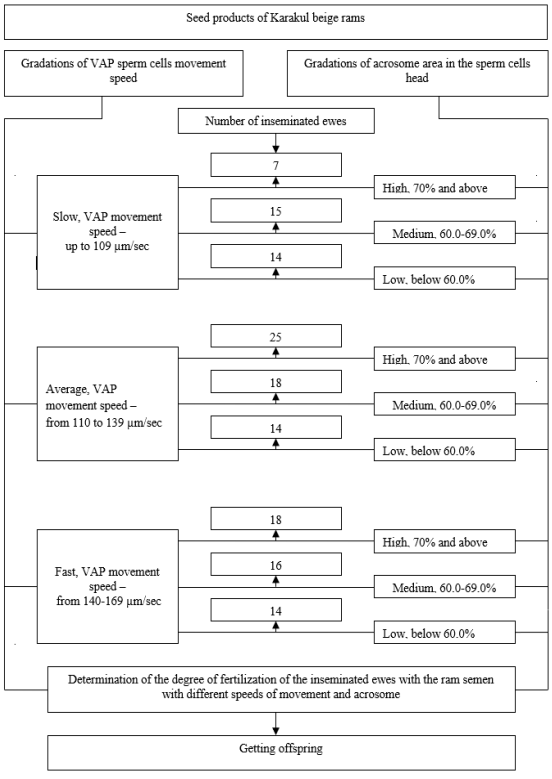

Fig. 1: Scheme for using ram semen from producers of beige-colored Karakul sheep

Results and Discussion

Sperm volume (VOL) - the amount of ejaculate, sperm concentration (CONC), total sperm count (TOT), motility of frozen and thawed sperm (MOT), and straw production (STR) were evaluated directly during collection (Isnaini *et al*., 2021). Sperm samples were diluted with egg yolk citrate diluent, then cooled and loaded into straws, and subsequently placed in a programmable freezer for freezing. Frozen sperm straws were stored in a tank with liquid nitrogen. The frozen sperm straws were then thawed at 15°C for 5 min and further evaluated after thawing from 1-15 min during in vitro (Mphaphathi and Nedambale, 2021). After sperm defrosting, sperm motility, and morphology were evaluated (Tshabalala *et al*., 2021).

32 ejaculates selected from 6 rams of beige Karakul sheep were examined for the speed of movement of the head along the average trajectory (µm/sec) - VAP and the area of the head occupied by the acrosome (Table 2).

Assessment of the speed of movement and the presence of acrosomy in sperm showed that the speed of movement of sperm averages 125.27±0.442 µm/sec and ranges from 80-169 µm/sec and the number of sperm with an acrosome area averages 63.05±2.049 percent and ranged from 40-86% with a coefficient of variation of 18.9 and 18.4%, respectively.

Based on the determination of the proportion of progressively mobile sperm cells, the resulting ejaculates are divided into 3 groups according to the speed of movement of spermatozoa: “Slow” with a movement speed of up to 109 µm/sec, “medium” with a movement speed ranging from 110-139 µm / sec and “fast” with a movement speed of -140 µm/sec and higher.

The results of the analysis of the distribution of the movement speed of sperm VAP show that among the studied groups the maximum specific weight is occupied by "medium" with a speed of movement ranging from 110-139 µm/ sec -53.1%, “fast” with a speed of movement over 140 µm/sec and “slow” with a movement speed of up to 109 µm/sec occupies 21.9 and 25%, respectively (Fig. 2).

Similarly, each group of sperm in the ejaculate, depending on the area of the acrosome, is also divided into 3 groups: (I) Group “high” with the content of the acrosome area in sperm ranging from 70.0% and above, (II) Group “medium” with the content of the area acrosomes in sperm from 60.0-69.0% and group (III) “Low” with acrosome area content in sperm up to 60.0%.

Table 2: VAP indicators and acrosomy of sperm cells of Karakul sheep of beige color (n-32 ejaculates)

Indicators	Unit	M ± m	Cv, %	Min	Max
Sperm head speed - VAP	µm/sec	125.27 ±0.442	18.9	80	169
Acrosome head area	%	63.05±2. 049	18.4	40	86

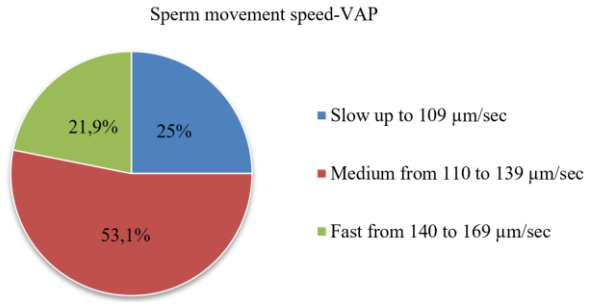

Fig. 2: VAP sperm velocity distribution

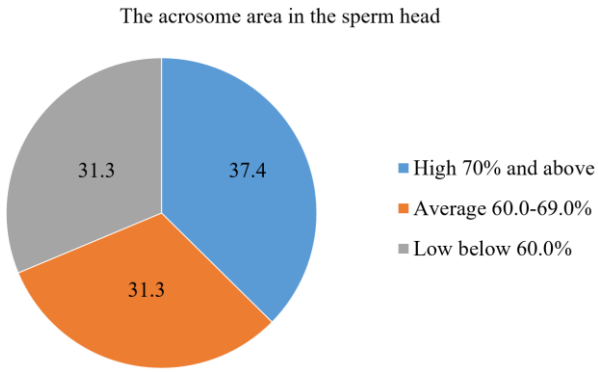

Fig. 3: Distribution of acrosome area in the sperm head

According to the area of the acrosome in the head of the sperm, the groups were distributed as follows: “High" with an area of 70% and above -37.4%, "medium" with an area of 60.0-69.0%, and "low" with an area below 60.0, 31.3% each (Fig. 3).

Analysis of Table (3) shows that the number of sperm with an acrosome in the studied ejaculates, depending on their speed of movement, were different and this indicator in different groups ranged from 3.1-21.9%.

It should be noted that among ejaculates with high specific gravity, semen production IV groups-21.9% and V groups 18.8%. The minimum number of ejaculates is characteristic of the I and VIII groups whose specific gravity is 3.1% of the total number of ejaculates.

Thus, the integration of the speed of head movement along the average trajectory µm/sec and the area of the acrosome in the head of the sperm showed a varied level of occurrence in the seed production of the population of beige-colored Karakul rams.

Based on the results of studies of 32 ejaculates from rams of the Karakul breed of beige color, according to the criteria of VAP gradation and acrosomy, 9 groups were subdivided (Table 3).

The fertilizing ability of sperm in ejaculation with different parameters of movement speed and the presence of sperm with different acrosomes was tested by artificial insemination on black ewes (Table 4).

Table 3: Distribution of ejaculates according to the speed of sperm movement and the area of the acrosome in the head

Groups			Number of ejaculates	
Group number	VAP movement speed	Acrosome area	n	%
I	Slow (up to 109 µm/sec)	High (70% and above)	1	3.1
II	Slow (up to 109 µm/sec)	Medium (60.0-69.0%)	3	9.4
III	Slow (up to 109 µm/sec)	Low (below 60.0%)	4	12.5
IV	Medium (from 110-139 µm/sec)	High (70% and above)	7	21.9
V	Medium (from 110-139 µm/sec)	Medium (60.0-69.0%)	6	18.8
VI	Medium (from 110-139 µm/sec)	Low (below 60.0%)	4	12.5
VII	Fast (from 140 µm/sec and above)	High (70% and above)	4	12.5
VIII	Fast (from 140 µm/sec and above)	Medium (60.0-69.0%)	1	3.1
IX	Fast (from 140 µm/sec and above)	Low (below 60.0%)	2	6.2
Total			32	100

Table 4: Results of artificial insemination of Karakul sheep

Groups			Indicators		
Group number	VAP movement speed	Acrosome area	Inseminated sheep, heads	Embraced ewes, heads	Fertility, %
I	Slow (up to 109 µm/sec)	High (70% and above)	7	5	71.4
II	Slow (up to 109 µm/sec)	Medium (60.0-69.0%)	15	9	60
III	Slow (up to 109 µm/sec)	Low (below 60.0%)	14	5	35.7
IV	Medium (from 110-139 µm/sec)	High (70% and above)	25	24	96
V	Medium (from 110-139 µm/sec)	Medium (60.0-69.0%)	18	15	83.3
VI	Medium (from 110-139 µm/sec)	Low (below 60.0%)	14	7	50
VII	Fast (from 140 µm/sec and above)	High (70% and above)	18	18	100
VIII	Fast (from 140 µm/sec and above)	Medium (60.0-69.0%)	16	16	100
IX	Fast (from 140 µm/sec and above)	Low (below 60.0%)	14	11	78.6
Total			141	110	78

From the data in Table 4, it is clear that the fertility of ewes depends not only on the speed of sperm movement in the ejaculate but also on the presence of acrosome area in the head of the sperm. For example, high fertility was observed in the group of ejaculates, where sperm motility and head area with acrosomes were "medium-medium" - 83.3%, "medium-high" 96.0%, “fast-medium" and "fast-high" - 100%.

The lowest fertility of ewes was obtained in the groups “slow-low”, “medium-low” and “slow-medium”, which ranged from 35.7-60%.

In the remaining groups of seed production, the fertilization rate was in the range of 71.4-78.6%. The average values of the fertility rate of ewes between groups are significant according to the Student's test in the range from $p<0.05$-$p<0.001$.

Subsequently, the method of variance analysis was used to calculate the share of influence of the studied factors on the fertility of ewes. It was found that the influence of the speed of sperm movement on the fertility of ewes ranged from 10.8-18.2% and the influence of the acrosome area in the sperm head was significantly higher than the speed of sperm and amounted to 21.7-28.5%. This means that the faster the speed of sperm movement and the larger the area of the acrosome in the head, the higher the fertility of ewes. In general, the data obtained once again convincingly demonstrate the need for a comprehensive and objective assessment of sperm parameters in terms of speed of movement and area in the sperm heads in ram ejaculates.

Thus, based on the research, criteria for selecting sheep ejaculate with high fertilizing ability have been determined, increasing the efficiency of insemination of animals of rare colors, while the speed of forward movement and the proportion of acrosomes in the head of sperm must be at least 110 μm/se and 60%, respectively. The established selection criteria for ram ejaculate increase the efficiency of insemination and genetic progress in populations of Karakul sheep of rare colors from 12.0-22.0%, which confirms the high fertilizing ability of sperm and proves the possibility of using a new method of sperm selection in practice.

Sperm cryopreservation is one of the effective tools for the conservation and use of genetic resources of farm animals.

In this regard, the influence of cryopreservation on the quantitative and qualitative indicators of seed production of beige Karakul rams was studied.

The fertilizing ability of frozen ram sperm was assessed after thawing (Table 5).

As the data in Table (5) shows, the cryogenic factor has a significant impact on sperm motility, regardless of the initial status (in the native state) in terms of speed of movement and the presence of acrosome area. Moreover, the number of sperm with forward movement in the studied ejaculate ranged from 11.9-65.4%, sperm with non-forward movement ranging from 11.1-52.5%, and immobile sperm from 20.2-50.9%. It should be noted that the frequency of occurrence of sperm with forward movement was higher in ejaculate with sperm motility and acrosomy status “medium-high” - 57.9%, “medium-medium” - 44.1%, “fast-high” - 65.4% and “fast-medium” -60.0%. The difference in sperm motility between these and other groups of ejaculate statistics is highly significant ($p<0.001$).

VAP movement and the area of the acrosome in the head of spermatozoa after freezing and thawing of various groups of freshly obtained sperm in beige-colored Karakul rams were studied (Table 6).

The results of the analysis of Table (6) show that in frozen-thawed sperm products the average speed of movement of the head along the average trajectory decreases by 30.7-97.4 μm/sec and the area of the head occupied by the acrosome by 9.2-36.7% and is respectively in the range of 49.62-68.67 μm/sec and 17.4-65.0%, that is, cryopreservation has a significant impact on the normal physiology of sperm, causing a decrease in the quality parameters of semen production. The impact of the detrimental effects of cryopreservation on the quality of seed products has been noted by many researchers and research is being undertaken to address this issue (23-24).

Table 5: Motility of frozen-thawed ram sperm

Groups			Indicators of frozen-thawed sperm by mobility, %			
Fresh sperm status			Sperm count	Forward movement	Non-progressive movement	Motionless
Group number	Movement speed VAP	Acrosome area				
I	Slow (up to 109 μm/sec)	High (70% and above)	516	23.8	27	49.2
II	Slow (up to 109 μm/sec)	Medium (60.0-69.0%)	618	19.4	37.2	43.4
III	Slow (up to 109 μm/sec)	Low (below 60.0%)	941	11.9	52.5	35.6
Average			2075	17.1	41.6	41.3
IV	Medium (from 110-139 μm/sec)	High (70% and above)	299	57.9	11.7	30.4
V	Medium (from 110-139 μm/sec)	Medium (60.0-69.0%)	361	44.1	20.2	35.7
VI	Medium (from 110-139 μm/sec)	Low (below 60.0%)	524	26.3	33	40.7
Average			1184	39	23.7	37.3
VII	Fast (over 140 μm/sec)	High (70% and above)	263	65.4	14.4	20.2
VIII	Fast (over 140 μm/sec)	Medium (60.0-69.0%)	272	60	14.3	25.7
IX	Fast (over 140 μm/sec)	Low (below 60.0%)	371	38	11.1	50.9
Average			906	52.5	13	34.5
Total			4165	31.1	30.3	38.6

Table 6: Comparative assessment of the speed of movement of spermatozoa with acrosomes before and after freezing

Groups			Indicators			
Condition of sperm before freezing			Condition of sperm after freezing			
Group number	Movement speed VAP	Acrosome area	Average movement speed, µm/s	Degree of reduction, µm/sec	Sperm with acrosomes, %	Percentage reduction
I	Slow (up to 109 µm/sec)	High (70% and above)	58.77±0.674	48.4	38.7±5.44	36.7
II	Slow (up to 109 µm/sec)	Medium (60.0-69.0%)	63.68±0.794	36	30.7±6.31	35.5
III	Slow (up to 109 µm/sec)	Low (below 60.0%)	68.67±0.706	30.7	17.4±5.59	32.7
	Average (100.3)	Average (58.9)	62.77±0.522	37.5	30.7±3.45	28.2
IV	Medium (from 110-139 µm/s ec)	High (70% and above)	50.83± 0.339	71.6	58.3±3.03	18.5
V	Medium (from 110-139 µm/sec)	Medium (60.0-69.0%)	59.66±0.755	70.6	53.9±3.88	12.5
VI	Medium (from 110-139 µm/sec)	Low (below 60.0%)	62.81±0.479	60.8	39.6±6.72	14.5
	Average (125.3)	Average (67.7)	55.42±0.403	69.8	54.7±2.20	13
VII	Fast (over 140 µm/sec)	High (70% and above)	49.62±0.479	97.4	65.0±2.06	17.8
VIII	Fast (over 140 µm/sec)	Medium (60.0-69.0%)	58.85±0.350	84.5	59.6±3.07	9.2
IX	Fast (over 140 µm/sec)	Low (below 60.0%)	60.77±0.671	84	48.4±6.34	10.7
	Average (145.8)	Average (73.6)	54.64±0.280	91.1	61.0±1.98	12.6
	Overall average -124.6	Overall average -67.2	56.07±0.230	68.5	54.4±1.38	12.8

Table 7: Fertility of ewes with frozen-thawed sperm

Group number	Groups				Indicators	
	Before freezing		After defrosting		Inseminated ewes, heads	Fertilization rate,%
	Movement speed VAP	Acrosome area	Sperm movement speed, µm/sec	Acrosome area,%		
I	Slow (up to 109 µm/sec)	High (70% and above)	58.7±0.67	38.7	9	33.3
II	Slow (up to 109 µm/sec)	Medium (60.0-69.0%)	63.6±0.79	30.7	9	22.2
III	slow (up to 109 µm/sec)	Low (below 60.0%)	68.6±0.70	17.4	9	-
Average			62.8±0.52	30.7	27	18.5±7.5
IV	Medium (from 110-139 µm/sec)	High (70% and above)	50.8±0.34	58.3	9	88.9
V	Medium (from 110-139 µm/sec)	Medium (60.0-69.0%)	59.7±0.75	53.9	10	70.0
VI	Medium (from 110-139 µm/sec)	Low (below 60.0%)	62.8±0.48	39.6	9	44.4
Average			55.4±0.40	54.7	28	67.9±8.8
VII	Fast (over 140 µm/sec)	High (70% and above)	49.6±0.48	65.0	11	72.7
VIII	Fast (over 140 µm/sec)	Medium (60.0-69.0%)	58.8±0.35	59.6	10	80.0
IX	Fast (over 140 µm/sec)	Low (below 60.0%)	60.9±0.67	48.4	10	50.0
Average			54.6±0.28	61.0	31	67.7±8.4
Total			56.1±0.23	54.4	86	52.3±5.4

The main indicator in assessing the fertilizing ability of frozen-thawed ram semen is the fertility of ewes. Therefore, we studied the degree of fertilization of the uterus depending on the speed of sperm movement and the indicator of the acrosome area in the head (Table 7).

An analysis of the fertility results of ewes with frozen-thawed semen shows that groups IV, V, VII, and VIII have relatively high fertility rates and are respectively 88.9, 70.0, 72.7, and 80.0%.

Summary

When cryopreserving the semen of Karakul sheep of beige color, assessment of the speed of sperm movement and the area of the acrosome of the head are of decisive importance when breeding livestock of rare colors that are on the verge of extinction.

Thus, one of the effective tools in increasing the fertilizing ability of seed production in rams of beige Karakul sheep is the integration of the speed of movement of the head along the average trajectory (µm/sec) - VAP and the area of the head occupied by the acrosome, is the selection of seed, the indicators of which are based on the speed of movement the head is at least 110 µm/sec and the area of the head occupied by the acrosome is more than 60%.

Conclusion

A method has been developed for determining the fertilizing ability of sperm in Karakul sheep, including a visual assessment of the quality of the ejaculate, qualitative and quantitative indicators of sperm on a sperm analyzer, characterized in that, in order to increase the effectiveness of the method, the speed of movement of VAP spermatozoa and the acrosome area are additionally evaluated and for artificial insemination and cryopreservation, those freshly obtained ejaculates in which the spermatozoa have translational motion at a speed of at least 110 microns/s, containing 60% or more of spermatozoa with acrosomes.

A method for selecting sheep sperm with new morphological criteria, movement speed parameters, and acrosome area in the sperm head has been developed, contributing to increasing the genetic progress of beige reproduction in Karakul sheep populations by 12.0-22.0% more than the traditional method of selecting sheep seeds

and proves the need to introduce a new selection method into artificial insemination technology in karakul farming.

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Author's Contributions

Aigerim Turekyzy Bigarayeva: Responsible executor, experimental part of the research. Preparation of the manuscript. Share of implementation and contribution to the preparation of the article.

Nuradin Alibaev: Author of the idea, analysis, and generalization of the obtained data, Share of implementation, and contribution to the preparation of the article.

Arkadiy Kanurovich Natyrov: Performer, analysis of experimental data. Share of implementation and contribution to the preparation of the article.

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Ethics

During the research, ethics were observed in the process of studying the milk productivity of experimental animals of the studied breed. The authors of the article confirm the absence of a conflict of interest with third-party organizations.

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