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QUALITATIVE INDICATORS OF RAM-SIRES THAWED SPERM, FROZEN IN DIFFERENT PERIODS OF SEXUAL ACTIVITY

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The purpose of our research was to study qualitative indicators of sperm productivity and its fertility depending on seasonal activity of such Transcarpathian and Carpathian breeds as Ukrainian Carpathian Mountain and Precose, and local crossbred rams (3/4 Suffolk x 1/4 Precose genotype) during breeding and non-breeding seasons. Qualitative indicators of ram thawed sperm, its activity in particular, taking into account the kinetic parameters using a computer program «Sperm Vision», the sperm resistance to freezing, surviving and the absolute survival indicator, fertility of the semen which was frozen in different periods of sexual activity, have been studied. It is established that the thawed sperm of ram-sires from the Ukrainian Carpathian Mountain breed, the breed Precose and local sires of 3/4 Suffolk x 1/4 Precose, which has been collected and frozen during breeding and non-breeding seasons, has good morphological quality and high ability for ewes fertilization. However, the sperm conserved during the breeding period, has slightly higher fertility during the first insemination of sheep by 3.7–4.7 %, and by 2.6–3.1 % during all inseminations.

Sperm quality and fertility indicators of the Ukrainian Carpathian Mountain breed of ram-sires have been discovered to be slightly higher than those of the Precose breed and local sheep 3/4 Suffolk x 1/4 Precose ram-sires, which is most likely a result of the local sheep being more adapted to natural climatic conditions of the Carpathian region.

Keywords: SEASONALITY OF SEMEN PRODUCTION, CRYOPRESERVATION, FROZEN-THAWED SPERM, INSEMINATION, FERTILITY, LAMBING, LAMB SURVIVAL

ЯКІСНІ ПОКАЗНИКИ ДЕКОНСЕРВОВАНОЇ СПЕРМИ БАРАНІВ-ПЛІДНИКІВ, ЗАМОРОЖЕНОЇ У РІЗНІ ПЕРІОДИ СТАТЕВОЇ АКТИВНОСТІ

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Сезонні кліматичні чинники призводять до зниження статевої активності та погіршення кількісних і якісних показників спермопродуктивності у плідників. Метою досліджень було вивчити якісні показники спермопродуктивності та запліднюючу здатність сперми залежно від сезонної активності плідників у парувальний і непарувальний періоди районованих порід Закарпаття та Прикарпаття — української гірськокарпатської породи, породи прекос та помісних баранів генотипу 3/4суффолк x 1/4 прекос.

Вивчено якісні показники деконсервованої сперми баранів-плідників, зокрема її активності, з врахуванням кінетичних показників за комп'ютеризованою програмою CASA (Computer Assisted Sperm Analysis) — Sperm Vision, стійкості спермій до заморожування, виживаності і показника абсолютного виживання, а також запліднюючої здатності сперми, яка була заморожена у різні періоди статевої активності. Встановлено, що деконсервована сперма баранів-плідників української

гірськокарпатської породи, породи прекос і помісних плідників 3/4 суффолк х 1/4 прекос одержана і заморожена у парувальний і непарувальний період, володіє достатньо високими якісними показниками та запліднюючою здатністю. Однак сперма кріоконсервована у парувальний період, має децю вищу запліднюючу здатність при першому осіменінні овець на 3,7–4,7 %, при всіх осіменіннях — на 2,6–3,1 %.

Виявлено децю вищі показники якості та запліднюючої здатності сперми у баранів-плідників української гірськокарпатської породи порівняно з плідниками породи прекос і помісними баранами 3/4 суффолк х 1/4 прекос, які, очевидно, детермінуються з кращою пристосованістю місцевих овець до природно-кліматичних умов карпатського регіону.

Ключові слова: СПЕРМОПРОДУКТИВНІСТЬ, КРІОКОНСЕРВУВАННЯ, ДЕКОНСЕРВОВАНА СПЕРМА, ЗАПЛІДНЮЮЧА ЗДАТНІСТЬ, ОКОТИ, ЗБЕРЕЖЕНІСТЬ ЯГНЯТ

КАЧЕСТВЕННЫЕ ПОКАЗАТЕЛИ ДЕКОНСЕРВИРОВАННОЙ СПЕРМЫ БАРАНОВ-ПРОИЗВОДИТЕЛЕЙ, ЗАМОРОЖЕННОЙ В РАЗНЫЕ ПЕРИОДЫ ПОЛОВОЙ АКТИВНОСТИ

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Сезонные климатические факторы приводят к снижению половой активности и ухудшению количественных и качественных показателей спермопродуктивности в производителей. Целью исследований было изучить качественные показатели спермопродуктивности и оплодотворяющую способность спермиев в зависимости от сезонной активности производителей в случной и неслучной периоды районированных пород Закарпатья и Прикарпатья — украинской горнокарпатской, прекос и поместных баранов генотипа 3/4 суффолк х 1/4 прекос.

Изучены качественные показатели деконсервированной спермы баранов- производителей, в частности активность спермиев с учетом кинетических показателей по компьютеризированной программе CASA (Computer Assisted Sperm Analysis) — Sperm Vision, их устойчивость к замораживанию, выживаемость и показатель абсолютного выживания, а также оплодотворяющую способность спермиев, замороженных в разные периоды половой активности. Установлено, что деконсервированная сперма баранов украинской горнокарпатской породы, породы прекос и поместных производителей 3/4 суффолк х 1/4 прекос, полученная и замороженная в случной и неслучной периоды, обладает достаточно высокими качественными показателями и оплодотворяющей способностью. Однако сперма, кріоконсервованная в случной период, имеет несколько более высокую оплодотворяющую способность при первом осеменении овец на 3,7–4,7 %, при всех осеменениях — на 2,6–3,1 %.

Виявлено несколько высшие показатели качества и оплодотворяющей способности спермы у баранов-производителей украинской горнокарпатской породы по сравнению с производителями породы прекос и поместными баранами 3/4 суффолк х 1/4 прекос, которые, вероятно, детерминируются лучшей приспособленностью местных овец к природно-климатическим условиям карпатского региона.

Ключевые слова: СПЕРМОПРОДУКТИВНОСТЬ, КРІОКОНСЕРВИРОВАНИЕ, ДЕКОНСЕРВИРОВАННАЯ СПЕРМА, ОПЛОДОТВОРЯЮЩАЯ СПОСОБНОСТЬ, ОКОТ, СОХРАННОСТЬ ЯГНЯТ

The modern development of sheep breeding is characterized by a wide use of biotechnological methods of breeding which provide a large number of descendants from one ram-sire and, therefore, its influence as a carrier of the genetic basis on breeding, productive and technological qualities of significant groups of sheep or their flocks is represented. As a result, objective assessment of ram-sires' reproductive function as well as their use in breeding and commercial flocks becomes very important. However, in order to make maximum use of the mentioned genetic feature, it is necessary to take into account both external and internal factors for a ram-sires' breeding function [1–3].

According to literature sources, it is clear that sperm production and secretion of the sex hormone testosterone of ram-sires of all breeds are continuous under favorable conditions of keeping and balanced feeding. However, numerous studies and practical observations show that seasonal climatic factors lead to a decrease in sexual activity and deterioration of quantitative and qualitative indicators of ram-sires' sperm production. Yet, such dependency manifests itself differently in different natural climatic zones [4–12].

Based on the fact that in Ukraine research into the influence of genotypic and paratypical factors on sperm productivity and fertility of Askanian fine-wool, Askanian karakul multifetal type, Caucasian and Australian Merino breeds was mainly conducted in southern regions, the purpose of our research was to study qualitative indicators of sperm production and its fertility depending on seasonal activity of such Transcarpathian and Carpathian breeds as Ukrainian Carpathian Mountain and Precose, as well as local crossbred rams of 3/4 Suffolk x 1/4 Precose genotype during breeding and non-breeding seasons of the year.

Materials and methods

The study of quantitative and qualitative indicators of sperm production and its fertility which depends on ram-sires seasonal reproduction activity during breeding

(April–July) and non-breeding (August–October) periods was conducted in the gene pool laboratory of Lviv Scientific-Production Center (LSPC) «Zahidplemresursy», Educational-Scientific-Production Center (ESPC) «Komarnivsky», peasant farm organization (PFO) «Saldomash». 17 mature ram-sires were used in the experiment, among which there were 6 heads of Ukrainian Carpathian Mountain sheep (UCMS), 6 heads of the Precose breed and 5 — local rams of 3/4 Suffolk x 1/4 Precose.

Freshly collected sperm was estimated according to its volume, sperm concentration, total amount of sperm in the ejaculate, sperm motility and speed of motion, the amount of sperm with a progressive motion and resistance by using common methods. Ram sperm with motility of at least 80 % and concentration of not less than $2.5 \cdot 10^9$ /ml was used for dilution and freezing. The diluted sperm was packaged in straws and cooled for 3–4 hours at a temperature of +2–4 °C equitainer (Minitub Germany). After that the straws were plunged into vapour of nitrogen (-120 °C) for 20 minutes and then plunged into liquid nitrogen.

The frozen-thawed sperm was analysed for sperm motility after equilibration and cryopreservation, sperm kinetic characteristics, sperm resistance to freezing, sperm survival and the absolute survival indicator (Sa). Sperm resistance to freezing was determined as the ratio of the number of active gametes with a straight-forward motion after thawing to the number of sperm with a straight-forward motion after equilibration. Thawed sperm survival rate Thawed sperm survival rate was determined under the microscope (x200) at 38 °C every 0.5 hour until no motile spermatozoa were observed. The sperm absolute survival indicator at +38 °C was calculated using a simplified formula: $Sa = a^{1/2} + \sum (a \times t) \times n$, (where Sa — absolute survival indicator; \sum — sum; a — assessment of sperm motility in examination points; t — indicator of time interval between the previous and the next test of sperm; n — number of observations).

With the aim of a comparative study of ram-sires' sperm fertility of the UCMS, the Precose breed and local crossbred rams of 3/4 Suffolk x 1/4 Precose collected during different periods of seasonal activity, the thawed sperm frozen in May (non-breeding season) and in September (breeding season) was used for sheep insemination. To achieve this, in ESPC «Komarnivsky» and PFO «Saldobush», three groups of ewes were selected according to an analogue principle: the first group included 86 head of the UCMS; the second group included 66 head of the Precose breed and the third group included 52 head of local crossbred ewes of 3/4 Suffolk x 1/4 Precose breed, which were analogues by breed, age, body weight, fatness and physiological status. Each group was divided into 2 subgroups. One of them was inseminated with the sperm frozen during the breeding season, the other one — with sperm from the non-breeding season.

With the help of visual observation and ram samples, clinically healthy sheep of average fatness with signs of sexual hunting were selected for artificial insemination and were inseminated using a laparoscope of Wolf (Germany) production. The laparoscope is put through incision in the belly of ewe to look at female uterus. On the opposite side is put

through insemination pipette. Under a visual control using the laparoscop, the wall of the uterus horn was punctured with a pipette needle 0.04 mm in diameter and 0.125 ml of sperm was placed into each uterine horns.

In total, 204 ewes were inseminated. Just after lambs were born, their sex and body weight were determined and inventory numbers were given.

Results and discussion

An important indicator of the quality of thawed sperm is its activity and survival. This studies found that the period of seasonal activity and the animal breed directly affect quality of collected sperm and its usefulness for freezing (tabl. 1).

Therefore, during the breeding period (July–November) compared to the non-breeding period (April–June), ram-sires of the investigated breeds and genotype were observed to have a higher activity of sperm after equilibration by 7.3–16.5 %, while that of the frozen-thawed ones — by 12.4–24.2 %. Sperm resistance before cryopreservation was also higher during the breeding period by nearly 2.3–3.2 %

Table 1

Sperm activity and resistance of ram-sires before and after cryopreservation (M±m)

Indicators	Period of seasonal activity					
	I (non-breeding)			II (breeding)		
	breed, genotype					
	UCMS	Precose	3/4 Suffolk x 1/4 Precose	UCMS	Precose	3/4 Suffolk x 1/4 Precose
No. of rams	6	6	5	6	6	5
No. of ejaculates	48	54	50	48	54	50
Motility of collected semen, %	91.4±0.14	81.2±0.18	81.0±0.28	97.4±0.08**	92.3±0.11***	93.2±0.10***
Motility of semen after equilibration, %	82.0±0.29	71.1±0.25	70.2±0.2	88.0±0.12*	82.8±0.13***	81.5±0.12***
Motility of frozen-thawed semen, %	40.2±0.1	34.0±0.21	31.8±0.14	45.2±0.16*	42.0±0.11**	39.5±0.10***
Sperm resistance to freezing, %	49.02±2.02	47.81±3.57	45.30±2.14	51.36±1.56	50.72±1.33	48.47±1.49

Note: Variance in this and the following tables: * — p<0.05; ** — p<0.01; *** — p<0.001

In order to obtain a complete description of sperm dynamic properties in

addition to the motility and activity of sperm with a straight-forward movement, we studied

sperm kinetic indicators using computer system CASA (Computer Assisted Sperm Analysis) — «Sperm-Vision» which allows the user to completely describe their motion parameters with the help of technological equipment produced by the German company «Minitub» (tabl. 2).

While analyzing sperm kinetic indicators, such sperm motion parameters were found as: curvilinear velocity (VCL), average path velocity (VAP), straight line velocity (VSL). The parameters in terms of VCL were

higher by 12.8–17.0, while those in terms of VAP — by 15.5–25.04 and VSL 12.7–20.29 %, respectively. At the same time, no significant difference in terms of the linearity degree (LIN=VSL/VCL) between the studied periods was found. However, a slight decrease in the degree of sperm straightness (STR=VSL/VAP) by 2.0–3.4 % and a slight increase in wobble (WOB=VAP/VCL) by 1.3–3.2 percent was stated.

Table 2

Kinetic indicators of ram-sires' thawed sperm (M±m)

Indicators	Period of seasonal activity					
	I (non-breeding)			I (breeding)		
	breed, genotype					
	UCMS	Precose	3/4 Suffolk x 1/4 Precose	UCMS	Precose	3/4 Suffolk x 1/4 Precose
No. of rams	6	6	5	6	6	5
No. of ejaculates	48	54	50	48	54	50
Motility of frozen-thawed semen with straight-forward movement, %	51.9±3.11	46.1±3.03	43.9±3.29	56.4±2.09	54.8±1.98*	53.2±1.92*
	40.2±1.0	34.0±2.1	31.8±1.4	45.2±1.6*	42.0±1.1**	39.5±1.0***
VCL, µm/s	149.3±3.2	140.4±1.94	131.1±1.21	168.4±1.36***	162.2±1.80***	153.4±1.69***
VAP, µm/s	76.5±1.72	67.8±2.31	60.3±1.55	88.4±0.98***	81.7±1.45***	75.4±1.12***
VSL, µm/s	64.1±1.27	59.2±2.03	54.7±1.54	72.3±1.5**	68.7±1.73**	65.8±1.30***
LIN, %	42.9±0.99	42.2±1.63	41.7±1.25	42.9±0.91	42.4±1.19***	42.9±0.96
STR, %	83.8±1.5	87.3±1.03	90.7±0.58	81.8±1.38	84.2±1.05*	87.3±0.87**
WOB, %	51.2±1.69	48.2±1.66	46.0±1.25	52.5±0.7	50.4±1.16	49.2±0.97*

Moreover, fluctuations in sperm motion between the studied ram-sire species and genotype in terms of the specified parameters were determined. Thus, the rams of the Ukrainian Carpathian Mountain breed during the breeding period showed a curvilinear velocity of 168±1.36 µm/s which is higher than that of Precose breed by 3.8 % and of local rams 3/4 Suffolk_x 1/4 Precose by 9.8 %. Similarly, the rams of the Ukrainian Carpathian Mountain breed had a higher average path velocity compared to ram-sires of the Precose breed and local rams by 8.2 and 17.2 percent respectively. Similar results of the straight line velocity were obtained. While this parameter of Ukrainian Carpathian Mountain rams accounted for 72.3±1.5 µm/s, the one of Precose breed sires — 68.7±1.73µm/s, and

that of local rams — 65.8±1.30 µm/s or less, by 5.2 and 9.9 percent respectively.

Identical fluctuations in terms of the analyzed sperm motion parameters were found between ram species and genotype during the non-breeding period. They were highest for Ukrainian Carpathian Mountain breed of rams and lowest for the local rams. On average, the difference in the curvilinear velocity was 6.3–13.9 %, while the one in the average path velocity was 12.8–26.8 %, and the straight line velocity accounted for 8.2–17.2 %. The figures obtained for the studied rams were similar to those obtained during the breeding period, in terms of the degree of linearity, the difference was 0.7 and 1.2 %, while in terms of the degree of sperm movement straightness — 3.5

and 6.9 % and in terms of the degree of deviation — 3.0 and 5.2 percent.

Consequently, according to the periods of seasonal activity, rams and genotype experienced quite high fluctuations not only in sperm motility and activity, but also in terms of the kinetic parameters, whose determination allows for analysis of ejaculate much faster, and automatically calculates the volume of diluent and the exact sperm dose.

By assessing the frozen-thawed sperm according to sperm survival and the absolute survival indicator, it was found that during the breeding season, sperm survival rate at +38 °C was 7.05±0.11 h for Ukrainian Carpathian Mountain breed rams and 6.46±0.14 for Precose breed, and 6.02±0.25 for the 3/4 Suffolk x 1/4 Precose genotype, which was higher than during the non-breeding season by 8.7, 16.6, 18.0 % respectively (tabl. 3).

Table 3

The studied ram-sires thawed sperm survival (M±m)

Indicators	Period of seasonal activity					
	I (non-breeding)			I (breeding)		
	breed, genotype					
	UCMS	Precose	3/4 Suffolk x 1/4 Precose	UCMS	Precose	3/4 Suffolk x 1/4 Precose
Number of sperm dose	48	54	50	48	54	50
Sperm surviving rate, hours	6.48±0.23	5.54±0.14	5.10±0.32	7.05±0.11*	6.46±0.14***	6.02±0.25*
Absolute survival indicator, S.U	12.95±0.25	11.45±0.27	11.12±0.12	14.12±0.33*	13.21±0.15***	13.15±0.17***

The absolute survival indicator of the studied sires was also higher in the breeding period — by 9.0; 15.4; 18.3 percent respectively.

At present, there is a sufficient number of references available which prove that, along with individual, age and seasonal factors there are natural fluctuations in sperm productivity and resistance before cooling and cryopreservation. Therefore, we have analyzed the above indicators of ram-sires sperm of the studied rams and genotypes and found that a higher activity after equilibration and cryopreservation was shown by the Ukrainian Carpathian Mountain breed compared to the Precose breed and the local rams during both breeding and non-breeding periods (tabl. 1). The sperm activity after adaptation in the breeding period was higher by 6.3 and 8.0 % respectively, after freezing and thawing — by 7.6 and 14.4 % in the non-breeding period these figures were higher by 15.3 and 16.8 % and by 18.2 and 26.4 percent respectively.

The sperm of Ukrainian Carpathian Mountain breed rams has a higher resistance rate to freezing (in both of the studied periods) compared with Precose breed rams and local

rams, in the breeding season by 0.7–2.9 %, while in the non-breeding season — by 1.2–3.7 percent. The sperm surviving at t +38 °C and the sperm absolute survival indicator of the Ukrainian Carpathian Mountain breed rams were also higher in both periods and accounted for 6.48±0.23 h and 12.95±0.25 S.U. In terms of sperm survival, this figure exceeded the Precose breed rams' figures by 9.1 % and the local rams' by 17.1 % during the breeding period, whereas by 17.0 and 27.1 % respectively during the non-breeding period; in terms of the absolute survival indicator — by 6.9 and 7.4 % and by 13.1 and 16.5 % respectively.

Thus, the study showed that the sperm of different rams according to the direction of breed and genotype productivity can be frozen and accumulated in the breeding and the non-breeding periods with the aim of maximizing the use of valuable ram-sires by using cryopreserved sperm for sheep insemination in both their breeding areas and in other areas of the country.

In order to determine the effectiveness of sheep insemination by cryopreserved ram-sires sperm collected and frozen in the breeding and the non-breeding periods of sexual activity, we carried out scientific

practical research in PFO «Saldobush» and ESPC «Komarnivsky». The results of sheep insemination by thawed sperm, frozen in the breeding and the non-breeding periods, were slightly different in terms of fertility, periods of cryopreservation and breed or crossbred.

After the first insemination of sheep with sperm frozen in the breeding period, 72.7

percent of females of the Ukrainian Carpathian Mountain breed, 65.6 percent of females of the Precose breed and 64.0 percent of local females of the 3/4 Suffolk x 1/4 Precose were fertilized, which is by 3.7, 3.8 and 4.7 percent higher than of the females inseminated by the cryopreserved sperm of the same rams, but collected in the non-breeding period (tabl. 4).

Table 4

Analysis of the sheen fertility after insemination by thawed ram sperm frozen in the breeding and the non-breeding periods

Indicators	Period of seasonal activity					
	I (non-breeding)			I (breeding)		
	breed, genotype					
	UCMS	Precose	3/4 Suffolk x 1/4 Precose	UCMS	Precose	3/4 Suffolk x 1/4 Precose
No of inseminated sheep	42	34	27	44	32	25
Fertilization rate of the ewes after first insemination, heads, %	29–69.0	21–61.8	16–59.3	32–72.7	21–65.6	16–64.0
Fertilization rate of the ewes after all inseminations, %	92.9	91.2	88.9	95.5	93.8	92.0
Number of ewes that have lambed, heads	39	31	24	42	30	23
No of lambs lambed	45	44	34	48	42	32
Fertility index, %	107.1	122.4	125.9	109.1	131.3	128.0
Number of lambs weaned, heads	41	39	31	44	37	29
Fertility index after weaning, %	97.6	114.7	114.8	100.0	115.6	116.0
Lamb survival, %	91.1	86.7	91.2	91.7	88.1	90.6

The fertilization rate of the ewes after all inseminations with ram sperm frozen in the breeding season accounted for 95.5 % of those of the Ukrainian Carpathian Mountain breed, 93.8 % of Precose breed and 92.0 % of the local rams, which is by 2.6 and 3.1 % higher than after insemination with the sperm collected and frozen in the non-breeding season. As a result, the thawed sperm of the studied ram-sires, which was collected and frozen in the breeding and the non-breeding periods has a quite high fertilizing ability. However, the sperm cryopreserved in the breeding period has a slightly higher fertilizing ability. A slight difference of two percent was observed in the fertility of the females, inseminated with the sperm frozen in the breeding as compared to the non-breeding periods as well as the lamb birth rate and weaning. However, no difference between the

lamb survival and the insemination with the sperm frozen in the breeding and the non-breeding periods was found.

Therefore, one of the important reserves of the increase in the efficient use of highly productive ram-sires is the prolongation of their exploitation period by freezing and accumulating sperm in the breeding period. The conducted research demonstrates that by ensuring proper conditions of feeding and keeping, Ukrainian Carpathian Mountain breed and Precose breed ram-sires, as well as the local rams of 3/4 Suffolk x 1/4 Precose, produce high-quality sperm suitable for cryopreservation not only in the breeding period, but also the non-breeding period. This is of practical importance for preserving the gene pool of valuable rams, and enables use of such sires either for deeper selection breeding work, or for mass productivity increase.

Conclusions

1. The studied ram-sires' thawed sperm collected and frozen in the breeding and non-breeding periods has quite high qualitative indicators and fertility rates. However, the sperm cryopreserved in the breeding period shows a slightly higher fertility rate after the first sheep insemination by 3.7–4.7 %, and after all inseminations — by 2.6–3.1 percent.

2. It was found that by ensuring proper conditions of feeding and keeping, Ukrainian Carpathian Mountain breed and Precose breed ram-sires, as well as the local rams of 3/4 Suffolk x 1/4 Precose produce high-quality sperm suitable for cryopreservation in the breeding and the non-breeding periods. This is of practical importance for preserving the gene pool of valuable rams, and enables use of such sires either for deeper selection breeding work, or for mass productivity increase.

3. Higher sperm quality indicators and fertility of ram-sires of the Ukrainian Carpathian Mountain breed compared to sires of the Precose breed and local rams of 3/4 Suffolk x 1/4 Precose are most likely the result of the local sheep being better adapted to natural climatic conditions of the Carpathian region.

Perspectives of further research.

Results will serve as a basic for further research on improving the quality indicators of ram frozen-thawed semen

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