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EFFECTIVENESS OF REPRODUCTIVE BIOTECHNOLOGICAL METHODS APPLICATION IN UKRAINIAN CARPATIAN MOUNTAIN SHEEP BREED

Наведено результати використання біотехнологічних методів відтворення у розведенні овець української гірськокарпатської (УГК) породи. Встановлено високу ефективність застосування розроблених нами методів стимуляції ранніх окотів, статевої охоти в анестральний період, синхронізації еструсу в парувальний сезон; апробовано в практику гірськокарпатського вівчарства лапароскопічний метод штучного осіменіння овець. Удосконалений метод кріоконсервування сперми (середовище, технологічні параметри заморожування) забезпечив підвищення якості спермiїв баранів УГК породи.

Ключові слова: вівці, українська гірськокарпатська порода, стимуляція, синхронізація, кріоконсервація, сперма.

Statement of the problem. The sheep breeding in the Carpathian mountains districts is specific. On this territory there is possibility of breeding practically only mountain breeds of sheep, in particular Ukrainian Carpathian Mountain sheep, that is well adapted to the local conditions [1, 2]. A not simple situation that we have in the stock-raising of our state and, in particular, in the sheep breeding, induces to the search and introduction of new, non-standard approaches and methods in the industry. Therefore development of fundamentally new approaches in breeding- work, in particular, with the use of the last achievements of biotechnology will give an opportunity to perfect the existing breeds of animals, promoting their productivity and adjusting to industrial technologies [3, 4].

Analysis of last research and publications. It is experimentally well-proven that application of biotechnological methods of reproduction allows considerably to accelerate the breeding effect and the reconstruction of valuable genetic material [5]. Seasonal sexual activity of UCM breed ewes results in a volume, that lambs give birth in the end of January – February that does not give an opportunity to get lamb in a period of high demand – in spring (so-called "Easter lambs") [6]. Introduction of biotechnological of intensification of the sheep breeding needs development of methods of governing the reproductive functions of sheep. The special place among them belongs to the method of stimulation of oestrus in a pairing and anoestrus periods in the optimally short terms and birth of even-aged lambs and increases their survival [7, 8].

The research aim was to study the efficiency of the use of the newest biotechnological methods of reproduction in breeding Ukrainian Carpathian Mountain sheep.

Materials and methodology of research. Our research was conducted during 2008–2013 on 3–4-years-old ewes of UCM sheep weighting 40–45 kg at the "Saldobosh" farm in the Khust district of Transcarpathion area. For stimulation early lambing of experimental animals for rapid fallowing oestrus was stimulated by means of vaginal sponges with progesterone "Hronogest" (Netherlands) and sheep-inseminators insertion three days prior to taking out or during taking out vaginal sponges.

With the aim of oestrus synchronization in a pairing period (August–September) the vaginal sponges "Hronogest" were inserted into ewes, and were taken out after 14 days. On day 16 the analogue of gonadotropin-releasing hormone (GnRH) – Surfagon in a dose 15 mg was intramuscularly injected and artificial insemination was made by laparoscope method.

For induction of oestrus in an anoestrus period (April–July) vaginal sponges with progesteron "Hronogest" were inserted to ewes on the14-th day. After taking out sponges the experimental animals were intramuscularly injected with 350 IU of pregnant mare serum gonadotropin (PMSG, Foligon) containing bioactive substances (BAS, Unitiol – 10 mg, Inosinum – 250 mg, Insolvit – 3 ml,

dimethylsulphoxid – 10 %). On day 16 during laparoscope insemination the analogue GnRH of Surfagon in a dose 10 mg was intramuscularly injected.

During experiments the laparoscopic estimation of ovaries was conducted, the concentration of progesteron and estradiol as well as fertilization, amount and quality of the sapling were determined.

With the aim of UCM sheep gene fund preservation, we improved the method of rams sperm cryopreservation. Rams sperm was got with the help of artificial vagina two times within a week. For rams sperm with mobility not below 8 points and with concentration not less than 2,5 milliards/of ml a dilution was used. After sperm estimation lactose-yolk-tris-citrate-glycerine (LYCTG) medium was immediately diluted in a ratio 1:3, so that in one dose of deconserved sperm there was not less than 60–80 million spermatozoa with rectilineal-forward motion. To improve the medium, the renewed glutathione (50 μM) and bovine serum albumin (BSA, 15 mg/ml) were added. Diluted sperm was packaged, cooled during 3–4 hours to temperatures +2–4 °C retained for 20 min above the nitrogen vapor (–120 °C) and put into liquid nitrogen. The frozen sperm was kept in metallic tubas in the Dewar flask with liquid nitrogen.

Then the unfreezed sperm was determined by survivability, index of absolute survival, damage of acrospermia and the impregnating ability. The obtained digital data was processed statistically by means of Microsoft Office Excel.

Results and discussion. Analysing the results of stimulation early lambing, it should be noted that application of vaginal sponges with progesteron provided the displays of oestrus for all ewes during three weeks (Table 1). However, different time of introduction of rams in the herds of experimental animals resulted in the different amount of ewes in the state of oestrus. In particular, introduction of ram three days prior to taking out vaginal sponges provided most activity in the first week – 47,7 %, and in the day of taking out sponges – on the second week (46,0 %).

Table 1 – **Ewes in oestrus**

Time of coming ewes in to heat	The number of sheep in groups, n – %	
	1 experimental, n = 50	2 experimental, n = 65
1 week	17–34,0	31–47,7
2 week	23–46,0	22–33,8
3 week	10–20,0	12–18,5

Application of biotechnological method of stimulation of sexual function render substantial influence not only on the displays of oestrus for ewes but also on fertility which in the first experimental group of sheep amounted 118,0 %, in the second one – 121,5 %, that, accordingly, by 10,0 % and 13,5 % more, as compared with control.

Thus, application of the offered method of stimulation early lambing of sheep provides the satisfactory results of displays of oestrus and allows to grow young lambs with living mass over 15 kg to the end of March and thus to provide demand on young lamb in a spring period.

The test of synchronization method for sexual heat in a pairing period on ewes of sheep of breed confirmed high efficiency of our method. Practically all experimental animals showed oestrus and they were inseminated by a laparoscope method. It is proved using the laparoscope estimation of the morpho-functional state of ovaries, that the number of preovulatory follicles ewes of control and experimental groups was almost identical, and the number of yellow bodies – almost half as less ($p<0,05$), that it is caused by impact of analogue of GnRH on synchronization of ovulation. After ewes inseminating the level of impregnation for experimental and control animals did not almost differ and presented accordingly 73,7 and 68,4 %. It points to the possibility of the use of method of synchronization of oestrus for UMC sheep in a pairing period.

The use of our stimulation method of sexual heat in oestrus period showed high efficiency – one could see oestrus in all experimental animals. It is establishet by laparoscopic estimation of the morpho-functional state of ovaries, that the number of UCM ewes preovulatory follicles in the experimental group was by 19,9 % more than in control animals. It testifies the strengthening of folliculogenesis under complex impact of PMSG and BAS (Table 2). At the same time the number of yellow bodies in the ovaries of experimental animals was by 36,5 % ($p<0,01$) less, as compared with control ewes, that specifies on more exact synchronicity of ovulations after using the analogue of GnRH.

Table 2 – **Functional status and ovarian steroid hormone concentrations in the blood of ewes**, M±m, n=20

Index	The group of animals	
	control	experimental
Number of follicles per head	3,52±0,27	4,22±0,21
Number of corpora lutea per head	0,52±0,06	0,33±0,03**
Progesterone ng / ml	1,5±0,19	2,5±0,22*
Estradiol-17β, pg / ml	12,3±1,17	8,6±0,88*

Remark: in this and the following tables * – p<0,05; ** – p<0,01; *** – p<0,001 – there is significant difference between the experimental and control groups.

The results of research on steroid hormones concentration in blood of ewes and young ones on 5-th day after laparoscopic insemination confirm the functional state of ovaries. The concentration of progesteron in blood of UCB ewes in the experimental group was by 66,7 % higher (p<0,05), and that of estradiol-by17β – 36,0 % (p<0,05) less than in control animals. It is obviously related to the greater amount of preovulation follicles which under the impact of separate BAS and analogue of GnRH synchronously ovulated and stimulated the rise of functional yellow bodies up to the fifth day of sexual cycle.

After laparoscopic insemination ewes a level of impregnation in the experimental group was 10 % higher then that of for control animals and riched 80 %, that confirms efficiency of the offered method of sexual heat stimulation in anoestrus period for the UCM sheep.

In the process of implementation of the program of maintenance of UCM sheep gene fund we improved the method of cryopreservation of rams sperm, which means modification of environment for freezing. It is set that adding of renewed glutathione and BSA to the experimental environment comparatively with control increased activity of spermatozoa after equilibration by 10,6 % (p<0,01), unfreezing by 11,0 % (p<0,001), vitality of spermatozoa to freezing by 6,0 %, storing abilities of acrosomes by 9,5 %, survivability of spermatozoa, by 23,9 % (p<0,05) and index of absolute survival by 21,8 % (p<0,05) compared with control (Table 3).

Table 3 – **The quality of the rams sperm for the use of the improved medium for sperm freezing**, M±m, n=10

Index	The medium	
	control	experimental
The activity of sperm after equilibration, %	73,82±2,35	84,46±1,52**
Activity thawing sperm, %	41,24±1,31	52,26±2,13***
Number of deconcerved sperm with damaged acrosome, %	27,50±2,85	18,00±1,65*
The survival rate of sperm, head	7,10±0,48	8,80±0,47*
Index of absolute survival, cu	13,24±0,96	16,12±1,15*
Insemination ewes, head	52	47
Fertility from 1st insemination, head, %	37–71,1	39–83,0
Fertility from all inseminations, %	94,2	97,9

The fertilization of ewes, inseminated by thawing sperm which had been frozen in the improved medium, was 12,0 % higher comparatively with animals, inseminated by the sperm frozen in a control medium. The fertilization of all inseminations also was 3,7 % high then that of inseminated by sperm frozen in the improved medium.

Thus, received results testify to high efficiency of the proposed biotechnological methods of reproduction (stimulation of early lambing, sexual hunt in a anoestrus period, synchronization of oestrus in a paring season) in breeding of UCM sheep. The laparoscopic method of artificial insemination sheep was tested and put in practice of the UCM sheep breed. The improved method of cryopreservation of sperm of rams (environment, technological parameters of freezing) is proposed for the program of maintenance of gene fund of UCM sheep.

Conclusions and prospects of further research. Stimulation of oestrus in UCM sheep in anoestrus period by using vaginal sponges with progesterone and introduction of rams in to the herd of ewes secures early lambing (December). Application of the improved chart of stimulation of sexual hunt in a anoestrus period for UCM sheep provides the high level of fertilization of animals, that is confirmed by the corresponding concentration of steroid hormones in blood. The

improvement of environment for cryopreservation of rams sperm provided the increase of quantitative and qualitative indexes of semen. Usage of the indicated methods of reproductive biotechnology has a prospect of broad implementation in projects aimed at the improvement of breeding work and maintenance of valuable breeds of sheep.

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Эффективность использования биотехнологических методов воспроизводства в разведении овец украинской горнокарпатской породы

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Приведены результаты использования биотехнологических методов воспроизводства в разведении овец украинской горнокарпатской (УГК) породы. Установлена высокая эффективность применения разработанных нами методов стимуляции ранних окотов, половой охоты в анестральный период, синхронизации эструса в сезон спаривания; апробирован в практику горнокарпатского овцеводства лапароскопический метод искусственного оплодотворения овец. Усовершенствованный метод криоконсервирования спермы (среда, технологические параметры замораживания) обеспечил повышение качества спермиев баранов УГК породы.

Ключевые слова: овцы, украинская горнокарпатская порода, стимуляция, синхронизация, криоконсервация, сперма.

Effectiveness of reproductive biotechnological methods application in Ukrainian carpatian mountain sheep breed

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The results of reproductive biotechnological methods application in breeding of Ukrainian Carpathian Mountain (UCM) sheep breed are being shown. Increased effectiveness of the developed methods by early lambing stimulation, sexual heat in anoestrus period, oestrus synchronization in heat season were established, also integrated in daily practice laparoscopic method of artificial insemination of Carpathian Mountain sheep. An improved method of semen cryopreservation (extender, technological parameters of freezing) provided improvement of thawed ram semen quality of UCM breed.

Key words: Ukrainian Carpathian Mountain sheep, sexual heat stimulation, synchronization, cryopreservation, sperm.

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