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РЕЗЮМЕ

В статье представлены результаты исследований влияния генетических факторов на оплодотворяющую способность и показатели качества спермопродукции быков-производителей мясных пород.

Основная цель работы: определение влияния породы и страны происхождения на количественные и качественные показатели спермапродукции быков-производителей мясных пород с различным генотипом и взаимосвязь исследуемых селекционных признаков.

Научно-исследовательские работы проводились в Акмолинской области. Объектом исследования являются быки-производители казахской белоголовой, абердин-ангусской и герефордской породы. Практическая часть работы осуществлялась на базе АО "Асыл түлік" на здоровом поголовье с соблюдением всех ветеринарно-санитарных требований. Для исследования оплодотворяющей способности спермы быков получены показатели 18 быков: по казахской белоголовой породе-6 быков, абердин-ангус-6 быков, герефорд - 6 быков. Осеменено 5610 коров и телок: по казахской белоголовой породе-2190, абердин-ангус-2160, герефорд - 1260.

По результатам исследования установлено, что оплодотворяющая способность быков казахской белоголовой породы колеблется в пределах 47-60%; абердин - ангусов – в пределах 30-59%; герефордской породы - в пределах 45-60% и на показатели спермопродукции быков-производителей влияет 38% генетических факторов. Из них 22,0% по генотипу, 14,0% по породе и 1,5% по стране происхождения.

ТҮЙІН

Бұл мақалада етті бағыттағы тұқымдық бұқалардың ұрықтандыру қабілетіне және ұрық өнімінің сапалық көрсеткіштеріне генетикалық факторлардың әсерін зерттеу нәтижелері көрсетілген.

Жұмыстың негізгі мақсаты – әртүрлі генотипті етті бағыттағы тұқымдық өндіруші бұқалардың шәует өнімділігінің сандық және сапалық көрсеткіштеріне мал тұқымы мен шыққан елінің әсерін және зерттелетін селекциялық белгілердің байланысын анықтау.

Ғылыми-зерттеу жұмыстары Ақмола облысында жүргізілді. Зерттеу нысаны ретінде қазақтың ақбасы, абердин-ангус және герефорд тұқымдарының асыл тұқымды бұқалары алынды. Жұмыстың тәжірибелік бөлігі «Асыл Түлік» АҚ базасында барлық ветеринариялық-санитариялық талаптарды сақтай отырып, сау мал басы бойынша жүргізілді. Бұқа шәуетінің ұрықтандыру қабілетін зерттеу үшін 18 бас бұқаның көрсеткіштері алынды: қазақтың ақбас тұқымы үшін – 6 бұқа, абердин ангус – 6 бұқа, герефорд – 6 бұқа. 5610 сиыр мен құнажын ұрықтандырылды: қазақтың ақбас тұқымы бойынша – 2190, абердин-ангус – 2160, герефорд – 1260.

Зерттеу нәтижелері бойынша қазақтың ақбас тұқымының бұқаларының ұрықтандыру қабілеті 47–60% аралығында; Абердин-ангус бұқаларында – 30–59% арасында; Герефорд тұқымында – 45–60% және 38% диапазоны аралығында аталықтардың сперматозоидтарының түзілуіне генетикалық факторлар әсер еткен. Оның 22,0%-ы генотипі бойынша, 14,0%-ы тұқымдылығы бойынша және 1,5%-ы шыққан елінетіесілі болды.

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THE QUALITY OF BIOLOGICAL MATERIAL IN EMBRYO TRANSPLANTATION

ANNATATION

This article presents the results of embryo transplantation in Pobeda LLP, one of the largest farms in the Pavlodar region. New economic conditions dictate the need for identification of selection works to the improvement of breeding and productive qualities of animals. This in turn requires improving the efficiency of artificial insemination method and wider use of embryo transplantation. The main objective of the embryo transplantation method is obtaining maximum number of valuable genotypes of animals from highly productive mothers and fathers, evaluated by the quality of offspring.

In this regard, there is an urgent need to rethink the definition of old and new, original approaches to research in the chosen field of knowledge with the creation and use of fundamentally new methods. However, modernization prospect and practical application of biotechnological method of embryo transplantation for the purpose of overcoming problems in dairy cattle breeding and further development of the industry is impossible without establishment of modern theoretical concepts, systematization, analysis and generalization of the accumulated in the last twenty years, experimental data and manufacturing experience.

Therefore, the embryo transplantation method has been used at the farm "Pobeda" to solve these problems and our goal was to develop and improve the technology to produce not less than 6 embryos per retrieval per donor, to study their qualitative characteristics and to achieve a high engraftment of embryos during transplantation and that experience is summarized here.

Key words: *Biotechnology, transplantation, donor, recipient, ovulation, synchronization, morula, blastocyst.*

Introduction. An effective way to increase productivity in dairy and beef cattle breeding is the use of biotechnological methods of accelerated reproduction of the most valuable parental genotypes [1,2,3].

Notable progress has been shown over the past 20 years in implementation of the assisted reproductive technologies in cows in advance with the study of stages of embryo development, qualitative characteristics of embryos with the purpose of determining suitability for transplantation. Modern biotechnological methods of cattle reproduction include artificial insemination of cows, deep freezing of semen, the technology of embryo transplantation. The main advantages of the latter are, firstly, the possibility of regulation of multiple pregnancies and increasing the intensity of selection replacements; secondly, there is a possibility of quick reproduction of highly productive animals, including donor herd of cows, which directly have characteristics of high productivity [4].

A cow can reproduce up to 6–8 calves during its life and with the use of transplantation technology, tens or hundreds of descendants with highly economically valuable traits. The use of this method makes it possible to obtain 50–60 embryos suitable for transplantation from one donor cow per year, or 25–30 newborn calves [5].

Currently the work of livestock breeders is very difficult and demanding as it needs using new technologies in order to improve productivity. Thus, biotechnology in the process of cattle reproduction and breeding is of particular importance, since this type of animals belongs to the singleton species of mammals, while cow ovaries contain hundreds of thousands of immature eggs - oocytes, representing a huge genetic reserve [6,7].

In Kazakhstan the work on embryo transplantation based on global experience began in the last years. Embryo transplantation is regarded not as a substitute but as a complement to the method of artificial insemination. Using this method differently can significantly increase the genetic value of farm animals, successfully overcome some forms of infertility, provide favorable conditions for the implementation of special breeding programs [8,9,10].

Expansion of embryo transplantation technology has allowed to conduct some series of studies to improve and control the processes of separation and freezing, zygote transplantation and determine the time and place of their application. These achievements are very valuable in the study of genetic parameters and functions of farm animals reproduction [11].

The main stages of embryo transplantation using the *in vivo* technique is superovulation, egg fertilization, zygote formation, and then its fragmentation and blastomerization. Knowledge of the oocyte morphology makes it possible to control the development of the donor cow embryos, starting from the process of obtaining embryos by washing, and thus allows the assessment of the embryo and determining its further development [12,13,14].

In this regard, the introduction of embryo transplantation technology for the development of cattle breeding is justified. Practical application of this method in the dairy and beef cattle provides intensive breeding of animals with a high genetic value, accelerates obtaining of valuable breeding bulls, whose mothers are outstanding ancestors, enhances the effectiveness of breeding, recovery of herds from several diseases [15,16,17].

Pavlodar region is the region with the developing cattle breeding, where administration in cooperation with academic institutions developed and implemented a program of state support and working on the study of adaptive and productive qualities of cattle imported breeds of foreign selection. In these favorable conditions for the development of animal husbandry in the region implementing embryo transplantation technology based on imported breeds of cattle with the aim of obtaining valuable genotypes is justified and forward-looking [18,19,20].

Research material and methodology. The study was carried out from 2020 to 2021 in the Pobeda LLP of the Pavlodar region (Republic of Kazakhstan). 10 heads of record-breaking cows of the Simmental breed were selected as donors. The donor cows were selected according to zootechnical data analysis. These were healthy animals with no gynecological diseases, weighing 550–650 kg, giving 6000–8000 kg of milk per lactation, and having from 2 to 5 lactations. To induce superovulation in cows, a folliclestimulating hormone was used (Pluset, Laboratorios Calier, S.A., Spain). Hormone injection was performed intramuscularly twice in decreasing doses for 4 days (10 mL in total). The order of administration of the hormone was as follows: On the first two days, 1.5 mL in the morning and evening, and on the third and fourth day, 1 mL. In conclusion, 4 mL of prostaglandin (Magestrophan, Mosagrogen JSC, Russia) was injected in the morning and evening for the rapid manifestation of cow heat. The superovulation pattern of donor cows is shown below.

Table 1 – Donor Superovulation Pattern

The time of the sexual cycle	Pluset hormone (KDAG)	
	It's morning 06:00	o'clock in the evening 18:00
0 day	Arrival of donors	
11 day	1,5 mL	1,5 mL
12 day	1,5 mL	1,5 mL
13 day	1,0 mL	1,0 mL
14 day	1,0 mL +2,0 mL prostaglandin (Magestrophan)	1,0 mL +2,0 mL prostaglandin (Magestrophan)
Day 16 or cycle day 0 (due date)	Artificial insemination (with 2 amounts of semen)	Artificial insemination (with 2 amounts of semen))
7 days of the cycle	embryo abortion	

At the end of hormonal treatment, the manifestation of heat was detected in each cow under study, according to external signs and behavioral changes. Artificial insemination was carried out twice (in the morning and the evening, two doses each) with the sperm of a bull assigned to each

donor cow. On the 7 th day after artificial insemination, the embryos were washed out of the uterine horns with Dulbecco solution, which was collected into a container after treatment, controlling the volume of fluid.

After the embryos were deposited on the bottom of the bottle, the upper part of the solution was drained using the siphoning effect. The remaining solution at the bottom of the bottle with a volume of up to 5 cm³ was gently shaken and poured into Petri dishes. Embryo detection was performed using a Nikon SMZ-745 microscope, with a magnification of $\times 50$ –100 or more. The quality of the embryos was assessed according to morphological parameters by stereomicroscopic examination.

Using of embryo transplantation method we have tried to solve another problem – obtaining valuable adaptive genotypes of donor animals of imported breeds, transplants from highly productive mothers and fathers. The local cows that carry embryos are recipients. Many imported breeds poorly adapt and do not realize their genetic potential when being brought into new conditions.

Research results discussions. Morphological assessment of embryos is one of the main methods of integrated assessment. Its aim is embryo selection suitable for conservation and transplantation, culling degenerated embryos and identification of anomalies in their development. Decisive importance in assessing the quality of transplanted frozen - thawed embryos is their viability, checked by culturing in vitro or transplantation to recipients.

Table 2 – Quantity and quality of embryos received from Simmental breed

Nickname, cow's number	Quantity of received embryos		Embryo quality			
	Total		Suitable		Unsuitable	
	n	%	n	%	n	%
23123	4	100	4	100	-	-
70775	23	100	16	69,5	7	30,5
16533	15	100	10	66,6	5	33,4
91773	10	100	6	75,0	4	25,0
97621	8	100	6	75,0	2	25,0
06935	7	100	4	57,1	3	42,8
84162	4	100	3	75,0	1	25,0
85085	4	100	3	75,0	1	25,0
80223	0	0	-	-	-	-
02136	0	0	-	-	-	-
Total	75	100	52	69,3	23	30,7

According to our research out of 10 donors only 8 cows reacted. The results of embryos quality evaluation showed that 75 embryos were flushed from those donor cows out of 10 donor cows, the embryos from 2 cows were not received, 52 embryos turned out to be suitable for transplanting which is 69.3 %. The number of flushed embryos varies from 0 to 23, the results obtained indicate that the total number of produced embryos also depends on the individual characteristics of the donor.



Figure 1 – Determination of embryo quality assessment

The efficiency of transplantation is largely determined by the method of extraction of the embryos. Fertilized eggs from superovulated donor cows can be extracted in three ways: after the slaughter of donor cows; surgical; non-surgical. The easiest and most reliable method of extraction of the embryos is slaughter of donor cows. This method was practiced only in the early stages of development of the method of transplantation. At the present time it is not used because of the loss of genetically valuable donor cow.

The important point, ensuring the efficiency of extraction of embryos, is the definition of the stage of their development and location in the genital tract of the donor cow.

At the farm "Pobeda" we extracted the embryos using a non-surgical way as the part of our research work. The main advantage of non-surgical method of extraction of the embryos is the ease of manipulations.

It does not require special operating room. Washing-out was repeated 5–8 times. The main part of the embryos was extracted in the first three or four wash-outs. It took approximately 50 minutes to wash-out both horns of the uterus, including the establishment of catheters. During this time, more than 50 % of the embryos were extracted at the stage of morula or blastocyst.

After extracting and assessing the viability we transferred embryos in a nutrient medium with the temperature of 37 degrees. Most of the nutrient mediums where the embryos were cultivated and stored, included solutions of salts, amino acids with bicarbonate ion as a buffering agent providing a pH in the range of 7.2 and 7.6. Our studies showed that the duration of cultivation without loss of biological qualities of embryos is possible up to 95 hours.

The indicators of morphological assessment are very important, because the visible diversity is an indicator of internal physiological and biological inferiority of embryos.

Special attention is paid to the fluctuation of the volume of embryos, their shape, integrity, state of the transparent membrane and protoplasm.

The biologically usable are considered to be the embryo which has a spherical shape, homogeneous cytoplasm, undamaged transparent membrane, equally sized blastomeres with tight intercellular contact and must comply with the level of crushing, the age from the moment of fertilization until its extraction.

The normal morula has the shape of a sphere. Doubling the number of blastomeres in morula occurs every 24 hours. The degree of fragmentation of embryos in the early period may be due to the lengthiness of ovulation. Some morula have individual cells that can bulge, they may have small bubbles on their surface.

Along with the assessment of the quality of embryos, the stage of embryo development was studied, as embryo's settling down depends on its development.

Table data on stages of development show that 52 embryos were transplanted, 5.8 % of which was at the early stage of morula, 57.7 % at the stage of compact morula, 28.8 % at the early stage of blastocyst and 7.7 % at the stage of blastocyst.

Table 3 – Stage of embryo development of cows of Simmental breed

Number of the donor-cow	Total number of embryos		Stages of embryo development									
			Early morula		Compact morula		Early blastocyst		Blastocyst		Expanded blastocyst	
	n	%	n	%	n	%	n	%	n	%	n	%
23123	4	100	-	-	2	50	2	50	-	-	-	-
70775	16	100	-	-	10	62,5	3	18,7	3	18,7	-	-
16533	10	100	2	20	5	50	3	30	-	-	-	-
91773	6	100	-	-	4	67	2	33	-	-	-	-
97621	6	100	1	20	3	50	2	30	-	-	-	-
06935	4	100	-	-	2	50	2	50	-	-	-	-
84162	3	100	-	-	1	33,3	1	33,3	1	33,3		
85085	3	100	-	-	3	100	-	-	-	-	-	-
Total	52	100	3	5,8	30	57,7	15	28,8	4	7,6	-	-

The data on the assessment and development of embryos corresponds to the requirements of transplantation which will provide a high percentage of embryo survival and receiving of transplant calves.

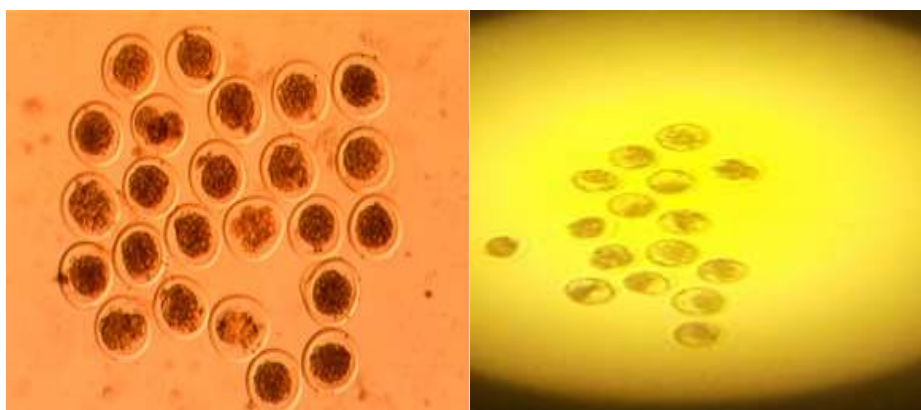


Figure 1 – Viable embryos for transfer

As it has been noted above, the embryos were transplanted to healthy heifers of local black - motley breed with a live weight of 380 to 420 kg above the average fatness in order to obtain the adaptive animal. The main indicator of recipient selection was the absence of gynecological disorders, and productive, breeding and breed qualities was not so important. However, recipients with poor body condition, low fertility after the first insemination, may have problems with settling down the embryos.

On average, 5–6 recipients were collected for each donor. Most experts believe that physiologically developed heifers with good breeding conditions are more suitable as recipients.

The basic condition of good implantation of embryos is synchronous manifestation of sexual hunting of donors and recipients. The time difference in the manifestation of sexual hunting should not exceed 24 hours, the optimal results are obtained when the difference is not more than 12 hours. The modern level of techniques of transplantation recommend to transplant embryos immediately after their extraction from the horns of the uterus of the donor and evaluation.

Animal genetic resources are valuable and strategically important capital of any country, since they are connected with the problem of providing the population with food, industry with raw materials.

According to Boettcher P., Gandini G. to preserve the gene pool of farm animals is necessary to carry out activities at two levels: the population and cell biotechnology.

At the population level relic farms should be established in different regions for the conservation of endangered species and populations of animals in genetic pool preserving farms; obtaining from them a sufficient number of gametes and embryos and their further cryopreservation; accelerated reproduction of valuable species by transplanting embryos; genetic expertise of existing in the country and imported into the country breeding animals to protect genetic resources of Kazakhstani livestock from drift of undesirable genes. The need for these activities is due to the possibility of harmful genes importation, fraught with the most unpredictable and undesirable consequences.

According to M. Ayatkhanyuly, T. Bekseitov one of the key points for rapid multiplication of valuable genotypes of animals is the development of fundamental and applied bases of biotechnological techniques such as embryo transplantation, aimed primarily at maximizing the use of reproductive abilities of donor cows. For a more complete use of this huge genetic potential as an indispensable tool serves a biotechnological method - embryo transplantation in combination with hormonal induction of superovulation.

Zh. Almantay notes that embryo transplantation plays an important role in dairy and beef cattle of the country, and its importance will continue to grow, as it allows a better use of biological reserve of females to improve livestock production.

T. K. Bekseitov, R. B. Abeldinov note that Kazakhstan has taken timely measures to solve fundamental problems of conservation and accelerated reproduction of valuable and rare genotypes.

Conclusions. The results of studies on the stages of embryo development, assessing the embryo quality meet the physiological parameters, the results will increase the percentage of embryos engraftment and obtain a large amount of valuable transplant-calves.

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ТҮЙІН

Бұл мақалада Павлодар облысындағы ірі шаруашылықтардың бірі «Победа» ЖШС-да эмбрион трансплантациялау жұмыстарының нәтижелері көрсетілген. Жаңа экономикалық жағдайлар жануарлардың асыл тұқымдық және өнімділік қасиеттерін жақсарту үшін селекциялық жұмыстарды анықтау қажеттілігін туындатады. Бұл өз кезегінде қолдан ұрықтандыру әдісінің тиімділігін арттыруды және эмбриондарды трансплантациялауды кеңінен қолдануды талап етеді. Эмбриондарды трансплантациялау әдісінің негізгі мақсаты – ұрпақтарының сапасымен бағаланатын, өнімділігі жоғары аналықтар мен аталық малдардың құнды генотиптерінің максималды санын алу.

Осыған байланысты тандалған білім саласындағы зерттеулерге ескі және жаңа, өзіндік көзқарастардың анықтамасын түбегейлі жаңа әдістерді құру және қолдану арқылы қайта қараудың шұғыл қажеттілігі туындайды. Алайда, сүтті мал шаруашылығындағы проблемаларды еңсеру және саланы одан әрі дамыту мақсатында эмбриондарды трансплантациялаудың биотехнологиялық әдісін соңғы жиырма жылда, тәжірибелік деректер мен өндірістік тәжірибелерді модернизациялау перспективасы мен практикалық қолданылуы қазіргі теориялық тұжырымдамаларды, жүйелеуді, талдауды және жинақталғанды жалпыламай мүмкін емес.

Сондықтан осы мәселелерді шешу үшін «Победа» жауапкершілігі шектеулі серіктестігінде эмбриондарды трансплантациялау әдісін қолдану арқылы бір донордан орташа 6 эмбрион алуға болатындығын, алынған эмбриондардың сандық және сапалық көрсеткіштерін бағалап, талдау жұмыстары жүргізілді.

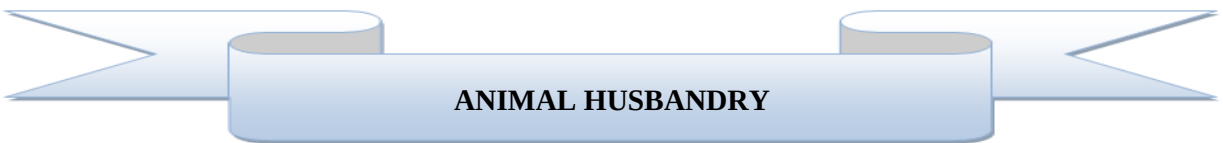
РЕЗЮМЕ

В данной статье представлены результаты работы по трансплантации эмбрионов в ТОО «Победа», одном из крупнейших хозяйств Павлодарской области. Новые экономические условия диктуют необходимость выявления селекционных работ по улучшению племенных и продуктивных качеств животных. Это, в свою очередь, требует повышения эффективности метода искусственного осеменения и более широкого использования трансплантации эмбрионов. Основной задачей метода трансплантации эмбрионов является получение максимального количества ценных генотипов животных от высокопродуктивных матерей и отцов, оцениваемых по качеству потомства.

В связи с этим возникает острая необходимость переосмысления определения старого и нового, оригинальных подходов к исследованиям в выбранной области знаний с созданием и использованием принципиально новых методов. Однако перспектива модернизации и практическое применение биотехнологического метода трансплантации эмбрионов с целью преодоления проблем в молочном скотоводстве и дальнейшего развития отрасли невозможны без создания современных теоретических концепций, систематизации, анализа и обобщения накопленных за последние двадцать лет экспериментальных данных и производственного опыта.

Поэтому для решения этих задач в хозяйстве «Победа» был использован метод трансплантации эмбрионов, целью которого было разработать и усовершенствовать технологию получения не менее 6 эмбрионов за одно извлечение на одного донора, изучить их качественные характеристики и добиться высокой приживляемости эмбрионов при трансплантации, и этот опыт обобщен в данной работе.

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