

Quality of biological material in embryo transplantation

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SUMMARY

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 "Irtyshskoye" 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.

Keywords: Biotechnology, transplantation, donor, recipient, ovulation, synchronization, morula, blastocyst

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INTRODUCTION

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.

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.

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.

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.

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.

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.

MATERIAL AND METHODS

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

Some farms, which imported animals of Simmental breed from abroad, faced with this problem. For example, in 2012 - 2013 at the farm "Irtyskoye" in Pavlodar region 87 cows out of imported 489 cows died for various reasons, mainly due to disease of limbs.

An important link in modern biotechnology transplantation of large horned livestock embryos is hormonal induced superovulation in donor cows. To stimulate multiple ovulation, we have used the follicle-stimulating hormone "Plyusset" in doses of 42 and 52 mg. The treatment was performed at 8-12th day of the sexual cycle. To synchronize cows-recipients chasing a 4-day scheme of "megaestrofan" hormone drug treatment at a dose of 500 micrograms was used. This method, according to research of J. Almantay allows inducing superovulation in about 70% of cows.

RESULTS

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.

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.

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.

Table 1. Quantity and quality of embryos received from German Simmental breed

Cow 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

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 "Irtyskoye" 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 2. Stage of embryo development of cows of German Simmental breed

Donor cow number	Total		Stages of embryo development									
	number of embryos		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	-	-

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. 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.

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 recommends transplanting embryos immediately after their extraction from the horns of the uterus of the donor and evaluation.

DISCUSSION

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. Ayatkhanuly and 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 and 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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