Results of research on group formation donor cows and embryos transplantation

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Abstract

The article presents the results of research on the formation of a group of donor cows and embryo transplantation of Ukrainian red-spotted breed of cattle on the basis of the State Enterprise of Research Farm «Khrystynivske» of the Institute of Animal Breeding and Genetics named after M. V. Zubzya of NAAS (Cherkasy region). In our studies, high donor cow embryo productivity was established. This is confirmed not only by the fact that almost 91% of the washed Melody 3142 donor embryos were evaluated as transplantable, but also by the fact that, for one flushing, the response to superovulation is a record – 22 embryos. According to the results, such indicators are due to the optimal preparation of donors for superovulation and their clinical health. The wash efficiency we have obtained is quite high. The decrease of this indicator is possible due to the lack of qualification of the technician. In addition, it is believed that a certain number of eggs with a large increase in the ovaries do not enter the funnel of the oviduct.

As a result of research from a group of donors obtained a high yield of suitable for transplantation of embryos at the level of 86.8%. In the study, 33 full-fledged embryos were transplanted to 24 recipients, their pregnancy rate was 45.8% and 10 transplant heifers were born.

Key words: Ukrainian red-spotted breed, donor cows, recipient heifers, non-surgical embryo wash, morphological assessment, transplantation.

Introduction

The effectiveness of the method of embryo transplantation largely depends on the clinical and physiological condition of cows, their ability to respond to exogenous gonadotropins and to allocate a sufficient number of quality embryos (Kovtun et al., 2012). The study of anatomical and physiological features of the reproductive function of cows and heifers is a fundamental principle of managing the reproduction of the herd and, ultimately, increase the profitability of the entire livestock industry.

Against the current background of significant achievements of global transplantation of cattle embryos as part of reproductive biotechnology, the issues of improving methods of selection of animals with technologically high and stable properties for the production of early quality embryos remain unresolved (Melnik and Sidashova, 2013).

Today, despite the significant achievements of reproductive biotechnology in cattle, there is an

acute problem of constant decline in fertility of the uterus in all countries with developed dairy farming (Madison, 2019).

The most practical use among biotechnological methods associated with the manipulation of embryos, is their transplantation to recipient heifers. Constraining factors of intensification of the method are insufficient number of embryos, morphologically suitable for transplantation, insignificant or absent super ovulation reaction in donor cows during its stimulation by exogenous gonadotropic hormones. Therefore, the development of methods for selecting cows for donors is relevant because it allows to increase the economic and selection efficiency of the method of embryo transplantation (Sidashova, 2013).

The target parameters of the strategy for the development of animal husbandry in Ukraine until 2030 are to increase the number of cows to 2.6 million heads and significantly increase their productivity to 7600 kg in agricultural enterprises (Livestock of Ukraine: state, problems, ways of development – 1991–2017–2030, 2017). To achieve such indicators, including through their own reproduction of genetically valuable cows, embryo transplantation should be preferred and used as one of the ways to increase the population (Gladiy et al., 2018).

Given the significant global trend of biotechnology of cattle embryo transplantation in developed countries in recent years, the urgency of the problem of studying the factors influencing the effectiveness of non-surgical transfer of embryos to recipients is growing significantly (Budevich, 2004).

Currently, the selection of cows is carried out on the basis of predicting the results of superovulation after hormonal treatment of donors. The ovaries of female cattle, as key organs of the reproductive system, have been studied for many years (Embrose, 2015; Lyuta, 2016). Some authors consider the function of paired gonads to be symmetrical and interchangeable, others give results on the anatomical and morphological asymmetry of cattle gonads (Kovtun and Sidashova, 2018; Sidashova, 2014), but data from different sources are quite contradictory.

An important additional diagnostic test for the detection of ovarian pathology in cows in order to increase the efficiency of selection of potential embryo donors is the intradermal administration of steroid hormones (progesterone, estradiol dipropionate and folliculin) (Madison, 2019).

A significant number of different methods for determining the morpho-functional state of the ovaries have been developed, which makes it possible to predict the response of animals to the introduction of gonadotropic hormones (Viana, 2018). Thus, the selection of donors with a concentration of estradiol in the blood on the 6th day of the sexual cycle at 2.3 ng/ml and its subsequent reduction on the 7th day by 1.5–4.5 times is a guarantee of good results of superovulation (Bugrov, 2014; Sheremeta, 2010).

A method for selecting donor cows for an allergic reaction to intradermal administration of certain steroid hormones (estradiol and progesterone) has been developed, which indicates certain ovarian pathologies that correlate with the hormonal background of the body (Sidashova, 2013).

As a result of research (Koshovy, 2011) it was found that donor cows, in which before the synchronization of sexual hunting the tip of the corpus luteum went beyond the ovary by 0.5 cm or more, respond better to GSHK and they can be obtained by 26–36% more embryos suitable for transplantation.

The proposed methods of selection of cows for donors require the use of the functional state of the ovaries, which is assessed by the dynamics of sex hormones and allergic reactions to their intradermal injection. These time-consuming methods require additional funding and the use of laboratory equipment (Yulewitch et al., 2010).

There is currently no reliable method of selecting cows for embryo donation, as this indicator has a significant individual difference. Numerous factors affecting the level of embryo productivity and quality of embryos of donor cows have been studied for a long time, but cows remain numerous biological patterns of the process of follicular, luteo- and embryogenesis have not yet been elucidated, the understanding of which

may increase the efficiency of cattle reproduction, including embryo transplantation methods (Vorobyev, 2012; Kabanova, 2016).

On the basis of the State Enterprise Research Farm «Khrystynivske» of the «Institute of Animal Breeding and Genetics named after M.V. Zubzya NAAS» a laboratory for embryo transplantation of Ukrainian red-spotted dairy cattle is created as a training and demonstration site of NAAS. The aim of our research was to obtain embryos from five cows after their first hormonal treatment, to perform their morpho- and cytogenetic analysis, transplantation to recipient heifers. This made it possible to form a group of donor cows in the embryo transplantation system to increase the number of livestock and increase its productivity.

Materials and methods of research

The selection of donor cows were guided not only by indicators of milk productivity, but the type of animals on a set of breeding traits, ease of calving, viability of the offspring and so on were taken into account.

We performed the first hormonal treatment of five cows of the Ukrainian red-spotted breed to cause them to super ovulate with follicle-stimulating hormone (drug «FSG-super») on a 4.5-day schedule. For artificial insemination of these cows the semen of two bulls of Holstein breed Jornado 6108 and Rizvy 7970 were used. Insemination of animals was carried out 2 times with a double dose of semen at intervals of 12 hours.

The effectiveness of hormonal treatment largely depends on the physiological state of the ovaries, so they were palpated for the presence of corpus luteum.

The effectiveness of the method of embryo transplantation depends on the method of embryo wash, the qualifications of specialists, the skillful use of tools and devices.

Embryo wash was performed on day 7–8 after the first insemination using a non-surgical method. The obtained bovine embryos were washed in Dulbeco medium with the addition of 0.075 mg/ml kanamycin sulfate from 20% fetal calf serum. Containers with wash liquid were defended (20–30 minutes) and the top layer of liquid was removed according to the siphon principle, leaving 70–90 ml with embryos settled to the bottom, then under the MBS-9 microscope for magnification of 28 and 56 times performed search, evaluation and others manipulation of embryos.

Tools and equipment of the company «Minitub» were used for non-surgical embryo transplantation. Fresh nutrients (1.0–1.3 cm), then air (0.5 cm) and then under the control of a microscope – the main volume of the medium with the embryo (2–3 cm) are collected in the straw. Then a small amount of air (0.5 cm) and nutrient medium (1.0–1.5 cm) are sucked.

As, recipients were selected 24 heifers that are less valuable in breeding. Recipient heifers had normally developed and well-functioning reproductive organs, were clinically healthy, the minimum age of heifers was 18 months, and the live weight was at least 350 kg.

The sexual cycle of recipient heifers was synchronized with the sexual cycle of donor cows due to the introduction of FSH for 5 days. Recto genital palpation of the ovaries in heifers was performed before transplantation. The presence or absence of the corpus luteum of the sexual cycle was assessed and the ovary on which it was formed was recorded. The quality of the corpus luteum was also determined. Animals with ovarian pathology were not transplanted with embryos.

Animals that showed a positive response to the hormone were given epidural anesthesia to relieve the reproductive pain and rectal tension. To do this, 5 ml of a 2% solution of novocaine was injected.

Diagnosis of pregnancy in recipients was performed rectally 2 months after embryo transfer.

Cytogenetic analysis of embryos unsuitable for transplantation was performed after preparation of drugs from them by a modified method of A. Tarkovsky (1966). The preparations were stained using a 2% solution of Giemsa dye and analyzed under a light microscope «Carl Zeiss».

Results and discussion

The effectiveness of the transplant method significantly depends on the method of embryo removal. We used a non-surgical method of obtaining embryos through the cervix using special equipment from «Minitub». This method is relatively simple and reliable, allows you to successfully obtain embryos directly on a livestock farm, using the donor many times without reducing its reproductive potential. Early blastocysts can be used for transplantation, so embryos are washed out between 7 and 8 days after the first artificial insemination. It is at this stage of development that it is best to obtain and transplant embryos. In addition, at this stage of development, embryos tolerate freezing-thawing well.

But on the basis of morphological assessment of embryos it is impossible to draw a final conclusion about their viability. It all depends on many factors, in particular, on their development in the process of cultivation and engraftment in the uterus of the recipient heifer (Duvanov, 2011).

In our studies, among the five hormonally treated high-yielding cows, three were selected for the donor group (Nymph 9025, Melody 3142, Rick 8607), because only they, along with a positive response to superovulation, received an average of 12.7 embryos for the first wash (Table 1, Fig. 1).

Also, the high yield of embryos suitable for transplantation (only 38 pieces) at the level of 86.8% confirms their use as donors. The obtained results of embryo productivity of the above cows are consistent with the results of studies by other authors (Vorobyev, 2012).

Although only three cows are currently effective embryo donors in our studies, the average number of embryos obtained that are suitable for transplantation at 11% is quite high.

According to the results, such indicators are determined by the optimal preparation of donors for superovulation and their clinical health. The leaching efficiency obtained by us is quite high. Reduction of this indicator is possible due to insufficient qualification of the technician. In addition, it is believed that a number of eggs with a large enlargement of the ovaries does not fall into the funnel of the fallopian tube.

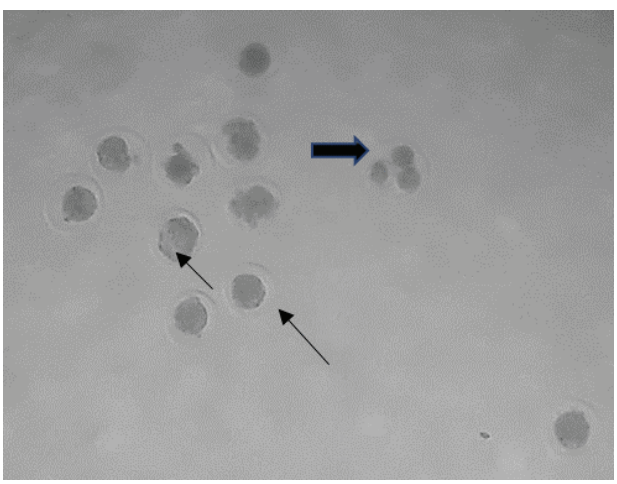

Fig. 1. Embryos of donor Melodiya № 3142 under a microscope.

In some noticeable abnormalities of normal development:

degeneration of individual blastomeres

Cleavage block(increase 28 times)

Table 1. The effectiveness of superovulation in lactating cows using the drug «FSG-super»

Indexes	Lactating cows
Processed cows, heads	5
Reacted to superovulation, heads. (%)	3 (60.0)
Positive wash of embryos, heads. (%)	3 (100.0)
On average, per donor of embryos received, pcs., M ±m	12.7 ± 4.98
including suitable for transplantation, stage of development – late moruli – late blastocysts, pcs., M ±m	11.0 ± 4.58
The yield of suitable for transplantation of embryos, %	86.8

In the course of our research, high embryo productivity of donor cows was established (Table 2).

This is confirmed not only by the fact that almost 86.8% of washed embryos are rated as suitable for transplantation, but also by the fact that in one wash the reaction to superovulation is a record – 22 embryos. Although there are cases of obtaining 40–60 embryos and eggs per wash, which indicates a significant reproductive potential of the ovaries of cows (Duvanov, 2011), in our studies, 22 embryos from the donor Melody 3142 and the birth of 6 transplant heifers indicates the correctness of the chosen scheme of embryo transplantation. It is important to note that 33 full-fledged embryos were transplanted to 24 recipients, their pregnancy rate was 45.8% and 10 transplant heifers were born.

It should be noted that the donor cow Nimpha 9025 (born on April 28, 2010) with a capacity of 8,801 kg for five lactations had one heifer and four bulls. With the help of embryo transplantation, only in 2018, three heifers were obtained from her, and further embryos from this donor cow will be continued.

Promising as an embryo donor is the cow Melody 3142 (born on January 7, 2009, productivity – 5,747 kg of milk). Six transplant calves were obtained from 20 full-fledged embryos in one wash and 50% pregnancy of the recipients. Although the percentage of offspring born from the number of transplanted embryos does not exceed 30% (no twins were obtained), all born calves are heifers. During the use of this cow on the farm for four lactations, only one heifer was received from her.

Although the use of bovine embryos obtained outside the body (in vitro) for transplantation is currently becoming widespread, for example, in 2018, 1,029,400 units were obtained in vitro against 469,967 units washed from donor cows. In our research, we used to wash them from cows in order to master this method and increase the efficiency of its use. Also in European countries in 2018, the traditional method of obtaining embryos from donor cows was used at the level of 79.3% (Viana, 2018).

As mentioned above, of the 38 embryos obtained, 33 were transplanted to recipients, and cytogenetic preparations were made from the remaining five unsuitable for transplantation and the status of their chromosomal apparatus was analyzed. Such cytogenetic analysis is necessary to identify chromosomal abnormalities, helps to determine the stages of embryo development. In our studies, the analysis of cytogenetic drugs confirmed that one embryo stopped its development at the stage of three cells, the other four - from 16 to 32 cells. The nuclei of almost all embryonic cells were degenerated, they were characterized by compacted chromatin and the inability to identify the nucleoli.

Conclusions

Thus, the effective implementation of the embryo transplantation system in farms requires

Table 2. Quantitative and qualitative characteristics of donor cowsfor embryo transplantation

Alias №	Embryos obtained		Recipients, heads	Pregnant recipients, heads (%)	Calf grafts***, heads
	total, pcs.	suitable for transplantation, pcs. (%)			
Nimpha 9025	11	8 (72.7)	7*	3 (42.8)	3
Melodiya 3142	22	20 (90.9)	12**	6 (50.0)	6
Rica 8607	5	5 (100.0)	5	2 (40.0)	1

* *– two embryos were transplanted into one recipient heifer;*
** *– eight recipient heifers were transplanted with two embryos each;*
*** *– all born calves are heifers.*

qualified veterinarians and artificial insemination technicians who, together with a group of embryologists, are able to establish their own pedigree transplant heifers and generally refuse to import livestock. With the support and understanding of the state, it is necessary to increase our own breeding stock from the formed breeding nucleus of highly productive cows on the basis of embryo transplantation.

Also, the prospect of our further research is that embryo transplantation is indispensable for obtaining offspring due to the increase in the number of bulls from mothers with record milk productivity. In the United States and Canada, more than 70% of bulls working at artificial insemination stations obtained by this method.

In addition, the resulting transplant heifers are a successful breeding material for the selection of new generation record holders among them and their use as mothers of bulls.

We need to increase the efficiency of embryo transplantation, given that this increases the efficiency of selection work. In artificial insemination of cows, the calf inherits only part of the useful properties of the bull and the inheritance of traits from the cow is preferred, and in embryo transplantation, the effect of recipients on the offspring is almost absent.

Countries in the world with developed livestock effectively use embryo transplantation because of its great value for the accelerated creation of certain families, lines and types of animals in herds, the spread of mutant genes (eg, disease resistance) or, conversely, the detection of carriers of recessive genes and timely culling of such animals. herds.

The advantage of embryo transplantation is also that in the case of breeding new breeds or improving existing ones, international animal exchange is used. Embryo exchange reduces the cost and simplifies the problem of transporting animals. In this case, the need for complex quarantine veterinary requirements is almost completely eliminated, as embryos are virtually free of bacteria and fungi due to repeated washing in sterile environments enriched with antibiotics, embryo transplantation is an effective method of acclimatization of animals. This method is of great importance for the recovery of dairy herds from leukemia, infectious rhinotracheitis, viral diarrhea in cattle, as the viruses of these diseases are not transmitted through embryos.

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