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The Influence of Controllable Factors on Successful Embryo Implantation After Transfer

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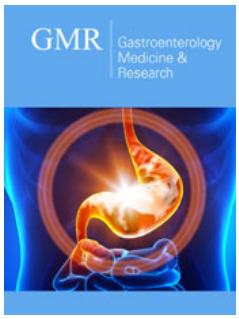

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Abstract

The introduction of reproductive technologies into production is a multi-stage and is a rather complicated process, therefore, in practice, specialists often encounter low-efficiency results. It is noted that for multiple embryo production, precise execution of technological techniques is necessary. At the same time, their successful transplantation largely depends on the professionalism of the performers. It has been noted that obtaining high-quality embryo products from a donor does not yet guarantee the successful production of offspring from a recipient. This is due to a complex of objective and subjective reasons, of which the factors that can be directly controlled by embryo transfer specialists deserve special attention. In the presented work, as a result of an analytical study, the main reasons for low results in the practice of transplantation of preimplantation embryos were studied to optimize the management of processes to improve the efficiency of applied use of reproductive technologies in cattle breeding. The significance of such factors as the readiness of the morphophysiological state of the recipient's reproductive system for embryo implantation at the time of transplantation and the efficiency of estrous cycle synchronization systems was determined. Particular importance was given to the results of transplantation depending on the place of embryo application in the uterine horn, which is associated with the need for professional experience among specialists and is a significant threshold for limiting the level of embryo engraftment. In this regard, the effectiveness of using modified instruments for deep embryo application was noted. The dependence of the engraftment of depreserved embryos on the methods of cryo- and depreservation during non-surgical transfer to recipients was determined. In the overall analysis of our own research, morphological quality assessment was carried out on more than 12,000 embryos, of which about 8,000 underwent cryo- and deconservation procedures, and the total number of transplants of freshly obtained and frozen-thawed embryos amounted to more than 9,000, which together indicates the high significance of the results obtained.

Keywords: Reproductive system; Estrous cycle; Embryo; Transplantation; Synchronization; Engraftment; Implantation; Application site; Cryopreservation; Deconservation

Introduction

Today, in the biology of cattle reproduction, two directions have been defined, related to the accelerated replication of the genetic potential of the best cows. Today, the MOET (multiple ovulation and embryo transfer) biotechnology is widely used in practice. It is based on the mass production of preimplantation embryos, the development of which occurs in the mother's body - the *in vivo* (IVD -embryos). In recent years, the technology has been intensively developing *in vitro* - production of embryos from oocytes extracted from cow follicles (IVP embryos) - the growth and development of such embryos is carried out artificially in laboratory conditions. The advantages of reproductive technologies in comparison with traditional methods of reproduction allow to significantly increase the number of offspring from cows with high genetic potential in an accelerated time. Technological stages of the relationship in the system "donor-embryo-recipient-offspring" in technologies in *in vivo* and in *in vitro* determine their main practical tasks. Moreover, if the mass production of embryos

from outstanding donor mothers is carried out in different ways, then embryo transplantation, as the target and final stage, namely the production of direct descendants from donors through recipient mothers, is carried out by methods that are common to both technologies. The introduction of reproductive technology methods into production is a rather complex process, so practicing specialists often encounter low efficiency results, which is due to various, but often obvious and determinable reasons. It should be noted that if at the stage of obtaining embryos, success is mainly based on the precise implementation of technological techniques, then successful embryo transplantation depends to a greater extent on the professionalism of the performers. Specialists are forced to acknowledge the fact that obtaining high-quality embryo products from donors does not yet guarantee the success of effective offspring production.

To ensure the conditions for the effectiveness of embryo transfer, it is necessary to take into account a set of objective and subjective factors, which includes requirements for recipients, technological aspects of embryo preparation and methods of their transplantation. Of great importance is the general physiological state and the state of the reproductive system of animals, related to the conditions of maintenance and feeding, in particular heat, thirst, hunger, illness and fatigue, as well as inflammatory processes in the recipient's body such as mastitis, pneumonia, lameness and diseases of the reproductive organs. There are factors that cannot be determined in practice. But their probability and frequency of occurrence are questionable. This is the inability of the embryo to implant in the uterus, associated with the impossibility of its hatching, or the exit of the embryonic complex from the pellucid zone, interrupted biochemical interaction between the embryo and the recipient's uterus, genetic aspects of their relationship and various chromosomal abnormalities. Particular attention should be paid to the study of the causes that are directly controlled by biotechnologists. These include methods of preparing recipients and determining the readiness of their reproductive system for embryo implantation, the technology of processing embryos and preparing them for transplantation, as well as the technique for performing the transplantation procedure itself.

Purpose of the study

The search for and analysis of the reasons for low results in the practice of embryo transfer to determine effective ways to optimize the management of procedures for preparing recipients, processing embryos during cryo- and deconservation for transplantation to recipients are today in the category of priority tasks for increasing the effectiveness of reproductive technologies, which is the goal of this analytical study based on our own experiments and data from other authors.

Materials and Methods

This work is an analytical study based on the results of our long-term experiments on embryo transfer biotechnology at the Brest Biotechnology Center of the Republic of Belarus (1987-2003), at the embryo production center of Betagran Lipetsk LLC (2014-2016), Batu Plus peasant farm of the Republic of Kalmykia (2016-2018) and B.B. Gorodovikov Kalmyk State University from 2018 to the present, as well as data from other authors. The experimental materials considered in this paper used the results of our own experiments and data from other authors on the practical application of reproductive biotechnologies, including methods for preparing recipients, including the use of systems for synchronizing the sexual cycle, morphological assessment of quality, cryopreservation and transplantation of freshly extracted and depreserved embryos using generally accepted and developed methods. Suitable 7-8 day old embryos obtained from donor cows of various breeds using in technology vivo (MOET) were used in transplantation programs freshly obtained or after deep freezing. The technology of deep cryopreservation of embryos included procedures of embryo saturation by equilibration in solutions of penetrating cryoprotectors glycerol and ethylene glycol of increasing concentrations. Glycerol was used to freeze embryos for classical indirect transplantation, ethylene glycol for direct transplantation. Embryos of excellent and good quality at the stage of morula-blastocyst development were selected for cryopreservation. Deep cooling to a temperature of -196 °C was carried out on the ZEM-4 and CL5500 programmable freezers. Embryo deconservation was carried out in two ways. For indirect transplantation after thawing in a water bath, stepwise removal of glycerol was carried out by equilibration in solutions of decreasing concentration with an assessment of the quality and safety of the embryos after deconservation with subsequent transfer to recipients. For direct transplantation, the cryoprotectant ethylene glycol was not removed, the condition of the embryos was not checked after thawing, and direct transfer to recipients was performed. Cows and heifers of breeding age, selected in spontaneous heat or after hormonal synchronization of the estrous cycle according to the recommendations of A.V. Makarov and S.V. Shadrin "Synchronization of estrus in cows and heifers using hormonal drugs", 2020, by double administration of estrofan with an interval of 11 days, were used as recipients for embryo transfer. Embryo transfers were performed rectocervically using Kassou catheters and a developed instrument for deep application of embryos (patent of the Republic of Belarus). for the invention "Device for embryo transplantation" No. 1796171, 1993).

Statistical processing of experimental data was carried out using the office software package "Microsoft Office" using the program "Excel" ("Microsoft", USA) with data processing in " Statistica 9.0" (" Stat Soft Inc. , USA).

Results and Discussion

To ensure the conditions for the effectiveness of embryo transfer, it is necessary to take into account a set of objective and subjective factors, which includes requirements for recipients, technological aspects of embryo preparation and methods of their transplantation. In the embryo transfer program, the basis for achieving the final goal - the accelerated production of genetically valuable offspring

from elite mothers - is the selection of animals as recipients. At this stage, the beginning of the goal is formed. Cows and heifers can be used as recipients for embryo transplantation. Cows are subject to significantly higher requirements than heifers. Cows can be suitable recipients if they have no reproductive problems and have had easy calvings. Embryo transfer is performed after the uterus has fully recovered, no earlier than 2 months after calving and the sexual cycles have recovered. However, there is a risk of chronic diseases, in particular endometritis, and morpho functional disorders in the ovaries are common. Therefore, according to our data and the data of other authors, embryo survival after transplantation in cows is usually lower than in heifers [1-5]. In 1987-2003, we conducted practical studies on transplantation of freshly extracted and frozen-thawed embryos using heifers of breeding age as recipients at the Brest Regional Center for Embryo Transplantation "Litvinovo" in the Republic of Belarus. The data of long-term studies conducted on a large amount of factual material showed the possibility of achieving highly effective results [6-10]. The average pregnancy rate of recipient heifers after transplantation of freshly obtained embryos was 54.7%, a similar figure when using embryos after deconservation was 48.0% (Table 1). It should be taken into account that embryos of different quality categories, as well as embryos from experimental groups, were used for transplantation, which is inevitable with a large population of recipients, taking into account the direction and volume of work.

Table 1: Survival of fresh and preserved embryos in recipient heifers.

Condition of Embryos	Number of Recipients	Pregnant Recipients	Pregnancy, %
Freshly received	1065	583	54.7
Decanned	1030	494	48

A mirror result (54.7% pregnancy) on recipient heifers with freshly obtained embryos was obtained in the studies of Duvanov AV [3] - out of 95 heifers, pregnancy was established in 52 animals. As was said, the use of cows as recipients is characterized by lower efficiency. Thus, the same author, under similar conditions on a large herd of recipient cows (2797 heads), for the period from 2018 to 2021 after transplantation of depreserved embryos, obtained 1086 pregnancies, which amounted to 38.8% with fluctuations over the years from 33.6 to 42.8% [4]. Our experiment, conducted in 2002 at the Snov Agricultural Production Cooperative in the Republic of Belarus on the transplantation of frozen-thawed embryos, also showed a lower pregnancy rate in cows compared to heifers - 33.3 versus 45.2%, respectively [1,2] (Table 2). There is no doubt that, in addition to the influence of age and gender, the results of embryo transplantation are directly dependent on the general health and reproductive system of the recipients. Therefore, the preparation of potential recipients begins with the selection of animals that meet the necessary morphophysiological requirements [11-13]. The next necessary condition is the synchronicity of sexual estrus of the donor and recipient. This factor ensures the maximum level of embryo engraftment. At the same time, the asynchrony in the time of manifestation of estrus in donors and recipients should not exceed +/- 12 hours [14,15]. When preparing groups of animals for artificial insemination and embryo transfer, the system of estrus synchronization has become widespread. It allows using a large number of animals in a short time, postponing mass calving to another time, inseminating and transferring embryos when it is difficult to detect estrus. According to the University of Pennsylvania, many estrus synchronization protocols can induce ovulation in 75-90% of animals over a 5-day period [11]. In this context, there are both positive and negative aspects. Hormonal estrus stimulation schemes lead to suppression of the endocrine organs that ensure the functioning of the reproductive system with the unsystematic use of hormonal drugs. In addition, Makarov AV and Shadrin IV cite the following data: "... when using synchronization schemes, pregnancy will occur only in 45-50% of cases of the total number of inseminated cows" [16,17]. Similar results are noted by other authors [11,13].

Table 2: Efficiency of transfer of depreserved embryos in recipient cows and heifers.

Indicators	Animal Groups	
	Heifers	Cows
Number of recipients	62	21
Pregnant recipients	28	7
Pregnancy rate, %	45.2	33.3

What is the reason for the low level of animal fertilization? When synchronizing the sexual cycle, one of the fundamental requirements is the time of animals' coming into heat to determine the time of insemination or embryo transfer. As a rule, with strict observance of all other conditions for the use of animals in reproduction, the significance of deviations in the timing of the manifestation of sexual heat from those expected according to the scheme remains "outside the brackets". In our context, "short deadlines" mean the animals coming into heat within a 5-day period, as discussed above, rather than within 24 hours, which would meet the necessary requirements of embryo transfer technology [12,18-22]. However, tour synchronization of the estrous cycle involves insemination or embryo transfer according to the predicted date of heat, which is its main goal. Fertilization losses in animals are relegated to the background, which limits the efficiency within 45-50% of pregnancy. A shift in the timing of the manifestation of heat acts as a negative factor, significantly limiting the efficiency of artificial insemination and successful implantation of embryos . Accordingly, tour embryo transfer associated with synchronization of estrus in recipients is usually accompanied by low results [23]. In Western countries, where embryo transfer is used on a

large scale, the pregnancy rate in a large number of transplants is limited to 45-55% [24,25]. Thus, it is necessary to take into account that the logistics of synchronization methods are limited by the dilemma of choice. On the one hand, there are losses in the level of pregnancy due to difficulty or impossibility of detecting sexual heat, but at the same time, the possibility of carrying out a large number of transplants in a short time. On the other hand, there are difficulties in carrying out mass transplants in a limited time, but higher efficiency in recording heat by any possible means, in particular when forming small groups of animals. Practice shows that in groups of up to 30 heads, detecting animals in heat does not have difficulties. At the same time, today there is a system Heatime - automated detection of heat in cows with an accuracy of up to 97% [11]. The system detects heat during the entire period of activation of sexual function (48-120 hours), which, accordingly, requires multiple approaches to animals of a synchronized group. At the same time, unlike artificial insemination, embryo transfer can be adjusted based on the results of selection of recipients in heat, since it is carried out 7-8 days after heat. Taking into account the age of the embryos and the tolerance of +/- 12 hours, the animals included in the embryo transfer group were in heat for 3 days: day 1 - after lunch, day 2 - the whole day, day 3 - before lunch. This allows for a sufficiently full use of the effect of synchronization of large groups of recipients using the Heatime system - over 80% of the treated animals come into heat within three days. The optimal level of detection of sexual heat is at least 70-80% [16] and even 75-90% [11]. The ambitious nature of the tasks of transplanting many dozens, and sometimes hundreds of embryos in a limited time frame with adjustments not based on the actual date, but on the predicted date, is often accompanied by low results, as mentioned above, and is one of the reasons for discrediting the embryo transfer technology. Thus, in the practice of embryo transfer, recording the recipients' estrus is of great importance. The practical significance of this factor is confirmed by studies conducted from 1987 to 1993 at the embryo transplantation center of the experimental base "Maisk" of the Brest Scientific and Production Association for Agriculture of the Republic of Belarus. After transplanting deconserved embryos to recipient heifers based on the predicted estrus in the presence of the Corpus Luteum (CL) in the ovary, the pregnancy rate was 45.2%, and significantly higher if the estrus was recorded (54.3%). This result was not inferior to the transfer of fresh embryos under the same conditions on a large population of recipient animals with fixed estrus – after the transfer, pregnancy was established in 663 of 1183 recipients (56.0%) [6]; (Table 3).

Table 3: Dependence of engraftment embryos from taking into account the estrous cycle of recipients.

Embryo Groups	Fixation of Hunting	Indicators		
		Transfers	Bedridden	%
De-canned.	Eat	81	44	54.3
De-canned.	No	62	28	45.2
Fresh	Eat	1183	663	56

Successful implantation of the transplanted embryo depends on the stage of the recipient's estrous cycle, when the state of his reproductive system corresponds to the stage of development of the embryo that has undergone a similar period of development in the donor's uterus. Preimplantation embryos aged 6-8 days are transplanted to recipients at the appropriate stage of the sexual cycle. In this case, the recipient's ovary must have a functioning PT. In practice, this is determined by rectal palpation of the ovaries or using an ultrasound scanner. The presence or absence of PT indicates the readiness or unreadiness of the uterus for embryo implantation [26-28]. However, its presence does not guarantee an exact match of the sexual cycles of donors and recipients if the exact time of the animal's onset of sexual heat is not known. It is impossible to determine the stage of PT development by morphological features, and the asynchrony of the sexual cycle stage with the age of the embryos by more than 12 hours leads to a decrease in the engraftment of embryos [14,15,29]. Thus, the need for strict recording of the time of the recipient's onset of sexual heat becomes obvious. Therefore, when selecting potential recipients, animals that come into heat earlier or later than the specified time period are eliminated immediately, without assessing the condition of the ovaries. This is necessary when using freshly obtained embryos, but when using frozen embryos, the transplant procedure can be postponed to another time in accordance with the age of the embryos. The absence of VT in the presence of an unovulated follicle indicates a delay in ovulation due to disturbances in neurohumoral regulation of intraovarian processes. In this case, the uterus is not ready for embryo implantation and pregnancy cannot occur. Such recipients should be rejected. Identical in size and, as a rule, reduced ovaries may indicate hypofunctional disorders or an error in detecting estrus. Recipients whose ovaries show cystic changes, palpable as large formations with tight fluctuation, are also subject to rejection [29,30]. Thus, an important component of successful embryo transplantation is the physiological correspondence of the reproductive system to the stage of the recipient's sexual cycle, provided that the morpho functional state of the ovaries is accurately determined, which ensures optimal conditions for embryo implantation.

Of decisive importance is the technical execution of the embryo transfer procedure itself, which is carried out non-surgically. Unlike the technique of artificial insemination, when the semen is sufficiently delivered to the animal's uterus, embryo transplantation is carried out in the horn of the uterus, which is a complex procedure, especially in heifers, and requires experience

associated with long-term training of the specialist. At the same time, the instruments used for embryo transfer, due to the peculiarity of the reproductive tract of cows and heifers, by analogy with catheters for artificial insemination, have a rigid design. Thus, the most frequently used Kassou catheter and its modifications allow for 50-60% engraftment in experienced specialists [5,6,17, 28, 31,32]. It should be taken into account that with such embryo transfer there is a high risk of microtrauma of the endometrium of the uterine horn with the release of blood toxic to the embryos, which reduces the likelihood of their implantation [6,33,34]. The transplant procedure is associated with the implementation of fairly complex manipulations for the rectocervical insertion of a catheter through the genital tract to the optimal place for implantation of the uterine horn. Reducing this risk to a minimum requires professional experience, which is achieved over the years and depends on the individual abilities of a particular performer. This is confirmed by the results of transplantation by an experienced specialist (58% pregnancy) and a novice - only 35% [35].

At present, the fact of dependence of the engraftment of the transplanted embryo on the place of its application in the horn of the uterus of the animal has been established. Thus, in the studies of Niemann H [36] showed that the apex of the uterine horn is the most optimal location. A number of other authors confirm this point of view [20,35,37,38]. In cases where the embryo is located closer to the apex of the uterine horn, the most frequent occurrence of pregnancy is noted, where optimal conditions for implantation and development of the transplanted embryo are created by neurohumoral regulation of intraovarian processes [39-42]. It is also noted that the necessary concentration of progesterone ensures uterine secretion precisely in the apex of the horn [27,20,43]. According to some early literature data, the pregnancy rate when transplanting embryos into the middle of the uterine horn is 25-37.5%, and when transplanting into the top of the horn it reaches 40-50% or more [44]. Therefore, in order to increase the pregnancy rate, it is necessary to strive to carry out the application of embryos directly into the top of the horn, which, as mentioned above, is technically a complex procedure and requires highly qualified specialists [45]. The influence of qualification on the efficiency of transplantation is also noted by other authors [6,46,47]. Thus, the limiting factor for high embryo engraftment is the available depth of embryo application. The negative impact of complex manipulations during catheter insertion into the recipient's uterine horn apex can be significantly simplified by using specially designed instruments. In recent years, various modifications of catheters for transplantation have been developed, the design of which allows for the application of embryos directly into the uterine horn apex with minimal risk of endometrial injury. In 2022, a new device XtremiA, manufactured in France by Elexinn, for deep artificial insemination appeared on the biotechnology equipment market, which is also positioned as a revolution in the field of embryo transfer. However, the effectiveness of this device is questionable, since it is based on the use of a super-thin tube, similar to an endotracheal catheter, to advance it deep into the cavity of the uterine horn. In our opinion, all devices with a similar principle of operation have a significant drawback, proven in practice - when advancing a very thin tube into the cavity of the uterine horn, there is a risk of the tip of the tube getting caught between the caruncles lining the mucous membrane of the horn [19]. In this case, the tube does not advance into the cavity of the horn but forms a loop and when pushing out the embryo, instead of the top of the horn, ends up in the place of the hook. In this case, the operator may not be aware of the violation that has occurred, and the frequency of such violations is unknown. These deviations do not affect the results of artificial insemination, since the semen enters directly into the cavity of the uterine horn. As for the transplanted embryo, based on the above, its ability to implant when applied to the base or middle of the horn is reduced. In our research, a special catheter with a flexible sliding cover was developed, which, after passing through the cervical canal of the cervix, ensures the delivery of the embryo to the apex of the uterine horn without the risk of microtrauma to the endometrial mucosa (USSR A.S. for the invention "Device for embryo transplantation" No. 1796171, 1993).

Carrying out transplantation with this instrument does not require high qualifications of the performer and long-term practical experience. Professional skills of the artificial insemination technician are sufficient. The influence of the design features of the modified instrument for deep application on the efficiency of transplantation can be seen from the experiment below. Comparative results obtained after embryo transfer using the Kassou catheter and the new modification of the instrument confirm the existing opinion about a higher pregnancy rate when embryos are applied to the apex of the uterine horn (Table 4). Thus, when transferring freshly extracted embryos into the apex of the uterine horn using the new instrument, it was possible to achieve a pregnancy rate of 71.4% (transfers/pregnancies - 70/59), which was 19.5% higher than when transferring into the middle of the horn using the Kassou catheter - 51.9% (661/343) at a high level of reliability ($P < 0.001$). A similar trend was observed when transferring depreserved embryos - the difference in pregnancy rate was 13.8% in favor of transferring with the new instrument versus the Kassou catheter - 59.2% (108/64) versus 45.4% (603/274), respectively, at a level of reliability of $P < 0.01$ [48,49]. Similar results were obtained in studies by other authors using a tool of their own design, allowing for deep application of embryos (RU Patent for Utility Model "Device for Application of Bovine Embryos" No. 154919, 2015). The pregnancy rate when transplanting embryos into the apex of the horn using the new tool was fresh - 68.2% (transfers/pregnancies - 41/28), frozen-thawed - 58.1% (43/25). Similar indicators when transplanting into the middle of the horn using the Kassou catheter were noticeably lower: fresh - 44.3% (97/43); frozen-thawed - 36.3% (113/41) [23].

Table 4: Embryo engraftment depending on the location in the recipient's uterus.

Condition of Embryos	Catheter	Place in the Horn of the Uterus	Indicators		
			Transfers	Steln.	%
Fresh	Cashier	Middle	661	343	51.9
	New	The top	70	50	71.4***
Deconserved.	Cashier	Middle	603	274	45.4
	New	The top	108	64	59.2**

**P <0.01 ***P <0.001

Deep freezing of embryos is of particular importance in reproductive biotechnology. Its use allows for unlimited preservation of valuable genetic resources in the form of frozen embryos, and also eliminates the need to maintain large groups of recipients; transplants can be performed at times independent of the time it takes to wash out embryos from donors, which has a positive effect on logistics and production profitability. It is generally accepted that the engraftment rate of depreserved embryos is lower than that of freshly extracted embryos. Today, the survival rate of depreserved embryos exceeds 90%, and the engraftment rate after transplantation reaches 50-55% [6,50,51]. However, this is far from the limit. With the current level of development of cryo- and depreservation methods for embryos, as well as taking into account a number of other factors, it is possible to obtain equivalent engraftment results when transplanting frozen-thawed and freshly extracted embryos.

When transplanting freshly obtained and depreserved embryos, both similar and diametrically opposite results can be obtained, associated with a number of technological and physiological factors. In particular, the quality and breed of embryos, associated with the fertility of donors, as well as the fertility of recipients, make it possible to increase the engraftment of depreserved embryos to a level comparable to the transplantation of freshly obtained embryos. In our studies, when studying the dependence of engraftment on the quality of embryos, the following results were obtained. In 1998, in the Brest Regional Center for Biotechnology, Litvinovo LLC, Republic of Belarus, the pregnancy rate was 54.4% on a large sample - out of 913 recipients, pregnancy was established in 497 animals [6]. At the same time, in 2012, in Vaganovo OJSC, Kemerovo Region, after an experiment on intrabreed transplantation of freshly extracted embryos, the engraftment rate was 11.0% higher and was 65.4% - out of 26 recipient heifers, pregnancy was obtained in 17 heads. The results obtained are reflected in Table 5. In this case, the noticeable difference in the results obtained is explained by the quality of the embryos. At Vaganovo OJSC, only excellent and good quality embryos without visible morphological abnormalities were used in a small volume of transplants. At the same time, all other things being equal, at Litvinovo LLC, where the work was carried out in stages over a number of years using a large number of recipients, embryos of different quality categories were used for transplantation, including satisfactory and conditionally suitable with a lower potential for implantation.

Table 5: Average engraftment rate of fresh embryos of different quality categories.

Indicators	Results	
	LLC "Litvinovo"	JSC Vaganovo
Number of recipients	913	26
Fresh embryos transferred	913	26
Pregnant recipients	497	17
Embryo survival rate, %	54.4	65.4

A number of experiments have studied the influence of reproductive qualities, fertility of recipients and embryo donors on the efficiency of transplantation of de-preserved embryos. Thus, in 2017-18, at the PZ AO Ababkovskoye in the Nizhny Novgorod Region and the KFH Batu Plus in the Republic of Kalmykia, we conducted studies on the interbreed transplantation of de-preserved embryos of the Krasnogorbatovskaya beef and dairy and Aberdeen Angus breeds to recipient heifers of the Kalmyk beef breed [7,9]. The engraftment rate of Krasnogorbatov embryos was 62.4% (out of 117 recipients, pregnancy was established in 73 animals). High results were also obtained with the transplantation of Aberdeen Angus embryos - 65.6% pregnancy (40 pregnant out of 61 recipients). In both cases, the pregnancy rate was not inferior to the transplantation of freshly extracted embryos (Table 6). At the same time, it is highly likely that, in addition to other factors (the properties of the cryoprotectant used for indirect transplantation, the assessment of the quality of embryos after deconservation and the accuracy of recording the estrus in recipients), the breed fertility of embryo donors and recipients is also of great importance. In our context, the Red Gorbatov and Kalmyk breeds were distinguished by high fertility [9].

Table 6: Efficiency of interbreed transplantation of deconserved embryos of Krasnogorbatovskaya and Aberdeen Angus breeds.

Indicators	Results	
	Krasnogorb.	Angus
Transfers performed	117	61
Transferred depreserved embryos	117	61
Pregnant recipients, n - %	73 – 62.4	40 – 65.6

In non-surgical transfer of de-preserved embryos to recipients, the technique of performing the transplant procedure itself is of great importance. Currently, direct and indirect methods are used for this purpose in cattle, which do not differ in technical execution. However, there are a number of differences, in particular in the methods of preparing embryos for transplantation, the possibility of identifying the recipients' sexual heat, the difference in the cryoprotectors used and the methods of de-preservation, which together determines the level of their effectiveness. The main advantage of indirect transfer is a higher level of engraftment of preserved embryos compared to direct transfer, which is mainly due to the procedures of cryopreservation and depreservation of embryos for transfer and the accuracy of recording the recipients' sexual desire. Currently, glycerol and ethylene glycol are the most widely used cryoprotectants in embryo cryopreservation. For indirect transplantation, glycerol is used as a cryoprotector, which is less toxic to embryos than ethylene glycol [50-53]. This allows for an increase in the time of manipulations with embryos during cryopreservation with a minimal risk of reducing their viability (when using ethylene glycol, the time is limited to 10 minutes). During deconservation, immediately after thawing, the embryos undergo an equilibration procedure to remove glycerol, followed by a morphological assessment of the embryos for cryodamage to the zona pellucida and the embryonic complex. Thus, with indirect transplantation, the likelihood of transferring critically damaged embryos is excluded. The time for performing the transplantation procedure itself has no strict limit, since the embryo, in the absence of the toxic effect of the cryoprotector, is able to remain viable longer.

Indirect transplants (limited number of recipients) facilitate the selection of animals in heat, which allows for more precise synchronization of the recipients' sexual cycle stage with the embryo development stage to increase their engraftment rate. Comparative studies of indirect and direct transplantation of fresh and de-preserved embryos in Litvinova LLC of the Republic of Belarus showed the dependence of recipient pregnancy on the accuracy of synchronization of the sexual cycles of donors and recipients with fixed estrus and determination of the viability of embryos after thawing. Thus, the pregnancy rate with indirect transplantation with fixed estrus was 9.1% higher compared to direct transplantation without assessing the quality of embryos and fixing the recipients' estrus, and amounted to 54.3% versus 45.2%, respectively [6] (see data in Table 3). Similar in significance are the results we obtained at Litvinovo LLC three years earlier, in 1995, after indirect transplants, which showed a 50.7% engraftment rate of depreserved embryos – out of 258 transplanted embryos, 131 successfully engrafted [8] (Table 7). In studies at the Batu Plus peasant farm in Kalmykia, as a result of indirect transplantation of de-preserved embryos with an assessment of their safety after cryopreservation in glycerol to heifers of the Kalmyk breed, which are distinguished by high fertility, the engraftment rate was comparable to the transplantation of freshly obtained embryos - pregnancy was established in 40 animals out of 61, which amounted to 65.6%. [9] (Table 8). Thus, the effectiveness of frozen-thawed embryo transplantation can be significantly increased by taking into account the factors that are positive for successful implantation. The disadvantage of the indirect transplantation method is the difficulty of performing mass transplants at one time. This is due to the time required to perform cryoprotectant removal procedures and morphological assessment of the embryos after deconservation. However, as follows from the above, a higher level of recipient pregnancy, in particular associated with the assessment of embryo viability after deconservation, can compensate for this disadvantage.

Table 7: Results of frozen-thawed embryo transfer.

Number of Recipients	Embryos Transferred	Pregnant Recipients	Embryo Survival Rate, %
258	258	131	50.7

Table 8: The engraftment rate of depreserved embryos depending on the cryoprotectant, fixed estrus and recipient fertility.

Indicators	Result
Transfers performed	61
Frozen-thawed embryos transferred	61
Pregnant recipients, n - %	40 – 65.6

The main advantage of a direct transplant is the possibility of a one-time conducting mass transplants. After thawing, the embryos are not equilibrated to remove the cryoprotector. Therefore, the transplant is carried out by direct transfer of the embryo from the thawed straw directly into the recipient's uterus. This reduces the time spent on manipulations with embryos, which makes it possible to transplant a large number of embryos at a time. In 2016, specialists from Betagran-Lipetsk LLC in the Republic of Bashkortostan as a result of direct transplants without assessing the quality of embryos after thawing, the pregnancy results were 35.8% - out of 95 recipients, pregnancy was established in 34 heads. The same specialists at the PZ za mir i trud agro-industrial complex in Krasnodar Krai performed direct and indirect transplants on recipient heifers (Table 9). The result of direct transplants was 39.4% pregnancy, which is also not indicative. At the same time, indirect transplants showed very high efficiency - 71.4% [54]. obtained by a group of other researchers are close in significance. in 2018 at Kubansky MTK LLC in Krasnodar Krai after direct transplantation to recipient cows without precise recording of estrus [3]. One group of recipients underwent transplantation in the summer during the period of heat stress with a pregnancy rate of 36.8%, while the second group received embryos in the spring-summer period with a pregnancy rate of 41.2% (Table 10). In both

cases, the efficiency of the transplants was relatively low. Despite the presence of a negative temperature factor in the first case, its absence in the second was not significant. Thus, there is a general trend towards a decrease in the engraftment of embryos during direct transplantation.

Table 9: Efficiency of direct and indirect transfers of depreserved embryos into recipient heifers.

Indicators	Krasnodar Region	Bashkortostan
Direct transfers, goals	94	95
Of which pregnant, heads - %	37 – 39.4	34 – 35.8
Indirect transfers, goal.	35	-
Of which pregnant, heads - %	25 – 71.4	-

Table 10: Efficiency of direct transfer of depreserved embryos to recipient cows.

Indicators	Heat Stress Period	Comfortable Season
Number of embryo transfers	690	165
Number of pregnant women	254	68
Pregnancy rate, %	36.8	41.2

When cryopreserving embryos for direct transfer, ethylene glycol is used as a cryoprotectant, which is more toxic to embryos than glycerol. To avoid the risk of reducing the viability of embryos, the time for manipulations with them in procedures preceding cryopreservation is limited to 10 minutes [15,55]. After thawing, for the same reason, equilibration of the embryos to remove the cryoprotectant is not carried out; the straw is immediately loaded into the catheter for direct transfer to the recipient without assessing the quality of the embryo. Since there is no control over the viability of the embryos, the risk of transplanting embryos that have lost viability cannot be ruled out. Due to the toxicity of ethylene glycol, the time from thawing to the actual transplant is also limited to 10 minutes. The disadvantages of the direct method for mass transfers also include the time limit for performing the transfer procedure, which is associated with the time frame of the synchronization protocol. After synchronization, animals come into heat within 24-120 hours, the majority after 48-72 hours. According to the predicted heat, artificial insemination is carried out after 60-72 hours, when about 70% of animals come into heat [12,21,22]. Therefore, the error in the timing of insemination is 30% or more, which limits the effectiveness of tour insemination to 45-50%, which we discussed above. However, the transfer of embryos within these limits, as discussed above, unlike artificial insemination, leads to an error in the coincidence of the recipient's sexual cycle stage with the embryo development stage within unacceptable limits (+/- 12 hours). Therefore, to achieve acceptable results of embryo transfer, it is necessary to strictly account for the time of arrival of recipients in heat, which is difficult with a large number of animals during a tour transfer. This can be done using the system Heatime, automated detection of heat in cows with an accuracy of up to 97% [11]. But even in this case, from among the animals that came into heat at different times, it is necessary to form groups of recipients with the time of manifestation of heat linked to the stage of embryo development.

Conclusion

When analyzing the results of our own research and data from other authors, we identified a set of factors that influence the effectiveness of non-surgical embryo transplantation.

1. A comparative assessment of the efficiency of using cows and heifers of breeding age as recipients was conducted. It was found that the engraftment of embryos in cows shows lower results than in heifers. When transplanting depreserved embryos to a large sample of recipient cows, the average pregnancy rate was 38.8%, while in heifers it was 48.0%. Based on economic feasibility, cows should be used as recipients only in the event of a shortage of recipient heifers.

2. Recording sexual hunting during recipient preparation is a mandatory condition. In the practice of embryo transfer, successful implantation depends on the synchronicity of the stages of the estrous cycles of donors and recipients. Thus, with direct transplantation of depreserved embryos with an allowance for an error in the coincidence of the stage of the recipient's sexual cycle with the age of the embryos, pregnancy is 35.8-42.8%. At the same time, taking these indicators into account allows this level to be increased to 45.2-65.6%.

3. It has been confirmed that the optimal place for embryo implantation during the technical implementation of the transplantation procedure is the apex of the uterine horn. However, for Kassu catheters, the available depth of application is a limiting factor for high embryo engraftment. The complexity of the embryo transfer procedure requires high qualifications - the pregnancy rate for an experienced specialist is 58%, while for a beginner it is 35%. It has been proven that false manipulations during catheter insertion into the apex of the uterine horn can be simplified by using the developed instruments for deep application. Thus, the use of two modifications of instruments for transplantation made it possible to increase the engraftment rate of fresh embryos to 71.4 and 68.2% and of depreserved embryos to 59.2 and 58.1%. Establishing the production of instruments for deep embryo application will significantly increase the profitability of existing ART laboratories. It has been established that the level of engraftment and viability of embryos have a direct connection with the fertility of animal breeds in the "donor-embryo-recipient-offspring " system. Experiments on interbreed transplantation of deconserved embryos show that with high fertility of the breeds used in the transplantation program, the result of embryo engraftment can be comparable to the transplantation of freshly extracted embryos. In studies on the transplantation of deconserved embryos of the Krasnogorbatovskaya breed to recipients of the Kalmyk breed, this figure was 62.4%. Transplantation of deconserved Aberdeen Angus embryos to recipients

of the Kalmyk breed made it possible to achieve a level of engraftment of 65.6%. The Krasnogorbatovskaya and Kalmyk breeds that participated in the experiments are distinguished by high reproductive qualities. In a comparative aspect, during intrabreed transplantation of deconserved embryos of the Holstein breed, which is characterized by low fertility, the engraftment rate was significantly lower and amounted to 54.4%. Thus, in the embryo transfer program, the breed fertility of the donor cows - mothers of the embryos - and the fertility of the recipient animals should be considered a positive factor.

4. Currently, direct and indirect methods are used for the transfer of depreserved embryos. The well-known indirect method involves the transfer of embryos after assessing their quality and safety after thawing. When using the direct method, embryos are transferred without assessing their viability. A comparative analysis of the effectiveness of the practical application of the two methods shows the following results. Embryo transfer using the indirect method allows for 45.4 to 65.6% pregnancy, while using the direct method, the pregnancy rate drops to 35.8-42.8%. An analysis of numerous studies has shown that with a comprehensive consideration of the maximum possible number of factors of varying degrees of influence on the successful implantation of embryos, the likelihood of obtaining high results increases when using reproductive technologies in the donor-embryo-recipient-offspring system in practice [56-58].

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