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Effect of Cryopreservation and Type of Cryoprotector on the Transplant Calves and the Gender Ratio of Kazakh White-headed and Red Steppe Cattle Breeds

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Abstract

The study was conducted in the Republic of Kazakhstan with embryos of Kazakh white-headed and red steppe cattle breeds. This article presents the results of studying the effect of cryopreservation and type of cryoprotector on the transplant calves crop. The aim of the research was to obtain preliminary information for future studies that would give scientific credence of the ways to increase beef cattle production due to a fuller realization of the animals' genetic potential.

Keywords

Embryo transfer; Calf crop; The gender ratio

Introduction

As reported Voronin and Petrikh; Kikebaev *et al.* [1,2], embryo transfer is a biotechnological method of accelerated reproduction of highly productive animals, allowing more efficient use of the genetic potential of record cows.

According to the research Polyantsev and Afanasev; Mikhailov; Kugonza *et al.* [3-5], the essence of this method consists in extraction of embryos at early development stages from genital tract of the female donor and transfer to the genital tract of the female recipient.

The most important task of the embryo transfer is a reproduction of the descendants of prominent parents in order to select among them and breed subsequently offspring of even higher quality. Thus, the embryo transfer is a systematic biotechnological method of accelerating breeding process to improve livestock in a number of generations.

As noted Nekrasov and Sumanova [6], the embryo transfer method involves a number of stages, such as the production of embryos, their evaluation, storage and transportation, preparation of donors and recipients, synchronous in terms of sexual cycle phases, and, finally, the transfer of embryos into the recipient's reproductive tract.

Today's embryo transfer technology allows obtaining a few dozen of calves from the record cow during cow's life. Ernst and Varnavskiy [7] claim that in France a single donor cow gave 80 calves during 1 yr, while in Canada this number amounted to 60 for the same period.

In order to transfer embryos in the most optimal time of the year, maintain a limited number of recipients, or simplify the embryos export and import Gavrikov [8], suggests one to use the low temperature embryo cryopreservation. One can find the information about the first calves, obtained from frozen embryos by Wilmut and Rowson in 2011, in the article by John [9].

According to Goncharov and Karpov [10], this technique still needs to be improved, though it opens up great opportunities and prospects in the dairy and beef cattle breeding.

One of the main tasks in cryobiology, according to Krasil'nikova and Tikhomirov [11], is the search for methods of artificial protection of tissue cells against the damage during freezing and thawing. Besides the protective effect, cryoprotectors also have toxic impact. When binding water, they inhibit normal hydration of the proteins and other substances.

According to some reports, freezing process reduces the viability of embryos as compared with fresh embryos, due to changes in their functional and morphological state [12]. Madison [13] reported that the percentage of engraftment of frozen and thawed bovine embryos ranged within 45-55%, while the similar factor reported in Youngs [14] was 60-70%. Seidel [15] reported that the percentage of damaged high quality embryos when freezing–thawing varied within the range of 20-40%. This can be explained by the fact that the ice formed inside the tissue cells during the cryopreservation of embryos damages the intracellular structure. Besides, according to the existing data, bovine embryos are very sensitive to thermal stress. Consequently, the effect of cryopreservation on the viability of the embryos is undeniable.

The aim of the present study was to investigate the effect of cryopreservation and type of cryoprotector on the crop and the gender ratio of transplant calves.

Methods

Investigations were carried out on three groups of cattle embryos of excellent and good quality. The embryos from the first group were transplanted to recipients on the 7th day of the sexual cycle immediately after leaching of embryo in donor cows and their morphological evaluation. The second group of embryos was subjected to cryopreservation using ethylene glycol as cryoprotector.

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Frozen–thawed embryos were transplanted into recipient heifers on the 7th day of the sexual cycle. The third group of embryos was exposed to cryopreservation using glycerol 1.4M as a cryoprotector followed by equilibristic, both at saturation and directly after thawing with removal of cryoprotector immediately before transplantation.

Donors were highly productive healthy cows, in terms of their gynecology, with two to four lactations. Recipients were selected according to the analog-based principle, i.e., healthy breeding age heifers having no essential selection value with a weight of not less than 350-380 kg. Nondescript cattle served as recipients.

Selection of recipients that, according to Dawson [16] is very important procedure, was determined, as in most cases [17], based on an evaluation of the corpus luteum and progesterone level. All recipients were synchronized using the synthetic functional drug analog of prostaglandin F2α (άλφα) (Alpha).

Transfer was performed on the 7th day of the sexual cycle depending on availability of an active corpus luteum of the 2nd and 3rd quality.

The total number of transplants was 347 embryos from 63 donor cows.

All studied embryos were obtained and transferred in the economic entities of the Republic of Kazakhstan, such as Limited Liability Companies "Taiynsha-Astyk" and "Homeland", as well as "Yenta", "Batyrgerey", and "Hafiz" farm households.

Engraftment of embryos was determined by means of a veterinarian ultrasonic diagnostic apparatus on the 45th day of the sexual cycle and on the 2nd month of pregnancy. Surveys of calves were conducted upon their birth in terms of their sexual character.

- 107 freshly born embryos were transferred to the 1st group of recipients.
- 124 frozen–thawed embryos were transferred to the 2nd group, where ethylene glycol was used as a cryoprotector.
- 116 frozen–thawed embryos were transferred to the 3rd group, where ethylene glycol 1.4M was used as a cryoprotector.

Results and Discussion

It was determined that the engraftment of embryos was disparate and varied depending on the use of cryopreservation and the type of cryoprotector. At the same time, there was a difference in the sex ratio of calves at birth.

When conducting research to determine the effect of cryopreservation on the gender predominance among the accepted bovine embryos, it was obvious that the result of embryos engraftment and further development of the fetus followed by calving varied depending on whether the embryo was exposed to cryopreservation or not.

Engraftment of fresh-received embryos, which followed by calving, reached 57%; among newborn calves, there were 32 female and 29 male calves (52.5% and 47.5%, respectively). When applying cryopreservation technique using ethylene glycol as a cryoprotector, the corresponding figures were 47.6%, 35%, and 24% (59.3% and 40.7%), respectively, while using glycerol 1.4M as a cryoprotector resulted in figures equaled to 31 females and 20 males, which corresponds to 61% and 49%, respectively.

The efficiency of transfer on getting calves is given in Table 1 for various breeds.

As it is obvious from the data presented in Table 1, the embryo transfer efficiency is higher in beef cattle breeding. At that, ethylene glycol is more benign cryoprotector as compared with glycerol.

Effect of cryopreservation on gender predominance in engraftment bovine embryos depending on the breed is presented in Table 2.

As is clear from the data shown in Table 2, female calve crop is higher as compared to the male calves; crop percentage increases when applying a more aggressive action on the embryos.

Breed	Number of transplanted embryos				Number of obtained calves			
	Transplanted in total	I	II	III	Born in total (%)	I/%	II/%	III/%
Kazakh white-headed breed	189	61	72	56	96/50.79	35/36	38/40	23/24
Red Stephen breed	158	46	52	60	75/47.47	26/35	21/28	28/37

Table 1: Efficiency of transfer on getting calves for various breeds

Group	Gender ratio of newborn calves depending on the breed			
	Female calves		Male calves	
	Number	%	Number	%
Kazakh white-headed cattle breed				
I	18	51.4	17	48.6
II	23	60.5	15	39.5
III	14	60.9	9	39.1
Red steppe cattle breed				
I	14	53.8	12	46.2
II	12	57.1	9	42.9
III	17	60.7	11	39.3

Table 2: Effect of cryopreservation on gender predominance in engraftment bovine embryos depending on the breed.

Conclusion

The efficiency of embryo transfer is higher in beef cattle breeding production. Also it should be noted that the use of cryopreservation techniques effects not only on the embryos engraftment but also on calve sex.

Higher crop of female animals among the transplant calves can be explained by the fact that embryos with the Y-chromosome, which determines male sex, are more prone to internal mutations, associated with destructive changes in cells, and are less resistant to environmental conditions, demonstrating a lower engraftment.

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