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EMBRYO PRODUCTIVITY OF THE DONOR COWS INSEMINATED BY UNISEXUAL AND BISEXUAL SEMEN*

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Abstract

The aim of the study was to determine the quantity and quality of embryos and effectivity of embryo transfer obtained from Simmental donor cows by artificial insemination with various semen types. The study indicated that cows fertilized with bisexual sperm had a higher fertilization efficiency than those fertilized with unisexual sperm. From one cow fertilized by bisexual sperm, an average of 10.0±2.25 transplantable and 2.8±1.65 was non-transplantable embryos were obtained. When used the unisexual semen, 6.2±4.30 and 4.0±2.02 embryos respectively were obtained. Thus, using bisexual semen resulted in an average of 2.6 more embryos.

The superovulation of two donors fertilized by unisexual sperm was unsuccessful. The above suggests that conditions close to natural ones created greater survival possibilities for a higher number of transplanted embryos which means more successful reproduction.

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Introduction

Embryo transplantation as a biotechnological method for accelerated reproduction opens up enormous opportunities for realizing the reproductive and biological potential of animals, with the use of an individually targeted genetic reserve with economically useful features, given phenotypic and genotypic characteristics, as well as their subsequent maximum replication in herds of recipients with the least valuable indicators. Despite the successes achieved in the technology of embryo transplantation, the issues of finding new methods for the selection of donors and recipients, induction of superovulation with a higher yield of embryos, reducing the complexity of the process, and animal stress – are still valid (HANSEL et al. 1986, GORDON 2003, MAPLETOFT et al. 2016).

In order to increase the production of milk and meat products, it is necessary to use biotechnological methods to improve animal husbandry, as well as to conduct correct selection and slaughter of animals based on their genetic potential (THORNE 2013).

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 (BAYMISHEV 1999, BABINTSEVA et al. 2012, POLYANTSEV et al. 2012).

A breeding cow gives birth to about 6–8 calves during its lifetime. Embryo transplantation is the only selection method to obtain hundreds of calves with good breed characteristics. Studies have shown that this method produces 50–60 transplant embryos obtained from one sample per year. This means that a high-yielding cow can gave a minimum of 25–30 calves embryos (AIATHANULY and SANJJAVYN 2012). Due to the transplantation of cattle embryos, it is possible to improve the breeding stock reproductive activity and to secure high productivity as soon as possible.

Following the experts, the X chromosome semen is demanded by the dairy livestock sector, and the Y chromosome semen by the beef cattle breeding sector. Fertilization of ova with sexually separated sperm, as opposed to normal sperm, allows obtaining individuals of the desired sex with a probability of 93%. When transplanting embryos not divided by sex, the birth rate of heifers is up to 55%, which is of no small importance in dairy cattle breeding, where there is a high demand for heifers with high milk production (DMITRY 2017).

Transplantation of unisexual embryos results in a major economic impact, for example:

– growth of heifers up to 95 pieces per 100 pieces. It is used to form a herd of high-yielding breeding cows without buying expensive animals from abroad;
– high productivity of cows born through the transplantation: increase average annual gross milk yield which can reach 16–20 thousand kg (compared with 4–8 thousand kg of the usual ones);
– the cost of livestock management is reduced several times due to reducing the duration of the service period (this is facilitated by the high fertility of the sexed semen and high survival ability of the transplanted embryos). Due to the reduction in the number of difficult calving and birth of dead calves, the economic losses are reduced;
– the average daily gain in live weight of male cattle generated by this technology significantly exceed the average weight gain of those of the "local" herds and increased the profitability of livestock meat farms.

The development or non-development of embryos in the uterus of recipients directly depends on their quality. During transplantation to recipients, based on their morphological characteristics, the embryos assigned to the highest category developed in 70%, while those assigned to the satisfactory group in 44% (ANZOROV et al. 2005, BAYTLESOV et al. 2007, GAVRIKOV 2012).

Proper evaluation of the embryos directly influences the results of transplant biotechnology. The use of modern scientific achievements and the latest technologies is a priority for the development of a competitive livestock industry. Therefore, in the North-Eastern region of Kazakhstan, research began determined of the embryo productivity of Simmental through fertilization by unisexual and bisexual semen.

The aim of the study was to determine the quantity and quality of embryos and effectivity of embryo transfer obtained from Simmental donor cows by artificial insemination with various semen types.

Materials and Methods

The research were carried out within the period from 2017 to 2019 in the "Galitskoye" LLP and "Pobeda" LLP in the Pavlodar region. The cows were kept in the barn all year round and fed in the TMR system. In the ration, roughage preserved in a mixed form was used, i.e. maize silage, haylage, concentrated mix, molasses, and mineral premix. Additionally, selenium licks were used. Cows were divided into 3 feeding groups depend-

ing on their yield, i.e. with the highest yield (more than 40 kg of milk), with average yield (20–30 kg of milk) and the lowest yield (less than 20 kg of milk). The fourth group consisted of dry cows that did not receive concentrated feed.

Healthy dairy cows without gynecological diseases were selected for the study, with milk production of 6000–8000 kg per lactation, an average live weight of 500–650 kg, and from 2 to 5 lactations. Thirteen Simmental animals with higher milk yields were selected. Selected donor cows were subjected to gynecological examination to determine their basic health, mainly the function of the sexual organs, and to determine the moment of egg collection. In addition, donor cows were selected based on zootechnical characteristics.

Pluset hormone (follicle-stimulating hormone; Calier, Spain) was used to activate superovulation of donor cows. The donors achieved superovulation within 11 days after hormone administration. For this purpose, the Pluset hormone was intramuscularly injected twice (morning and evening) for 4 days (in lowering doses). The hormone was injected as follows: on the first two days 1.5 ml in the morning and evening; on the third and fourth day – 10.0 ml twice per 1.0 ml. After all, cows were inoculated, 4.0 ml of prostaglandin (magestrofan) were injected additionally in the morning and the evening in order to rapidly ovulate and collect the embryos. After the hormonal treatment, signs of ovulation appeared in each of the test cows were defined based on external characteristics and behavioral changes. Then, double artificial insemination was performed with bull semen assigned to each donor (2 doses in the morning and 2 doses in the evening). In an aim to determine the embryo quantity and quality of the donor cows, two groups were identified. The first group was artificially inseminated by the unisexual sperm (X sperm), the second one with bisexual one (X, Y sperm). On the seventh day after artificial insemination of donors, the embryos were washed with Dulbecco saline from the uterine horns. The rinsed saline from the uterine horns along with the outflowing contents were collected into a container and the volume of the injected and withdrawn saline was examined. The container with the saline was kept in a laboratory with a temperature not lower than +20 degrees, settled for 15–20 minutes, and, after the embryos had been settled on the bottom of the bottle, the upper layer of the saline. The saline solution which had collected at the bottom of the bottle, up to 5 cm in volume, was carefully shaken and poured into the petri dish. The embryos were detected by microscopy (Nikon SMZ-745, magnification 0.67–5). After detection, each embryo was subjected to stereomicroscopic examination at 50–60 magnification. The quality of the embryos was assessed with the use of morphological indicators.

The obtained data on the quantity and quality of embryos obtained from donor cows were statistically analyzed. The arithmetic means (X) and standard deviations (Sd) were calculated.

The results

Selected donors which had superovulation were artificially inseminated with bisexual and unisexual semen. 8 donors were artificially inseminated with bisexual semen and 5 donors with same-sex semen. 4 portions of semen were used per animal. The embryo quantity of donors artificially fertilized with different sperm is shown in Table 1 and the diagram.

Table 1

Influence of bisexual and unisexual spermatozoids on donor embryo quantity and quality

#	Semen type	Number of donors (*n*)	Quantity of embryos obtained [%]		Quantity of transplantable embryos [%]		Quantity of non-transplantable embryos [%]	
1	bisexual sperm (X,Y)	8	102	100	80	78.4	22	21.6
2	unisexual sperm (X)	5	51	100	31	60.8	20	39.2
Total		13	153	100	111	78.4	42	21.6

As can be perceived from the above Table 1, 102 embryos were washed out of 8 donors artificially inseminated with bisexual sperm. 78.4% of the obtained embryos were transplantable, the remaining 21.6% were non-transplantable. Of all 5 donors artificially fertilized with same-sex sperm, 51 embryos were washed away. 60.8% of the embryos were suitable for transplantation and 39.2% were not suitable for transfer. If we compare the transplantability of the embryos washed out from donors, the donors impregnated with bisexual sperm have 17.6% more embryos, and the non-transplantable embryos fertilized with unisexual sperm have 2 times more embryos. 153 embryos were obtained from all cows. Of these, 111 were transplantable and 42 were without transplants.

The quantity and quality of embryos obtained through superovulation are presented in Table 2.

Table 2

Quantity and quality of embryos obtained from donor cows

#	Donor number	Embryos quantity		Embryos quality			
		total		transplantable		non-transplantable	
		n	%	*n*	%	*n*	%
Inseminated by bisexual sperm							
1	KZS178874122	20	100.0	20	100.0	–	–
2	KZS178685616	14	100.0	12	85.7	2	14.3
3	KZS178865888	1	100.0	1	100.0	–	–
4	KZS178863784	19	100.0	16	84.2	3	15.8
5	KZS178873964	7	100.0	7	100.0	–	–
6	KZS178863784	10	100.0	9	90.0	1	10.0
7	KZS178779002	14	100.0	12	85.7	2	14.3
8	KZS178777715	17	100.0	3	17.6	14	82.4
Total		102	100.0	80	78.4	22	21.6
Inseminated by unisexual sperm							
1	KZS178924313	–	–	–	–	–	–
2	KZS178865458	28	100.0	23	82.2	5	17.8
3	KZS178865471	14	100.0	3	21.4	11	78.6
4	KZS178780636	–	–	–	–	–	–
5	KZS178780424	9	100.0	5	55.6	4	44.4
Total		51	100.0	31	60.8	20	39.2

Through artificial insemination with bisexual sperm from 8 donor cows, a total of 102 embryos were obtained. It is reported that on average 12.8 embryos can be obtained from one cow. The number of embryos received from each cow was different. For example, the largest number of embryos (20) was received from the KZS178874122 cow, while the smallest embryo (1) from the KZS178865888 cow. 78.4% of the washed out embryos were allocated transplantable, 21.6% non-transplantable. The developmental stages of embryos obtained from each cow are found in different proportions. In general, the proportion of transplantable embryos varied from 17.6 to 100.0%, while the proportion of non-transplantable embryos – from 10.0 to 82.4%.

In the results of the superovulation of donors inseminated with same-sex sperm induced by the gonadotropin hormone Pluset, in the order mentioned above. We confirmed that out of 5 experimental cows, 51 embryonated eggs were obtained and an average of 10.2 embryos from one cow. 60.8% of all embryos washed from the uterus were transplant embryos of

normal structure and development. The percentage of embryos unfit for transplant, whose development was late or morphologically changed, was 39.2%.

A variety of indicators of superovulation proves that individual characteristics and physiological differences in donor cows artificially inseminated by unisexual sperm were also formed at a fairly high level. The number of embryos, cells obtained from each cow, and the proportion of transplantable and non-transplantable embryos were different. For example, the number of embryos washed out from each cow was calculated in the range of 9–28, the number of transplantable embryos was 3–23, and the number of the non-transplantable ones was 4–11.

One we can conclude that superovulation was unproductive in 2 donors subjected to the treatment. It means that the effect of the hormone on donors is different. Table 3 presents the results of a comparison of the indicators of superovulation of the Simmental donor cows artificially inseminated by unisexual and bisexual sperm. As indicated in the Table, an average of 12.8 embryos were received from a single donor fertilized with bisexual sperm, including 10 transplantable and 2.8 non-transplantable embryos.

Table 3

Average quantity and quality of embryos received from donor cows (superovulation was provoked)

Semen type	Number of donors (*n*)	Number of embryos received, total	Average number of embryos per cow	Transplantable embryos	Non-transplantable embryos
		n	X±Sd	X±Sd	X±Sd
Bisexual (X, Y)	8	102	12.8±2.28	10.0±2.25	2.8±1.65
Unisexual (X)	5	51	10.2±5.68	6.2±4.30	4.0±2.02

As a result of superovulation, on average 10.2 embryos per specimen were received from the Simmental donor cows inseminated by unisexual sperm, of which 6.2 are transplantable, 4.0 non-transplantable.

Now let us compare the results of the superovulation, fertilized by bisexual sperms in this experiment. On average 12.8 embryos were received from the donor cow fertilized by bisexual sperm, of which 10.0 were transplantable, 2.8 non-transplantable. It was reported that superovulation of the Simmental breed cows, fertilized by unisexual sperm, resulted in high rates of 10.2; 6.2; 4.0. All this highlights that the number of the embryos received from a Simmental cow fertilized by bisexual sperm

is 2.6 more, the number of transplantable embryos is 3.8 more, while in unisexual embryos the number of non-transplantable embryos is conversely 1.2 lower.

Statistical processing of the obtained data indicates that the difference between the number of transplantable, non-transplantable embryos and those obtained from bisexual and unisexual semen undoubtedly varies. The mentioned data indicates that insemination with various sperm types significantly affects the result of cattle fertilization. This demonstrates that cattle fertilization directly depends on various types of spermatozoids. The number of spermatozoids contained in unisexual sperm is 10 times lower than in bisexual sperm. This indicates that the donors artificially inseminated by unisexual sperm have a large number of non-transplantable embryos.

The development of transplanted embryos in the uterus of recipient cows directly depends on their quality. It was confirmed by many experiments that according to the morphological parameters, the highest-category embryos develop at 70%, whereas in satisfactory, middle groups the indicators do not exceed 44%. Therefore, the correct assessment, as the embryos develop, has a great influence on the results of embryo transplant biotechnology.

After the artificial insemination of superovulated cows, we washed out the 7-day-old embryos that descended from the oviduct into the uterus horn cavity using a Foley catheter. Only those embryos are transplanted to the recipient whose development corresponded to the natural development of the embryo at this stage. Only in this case, the fetus would continue to develop after transplantation.

Table 4

Stages of development of embryos obtained from donor cows inseminated by bisexual sperm

Donor number	All embryos		Embryos developmental stage							
			early morula		compact morula		early blastocyst		non-transplantable	
	n	%	*n*	%	*n*	%	*n*	%	*n*	%
KZS178874122	20	100	–	–	2	10.0	18	90.0	–	–
KZS178685616	14	100	–	–	3	21.4	9	64.3	2	14.3
KZS178865888	1	100	–	–	–	–	1	100	–	–
KZS178863784	19	100	1	5.3	2	10.5	14	73.7	2	10.5
KZS178873964	7	100	–	–	3	42.8	4	57.2	–	–
KZS178863784	10	100	–	–	4	40.0	5	50.0	1	10.0
KZS178779002	14	100	1	7.1	2	14.3	10	71.5	1	7.1
KZS178777715	17	100	7	41.2	3	17.6	–	–	7	41.2
Total	102	100	9	8.8	19	18.6	61	59.8	13	12.8

As shown in Table 4, 8.8% of the embryos obtained from the donor cows were early morula, 18.6% – compact morula, 59.8% – early blastocyst, and 12.8% – unfertilized eggs. Consequently, the morula embryos made up 27.4%, and the blastocyst embryos – 59.8%. In contrast, the morula embryos showed that the level of development from the blastocyst stage was lower.

This means that the obtained embryos developed at different stages. The degree of the embryo's development did not depend on the number of embryos obtained but on the individual characteristics of the development of animals. Most embryos were early blastocysts. It can be noted that at the stage of the development of embryos obtained from each of the experimental animals, there are some deviations. For example, early morula embryos deviate by 5.3–41.2%, compact morula – by 10.0–42.8%, early blastocyst – by 50.0–100.0%, unfertilized egg – by 7.1–41.2%. In comparison with the processes running in natural conditions, we assume that this is due to a large amount of foaming in heterogeneous uteruses, as well as their slow maturation and prolonged course of ovulation. Endocrinological regulation of processes in the gonads and the function of the fallopian tube after the hormonal treatment show what promotes the development of eggs. Table 5 shows the results of the classification of embryos by developmental stages obtained from donor cows fertilized by unisexual sperm.

Table 5

Developmental stages of embryos obtained from donor cows fertilized by unisexual sperm

Donor number	All embryos		Embryos developmental stages							
			early morula		compact morula		early blastocyst		unfertilized eggs	
	n	%	*n*	%	*n*	%	*n*	%	*n*	%
KZS178924313	–	–	–	–	–	–	–	–	–	–
KZS178865458	28	100	2	7.1	8	28.6	15	53.6	3	10.7
KZS178865471	14	100	2	14.3	–	–	3	21.4	9	64.3
KZS178780636	–	–	–	–	–	–	–	–	–	–
KZS178780424	9	100	1	11.1	2	22.2	3	33.3	3	33.4
Total	51	100	5	9.8	10	19.6	21	41.2	15	29.4

In this case 9.8% of the embryos obtained from donor cows were early morula, 19.6% compact morula, 41.2% early blastocyst, and 29.4% – unfertilized eggs. In both donors fertilized by unisexual sperm, superovulation was unproductive. The number of embryos at different stages of development and their proportion for experimental cows were different. For example, the minimum and maximum percentage difference of the early morula

varies from 7.1 to 14.3, the compact morula from 19.6 to 28.6, the early blastocyst from 21.4 to 53.6, and the unfertilized egg from 10.7 to 64.3. Donors receiving a relatively large number of embryos, and cows receiving a smaller number of embryos, demonstrated the priority development of early blastocyst. In addition, it should be noted that in the Simmental cows a large follicle formation inhibited the development of the embryo. Inversely, a small follicle formation accelerates its formation. It can be assumed that the delay and acceleration of the development of the embryo is a phenomenon arising from the simultaneous rupture of the bubbles formed in the eggs, and the uneven release of the eggs.

Table 6

Comparison of developmental stages of embryos obtained by fertilization with bisexual and unisexual semen

Spermatozoids types	Number of specimens	Quantity of embryos obtained, total		Early morula		Compact morula		Early blastocyst		Unfertilized eggs	
		n	%	*n*	%	*n*	%	*n*	%	*n*	%
Bisexual sperm	8	102	100	9	8.8	19	18.6	61	59.8	13	12.8
Unisexual sperm	5	51	100	5	9.8	10	19.6	21	41.2	15	29.4

A donor fertilized with bisexual sperm has an average of 8.8% early morula, 18.6% compact morula, 59.8% early blastocyst, and 12.8% unfertilized egg (Table 6). As for the Simmental cow, fertilized by unisexual sperm, the development of the early morula embryos is shown by 9.8%, compact morula 19.6%, early blastocyst 41.2%, unfertilized egg 29.4%.

If we compare the embryos fertilized by bisexual semen, each embryo has the early blastocyst developed in maximum quantity (bisexual – 59.8%, unisexual – 41.2%). The next stage of the development of embryos is a compact morula, which shares 18.6% in the bisexual scenario and 19.6% in the unisexual one. The most poorly formed embryo species are as follows: early morula in two breeds makes up 8.8% in the bisexual scenario, and 9.8% in the unisexual one. As for the unfertilized egg, the percentage of embryos fertilized by unisexual sperm was 2.3 times higher (in the bisexual scenario 12.8%, the unisexual one 29.4%). In this narrow tube of 0.25 ml, one dose of sperm contains at least 15–25 million germ cells. And a single dose of unisexual sperm of 0.25 ml contains only 2 million germ cells.

The actual difference lies in the fact that in the cows inseminated by bisexual sperm, in contrast to donors fertilized by unisexual sperm, the proportion of unfertilized eggs is around 16.6% more (29.4% and 12.8%). Early blastocyst embryos in bisexual embryos were more than 18.6% greater (in unisexual embryos 41.2, in bisexual 59.8). Besides, it was observed that the early morula and compact morula embryos did not have many differences, i.e. their share is only 1% (early morula 8.8 and 9.8%, compact morula 18.6 and 19.6%).

Pregnancy rates after embryo transplantation rarely exceed 50%, and in most cases are even lower. According to the reports, the onset of pregnancy at the transplantation of embryos into the lower and middle third of the uterine horn is 25–37.5%, and when they are transplanted into the upper third, it reaches 40—50% or more. According to BRIGIDA (2017), such difference in the embryo engraftment is unlikely to be related to the technique of transplantation or the quality of embryos, since in all cases these indicators were the same; at the same time, the optimal location of the seven-day embryo has a direct effect on the hormone-mediated signaling system by the feedback mechanism.

In this regard, it can be assumed that the upper third of the uterine horn is an optimal site for implantation of a seven-day embryo (OVCHINNIKOV and SMYSLOVA 1985, SREENAN 1976). The results of the study are presented in Table 7.

Table 7

Results of the embryos implantation

Farm name	Embryos transplanted in the upper part of the uterus horn		Implanted embryos		Non-implanted embryos	
	n	%	*n*	%	*n*	%
unisexual embryos						
"Galitskoye" LLP	7	100.0	3	42.8	4	57.2
"Pobeda" LLP	5	100.0	4	80.0	1	20.0
Total	12	100.0	7	58.3	5	41.7
bisexual embryos						
"Galitskoye" LLP	27	100.0	15	55.6	12	44.4
"Pobeda" LLP	15	100.0	10	66.7	5	33.3
Total	42	100.0	25	59.5	17	40.5

Table 7 above shows that out of 12 unisexual embryos 7, or 58.3%, continued to develop in the uterus of the recipient heifers, the remaining 5 embryos, or 41.7%, stopped their development. However, the results of

the embryo transplantation in different farms were different, amounting to 42.8–80.0%. Bisexual embryo transplantation studies show that 25, or 59.5%, of the 42 embryos continued to develop, while the remaining 17 embryos, or 40.5%, stopped developing. The results of the study on embryo transplantation on different farms show a difference of about 11%. Based on these data, we conclude that the development of unisexual and bisexual embryos is approximately the same. The remaining transplantable 38 bisexual and 19 unisexual embryos were kept deep-frozen in liquid nitrogen at –196ºC.

From the obtained data it was found that the results of transplantation at the localization of implanted embryos in the middle third of the uterine horn do not have significant differences.

Discussion

The current study resulted in new, theoretically substantiated, and tested in practice data on the morphology of ovaries and embryos obtained from donor cows at the induction of polyovulation, extraction, collection, and transplantation of embryos. All studies proved that most of the embryos washed out from the donor uterus formed compact morula and early blastocyst. However, it does not exclude that there are early morulae and expanded and released blastocysts. Comparing with natural conditions, such rapid and late development of embryos in the eggs of females is associated with a variety of bubbles formed in excess, and prolonged ovulation. Many bubbles formed in the egg cannot develop evenly. Some of them develop normally, the others earlier, the next ones – late. Because of this, the maturation of the bubbles varies. Due to various maturated bubbles, the eggs gradually decrease and the ovulation process lasts 4–12 hours (HASLER et al. 2003).

The combination of spermatozoids with eggs prolongs the process of fertilization for a while. Besides, the body increases the estrogen hormone released from a large number of bubbles. Due to this, the embryo moves much faster along the oviduct to the cavity of the uterine horns than it does in a natural. As a result of the imbalance of hormones in the organism, normal conditions inside the uterus also change. For these reasons, we can conclude that the development of embryos obtained from superovulated cows is also changed. In addition, we can say that the influence of such factors as the hormone type, cattle breed, time and method of producing embryos, reproductive cycle, repetition of superovulation, is significant.

As a result of research by ERNST and SERGEYEV (1989) and SERGEYEV and AMARBAYEV (1987), 1512 embryos were received from donors within 6 and 7 days after fertilization. The morphological assessment was performed. According to the authors, 27.4% of all embryos taken on the 6th day were early morula, 69.3% morula, 3.3% early blastocyst. On the 7th day, the number of morulae decreased, while the proportion of blastocyst species increased. In particular, the early morula changed for 9%, morula for 10.2%, early blastocyst for 79.5%, expanded blastocyst for 1.3%. It is described that 38.2% of all examined embryos were morphologically normal and at appropriate developmental stages. The degenerated embryos accounted for 24.1%, unfertilized eggs – 37.7%. The number of embryos transplanted from adult cows was higher compared to heifers (33.6% and 46.8%, respectively). In heifers, the incidence of unfertilized eggs is higher (42.8% and 28.4%).

In Bavaria (Germany), AYATKHANULY et al. (2010) received 791 embryos from 47 donors of the Simmental race and determined the stages of their development. 63.8% of the embryos obtained as part of the study were suitable for transfer, 15.1% were unfit for transplantation, and 21.1% of the eggs were unfertilized. The embryos obtained on the 7th day after fertilization were classified according to the stages of their development. The results were as follows: 22.8% – early morula, 49.62% – morula, 16.26% – early blastocyst, 11.32% – expanded blastocyst.

Another study proved that 69.9% of embryos obtained from 202 donor cows with the use of follitropin were suitable for transplantation. Of these, 76.6% were morules, 23.4% were blastocysts (WILLETT et al. 1951).

Scientists at the Kazakh Research Institute of Animal Breeding and Feed Production conducted research to generate the calf of the required breed through the in vivo and in vitro methods. As a result of the in vivo study, 38 same-sex embryos were obtained. Of these, 26 were transplanted fresh, the remaining 12 were cryopreserved (KARYMSAKOV et al. 2017). Scientists of the Pavlodar State University conducted comparative research to determine the quantity and quality of embryos received from 6–10 year old adult Simmental breed donor cows and 18–24 month old heifers. From 7 adult cows acquired 58 embryos, 29 embryos from heifers were received within the study. Of the embryos received from adult cows, 49 were transplantable, 9 non-transplantable. Of the embryos received from heifers, 16 were transplantable, 3 non-transplantable. Embryos from adult donor cows were in 6.9% at the early morula stage, 39.7% – compact morula, 27.6% – early blastocyst, 17.2% – blastocyst, 8.6% – expanded blastocyst. Embryos from heifers were in 10.3% at the early morula stage, 51.7% – compact morula, 34.5% – early blastocyst, 3.5% – blastocyst (AYAT-

KHAN et al. 2015). According to research by Brigida (2017), 1218 embryos came from 138 milk donors. That is on average 8.8 embryos were collected from one donor. Of these cases, 685 (56.2%) were transplanted, 98 (8.2%) were partially degenerated, 171 (14.0%) were degenerated and 264 (21.6%) were unfertilized eggs. In 2015, at the animal breeding association in Neustadt Aisch (Germany), a group of scientists headed by NOHNER received 3999 transplantable embryos from 386 donor cows, on average 10.4 embryos per donor (NOHNER 2016).

Conclusion

Based on the results of the research carried out in North-East Kazakhstan, it was found that: It was determined that the Simental breed of cattle significantly affects the result of superovulation. The cows fertilized by bisexual sperm form more embryos than those fertilized by unisexual sperm (12.8±2.28; 10.2±5.68). An average of 10.0±2.25 of a cow's embryos are transplantable, 2.8 ± 1.65 are non-transplantable; in the unisexual embryos are 6.2±4.30, 4.0± 2.02 washed out. Consequently, on average more than 2.6 embryos were obtained from bisexuals. 8.8% of all embryos obtained by fertilization with bisexual sperm developed to the early morula stage, 18.6% compact morula, 59.8% early blastocyst, 12.8% unfertilized egg. It can be noted that at the stage of development of embryos obtained from each of the experimental animals, there are significant deviations. For example, embryos at the early stage of morula deviate from 5.3 to 41.2%, compact morula from 10.0 to 42.8%, early blastocyst from 50.0 to 100.0%, unfertilized egg from 7.1 to 41.2%. As for the unisexual semen, 9.8% of all developed embryos were early morula, 19.6% compact morula, 41.2% early blastocyst, 29.4% unfertilized egg. In both donors fertilized by unisexual sperm, superovulation was unproductive. The survival rate in transplanted unisexual embryos was 58.3% but in bisexual 59.5%. However, the engraftment of unisexual embryos to the uterus of the recipient heifers in various farms ranged from 42.8–80.0%. In bisexual embryos, this indicator was 55.6–66.7%.

It is noted that for successful transplantation, embryos must reach a certain stage of development. Creating conditions similar to the natural ones improves opportunities to successfully breed and increases the number of transplanted calves.

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