

Original Research Paper

Effect of Semen on the Embryo Productivity of Donor Cows and the Development of Transplant Calves

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Abstract: In animal husbandry, more heifers are required for the expanded reproduction of the herd or milk, and more bulls are needed to obtain meat. One of the effective ways to artificially regulate the secondary (at birth) sex ratio is the technology of sexed semen. In this connection, the purpose of this study was to study the effect of sexed and native semen on the embryonic productivity of donor cows, the survival rate of embryos in recipient cows, and the development of transplant calves. The studies were carried out on Simmental cows in the period from 2017 to 2019 in the Galitskoe LLP and Pobeda LLP in the Pavlodar region (Republic of Kazakhstan). It was found that 80 (78.4%) of 102 embryos were suitable for transplantation in cows fertilized with native sperm and 31 of 51 embryos (60.8%) in cows inseminated with sexed sperm. At the same time, embryos at the stage of compact morula and early blastocyst prevailed in both groups of cows. Later, at the time of embryo transplantation to recipient cows, the survival rate was 59.5% for two-sex and 58.3% for single-sex transplants. During the analysis of postnatal development of different-sex transplant calves, it was found that although they were characterized by different growth dynamics depending on sex, they generally exceeded the calves of the control group in this parameter. The detected differences in superovulation and embryo productivity of cows of the Simmental breed show that the individual characteristics of donor cows are decisive regardless of which sperm (native or sexed) was used for artificial insemination. At the same time, in all cases, donor cows fertilized with native semen were distinguished by the best embryo productivity, although the differences with cows inseminated with sexed sperm were not significant.

Keywords: Superovulation, Artificial Insemination, Native and Sexed Semen, Embryo, Morphogenesis

Introduction

An effective way to increase productivity in dairy and beef cattle breeding is the use of biotechnological methods of accelerated reproduction of the most valuable parental genotypes (Kurmanov and Baluanov, 2012; Thorne *et al.*, 2013; Ateikhan *et al.*, 2019).

A cow can reproduce up to 6-8 calves during its life and with the use of transplantation technology, tens or hundreds of descendants with highly economically valuable traits. The use of this method makes it possible to obtain 50-60 embryos suitable for transplantation from one donor cow per year, or 25-30 newborn calves (Ayatxanuli and Sanjjavin, 2012).

An even greater economic effect is achieved with the use of transplantation of single-sex embryos obtained using sexed semen.

In particular, this procedure makes it possible to replace the herd with breeding stock in the shortest possible time, reduce the cost of keeping cows by reducing the duration of the service period, reduce the number of difficult calving and stillborn calves and significantly increase the productivity of the livestock used (BULL Selectservice, 2022).

Unlike native sperm, the fertilization of eggs with sexed semen makes it possible to obtain calves of the desired sex with a probability of up to 93%. When

transplanting embryos that are not separated by sex, the birth rate of bulls can reach up to 55% (Mashtaler, 2017).

Sexed semen is mainly used in dairy farming, where the share of its use is 95.6% (IETS, 2022). Accordingly, cows with high milk yields and valuable breeding qualities are inseminated with sexed semen (gametes with an X chromosome), followed by transplantation of the resulting embryos to mothers of medium or low productivity.

It is of interest to compare the results of the insemination of donor cows with native and sexed sperm. This applies not only to the number of embryos suitable for transplantation but also to their qualitative characteristics since the correct assessment of embryos determines the effectiveness of their further transplantation and survival. Taking into account the fact that the age dynamics of live weight are an important indicator of the growth and development of animals, a comparative analysis of the live weight gain of calves obtained by embryo transplantation and naturally is of particular interest.

In connection with the above, the purpose of our research was to study the effect of sexed and native semen on the embryonic productivity of donor cows, the survival of embryos in recipient cows, and the development of transplant calves.

Materials and Methods

The study was carried out from 2017 to 2019 in the Galitskoe LLP and Pobeda LLP of the Pavlodar region (Republic of Kazakhstan). 13 heads of record-breaking cows of the Simmental breed were selected as donors. The donor cows were selected according to zootechnical data analysis. These were healthy animals with no gynecological diseases, weighing 550-650 kg, giving 6,000-8,000 kg of milk per lactation, and having from 2 to 5 lactations.

To induce superovulation in cows, a follicle-stimulating hormone was used (Pluset, Laboratorios Calier, S.A., Spain). Hormone injection was performed intramuscularly twice in decreasing doses for 4 days (10 mL in total). The order of administration of the hormone was as follows: On the first two days, 1.5 mL in the morning and evening, and on the third and fourth day, 1 mL. In conclusion, 4 mL of prostaglandin (Magestrophan, Mosagrogen JSC, Russia) was injected in the morning and evening for the rapid manifestation of cow heat.

At the end of hormonal treatment, the manifestation of heat was detected in each cow under study, according to external signs and behavioral changes. Artificial insemination was carried out twice (in the morning and the evening, two doses each) with the sperm of a bull assigned to each donor cow. To assess experimentally the embryo productivity of cows, they were divided into two groups: The first one was artificially inseminated with native sperm and the second one was inseminated with sexed sperm (gametes with an X chromosome). On the 7th day after artificial insemination, the embryos were

washed out of the uterine horns with Dulbecco solution, which was collected into a container after treatment, controlling the volume of fluid.

After the embryos were deposited on the bottom of the bottle, the upper part of the solution was drained using the siphoning effect. The remaining solution at the bottom of the bottle with a volume of up to 5 cm³ was gently shaken and poured into Petri dishes. Embryo detection was performed using a Nikon SMZ-745 microscope, with a magnification of $\times 50$ -100 or more. The quality of the embryos was assessed according to morphological parameters by stereomicroscopic examination.

For a comparative study of the growth and development of the born calves, two groups were formed: A control and an experimental group of 5 bulls and 7 heifers each. The experimental group was represented by transplant calves obtained using native semen. The animals were selected according to the principle of analogs in age and live weight. The care and maintenance of the experimental calves were the same. The calves were weighed in the morning before watering and feeding. Live weight at birth, at the ages of 3 and 6 months was determined by the method, described by Sakar *et al.* (2020) and Ashwini *et al.* (2019). Based on the weighing data, the average daily, absolute, and relative weight gain of calves was calculated.

Statistical analysis of sample data was carried out according to Student's t-test with a preliminary determination of the normality of the distribution according to the Kolmogorov-Smirnov and Shapiro-Wilk criteria. In case of non-fulfillment of the assumption of a normal distribution of the populations from which the compared samples were taken, the nonparametric Mann-Whitney U-test (M-W U-test) was used, otherwise, Student's t-statistics (Triolo *et al.*, 2018).

As an indicator of the variability of the mean value of the trait (X), the standard error (Sx) was determined. Grouping of primary data and biometric calculations were carried out using Excel Microsoft and STATISTICA programs.

Results

The results of the embryo productivity of donor cows are shown in Table 1. A total of 153 embryos were obtained from all cows, of which 111 were suitable for transplantation.

As follows from the data presented in Table 1, 17.6% more embryos suitable for transplantation were obtained from donor cows inseminated with native sperm than from the animals inseminated with sexed sperm. However, the difference was not significant. In total, out of 102 embryos from cows inseminated with native sperm, 78.4% were found suitable for transplantation and 60.8% of 51 embryos were found suitable in cows inseminated with sexed sperm. Moreover, this indicator varied depending on the individual characteristics of cows (Table 1).

On average, when cows were inseminated with native sperm, there were 10 embryos suitable for

transplantation per head, and in cows inseminated with sexed semen -6.2 (Table 1).

The proportion of embryos suitable for transplantation in different cows varied from 17.6 to 100.0% (insemination with native sperm) and from 21.4 to 83.2% (insemination with sexed semen).

Since the recipient cow receives only those embryos that correspond to the natural development of embryos at this stage, part of our study was aimed at evaluating the morphology of the embryos obtained (Table 2). It can be seen that the stages of embryo development from a particular cow were characterized by variability.

In total, embryos in the morula stage accounted for 27.4% of the embryos obtained from the donor cows inseminated with native sperm, and for those in the blastocyst stage, this number equaled 59.8% and in cows fertilized with sexed semen -29.4 and 41.2%, respectively.

As for cows fertilized with sexed semen, the proportion of embryos at the early stage of morula was 9.8%, while 19.6% were at the compact morula stage, 41.2% at the early blastocyst stage, and 29.4% at the unfertilized egg stage.

Although the degree of embryo development was determined not by their number, but by the individual characteristics of animals, in general, embryos at the compact morula and early blastocyst stages prevailed in both groups. Greater development of the follicle slows down the development of the embryo and a smaller one accelerates it.

An important point of embryo transplantation is their survival rate. Studies on the transplantation of different-sex embryos show that out of 42 transplants, 59.5% continued development, while the remaining 40.5% of embryos stopped development. In turn, of the 12 single-

sex embryos transplanted to recipient cows, 58.3% continued their development in the uterus and the remaining 41.7% stopped developing.

The next important indicator characterizing the postnatal development of calves obtained by transplantation is the dynamics of live weight.

As can be seen from the results presented in Table 3, at birth, the bulls of the experimental group significantly exceeded the bulls of the control group by 1.6 kg, at 3 months by 4.4 kg, and at 6 months by 10.4 kg.

A similar picture can be observed for heifers, only with the difference that, compared with the bulls, the live weight of the born heifers of the experimental group exceeded the animals in the control group by 2.4 kg and at 3 and 6 months by 2.9 and 7 kg, respectively.

The average daily gain in bulls of the experimental group aged from birth to 3 months was 31.1 g more in comparison with the bulls of the control group, while at the age from 3 to 6 months the difference in this indicator was already 66 g and taken for the entire growing period it equaled 48.8 g. At the same time, when comparing the heifers between groups, only at the age of 3-6 months, there were significant differences in the average daily weight gain of experimental animals from control animals at the level of 46 g.

On the contrary, when calculating the relative gain, significant differences were not found in the bulls of the experimental and control groups and when comparing heifers from 3 to 6 months, significant exceedance in the experimental group compared to the control group amounted to 2.6%.

Table 1: The effect of native and sexed sperm (gametes with X chromosome) on the embryo productivity of donor cows ($X \pm S_x$)

Item	Cow number	Embryos					
		Total		Suitable for Transplantation		Unsuitable for transplantation	
		n	%	n	%	n	%
Native sperm							
1	KZS178874122	20.0	100.0	20.0	100.0	-	-
2	KZS178685616	14.0	100.0	12.0	85.7	2	14.3
3	KZS178865888	1.0	100.0	1.0	100.0	-	-
4	KZS178863784	19.0	100.0	16.0	84.2	3	15.8
5	KZS178873964	7.0	100.0	7.0	100.0	-	-
6	KZS178863784	10.0	100.0	9.0	90.0	1	10.0
7	KZS178779002	14.0	100.0	12.0	85.7	2	14.3
8	KZS178777715	17.0	100.0	3.0	17.6	14	82.4
Average per head		12.8±2.28		10.0±2.25		2.8±1.65	
Total		102.0	100.0	80.0	78.4	22	21.6
Sexed sperm							
1	KZS178924313	-	-	-	-	-	-
2	KZS178865458	28.0	100.0	23.0	83.2	5	17.8
3	KZS178865471	14.0	100.0	3.0	21.4	11	78.6
4	KZS178780636	-	-	-	-	-	-
5	KZS178780424	9.0	100.0	5.0	55.6	4	44.4
Average per head		10.2±5.68		6.2±4.30		4.0±2.02	
Total		51.0	100.0	31.0	60.8	20	39.2

Table 2: Stages of development of embryos obtained from donor cows inseminated with native and sexed sperm (gametes with X chromosome)

Donor number	Stage of embryo development									
	All embryos		Early morula		Compact morula		Early blastocyst		Unfertilized eggs	
	n	%	n	%	n	%	n	%	n	%
Native sperm										
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	100.0	--	
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
Sexed sperm										
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

Table 3: Change in live weight of transplant calves (two sexes) with age, kg ($X \pm Sx$)

Age and age period months	Control group		Experimental group	
	Young bulls (n-5)	Young heifers (n-7)	Young bulls (n-5)	Young heifers (n-7)
Live weight, kg				
At birth	36.2±01.240	31.3±00.420	37.8±1.42*	33.7±1.19*
3	104.4±01.020	98.8±00.790	108.8±1.68**	101.7±0.96***
6	172.6±02.710	150.7±00.920	183.0±1.22**	157.7±1.77***
Average daily gain, g				
From birth to 3	757.8±07.380	750.7±06.360	788.9±14.48*	753.9±12.93
3 - 6	757.8±24.190	576.2±16.520	824.4±23.67***	622.2±20.98**
From birth to 6	757.8±11.190	663.5±6.0400	806.6±7.11***	688.9±14.75
Relative gain, %				
From birth to 3	189.4±07.560	216.2±02.990	189.0±08.85	203.9±09.98*
3 - 6	65.3±01.980	52.5±01.860	68.3±02.91	55.1±02.14*
From birth to 6	378.3±12.560	382.3±07.630	386.6±16.58	372.2±20.30
Absolute gain, kg				
From birth to 3	68.2±0.6600	67.5±00.570	71.0±1.30*	68.0±0.21
3 - 6	68.2±2.1700	51.9±01.490	74.2±2.13***	56.0±1.89***
From birth to 6	136.4±2.0100	119.4±01.090	145.2±1.28***	124.0±2.65

Note: Confidence: *p<0.05; **p<0.01; ***p<0.001

In terms of absolute weight gain, the bulls of the experimental group exceeded the control bulls in the age period from 3 to 6 months by 6 kg and from birth to 6 months by 8.8 kg. In heifers, a significant difference in experimental animals compared to the control group was noted only at the age of 3 to 6 months and equaled 4.1 kg.

That is, although the animals of the experimental groups, regardless of gender, were characterized by different growth dynamics, according to certain indicators, either in all or in certain age periods, they surpassed the animals of the control groups and calves obtained from transplanted embryos were characterized by better growth dynamics.

Discussion

Embryo transplantation, as a biotechnological method of accelerated reproduction, opens up great opportunities for the practical realization of the genetic potential of valuable animals due to their multiple replications in descendants. In dairy farming, the transplantation of cattle embryos has one main goal, namely, to receive the largest number of calves with the genetic capabilities of the mother from the donor cow.

The differences in the indicators of superovulation and embryo productivity of cows of the Simmental breed found in our study show that individual characteristics and

physiological differences in donor cows are decisive regardless of which sperm (native or single-sex) was used for artificial insemination.

Our results are largely consistent with the data of other authors. For example, in our case, the average number of embryos from one donor fertilized with native sperm was 12.8 and in the case of single-sex embryos, their number equaled 10.2. According to foreign researchers, this indicator can vary from 8.8 to 16.8 (BVN, 2022; Ayatkhan *et al.*, 2010; Brigida, 2018). In turn, the number of embryos suitable for transplantation according to our data was 78.4% (native sperm) and 60.8% (sexed sperm). According to other information, the variability of this parameter is 38.2-69.9%, of which the proportion of embryos suitable for transplantation and classified as the highest category can be up to 70% and satisfactory up to 44% (Willett *et al.*, 1951; Anzorov *et al.*, 2005; Baitlesov *et al.*, 2007; Ayatkhan *et al.*, 2010; Brigida, 2018).

The development of transplanted embryos in the uterus of recipient cows directly depends on their quality. In particular, in the works of individual authors, as in our case, on the 7th day, the number of morulae decreased and the proportion of blastocysts increased. At the same time, embryos undergoing degeneration accounted for 24.1%, and unfertilized eggs for 37.7% (Ernst and Sergeev, 1989).

According to other data, the embryos obtained on day 7 after fertilization were distributed according to the stages of development as follows: Early morula: 22.8%, Morula: 49.62%, Early blastocyst: 16.26%, Expanded blastocyst: 11.32% (Ayatkhan *et al.*, 2010).

According to the results of the work of scientists of the Pavlodar State University (Republic of Kazakhstan), the dependence of the degree of embryo development on the age of the donor was established. For example, in heifers, compared with adult cows, the stages of morula and early blastocyst prevailed in embryo development (Ayatkhan *et al.*, 2015).

Many factors can affect the pregnancy of recipients. The greatest problem here is the survival of embryos. This is one of the main problems in embryo transplantation, manifested in rejection and death in the recipient's body. For example, the survival rate may depend on the condition of the semen: 45% for thawed and up to 55% for freshly obtained embryos (Madison and Madison, 2018). However, in general, the causes of this phenomenon are not fully understood. In this connection, according to many authors, special attention should be paid to the selection of recipient cows. The success of transplantation depends on the reproductive health of recipients.

In particular, some publications provide information that anatomical and physiological features of blood supply and neurohumoral regulation of introverted processes in cows influence the development and implantation of a transplanted embryo. In this regard, it

can be assumed that the upper third of the uterine horn is the optimal place for the implantation of a seven-day embryo (Sreenan, 1976). The age factor is also not excluded. For example, it was noted that the number of suitable embryos in adult cows was higher compared to heifers (46.8 vs. 33.6%) and on the contrary, the occurrence of unfertilized eggs in heifers was higher (42.8 vs. 28.4%) (Ernst and Sergeev, 1989).

The differences in the semen (native and sexed) used are superimposed on the individual differences of the animals. According to our data, it follows that superovulation was less productive in cows inseminated with sexed sperm, and more unsuitable embryos were obtained in cows of this group.

The number of spermatozoa contained in single-sex sperm is 10 times lower than in two-sex sperm. Thus, one dose of 0.25 mL of native sperm contains at least 15-25 million germ cells and one dose of the single-sex one contains only 2 million germ cells.

In cows inseminated with native sperm, unlike the donors fertilized with sexed semen, the proportion of unfertilized eggs was about 16.6% less and the number of embryos in the early blastocyst stage was 18.6% more.

Studies confirm that most of the embryos washed from the uterus of a donor cow form a compact morula and an early blastocyst, but do not exclude early morula, expanded, and released blastocysts. At the same time, a lot of bubbles formed in the egg cannot develop evenly. Some of them develop earlier, others later, which means that the maturation of the bubbles varies. Due to various mature vesicles, the eggs gradually decrease and the ovulation process lasts 4-12 h (Hasler *et al.*, 1987).

Pregnancy rates after embryo transplantation rarely exceed 50% and in most cases are even lower. Of course, these indicators are influenced by numerous factors, including the localization of the embryo implanted in the uterine horn.

Thus, the breed of cattle, age, type of hormone used, repetition of superovulations, time and method of obtaining embryos, and sexual cycle are additional factors that have to be taken into account during the artificial insemination of cows and embryo transplantation.

Accordingly, considering the results obtained in Simmental cows, we believe that the technology of semen separation by sex, followed by artificial insemination of valuable animals and transplantation of embryos to recipient cows is a promising method to accelerate reproduction and increase productivity in animal husbandry with the opportunity of predominantly obtaining bulls or heifers, depending on the specialization of the economy.

Conclusion

Based on the results of the study carried out, the following conclusions can be drawn:

- 1) The quality of sperm (native or sexed) significantly affects the result of superovulation, which is less productive in cows inseminated with sexed semen. Cows fertilized with native sperm form a larger number of embryos per head (12.8 ± 2.28) than those fertilized with sexed sperm (10.2 ± 5.68) and more embryos suitable for transplantation (10.0 ± 2.25 and 6.2 ± 4.30 , respectively). However, the revealed differences are non-significant
- 2) The quality of embryos obtained during insemination of donor cows with native and sexed sperm does not differ significantly, except for the early blastocyst stage and the number of unfertilized eggs. In the first case, 59.8% of all embryos were at the early blastocyst stage and 12.8% were unfertilized eggs. In the second one, 41.2% of all developed embryos were early blastocysts and 29.4% were unfertilized eggs. At the same time, there were significant differences in individual animals
- 3) The survival rate of transplanted embryos was 59.5% from cows inseminated with native sperm and 58.3% from cows fertilized with sexed sperm
- 4) Calves obtained from transplanted heterosexual embryos, compared with the ones born naturally, had better growth dynamics

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Author's Contributions

All authors equally contributed to this study.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues are involved.

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