

Results of transplantation of embryos of the Simmental breed

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Abstract

The selection of offspring from the desired sex can be one of the determining factors to increase the genetic progress and farmer's profitability in either beef or dairy cattle. This study's objective was to determine the impact of two approaches of artificial insemination on embryo-productivity and transplantation of cow embryos. We used as a model the symmental breed, bred in Kazakhstan's Northeast region. The quantity and quality of embryos obtained by artificial insemination of donor cows with same-sex and ambisexual semen were evaluated. In particular, the study examined the embryo transfer process from artificial insemination of ambisexual semen, embryo transfer from cow donors, washing out, evaluation, and transfer to recipients. This study shows that ambisexual insemination resulted in an average 61.3% more suitable embryos than same-sex insemination. Additionally, embryos from ambisexual fertilization are more likely to reach the early blastocyte stage (59.8±4.18%). Despite that, there is no change in the success of embryo transplantation. In summary, creating conditions close to natural ones improves the success rate of embryo transfer.

Keywords: ambisexual semen, blastocyte, embryo transfer, in vivo embryo production, sex sorting, symmental.

Introduction

Pre-selection is the most sought-after reproductive biotechnology of all time (Ateikhan et al., 2019; Garner & Seidel, 2008; Kurmanov & Baluanov, 2012). The key motivation for incorporating sex-sorted semen in the process is to achieve the desired sex in the resulting progeny (Chowdhury et al., 2018; Hand et al., 2016). Adopting this technology increases the supply of replacement heifers and the consequent hastening of genetic gain (Babintseva et al., 2012; De Vries et al., 2008). It has also been observed that the gestation of a female calf leads to an increase in milk production in cases when the daughter's pregnancy occurs at the first parity (Hinde et al., 2014; Moore & Hasler, 2017). Therefore, increase milk and meat products, it is necessary to use biotechnological methods of accelerated reproduction of highly productive stock and selection of animals with high genetic potential (Garner & Seidel, 2008).

Embryo transfer represents a small proportion of the total commercial market for assisted reproduction,

but recent records indicate an increase in technology adoption (Ferré et al., 2020; Polyantsev & Afanasyev, 2012). It is widely reported that chances of multiple calves production are increased by transfer of embryo using sexed semen for fertilization (Bylesjö et al., 2006; Hayakawa et al., 2009). Fertilization of ovum with sperm divided by sex, instead of conventional fertilization, increases the probability to obtain individuals of the desired sex by up to 93% (Betteridge, 2003; Itze-Mayrhofer & Brem, 2020). Also, sex sorting increases bulls' birth rate by 55% (Itze-Mayrhofer & Brem, 2020). This increase has immense benefits in dairy cattle breeding since the farmers can easily replace heifers to boost milk production (Hayakawa et al., 2009).

Globally, dairy producers have adopted frozen-thawed semen for artificial insemination (A.I.) due to the high probability of producing a male calf compared to natural mating (Berry & Cromie, 2007; Phillips & Jahnke, 2016), as does the use of frozen

semen (Roche et al., 2006; Willett et al., 1951). However, insemination's optimal time depends on spermatozoa's lifespan and oocyte viability (Lestari et al., 2019; Sá Filho et al., 2014). Our research aims to determine the impact of artificial fertilization with same-sex semen on embryo-productivity. To achieve that, we used of cow donors of symmental breed in Kazakhstan's northeastern region.

Materials and Methods

Animal management

Our research was conducted from 2017 to 2019 in LLP Galitskoye and Pobeda Pavlodar region. The study had thirteen Symmental cattle that met the study criteria. The study criteria were that the herd must be donor healthy, without gynecological diseases, and have dairy productivity per lactation 6000-8000 kg. Their live weight ranged between 550-650 kg, and they have engaged in 2 to 5 lactations in the last week. The selected donor cows were rectally examined to reveal their structure, functionality of their genitals, and hunting signs. Also, donor cows were selected based on zootechnical data (Moore & Hasler, 2017). The experiments were conducted following the institutional and national standards for the care and welfare of animals. The researchers used appropriate measures to minimize the pain or discomfort of the animals used in the study.

The hormone Pluset (follicle-stimulating hormone) manufactured in Spain (Calier Spain) was used to induce super donor humidity. The double administration method (morning and evening) of the hormone Placed intramuscularly for four days, 10.0 ml (in decreasing doses) was used for this purpose (De Vries et al., 2008). In the first two days, 1.5 ml of pluset hormone was administered in the morning and evening, followed by 1.0 ml of the hormone on the third and fourth day. Also, the cows received the latest vaccination and a dose of 4.0 ml of prostaglandin (magestrophan) in the morning and evening for fast hunting.

Embryo-productivity of cow donors

At the end of hormonal treatment, the cows were hunted according to external signs and behavioural changes. The artificial insemination assigned to each donor was performed twice (two doses in the morning and two doses in the evening). Two groups were allocated to determine the embryo-productivity of cow donors. The first group was artificially inseminated with same-sex sperm (X sperm), and the second

group was artificially inseminated with ambisexual sperm (X, U sperm). On the seventh day after artificial insemination, the donor horns' embryos were washed with Dulbecco solution. The solution flowing out and washing the uterine horns was collected in a container, and the volume of the injected and excreted solution was monitored. The container with the solution was stored in a laboratory with temperature not lower than +20 degrees Centigrade. It was defended for 15-20 minutes after embryos were deposited on the bottom of the bottle, and the upper part of the solution was drained using the siphoning effect.

The remaining solution at the bottom of the bottle volume up to 5 cm was shaken and poured into Petri dishes. Detection of embryos was performed microscopically (Nikon SMZ-745), with magnification from 50-100 times. After detection, each embryo was evaluated by stereomicroscopic examination. The quality of the embryos was assessed using morphological indicators (Hand et al., 2016).

Statistical analyses

All statistical analyses were performed using R version 4.0.3 (R Foundation for Statistical Computing) with the standard packages for statistical analysis. Data were presented as means \pm standard deviation. Differences between two groups in the numbers of embryos, suitable embryos, and unsuitable embryos were compared using student's t-test. Evaluation of stage of embryo development (early morula, compact morula, early blastocyte, unfertilized) and the results of embryo transplantation (survived embryos, uninhabited embryos) were analyzed by Fisher's exact test. After finding a significant relationship, we performed a multinomial regression to determine the log odds of the outcomes are modeled as a linear combination of type of fertilization. Significant differences were defined when p-value was less than 0.10, similarly as performed by Albuquerque et al. (2012).

Results and Discussion

Artificially inseminated eight heads of donors with ambisexual sperm, 5 with same-sex sperm. For each donor, four doses of semen were used. The results of the quantity and quality of the embryos obtained by inducing superovulation are presented in Table 1.

Table 1 – Average quantity and quality of received embryos from cow donors (super-population was caused)

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Types of men	Number of donors, n	Number embryos received, total. n	Average number	Suitable embryos	Unsuitable embryos
			embryos per cow		
			mean±SD	mean±SD	mean±SD
Ambisexual (X, y)	8	102	12.8±2.28a*	10.0±2.25a	2.8±1.65a
Same-sex (X)	5	51	10.2±5.68a	6.2±4.30b	4.0±2.02a

*Different letters on the same column show different rates (p < 0>

The type of semen did not show any influence in the average number of embryos (p>0.10). Artificial insemination with ambisexual semen from 8 cow donors resulted in 102 embryos with an average of 12.8 embryos. For cow-donors inseminated with same-sex sperm, the superovulation results on average per cow revealed 10.2 embryos. Previous studies showed that the number of embryos washed from each cow varied between 9-28 and the number of usable embryos was between 3-23, and the number of useless embryos was between 4-11 (Phillips & Jahnke, 2016).

The two types of insemination affected the quality of embryos obtained. The statistical analysis suggested

that cows inseminated with same-sex sperm are less likely to produce suitable embryos (Table1). Ambisexual insemination resulted in an average 61.3% more suitable embryos than same-sex insemination. In contrast, in same-sex embryos, the number of unsuitable embryos is 42.8% higher. In agreement with the current findings, previous studies showed that donors artificially inseminated with same-sex semen have many unsuitable embryos (Niles et al., 2019).

Only those embryos whose development corresponds to the embryo's natural development at this stage are transferred to the recipient. The research results are shown in the Tables 2.

Table 1: Average quantity and quality of received embryos from cow donors (super-population was caused)

Type of semen	Donor number	All embryos		Stage of embryo development							
				Earlymorula		Compactmorula		Early blastocyte		Unfertilized	
		n	%	n	%	n	%	n	%	n	%
Ambisexual (X, Y)	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/Average	102	100	9	8.8	19	18.6	61	59.8	13	12.8	
Same-sex (X)	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/Average	51	100	5	9.8	10	19.6	21	41.2	15	29.4	
Exact Fisher Test		p-value = 0.06									

From the embryos obtained from cow donors, 8.8% were early morula, 18.6% compact morula, 59

Conclusions

The quality of the sperm significantly influences the superovulation result. Cows fertilized with ambisexual

sperm formed more embryos suitable for transplantation (10.0±2.25). Additionally, we found an association between embryos development stages

and the type of semen used. In summary, embryos obtained by ambisexual fertilization are 1.5 times more likely to reach the early blastocyte stage than the embryos obtained by same-sex fertilization. Finally, the transplanted same-sex survival rate was 58.3%, but that of the bipeds was 59.5%. However,

same-sex embryos' survival rate to the uterus of heifer-recipients in different farms varies between 42.8-80.0%. In ambisexual embryos, the rate was 55.6-66.7%. Consequently, same-sex fertilization is a reliable technique for symmental cows, but with a reduced quantity and quality of embryos.

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