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The Effect of Conventional Semen, Sexed-Semen, and Embryo Transfer on Pregnancy Rate in Holstein Dairy Cows

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Abstract

In this study, it was aimed to detect the effects of conventional semen, sexed-semen and embryo transfer on pregnancy rates in Holstein dairy cows. In the study, a total of 139 healthy cows with the serum progesterone > 8 ng/ml were used as animal material. Estrus synchronization protocol was applied and cows were divided into three different groups as conventional semen group (G1, n=46), sexed semen group (G2, n=47), and embryo transfer group (G3, n=46) considering age, body condition score, lactation number, and body weight. Cows in G1 and G2 were inseminated with conventional semen and sexed-semen, respectively. Embryo transfer was performed to cows in Group 3 (n=46) 7th day after estrus. The embryos were transferred to recipients as freshly. Pregnancy examinations for the cows were conducted on the 30th and 60th days of gestation. The statistical analysis of the obtained results was performed. Pregnancy rates were detected as %50, %46,8 and %69,56 in G1, G2, and G3 respectively on 30th day. On 60th day, however, pregnancy rates were %45.60, %42.55 and %67.39 in G1, G2, and G3 respectively due to embryonic losses. The pregnancy rate was significantly higher in the embryo transfer group (G3) compared to the conventional semen (G1) and sexed-semen (G2) group ($p<0.05$) on both 30th and 60th days. There was no significant difference between G1 and G2 ($p>0.05$). Findings led to the conclusion that higher serum progesterone level may increase pregnancy rate in cattle. In addition, it is evident that embryo transfer has the potential for widespread use in the field of veterinary medicine in terms of genetic progress.

Keywords: cattle reproduction, embryo transfer, sexed-semen, conventional semen, theriogenology

Introduction

Genetic progress in economic traits such as milk and meat is the main goal of cattle breeding. Genetic improvement of economic traits relies on phenotype and pedigree information of artificial insemination (AI) bulls the for many years.[1] During the last two decades, with the development of genomic selection (GS), which relies on genetic markers such as single nucleotide polymorphisms (SNP), it has been focused on the selection of AI bulls using their genotype and phenotype information. Selection of AI bulls using GS has enabled a dramatic decrease in generation interval and increased genetic progress especially in dairy cattle. [2]
AI is a common reproductive tool in dairy cattle and has revolutionized cattle breeding during the last century. The first application of AI dates from the 1900s.[3] In conventional AI, semen is not evaluated for the sex of spermatozoon. Sexed-semen technology was first developed in the 1980s. This technology enables to discrimination of X or Y-carrying spermatozoon using a fluorescence-activated cell sorter based on differences in their DNA content. [4] Sexed-semen may decrease the economic cost of replacement heifers, enables the production of valuable females, and accelerate the expansion of the growing herd. Although sexed-semen represents a small proportion of the AI market, there is an increasing demand for sexed-semen. Studies indicate that using sexed semen in AI may affect fertility in dairy cattle. [5-8]

Embryo transfer is another important biotechnological tool in cattle breeding. Embryo transfer contributes to an increase in genetic progress in cattle. [1,9] In embryo transfer, embryos collected from valuable donor animals are transferred to recipients aiming to have several offspring in the

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same breeding season.[10] The primary use of this tool is to improve the reproductive traits of females with genetic superiority. [11, 12] Different factors such as embryo quality, suitability of recipient, and interaction between embryo and recipient may affect the success of embryo transfer. [11-14]

The success of using conventional semen, sexed-semen, and embryo transfer is evaluated by taking into account the pregnancy rates. A high pregnancy rate is an important matter for the sustainability and profitability of animal production in dairy cattle. Therefore, herein we investigated the effects of conventional semen, sexed-semen, and embryo transfer on pregnancy rates in Holstein dairy cows.

Material and Methods

Ethical Approval

This study was performed by the approved ethical rules of Bursa Uludağ University (Protocol no. 2021-13, Decision number: 02).

Animals and Management

In the study, a total of 139 healthy cows were used as animal material. Because serum progesterone level may effects pregnancy rates[12], cows with the > 8 ng/ml serum progesterone were selected as animal material. Cows were divided into three different groups conventional semen group (G1, n=46), sexed-semen group (G2, n=47), and embryo transfer group (G3, n=46) considering age, body condition score, lactation number, and body weight. Embryos were collected from five valuable donor cows and transferred to 46 recipient cows in G3. Donor cows were 3-4 years old, with 2,75-3.25 body condition scores and regular estrus. On the other hand, healthy recipient cows were, 2-3 years old, with 400-500 kg body weight, and had never been inseminated at postpartum. The corpus luteum was detected with rectal palpation and 5 ml prostaglandin F (PGF) (Dinolytic, Zoetis) was injected intramuscularly. Then, estrus was detected and cows were inseminated with conventional semen and sexed-semen of the same bull in G1 and G2 respectively. All cows in the study groups were fed according to NRC (National Research Council) recommendations. The chemical composition of the ratio of cows were given in Table 1.

Table 1. Chemical composition of animal ration

Nutrients	
Dry matter (DM)	23,5 kg
Crude Protein of DM (%)	13,6
Ash of DM (%)	5,5
Starch of DM (%)	25
Non-fiber carbohydrate of DM (%)	38,5
Neutral detergent fiber of DM (%)	33
Acid detergent fiber of DM (%)	19,5
Calcium of DM (%)	0,78
Phosphorus of DM (%)	0,39
Net energy lactation (Mcal/kg DM)	1,39

Collection of Blood Serum Samples and Progesterone Hormone Analysis

Blood samples were obtained from the coccygeal vein on the 7th day following artificial insemination in G1 and G2, and the 7th day after estrus in G3. Subsequently, the collected blood samples underwent centrifugation at 3000 rpm for 15 minutes. Blood serum hormone levels were determined with the chemiluminescence immunoassay technique, employing the ADVIA Centaur Progesterone test kit (Siemens, USA).

Superovulation, Estrus Synchronization Protocol and Embryo Collection

The superovulation and estrus synchronization protocol were summarized in Table 2. Embryos were collected from donors by the uterus flushing, a non-surgical method, on the 7th day of AI.[12] For this purpose, epidural anesthesia was performed using a 4 ml lidocaine injection. Then Foley catheter was passed through the cervix and fixed by inflating the balloon in the uteri. One liter of lactated Ringer's solution with 1.5% fetal calf serum (FCS) was used as the flushing solution. The flushing solution was first transferred to 2/3 of the corn, then it was vacuumed again into the filter system. The same procedure was repeated 4-5 times. The same flushing procedure was performed on the other cornu uteri.

Table 2. Superovulation and estrus synchronization protocol

Days	Donor Cows			Recipient Cows
	Application		Dosage	Application
-3				PGF2α
0	CIDR + GnRH		2,5 ml	
7	FSH	Morning	2 ml	
		Evening	2 ml	
8	FSH	Morning	1,5 ml	
		Evening	1,5 ml	PGF2α
9	FSH+PGF	Morning	1 ml	
	FSH+CIDR removal	Evening	1 ml	
10	FSH	Morning	0,5 ml	
		Evening	0,5 ml	
11	AI (least 2 times with an interval of 12 hours)			Estrus observation
18	Uterus flushing			Embryo transfer

Embryos were detected using a stereo microscope following the flushing procedure and transferred to a petri dish containing TL- HEPES immediately. Then, embryos were evaluated for their quality assessment according to criteria described by IETS (International Embryo Transfer Society). Finally, grade 1 embryos at the blastocyst stage were taken into 0.25 ml straws.

Embryo Transfer

Collected embryos were transferred to recipients as freshly (Table 2). Synchronized recipient cows were examined for corpus luteum detection using ultrasound (HASVET 838, Turkey) and ovaries containing corpus luteum were determined. Embryo straws were placed in the embryo transfer catheter. The catheter was pushed forward into the cornu uteri on the side of the corpus luteum and the embryo transfer process was completed by pushing the catheter pistole.

Pregnancy Detection

Following artificial insemination and embryo transfer, pregnancy examinations were performed on 30th and 60th day of gestation in G1, G2 and G3 using ultrasound. Ultrasonography was carried out by a single experienced operator 30th and 60th day.

Statistical analysis

SPSS (SPSS 23.0 for Windows; SPSS, Chicago SPSS 23) program was used for statistical evaluation of the results. Pregnancies between the groups were compared using the Chi-square test. P-values less than 0.05 were considered to be statistically significant.

Results

Pregnancy rates in G1, G2, and G3 were given in Table 3. Pregnant cow number were 24, 23 and 34 in G1, G2 and G3 respectively on 30th day. However, two embryonic loss in G1 and G2 and one embryonic loss in G3 were detected on 60th and 30th (Table 3).

Table 3. Pregnancy rates on the 30th and 60th day in conventional semen, sexed-semen and embryo transfer

Groups	n	Pregnant	Non-Pregnant	Pregnancy rate (%)
				On 30th day
Conventional semen (G1)	46	23	23	50[a]
Sexed-semen (G2)	47	22	25	46.80[a]
Embryo transfer (G3)	46	32	14	**69.56[b]**
				On 60th day
Conventional semen (G1)	46	21	25	45.60[a]
Sexed-semen (G2)	47	20	27	42.55[a]
Embryo transfer (G3)	46	31	15	**67.39[b]**

Different upper letters ([a, b]) show statistically significant difference ($p<0.05$)

Results show that the pregnancy rate was significantly higher in the embryo transfer group (G3) compared to the conventional semen (G1) and sexed-semen (G2) group ($p<0.05$) on both 30th and 60th days.

Discussion

In this study, we have revealed that the pregnancy rate was significantly higher in the embryo transfer group (G3) than in conventional semen (G1) and sexed-semen (G2) both on 30th and 60th days. There was no significant difference between G1 and G2 in pregnancy rate.

The embryo transfer is an important biotechnological tool that enables to transfer of embryos to healthy donor animals where embryos complete their development.[15] This approach is one of the most important breeding methods in dairy cattle breeding and contributes to the acceleration of genetic progress by increasing the number of offspring with a high genetic value from elite females in a breeding season. [9] Because of its high economic cost, embryo transfer is generally used for the transfer of embryos with high genetic value. [12] Therefore, the success of embryo transfer is an important factor affecting genetic improvement and therefore production and profitability in dairy cattle.

Different factors may affect the success of embryo transfer. These are the quality of the embryo[16], serum progesterone level of recipients[12] and interaction between embryo and recipient. [11] Progesterone is an important regulator of endometrial functions. Studies show that progesterone cont-

rols the expression of the genes associated with uterine receptivity, embryonic development, and innate immune response in pregnant cows.[17, 18] Serum progesterone level is an important factor that affects pregnancy rate in the success of embryo transfer. Alçay et al.[12] reported that the pregnancy rate increased in cows with high serum progesterone levels (>8 ng/ml). On the other hand, low serum progesterone levels delayed the establishment of uterine receptivity to implantation[19], and pregnancy rate in embryo transfer.[12] Therefore, in this study, recipient cows with high serum progesterone levels were preferred for embryo transfer. It was reported that estrus synchronization before the transfer of d-7 embryos increased the pregnancy rate.[15] Another factor that affects the pregnancy rate is embryo quality. It was reported that the pregnancy rate was higher when fresh embryos were used in transfer compared to frozen embryos.[13] In a similar study, it was found that a high rate of pregnancy was achieved when embryo transfer was performed.[20] In this study, it was used fresh and high-quality (grade 1) embryos (at blastocyst stage, d-7) for embryo transfer. Grade 1 embryos are excellent for transfer due to their quality aspect. These embryos are consistent with their expected developmental stage and have intact and viable embryonic mass. Grade 1 embryos can also well survive during freezing/thawing procedures.[21] Therefore, using of high-quality fresh embryos in this study may be related to a significantly higher pregnancy rate in the embryo transfer group (G3).

Sexed-semen is preferred by farms that aim to have female or male calves. In dairy farming, female calves are important for the sustainability and expansion of herds, so male calves have not been desired. In addition, male calves may cause dystocia and economic losses.[22] Different studies show the effects of sexed-semen on the pregnancy rate in Holstein cows. The pregnancy rate was detected as %31,8 and %40,9 in sexed-semen and conventional semen respectively in synchronized Holstein cows without significant difference.[6] Similarly, in another study, pregnancy rates were detected as %42,8 and %50 in sexed-semen and conventional semen respectively.[7] Bayrıl, [8] reported pregnancy rates of Holstein heifers %70,7 and %77,8 in sexed-semen and conventional semen respectively. However, Norman et al. [5] reported a pregnancy rate of %25 in sexed-semen in the US. In this study, pregnancy rates in sexed-semen (G2) and convectional semen (G1) were %46,8 and %50 respectively, and there was no significant difference between G1 and G2. These results were similar to findings of Karakaya[6] and Serim[7].

A similar pregnancy rate between sexed-semen and conventional semen in synchronized cows is an important matter for profitability in dairy cattle breeding.[23] Serim, (2022)[7] reported that the birth rate of female calves is %91 in sexed-semen and is significantly higher compared to conventional semen. Therefore, even if sexed-semen is more expensive than conventional semen, the higher birth rate of female calves may be more profitable considering long-term breeding. It was emphasized that the widespread application of sexed-semen may decrease the economic burden of replacement heifers, and accelerate the expansion of growing herds.[1] On the other hand, it should note that sexed-semen still represents a small proportion of the AI market.[24] Nevertheless, there is an increasing demand for sexed-semen in AI. Oikawa et al.[25] reported that the percentage of use of sexed semen in AI and pregnancy rates gradually increased from 2012 to 2016 in Japan. However, a straw of sexed-semen is 15-50 US dollars more expensive than those conventional semen.[26] Some researchers emphasize that sexed-semen may decrease the price of female offspring. Therefore, over expansion of herds may result in a dramatic decrease in milk prices and negatively affect the dairy industry. It was also reported that the quality of embryos produced with sexed-semen was lower compared to those of conventional semen.[26] Nevertheless, accumulative results of studies and our findings support that sexed-semen could be used to obtain favorable pregnancy rate in cattle.

In summary, we have detected that the pregnancy rate was significantly higher in the embryo transfer group compared with the conventional semen and sexed-semen group. Findings led to the conclusion that higher serum progesterone level may increase pregnancy rate. Therefore, the measurement of the serum progesterone levels may be beneficial in veterinary field. In addition, embryo transfer may be used more widely to increase genetic progress in dairy cattle.

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