Original Article

Effect of Embryo Transfer Seven Days after Artificial Insemination with Sexed and Conventional Semen from Superovulated Cattle

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ABSTRACT Sexed sperm can contribute to increase the profitability of the cow industry through the production of offspring of the craved sex, such as males for meat or females for dairy production. Therefore, the utilization of sexed sperms plays a very important role in the production of offspring of superior cattle. In this study, we examined the pregnancy rates and calves sexing proportion of male and female calves produced using AI, both performed using sexed and conventional sperm. In the result, the conception rates after ET were 73.3% (33/45) sexed semen and 52% (55/104) conventional semen. Thus, the sex ratio for sexed-semen inseminations was 70% (21/30) females for singleton births within a 272 to 292 day gestation interval. The sex ratio for conventional semen was 61% (34/56) females for births. As a result, it is suggested that the use of sex classification sperm will play a very important role in the offspring production of Korean bovine.

Keywords: bovine, embryo, gestation, offspring, sexed sperm

INTRODUCTION

Sexed sperm is now widely used in cattle breeding to enhance industrial competitiveness and achieve high female fertility (Seidel, 2003; Rath et al., 2008).

The production rates of fresh viable embryos using conventional semen with the sexed methodologies of embryo transfer (ET) and superovulation do not yield acceptable results. Accordingly, it is needful to use a greater number of doses of semen per superovulated cow to obtain high embryo production, including a change in the artificial insemination (AI) time (Baruselli et al., 2006; Dell'Aqua Jr et al., 2006). Sperm sexing does not affect the sperm's

ability to fertilize the oocytes, but it can affect the ability of embryos to develop normally after fertilization (Morton et al., 2007; Underwood et al., 2010).

Sexed sperm can contribute to increase the profitability of the cow industry through the production of offspring of the craved sex, such as males for meat or females for dairy production. Other applications would allow cattle breeders and AI sperm companies to test elite bulls on small numbers of females (Hossein-Zadeh et al., 2010).

Therefore, advancement of reproductive biotechnologies seems to have reached a limit, and its implementation relies on basic knowledge of the embryos and biology of gametes. The limiting factors provide an opportunity

for unification of epigenetics knowledge. Embryos and gametes must pass through a broad epigenetic reprogramming process to be able to be fertilized and form a viable embryo (Reik, 2001; 2007).

ET in cattle can be an important tool for genetic modification and can improve pregnancy rates compared with AI when fertility is low, such as during heat stress or in repeat breeder cows (Hansen, 2004; 2007; Block et al., 2010; Stewart et al., 2011). In addition, many reports demonstrating a reduction in the number of transferable *in vivo* embryos when sexed semen is used, the quality grades of embryos are included in the analysis in only a few reports. A compromised fertilization rate in cows inseminated with sexed semen is obvious, but with respect to the proportion of quality grade 1 embryos, either no difference or a decrease has been reported (Sá Filho et al., 2008; Hayakawa et al., 2009; Peippoet al., 2009; Larson et al., 2010). AI using sexed semen (sexed AI) has a pregnancy rate lower than that using conventional semen (Carvalho et al., 2010; DeJarnette et al., 2011).

We bought a “Whole-mom” from the Nuri Science Co., Ltd company. Sperm sexed by “Whole-mom”, with purities of over 80% of X-bearing sperm, can be used for AI with subsequent transfer of the embryos produced. Therefore, the objective of this study was to evaluate the pregnancy rates and calves sexing proportion of male and female calves produced using AI, both performed using sexed and conventional semen.

MATERIALS AND METHODS

Animals

The study was carried out spring to autumn season (March to October) on 149 Hanwoo bred on a farm located in Southern Korea. The Hanwoo were housed in free stall facilities, fed complete rations, and allowed free access to mineralized salt and water. The Hanwoo were included in the study based on a clinical examination including good ovary and normal uterus, were selected.

Estrus synchronization

For the donor cows, four days after the insertion of Progesterone Releasing Intravaginal Device (CIDR; Hamilton, New Zealand) into the vagina of cows using a CIDR injector, estrus cycle was induced by the administration of FSH (Kyoritsu seiyakucorporation, Japan) for 4 days with an interval of 12 hr between each FSH injection. PGF_{2α} (Zoetic, Belgium) was administered 2 days after FSH injection. Three days later, CIDR was removed. AI was carried out using sexed and conventional semen, 3 times with a 12hr interval between the procedures, after the injection of GnRH (250 µg, DongBang, Korea).

Sexing of semen (sperm) and AI

In all superovulated donors, inseminations were initiated 12 hours after the onset of standing estrus. All six batches of frozen-semen used came in 0.5 mL straws. For experiments cattle were used as embryo donors and were treated in a same manner (Table 1) in 2016. Semen were

Table 1. Procedures for embryo recovery of donor and embryo transfer to recipients

Treatment day	Donors		Recipients	
	AM	PM	AM	PM
0	P4 device insertion 50 mg P4 1 mg E2	–	P4 device insertion 50 mg P4 1 g E2	–
4	6 mg FSH	6 mg FSH	–	–
5	5 mg FSH	5 mg FSH	–	–
6	4 mg FSH 30 mg PGF _{2α} (Dinoprost)	4 mg FSH, 15 mg PGF _{2α} (Dinoprost)	0.625 mg PGF _{2α} (Croprostenol)	–
7	P4 device removal 3 mg FSH	3 mg FSH	P4 device removal	–
8	Estrus	AI 100 µg GnRH	Estrus	250 µg GnRH
9	AI	AI	–	–
15	Embryo Recovery		Embryo Transfer	

thawed by first holding them in the air, at room temperature (24°C) for 5 s and then plunging them into a water bath at 37°C for 20 s. In this experiment, sexed semen was mixed with “Whole mom (Nuri Science Co., Ltd company)” in a water bath at 37°C for 20 minutes. The sexed and conventional semen was deposited in the body of the uterus.

Embryo collection and evaluation

Embryos were collected 7 days after inseminations by transcervical uterine flushing using a Dulbecco's phosphate-buffered saline (D-PBS) 2% FBS supplemented media. After collection, embryo morphology was assessed under a stereomicroscope (60 magnification), and recovered embryos were evaluated according to the International Embryo Technology Society (IETS) classification guidelines for the developmental stage (4=compact morula, 5=early blastocyst, 6=blastocyst, 7=expanded blastocyst, 8=hatched blastocyst, and 9=expanding hatched blastocyst) and quality (grades 1-2) by experienced embryo transfer practitioners.

Transfer of embryos

Selection of recipients was performed immediately before ET. Recipient acceptance focused on time elapsed from standing estrus (6.5 to 7.5 days), quality of corpus luteum (CL) and serum levels of P4 on Day 0 and Day 7, as previously reported in Hidalgo et al (Hidalgo et al., 2004). Embryos were nonsurgically transferred to the uterine horn ipsilateral to the CL, under epidural anesthesia (4 mL, 2% lidocaine chlorhydrate, Laboratorios Ovejero, Leon, Spain). Grades 1-2 quality fresh embryos were washed twice in embryo D-PBS medium with 20% FBS and then loaded into straws in the same medium. Collected Day 7 embryos were transferred to synchronous mixed breed recipient cows. A total of 149 cows were used as recipients.

Table 2. Calving rate after ET derived from sexed or conventional semen in Hanwoo

Semen type*	No. of transferred cows	No. of pregnant Cows (%)	Calving rate (%)
Sexed	45	33 (73.3)	30 (66.7)
Conventional	104	55 (52.8)	55 (52.8)

*Sexed semen is mean used ‘whole mom’ semen and conventional is control group.

Pregnancy diagnosis, fetal sex and calving

Two months after embryo transfer, a rectal palpation was performed to evaluate pregnancy. Fetal sex was judged by confirming the birth fetus.

RESULTS AND DISCUSSION

In the result, the conception rates after ET were 73.3% (33/45) sexed semen and 52% (55/104) conventional semen (Table 2). And the sex ratio for sexed-semen inseminations was 70% (21/30) females for singleton births within a 272 to 292 day gestation interval. On the other hands, sex ratio for conventional semen was 61% (34/56) females for births (Table 3).

The use of X-sexed sperm, already widely employed for AI in dairy cattle (Norman et al., 2010), has the potential to alter the structure of the dairy industry by increasing the replacement heifer supply, creating opportunities for using a proportion of the dairy herd for producing beef animals, and improving the rate of genetic selection (De Vries and Feleke, 2008). Use of X-sexed sperm in ET programs represents another use of this technology.

Conception rates after ET achievable with sexed semen vary significantly across studies, which emphasizes the influence of on farm factors. Conception rates after ET can be affected by parity, body condition, sire selection, and accuracy of estrus detection (Seidel and Schenk, 2008). In this study, we investigated the farmers who are breeding through the ET technique and found that the pregnancy rate of sexing sperm was not lower than that of conventional sperm. These results suggest that the sexed semen by Whole-mom has no effect on the decrease of fertilization rate and the decrease of pregnancy rate. On the other hand, pregnancy rate was rather 73.3% in the case of sexed semen, which appeared higher than 52.8% of ordinary semen, which is similar to the research results of

Table 3. Effect of sexed and conventional semen on calving and gestation periods

Semen type*	Calf sex	No. born (%)	Gestation period (days ± SE)	No. Abortion (%)
Sexed	Female	21 (70.0)	280.4 ± 4.8	3 (0.1)
	Male	9 (30.0)	279.4 ± 5.4	
Conventional	Female	34 (61.0)	276.6 ± 7.7	0 (0.0)
	Male	22 (39.0)	280.3 ± 6.2	

*Sexed semen is mean used ‘whole mom’ semen and conventional is control group.

Seidel and Schenk considered to be due to the environmental condition and nutritional condition of each cows.

The sex ratio for conventional semen was in accordance with most trials, which achieve approximately 50 to 52% males (Tubman et al., 2004; DeJarnette et al., 2009). However, Norman et al. (2010) observed only 48.5% single males born to heifer dams. According to Nuri Science, in the case of the 'whole mom' used in this experiment, Y-sperm agglutination protein is used to inhibit the fertilization of Y-sperm to induce the regulation of sex, in this case, about 70% was regulated.

The results for gestation length were in accordance with several studies reporting that gestation length in dairy cattle is affected by parity, season, sire, the incidence of twins, and calf sex (Fisher and Williams, 1978; DeJarnette et al., 2009; Norman et al., 2009). Apart from the effect on calf sex, semen sexing did not significantly attenuate or prolong gestation length, in agreement with Tubman et al. (2004).

In conclusion, according to this study, when a sex-controlled sperm was compared with a normal sperm, a whole mom-method of obtaining a sex-adjusted calf of about 70% without decreasing the pregnancy rate and increasing or decreasing the duration of pregnancy, It is expected to play a very important role in production.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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