

Induction of twinning in Holstein and Japanese Black cows by ipsilateral frozen embryo transfer

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Abstract

Induction of twinning by ipsilateral nonsurgical transfer of two frozen–thawed Japanese Black bovine embryos to each of 20 Holstein and 26 Japanese Black cows, that had been kept under a stable in private farm conditions, was examined. The cows were monitored every 20 days from Day 25 to Day 65 of gestation for pregnancy and fetus survival (estrus is Day 0). Seventy-five per cent (15 of 20), 65.0% (13 of 20) or 60.0% (12 of 20) and 61.5% (16 of 26), 53.8% (14 of 26) or 50.0% (13 of 26) of Japanese Black cows were diagnosed pregnant at 25, 45 and 65 days after transfer by ultrasonic echography. Embryonic losses were observed between Days 25 and 65 in 29.2% (7) Holstein and 31.8% (7) Japanese Black cows. The twin pregnancy rate in Holstein and Japanese Black cows decreased with time; 60.0% (9 of 15) vs. 37.5% (6 of 16) at Day 25; 53.8% (7 of 13) vs. 28.6% (4 of 14) at Day 45 and 41.7% (5 of 12) vs. 15.4% (2 of 13) at Day 65. At calving, Holstein cows produced five sets of twins and seven single calves, and Japanese Black cows two sets of twins and 11 single calves. The twinning rate in Holstein cows was higher ($P < 0.05$) than that in Japanese Black cows, 41.7% (5 of 12) vs. 15.4% (2 of 13). The calf birth weight in Holsteins was heavier ($P < 0.05$) than that in Japanese Black dams (24.5 kg, 33.6 kg vs. 19.3 kg, 25.5 kg for twin and single calves). The placental weight in Holstein dams calving twins was heavier than that in Holstein dams calving a single calf or in Japanese Black dams calving either twins or a single calf (6.6 kg vs. 3.5 kg, 4.6 kg or 2.8 kg). The number of placentome in Holstein dams calving twins was also higher ($P < 0.05$) than that in Holstein dams calving a single calf or Japanese Black dams (103.5 vs. 41.8, 67.9, 33.0). The number of placentome was approximately double in dams calving twins than that of dams calving a single calf.

Keywords: Frozen embryo transfer; Twin pregnancy; Embryo mortality; Placentome number; Bovine

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1. Introduction

Production costs in beef herds are associated mainly with the maintenance of cattle. Thus, cows producing twins are cheaper to maintain than those producing single calves. Several investigators have found twin pregnancy in a high proportion of recipients following bilateral embryo transfer (Rowson et al., 1971; Sreenan et al., 1975; Anderson et al., 1978). However, ipsilateral transfer to the corpus luteum is a much simpler and convenient method compared to bilateral transfer, especially with frozen–thawed embryos (Sreenan et al., 1975; Niemann et al., 1986). Dairy cows have a higher twinning rate than beef cows because of treatment by artificial insemination (Gordon et al., 1962; Scanlon et al., 1974). However, there have been no reports on which method is most likely to produce twins. The incidence of embryonic loss in the early stages of gestation between dairy and beef cows in ipsilateral pregnancies, in particular, has not been subjected to sequential observation. Nor has the number of placentome in dairy and beef cows been counted after calving.

The present experiment was undertaken to determine, (1) the twinning rate following transfer of two frozen–thawed embryos to the ipsilateral horn, (2) the ability of Holstein or Japanese Black cows to carry twins to term, and (3) complications associated with twin pregnancy.

2. Materials and methods

2.1. *In vivo* embryos

Japanese Black cows ($n = 10$) from 3 to 5 years of age, were superovulated with follicle stimulating hormone (FSH, Denka Pharmaceutical Co., Ltd., Japan) in a total dose of 28 mg given by intramuscular injection (6, 6 mg; 5, 5 mg; 3, 3 mg at 12 h interval during 3 days) followed by an injection of 15 mg prostaglandin F₂ α -THAM salt (PGF₂ α , Dainihon Pharmaceutical Co., Ltd., Japan) 48 h after the first FSH treatment.

Donors were single inseminated with frozen–thawed semen at the time of estrus. Embryos were collected nonsurgically from donors on Day 7 (estrus is Day 0) by flushing the uterine horns with modified phosphate buffered saline (m-PBS; PBS supplemented with 3 mg ml⁻¹ bovine serum albumin) according to procedures previously described (Suzuki et al., 1984). The quality of the embryos was assessed using a stereo microscope and only excellent and good quality (Lindner and Wright, 1983) embryos were used in this experiment.

2.2. *Embryo freezing*

Ninety two *in vivo* Japanese Black embryos were suspended in 1.4 mol glycerol containing 0.2 mol sucrose at room temperature, added in a one-step manner. Embryos were loaded into 0.25 ml plastic straws (two embryos per straw), placed directly into a 0°C programmable freezer (Tokyo Rika Kikai Co. Ltd. Japan) and held for 2 min. The embryos were cooled from 0°C to -7°C at 1°C min⁻¹ and seeded at -7°C. Straws

were held for 10 min at -7°C , and cooled at a rate of $0.3^{\circ}\text{C min}^{-1}$ to -30°C . Finally, the straws were plunged and stored in liquid nitrogen. Recipients were made up for Holstein cows ($n = 26$) and Japanese Black cows ($n = 20$) 3 to 5 years of age. After 1–6 months of storage in liquid nitrogen the cryopreserved embryos were plunged into a 30°C water bath for thawing. After thawing, embryos were drained in m-PBS for evaluation under a microscope, and then two whole embryos were placed in a straw and transferred ipsilaterally to the synchronized recipients. The recipients were prepared by injection of 15 mg Prostaglandin $\text{F2}\alpha$ -THAM salt.

Pregnancy diagnoses were performed using an ultrasonic scanning instrument (SSD-210DX, Aloka Co., Tokyo). The detection of pregnancy and subsistence of embryo was confirmed at 25, 45 and 65 days after transfer according to the previous report (Pierson and Ginther, 1984; Reeves et al., 1984).

The twinning rate was determined from the number of calves born. At calving, calf birth weight, placental weight, number of placentome and sex were recorded.

Data were analyzed by *t*-test. A probability of $P < 0.05$ and $P < 0.01$ were considered to be statistically significant.

3. Results

The percentage rate of all pregnancies in Holstein and Japanese Black cows diagnosed on Days 25, 45 and 65 after the transfer is as follows:

Day	Holstein		Japanese Black	
	Actual	(%)	Actual	(%)
25	15 of 20	25.0	16 of 26	61.5
45	13 of 20	65.0	14 of 26	53.8
65	12 of 20	60.0	13 of 26	50.0

There was no statistically significant difference between the pregnancy rate in Holstein and Japanese Black cows. In 29.2% (7 of 24) Holstein cows and 31.8% (7 of 22) Japanese Black cows, embryonic losses were observed between Days 25 and 65. Twin pregnant Holstein and Japanese Black cows decreased with time; 60.0% (9 of 15) vs. 37.5% (6 of 16) on Day 25; 53.8% (7 of 13) vs. 28.6% (4 of 14) on Day 45 and 41.7% (5 of 12) vs. 15.4% (2 of 13) on Day 65. On the other hand, the number of single pregnancies increased with time; 33.3% (5 of 15) vs. 62.5% (10 of 16) on Day 25, 46.2% (6 of 13) vs. 71.4% (10 of 14) on Day 45 and 58.3% (7 of 12) vs. 84.6% (11 of 13) on Day 65 (Fig. 1). There are significant differences ($P < 0.05$) between both twin and single pregnancies in Holstein and Japanese Black cows.

At calving, 12 Holstein cows and 13 Japanese Black cows produced 83.4% (5) and (30.8%) (2) sets of twin calves, respectively, and (58.3%) (7) and (84.6%) (11) single calves, respectively (Fig. 2). The calving rates (number of calves per pregnancy) of Holstein and Japanese Black cows were 1.7 and 1.4 at Day 25, 1.5 and 1.3 at Day 45, 1.4 and 1.2 at Day 65. The twinning rate in Holstein cows was higher ($P < 0.05$) than that in Japanese Black cows, 41.7% (5 of 12) vs. 15.4% (2 of 13). In contrast, the single

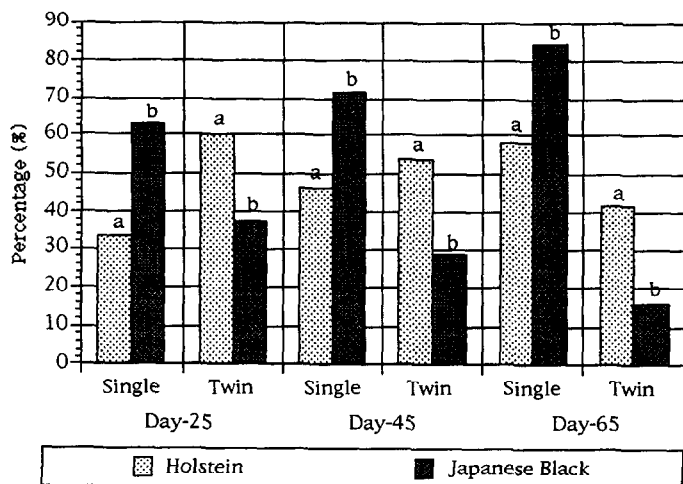


Fig. 1. Pregnancy rate of frozen-thawed embryos after ipsilateral non-surgical transfer in Holstein and Japanese Black cows (a–b, $P < 0.05$).

calf rate in Holstein cows was lower ($P < 0.05$) than that in Japanese Black cows, 58.3% (7 of 12) vs. 84.6% (11 of 13). The percentage of total calves produced from Holstein dams was higher ($P < 0.05$) than that from Japanese Black dams, 141.7% (17 of 12) vs. 115.4% (15 of 13).

Fig. 3 shows the total calf birth weight and placental weight in Holstein and Japanese Black dams. The total calf birth weight of both twin and single calves was heavier ($P < 0.05$) in Holstein than in Japanese Black dams (49.0 kg, 33.6 kg vs. 38.5 kg, 25.5 kg). Placental weight in Holstein dams calving twins was heavier than that in Holstein

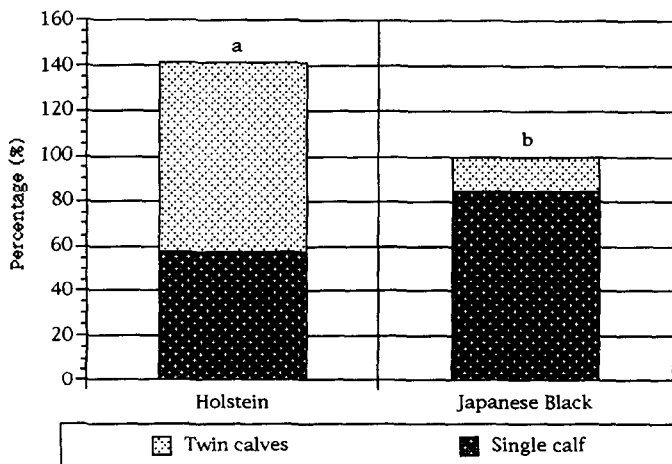


Fig. 2. Total calves obtained after non-surgical ipsilateral transfer of frozen-thawed bovine embryos (a–b, $P < 0.05$).

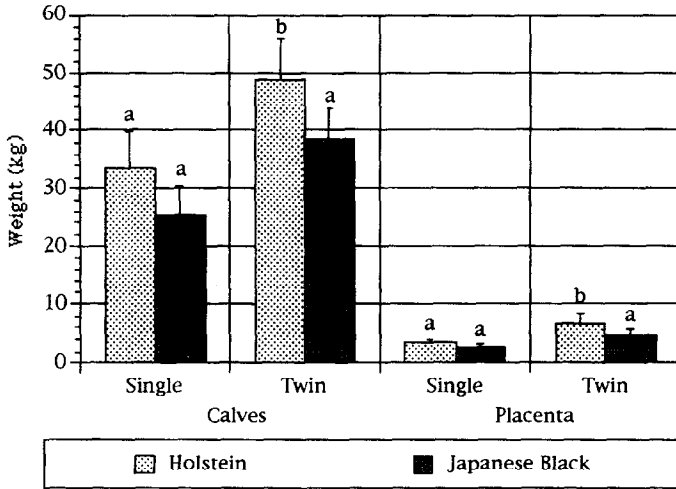


Fig. 3. Calves birth weight and placental weight in Holstein and Japanese Black dams (a–b, $P < 0.05$). Means \pm SEM.

dams calving a single calf (6.6 kg vs. 3.5 kg) or Japanese Black dams calving either twins or a single calf (4.6 kg or 2.8 kg) (Fig. 3).

The number of placentome (Fig. 4) in Holstein dams calving single or twins was higher ($P < 0.05$) than that in Japanese Black dams calving a single or twins calves (41.8 vs. 33.0 for single calf and 103.5 vs. 67.9 for twin calves). The number of placentome was approximately double in dams calving twins than in dams calving a single calf.

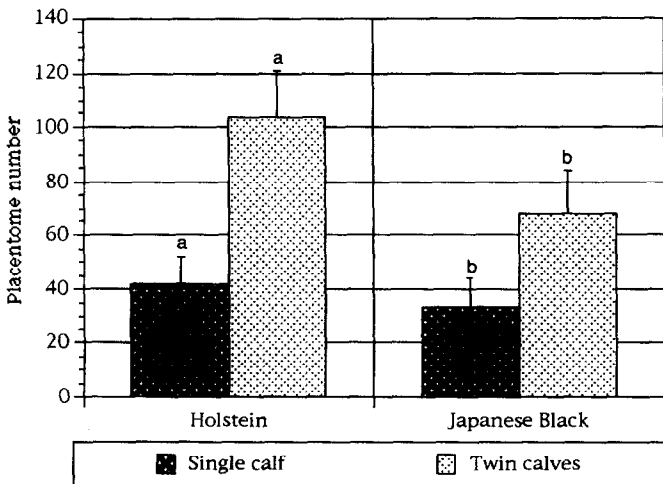


Fig. 4. Number of placentome in Holstein and Japanese Black dams (a–b, $P < 0.05$). Means \pm SEM.

4. Discussion

Induction of twinning by means of nonsurgical transfer of two frozen–thawed embryos was attempted in 20 Holstein cows and in 26 Japanese Black cows.

No significant difference was observed between the pregnancy rate in Holstein and Japanese Black recipients. However, the number of twin pregnant recipients was reduced with time between Days 25 and 65. On the other hand, the number of single pregnant recipients increased. In other words, embryonic losses of 29.2% and 31.8% were observed between Days 25 and 65 in Holstein and Japanese Black cows, respectively. There were no further prenatal losses. The embryonic loss of *in vitro* produced embryo was reported within 14 days after transfer (Farin and Farin, 1995). As described by Izaïke et al. (1988), the embryonic mortality in twin pregnancies was observed in two categories, where one of the two embryos becomes extinct while the other continues to survive, or where both of the embryos disappear. In this case, embryonic mortality was not detectable by rectal palpation, because slipping of the fetal membrane was felt, as in the case of normal pregnancy, until the resumption of estrus. Anderson et al. (1978) reported similar cases of embryonic mortality in which pregnant cows diagnosed by rectal palpation between Days 45 and 60, returned to estrus between Days 76 and 232 of gestation without visible abortion. Embryonic loss after transfer has been estimated to be 17.8%–19.0% between Days 25 and 60 and 19% between Days 25 and 48 (Markette et al., 1984; Suzuki et al., 1989). However, in the present study, embryonic loss occurred more frequently during 25 to 65 days after transfer, depending on the capacity of the uterus. The cause of embryonic loss may be reduced uterine capacity or a reduced surface area of contact between the conceptus and the uterine wall (Rawson et al., 1971), and it may also be concerned with the number of placentome.

However, the twinning rate in Holstein dams was significantly higher ($P < 0.05$) than that in Japanese Black dams. Suzuki et al. (1986); Suzuki et al. (1989) reported that the pregnancy and twinning rates following nonsurgical transfer of two fresh and frozen–thawed embryos in Holstein heifers were higher than those in Japanese Black cows. The effect of genotype of fetus of birth weight of calves from conventional embryo transfers is especially evident when the breeds of embryo donor and recipient are different (Guilbault et al., 1990). In the present study, the calf birth weight of twins and single calves in Holstein dams was also heavier than those in Japanese Black dams. These findings suggest that Japanese Black cows are not suitable as a recipient for induction of twinning because of a small uterus capacity and small number of placentome.

Thatcher et al. (1980) reported a positive curvilinear relationship between calf birth weight and milk production in Holstein and Jersey cows. Gestation length and calf birth weight are affected by the genotype of fetuses (Guilbault et al., 1985; Key et al., 1976). In the current study, placental weight was closely associated with calf birth weight. In both dams, placental weight was increased with increasing calf birth weight. These findings suggest that placental weight is positively correlated in Holstein.

Anthony et al. (1985) reported that the sire affected the prenatal development of the calf and related placental characteristics. Total placentome weight and placental fluid volume were also closely associated with fetal calf weight.

5. Conclusion

In the present study, the number of placentome in Holstein dams calving twins was higher ($P < 0.05$) than that in Japanese Black dams. These findings show that average calf birth weight, placental weight and placentome numbers are affected by the breed of dam in the case of twin births. The overall findings also suggest that it is possible to transfer Japanese Black cattle embryos to Holsteins to produce twin births of Japanese Black cattle.

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