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PRODUCTION AND DEVELOPMENT OF CALVES FROM SEXED-BISECTED BOVINE EMBRYOS

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Key words: bovine, chromosomal analysis, sexed-bisected embryo.

ABSTRACT

Sixty-five chromosomal preparations from bisected bovine embryos were examined and 43 embryos (66.2%) with metaphase plates were observed; however, only 25 embryos (38.5%) were sexed. Fifteen of the bisected embryos sexed by chromosomal analysis were transferred to each of the 15 recipients, and 5 recipients became pregnant. Female calves were born on December 28, 1985, August 2, 1986 and July 18, 1988. The first female calf born from a sexed-bisected embryo (December 28, 1985) was the first case in Japan. The gestation lengths and birth weights of these calves were 277, 278 and 274 days, and 42.0, 44.0 and 37.0 kg, respectively. These two calves grew within the range of the "Standard developmental growth curve of the Holstein heifer" of the Japanese Holstein Association. The milk yield from 2 sexed-bisected cows were recorded. In the first cow (Case 1), she produced 8,575 kg of milk, 5.0% fat and 9.4% solids-non-fat (SNF) per year. In the other cow (Case 2), her expected milk volume was 7,800 kg per year. Confirmation of parentage was done by blood typing, and certified by the Japanese Livestock Animal Improvement Association.

INTRODUCTION

There have been many reports concerning bovine chromosomal analysis. Chromosomal analysis studies for sex determination of bovine embryos began with the use of Day 12–15 embryos.^{1,12,15)} It was reported that it was possible to determine the sex from biopsies of some trophoblasts. But these embryo cell stages were not suitable for routine embryo transfer procedure, because embryos were usually collected at 7 days after estrus. However, MOUSTAFA and HAHN (1978) reported a high

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sexing rate from biopsies of 7–10 trophoblasts of Day 7 bovine morulae and blastocysts.⁷⁾ Recently, PICARD et al. (1984, 1985) obtained a sexing rate of 4.3–60% and a pregnancy rate of 37.5% from bisected embryos treated with colcemid and cultured for different lengths of time.^{4,5)} RALL and LEIBO (1987) also obtained sexing and pregnancy rates of 62.1% and 40.0%, respectively, from bisected bovine embryos.⁹⁾

In previous papers, we reported the production of calves and identical twins from bisected embryos.^{10,14)} In order to obtain more benefits from the bisection technique, chromosomal analysis on one of each pairs of bisected embryos was done and the other half was transferred to the recipient heifers.

The objectives of this paper were to produce and examine the development of calves from bisected bovine embryos that had been sexed by chromosomal analysis.

MATERIALS AND METHODS

Collection of Embryos

Embryos were collected nonsurgically from superovulated Holstein or Holstein × Ayrshire cows 7 days after estrus. Superovulation was done as described in our previous reports.^{10,11)} Dulbecco's phosphate-buffered saline (PBS; GIBCO Lab. Life Tec. Inc., Grand Island, NY) supplemented with 1% heat-inactivated calf serum (CS; GIBCO) was used as a flushing medium. The collected embryos were washed in PBS plus 20% CS and maintained in sterilized plastic petri dishes (Falcon; 3001, Lincoln Park, NJ) containing the same medium. These dishes were placed on a 37°C warm-plate.

Bisection and Chromosomal Preparation

Only normal embryos classified as good to excellent were used for bisection. Using a modification of the technique of OZIL et al. (1982) embryos from the morula to the blastocyst stage were bisected.³⁾ One of each pair of demi-embryos was examined by chromosomal analysis, while the other was cultured overnight with Ham's F-10 in 5% CO₂ and 95% air at 37°C until transfer to the recipient.

Demi-embryos were precultured 2–4 hours before colcemid treatment. Colcemid treatment of demi-embryos was based on the method of HISHINUMA et al. (1984).²⁾ Demi-embryos were cultured for 2–8 hours in Brinster's medium (BMOC-3, GIBCO) containing 0.05–0.025 µg/ml colcemid (GIBCO).

Chromosomal preparation of demi-embryos was made by the air-drying method of MIKAMO (1981).⁶⁾ After colcemid treatment, demi-embryos were placed in a hypotonic solution of 1% sodium citrate for 15 minutes. The fixation procedure was done in three steps: the first step (methanol, acetic acid and distilled water=5:1:4) took 5–10 minutes, the second step (methanol and acetic acid=3:1) 20 minutes and the third step (methanol, acetic acid and distilled water=3:1:1) 1 minute. The preparations were stained with 4% Giemsa, pH 6.8, and examined under a

stereomicroscope ($\times 1,000$) to confirm the presence of XX and XY chromosomes. Figure 3 shows a metaphase plate and XX chromosomes from the bisected embryos.

Transfer and Calf Development

For the production of sexed calves, one of each pair of demi-embryos was put into a 0.25-ml plastic straw and transferred nonsurgically using a Cassou gun (IMV, L'Aigle, France). The virgin Holstein heifer recipients used were either synchronized or natural-cycle heifers whose estrus was ± 1 day of the donor's. Pregnancy diagnosis was confirmed by ultrasonography and rectal palpation on the 40th and 60th day of gestation. Gestation length was calculated by assuming that pregnancy was initiated when artificial insemination was done in the donor, and that it terminated with parturition. The calves from sexed-bisected embryos were weighed on the 20th day of each month for 15 months, and their live weights were compared with the "Standard developmental growth curve of the Holstein heifer" of the Japanese Holstein Association. Milk yield was recorded according to the method of the Hokkaido Milk Certificate Association. Confirmation of parentage tests were done by blood typing and certified by the Japanese Livestock Animal Improvement Association.

RESULTS

From 65 chromosomal preparations of bisected bovine embryos, 43 embryos (66.2%) with metaphase plates were observed, but only 25 of the embryos (38.5%) were sexed. Sixteen embryos were female and 9 embryos were male. The results are presented in Table 1.

Fifteen of the bisected embryos sexed by chromosomal analysis were transferred to each of the 15 recipients, and 5 recipients became pregnant. Although three recipients each calved a normal calf, 2 recipients aborted at about 70–80 days of gestation. The 3 calves (2 Holstein, 1 Holstein \times Ayrshire) were all female, which was in agreement with the results of the chromosomal analysis. These female calves were born on December 28, 1985, August 2, 1986 and July 18, 1988. The births of these sexed-bisected female calves are indicated in Table 2. The gestation lengths and birth weights of these calves (2 Holstein, 1 Holstein \times Ayrshire) were 277, 278 and 274 days, and 42.0, 44.0 and 37.0 kg, respectively. The calves obtained from sexed-bisected bovine embryos are shown in Figure 4.

Figure 1 shows the growth curves of the 2 Holstein calves. Their live weights show very similar patterns of development for 15 months, and both calves grew within range of the "Standard developmental growth curve of the Holstein heifer" of the Japanese Holstein Association. The milk yield from 2 sexed-bisected cows were recorded and is shown in Figure 2. In her first lactation period (Case 1), she produced 8,575 kg of milk with 5.0% fat and 9.4% SNF per year. The milk yield of the other cow (Case 2) was also recorded for 7 months, and her expected milk volume was 7,800 kg per year. Confirmation of parentage tests were done by blood typing,

Table 1. Results of sexing rate and pregnancy rate from Day-7 bovine bisected embryos

Item	Number (%)
Bisected embryos	65
Metaphase plates	43 (66.2)
Sexed demi-embryos	25* (38.5)
Transferred demi-embryos	15
Pregnant heifers	5** (33.3)

* Sixteen were female, 9 were male.

**Two recipient heifers aborted at 70-80 days of gestation.

Table 2. Birth date, birth weight and gestation length of sexed-bisected embryos

No.	Transfer date	Birth date	Birth weight	Gestation length	Breed
1	Apr. 2, 1985	Dec. 28, 1985	42 (kg)	277 (days)	Holstein
2	Nov. 4, 1985	Aug. 2, 1986	44	278	Holstein
3	Oct. 24, 1987	Jul. 18, 1988	37	274	Cross-breed

and certified by the Japanese Livestock Animal Improvement Association.

DISCUSSION

In our study, the percentage of the successful sexing rate was 38.5% (25/65). This sexing rate was in agreement with the findings of SINGH and HARE (33%) 1980¹²⁾, YOSHIZAWA et al., (36.1%) 1986¹⁶⁾ and HISHINUMA et al. (28.6%) 1984²⁾. However, higher sexing rates of 53-63%, 60% and 70% were reported by MOUSTAFA and HAHN (1978),⁷⁾ PICARD et al. (1984, 1985)^{4,5)} and USHIJIMA et al. (1985),¹³⁾ respectively.

It seems that these differences in the sexing rates may be due to the colcemid or colchicine concentration used, the length of the culture period, and the method of chromosomal preparation. The main reasons for the lower sexing rate (38.5%) in this

study may be the presence of few metaphase plates and the absence of a distinct Y chromosome.

The pregnancy rate of sexed-bisected embryos was 33.3% (5/15). This was in agreement with the findings of NAKAGAWA et al. (38.9%) 1985⁸⁾, PICARD et al. (37.5%) 1985⁵⁾ and RALL and LEIBO (40.0%) 1987⁹⁾. However, these pregnancy rates were lower than our previous results with bisected embryos 72.6%¹⁰⁾. These differences in the results may be due to the different length of culture period. This suggests that it is necessary to improve the culture system in order to obtain a high pregnancy rate.

We obtained three female calves from the sexed-bisected embryos transferred, and their sex was in complete agreement with the results of the chromosomal analysis. The gestation lengths and birth weights of these calves were 277, 278 and 274 days, and 42.0, 44.0 and 37.0 kg, respectively. There seemed to be no observable difference between the gestation lengths and birth weights of these calves, and those calves born from artificial insemination reported in our previous studies¹¹⁾. However, it is difficult to make a comparison because of the limited number of calves obtained in this study.

The growth curves of 2 Holstein calves are shown in Figure 1. Both calves grew within the range of the "Standard developmental growth curve of the Holstein heifer" of the Japanese Holstein Association and their growth curves suggest normal development. The weight of one calf (Holstein×Ayrshire) was recorded monthly, but her live weight could not be compared because there is no "Standard developmental curve" for the Holstein×Ayrshire cross.

The milk yield from 2 sexed-bisected cows were recorded. In her first lactation period (Case 1), she produced 8,575 kg of milk with 5.0% fat and 9.4% SNF per year. In other cow (Case 2), her milk record for 7 months indicated an expected milk volume of 7,800 kg per year. The data concerning the growth curves and milk yield from sexed-bisected calves or cows were the first studies done in Japan.

We concluded that it is possible to obtain sexed-bisected calves, and that these calves could grow and develop into mature cows with normal reproduction and lactation.

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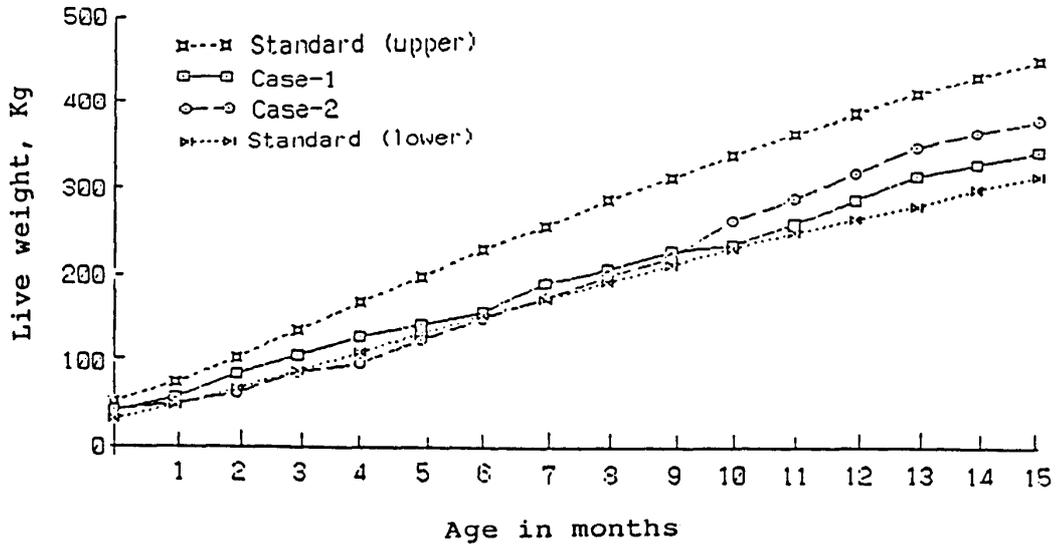


Figure 1. Growth curves of calves obtained from sexed-bisected embryos.
Case 1: first calf and Case 2: second calf.

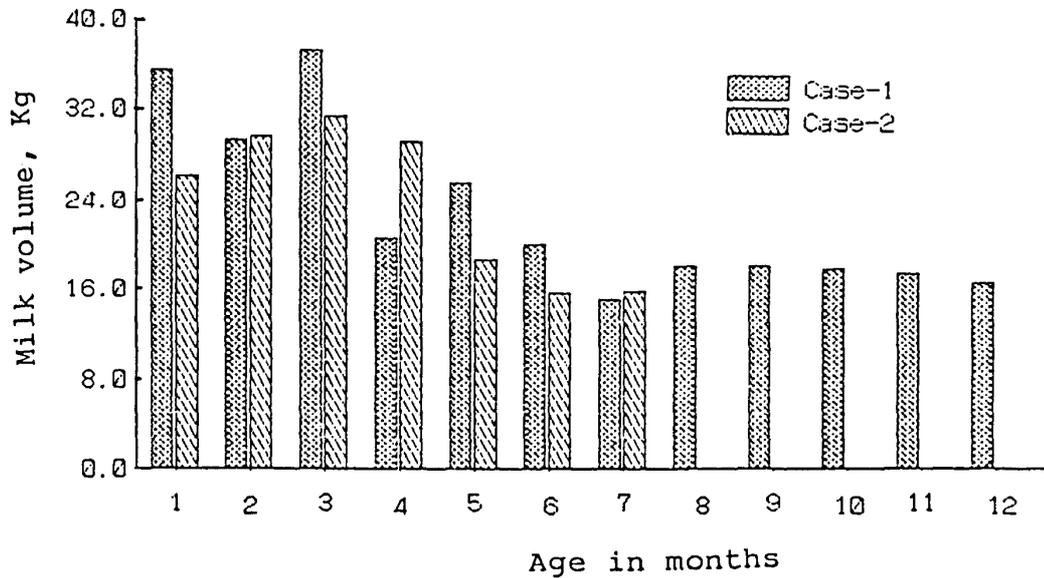


Figure 2. Lactation curves of cows obtained from sexed-bisected embryos.
Case 1: first calf and Case 2: second calf.

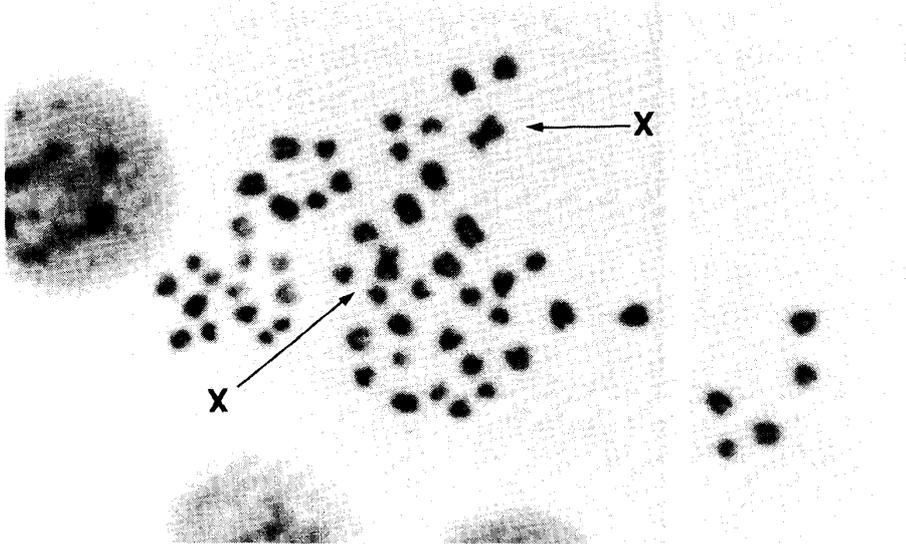


Figure 3. A metaphase plate and XX chromosomes from a bisected embryo.
($\times 1,000$)



Figure 4. The first calf from a sexed-bisected embryo (December 28, 1985)
and her recipient cow.

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