PREGNANCY IN CATTLE AFTER TRANSFER OF BISECTED BLASTOCYSTS OBTAINED FROM IN-VITRO FERTILIZATION OF OOCYTES MATURED IN-VITRO

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Summary

Bovine blastocysts were obtained by in-vitro culture of embryos derived from in-vitro fertilization of oocytes matured in-vitro. These blastocysts and blastocysts from inseminated donors were bisected by a simple method (without a holding pipette) using a microblade operated by a micromanipulator.

A pair of demi-embryos was transferred nonsurgically into each uterine horn of a recipient cow 6 or 8 days after estrus. Pregnancy resulted from the third transfer. Ultrasound examination done 52 days after estrus (46 days after transfer) confirmed the presence of at least one fetus in the each uterine horn.

This is the first report to show the viability of bisected bovine blastocysts obtained from in-vitro culture of embryos derived from in-vitro fertilization of oocytes matured in-vitro. In addition, a simple method to bisect bovine embryos is described.

(Key Words: In Vitro, Fertilization, Blastocyst, Bisection, Cattle)

Introduction

Splitting of bovine embryos is an effective means for increasing the number of calves per embryo (Williams et al., 1984; Takeda et al., 1988; Matsumoto et al., 1987) and for producing identical twins (Willadsen et al., 1981; Ozil et al., 1982; Lambeth et al., 1983). This method however has never been applied to bovine embryos obtained from in-vitro fertilization.

We (Goto et al., 1988) have developed a method to obtain calves from in-vitro fertilization of cow oocytes matured in-vitro. In the experiment described here we tested the viability of bisected bovine balstocysts obtained from our in-vitro fertilization method by transferring them into cow uteri. In addition, we have devised a simple method to bisect bovine embryos without using a holding pipette.

Materials and Methods

Blastocysts. Bovine blastocysts were obtained either from culture of embryos obtained by in-vitro fertilization of oocytes matured in-vitro or from superovulated cows. Superovulation was induced by FSH-PG treatment and the embryos were collected nonsurgically as described by Goto et al. (1987). The precise method of bovine in-vitro fertilization was previously reported (Kajihara et al., 1987; Goto et al., 1988).

Bisection of blastocysts. Only normal blastocysts were selected for bisection. Bisection was done in a microdrop of medium (TCM 199 with 25 mM HEPES plus 5% neonatal calf serum (GIBCO, Lot. 22P-4457)) covered with liquid paraffin in a 35 mm polystyrene dish (Termo, Tokyo, Japan).

Bisection was performed using a microblade (Bisecting Blade, No. 62, Feather Ind. LMD. Gifu, Japan) attached to the micromanipulator (MO-102, Narishige Scientific Instrument Lab, Tokyo, Japan).

A small groove (about 10 μM width) was made with the microblade on the bottom of the dish. The embryo to be bisected was placed in this groove which was sufficient to stabilize the embryos so that it could be bisected without a holding pipette.

With a lateral motion of the point of the blade, the embryo (blastocyst) was oriented in such a way that the inner cell mass could be divided in two equal portions. The blade was then moved

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above the embryo and with a tilting motion of the micromanipulator the blade was brought quickly through the zona pellucida, the final motion being a forward cutting of the embryonic tissue along the bottom of the dish.

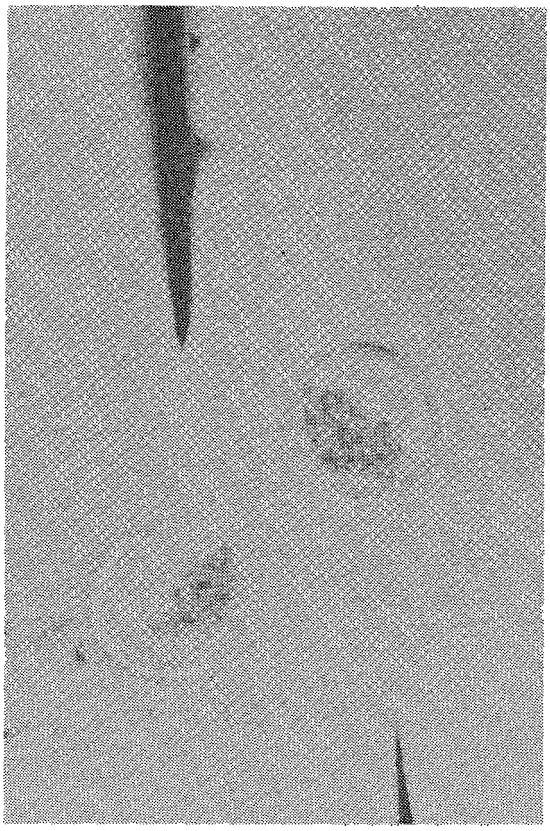

Figure 1. Bisection of bovine embryo (blastocyst) by a microblade.

Transfer of demi-embryo. One resulting pair of demi-embryos from bisection of blastocysts obtained from in-vitro fertilization were immediately transferred to each uterine horn of a recipient cow. The demi-embryos were within their halved zona pellucida at least at the time when they were pipetted into a straw for transfer (figure 1). Transfers were conducted nonsurgically on Day 6 or Day 8 of estrus (Day 0=estrus). One cow was repeatedly used as a recipient at 3 consecutive estrus cycles and at the third transfer, the recipient cow became pregnant. Pregnancy diagnosis was done by palpation per rectum and by ultrasound at 52 d.

Results

Table 1 shows the result of the bisection by a microblade of embryos obtained from super-

TABLE 1. BISECTION OF BLASTOCYSTS[1] BY A MICROBLADE

Surface of dish	No. of blastocysts bisected	No.(%) of blastocysts cut into 2 equal portions
Without groove	15	10 (67)
With groove[2]	17	16 (94)*

[1] Blastocysts recovered from superovulated donor cows were used.
* $P < 0.05$ (X^2-test).
[2] A small groove was made by a microblade on the bottom of a dish and the embryo was placed on it during bisection.

TABLE 2. RESULT OF EMBRYO[1] TRANSFERS

Recipient[2]	Day after estrus (Day 0=estrus)	Side of uterine horn	Demi-embryos transferred	Pregnancy diagnosis	Presence of fetus
1	8	R	1 Pair	Not pregnant	–
		L	–		
2	8	R	1 Pair	Not pregnant	–
		L	1 Pair		
3	6	R	1 Pair	Pregnant	R two fetuses
		L	1 Pair		L one fetus

[1] Blastocysts obtained from in-vitro fertilization were used for bisection.
[2] Cow No.1644 was repeatedly used as a recipient during 3 consecutive estrous cycles.
R = right, L = left

ovulated cows. A small groove made on the bottom of the dish by a microblade significantly ($P < 0.05$) increased the percent of embryos bisected into 2 equal portions.

Table 2 shows the result of embryo transfers. Recipient cow No. 1644 which received one pair of demi-embryos in each uterine horn at three consecutive estrus cycles became pregnant at the third transfer. At rectal palpation there seemed to be one fetus in the left uterine horn and two fetuses in the right horn. On ultrasound examination one fetus with a heatbeat was detected in each uterine horn. Thoroughness of the examination was limited so as to avoid the chance of

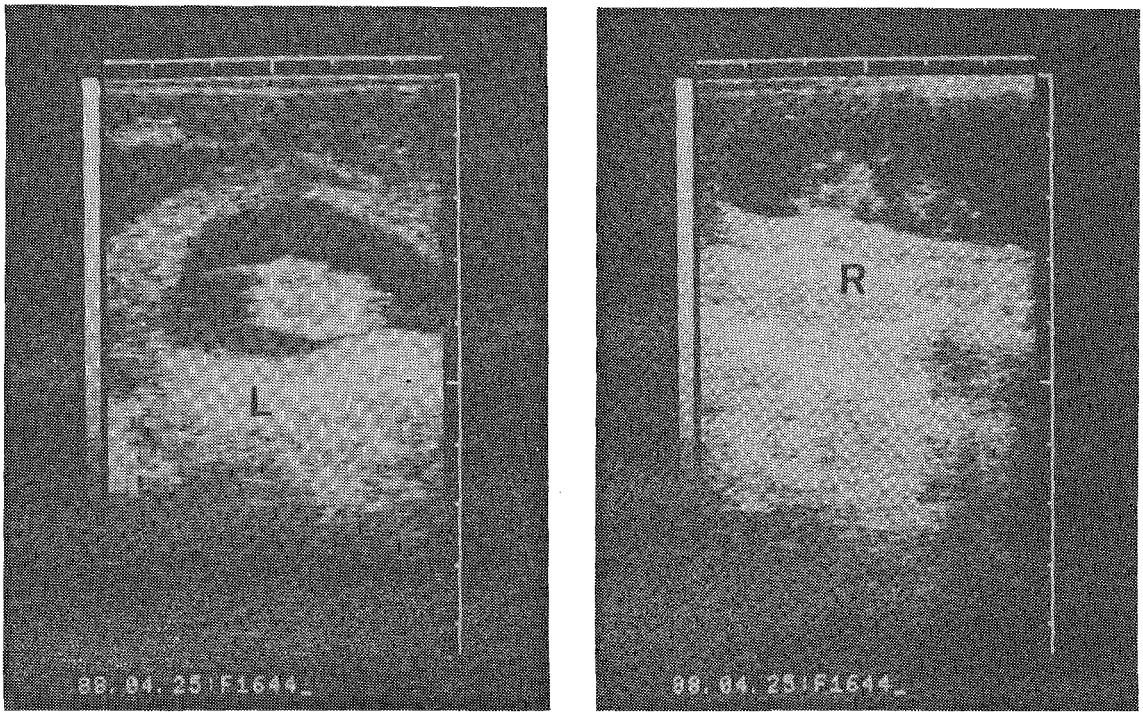

Figure 2. Ultrasound image of pregnancy at 46 days after transfer (52 days after last estrus).
L: Left uterine horn R: Right uterine horn

inducing abortion or resorption of the fetuses. Figure 2 shows the ultrasound image of each fetus at 52 d after estrus.

Discussion

We have previously reported pregnancies from the transfer of whole blastocysts derived by our in-vitro fertilization method (Goto et al., 1988: Goto, 1988). The transfer of fresh blastocysts to 6 recipients resulted in 3 pregnancies (50%) and the transfer of frozen-thawed blastocysts to 11 recipients resulted in 5 pregnancies (45%) (Goto et al., 1988). Normal calves were born to these recipients. The establishment of pregnancy with demi-blastocysts further verifies the excellent quality of blastocysts produced by our in-vitro fertilization methods.

The methods described (Goto et al., 1988) for in-vitro production of blastocysts are cheap and practical. Experiments using our in-vitro techniques with larger numbers of recipients are necessary to confirm the viability of demi-embryos and to establish the cost and practicability compared to whole embryos.

The observations on bisection of embryos indicate that the technique can be simplified by placing the embryo in a small groove on the floor of the dish. This eliminates the need for a holding pipette.

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(Appendix)

Premature delivery occurred at 211 days of preganancy. Two male fetuses (9.0 and 9.1 kg) were expelled from the right uterine horn. The body weight of the fetuses was within normal range of this stage but they were already dead when pulled out.

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