

# NORMALITIES OF CALVES OBTAINED FROM THE TRANSFERS OF BLASTOCYSTS PRODUCED BY TOTALLY IN-VITRO TECHNIQUE

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## Summary

Bovine blastocysts were obtained by totally in-vitro technique and then transferred to recipient cows. Total of 15 calves (including 4 premature calves) were obtained from 11 recipients. Four calves were obtained from the transfers of fresh blastocysts and 9 calves were obtained from the transfers of frozen-thawed blastocysts. Two calves were obtained from the bisected fresh blastocyst.

Ten males and 5 females were delivered. Birth weight of calves was within normal range except one female, and all calves appeared to be completely normal.

(Key Words: Bovine Blastocyst, In-vitro Technique)

## Introduction

Fertilization in-vitro of bovine oocytes was first achieved in 1977 (Brackett et al., 1977; Iritani and Niwa, 1977). Calves resulting from bovine in-vitro fertilization have been reported (Brackett et al., 1982, 1984; Hanada et al., 1986; Lambert et al., 1986). Recently calves were obtained from the transfers of blastocysts obtained from totally in-vitro technique (Goto et al., 1988a; Lu et al., 1988; Eyestone and First, 1989).

In the experiment described here we examined the normalities of calves obtained from our totally in-vitro technique.

## Materials and Methods

### Blastocysts

Bovine blastocysts were obtained from totally in-vitro technique (Kajihara et al., 1987; Goto et al., 1988a).

### Cryopreservation of Blastocysts

The cryoprotectant used was glycerol at 10% final concentration in TCM 199 (25 mM HEPES + 10% calf serum). The embryos were exposed to increasing concentrations of the cryoprotectant in 3 steps (3.3, 6.7, 10%; 7-9 min each) at room temperature. The embryos were then transferred to 0.25 ml straws. Freezer ( $-40^{\circ}\text{C}$ ) was used for cooling embryos (Oku et al., 1985). The straws were placed in a triangular flask (height, 10 cm; top diameter, 2 cm; bottom diameter, 6 cm) filled with ethanol (volume, 120 ml; height, 8 cm) and the one end of straws was kept 4-5 cm above the surface of ethanol to allow natural seeding. When the temperature of ethanol reached to  $-7^{\circ}\text{C}$ , the flask was transferred into a plastic vessel (diameter, 8.2 cm; height, 14 cm) containing 200 ml ethanol in order to slow down the cooling rate. When the temperature of ethanol reached to  $-38^{\circ}\text{C}$ , the straws were plunged into liquid nitrogen. The cooling rate obtained from this method was approximately  $1^{\circ}\text{C}/\text{min}$  (room temperature to  $-7^{\circ}\text{C}$ ),  $-0.38^{\circ}\text{C}/\text{min}$  ( $-7^{\circ}\text{C}$  to  $-32^{\circ}\text{C}$ ) and  $-0.17^{\circ}\text{C}/\text{min}$  ( $-32$  to  $-38^{\circ}\text{C}$ ). Embryos were thawed by plunging straws into a  $35-38^{\circ}\text{C}$  water bath for about 10 seconds. Cryoprotectant was removed in 3 or 6 steps (8.3, 6.6, 4.9, 3.2, 1.5, 0% glycerol; 5-10 min each) at room temperature.

### Bisection of Blastocysts

Bisection of bovine blastocysts was previously described (Goto et al., 1988b).

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Received April 28, 1989

Accepted September 18, 1989

### Transfer of Embryos

Blastocysts were nonsurgically transferred to the uteri of recipient cows at Day 6-8 (Day 0 = estrus). Fresh, bisected and frozen-thawed blastocysts were used for transfers (2-4 blastocysts/recipient).

### Results

Table 1 shows the results of this experiment. Fifteen calves were obtained from 11 recipients. One recipient delivered triplets but they died

during delivery. The birth weight of triplets was 14.5, 20.0 and 16.5 Kg and considered to be normal weight as triplets. This recipient had kept in the mountain and we could not help her deliver due to strong wind and heavy rain. By autopsy, three calves found to be normal.

One recipient that was transferred frozen-thawed blastocysts prematurely delivered twins at 257 days of pregnancy. The birth weight of twins was 9.0 and 27.0 Kg and they died soon after birth. One calf was smaller than normal but they had no abnormalities.

TABLE 1. BIRTH WEIGHT AND SEX OF CALVES OBTAINED FROM THE TRANSFERS OF BLASTOCYSTS PRODUCED BY TOTALLY IN-VITRO TECHNIQUE

Birth No.	Breed of recipient	Embryos transferred	Birth weight (kg)	Sex (No.)	Appendix
1	JB	Fresh	41.0	F(1)	
2	JB	Fresh	14.5,20.0,16.5	F(2), M(1)	Died during delivery
3	H	Frozen	35.0	M(1)	
4	H	Frozen	33.0	M(1)	
5	F1	Frozen	36.5	M(1)	
6	H	Frozen	50.0	F(1)	
7	H	Frozen	9.0,27.0	F(1), M(1)	Premature delivery at 257 d
8	JB	Fresh (Bisected)	9.0,9.1	M(2)	Premature delivery at 211 d
9	F1	Frozen	36.0	M(1)	
10	F1	Frozen	32.0	M(1)	
11	H	Frozen	30.0	M(1)	

JB = Japanese Black, H = Holstein, F1 = H x JB.  
F = Female, M = Male.

One recipient that was transferred bisected fresh-blastocyst prematurely delivered male twins (9.0, 9.1 Kg) at 211 days of pregnancy. Their body weight considered to be normal at this stage of pregnancy and they had no abnormalities by autopsy.

Eight calves born in healthy conditions and all of them appear to have no abnormalities.

### Discussion

Calves born after in-vitro fertilization had so far originated from in-vivo matured oocytes trans-

ferred surgically to recipient cattle (Brackett et al., 1982) or after using the rabbit oviduct as an incubator for 4-5 days (Lambert et al., 1986). Hanada et al. (1986) have reported the birth of calves after in-vitro fertilization of in-vitro matured oocytes and using the rabbit oviduct as a temporary incubator. Critser et al. (1986) used the sheep oviduct as a temporary incubator.

Recently calves were obtained from the transfers of blastocysts produced by totally in-vitro technique (Goto et al., 1988; Lu et al., 1988; Eyestone and First, 1989). Since the use of totally in-vitro technique in bovine in-vitro fertilization is

a new field, the normalities of calves obtained by this technique have been remained to be clarified before its wide use. In the present study, we observed 15 calves (including 4 premature calves) produced by our totally in-vitro technique. All calves found to be normal. First calf (female, 41 Kg, 74 cm) was born in March, 1988 and she became 338 Kg and 119.4 cm at her year age. She grew up above normal size. These results would encourage us the wide use of this technique for the production of calves.

This study was supported by Grant-in-Aid (63560269) from the Ministry of Education, Science and Culture of Japan and by grant from the Inamori Foundation to Dr. Goto. We thank Drs. K. Hamana and Y. Taura, School of Veterinary Medicine, Kagoshima Univ., Dr. K. Yanagita, Iriki Livestock Farm, Kagoshima Univ. We also thank to Mr. K. Hishiyama and Mr. Y. Koshiba, Marubeni Shiryō Co., Ltd., for help in conducting this study.

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