

PRODUCTION OF CHIMERIC CALVES DERIVED FROM PARTHENOGENETIC AND FERTILIZED BOVINE EMBRYOS

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The present study was aimed to examine whether parthenogenetic cells could contribute to the development of bovine embryos to term.

Chimeric blastocysts were produced by aggregation of parthenogenetic and *in vitro* fertilized bovine embryos at the 8-cell stage (Boediono and Suzuki, *Theriogenology* 45: 220, 1996), and nonsurgically transferred to synchronized recipient cows. To induce diploid parthenogenetic activation, matured oocytes were treated with 7% ethanol followed by 5µg/ml cytochalasin B or D (Boediono and Suzuki, *Theriogenology* 41: 166, 1994). Some of the blastocysts were cryopreserved in 1.8 M ethylene glycol plus 0.05 M trehalose supplemented with 10% polyvinylpyrrolidone (PVP) using a conventional slow cooling method. After being thawed and cultured on cumulus layer for 24 h, the re-expanded blastocysts were transferred to the recipients.

As shown in Table 1, after transfer of both the fresh and frozen-thawed blastocysts [1 or 2 embryo(s) per recipient], 8 out of 17 recipients (47.1%) were diagnosed pregnant by an ultrasonic scanning instrument (Aloka, Japan) on day 40 (estrus = day 0). However, 4 out of the 8 pregnant cows (50.0%) aborted from day 60 to 150. In addition, although 2 recipients delivered 3 males (twin and single), twin calves were stillbirth (day 234) and single calf died within 24 h after birth (day 261). But all of the males contained XX and XY chromosomes and also showed the evidence of chimerism by the hair color.

From these results, it seems to be difficult to obtain normal calves by transfer of chimeric blastocysts between parthenogenetic and fertilized embryos, which suggests that the paternal genome may be required for the proliferation of the extra-embryonic tissues (placentation).

Table 1. Summary of transferred embryos derived from aggregates between parthenogenetic and fertilized 8-cell embryos.

| | No. of recipient | No. of embryo | Stage of embryo | No. (%) pregnant | No. (%) abortion | No. (%) stillbirth | No. (%) parturition |
|---------------|------------------|---------------|----------------------|------------------|------------------|--------------------|---------------------|
| Fresh | 3 | 1 | Blastocyst (day 8-9) | 0 (0.0) | - | - | - |
| | 8 | 2 | Blastocyst (day 7-8) | 6(75.0) | 3 (37.5) | 1 (12.5)* | 0 (0.0) |
| Frozen thawed | 1 | 1 | Blastocyst (day 8) | 1 (100.0) | 0 (0.0) | 0 (0.0) | 1 (100.0)** |
| | 5 | 2 | Blastocyst (day 8-9) | 1 (20.0) | 1 (20.0) | 0 (0.0) | 0 (0.0) |
| Total | 17 | - | - | 8 (47.1) | 4 (23.5) | 1 (5.9) | 1 (5.9) |

*Twin (m, m); day 234, Chromosome XX+XY **Single (m); day 261, Chromosome XX+XY

At present, we have 2 pregnant cows after transfer of blastocysts derived from aggregates between 8-cell embryos from parthenogenetic (Holstein) and *in vitro* fertilized (Japanese brown breed).