Embryo Manipulation

AGGREGATION OF PARTHENOGENETIC AND FERTILIZED EMBRYOS FOR PRODUCING CHIMERA CALVES

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Chimeric blastocysts were produced by aggregation of parthenogenetic and fertilized demi-embryos from Japanese Brown breed and Holstein breed cattle, respectively. The fertilized embryos were obtained by in vitro maturation, fertilization and culture procedures (Boediono et al., Reprod. Fertil. Dev., 6: 261, 1994). To produce diploid parthenogenotes, matured oocytes were treated with 7% ethanol followed by 5 μg/ml cytochalasin D (Boediono and Suzuki, Theriogenology 41: 166, 1994). Aggregation embryos were produced by injecting a fertilized demi-embryo (8-cell stage) into a parthenogenetic demi-embryo (8-cell stage) and cultured in vitro. In experiment 1, the aggregation rate and development in vitro of micromanipulated embryos with or without embedding in 1.0% agar cylinder were compared. The aggregation rate of micromanipulated embryos in an agar cylinder was higher ($P<0.01$) than without agar (39/42, 93% for embedding in 1.0% agar and 27/51, 53% without agar, respectively). However, the development of aggregated embryos to blastocysts was not different (36/39, 92% for embedding in 1.0% and 23/27, 85% without agar, respectively). In experiment 2, the male fertilized demi-embryos (sexed by amplifying male specific DNA using the PCR) were injected into parthenogenetic demi-embryos to produce chimeric blastocysts. To prove the participation of cells derived from parthenogenetic and fertilized embryos during blastocyst formation, 28 embryos (hatching and hatched) were karyotyped, resulted in 15 embryos having XX and XY chromosome in the same plate, 7 embryos with XY and 6 embryos with XX chromosomes.

The chimeric blastocysts that produced by embedding in 1.0% agar (obtained on day 8, day 0 = day of insemination) were transferred to 6 recipients, one or two embryo(s) per recipient. Pregnancies were diagnosed in 3 recipients at day 40 (day 0 = estrus). The fetus disappeared in one recipient after day 60. Twin males (still born) and a single male calves were delivered from two recipients in which XX and XY chromosomes were detected. The skin color was also used as evidence of chimerism. From these results it can be verified that the parthenogenetic cells can contribute to normal development of aggregated embryos which ultimately resulted in chimeric calves.