MOLECULAR REPRODUCTION AND DEVELOPMENT 53:159–170 (1999)

Offspring Born From Chimeras Reconstructed From Parthenogenetic and In Vitro Fertilized Bovine Embryos

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ABSTRACT Chimeric embryos were produced by aggregation of parthenogenetic (Japanese Red breed) and in vitro fertilized (Holstein breed) bovine embryos at the Yamaguchi Research Station in Japan and by aggregation of parthenogenetic (Red Angus breed) and in vitro fertilized (Holstein breed) embryos at the St. Gabriel Research Station in Louisiana. After embryo reconstruction, live offspring were produced at each station from transplanting these embryos. The objective of this joint study was to evaluate the developmental capacity of reconstructed parthenogenetic and in vitro fertilized bovine embryos. In experiment I, chimeric embryos were constructed: by aggregation of four 8-cell (demi-embryo) parthenogenetic and four 8-cell stage (demi-embryo) IVF-derived blastomeres (method 1) and by aggregation of a whole parthenogenetic embryo (8-cell stage) and a whole IVF-derived embryo (8-cell stage) (method 2). Similarly in experiment II, chimeric embryos were constructed by aggregating IVF-derived blastomeres with parthenogenetic blsatomeres. In this experiment, three categories of chimeric embryos with different parthenogenetic IVF-derived blastomere ratios (2:6; 4:4, and 6:2) were constructed from 8-cell stage bovine embryos. In experiment III, chimeric embryos composed of four 8-cell parthenogenetic and two 4-cell IVF-derived blastomeres or eight 16-cell parthenogenetic and four 8-cell IVF-derived blastomeres were constructed. Parthenogenetic demi-embryos were aggregated with sexed (male) IVF demi-embryos to produce chimeric blastocysts (experiment IV). In the blastocyst stage, hatching and hatched embryos were karyotyped. In experiment V, chimeric embryos that developed to blastocysts (zona-free) were cryopreserved in ethylene glycol (EG) plus trehalose (T) with different concentrations of polyvinylpyrrolidone (PVP; 5%, 7.5%, and 10%). In experiment I, the aggregation rate of the reconstructed demi-embryos cultured in vitro without agar embedding was significantly lower than with agar embedding (53% for 0% agar, 93% for 1% agar, and 95% for 1.2% agar, respectively). The aggregation was also lower when the aggregation resulted from a whole parthenogenetic and IVF-derived embryos cultured without agar than when cultured with agar (70% for 0% agar, 94% for 1% agar, and 93% for 1.2% agar, respectively). The development rate to blastocysts, however, was not different among the treatments. In experiment II, the developmental rates to the morula and blastocyst stages were 81%, 89%, and 28% for the chimeric embryos with parthenogenetic:IVF blastomere ratios of 2:6, 4:4, and 6:2, respectively. In experiment III, the developmental rate to the morula and blastocyst stages was 60% and 65% for the two 4-cell and four 8-cell chimeric embryos compared with 10% for intact 8-cell parthenogenetic embryos and 15% for intact 16-cell parthenogenetic embryos. To verify participation of parthenogenetic and the cells derived from the male IVF embryos in blastocyst formation, 51 embryos (hatching and hatched) were karyotyped, resulting in 27 embryos having both XX and XY chromosome plates in the same sample, 14 embryos with XY and 10 embryos with XX. The viability and the percentage of zona-free chimeric embryos at 24 hr following cryopreservation in EG plus T with 10% PVP were significantly greater than those cryopreserved without PVP (89% vs. 56%). Pregnancies were diagnosed in both stations after the transfer of chimeric blastocysts. Twin male (stillbirths) and single chimeric calves were delivered at the Yamaguchi station, with each having both XX and XY chromosomes detected. Three pregnancies resulted from the transferred 40 chimeric embryos at the Louisiana station. Two pregnancies were lost prior to 4 months and one phenotypically-chimeric viable male calf was born. We conclude that the IVF-derived blastomeres were able to stimulate the development of bovine parthenogenetic blastomeres and that the chimeric parthenogenetic bovine embryos were developmentally competent. *Mol. Reprod. Dev. 53:159–170, 1999.* © 1999 Wiley-Liss, Inc.

Key Words: chimera; parthenogenetic; in vitro fertilization; cattle; offspring

Grant sponsor: Ministry of Education, Science and Culture, Japan; Grant number: 845160; Grant sponsor: Louisiana Agriculture Experimental Station, Louisiana State University Agriculture Center.

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Received 28 July 1998; Accepted 15 December 1998.