

Chimeric blastocysts produced by aggregation between parthenogenetic and fertilized bovine embryos

A. Boediono¹ and T. Suzuki¹

1) United Graduate School of Veterinary Sciences, Yamaguchi University, Yamaguchi 753, Japan

Abstract

Chimeric blastocysts were produced by aggregation of parthenogenetic and fertilized embryos. The normal fertilized embryos were obtained by *in vitro* maturation, fertilization and culture procedures. To produce parthenogenetic activation, matured oocytes were treated with 7% ethanol followed by 5 µg/ml cytochalasin D and cultured at 38.5° C under 5% CO₂ in air. Aggregation of embryos were produced by: (1) injection of 2 blastomeres of parthenogenetic embryo (16-cell stage) into fertilized embryo (4-cell stage), (2) injection of parthenogenetic demi-embryo (8-cell stage) into fertilized demi-embryo (8-cell stage), (3) injection of fertilized demi-embryo (8-cell stage) into parthenogenetic demi-embryo (8-cell stage) and (4) aggregation of whole parthenogenetic and fertilized embryo. The developmental rate of aggregated embryos produced by aggregation of whole embryos was significantly higher ($P < 0.05$) than by injections. Thirteen aggregated embryos were karyotyped, resulted on eight of embryos had XX and XY chromosome plate in the same sample. These results verified that the parthenogenetic cells can be contributed to the development of the embryo.

Introduction

Parthenogenetic development of embryos to give live offspring occurs naturally in many non-mammalian species (White, 1978) and can also be induced experimentally in non-mammalian vertebrates and invertebrates (Nagy *et al.*, 1979). Although the parthenogenetically activated diploid mammalian embryos develop normally through preimplantation, they rarely reach the forelimb bud stage (Kaufman *et al.*, 1977).

There are distinct differences between the paternal and maternal contribution to the embryonic development. The paternal genome appears to be more important for the proliferation of the extra embryonic tissues and the maternal genome plays a key role in preimplantation and early post-implantation development (Barton *et al.*, 1984).

Vital functional differences between the parental genomes of mammals have been demonstrated by embryonic lethality of parthenogenetic, gynogenetic and androgenetic uniparental genotypes (Solter, 1988). Cell derived from uniparental embryos may be rescued by integration with normally fertilized embryos, which results in chimeric organism (Stevens *et al.*, 1977; Mann and Stewart, 1991; Barton *et al.*, 1991). These phenomena may be relevant to the problem of mammalian parthenogenesis.

In the bovine oocytes, there are several effective ways induce diploid parthenogenesis mainly by exposure to ionophore A23187 (Ware *et al.*, 1989), ethanol, electric stimulation and combination of ethanol and electric stimulation (Yang *et al.*, 1994). Bovine parthenogenetic embryos have been transferred to recipient; in one study estrus did not occur until day 48 following transfer of single embryo (Fukui *et al.*, 1992) and

in another study estrus was delayed until day 67 following transfer of aggregated embryo, however pregnancy could not be maintained after this period (Boediono and Suzuki, 1994), it might be caused by early embryonic death.

In the present study, we investigated the viability of chimeric blastocysts produced by aggregation between diploid parthenogenetic and fertilized bovine embryos.

Materials and methods

The procedures for producing diploid parthenogenetic embryos were carried out as previously described (Boediono and Suzuki, 1994). Briefly, oocytes were aspirated from the ovaries collected from slaughterhouse and matured for 32 hours at 38.5°C under 5% CO₂ in air. To induce parthenogenetic activation, matured oocytes were suspended in culture medium containing 7% ethanol for 10 minutes followed by treatment using 5 µg/ml cytochalasin D (5 hours) to suppress polar body extrusion and to produce diploid parthenogenones. The oocytes were then washed and cultured *in vitro* on feeder layers of bovine cumulus cells for further development.

The normal fertilized embryos were obtained by *in vitro* maturation, fertilization and culture procedures (Boediono *et al.*, 1994). The follicular oocytes were matured for 22-24 hours at 38.5°C under 5% CO₂ in air. These matured oocytes were then fertilized by *in vitro* capacitated sperm and cultured *in vitro*.

Aggregation of chimeras were produced by the following methods: (1) injection of 2 blastomeres obtained from 16-cell stage of parthenogenetic embryo into 4-cell stage of fertilized embryo; 2/16P → 4/4F, (2) injection of parthenogenetic demi-embryo (8-cell stage) into fertilized demi-embryo (8-cell stage); 4/8P → 4/8F (Fig.1), (3) injection of fertilized demi-embryo (8-cell stage) into

parthenogenetic demiembryo (8-cell stage); 4/8F → 4/8P and (4) aggregation of whole parthenogenetic and fertilized embryo by physically pushing the two embryos together until the aggregate seemed sufficiently stable; 8/8P ↔ 8/8F. Aggregated embryos were then cultured on feeder layer of bovine cumulus cells in TCM - 199 containing 5% superovulated cow serum, 5 µg/ml insulin and 50 µg/ml gentamicine. Aggregated embryos produced by methods (1), (2) and (3) were cultured *in vitro* using zona pellucida and the aggregated embryos produced by (4) method were cultured without zona pellucida (Boediono *et al.*, 1993). All aggregates were rechecked after 6 hours to ensure that they had not drifted apart. Morphological examination had been done every 12 hours; both the unaggregated and aggregated embryos with extruded blastomeres were removed from the culture dish. The chimeric embryos were then left in culture until day 9 (day 0 = day of IVF or activation oocytes) and the embryos that had developed into morphologically normal blastocysts were recorded.

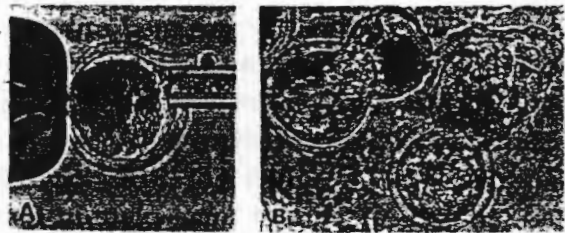


Figure 1. Chimeric blastocysts produced by (A) injection of parthenogenetic demi-embryo (8-cell stage) into fertilized demiembryo (8-cell stage) and (B) when developed into hatching blastocysts.

Results and discussion

Viability of aggregated embryos that developed into morula and blastocyst stage are shown in Table 1. Overall, the developmental rate of aggregated embryos, that were produced by the aggregation of parthenogenetic and fertilized embryos to morula and blastocysts was 56% and 45%, respectively. The developmental rate of aggregated embryos produced by aggregation of whole embryos was significantly higher ($P < 0.05$) than following injections. This was probably due to higher number of cells involved.

Table 1. Development of aggregated embryos to morula and blastocyst stage in vitro.

Aggregation ratio	No. of Aggreg. embryos	Number developed to	
		Morula (%)	Blastocyst (%)
2/16P → 4/4F	30	14 (47) ^a	9 (30) ^{ac}
4/8P → 4/8F	36	21 (58)	16 (44)
4/8F → 4/8P	37	17 (47) ^a	14 (38) ^a
4/8F → 4/8P*	37	27 (73) ^b	24 (65) ^{bd}
Total	140	79 (56)	63 (45)

* Aggregated embryos were cultured in vitro without zona pellucida

In order to prove the contribution of parthenogenetic derived embryonic cells in blastocyst formation, 13 aggregated embryos (hatching and hatched blastocysts) produced by methods (2), (3) and (4) were karyotyped according to the procedure described by Tarkowski (1966). Eight out of 13 blastocysts had XX and XY chromosome plate in the same sample (Fig. 2) and 5 blastocysts were detected as XX (female). These facts suggested that XX chromosome plate was obtained from parthenogenetic embryo or fertilized embryo and XY chromosome plate was obtained from fertilized embryo only. These results clearly demonstrated that the parthenogenetic cells also contributed to the development of the embryo, although the distribution rate could not be analyzed using the above procedure.

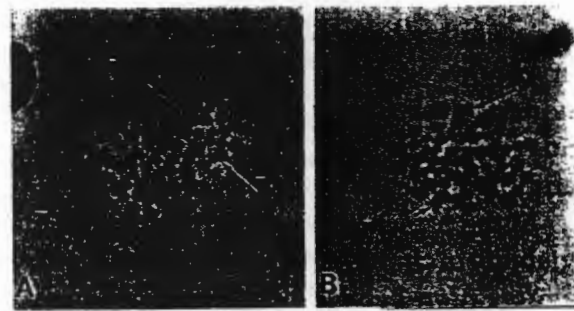


Figure 2. Methaphase spreads of chimeric blastocyst, (A) 60, XX, (B) 60, XY. Long arrows: X, short arrow: Y.

Means with different superscripts within columns are significantly different (a-b, $P < 0.05$; c-d, $P < 0.01$).

In the mouse egg, study of the postimplantation development of the parthenogenetic conceptus is restricted by infrequent development beyond implantation and the complete lack of development beyond placentation (Kaufman, 1983). The life and development of the parthenogenetically activated cells can be extended when chimeras are made between parthenogenetic and normally fertilized embryos (Steven *et al.*, 1977; Steven, 1978). Although their contributions of parthenogenetic cells to chimeras were low, not more than 20% of the total cell population (Surani *et al.*, 1977). The survival and integration of parthenogenetic cells in such chimeras are probably largely influenced by the environmental conditions determined by the cells from the fertilized embryo. The exact nature of this environmental influence remains unclear, but there are several probable ways in which such influences may be mediated. There is evidence for metabolic cooperation between genetically diverse cell types through permeable junctions that enable metabolically deficient cells to function normally (Pitts and Burk, 1976). Similar cellular interactions between parthenogenetic and fertilized cells may also ensure cooperation of parthenogenetic cells in normal development.

To our knowledge, this report is the first one to describe the contribution of parthenogenetic cells in embryo development in large animals. Results of this study indicated that chimeric blastocyst can be produced by aggregation of demi or whole embryos obtained from parthenogenetic and fertilized embryos. In an attempt to increase the participation parthenogenetic cells into the embryo proper, 2 blastomeres obtained from advanced stage (16-cell stage) of parthenogenetic embryo were injected into the less advanced stage (4-cell stage) of fertilized embryo. The concluding concept was the more advanced blastomeres (parthenogenetic embryo) would contribute to the ICM and the less advanced blastomeres (fertilized embryo) would develop to the trophectoderm.

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