CHIMERIC BLASTOCYST PRODUCTION USING IVF BOVINE EMBRYOS (HOLSTEIN-JAPANESE RED) WITHOUT ZONA PELLUCIDA

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The objective of this study was to produce chimeric blastocysts by the aggregation of IVF bovine embryos (Holstein - Japanese Red), without zonae pellucidae. The embryos were produced by the in vitro maturation and fertilization of oocytes were collected from a local slaughterhouse. Oocytes were taken from follicles of 2-5 mm diameter and those with multiple cumulus - cell layers, were matured in TCM-199 supplemented with 5 % superovulated cows serum (SCS, collected on day-7) and 0.01 mg/ml FSH containing 0.58 % luteinizing hormone (Denka, Pharmacheutical Co., Japan). Oocytes were cultured for 21 hours at 38.5°C under 5 % CO_2 in air, and then fertilized in vitro using frozen-thawed sperm.

Forty - eight hours after IVF, 8-cell stage embryos were used to produce aggregation chimeras. The zonae pellucidae were microsurgically removed using a microblade and a Narishige micromanipulator unit. Holstein embryos were aggregated with Japanese Red embryos after zona removal by hand manipulation in TCM-199, supplemented with 5 % SCS, then transferred into culture medium (TCM-199 supplemented with 5 % SCS and 5 ug/ml insulin) at 38.5°C under 5 % CO_2 in air. Embryos were evaluated 4 days after aggregation. Viability of aggregated embryos that developed into blastocysts is shown in Tab.1.

Tab. 1. Viability of Aggregated Embryos that Developed Into Blastocysts.

Trial	No. of embryos	Aggregated embryos (pairs)	Blastocysts	Degenerated
I.	10	4	4	0
II.	26	12	8	4
III.	12	5	3	2
IV.	12	5	3	2
V.	12	5	3	2
Total	72	31(86.1%)	21(67.7%)	10(32.3%)

These results indicate that chimeric blastocyst production by aggregation of zona-free embryos can be done in vitro.