FIRST ATTEMPT TO OBTAIN A BOVINE GYNOGENOTE USING POLAR BODY TRANSFER

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Gynogenesis is the development of an embryo originated from the fusion of two female gametes. The traditional way to create gynogenotes in vitro was by the transfer of a female pronucleus into the cytoplasm of a recipient zygote after male pronucleus extraction. Other attempts to induce gynogenesis in mice consisted in fusing two oocytes using Sendai viruses. Despite these studies have been unable to produce full-term pregnancies, the first report of a living gynogenote pup was originated from oocyte nuclear transfer. The aim of our study was to create a bovine gynogenote embryo using a Polar Body (PB) transfer technique.

Bovine ovaries were collected from a local abattoir. Cumulus oocyte complexes (COC) were obtained by follicular aspiration and in vitro matured in TCM-199 at 38.5°C under a 5% CO₂ and high humidity atmosphere. After 20-22 h, mature metaphase II oocytes (MII oocytes) were selected based on cumulus expansion, extrusion of the first PB and cytoplasm homogeneity. First PB of MII oocytes were extracted and injected into the cytoplasm of different MII oocytes using a micromanipulator (Naris Hige, Tokyo, Japan) under an inverted microscope (Nikon, Tokyo, Japan). After 1 h, transferred oocytes were activated chemically using Ionomycin (5 µM) and 6-DMAP (2.5 mM) followed by in vitro culture in SOF medium at 38.5°C under a 5% CO₂ and high humidity atmosphere. A control group consisted in parthenogenetic activation using the protocol described above. A 73.9% (17/23) of reconstructed oocytes developed in 2-4 cells on Day 2 (Day 0 = day of PB transfer) and 34.8% (8/23) in 8-16 cells on Day 5. Whereas a 82.3% (102/124) of the oocytes from the control group developed in 2-4 cells on Day 2, 26.6% (33/124) in 8-16 cells on Day 5 and 1.6% (2/124) in blastocyst on Day 8, respectively. In order to improve development rates, reconstructed oocytes (n=4) were cultured in an humid chamber under an atmosphere containing 5% CO₂, 5% O₂ and 90% N₂. Under these conditions, two oocytes cleaved on Day 2 and developed to hatched blastocyst on Day 8. This preliminary study is the initial attempt to create a bovine gynogenote. Although parthenogenesis may not be excluded as the underlying process in this embryo development, our data suggest that bovine oocytes are able to withstand PB transfer and to continue development up to blastocyst stage. Supported by Escuela de Graduados, FCV-UACH and DID-UACH S-2009-26.

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