Animal Reproduction

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Interspecific embryo production: In vitro fertilization of cow-buffalo hybrids.

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Resumo

Interspecific hybrid embryos are useful models both in research, allowing the study of species-specific maternal-fetal interactions, and in animal breeding for improvements in the environmental adaptation of cattle. The objective of this research is to compare the development of cow-buffalo hybrid embryos (hCxB) and bovine embryos (BxB) by in vitro fertilization (IVF). Three cycles were carried out where cow oocytes (n=84) and buffalo semen were used for hCxB and cow oocytes (n=74) and bull semen for the BxB group. Bovine ovaries were collected from slaughterhouses and transported to the laboratory at 25ºC to 30ºC. Cumulus-oocyte complexes (COCs) were aspirated with 18-gauge needles from follicles with a diameter of 2 to 5 mm and collected in Hepes-buffered Tyrode's albumin (Hepes-TALP). COCs were matured in vitro for 22 h in 100ul drops covered with mineral oil (M8410) of bicarbonate-buffered TCM-199 (31100-035; Gibco, Grand Island, NY, USA), containing 10% fetal bovine serum (013/07; Internegocios, Buenos Aires, Argentina), 10 mg/mL follicle-stimulating hormone (NIH-FSH-P1, Folltropin, Victoria, Australia), 0.3 mM sodium pyruvate (P2256), 100 mM cysteamine (M9768), and 2% antibiotic-antimycotic (ATB, 15240-096; Gibco). For the IVF cycles, the bull and buffalo sperm used were from the same individual, respectively. Frozen semen was thawed in 37ºC water bath 30 seconds. Motility of buffalo and bovine sperm was checked after thawing. Then both spermatozoa were centrifuged twice (490 g, 5 minutes) in Brackett-Oliphant (BO) medium and resuspended in BO supplemented with 5 mM caffeine (C4144) and 20 IU/mL heparin (H3149). Spermatozoa were adjusted to 16 x 10^6 /mL with BO containing 10 mg/mL fatty acid-free bovine serum albumin (A6003). COCs were exposed to the buffalo and bovine sperm suspension for 5 hours in 100 mL droplets at 39ºC under 5% CO_2 in humidified air. Presumptive zygotes were then washed three times in Hepes-TALP. Presumptive hybrid and intraspecific zygotes derived by IVF were cultured in 50 mL droplets of SOF medium supplemented with 2.5% fetal bovine serum at 39ºC under 6.5% CO_2 in humidified air. Cleavage rate was evaluated on Day 2 and blastocysts rate on Day 8. The percentage of blastocysts obtained was evaluated over the total number of oocytes. To evaluate the significance in the development of the hybrids and the control group embryos, a T-Student test was performed for independent samples. There were significant differences ($P<0.05$) in the number of cleaved zygotes (33% hCxB vs 87.3% BxB) and blastocysts (13% hCxB vs 42% BxB) compared to the initial number of oocytes obtained. As a consequence of these results, we can observe that interspecific IVF between cow and buffalo can be carried out successfully. It is noteworthy that the deviations observed between hybrid and bovine blastocyst rate may be due to a poorer buffalo semen quality and a difference of compatibility between the oocytes and sperm of both species.