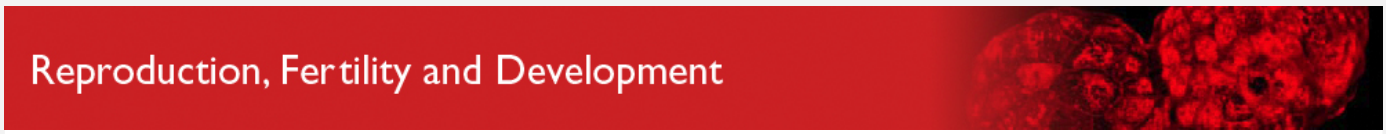

156 INVESTIGATION OF BOVINE HERPESVIRUS IN CUMULUS–OOCYTE COMPLEXES AND FOLLICULAR FLUID

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Abstract

The world market for bovine embryos has increased in the past few years. However, sanitary problems such as foot and mouth disease in Brazil, vesicular stomatitis in South America, and bovine spongiform encephalopathy (BSE) in North America and Europe have increased concerns regarding the risk of introducing exotic diseases and/or more virulent serotypes of endemic diseases by embryo transfer. Many countries are trying to develop and/or improve new techniques for infectious disease detection, with the scientific basis to support the import and export of animal germplasm. Therefore, the epidemiology of the diseases and the interaction between pathogens and cumulus–oocyte complexes (COCs), embryos, and semen must be investigated. Despite the many studies that have been carried out to evaluate the possibility of transmission of infectious agents by the embryo, few data are available regarding COC susceptibility (Tsuboi *et al*. 1992 J. Vet. Med. Sci. **54**, 1179–1181). The aim of this study was to evaluate the presence of bovine herpes virus serotype 1 (BHV-1) in COCs and follicular fluid (FF) collected from naturally infected animals in a low stress condition. Blood samples of non-lactating Gyr breed (*Bos indicus*) cows were collected and evaluated for BHV-1 antibodies by the serum neutralization microplate test, performed as described in the Manual for Standards for Diagnostic Tests and Vaccines (OIE, 1992). The cows were diagnosed as serologically positive (n = 38) or serologically negative (n = 8), and kept under grazing in *Brachiaria decumbens* pasture with mineral supplementation. The cows considered as positive showed titers greater than 1/4. COCs and follicular fluid (FF) were obtained by ovum pick-up (OPU) using sterile and disposable materials for each animal. Virus detection was performed by the PCR technique. PCR sensitivity was made using COCs and FF recovered from eight BHV-1 serologically negative animals. These samples were either artificially infected on plates with $10^{6.5}$ TCID in 50 µL of IBR Colorado 1 reference serotype (ATCC, VR-864) or used as a negative control. The PCR analitical sensitivity was $10^{0.5}$ TCID. The presence of BHV-1 in COCs and FF was not detected in any of the animals, despite the high sensitivity of the PCR technique. In the present *in vivo* model, results show that COCs collected from serologically BHV-1 positive cows presenting no clinical signs of the illness and managed in a low stress condition could be used as donors for *in vitro* fertilization procedures with minimal sanitary risks. Also, the absence of the virus in COCs and FF cannot be used as a predictor of BHV-1 infection status in bovine herds.

Keywords:

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