

## ABSTRACT

### **Successful Vitrification of In vivo Embryos Collected from Superovulated Japanese Black Cattle (Wagyu)**

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The aim of this study was to determine whether vitrification is an effective method when used for Japanese Black Cattle (Wagyu) in vivo-derived embryos, collected following a superovulation treatment and embryo transfer (MOET) programme. In vivo-derived morula and blastocysts collected on day 7 after artificial insemination, were vitrified using a modified droplet vitrification (MDV) procedure and subsequently warmed for transfer (ET) into synchronized recipients. Fresh embryos, and embryos cryopreserved using a standardized slow freezing procedure (direct thaw/direct transfer, DT) served as ET controls. Two different follicle-stimulating hormone (FSH) sources, Folltropin® Canada (FSH BAH, 24 donors) and a brand prepared by the Chinese Academy of Science (FSH CAS, 16 donors), were compared in a series of superovulation outcomes following well-established FSH administration protocols. Following data analysis, the total number of ovulations recorded at the time of embryo flushing (10.5 vs 8.5;  $p = 0.28$ ) and the total number of transferable embryos (6.2 vs 5.1;  $p = 0.52$ ) were similar between the two FSH sources. ET for MDV (39.7%,  $n = 78$ ), DT (35.2%,  $n = 71$ ) and fresh controls (47.1%,  $n = 34$ ) resulted in similar pregnancy rates ( $p > 0.05$ ). When MDV was used, a higher pregnancy rate (42.6%) resulted from the transfer of vitrified morulae, when compared to the DT counterparts (24.3%), ( $p = 0.05$ ). Transfer of vitrified morulae resulted also in higher pregnancy rate, when compared to the transfer of vitrified blastocysts (42.6% vs. 29.4%;  $p < 0.05$ ). Transfer of DT blastocysts resulted in higher pregnancy rate than morulae, similarly cryopreserved (47.1% vs. 24.3%,  $p < 0.05$ ). In conclusion, MDV is an effective alternative methodology for cryopreservation of in vivo-derived embryos. This study gives also indication that, compared to vitrified blastocysts, MDV of morula stage embryos results in higher pregnancy rates following warming and transfer into synchronized recipients.