



Production and Sex Ratio of Foals After Transfer of Frozen-thawed *In Vitro* Produced Embryos



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In vitro embryo production via intracytoplasmic sperm injection (ICSI) offers a possible solution for donor mares or stallions not suitable for conventional embryo transfer (ET), and embryo yield (1 per OPU) compares favourably to ET. However, the final outcome after transferring frozen-thawed *in vitro*-produced (IVP) embryos is not well described. The aim of this study was to determine the pregnancy and foaling outcomes after transfer of frozen-thawed IVP equine embryos in a clinical program, using fresh *in vivo*-recovered embryos as a reference point, and to examine the influence of rate of *in vitro* embryo development (time from ICSI to blastocyst formation). During 2015 and 2016, 213 *in vivo* embryos were flushed from Warmblood mares and 261 IVP blastocysts were produced by OPU-ICSI-IVP and cryopreserved by controlled-rate freezing; the interval (days) between ICSI and blastocyst formation was recorded. Embryos were transferred into recipient mares and the likelihoods of pregnancy, pregnancy loss, multiple pregnancy (twins/triplets) and foaling were calculated, and gestation length and sex of the foal were recorded. A logistic regression and mixed model was used to examine differences in pregnancy data after transfer of fresh *in vivo* versus frozen-thawed IVP embryos; differences in the sex-ratio of foals was examined using Fisher's exact test. The likelihood of pregnancy and foaling ($P < 0.001$) was 56% and 43% for frozen-thawed IVP embryos and

77% and 64% for fresh *in vivo* embryos. Neither the likelihood of pregnancy loss nor gestation length differed significantly between the groups. The incidence of multiple pregnancy after transfer of a single *in vivo* or IVP embryo was 0% and 1.6%, respectively. Unexpectedly, the sex ratio (colt:filly) of foals differed between IVP (2.5:1) and *in vivo* (1.2:1) embryos; i.e. the percentage of colts was significantly higher after transfer of IVP (72%) than *in vivo* embryos (56%). Within frozen-thawed IVP embryos, the rate of *in vitro* embryo development had an impact on foal production. Even though the likelihood of pregnancy was not significantly influenced by the ICSI to blastocyst formation interval, pregnancy losses tended to be higher for day 8 (29%) than day 7 (15%) IVP embryos, resulting in a significantly lower likelihood of a live foal after transfer of a day 8 (38%) than a day 7 (52%) blastocyst. Finally, the speed of blastocyst formation for IVP embryos appears to be sex-dependent because the sex ratio (colt:filly) of foals was 4.1:1 for day 7 ($n=51$) compared to 1.8:1 for day 8 blastocysts ($n=62$). To conclude, transfer of frozen-thawed IVP embryos results in a likelihood of foaling of approximately 45%, favours the production of colts, and occasionally results in a monozygotic multiple pregnancy. The rate of *in vitro* development affects the likelihood of foaling and may be sex-dependent.