



## Mare and Stallion Effects on Blastocyst Production in a Commercial OPU-ICSI Program

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In recent years, *in vitro* production of horse embryos by ovum pick-up (OPU) and intracytoplasmic sperm injection (ICSI) followed by culture to the blastocyst stage has become increasingly widespread in sport-horse breeding. However, while a mean of 1 blastocyst per OPU-ICSI cycle is achievable, there is considerable variation in success between treatment cycles. This study retrospectively examined the influence of mare and stallion on the success of OPU-ICSI, in terms of blastocyst production per treatment cycle. Between September 2014 and November 2017, 564 OPU sessions were performed on 349 privately-owned Warmblood mares. After collection, oocytes were shipped overnight in modified HEPES-buffered SOF medium at 22°C, for maturation and ICSI at a separate location. After arrival, the oocytes were matured for 24–28h and resulting MII oocytes were selected for ICSI. Following ICSI, embryos were cultured in SOF-IVC medium that was refreshed on days 4 and 6 of culture. Blastocyst formation was assessed on days 6–8. To examine the impact of the mare on blastocyst production, data for mares (n=70) on which OPU-ICSI was performed 3 or more times was analyzed. To examine the stallion effect, data was examined for animals (n=18) used for more than 5 ICSI sessions; in the latter analysis, only the first OPU-ICSI cycle for any given mare was included, to minimize confounding. Overall, 544 blastocysts were produced (i.e. 0.96 per

OPU). In the mares submitted for  $\geq 3$  OPU sessions, the mean number of blastocysts per session was 1.04; however, the per mare mean ranged from 0 to 3.3. With regard to blastocyst yield per injected oocyte, the per OPU-ICSI cycle mean for these mares was 13% but, as above, the range was large (0 to 41%). Moreover, in mares that yielded a blastocyst during the first OPU-ICSI cycle there was a 70% likelihood of success during subsequent attempts; conversely, when the first cycle was unsuccessful, the likelihood of failure (no embryo) in subsequent cycles was 69.5%. Age did not differ significantly between mares that yielded good or poor results. While there were also considerable between-stallion differences, with blastocyst yield per injected oocyte ranging from 4–27% (mean 12%) most stallions (16 of 18) clustered between 6 and 19%, and the number of blastocysts per OPU (mean 0.89; range 0.2–1.56) was less variable than among mares. However, an added complication when examining the stallion effect is that different straws of semen from the same stallion can yield very different success rates. In conclusion, while both mare and stallion influence the success of OPU-ICSI, mare identity appears to be the most reliable predictor of success; indeed, mares that yield high blastocyst percentages per OPU / injected oocyte did so irrespective of stallion used; for mares that did not yield blastocysts, changing stallion was not a reliable solution.