Using a Time-lapse System to Examine the Association between Morphokinetic Patterns and Bovine Embryo Gender

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Determining the gender of a preimplantation embryo is often a challenging procedure. Although pregnancy diagnostics are used for human embryos, these procedures have not been utilized in domestic animal reproductive management. In cattle, shifting the offspring gender ratio (50:50) might have high economic merit because it reduces the proportion of unwanted genders. Sex-sorted semen can be used to increase the desired embryo gender; however, it was found to reduce the pregnancy rate by 5-8%. Therefore, other approaches are required. The morphokinetics of the developing embryo have been used to assess its quality. Here, we examined whether the morphokinetic parameters throughout the early developmental stages are associated with the gender of the embryo.

Two sets of experiments were conducted using an *in-vitro* model of the production of bovine embryos. In the first set, cumulus oocyte complexes (COCs; n=416) were aspirated from ovaries collected at a local abattoir, and *in-vitro* matured for 22 h. COCs were fertilized by using Y- or X-sorted semen (Semex, Canada). Then, embryos were individually cultured in an incubator equipped with a time-lapse system (TLS) for ~190 h and the embryo morphokinetic parameters were monitored continuously. Blastocysts (n=25) were collected for gender validation; DNA was extracted (High Pure PCR Template Preparation Kit, Roche, Austria) from each single embryo, followed by polymerase chain reaction (PCR) using a Y-chromosome gene (TSPY and BOV97M). The proportion of oocytes that cleaved (74.1±7.3 vs. 70.8±7.1%; P=0.7) and those that developed to blastocysts did not differ between the Y- vs. X-sorted groups, respectively. Of the kinetic parameters, the time from fertilization to the 8-cell stage and to the morula stage was shorter for the Y- vs. the X-sorted group (87.5±4.9 vs. 107.5±4.5 hpf; P=0.02 and 119.3±1.9 vs. 129.6±5.1 hpf; P=0.03, respectively). Moreover, the time from fertilization to the early blastocyst stage tended (P=0.06) to be shorter in the Y-sorted group. No difference was found in the morphology of the embryos that were derived from the Y- vs. the X-sorted semen, throughout all the embryonic

developmental stages. Note that only the gender-validated blastocysts were analyzed, suggesting that male embryos differ from female embryos in their kinetics rather than in their morphological parameters. This understanding was taken into account in the second part.

In the second set, *in-vitro* matured COCs (n=466) were fertilized with non-sorted semen and cultured in an incubator equipped with TLS for ~190h. The morphokinetic parameters were evaluated continuously and blastocysts (n=112) were collected for gender validation based on DNA extraction followed by PCR. Multiple discriminant analysis (JMP 17 software) was conducted by combining kinetic parameters as covariates in accordance with PCR validation. By using the kinetic parameters, i.e., those parameters found to differ between genders in the first experiment, it was possible to predict that 59.7% of the embryos would be male and 49% would be female. Using a single kinetic parameter as a covariate, the time from fertilization to the 8-cell stage revealed a probability of 64.5% to predict a male embryo, whereas the time from fertilization to the early blastocyst stage revealed a probability of 60% to predict a female embryo.

We concluded that kinetic parameters can be used to select a preferred gender of *in-vitro*-derived embryos before transferring them. However, this selection might shift to some extent the offspring gender ratio without affecting the pregnancy rate.