Morphokinetics as a Tool to Identify Embryos with Superior Ability to Survive Cryopreservation

Tatiane S. Maia[1]; Masroor Sagheer[1]; McKenzie L. J. Haimon[1]; Dorit Kalo[2], Zvi Roth[2]; Peter J. Hansen[1]

[1]Department of Animal Sciences, University of Florida, Gainesville, United States
[2]Department of Animal Science, Faculty of Agriculture, Food and Environmental Quality Sciences, The Hebrew University of Jerusalem, Rehovot, Israel

Currently, in vitro-produced embryos represent over 2/3 of the global production of bovine embryos. The number of transfers of cryopreserved embryos has been increasing. However, pregnancy per transfer is lower for cryopreserved embryos than for fresh embryos. The objective was to use morphokinetic endpoints of development to identify embryos with superior ability for survival to cryopreservation. Survival was determined post-thawing during 72 h of culture. Embryos were produced in vitro and individually cultured in a time-lapse incubator (Miri®TL6, Esco Medical) from ~ 17 h after fertilization until day 7.5. Images were captured every 5 min in different focal planes and a final video was obtained for each embryo. Several morphokinetic features were evaluated, including timing of cell divisions, size of blastomeres at the 2-cell stage (equal *vs* unequal), type of first cleavage (1-cell to 2-cell, 1-cell to 3-cell, and reverse cleavage), appearance at various stages (including paleness of cytoplasm and occurrence of extruded cells), blastocyst grade and stage as well as duration of the lag-phase in temporary cessation in development occurring between the 4 to 16-cell stage (77% of lag phases were initiated between the 5 and 8-cell stage). On day 7.5, blastocysts (n=132) were vitrified using a Cryolock device. Subsequently, each blastocyst was thawed and cultured individually in the Miri®TL6 and survival characteristics (re-expansion of the blastocoele and hatching from the zona pellucida) were evaluated at 24, 48 and 72 h of culture. Data were analyzed using chi-square test and logistic regression procedures of SAS. The overall percent of blastocysts that re-expanded at 24, 48 and 72 h was 59, 59 and 54%, respectively and the percent that hatched was 14, 33 and 38%, respectively. Morphokinetic indicators were largely similar for time points after thawing. Here data at 48 h post-thawing are reported. Among the determinants of whether an embryo survived cryopreservation, whether measured as the percent that re-expanded or that hatched, was timing of first cleavage, timing of formation of the compact morula, duration of the lag-phase and blastocyst stage and grade at the time of cryopreservation. For example, the time of first cleavage was 28.3±0.5 h for embryos that re-expanded *vs* 30.4±0.6 h for embryos that did not re-expand (P=0.004). The time of compact morula formation was 134.5±1.3 h for embryos that hatched *vs* 140.3±1.5 h for embryos that did not hatch (P=0.004). The lag-phase duration was 34.3±1.2 h for embryos that re-expanded *vs* 39.0±1.2 h for embryos that did not re-expand (P=0.016). Embryos that were expanded blastocysts at time of vitrification were more likely to re-expand (79%) than blastocysts (51%) or early blastocysts (26%) (P<0.0001). Blastocysts that were classified as grade 1 prior to vitrification were more likely to hatch as compared to embryos of other grades (53% vs 26%; P=0.012). Size of blastomeres at the 2-cell stage, type of first cleavage, embryo paleness and degree of blastomere extrusion were not related to cryosurvival. Results demonstrate that timing of development and blastocyst characteristics at vitrification are important variables associated with survival post-thawing. Machine learning approaches and development of an in vivo model to characterize survival may further improve the accuracy of

morphokinetic endpoints for identifying embryos with superior cryosurvival. (Support: BARD IS-5474-22).