Sperm Capacitation Status Correlation With porcine IVF Cleavage Rates

Isabel Rodriguez[1], Alexandra Keller[1], Lindsey Jennett[1], Megan Johnson [1], Ian Shofner[1], Tyler Weide[1], Kourtney Jimmerson[1], Bethany Redel[2], Karl Kerns[1]

[1]Department of Animal Science, Iowa State University, Ames, IA 5001, USA

[2]USDA-ARS, Plant Genetics Research Unit, Columbia, MO 65211, USA

Sperm capacitation is a key biochemical and physiological change spermatozoa must undergo before fertilization. Differing percentages of sperm can undergo this process, which requires minutes to hours to complete depending on species and sire. Distinct localization patterns of zinc ions (Zn^{2+}) have been identified across various species, denoted as zinc signatures, which correlate with capacitation status. In the boar, four zinc signatures have been discovered. Zinc signature 1 is indicative of a non-capacitated sperm cell with Zn^{2+} localized across the head and whole tail. Signature 2 reflects early stages of capacitation with Zn^{2+} uniformly found in the head and midpiece of tail. Signature 3 is associated with intermediate to late capacitation stages with Zn^{2+} localized exclusively in the tail's midpiece. Lastly, Signature 4 reflects a fully capacitated or dead sperm cell, distinguished by the absence of Zn^{2+}. This study aims to determine if there is a correlation between sperm capacitation status and prediction of *in vitro* embryo cleavage rate. Fresh sow ovaries were collected from a local harvest facility. Follicles ranging from 3-5mm in size were aspirated. Oocytes containing at least two layers of cumulus cells were selected and placed in TCM-199 based maturation medium containing 10 ng/mL of EGF & FSH and 0.5 µg/mL of LH and incubated at 38.5º C in 5% CO2. After 40-44 hours, cumulus cells were removed by pipetting with 0.1% (wt/vol) hyaluronidase for 1 minute. Oocytes with a visible polar body were selected and placed in 50 µL droplets of modified Tris-buffered medium (mTBM) and co-incubated with sperm for 4 hours from 9 boars. After fertilization, potential zygotes were placed in MU4 culture medium at 38.5º C in 5% O_2 and CO_2. Cleavage percentages were checked on day 4 after fertilization. Boar samples used for *in vitro* fertilization (IVF) were analyzed by image-based flow cytometry to observe the zinc signature state before (0 hour) and after incubation in mTBM (1 and 4 hours) to observe what proportion of sperm cells capacitated. Sperm cells were labeled with zinc indicator FluoZin-3 AM; acrosome remodeling indicator lectin PNA-AF647; and live/dead cell, plasma membrane-integrity reflecting DNA stain propidium iodide to observe precise capacitation states. Sperm motility was recorded using a computer-assisted sperm analysis system. Correlation coefficients were calculated by using Python. Features with strong positive correlations include sperm progressive motility after 1 hour, signature 3 at 4 hours, and difference in signature 2 population from 4 hour to 1 hour, and difference in plasma membrane integrity from 4 hour to 0 hour. Features with a strong negative correlation include total motility changes from 4 hour to 1 hour, and signature 3 at 4 hours. Further statistical analysis will be performed with ongoing IVF trials. Work done here can help provide livestock producers with tools to predict a sire's potential fertility and help aid human assisted reproduction technologies. This project was supported by Agriculture and Food

Research Initiative Competitive Grant no. 2022-67015-36298 from the USDA National Institute of Food and Agriculture and National Pork Board Award no. PR005493.