UNIVERSITY of GUELPH

ONTARIO VETERINARY COLLEGE
Department of Biomedical Sciences

ANNOUNCEMENT

Interested members of the University community are invited to attend the final oral examination for the degree of **Doctor of Philosophy** of:

Kayla Perkel

of the Department of Biomedical Sciences on Monday, August 24, 2015 at 1:00pm in OVC Room 1642 (Seminar); OVC Room 3648 (Examination)

Thesis Title:

Non-Invasive Assessment of In Vitro Derived Bovine Embryos of Different Developmental Rates Using Metabolomics

Examination Committee
Dr. Pavneesh Madan
Dr. George Harauz
Dr. Thomas Koch
Dr. Glen Pyle – Chair
Dr. Andrew J. Watson (Western) – External Examiner

Advisory Committee
Dr. Pavneesh Madan
Dr. George Harauz
Dr. W. Allan King

ABSTRACT

In recent decades infertility in cattle has increased dramatically. Reproductive failure in cattle frequently occurs within the first three weeks following insemination, termed early embryonic mortality, and accounts for 75-80% of all embryonic and fetal deaths. The most common and preferred method for evaluating an embryo's developmental potential in vitro is by ascertaining the cleavage rate coupled with morphological assessment, in spite of the inherent limitations and inaccuracies of these types of evaluations.

A recent endeavor in the industry is to find alternative technologies, such as the study of metabolomics, to identify embryos with the highest chance of developmental success. Advanced analytical techniques such as proton nuclear magnetic resonance (1H NMR) and direct flow injection liquid chromatography mass spectrometry/mass spectrometry (DFI-LC-MS/MS) allow the culture media of a developing embryo to be analyzed for the presence and depletion of metabolites. From this, the metabolomic fingerprints of embryos differentially growing can be analyzed for the potential presence of biomarkers and bio-patterns.

We hypothesize that embryos developing at different rates differ in their metabolomic signature. The objective of this study was to determine the metabolomic signatures of fast (FG) and slow (SG) growing embryos at timed stages of development using 1H NMR and DFI-LC-MS/MS. Aspirated oocytes from slaughterhouse ovaries were matured and fertilized in vitro following standard protocols. Presumptive zygotes were placed individually for culture in 40 □l synthetic oviductal fluid drops. Media from FG embryos was collected at 2-cell (30 hours post fertilization or hpf), 4-cell (42 hpf), 8-cell (49 hpf), 16-cell (90 hpf), morula (144 hpf) and blastocyst (168 hpf) and from SG embryos which were defined as the embryos needing additional 12- to 24 hr to reach equivalent embryo stage. Plain media samples served as control. Samples were analyzed by 1H NMR or DFI-LC-MS/MS. Multivariate analysis of data suggests distinct differences in metabolomic signatures between slow and fast growing embryos.

Results of the 1H NMR analysis showed production and consumption of metabolites like pyruvate, lactate, alanine, glutamate, valine, leucine and isoleucine differed between pre and post maternal to embryonic transition phases among SG and FG embryos. Results of the DFI-LC-MS/MS analysis demonstrated production and consumption of metabolites belonging to several classes such as amino acids, biogenic amines, acylcarnitines, sugars, glycerophospholipids, and sphingolipids.

To our knowledge, this is the first study of its kind to characterize the metabolomic changes in the culture media of in vitro produced SG and FG preimplantation bovine embryos using 1H NMR and DFI-LC-MS/MS techniques. The results provide a positive first step towards the use of metabolomics for the development of a non-invasive tool for assessing embryo viability and the discovery of potential embryo viability biomarkers.

BIOGRAPHY

Upon completion of a BSc in Biological Sciences and Microbiology at the University of Guelph in 2010, Kayla joined Dr. Pavneesh Madan's lab as a research assistant. Her interest in bovine in vitro production, coupled with her pursuit for a higher education, led her to start her Masters with Dr. Madan, studying metabolomics as a method for non-invasive embryo viability assessment. When the possibility of a PhD transfer was offered, Kayla knew this was an opportunity she could not refuse and happily accepted. Her interests in assisted reproductive technologies have stretched beyond bovine, as she is currently employed as a junior embryologist at KARMA IVF, in Kitchener, Ontario, where she successfully puts into practice all of the valuable skills learned during her time as a graduate student.

PUBLICATIONS

Perkel, K.J., Tscherner, A., Merrill, C., LaMarre, J., Madan, P. 2015. The ART of selecting the best embryo: A review of early embryonic mortality and bovine embryo viability assessment methods. *Molecular Reproduction and Development*. Accepted July 10, 2015. Article in Press.

Perkel, K.J., Madan, P. 2015. Spent media metabolomic signatures of preimplantation embryos cleaving at different rates. *Zygote.* Submitted July 16, 2015. Submission number: ZYG-2015-0076.

Regiani, T., Bertuccez Cordeiro, F., do Vale Teixeira da Costa, L., Salgueiro, J., Cardozo, K., Melechco Carvalho, V., **Perkel, K.J.**, Zylbersztejn, D.S., Pereira Cedenho, A., Lo Turco, E.G. 2015. Follicular fluid alterations in endometriosis: label-free proteomics by MS^E as a functional tool for endometriosis. *Systems Biology in Reproductive Medicine.* Accepted: Ahead of Print. Pages 1-14.

Bertuccez Cordeiro, F., Regiani, T., **Perkel, K.J.,** do Vale Teixeira da Costa, L., Rochetti, R.C., Stevanato, J., Eberlin, M.N., Zylbersztejn, D.S., Pereira Cedenho, A., Lo Turco, E. D. 2015. Lipidomics analysis of follicular fluid by ESI-MS reveals potential biomarkers for ovarian endometriosis. *Lipids.* Submitted March 9, 2015. Submission number: LIPIDS-15-0066.

Roepke, J., Jean, T., **Perkel, K.J.**, Blom, T., Bozzo, G.G. 2013. Daminozide alters anthocyanin metabolism in bronze chrysanthemum (*Chrysanthemum morifolium* Ramat.) flowers. *Journal of Plant Growth Regulation.* 32:453-460.