EXAMPLE OF AN APPLICATION FOR THEME 3:
TRANSFORMATIONAL TECHNOLOGIES AND INNOVATION

Subject: Understanding microRNA-mediated regulation of aquaporin expression to improve cryopreservation in bovine oocytes.

Summary:

Advanced selection, breeding and reproductive strategies facilitate the rapid improvement of critical production traits in domestic animals. Cryopreservation of animal gametes and embryos is one key element in many such strategies as it allows the widespread inexpensive dissemination of animals that possess optimal genetics for desirable traits. Unfortunately, the cryopreservation of oocytes and many embryo stages from cattle remains problematic, as even those preserved with the most current strategies (ie. vitrification) often fail to develop normally. Aquaporins are cell-surface proteins that appear critical in the successful cryopreservation of cells, embryos and oocytes. They are regulated by osmotic changes, although the underlying mechanisms remain largely unknown. We hypothesize that specific members of a small RNA family known as microRNAs mediate the osmotic regulation of aquaporins in oocytes and, ultimately, that the levels of these miRNAs can be manipulated to effect successful oocyte cryopreservation. Using advanced molecular genetic tools and techniques, we will examine the relationship between microRNAs and aquaporins to help understand their roles in cryotolerance in bovine oocytes and embryos. If successful, this work should accelerate the implementation of novel genetic improvement strategies to overcome existing production constraints in cattle.

Description:

The dairy and beef industries of OECD member states contribute billions of Euros annually to the global economy and provide millions of jobs. Furthermore, dairy products are essential sources of protein for a significant proportion of the global population, the price of which depends heavily on production efficiency and costs. Overall, productivity in these important agricultural sectors is very heavily dependent on genetic factors that influence feed efficiency, disease resistance, milk production, protein and fat content of meat and milk. Strategies to overcome productivity constraints in these areas are highly dependent on bovine fertility because optimal fertility facilitates the dissemination of diverse genetic stock with optimal productivity traits. The cryopreservation (freezing) and subsequent dispersal of gametes and embryos represents the most efficient and effective means of rapidly propagating genetically superior animals, particularly when coupled with other advanced breeding tools (genomic profiling, SNP-based selection etc.) aimed at improving productivity. Despite many recent improvements, successful cryopreservation of oocytes and specific embryo stages remains challenging, as a significant proportion of embryos derived from these procedures fail to establish successful pregnancies. Many such failures are believed to result from freezing-induced disruption of the function or regulation of key molecules on the cell surface. Among the most important of these are molecules known as aquaporins (AQPs), which control water movement between the cell and the external environment. Aquaporins regulate water balance in multiple cell types and several are expressed in mammalian gametes and embryos (Edashige, 2016). Recent studies have suggested that aquaporin regulation is critical for cryotolerance in gametes and embryos (Tan et al., 2015), yet surprisingly little is known about mechanisms that regulate these proteins in this context. The host laboratory has established a strong, longstanding research program in cryobiology and the cryopreservation of gametes and embryos. One recent focus of the host laboratory has been the osmotic regulation of aquaporin expression prior to cryopreservation of oocytes and embryos.

In general, the regulation of aquaporins and other important genes involves a complex interplay between gene transcription, and post-transcriptional mechanisms that change RNA processing, RNA stability and/or translation. In gametes and embryos, post-transcriptional mechanisms are particularly important, as the transcriptional activity is often low in key developmental "windows". At the molecular level, post-transcriptional control mechanisms often involve very specific interactions between small non-coding RNAs (ncRNAs) called microRNAs and complementary "target" sequences in mRNAs (such as those encoding AQPs) leading to gene silencing. Targeted molecular suppression of miRNA pathways in oocytes, sperm and developing embryos often leads to subfertility or infertility in model animals such as mice. Furthermore, osmotic changes such as those that confer cryoprotection to embryos are recognized to rapidly change the expression of multiple miRNAs in different organisms (Flynt et al., 2009). Surprisingly, despite the importance of both microRNA pathways in general biology and aquaporins in the context of embryo development, very little is known about their relationship and importance in bovine oocytes or embryos and how they influence, or become altered by, cryoprotective strategies such as osmotic shock, or by cryopreservation itself.

The general hypothesis of this research project is: Aquaporin expression is regulated by microRNAs in maturing bovine oocytes and early embryos. Osmotically-regulated changes in the levels of these microRNAs levels alters aquaporin levels and influences cryopreservation success. We will begin to address this hypothesis through the following specific objectives:

1. Characterize the levels of specific aquaporins and microRNAs that target them in mature oocytes and early embryos.

Aquaporins 3 and 7 (AQP3 and AQP7) are variably expressed at different stages of gamete maturation and embryo development and are known to change in response to alterations in the osmotic environment. These aquaporins have also been specifically implicated in cryotolerance for gametes and embryos (Edashige, 2016). Computational predictions using TargetScan (www.targetscan.org) suggest that miRNA-34 and -148 family members, both of which are expressed at some level in bovine oocytes and embryos (Gilchrist et al., 2016) specifically target these AQP messenger RNAs. In studies to be performed over the first 6 weeks of the fellowship, the candidate will isolate RNA from in vitro matured oocytes using published techniques (Tscherner et al., 2014) and determine the relative levels of AQP3, AQP7 and miRNA-34 and -148 families to establish temporal and developmental relationships between miRNAs and their targets. Once the detection strategies have been optimized, we will extend the observations to subsequent stages of embryo development (2, 4, 8 cell, morula, blastocyst). Although the expression of some aquaporins and some miRNAs have been evaluated independently in the embryo context, any relationships between the two have not previously been reported nor has a comprehensive evaluation of their expression levels across different stages. Based on previous studies, we are highly confident that we will be able to detect the appropriate miRNAs and aquaporin mRNA targets. This should allow us to identify any relevant relationships over the developmental stages examined and will establish a strong base upon which to explore these relationships in the context of osmotic changes and cryotolerance.

2. Determine the effects of osmotic changes on the expression of AQP3, AQP7 and the miRNAs that target these transcripts.

Aquaporins are known to participate in cryotolerance in mammalian oocytes (Tan et al., 2015, Edashige, 2016). In cell and animal models, they are rapidly influenced by osmotic changes (Sugiyama et al., 2001). In order to begin to determine whether the regulation of oocyte and early embryonic aquaporin expression is mediated by the specific miRNAs we are examining, we will next examine correlations between osmotically-induced changes in AQP3 and AQP7 expression and changes in miR-34 (a,b,c) and -148 (a,b). To this end, once the relative levels of expression of relevant miRNAs and their aquaporin target mRNAs have been established during normal bovine oocyte maturation, we will assess the effects of osmotic shock on the expression of these miRNAs and their AQP targets. These studies will be performed in weeks 6 – 12 of the fellowship. Stages examined will include the MII oocyte, 2-, 4- and 8-cell embryos as these are the stages where osmotically induced miRNA changes should cause alterations in the AQP3 and AQP7 targets. We will also examine expression in morula and blastocysts to evaluate longer-term changes that ensue from osmotic shock. Oocytes and developing embryos at the specific stages will be treated with TCM199-HEPES supplemented with 20% FCS (Holding Medium, HM) containing different levels (0.25 M, 0.5 M, 0.75 M or 1 M) of sucrose, 10% GLY, 8% EG, 9.5% DMSO for 20 min. Groups treated with HM alone will be used as controls. RNA will be isolated from the samples and subjected to quantitative RT-PCR for AQP and miRNA analysis as described in objective one. These established techniques for osmotic manipulation are routinely employed in the M. laboratory. Should we establish a stage or temporal relationship between osmotic regulation and miRNA expression, we will subsequently establish a functional link between the two processes by antagonizing specific miRNAs using complementary sequences that inhibit their activity in collaborative studies performed in Dr. L.'s laboratory after the completion of the fellowship. Further subsequent collaborative work will then investigate the effects of osmotically-induced, miRNA-mediated changes on the cryotolerance and development of bovine embryos after cryopreservation.

Expected results and dissemination plan: During this fellowship, if awarded, it is anticipated that we will successfully characterize the patterns of aquaporin-3 and -7 expression in normal and osmotically challenged oocytes and embryos and the relationship of this expression pattern to that of microRNAs with strong regulatory potential for these genes. This will represent a strong basis for further study into the roles that miRNAs might play, and how they could ultimately be manipulated, to optimize cryopreservation strategies. We anticipate that the results of this research project will be published in at least one manuscript to be submitted to a solid impact journal in animal reproduction such as Biology of Reproduction, Reproduction or Theriogenology. Furthermore, we anticipate presenting this work at international meetings and producer specific meetings in Europe and North America.

Complementarity of Laboratory Groups: Dr. T. M. at the AUB is a recognized expert in the cryopreservation of gametes and embryos of domestic agricultural animals. Her laboratory continues to investigate the development and implementation of optimal strategies for oocyte and embryo cryopreservation and is actively funded. Recent studies by her group have begun to explore different molecular aspects underlying success and failure of cryopreservation strategies, focusing on aquaporins. She has invited (see letter) Dr. L. to bring his expertise in bovine embryonic gene expression and small RNA biology to investigate the issues described in this proposal with her lab group during his sabbatical leave. Dr. L. directs an active, well-funded research group in the Department of Biomedical Sciences at the University of Guelph

exploring the expression and function of small RNAs in the gametes and embryos of domestic species. After the completion of the project described, the two groups will continue to work collaboratively to identify small RNA regulated pathways that influence embryo viability and cryotolerance. Ultimately the aim is to collaboratively develop optimal techniques through which effective cryopreservation approaches can be implemented as part of economically viable strategies to improve the genetic traits influencing animal productivity.

Novelty and Significance: Taken together, the proposed studies should substantially enhance our understanding of the molecular processes that regulate aquaporin expression in oocytes and the developing bovine embryo. Once identified, there is substantial potential to manipulate these pathways in order to effect improvement in cryopreservation strategies, thereby enhancing the dissemination of optimal genetic traits and overcoming existing constraints on production in cattle populations. If successful strategies are developed and implemented based on these studies, significant economic benefits are likely to accrue due to enhanced efficiency and accelerated rates of genetic improvement in dairy and beef cattle.

References

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Where did you learn about the Programme?

Previous applicant for Fellowship in 2000.