

2017 OECD Summary Fellowship Report

Jonathan LaMarre, Project JA00094164

Understanding microRNA-mediated regulation of aquaporin expression to improve
cryopreservation of bovine oocytes

Theme 3: Transformational Technologies and Innovation

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1. Importance of project, hypothesis and objectives.

Importance: The cryopreservation of animal gametes and embryos is a key element to advanced animal breeding strategies as it allows the widespread dissemination of optimal genetics for key production traits (milk, meat production, etc.). Cryopreservation of bovine oocytes remains challenging and previously-frozen oocytes often fail to develop into embryos after fertilization. Understanding the factors that regulate gene expression in oocytes before and after freezing will facilitate the development of strategies that improve cryotolerance in bovine oocytes.

Hypothesis and objectives: We hypothesized that oocyte cryotolerance is influenced by the expression of aquaporins, which can be regulated by microRNAs. The objectives of this project were to: 1) Characterize the levels of specific aquaporins and microRNAs that target them in mature oocytes and early embryos and 2) To determine whether microRNAs (-34 and -148), which target aquaporins -3 and -7, are altered after osmotic changes in the media. Both of these aims are ultimately directed at improving cryotolerance. Although not described in the original application, an additional aim directly relevant to the long term objective of improving cryopreservation was also addressed – assessment of the effect of “pre-conditioning” with gp130 family cytokines on bovine oocyte cryotolerance.

2. Achievement of Objectives.

Both primary objectives, and the additional objective developed during the fellowship stay, are on the way to being achieved. Conditions for optimal identification of aquaporin protein (immunohistochemistry, western blot) and mRNA (RT-PCR) were examined, discussed, initiated and demonstrated by a graduate student in Dr. Mogas’ laboratory during the fellowship. Aquaporins are expressed in bovine oocytes and appear to demonstrate different levels between samples. These studies required multiple iterations and various modifications of techniques that have now been successfully employed. The current plan is for future collaborative studies between Dr. Mogas’ and Dr. LaMarre’s research teams to complete these experiments over the next year. Optimal small RNA (miR-34 and miR-148) isolation and analysis techniques have been developed and the techniques required to subject oocytes to osmotic stress have now been effectively transferred to Dr. LaMarre’s group in order to allow subsequent collaborative experiments on the effects of osmotic stress on specific miRNA and target (aquaporin) expression.

A third objective, in which the effects of gp130 cytokine treatment on cryotolerance were evaluated, was developed after discussions between the Drs. Mogas and LaMarre, along with a senior Ph.D. student. A series of initial studies suggests that strategic treatment with cytokines from this family during oocyte maturation significantly improves bovine oocyte cryotolerance. Due to the high possibility of immediate impact on oocyte cryopreservation, this is now the central focus of a collaborative research project between our laboratories.

Major Accomplishments:

- 1) Demonstration of induction of cryotolerance in oocytes by gp130 cytokines and the development of an ongoing collaboration based on this project.
- 2) Establishment of the optimal protocols to identify the protective cellular changes (miRNA and gene expression) induced in oocytes by cytokine treatment or extracellular (eg. osmotic) stress.
- 3) Knowledge transfer of culture molecular and cryopreservation techniques between the host and fellow’s laboratories and the implementation of a long term collaboration plan to continue the relationship between the groups.

Follow-up work.

Upon completion of the follow-up experiments planned over the next 6-12 months, at least one, and possibly several collaborative publications in peer-reviewed journals are envisioned from the studies described. As described in the results, this work has already led to follow-up collaboration between the two research groups involved, and in fact, has extended to an additional potential collaboration in Spain. If the preliminary findings described are confirmed and extended, it is likely that the use of “preconditioning” by cytokine treatment or osmotic shock will be incorporated as a routine component of cryopreservation protocols employed during oocyte freezing.

Potential impact of research results on national/international agro-food practices

As described in the preceding sections, this fellowship has allowed us to generate preliminary data and has facilitated an ongoing international collaboration between groups seeking to improve the cryopreservation of bovine oocytes based on a deeper understanding of the molecular pathways that influence cryopreservation outcomes. If successful, our collaborative studies should substantially improve the practice of oocyte preservation through freezing. This, in turn, would allow more widespread incorporation of bovine oocyte cryopreservation into breeding strategies that optimize productivity, disease resistance and dissemination of optimal genetics in a manner similar to that achieved with frozen semen in the past. This would have obvious benefits for national and international agro-food practices where genetic improvement of livestock can enhance productivity, decreasing costs and prices and increasing product accessibility.

Relevance of research to OECD Cooperative Research Program (CRP) objectives and themes

The primary overall objective of the CRP is to “strengthen scientific knowledge and provide relevant scientific information and advice that will inform future policy decisions related to the sustainable use of natural resources, in the areas of food and agriculture”. OECD support through the Fellowship program has allowed us to generate data and an ongoing collaboration that should ultimately inform agricultural practices in the area of breeding strategies to improve livestock genetics. Improved success in the cryopreservation of bovine oocytes would markedly accelerate the dissemination of optimal genetics that can be “tailored” to specific climatic, geographical or societal requirements for production. Policies could then be developed based in part on this knowledge that ultimately improve the success and economics of agricultural practices such as cattle breeding for improved production of milk and meat.

The research theme encompassing the studies conducted during this fellowship is Theme 3: Transformational Technologies and Innovation. Using advanced molecular concepts and techniques we have performed experiments and initiated a collaborative research program aimed at enhancing bovine breeding and genetics through the improvement of a technology (cryopreservation) that is presently limited and highly inefficient. This clearly falls under the descriptor: “Advanced breeding tools- genetics and genomic technologies”.

Satisfaction

Both the OECD Fellow and the host laboratory were highly satisfied with the fellowship. The administrative support was outstanding – highly professional and extremely helpful in the preparation and administration of the application and award. The objectives and expectations were clear and the requirements specific and well-articulated. Consultation with the Scientific Advisory Member for the relevant Theme (3) was also extremely helpful and informative. The Fellow and host are deeply appreciative of the award, which has opened up new international research opportunities and collaborative possibilities that would not have existed otherwise. The work will further expand the career opportunities of the Fellow by increasing his international understanding and exposure in this economically and socially important field. Absolutely no

practical problems were encountered and I can honestly offer no suggestions for program improvement as every aspect worked flawlessly from my perspective.

Advertising and Publicity

I was aware of the Co-operative Research Programme as a previous Fellowship holder (16 years ago). I also encountered several announcements in an internet search for international collaborative research support in agriculture. It is my impression that the programme is well-recognized and publicized internationally and that most eligible scientists in my field are aware of it.