Fellowship Summary Report

OECD Co-operative Research Programme: Sustainable Agricultural and Food Systems

Awardee: Christopher Grupen

Host institution: University of Murcia, Spain

Name of host collaborator: Dr Raquel Romar Andrés

Dates of fellowship: 24 June to 4 November 2022

Project title: "How do maternal reproductive biofluids affect the epigenome of developing embryos?"

Consent statement:
I, Christopher Grupen, consent to my report being posted on the Co-operative Research Programme's website.

1. What were the objectives of the research project? Why is the research project important?

   The specific objectives of the project were to determine the impacts that different maternal reproductive biofluids have on epigenetic reprogramming in porcine embryos produced in vitro.

   Over the past 40 years, approximately 9 million babies have been born worldwide through the clinical use of assisted reproductive technologies such as in vitro fertilisation. In the livestock industries, over half a million animals are produced annually worldwide using similar in vitro methods, and this number is increasing steadily every year. The viability of the embryos produced, and the health of the resulting offspring are known to be influenced by various factors that the gametes (eggs and sperm) and embryos are exposed to during their incubation. One of the developmental processes now believed to be significantly impacted by the early environment is "epigenetic reprogramming", which is required for correct gene expression in developing cells. Therefore, it is important to better understand how epigenetic reprogramming is altered in in vitro produced embryos. This project used the pig as a model species to study the early development of in vitro produced embryos. Groups of pig embryos were produced using either a completely defined in vitro system (artificial) or a system supplemented with sow reproductive tract biofluids to more closely resemble the maternal environment (natural).

2. Were the objectives of the fellowship achieved?
   - Or are they on the way to being achieved?
   - If not, for what reasons?

   Work towards achieving the objectives was successfully carried out, with new data generated in experiments that focused on the first steps of embryo in vitro production, namely oocyte (egg) in vitro maturation and in vitro fertilisation. The effects of supplementing the medium with follicular fluid were examined, with different aspects of oocyte quality, sperm penetration and embryo development assessed. Due to the extreme summer heat, access to abattoir-sourced ovaries for oocyte collection was limited, which prevented completion of the additional planned experiments. To help achieve the objectives, data generated previously by the Spanish group was used for epigenetic analysis. Analysis of this data is ongoing. Also, embryos generated from the experiments undertaken during the fellowship are in liquid nitrogen storage for further analyses.

3. What were the major achievements of the fellowship? (up to three)

The major achievements of the fellowship were:

- A/Prof Grupen formed a strong research relationship with the host researcher, A/Prof Romar, and other members of the Spanish group, including Prof Coy and Prof Gadea, facilitating continuation of the fellowship project and creating a solid platform from which to develop future projects.
- The results of experiments completed during the fellowship have revealed differences in the quality of embryos produced in vitro between the "artificial" and "natural" systems.
- A/Prof Grupen gained the skills needed to perform epigenetic analyses in oocytes and embryos.

4. Will there be any follow-up work?
   - Is a publication envisaged? Will this be in a journal or a publication? When will it appear?
   - Is your fellowship likely to be the start of collaboration between your home institution and your host?
   - Is your research likely to result in protected intellectual property, novel products or processes?

   The exchange of knowledge between the Sydney and Murcia researchers facilitated the establishment of a model system to produce groups of pig embryos in vitro under "artificial" and "natural" conditions. Embryos produced in experiments carried out during the fellowship have been stored in liquid nitrogen for further analyses. Follow-up experiments are planned in both the Sydney and Murcia laboratories with the intention of producing a journal article for publication. Recruitment of a research student for the follow-up work will make it possible for the publication to appear in late 2023. Both research groups are keen to further exploit the model system of artificial and natural embryo in vitro production and develop a long-term collaboration. This research is likely to result in intellectual property that involves the preparation and refinement of media formulations for embryo in vitro production in livestock species.

5. How might the results of your research project be important for helping develop regional, national or international agro-food, fisheries or forestry policies and, or practices, or be beneficial for society?

   The results of this research project will inform future improvements to embryo in vitro production systems in pigs and other livestock species. Increasing the efficiencies with which these systems generate viable, healthy embryos will promote their use in advanced breeding programs for genetic improvement. Ultimately, such genetic improvement schemes will benefit society through enhanced production in the animal industries and greater food security. In addition, the in vitro production of embryos with superior viability will improve the effectiveness of associated reproductive technologies, such as embryo cryopreservation, which is an essential part of programs that preserve the genetics of valuable and unique animals. Highly contagious viral diseases such as African Swine Fever and Food and Mouth Disease present major risks to livestock production worldwide, and effective gene banking programs are critical to ensure that breeding herds can be restored after a catastrophic disease outbreak. Likewise, improving the in vitro production of livestock embryos will assist efforts to generate genome edited animals. If such animals are approved for food production, there is the potential to improve animal health, welfare, and performance, as well as reduce the environmental footprint of production.

6. How was this research relevant to:
   - The objectives of the CRP?
   - The CRP research theme?

The in vitro production and cryopreservation of embryos is integral to advanced breeding programs of livestock species. These reproductive technologies facilitate genetic improvement to increase production efficiency and enable the banking of elite genetics to provide security against catastrophic losses from infectious disease outbreaks. The fellowship supported research to examine the impact of in vitro conditions on pig embryo viability, with the aim of increasing the capacity of in vitro production systems to yield healthy embryos. Hence, the fellowship research is relevant to the CRP objective of biosecurity preparedness in livestock production within the theme of "Managing Risks in a Connected World". The ability to produce healthier embryos in vitro is also important for advancing gene editing efforts in livestock species. Therefore, the fellowship research is also relevant to the CRP objective of developing new opportunities for animal improvement using advanced breeding tools/genetic and genomic technologies within the theme of "Transformational Technologies and Innovation".

7. Satisfaction
   - Did your fellowship conform to your expectations?
   - Will the OECD Co-operative Research Programme fellowship increase directly or indirectly your career opportunities? Please specify.
   - Did you encounter any practical problems?
   - Please suggest any improvements in the Fellowship Programme.

   The fellowship exceeded my expectations, as I developed an excellent research relationship with not only the host researcher, but also other senior researchers in her department. These research relationships will advance my career opportunities directly through the on-going experiments of the proposed fellowship project and more indirectly through the development and realisation of future projects.

   The main problem faced during the fellowship involved the accessibility of the abattoir-sourced material (pig ovaries) required for the project work. As collection of this material was limited by the extremely hot weather, nothing could be done to resolve this issue. Rescheduling the fellowship to the cooler months would have been the only way to avoid this problem. Also, while the host researcher and other members of the host group were extremely welcoming and helpful throughout the fellowship, their irregular availability due to busy work schedules and life events constrained the desired progress. Fortunately, I could seek the assistance of several senior researchers for different aspects of the project work. Therefore, I suggest that the Fellowship Programme could be improved by requesting the host researcher nominate a "reserve" host researcher who would be willing to assist with the project if needed.

8. Advertising the Co-operative Research Programme
   - How did you learn about the Co-operative Research Programme?
   - What would you suggest to make it more "visible"?
   - Are there any issues you would like to record?

   I learnt about the Co-operative Research Programme from a "Researcher Weekly" newsletter disseminated via email by the University of Sydney's Faculty of Science. The newsletter displayed a brief description of the fellowship scheme and a link to the CRP page on the OECD website. I believe this is an effective way of making the CRP visible, so the national correspondents should communicate with the research offices of their country's universities and institutes to ensure the fellowship scheme is advertised through their internal newsletters. Those who have recently completed a fellowship could also help to disseminate the CRP information via a short YouTube testimonial, targeting researchers of the Research Fellow's country.

One issue I experienced was a lack of support from the host institute's International Mobility Office. They didn't help me to obtain accommodation or give me any information about the visa requirements for my country. Whilst I received a small "welcome pack" on my arrival that had some local information, I would have appreciated a check on my welfare one or two weeks after arrival and a follow-up check mid-way through the fellowship. Fortunately, my host researcher and others in her group were always willing to help me and ensured my stay in Murcia was a wonderful experience.