Royal Veterinary College
University of London

Summary Minutes: AWERB meeting

Status: Chair approved

Meeting held: 26 January at 2pm by MS Teams

Present
Attendees: 8 plus 2 in attendance, 5 by invitation and 7 apologies.

1 **PRESENTATION ON APPLICATION FOR A NEW PROJECT LICENCE:**

An application for secondary availability at the RVC had been received for review. The aim of the project licence was to understand how cells choose and maintain specific fates during development and in the adult animal in specific biological systems, notably the gonads, pituitary and sexually differentiated tissues and how this leads to disease when the processes goes wrong. This work was important as during the development of an animal, cells have to undergo decisions of cell fate, choosing which path to follow. Similar decisions are made throughout life by stem cells and progenitors in many tissues. These decisions rely on intrinsic factors, such as transcription factors, and extrinsic signals, which together established gene regulatory networks that defined specific cell states. These have to be coordinated in time and space to generate functional tissues, organs and the animal, where little in the latter is static: cells constantly have to be replaced due to normal wear and tear and to cope with changing physiological states, trauma, and disease.

The main purpose of the work to be conducted was to provide fundamental knowledge on cell fate decisions in specific biological systems, notably the gonads, pituitary and sexually differentiated somatic tissues. They would focus on understanding how the gonad develops, matures, ages normally and its communication/interaction with the hypothalamus and pituitary gland to regulate reproductive activity and will assess the importance of sex chromosomes and sex hormones in the differentiation of secondary sexual characteristics.

Many situations where decisions of cell fate were aberrant or discordant could lead to disorders of sexual development (DSD). These could be associated with variation in genes, developmental programming and hormones and result in a large spectrum of congenital disorders often leading to infertility. Moreover some of these conditions were at increased risk for the development of cancer. Some were syndromic and affected multiple organs. There could also be aberrant responses to environmental factors. Improved understanding of underlying mechanisms could lead to improved diagnosis and/or novel forms of treatment.

The following queries/comments were raised by AWERB:

- The project licence was unclear about which protocols were being done at the RVC. The licence should be amended to make it clear why they were applying for secondary availability and the work that would be done here.
- Further detail was needed about how the chicks would be transported to the College. What precautions would be taken to ensure that animal welfare was not affected?

- The licence needed to include information about the defined points of care.
- One of the aims of the licence was to generate new lines. Had there been any general welfare scoring developed which might help when developing new models? Nothing had been developed so far though.
- Feedback from the lay panel members had expressed concern about the NTS saying it was too broad and complex and difficult to follow and they did not fully understand the project aims. This needed to be revised to make it more lay person friendly.
- More information was requested on the experimental design, as it would be useful to have examples embedded into the project licence. An example power calculation and EDA diagram was needed that the AWERB could review.
- The application lacked detailed description of the procedures which would be carried out during the next 5 years. The project was not focused around specific genes or pathways. The stage of development had not been specified and when the gene modification or editing would be carried out as the investigators had left it open to any new discoveries.
- There was already a lot of knowledge in the field, therefore, it would be helpful if the investigators were more specific about the novelty of the work.
- Considering the fundamental differences in the regulation of sex differentiation in the lower vertebrates which was controlled by oestrogen, as compared to human and other mammals, the investigators have provided a lot of text to emphasize the potential benefits of the project in human medicine. It was not clear how much of the data so far has been translated to clinical application in human?

The researcher was thanked for attending the AWERB meeting. A summary list of the queries raised by AWERB would be sent to her. Once the revised project licence had been received, it would be evaluated and either more information requested or the application approved for submission to the Home Office.

Once the researcher had left the meeting the following queries were raised:

- It was noted that one of the changes in phenotype related to weight loss but no details given of the percentage of animals this was expected in.
- There was uncertainty about the new lines and the potential side effects. The current wording was very broad and open to interpretation. More information and definition was needed on this area as well as information on the welfare score sheets and assessment sheets.
- It was noted that the primary establishment's AWERB were due to review this project licence in a couple of days. Usually project licence reviews involving secondary availability were carried out consecutively by AWERBs rather than simultaneously as had happened in this case. A question was asked whether AWERB's comments could be shared with the other AWERB. Was that permissible? AWERB confirmed that they were supportive of their comments being shared so long as the Establishment Licence Holder was happy with this.

2 UPDATE ON CAMDEN PONIES:

A briefing note had been provided to the meeting on the use of Camden teaching ponies, including a summary of their revised planned use during Term 2 (January to March 2021). As a result of two student cohorts' teaching being moved entirely online, the use of the animals would only be for 10 x 90-minute live anatomy sessions (reduced from 27 x 90-minute live anatomy teaching sessions). The pair of ponies would therefore now only be in Camden for a week rather than just over a month.

There had been two pony pairs that rotated between Camden and Hawkshead, however following behavioural changes in one of the ponies during the previous term, as she no longer tolerated her hind limbs being examined, AWERB had recommended at their previous meeting that she be replaced so that she could be retired from the anatomy demonstrations. This therefore meant that

currently there were only 3 ponies available. Permission was therefore being sought that the other pair of ponies be used at Camden for both Term 2 and Term 3, as they would only be in Camden for a short time for Term 2. AWERB confirmed that so long as the ponies did not seem distressed then in the circumstances they were happy to approve this. If, however, they showed any signs of stress, or if there was any deepening of lockdown, or there was a lack of BSU staffing due to sickness, then the ponies should be immediately transported back to Hawkshead.

Equine would be approached for advice on sourcing a new pony to replace the one being retired.

3 PRESENTATION ON APPLICATION FOR A NEW PROJECT LICENCE

An application for a new project licence had been received. The primary aim of the work was to develop new gene therapy vectors to treat common kidney diseases to prevent deaths and other major health care issues associated with acute and chronic kidney disease, in particular Nephrotic syndrome. This is a condition that caused kidneys to leak large amounts of protein into the urine, so leading to a range of problems, including kidney failure. It primarily affects young children. Along with a research colleague, a gene therapy in mice with a genetic form of FSGS had been developed that demonstrated that this could rescue the disease. There were no effective medical treatments for patients with genetic FSGS (with the rare exceptions), and these children commonly progress to end stage renal disease (ESRD), requiring dialysis and transplant. ESRD and dialysis was associated with significant cardiovascular morbidity long-term and greatly shortened life-span. Transplants have an average lifespan of about 15 years, and each successive transplant was more challenging as the patient was sensitised to antigens from previous transplants. As such, there was an unmet need for medical therapies for genetic FSGS.

AWERB discussed the proposed animal models. This included well established and characterized mouse models of human kidney disease to enable the optimal experiments to be designed, reducing the number of animals required and reducing pain, suffering and distress as the timeline of disease development was already known.

There were several comments/queries raised by AWERB including:

- There were several “copy and paste” errors that needed to be corrected
- Discussions were needed on an acceptable animal welfare scoring sheet to be used. Ideally a particular make of scoring sheet should not be specified as this could then limit how the animals were scored. Instead it was better to use an appropriate welfare assessment and give an idea of what was going to be assessed.
- As there was uncertainty about whether one of the proposed animal models would be used it was agreed that this model should be removed from the project licence for now.
- Concern was raised about the proposal to use tail tipping as a method of sampling. This method should not be used and was not encouraged. This method would be removed as there were alternative methods that could be used.
- The sections on using randomisation and blinding needed to be rewritten. It referred to the double blinding of the researcher and the double blinding of the technician, so it was not clear who was blinded for which.
- A query was raised about how regularly the blood samples would be collected for monitoring. A blood sample would be taken once protein in the urine was started to be seen followed by one to two more before the mouse was culled and then one for final measurement. It was suggested that the maximum volume and frequency should be explained in the project licence so that the Home Office Inspector was clear what the maximum number of samples would be. This was queried though as project licence

guidelines now advised to avoid specifying volumes and frequencies when there was no impact on animal welfare. It was argued however that when reviewing a project licence it was difficult for the reviewers to determine whether the sampling was going to impact on welfare, so the maximum should be provided. It was possible the change to the guidance had been made to avoid a potential breach of a licence if a researcher took one too many samples when they did not need to, but this did not have any impact on animal welfare. It was agreed that this should be raised with the Home Office Inspector generally for his advice. In the interim it was suggested that this information should be included in the animal experience section explaining that this was when expecting to take the blood samples and the reasons why and how many, rather than as part of a protocol step. It was good for AWERB to ask the question about the frequency but that it did not necessarily need to be included in the licence

- The estimated number of animals did not tally with the numbers specified in the protocols.
- Had alternatives to Freund Adjuvant been considered? There had been discussion with kidney experts and investors over the optimal models to use and which were the accepted models. There were a lot of animal models but a lot were not accepted as model of human disease, which was needed. For every stage they had tried to go for models that were already out there and were well characterised and well designed so that an initial set up period was not required to bring animals in that required a lot of ground work as the model was not fully understood. Most of the models being used were either commercially available and well established or they had in house already as they had designed them or were from collaborators who had already done the clinical history. It was suggested that for those coming in from collaborators a pilot study should be done to confirm the natural history of the disease. This could be done but would mean more animals being used at the beginning. It was recognised that it was a balancing act between using more animals initially to validate the model, but in the long run it should result in numbers being reduced.
- It was suggested in relation to refinements, that Labsand be used for the urine collection instead of metabolic cages. This was agreed.

The project licence holder was thanked for attending the meeting and answering the questions. There were a few changes that needed to be made. Once the revised project licence had been received it would be reviewed again and a decision made.

4 NVS REPORT: SUMMARY ITEMS

4.1 Ring tail lesions:

The group that had experienced ring tail lesions in their animals, had recently received a new batch of animals from their supplier, which already had signs of ring tail. This confirmed that the supplier was therefore the source of this problem. An investigation was being carried out and the results would be provided to AWERB. It was concerning that the supplier was sending out animals that were not up to specification.

4.2 BMS

The values in the system were still out of range. This had been raised again at a recent meeting with Estates. A regular maintenance schedule for the air handling units was needed.

4.3 Cardiac specialist talk

The cardiac specialist had recorded a talk for the technicians. A copy would be circulated to AWERB.

5 PROJECT LICENCES

AWERB noted that 3 new project licences had been granted by the Home Office and 3 licences amended.

6 REMAINING ITEMS ON THE AGENDA

As the meeting had now run out of time, it was agreed that the following items would be circulated as reports. Each AWERB member would provide e-mail confirmation that they had seen the reports and were happy with them:

- 3Rs
- NACWO report
- NVS report
- Mid term review.

7 ANY OTHER BUSINESS:

7.1 Date of next meeting: This was scheduled for 23 February 2021.

Secretary
1 February 2021