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**Minutes:** AWERB (PPL review meeting)

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**Status:** Chair approved

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**Meeting held:** 23 March 2021 at 2pm via MS Teams

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**Present**

Attendees: 11 plus 2 in attendance, 1 by invitation and 5 apologies

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**1 PROJECT LICENCE AMENDMENT REVIEW**

The project licence holder was welcomed to the meeting. The PPLH wanted to make two amendments to the licence:

- **Inclusion of a new protocol to enable them to spay dogs during oestrus in order to facilitate the IVF programme.**

Carrier females were already routinely spayed before they were rehomed. This was done under the Veterinary Surgeons Act (VSA), as it was not considered a regulated procedure, for it was required so that the dogs could be rehomed.

There was however now a need to spay dogs during oestrus. The research team were conducting an in vitro fertilisation (IVF) programme and they needed to have the oocyte from the carrier females in as mature a state as possible. As dogs have an unusual reproductive physiology that was different to other mammals their oocytes only go through the final stages of meiosis during oestrus so it meant they were much more easily fertilised at that stage. No one had achieved fertilisation of oocytes that have been harvested from ovaries obtained during anoestrus. It was conventional in the UK not to spay dogs during oestrus, as there were perceived increased risks associated with doing this such as a possibility of false pregnancy; as well as an ovariectomy procedure being potentially considered more of a surgical risk. However so long as appropriate surgical precautions were followed and a skilled veterinary surgeon was undertaking the procedure, that surgical risk should be minimal. The PPLH also advised that although they were not aware of any specific research that had examined these perceived risks, following advice from a canine reproductive specialist, they were considering using a prolactin antagonist (cabergoline) that could be administered on feed to prevent false pregnancy. They would be taking advice from their specialist through all of this.

Discussions had been held with the Home Office Inspector about whether this spaying would also be classed as recognised veterinary practice (RVP). The HOI had sought advice from colleagues and they had decided that it needed to be added to the project licence as an additional protocol as it was not conventional to spay dogs during oestrus, so could not be considered RVP. The spaying under a PPL, could be authorised, as there was a clear scientific purpose to generate usable eggs for IVF to breed GMN dogs, so helping to cover both the short term optimisation of the IVF procedure and then the longer term sourcing of eggs for IVF in the justification for a new fairly simple protocol.

The following queries were raised:

- **How was the sex/sperm sorting progressing?**  
They had achieved sex sorting of semen. This was being done with a commercial company, who were hoping to use the techniques for commercial purposes. They had achieved good sperm sorting for X & Y bearing sperm. The plan was to see if they could initially get matured fertilised blastocyst embryos, which if they could, would then be biopsied to confirm the sex as male and to also test them for mutation so confirming both male and DMD. This was still a little way off though but was the next aim.
- **What was the plan for implantation of these blastocysts?**  
The plan was to freeze the embryos using a vitrification process, which was standard in IVF technology. It was expected that the process would be the same for both human embryos and canine embryos though this did need to be tested. This could only be tested once they were getting mature blastocysts in culture.
- **Would freezing the blastocysts reduce their viability? Would there shortly be a further amendment to the project licence to enable the fertilised embryos to be implanted?**  
The initial aim was to validate and optimise the technique first, before progressing to implanting the embryos. Any implantation would be done via the vagina rather than via a laparotomy/laparoscopy so would be a minor procedure carried out in conscious/sedated bitches. The aim though was to confirm that they could do all the required steps, including biopsy of the embryos etc. It was not envisioned being in the position to be able to do that until at least a year to 18 months' time.
- **Who would carry out these surgeries?**  
There was a PhD student on the team who was a small animal vet and very experienced with ovariohysterectomy.
- **AWERB pointed out that as there was an increased risk of intraoperative bleeding, although it was only a perceived risk, it should still be added to the project licence.**  
This would be added.

The second amendment related to the modification of another protocol and related to the catheterisation. This was to enable them to place an IV cannula in an animal whilst under general anaesthesia. Advice had been sought from a senior anaesthetist, who had recommended that jugular catheters were placed in this way prior to the pharmacokinetic blood sampling to minimise risk to the animal and to help blood collection as it was felt that it was unlikely they would be able easily to maintain patent cephalic catheters during the period of blood collection or obtain sufficient blood via cephalic catheters. The jugular catheters would be placed the day before either under sedation or general anaesthesia.

The protocol was also being amended to state that the AAV-empty vector **might be** administered (rather than would be) because in the first instance they felt that they could achieve useful results by examining the total and parvovirus specific IgG in dogs following their routine vaccination rather than by administering AAV. This would therefore be a refinement to the protocol. They were also recommending that the dogs should be lone-housed in kennels when the IV cannulas were in place to prevent littermates or the dog itself biting them out.

The following queries were raised:

- **Would there be a cephalic catheter in addition to the jugular catheter?**  
The jugular catheter would replace the cephalic catheter. This would be clarified in the project licence.

- **Over what time period would blood samples be taken – up to 24 hours?**  
It would depend on how well the catheter was looking. If the catheter could be kept in for longer, then ideally they would look to take samples for up to 72 hours as that be better for the dogs and the researchers.
- **How often would the blood sampling be done?**  
Initially it would be done quite frequently and then reduced with samples taken for up to a week afterwards. The catheter would be kept in place for the frequent blood sampling and then when less frequent sampling was needed it would be taken via the jugular or cephalic via needle and syringe.
- There was discussion about the possible options of housing the dogs with intravenous catheters overnight.
- Clarification needed to be sought from the Home Office Inspector whether maintaining the jugular catheters would be classed as part of the procedure, so would require someone with a personal licence to do, or would be classed as animal husbandry.
- Clarity was needed on how long the catheter would remain in place as one sentence mentioned a maximum of 3 days and another said no longer than 24 hours. This would be checked.

The following comments/queries were also raised:

- **3Rs section – reduction:** mention should be made in this section about the IVF plan and that if this worked it would reduce the number of animals produced in this colony, which if it came to fruition would be a very valuable 3Rs addition.
- **For the natural history part of the project - would the successful use of IVF reduce the observed variation in terms of the development of the disease?**  
They were breeding from 12 carrier females so they were already quite closely related. It should therefore not reduce the variation, unless they managed to get a massive number of oocytes from one animal then it could be a possibility. The plan was to build into a mixed model the actual parents of the dogs to account for that potentially in the longitudinal natural history work that was being done. If the IVF programme was successful it would be used for treatment trials that they had planned.

This was queried for as the dogs were already quite closely related, if the IVF treatment was successful, they could have potentially have an even more inbred strain of the dogs. The PPLH however pointed out that there would be advantages with that for the way they randomised the treatment allocation was by using siblings. If they could get a whole group bred from the same mother and possibly father it would reduce variation.

- **Was there a risk of thrombus/thrombophlebitis if the catheters were kept in place for 3 days?**  
This would be added as a potential adverse effect.

The PPLH was thanked for attending the meeting. A recap of the requested changes would be sent so that the changes could be made and the project licence circulated for one final review.

After the PPLH had left the following query was raised:

- It was noted that at previous AWERB meetings there had been discussions about the need to improve the clarity of humane end points within the project licence. Had that happened? It was

confirmed that there had had long discussions about this and it had been decided that it was a matter of refining the processes and the approaches to the humane end points. There was now much better communication between the teams about each individual dog and how they were reacting. As each dog reacted differently the focus was on ensuring that the right thing was done for each dog.

## **2 MINUTES OF PREVIOUS MEETINGS**

The minutes of the meeting held on 12 March 2021 were confirmed as an accurate record.

## **3 ACTION LOG**

### **3.1 Item 4: Pan London 3Rs symposium (12 March 2021)**

Permission had been obtained from the poster presenters that their posters could be placed on the intranet.

### **3.2 Item 4: Tamoxifen (12 March 2021)**

It was being explained to researchers that where possible oral administration was now the preferred method to be used. IP delivery could only be used if there was a scientific justified reason for doing it that way.

### **3.3 Item 5.4: Anatomy pony (12 March 2021)**

The anatomy pony's lameness had resolved now she was on grass. The pony would be given a general health check before she returned to Camden in late April and shoeing options were being discussed with the farrier and whether it was better to use rubber or metal. A meeting was being arranged with anatomy to discuss whether it was feasible to rotate the animals more frequently (on a 3 week basis rather than every 6 weeks).

With regards to the facilities in Camden, the suppliers of the stable matting had been contacted to see what other options there were to make the ground softer.

### **3.4 Item 2: Update on 2nd anatomy pony (26 January 2021 meeting)**

This pony was doing well in Hawkshead. She was being trialled by Equine for teaching purposes, which she seemed to be enjoying.

### **3.5 Item 3: Proforma PPL comments form (11 February 2021 meeting)**

A meeting had been held. A guide was being developed to assess project licences which it was hoped would be ready for review at the next AWERB.

### **3.6 Item 8: Assessors list review (11 February 2021 meeting)**

The list was being updated.

### **3.7 Item 7.4: Breeding and Colony Management Resource (9 December 2020 meeting)**

Following some further advertising of this discussion group, take up to join had improved. A date for the first meeting had been arranged. The overarching aim of this session was for researchers and BSU staff to discuss how breeding and colony management practices at RVC met those of current best practice, and identify if there was any room for improvement. It would be covering both rodents and zebrafish.

### **3.8 Item 8: Mouse colony (9 December 2020 meeting)**

A copy of the extract from the previous AWERB minutes where this item had been previously discussed had been sent to the PPLH and a follow up conversation had now been held. The PPLH was now scheduled to attend the April AWERB meeting so that the project licence could be reviewed.

**3.9 Item 4.1: Ring Tailed Lesions (26 January 2021 meeting)**

The non-compliance had been discussed with the supplier. They would be ensuring that any future animals sent would have an additional check to ensure there were no ring tailed lesions. They had also checked all their animals on site and had not found any additional lesions similar to what had been seen here. For future animals that arrived, they would also be closely checked upon arrival and then monitored.

**4 DATES OF NEXT MEETINGS:**

These were scheduled for:

- 8 April 2021 at 1pm (standard agenda items)
- 27 April 2021 at 10am (PPL reviews meeting)

Secretary

01 April 2021