

Completion Report for BSAS Scholarships

Name and affiliation: Ruth Clamp, IBERS, Aberystwyth University

Award Name and value of the award: Early Career Award - £3000

Was any additional funding secured to support the activity?

(If yes, please state the value and source of funding): None

Start/end date of the award: June 2014

Summary of the award (Briefly describe the objectives and how was it undertaken):

(approximately 300 words)

The early career award was applied for to complete a project using a *Trueperella pyogenes* model of bovine endometritis to investigate both the immune response linked with *T. pyogenes* and the effects of two potential anti-inflammatory products as a treatment for endometritis. The study was designed as an addition to my current PhD project to enhance the data that I have produced on an *E. coli* model of endometritis.

The objectives were:

- Test Pre-implantation factor (PIF) as a possible anti-inflammatory peptide within a *T. pyogenes* cell culture model of endometritis.
- Test the use of crude and purified iminosugar cucumber extracts as potential anti-inflammatory compounds within a *T. pyogenes* cell culture model of endometritis.
- Assess the anti-inflammatory potential of these products by measuring the immune response from cultured endometrial cells, such as prostaglandin, cytokine and chemokine secretion and also gene expression of several innate immune components such as toll-like receptors (TLR), cytokines and antimicrobial peptides. By investigating these innate immune components, the effect of *T. pyogenes* on the uterine immune system will be further revealed.

The study was completed by first testing several different treatments of *T. pyogenes*, to assess which treatment gave a pro-inflammatory immune response in a bovine endometrial cell culture. Treatments used were live bacteria, bacterial free filtrate and heat killed bacteria. All treatments were tested on both epithelial and stromal primary cells isolated from the endometrium. Once this had been established, the treatment was used to produce a model of *T. pyogenes* endometritis, which the anti-inflammatory products could be tested in. Both products were tested using at least 2 concentrations. Samples were assessed for hormone production with radioimmunoassays, and cytokine production with ELISAs. Based on these results, genes of interest were examined with q-PCR.

Benefits of the Award:

This is the main part of the report and the two sections below should be approximately 1000 words in total. You may focus on benefits to yourself, to the animal science community, or both – depending on the nature of the activity undertaken.

Benefit of the award to you (e.g. new knowledge or skills, new contacts and collaborations):

The early career award has been of great benefit to me. Through working within another institute, I have learnt many new skills. Before going to Swansea University I had already set up a culture of bovine endometrial cells at Aberystwyth, however, I had little knowledge on how to use the cells to be able to attain the most reliable results from the culture. The cell culture technique has been used extensively by Martin Sheldon's group at Swansea for several years. Therefore, by working within the group I was able to improve my knowledge of the culture technique and learn the best ways to culture the cells and also how and when to use them for experimentation. Without the opportunity to be taught at Swansea, it would have taken an extensive amount of time troubleshooting in Aberystwyth to attain a culture that was as reliable as possible. Furthermore, I had little knowledge of microbiology work within the lab. From being taught at Swansea University, I can now prepare several different bacterial treatments for cell culture and also troubleshoot problems related to this. I also learnt other techniques that were not originally planned to be used within the award such as how to complete an MTT assay on cells and how to extract and culture macrophages from bovine whole blood. This experience will both benefit me within my PhD and in my future career.

Whilst working in Martin Sheldon's group, I have made several new contacts that will benefit me for the future. During my time spent in Swansea, there were also other visiting PhD students that were undertaking projects at the same time as me. Through this I have been able to network with students in my field from Brazil, Italy and Ireland, as well as those based in Swansea. In doing this I have made firm contacts for the future, and therefore potential collaborators. I would unlikely have met these people at this stage of my career. Through working in the lab I have demonstrated my abilities as a researcher to my host supervisor for this award, James Cronin. By doing this, I have built a good working relationship with the lab in Swansea. This has now carried on past the award. I am still in contact with James and also the PhD student that I was paired with and am always welcome to ask them for advice. Furthermore, whilst I was working in the lab, we were experiencing some problems with the cells culture. Since returning to Aberystwyth I have continued to help to troubleshoot these problems and work with the lab in Swansea to remedy these them. Furthermore, I am welcome to go back to complete more work if I feel it may benefit my project. I feel that this relationship will benefit me for future job prospects as Martin Sheldon's group is one of the most well-known groups within my field.

Therefore, this award has not only benefitted me for my future PhD work, but also for career prospects and collaborations after my PhD.

Benefit of the award to the animal science community, academic and industrial:

The award has allowed both further study of the immune response to *T. pyogenes* within the bovine endometrium and also an investigation into two potential treatments for endometritis.

Academic animal science community: In investigating the immune response to *T. pyogenes* mounted by the endometrium, the work completed has furthered current knowledge; how the bovine immune response deals with *T. pyogenes* endometrial infections. This benefits the animal science academic community as we must first understand how the endometrium is able to react to the pathogen causative of the disease before being able to research treatments or prevention methods. The data produced by this award, especially that looking at antimicrobial peptides, will further support other recently published data that has begun to elucidate the immune response induced by *T. pyogenes* within the uterus. This *in vitro* work will also be able to support the current knowledge of the *in vivo* situation, giving support to potential animal trials investigating endometritis in dairy cows.

Industrial animal science community: The current treatments for endometritis are undesirable or ineffective. Therefore a new treatment is needed, preferably one that is naturally derived in order to help prevent superbug resistance as seen with antibiotic usage. The work completed for the award tested two naturally derived potential treatments for endometritis in a *T. pyogenes* model of endometritis. No research has yet focused on a treatment within a specific model of *T. pyogenes* endometritis, thus giving the work a benefit for the animal science industry. In combination with the work completed as part of my PhD (looking at the treatments within an *E. coli* model of endometritis), the award helps to begin a thorough examination of both Preimplantation factor (PIF) and cucumber extracts as treatments for endometritis at an *in vitro* level. By completing this initial *in vitro* testing, the effectiveness and mechanisms of both PIF and cucumber extracts on the immune response within endometrial cells to bacterial models of endometritis was established. This then verifies the usage of animal trials to fully test the potential of PIF and cucumber extracts for endometritis treatments in dairy cows.

Other supporting information:

Click here to enter text.