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KTN/BSAS Summer Vacation Scholarship Award – Report

Mammalian IVP: The adaption of PGD for embryo selection

Student: Rebecca Jennings (University of Kent)
Supervisor: Dr Kara Turner (University of Kent)

Background and aims

It is believed that by 2050 the global population will reach 9 billion, and so the demand for livestock production will soar. With an ever-increasing need for agricultural supply, new methods of transportation and selection must be found. Techniques in *in vitro* production (IVP) and pre-implantation genetic diagnosis (PGD), originally developed for assisted reproduction in humans, offer a suitable solution for the selection of high quality embryos for transfer. This allows for the selection of high genetic merit animals as well as sex selection, which is particularly important in the pig and dairy breeding industry. Furthermore, this approach to livestock breeding enables the distribution of vitrified embryos as an alternative to the transportation of weanlings, which is both financially beneficial to livestock distributors and more environmentally friendly. Many advances have been made in animal IVP over recent decades (particularly in cows), however very limited attention has been paid to PGD. Moreover, recent advances in time-lapse technology, which has become a focus in human assisted reproduction, have been largely ignored as a tool in animal IVP. Through non-invasive time-lapse technology the morphokinetics of embryo development can be studied, which can enable the embryologist to predict the viability of a developing embryo much earlier. The aim of this study therefore was to utilise the PrimoVision time-lapse device in order to collect preliminary data that may assist in producing a platform that will improve embryo selection criteria, which will go on to benefit the agricultural industry. By incorporating advanced reproductive techniques into herd health and maintenance it can greatly enhance the selected genetics of a particular herd.

Description of work and skills gained over 6-week studentship

In vitro production involves the manual isolation of oocytes using a vacuum line and pump, *in vitro* maturation using defined media that has been optimised for a specific species, *in vitro* fertilisation and embryo culture. During my time in the laboratory I gained hands on experience of the full IVP process in two different mammalian models: porcine and bovine. In addition I gained experience of murine embryo culture from first cleavage stage up to hatched blastocyst. Consistent with current findings in the literature, mammalian IVP yielded varying levels of success across the three species, with murine being the most successful and porcine the least. In addition, I gained full training in embryo biopsy (bovine) at the blastocyst stage in order to establish the genetic status of the embryo by PCR. In order to obtain enough DNA for PCR analysis from the trophectoderm biopsy whole genome amplification must be employed. I performed a comparative study to assess the DNA yield from a number of commercially available whole genome amplification kits, including WGA4 GenomoPlex® which relies on PCR, and GenomiPhi® and RepliG, both of which utilise multiple displacement amplification (MDA), a technique that does not rely on thermal cycling, unlike PCR. By comparing results from whole blood, lymphocytes and single cells I was able to conclude that GenomiPhi® produced the highest DNA yield, whilst being the quickest and easiest protocol to follow. Once a consistent amplification method had been ascertained it was possible to test for the most reliable PCR primer pair that would accurately determine the sex of a known sample in a blind study. Due to length variation in the X-Y homologous amelogenin gene (AMELX and AMELY) AMEL I, II, III are widely used as primer pairs, in PCR amplification. Therefore these primers were utilised in a PCR reaction and their products run through agarose gel electrophoresis and capillary array electrophoresis to create different sized bands and peaks respectively providing the genetic status of the single cell sample. I found that AMEL I was the most reliable primer pair and produced the most accurate result. Finally, the use of PrimoVision during my summer placement to compare the morphokinetic parameters of developing embryos provided some interesting results. I established that bovine embryos appeared to enter a lag phase at the 8 cell stage, 53±1.7 hours post fertilisation, with a number failing to progress in the first study, however the blastulation rate in this study was 12.5% with embryos sharing a recorded 1st cleavage of 32 hours post fertilisation and 2nd cleavage of 42 hours 50 minutes post fertilisation. PrimoVision captured bovine and murine embryos at the expanded blastocyst stage and hatching, all of which provided data that helped form this preliminary study.