IETS Conference Report

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In cattle, reproductive technologies such as superovulation and *in vitro* maturation (IVM) have progressed substantially over the past decade, leading to increased production of embryos for breeding and research purposes. In addition, advances are being made in nuclear transfer and cryopreservation techniques. However, this technology has limitations due to the lack of large numbers of homogeneous oocytes for manipulation. Since the majority of follicular atresia (cell death) occurs during specific stages of development, obtaining a source of oocytes from immature follicles will not only provide larger numbers of oocytes, but may also allow oocytes of higher quality to be utilised. In addition, development of a culture system capable of supporting preantral follicles to a stage where their oocytes can be matured and fertilised in vitro will also allow identification of factors necessary for normal follicle development and acquisition of oocyte developmental competence. At this present time, no oocytes from immature bovine follicles developed in vitro have been competent to be matured or fertilised in vitro.

The aims of my project are to identify factors involved in early folliculogenesis in cattle, with the long term goal of obtaining developmentally competent oocytes from immature follicles in vitro. In order to do this, it is necessary to have knowledge of follicle development in cattle and other species, both in vivo and in vitro. The IETS conference was an important source of knowledge, as the emphasis was on domestic species, and the latest techniques for oocyte maturation and embryo transfer were discussed. For example, Hendriksen and colleagues correlated follicular development in the cow and the competence of matured oocytes to develop into an embryo following in vitro maturation and fertilisation. This seminar emphasised that rates of fertilisation from follicles at an advanced developmental stage are still very limited; therefore the work we are doing to provide a source of developmentally uniform oocytes will prove important in enhancing these techniques. In addition, cryopreservation studies were discussed, and although some success has been reported, there is a long way to go before such techniques can be used successfully and safely in domestic animals or humans.

The development of oocytes in vitro to a stage where they can support normal embryonic development is dependent on the oocyte reaching the appropriate stage of development to respond to the endocrine and paracrine signals responsible for the induction of maturation. In cattle, follicle development is a lengthy process, therefore it would be useful to identify non-invasive markers of follicle and oocyte development. For example, we have recently identified specific enzymes (metalloproteinases) which are secreted by follicles into the culture medium, and with morphological comparisons, have found a correlation between the presence of these enzymes and follicle health in vitro. So far we have been unable to link these factors to developmental competence of the oocyte. However, in a poster presented at the IETS conference, Robert and colleagues have identified potential competence markers using cDNA library subtraction and differential screening techniques. What was very interesting to us was that one of the genes which was associated with oocytes capable of undergoing in vitro fertilisation was a metalloproteinases inducer gene. This has led us to identify this gene within our cultured follicles, and experiments are ongoing to assess whether the presence of this factor will allow us to identify oocytes which are developing normally in vitro.

The lack of progress in applying assisted reproduction techniques to animals with follicles which undergo a long growth period is due to lack of knowledge of the basic mechanisms that control development in these species. The use of in vitro systems should help to advance our knowledge of these complicated processes. However, in order to do this, we must continue to communicate with one another in order to allow the many pieces of the jigsaw to fit together.