

Society of Reproduction and Fertility Vacation Scholarship 2012

Supervisor: Dr Amanda de Mestre

Project title: Regulation of trophoblast differentiation by TGF β signalling pathways

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Brief summary of the project: This 6 week summer project investigated the molecular pathways that regulate normal trophoblast development in the horse, a process critical to early embryo development and survival. Binucleate cells are a subtype of equine trophoblast that secrete equine chorionic gonadotrophin (eCG) and possess unique immunomodulatory properties. Mammalian trophoblast differentiation is tightly regulated through the expression of growth factors, transcription factors and components of their associated signalling pathways. Transforming growth factor β (TGF β) superfamily ligands are a large family of dimeric proteins abundantly expressed at the fetal-maternal interface. We have found that equine chorionic girdle trophoblast cells that give rise to binucleate trophoblast express type I, type II and accessory receptors specific for the TGF β ligand, BMP4. **This summer project** investigated the role of BMP4 in regulation of equine binucleate trophoblast differentiation. It focused on defining whether BMP4 signalling is induced through SMAD1/5 during chorionic girdle differentiation *in vivo*?

Main findings: Pregnancies were established in eight pony mares and conceptuses recovered between days 27 and 34 of pregnancy using non-surgical uterine lavage. The chorionic girdle was dissected free of surrounding tissue and snap frozen for protein lysates. Additional samples of chorionic girdle previously collected and stored at -80 were also utilised. Western blotting was performed using cross-reactive anti-human antibodies directed against phospho and Total SMAD1/5 and SMAD2 and B-Actin as a control. Preliminary western blot experiments confirmed that antibodies directed against human phospho SMAD1/5 and SMAD2 and human Total SMAD1/5 and SMAD2 were also able to detect a single equine protein of approximately 60 kDa confirming it was likely to be specific to equine SMAD proteins.

We found that phosphorylated SMAD1/5 expression, indicative of BMP signalling, was higher in the chorionic girdle when compared to the adjacent chorion. Phosphorylated SMAD1/5 expression in the chorionic girdle was higher at day 30-31 when compared with day 34, corresponding to the window when binucleate differentiation is initiated. Phosphorylated and total SMAD2 was similarly expressed in chorionic girdle and chorion and across the different time points studied. These observations confirmed that signalling through SMAD1/5 is induced during differentiation of chorionic girdle trophoblast cells *in vivo*. The results support our hypothesis that BMP4 induces terminal differentiation of binucleate trophoblast cells during a defined window in chorionic girdle development.


Student learning outcomes: This bursary has provided me with the opportunity to further establish laboratory and research skills and has given me the chance to apply content learned during my course and cement the information through practical, hands on learning. Having had previous laboratory experience, I felt comfortable with basic procedures at the start of this summer research project and was therefore able to immediately begin learning new techniques and protocols. One of the most important skills re-enforced during this project was the ability to problem solve. This is not always

something that is taught in a classroom, but it is crucial in any kind of research as well as many aspects of daily life. Conducting experiments and having them 'fail' is as significant as having them 'succeed'. While it can get frustrating at times, it requires critical thinking regarding the protocol, the science behind it, and ways in which it can be optimized. The ability to effectively and logically approach a problem is a skill that can be carried over into any field and will greatly aid me in my future career as a veterinarian. The importance of literature reviews is another point that was emphasized to me during this project. Not only are they necessary in order to gain the information needed to accurately conduct and interpret the experiments performed, they also present another way to supplement and expand upon knowledge gained in the classroom. Furthermore, literature reviews cultivate an appreciation for the work that goes into answering questions that many people now take for granted and inspire individuals to continually pursue a higher understanding of the world around them.

Future plans: The above project is a component of a larger project in the laboratory that aims to determine how TGF β signalling pathways are regulated during primary trophoblast differentiation. Current work aims to characterise the expression of Type I, Type II and accessory receptors during chorionic girdle development *in vivo* and *in vitro*. Tight regulation of these receptors, in particular the accessory receptors BAMBI and DRAGON, may explain the developmentally restricted activity of BMP4 signalling in chorionic girdle *in vivo*. The data generated by Alycia is publication quality. A manuscript reporting these findings is currently in preparation and will be submitted early in 2013. The data also contributed to an abstract that has been accepted for presentation at Fertility 2013.



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