

## Research Group: Oocyte Biology Group

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The Oocyte Biology Group investigates fundamental physiological aspects of oocyte-granulosa cell interactions in an effort to improve understanding of mammalian oocyte biology and to better implement assisted reproductive technologies. Ovarian folliculogenesis consists of a series of ordered maturation and differentiation events of both the somatic and germ cell components that culminates in ovulation and the production of a mature oocyte that is able to support early embryogenesis. The immature oocyte and the follicular granulosa cells are in intimate contact and normal growth and development of the oocyte is entirely dependent on this association. Oocyte-secreted growth factors have significant effects on the somatic cell compartment of the ovarian follicle. We are actively studying two of these proteins, namely growth differentiation factor 9 (GDF9) and bone morphogenetic factor 15 (BMP15), both of which appear to be essential for mammalian fertility. Our aim is to understand the functioning of these proteins and the detailed basis of the somatic/germ cell (oocyte) interactions in the ovary in order to provide the optimal environment for early embryonic development.

**Project title:** Understanding the Functioning of Oocyte-derived Growth Factors  
(Supervisors: Dr. David Mottershead & Dr. Robert Gilchrist)

Oocytes produce proteins of great importance for their ability to successfully be fertilized and develop into a viable embryo. The transforming growth factor  $\beta$  (TGF- $\beta$ ) superfamily of proteins consists of some 40 members in mammals, many of which are essential for normal development and maintenance of the adult organism. Growth differentiation factor 9 (GDF9) and bone morphogenetic factor 15 (BMP15) are two members of this family of proteins which are produced by oocytes and are critical for female fertility. The proteins GDF9 and BMP15 have great potential for improving currently used infertility treatments, as demonstrated previously by our group (see Yeo et al., 2008). In fact Dr. Gilchrist has patented this technology and has industry support for our research efforts on GDF9/BMP15. To take such work to the clinic/industry setting we need well characterized, stable preparations of these proteins. These and closely related proteins also have great potential in the area of stem cell research, both for the maintenance of stem cells in a pluripotent state as well as for directed differentiation of stem cells toward a particular lineage.

Potential projects include: (basic research projects)

1. Recombinant production and purification of human GDF9 and BMP15 derived from engineered mammalian cell lines. The aim of the project will be to determine if the pro-region of GDF9 or BMP15 can stabilize the bioactivity of the corresponding mature region.
2. Production of various mutants of GDF9 and BMP15, potentially including a stabilizing disulphide bond (Ser-Cys mutation), or mutating reported phosphorylation and glycosylation sites, to determine the impact on function of such mutations.
3. Use of a bacterially expressed recombinant GDF/BMP binding protein to create an affinity column to purify TGF- $\beta$  family members from physiological fluids, such as follicular fluid or seminal plasma. The aim of this project is determine in what forms do the proteins GDF9/BMP15 exist in such biological fluids, as such information could give insight into the mechanisms used *in vivo* to regulate the bioactivity of these proteins.

Techniques used can include:

- use of mammalian cell lines for recombinant protein production
- bacterial expression of proteins
- protein purification, especially affinity purification and HPLC/FPLC
- molecular biology, plasmid preps, PCR, transfection of mammalian cells
- growth factor assays, various types, especially transcriptional reporter assays
- use of transgenic GFP or luciferase reporter mice for growth factor characterization

**References:**

- Mottershead, DG et al., (2008) Characterization of recombinant human growth differentiation factor-9 signaling in ovarian granulosa cells. *Mol. Cell. Endocrinol.* **283**: 58-67
- Yeo, CX et al., (2008) Exogenous growth differentiation factor 9 in oocyte maturation media enhances subsequent embryo development and fetal viability in mice. *Human Reproduction* **23**: 67-73
- Gilchrist, RB et al., (2006) Molecular basis of oocyte-paracrine signaling that promotes granulosa cell proliferation. *Journal of Cell Science* **119**: 3811-3821.