

The mechanism of action of the FecB (Booroola) mutation

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Introduction

- Mechanisms of ovarian function - subject of intensive research since early 1980's - motivated by the extra-ordinary increase in ovulation rate (OR) of the Booroola?
- In 2001 - 3 research groups, showed the OR increase associated with a single-point-mutation in the intracellular domain of one of the receptors for **Bone Morphogenic Proteins (BMPR-1B)**
- BMP system had not formerly been recognised as influencing ovarian function.

Ovarian follicle development

- The Follicle (oocyte + granulosa cells) is the fundamental developmental unit of the mammalian ovary.
- Primordial follicles recruited for growth throughout life. ~ 100,000-250,000 of these follicles at birth.
- Follicles grow slowly until they have the ability to respond to the pituitary gonadotrophins (FSH and LH) at a diameter of around 250 μm .
- The responsive follicles grow to 2-4 mm some becoming dependent on FSH & LH.
- Many of the responsive degenerate (50-70% for follicles over 1 mm) and the **vast majority** (>99%) of follicles fail to ovulate

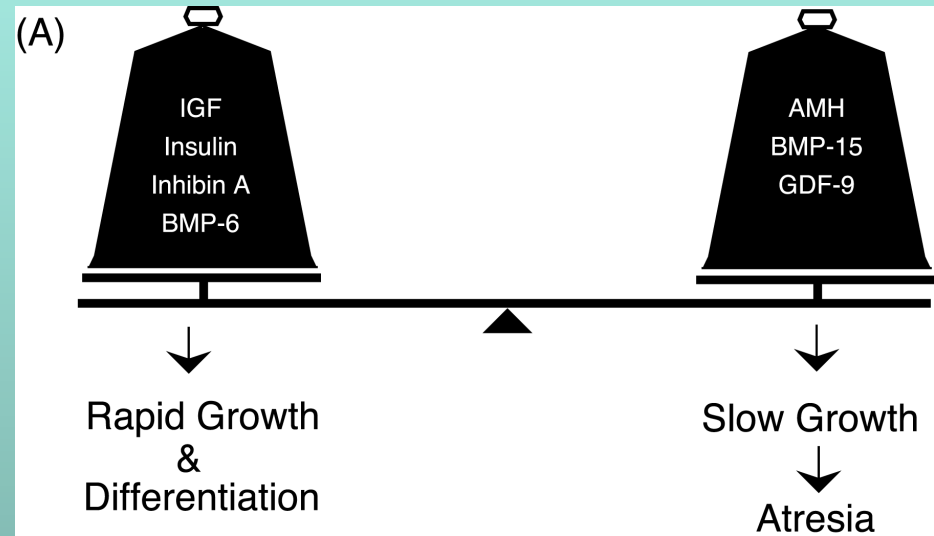
Follicle development

- Complex actions and interactions between locally produced hormones and growth factors.
- These systems include
 - the insulin/IGF system,
 - the inhibin/activin system and
 - the bone morphogenic system
- Recent studies indicate the **oocyte**, also secretes numerous factors that affect follicle development and ovarian function.
 - eg growth differentiation factor-9 (GDF-9),
 - bone morphogenetic protein –6 (BMP-6) and
 - BMP-15 and potentially a few others

Follicle selection?

The fate of individual follicles relies on the **balance between these conflicting local actions**

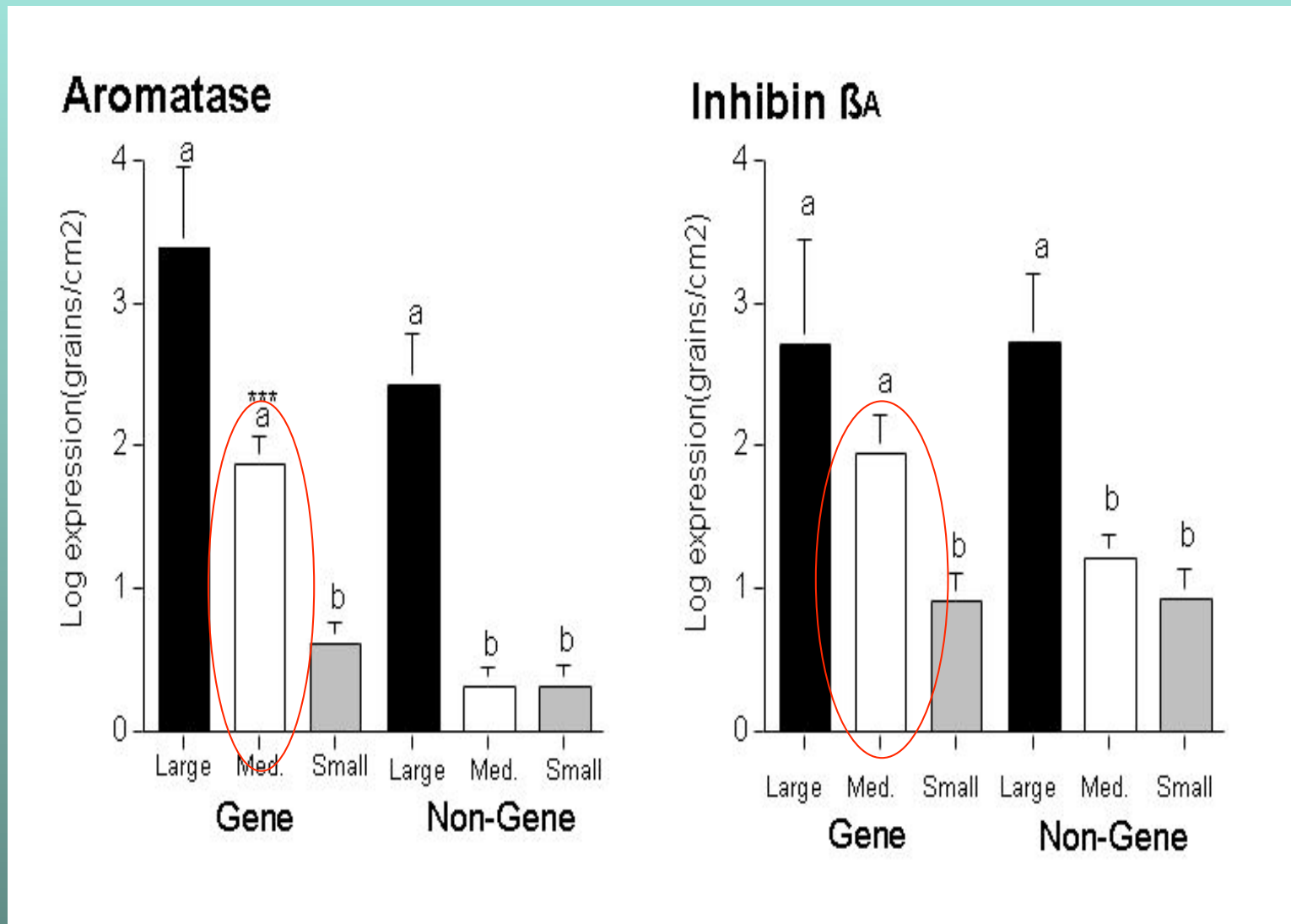
- Therefore possible to increase ovulation rate through :
 - an increase in circulating gonadotrophin conc.,
 - an increase in the activity of augmentors of gonadotrophic actions
OR
 - a decrease in the activity of attenuators of gonadotrophic actions



FecB gene carriers

- McNatty et al. comprehensive series of experiments showed
 - small antral follicles matured precociously in gene carriers, becoming “functional” at diameters of 2.5-3.5 mm compared to 4-6 mm in non-gene carriers
- Recently Campbell et al. showed that
 - expression of mRNA for both cytochrome P450 aromatase and the β A-subunit of inhibin/activin can be detected in much smaller follicles in FecB gene carrier compared to controls

Expression of mRNA in granulosa cells of antral follicles of different sizes in ewes with and without the FecB mutation (Campbell et al)



Therefore:

- Seems ovulation rate differences of the FecB gene carriers **are not** due to differences in the total number of antral follicles
- but to an **extended recruitment period** together with a **low incidence of atresia**, resulting in the ovulation of a large number of small ovulatory follicles.

Molecular basis of Booroola mutation

- Increase in fecundity associated with a single point-mutation in the intracellular domain of one of the **receptors for BMPs** (BMPR-1B/ALK6)
- The Fecundity gene is situated on Chromosome 6 in a locus corresponding to Chromosome 4 in the human
- In sheep demonstrated expression BMP receptors in ovarian somatic and oocytes & seems all components of this regulatory system are expressed in the sheep ovary.

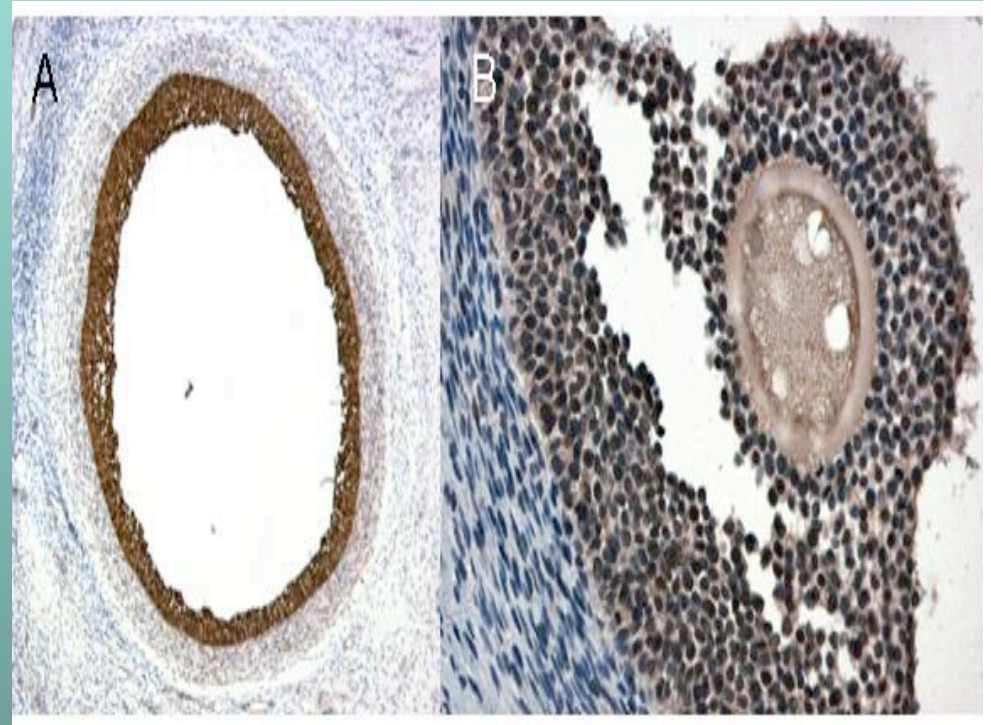
Mechanisms to ensure early maturation?

Suggested that the FecB mutation may act by either

- increasing the release of FSH from the pituitary or
- Increasing the sensitivity of follicular cells to FSH within the ovary.
- Some support for both but mostly the latter.
- Therefore the FecB mutation is acting at an ovarian level to modulate the sensitivity of somatic cells to gonadotrophic stimulation.

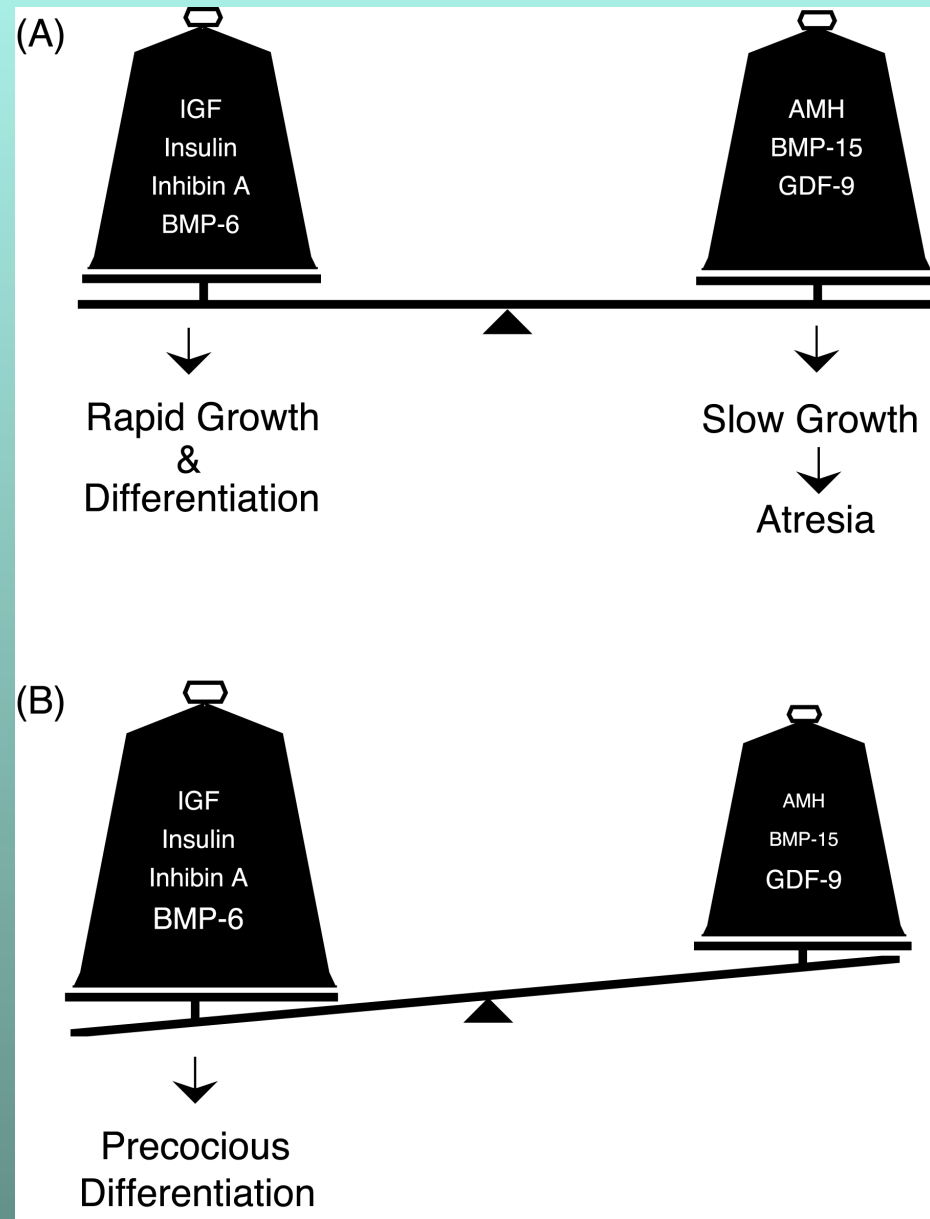
Potential ligands of the BMPR1B in ovarian somatic cells

- BMPR1B receptor interacts with a number of local factors which may act to **augment** or **attenuate** the actions of pituitary gonadotrophins.
- Now unequivocal evidence indicating both mRNA and protein expression in ovarian cells types in sheep has been observed for BMP-6, AMH (granulosa cells) GDF-9 and BMP-15.



At present the available evidence support gonadotrophic actions are amplified through either

- increased activation of an augmentor (BMP-6),
- decreased activation of attenuators (BMP-15, AMH) OR
- a combination of these two mechanisms.



Conclusions

- Mechanisms of action of the FecB mutation have been central to our understanding of the mechanism of follicle selection
- Increased prolificacy from this mutation is **not due** to an increase in the circulating FSH concentrations
- An **increased sensitivity** to FSH mediated by the action of intra-follicular local factors seems likely.
- The identity of these factors is still uncertain but **the BMP system** is important possibly via .
- Increased actions of **augmentors** (e.g. BMP-6) or decreased actions of **attenuators** (e.g BMP-15).



Breed

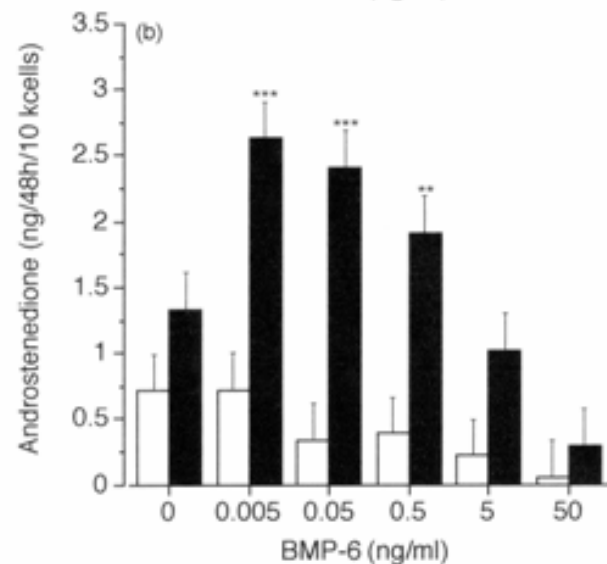
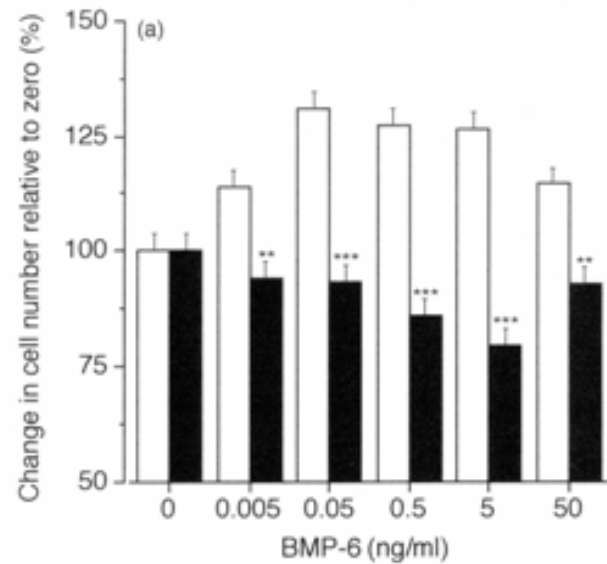
Total no.

Mean

% of Lambs

Lambs

Effect of FecB mutation on response of theca cells to BMPs



These data show cell number (a) and androstenedione production (b) by theca cells isolated from small antral follicles of Fec^{+/+} (open column) and Fec^{BF/F} (closed column) animals and cultured under serum free conditions for 144 h in the presence of increasing doses of BMP-6.