P-582 Cumulin and FSH cooperate to regulate inhibin B and activin B production by human granulosa-lutein cells in vitro

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Study question: Does the GDF9:BMP15 heterodimer termed cumulin regulate granulosa cell inhibin and activin production and does this require cooperation with FSH?

Summary answer: Cumulin, but not the homodimers GDF9 or BMP15, exerts paracrine control of FSH-induced regulation of inhibin B and activin B.

What is known already: There is genetic evidence that the oocyte-secreted factors bone morphogenetic protein 15 (BMP15) and growth differentiation factor 9 (GDF9) constitute key regulators of folliculogenesis, ovulation rate and fecundity in mammals. BMP15 and GDF9 interact functionally and it is hypothesised that this interaction may be mediated by formation of a GDF9:BMP15 heterodimer, termed cumulin. BMP15 and GDF9 regulate folliculogenesis by acting as paracrine growth factors in the follicle and are known to regulate inhibin expression, although the participation of cumulin in control of the inhibin-activin system is unknown.

Study design, size, duration: To assess the effects of cumulin versus GDF9 and BMP15, we bioengineered and purified wild-type non-covalent cumulin, as well as various covalent dimers of cumulin, GDF9 and BMP15, containing an introduced inter-subunit disulphide bridge. To assess the effect of the pro-domain, we engineered mature cumulin (without the pro-domain).

Participants/materials, setting, methods: Human granulosa-lutein (hGL) cells from IVF patients were cultured for 5 days, then treated for 24h ± FSH with various forms of recombinant cumulin (native and cysteine mutants, and with-/without the pro-domains), and the disulphide-subunit bridged forms of GDF9 or BMP15. Messenger RNA expression of the subunits of inhibins/activins (*INHA*, *INHBA*, *INHBB*), and secretion of inhibin A, inhibin B, and activin B protein by immunoassay into media were measured (n=3-7 biological replicates per experiment).

Main results and the role of chance: Mature and pro-forms of cumulin dose-dependently stimulated ($P<0.05$) *INHBB* mRNA expression (encoding the inhibin βB subunit of inhibin B or activin B), but did not alter *INHBA* (encoding the βA subunit of inhibin A). Correspondingly, cumulin stimulated ≥5-fold secretion of inhibin B and activin B ($P<0.05$), but did not alter inhibin A. In contrast to cumulin, GDF9 or BMP15 exhibited no significant effect on inhibin B or activin B expression ($P\geq0.05$). Furthermore, cumulin, but not GDF9 or BMP15, interacted synergistically (two-way ANOVA interaction, $P<0.05$) with FSH to increase *INHBB* mRNA and inhibin B expression. FSH markedly stimulated (≥16-fold) *INHA* expression, which encodes the α subunit of inhibin A/B, and suppressed activin B ($P<0.05$). Mature and pro-forms of cumulin ± FSH stimulated comparable effects on *INHBB*, *INHBA*, inhibin B, activin B and inhibin A ($P\geq0.05$), suggesting that the pro-domains of cumulin has a minimal role in its actions on granulosa cells.

Limitations, reasons for caution: In vitro study using granulosa-lutein cells from women undergoing stimulation for IVF.

Wider implications of the findings: Together these data demonstrate that the GDF9:BMP15 heterodimer cumulin exerts control of FSH-induced regulation of inhibin B and activin B, which may contribute to oocyte-secreted factor regulation of folliculogenesis and fecundity in women.

Trial registration number: not applicable