REGULATION OF MOUSE CUMULUS EXPANSION BY OOCYTE-SECRETED GROWTH DIFFERENTIATION FACTOR-9 (GDF-9)

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Oocyte paracrine signalling is necessary for mouse cumulus cell expansion, an important preovulatory process. The oocyte-secreted factor growth differentiation factor-9 (GDF-9) signals through the bone morphogenetic protein receptor-II (BMPR-II) and is currently the primary candidate molecule for the cumulus expansion enabling factor (CEEF). The present study was conducted to determine whether in the mouse GDF-9 is the CEEF. Cumulus oocyte complexes (COC) were collected from eCG-primed mice and the oocyte was microsurgically removed to generate an oocytectomised complex (OOX). An established scoring system was used to measure FSH-induced cumulus expansion; 0 (no expansion) to +4 (maximum expansion). OOX complexes treated with FSH alone failed to expand (score: 0), whereas expansion was significantly ($P < 0.05$) induced by either recombinant mouse GDF-9 (score; mean +/– SEM: 2.7 +/– 0.1), recombinant TGF-β1 (score: 2.6 +/– 0.2) or co-culture with oocytes (score: 2.3 +/– 0.2). A GDF-9 neutralising antibody mAb-53, raised against hGDF-9, was effective in neutralising the response of OOX complexes to GDF-9 (score: 0.1 +/– 0.1), but had no significant effect on the expansion of OOX complexes co-cultured with oocytes (score: 2.3 +/– 0.2). Likewise, a TGF-β antagonist neutralised ($P < 0.05$) TGF-β-induced, but not oocyte-induced, expansion of OOX complexes. A soluble portion of the BMPR-II ectodomain, a known GDF-9 antagonist, failed to neutralise oocyte-induced cumulus expansion ($P > 0.05$) at the highest dose implying that BMPR-II is not a critical receptor involved in regulating cumulus expansion. Using real-time RT-PCR, hyaluronan synthase-2 (HAS2) mRNA expression by OOXs was upregulated 6- to 7-fold by oocytes and GDF-9. The GDF-9 neutralising antibody mAb-53, partially neutralised GDF-9-induced OOX HAS2 expression, but not oocyte-induced HAS2 expression. This study provides evidence that like TGF-β1, GDF-9 can enable FSH-induced cumulus expansion, however more importantly demonstrates that neither GDF-9 nor TGF-β1 alone account for the crucial oocyte-secreted factor regulating cumulus expansion in the mouse.

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