93. IMMUNONEUTRALIZATION OF GROWTH DIFFERENTIATION FACTOR-9 REVEALS IT PARTIALLY ACCOUNTS FOR OOCYTE MITOGENIC ACTIVITY

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Paracrine factors secreted by oocytes play a pivotal role in promoting early ovarian follicle growth and in defining a morphogenic gradient in antral follicles, yet the exact identity of these oocyte factors remains unknown. The objectives of this study were to generate a specific growth differentiation factor-9 (GDF-9) neutralising monoclonal antibody, and to utilise this antibody to determine the extent to which the mitogenic activity of oocytes can be attributed to GDF-9. To do this, anti-GDF-9 monoclonal antibodies were generated. Based on epitope mapping, a clone was identified with very low sequence homology with related TGF-β superfamily members, including GDF-9B. 3D-peptide modelling of mature mGDF-9 suggested that the binding motif lies at the C-terminal fingertip. As predicted by the modelling, the antibody detected GDF-9, but not GDF-9B in a Western blot, and GDF-9 protein in oocyte extract and oocyte-conditioned medium. In a mouse mural granulosa cell (MGC) bioassay, the anti-GDF-9 antibody completely abolished the mitogenic effects of GDF-9, but had no effect on TGF-b1 or activin A-stimulated MGC proliferation. An unrelated IgG at the same dose had no effect on mGDF-9 activity. This GDF-9 neutralizing antibody (NAb) was then tested in an established oocyte-secreted mitogen bioassay [1], where denuded oocytes co-cultured with MGC promote cell proliferation in a dose-dependent manner. An increasing dose of GDF-9 NAb (0-160 μg/mL) dose-dependently decreased the mitogenic activity of oocytes, but only by ~45% at the maximum dose of NAb. The NAb at just 5 μg/mL neutralised 90% of recombinant mGDF-9 mitogenic activity, but only 15% of oocyte activity. This study has characterised a mGDF-9 neutralising antibody. This antibody was able to neutralise ~½ of oocyte bioactivity, demonstrating that GDF-9 is an important oocyte mitogen, but also that GDF-9 accounts for only part of total oocyte bioactivity.

[1] Gilchrist RB *et al.* (2001) *Developmental Biology* **240**: 289–298.

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