doi: 10.1387/ijdb.130044md



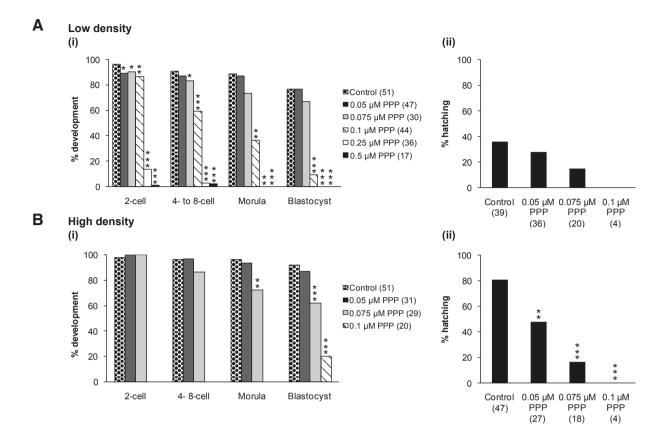
SUPPLEMENTARY MATERIAL

corresponding to:

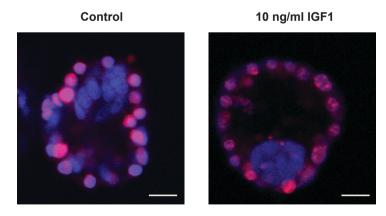
Insulin-like growth factor 1 acts as an autocrine factor to improve early embryogenesis *in vitro*

CHARMAINE J. GREEN and MARGOT L. DAY

*Address correspondence to: Margot Day. Department of Physiology, K25 - Medical Foundation Building, University of Sydney, NSW, 2006, Australia. Tel: +6-12-9036-3312 Fax: +6-12-9036-3316. e-mail: margotd@physiol.usyd.edu.au



Supplementary Fig. S1. Picropodophyllin decreases embryo development and hatching in a dose-dependent manner. Development of zygotes to the 2, 4-8, morula and blastocyst stage (i) and proportion of blastocysts hatching (ii) after culture in the absence or presence of 0.05-0.5 μ M PPP (A) at low density (1 embryo/100 μ l) or (B) in groups at high density (1 embryo/1 μ l). The results are displayed as the percentage of embryos developed to each stage or hatching, pooled from at least 2 experiments except for 0.5 μ M PPP which was pooled from 1 experiment as no embryos survived to the blastocyst stage when cultured in either 0.25 or 0.5 μ M PPP (n values in parentheses). Chi-square analysis was used to compare the development and hatching rate of the control to the treatment groups. * indicates P<0.05 ** indicates P<0.01 *** indicates P<0.001.



Supplementary Fig. S2. Differential staining of trophectoderm and inner cell mass. Scale bars represent 20 µm.

Supplementary video S1. Picropodophyllin disrupts spindle in and oocytes over six hours. Hoechst was used to stain the chromosomes. Oocytes were treated with 0.5 μ M PPP and imaged over 6 hours using the CellVoyagerTM CV1000 Confocal Scanner System, 405 nm laser at 5 % laser power. A Z-stack was taken through the embryo, at 1.7 μ m intervals, every 5 minutes.