BMP signalling in the human fetal ovary is developmentally-regulated and promotes PGC apoptosis

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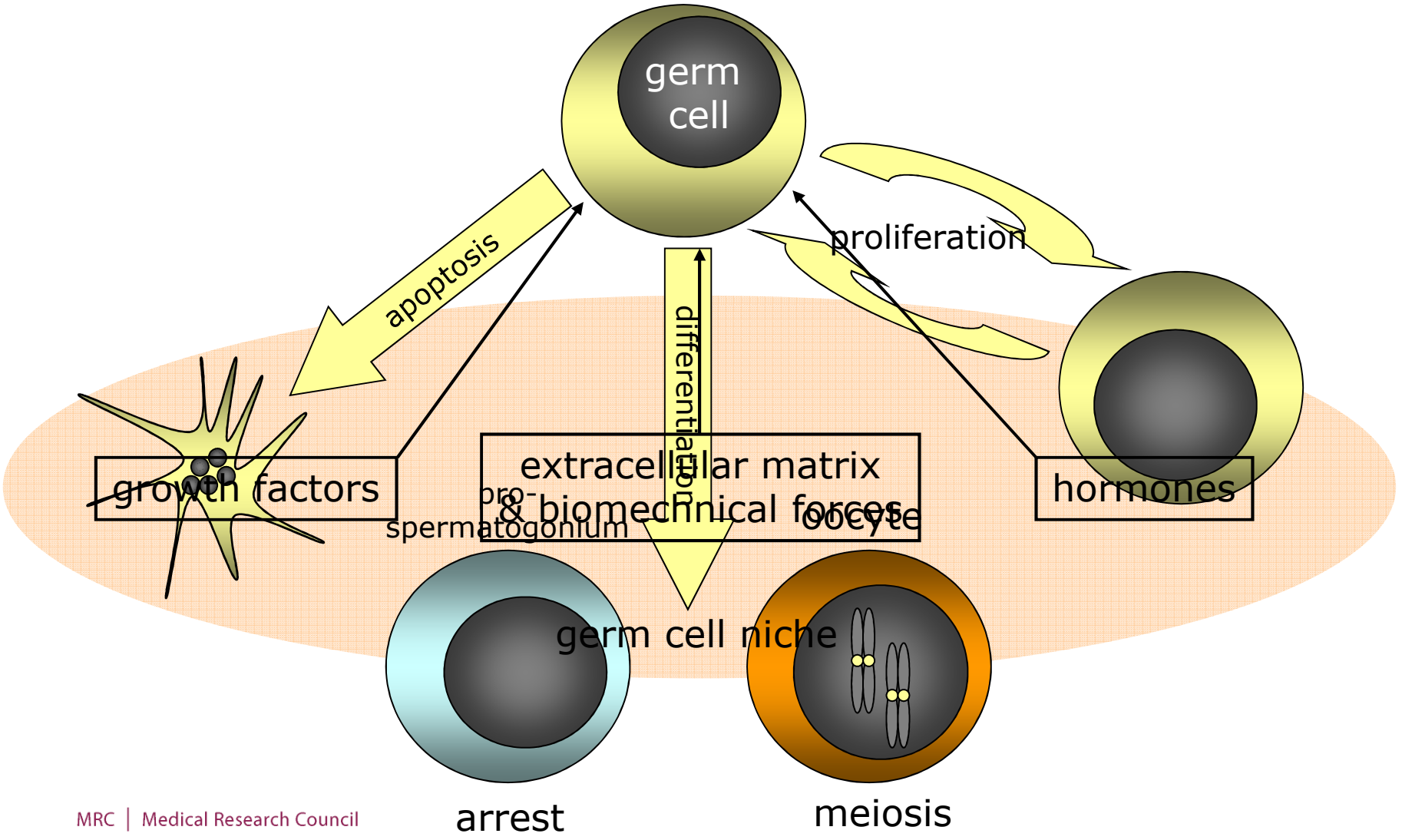
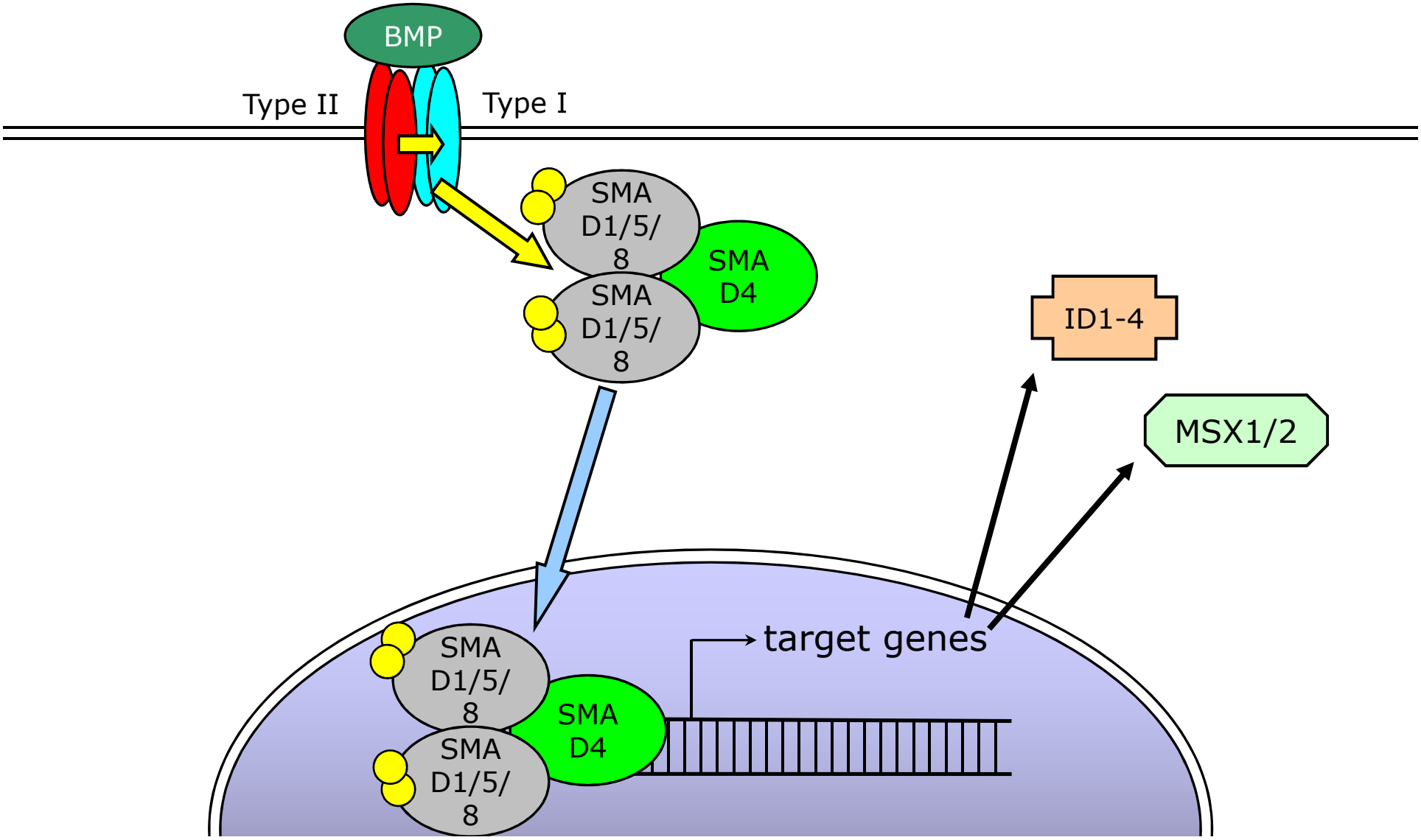
Extrinsic factors regulate germ cell fate decisions



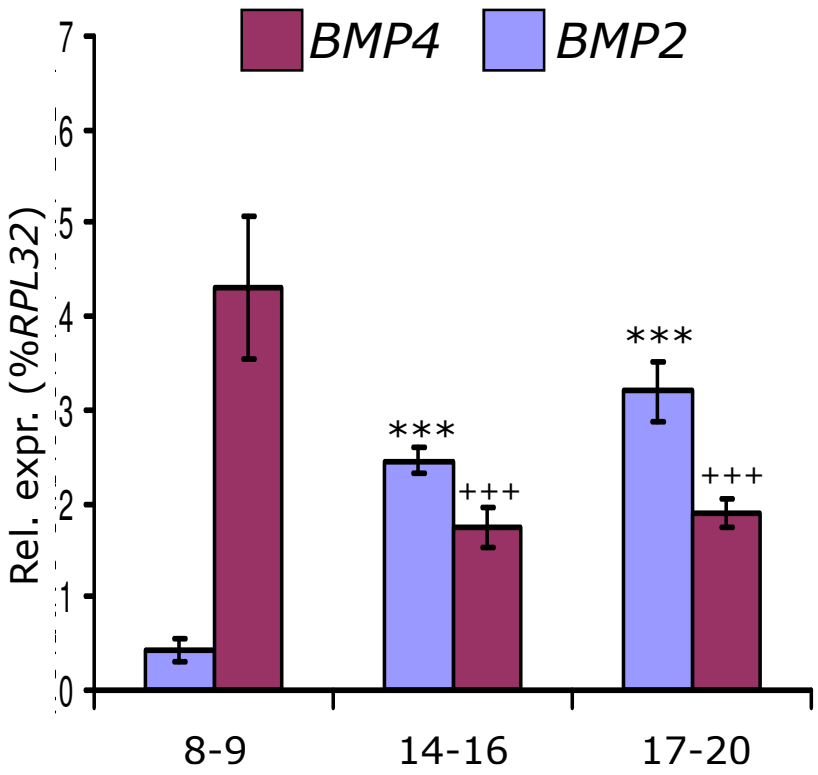
What is the function of BMP signalling in human fetal ovary?

- BMPs essential at multiple stages of gametogenesis
- BMP4 promotes proliferation of isolated mPGCs *in vitro*
  - Pesce *et al.,* 2002
- BMP4-treated fetal mouse ovaries contain fewer meiotic cells
  - Ross *et al*., 2003
- No known roles for BMP4 in regulating human PGCs

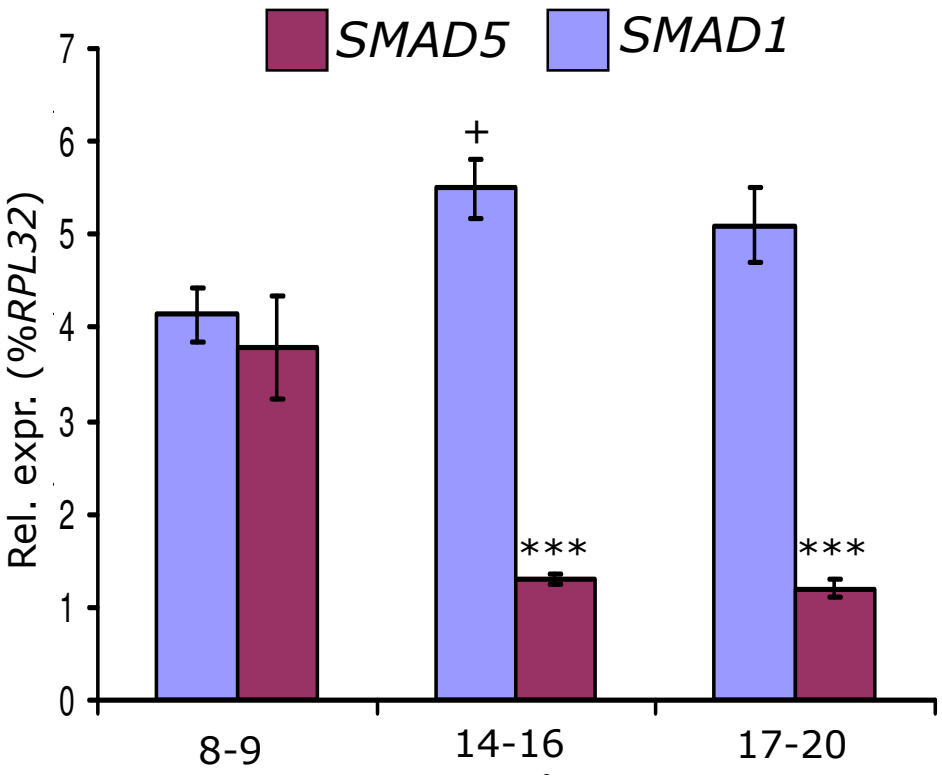
The BMP signalling pathway



Are BMPs expressed in the human fetal ovary?



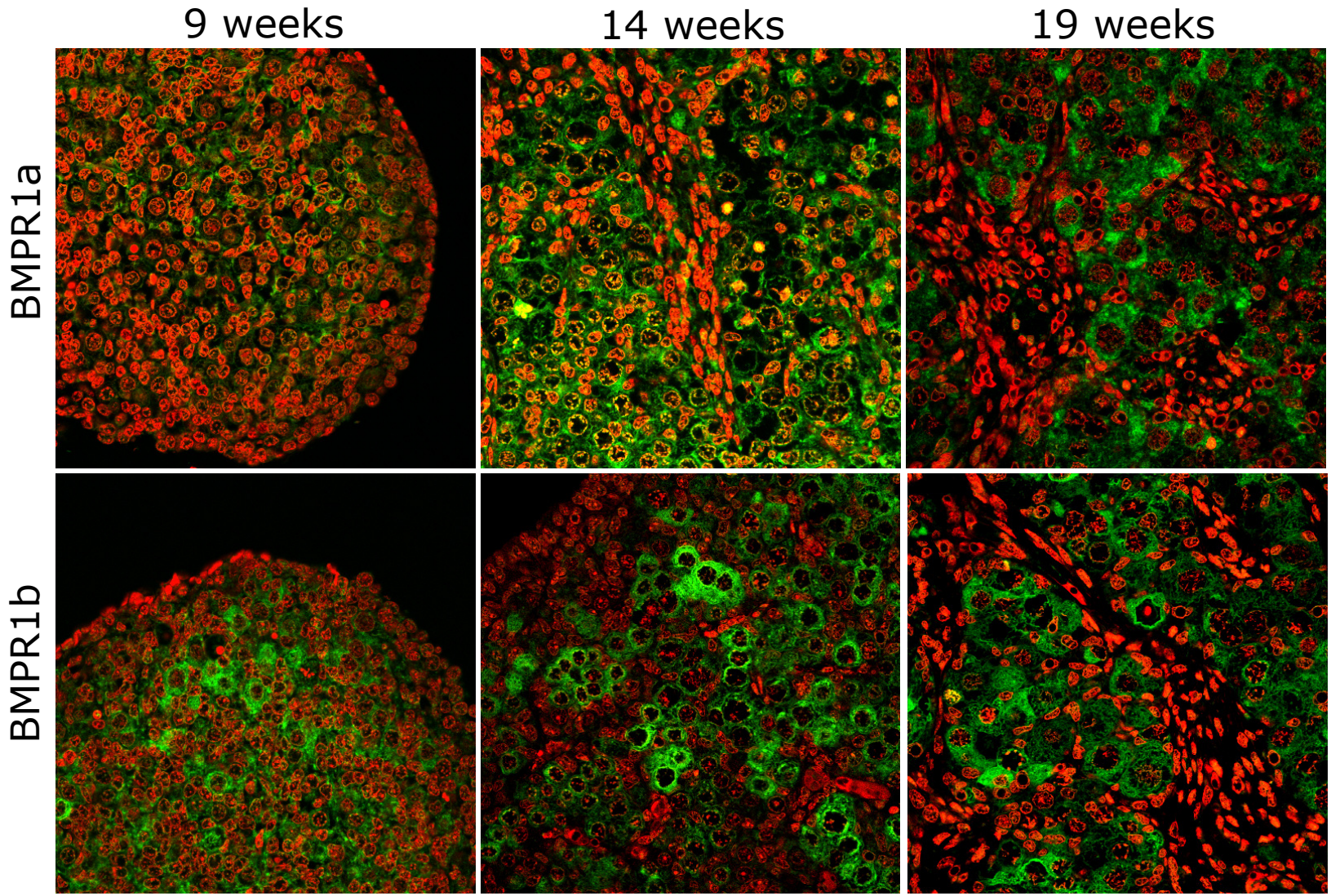
BMP6/7 extremely low



SMAD8 undetectable

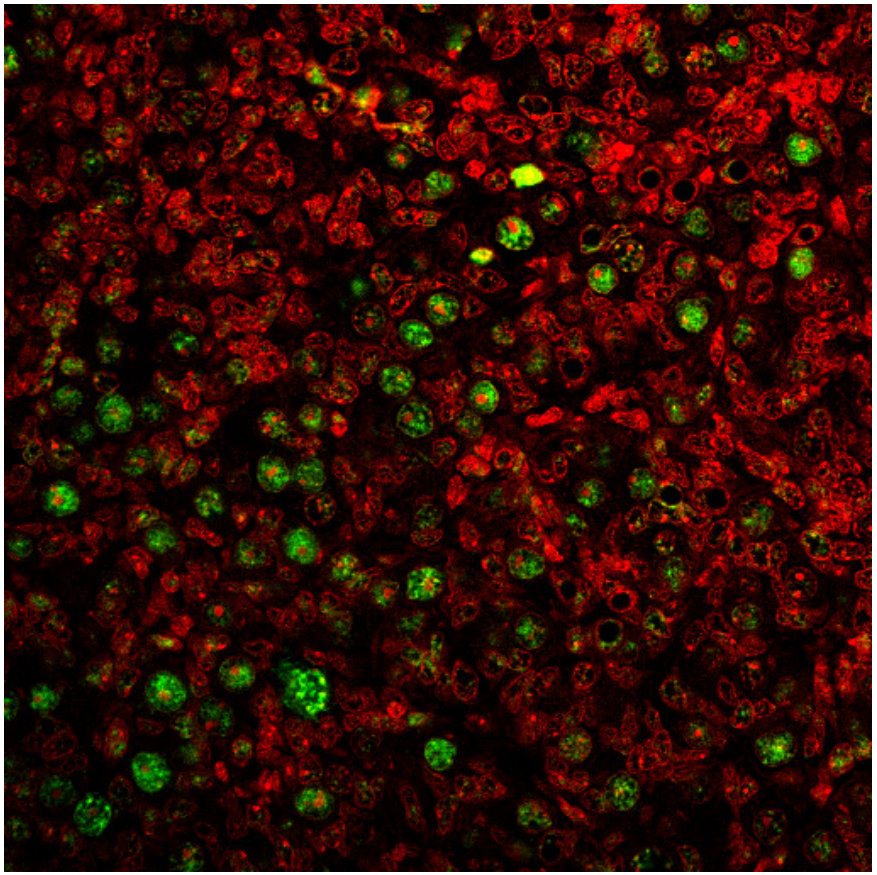
+p=0.05, 8-9 v 14-16 wks, $^{+++}$p=0.001, 8-9 v 14-16/17-20 wks, ***p<0.0001, 8-9 v 14-16 wks, n=5-6

What are the targets of fetal ovarian BMP signalling?

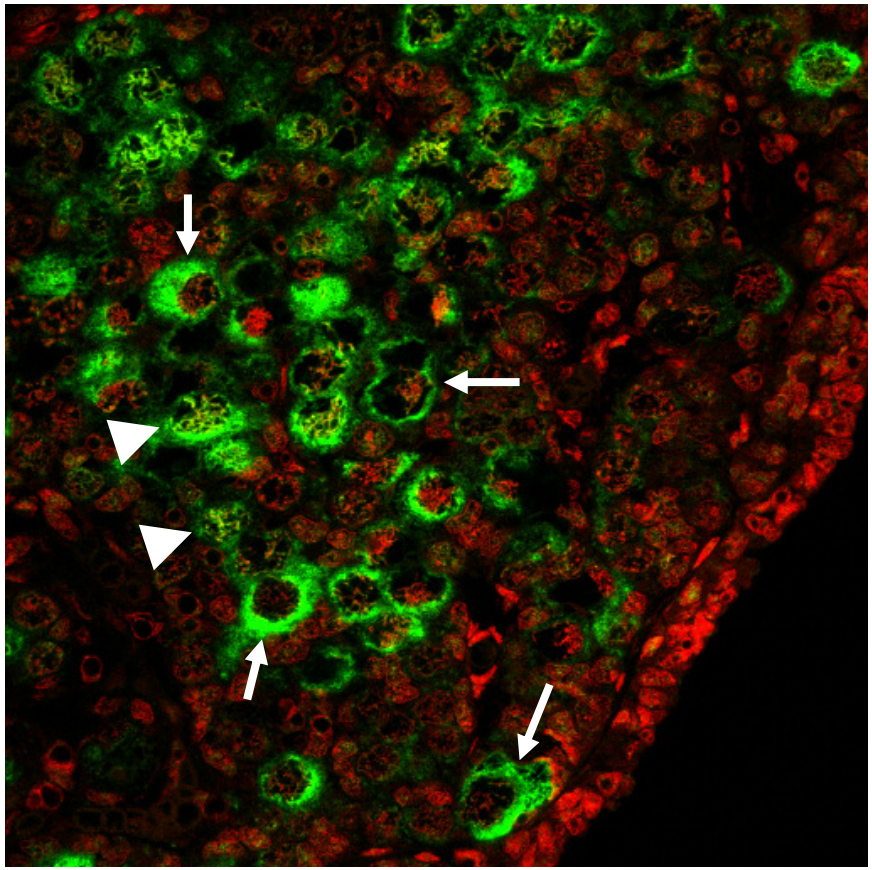


Red:propidium iodide, green:BMPR1a/b

Is BMP signalling active in the human fetal ovary?



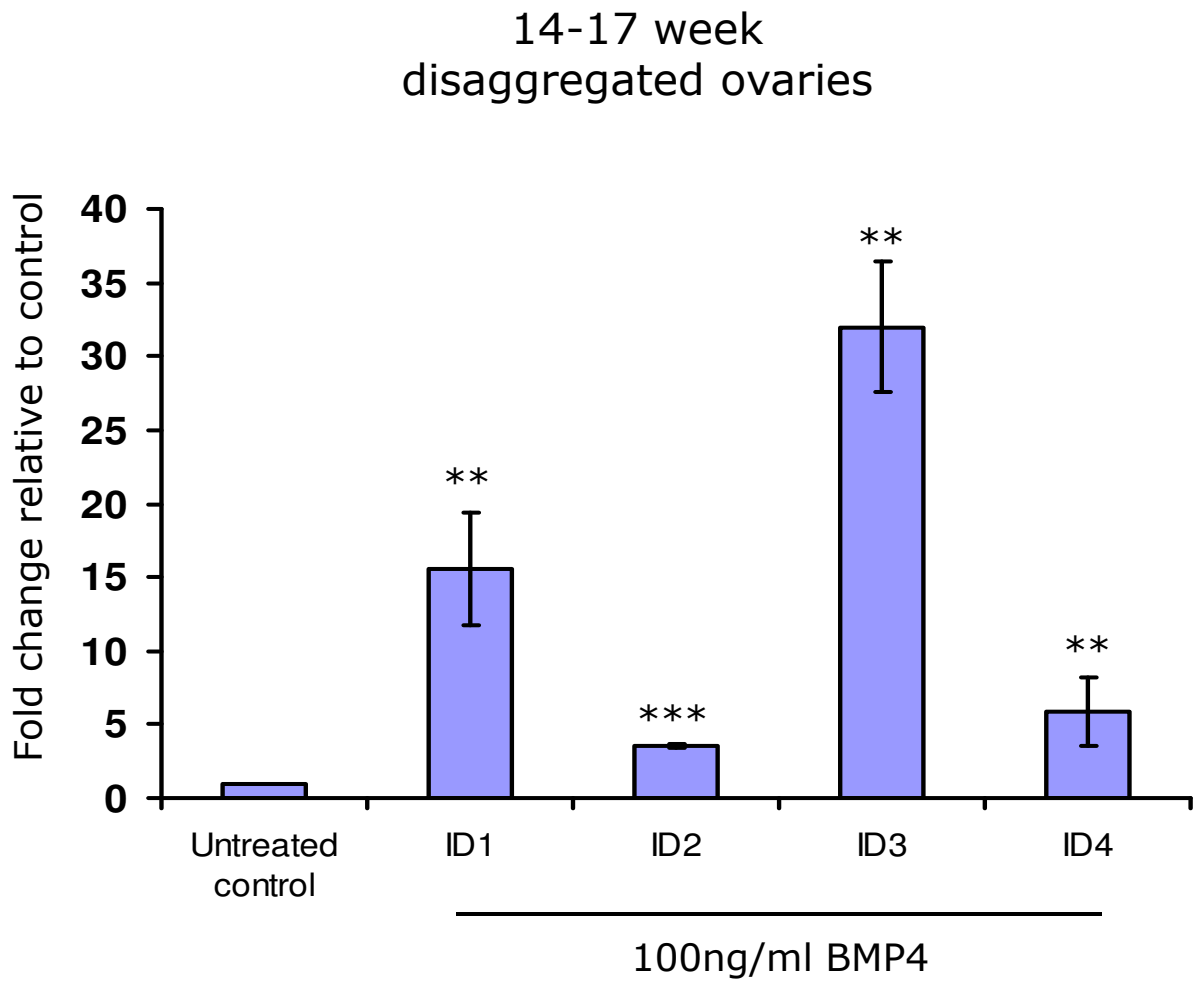
9 weeks



19 weeks

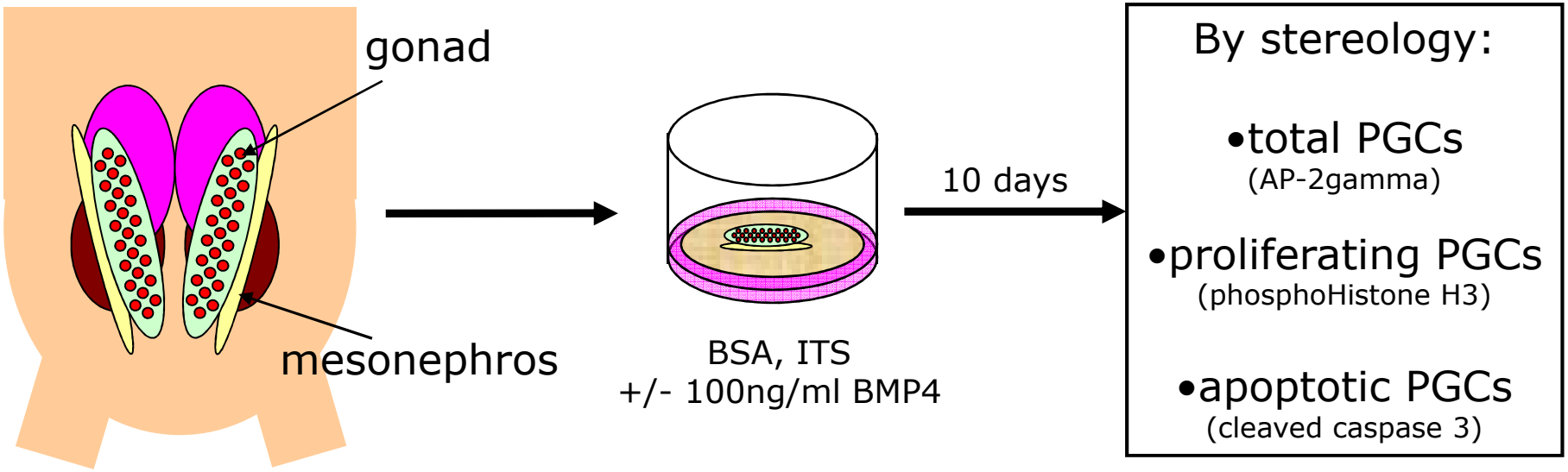
Red:propidium iodide, green:pSMAD1/5

Re-localisation of pSMAD1/5 does not impair germ cell responsiveness to BMP4



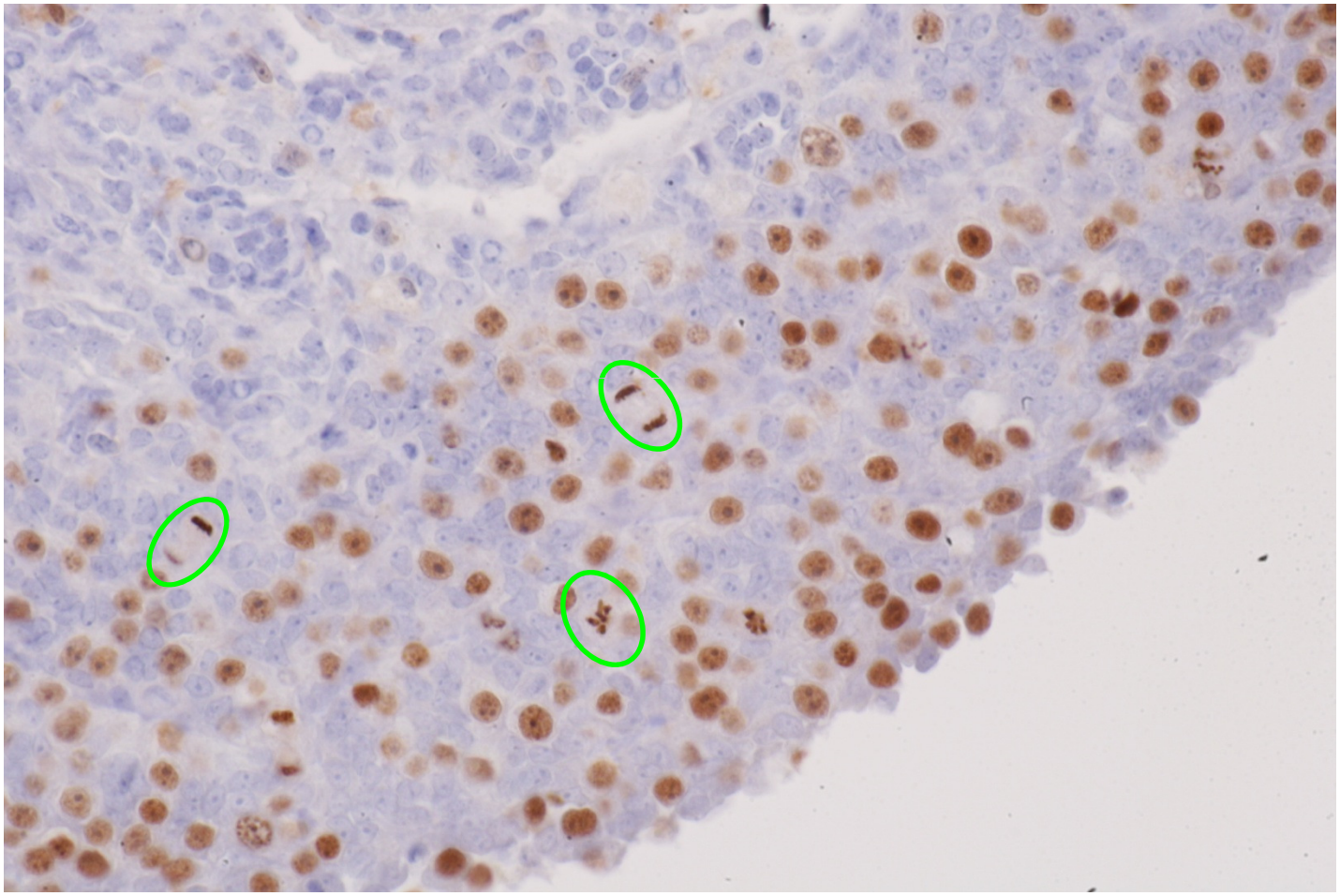
(**p<0.01,***p<0.001, n=3)

Determining the effects of BMP4 treatment on first trimester germ cells



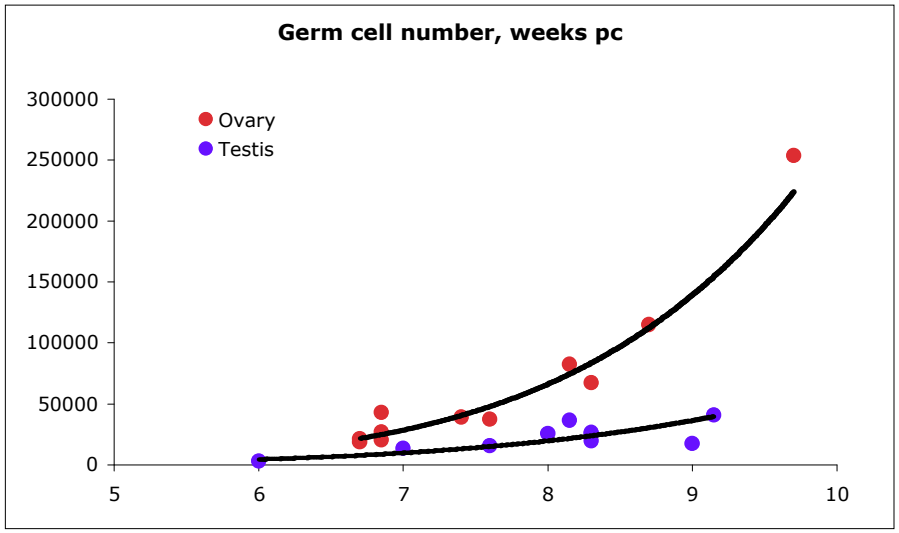
- retains germ-somatic cell interactions
- more accurately reflects the *in vivo* situation

Gonadal architecture is maintained *ex vivo* in the absence of growth factor support

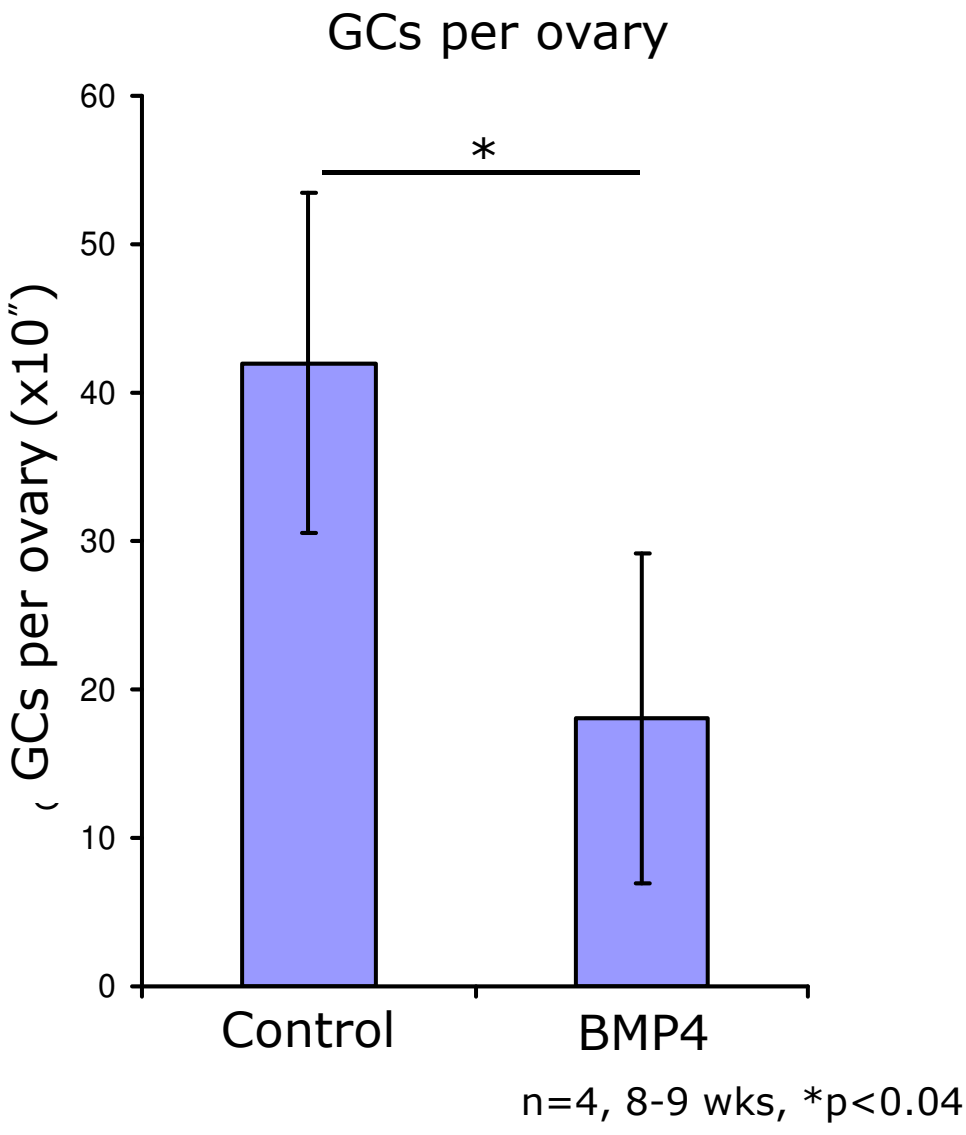


63d ovary +10d culture, GC marker: AP-2γ

Does BMP4 treatment affect germ cell number?



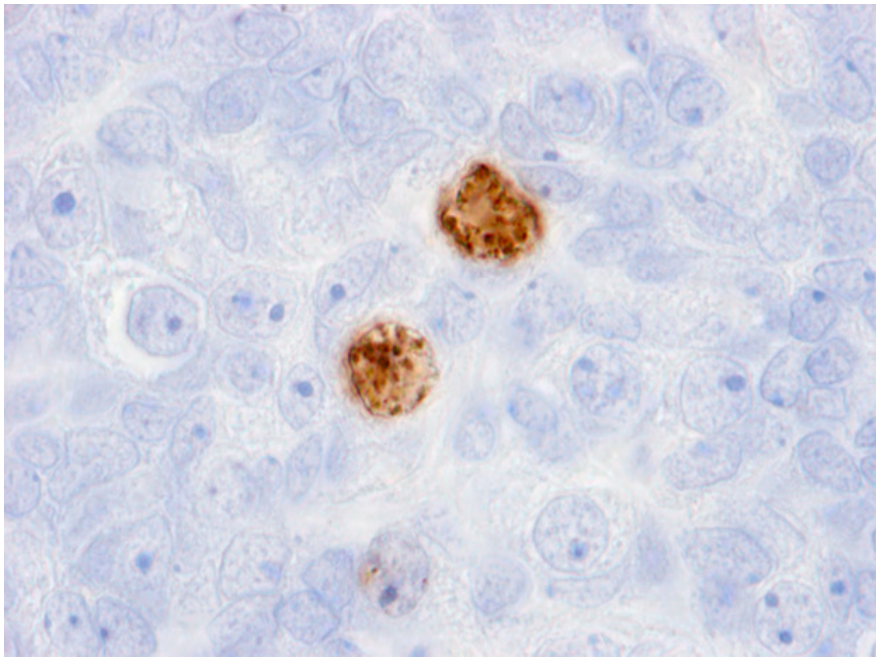
Data from Bendsen et al 2003, 2006



n=4, 8-9 wks, *p<0.04

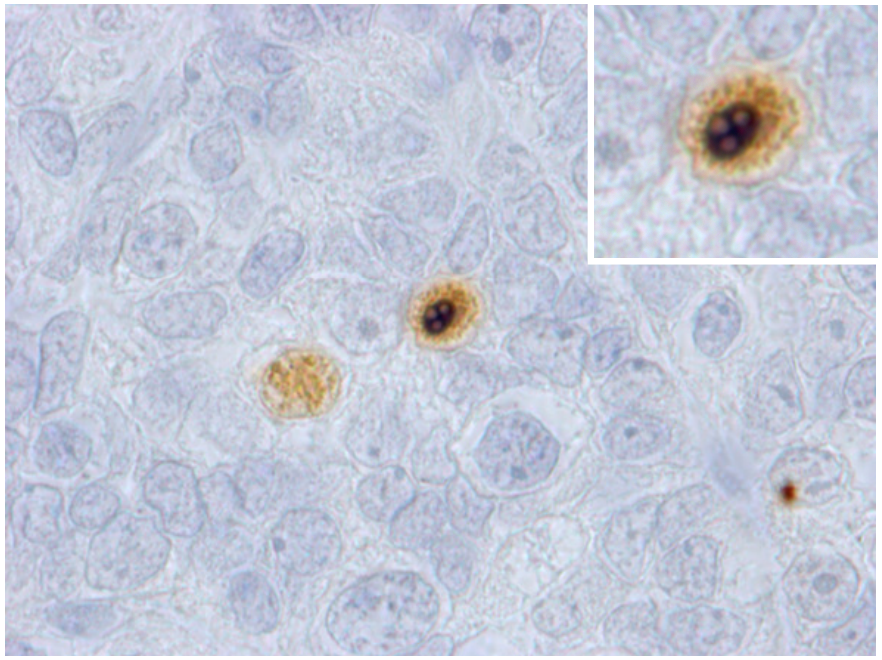
Does BMP4 inhibit proliferation or promote apoptosis?

Proliferation



phosphoHistone H3

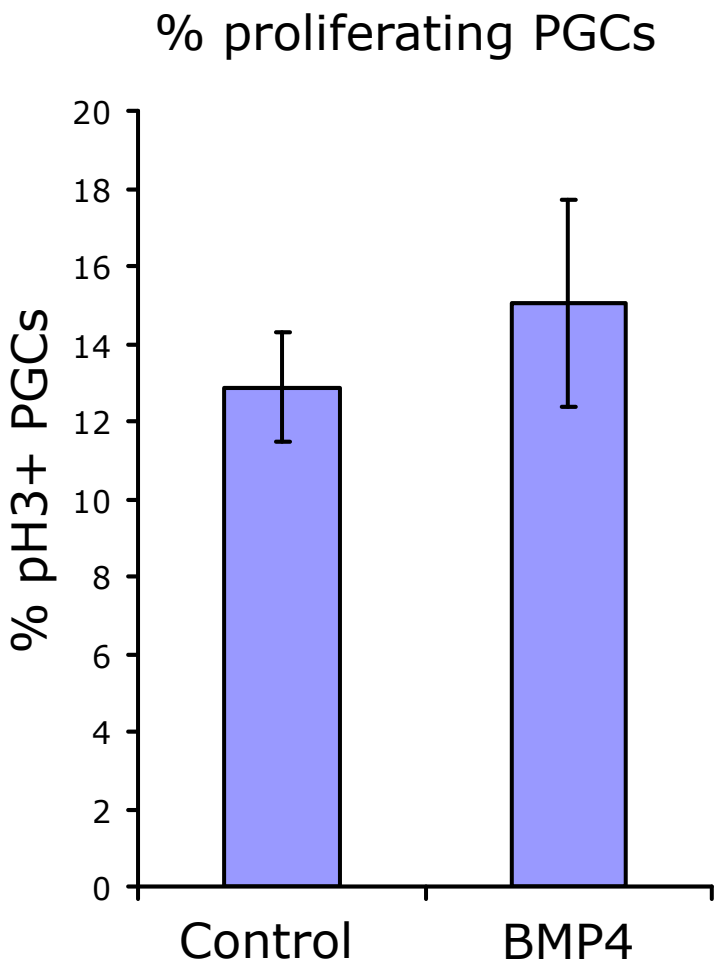
Apoptosis

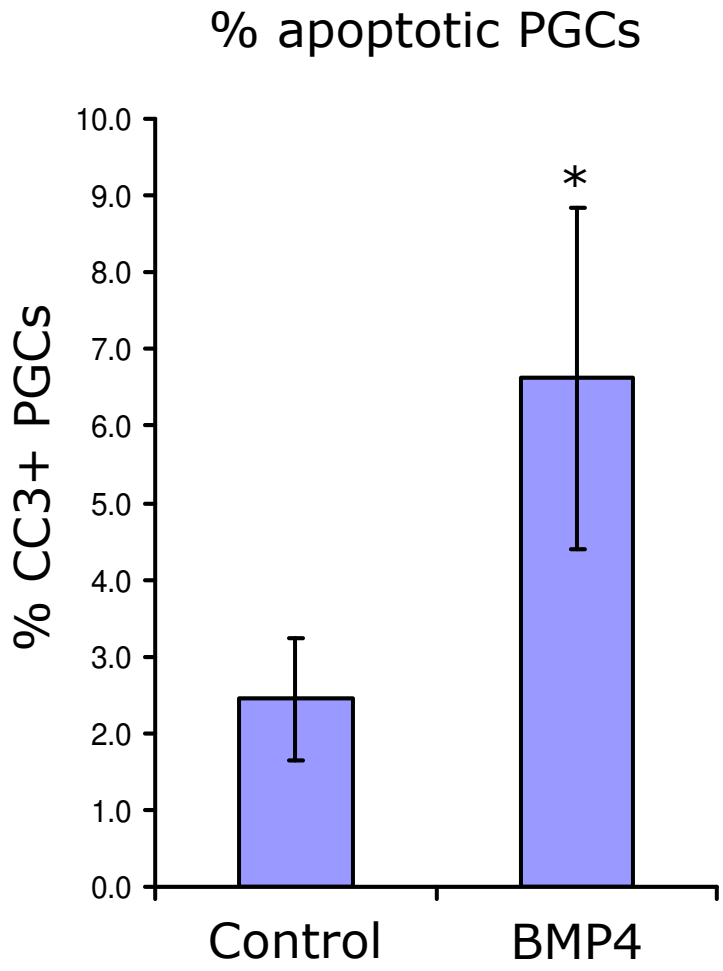


Cleaved Caspase 3

63d ovary +10d culture

Does BMP4 inhibit proliferation or promote apoptosis?



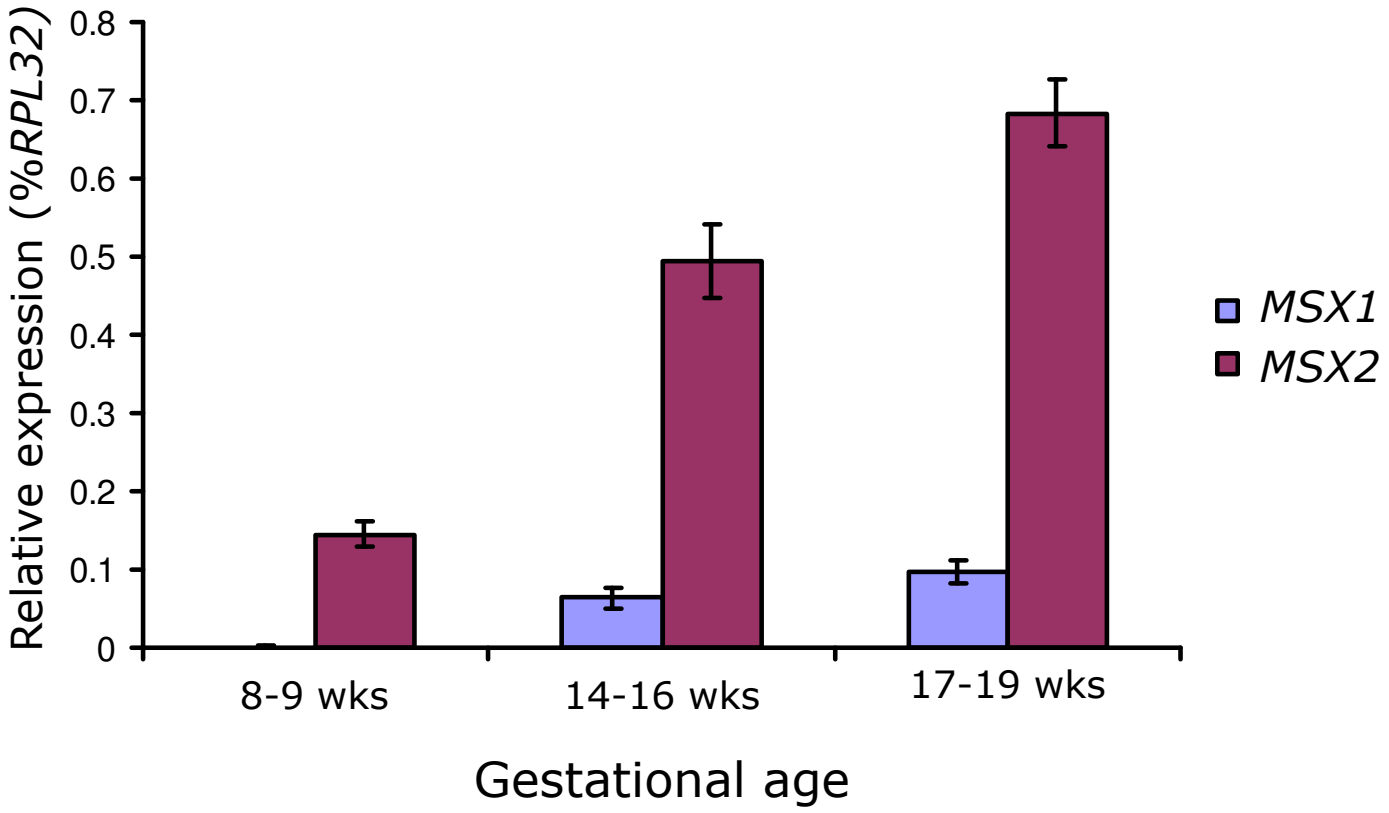


n=4, 8-9 wks, *p=0.03

BMPs have pro-apoptotic effects in diverse developmental contexts

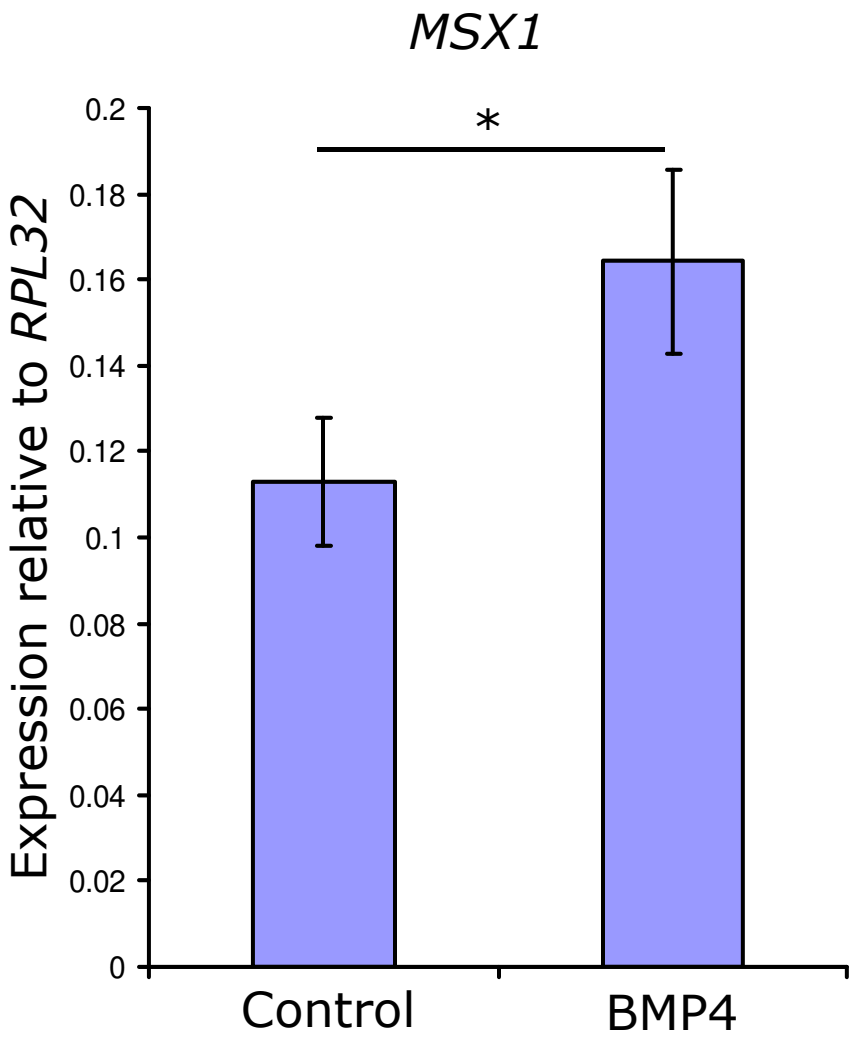
- Eye development
  - Trousse *et al*., 2001
- Capillary regression
  - Kimono and Shibuya, 2003
- Inter-digital apoptosis during limb morphogenesis
  - Zou and Niswander, 1996
- Focal apoptosis during brain development
  - Graham *et al.* 1993, 1994
- Promote apoptosis in association with MSX proteins
  - *Hox*-related developmental regulators
  - expression not previously described in the gonad

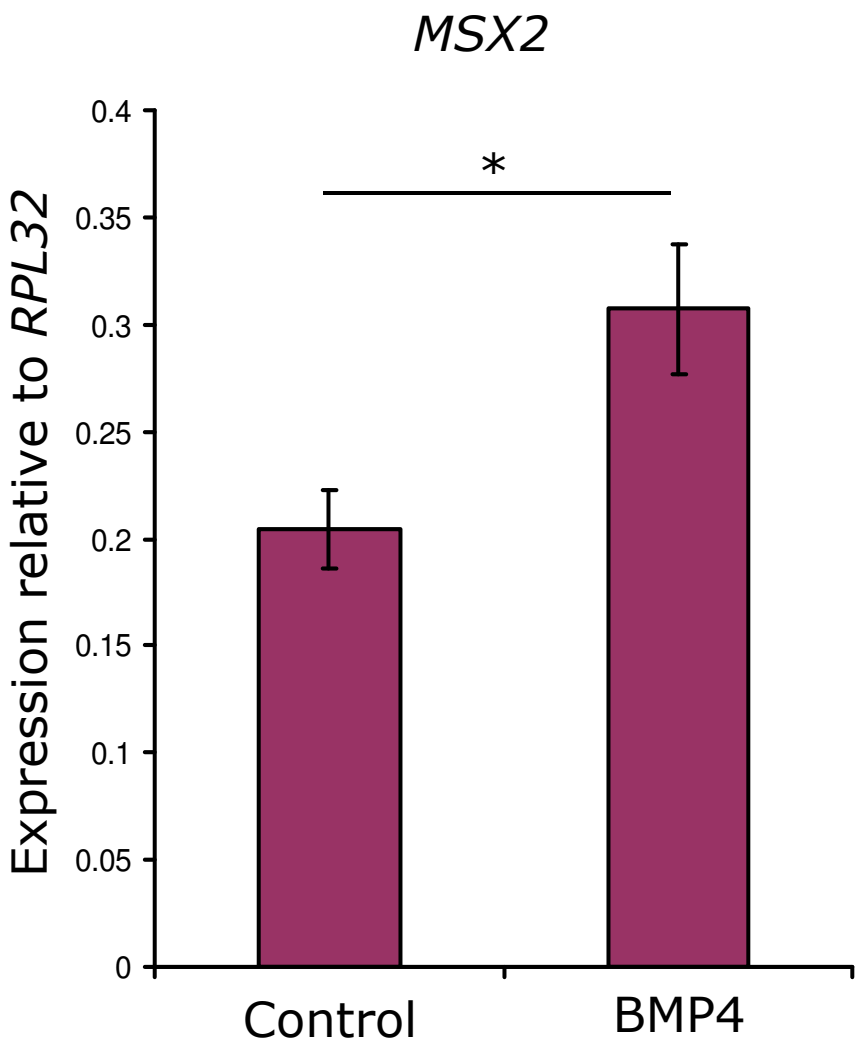
MSX genes are expressed in human fetal ovary



n=5-6 per group

Does BMP4 promote *MSX* gene expression in the human fetal gonad?





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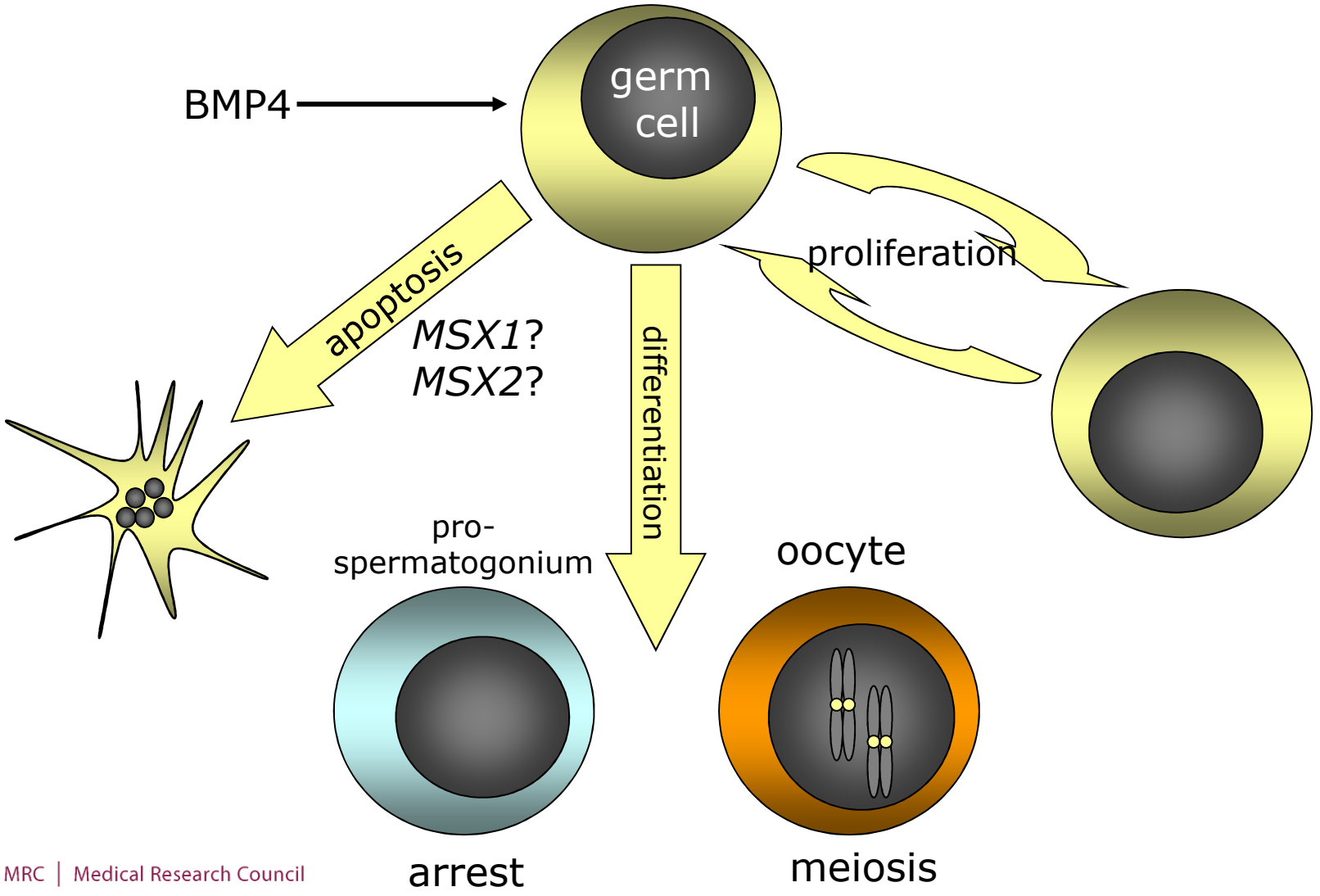
(n=14, mixed sex, 8-9 weeks, *p<0.05)

BMP4 promotes apoptosis in human PGCs



Conclusions

- Expression and functionality of BMP signalling is developmentally-regulated in human fetal ovary.

- BMP4 promotes apoptosis in human ovarian PGCs.
    - May explains the reduction in meiotic cells in BMP-treated fetal mouse ovaries.

- Organ culture vs. isolated PGCs?
    - Subtle differences in developmental stage?
    - Effect of feeder cells?

Acknowledgements

MRC HRSU

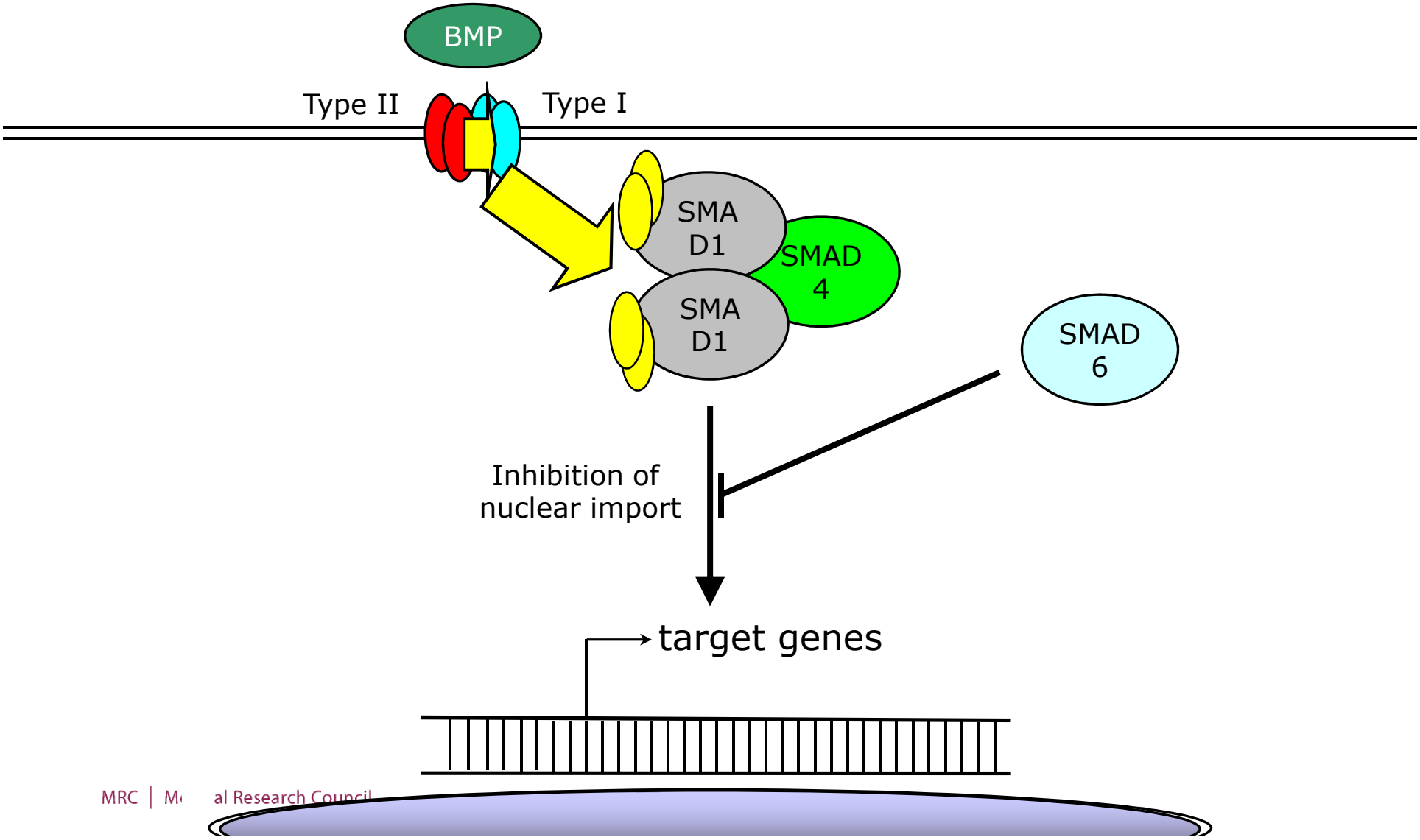
Hazel Kinnell
Craig Collins
Kirsten Hogg
Samira Tritton
Rosey Bayne
Richard Anderson

Alan McNeilly

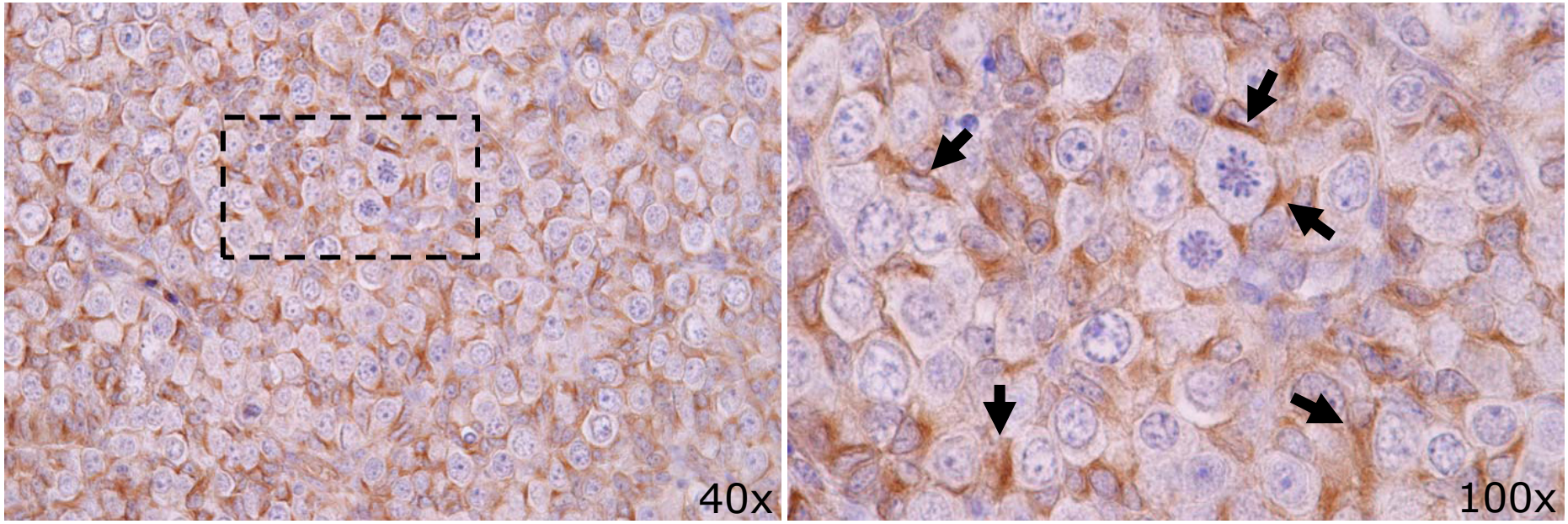
R.I.E.

Anne Saunderson
Joan Creiger
Isobel Morton
Bruntsfield Suite staff

SMAD6 can inhibit SMAD nuclear translocation by sequestering pSMAD1/5



Does SMAD6 inhibit pSMAD1/5 nuclear trans-localisation?

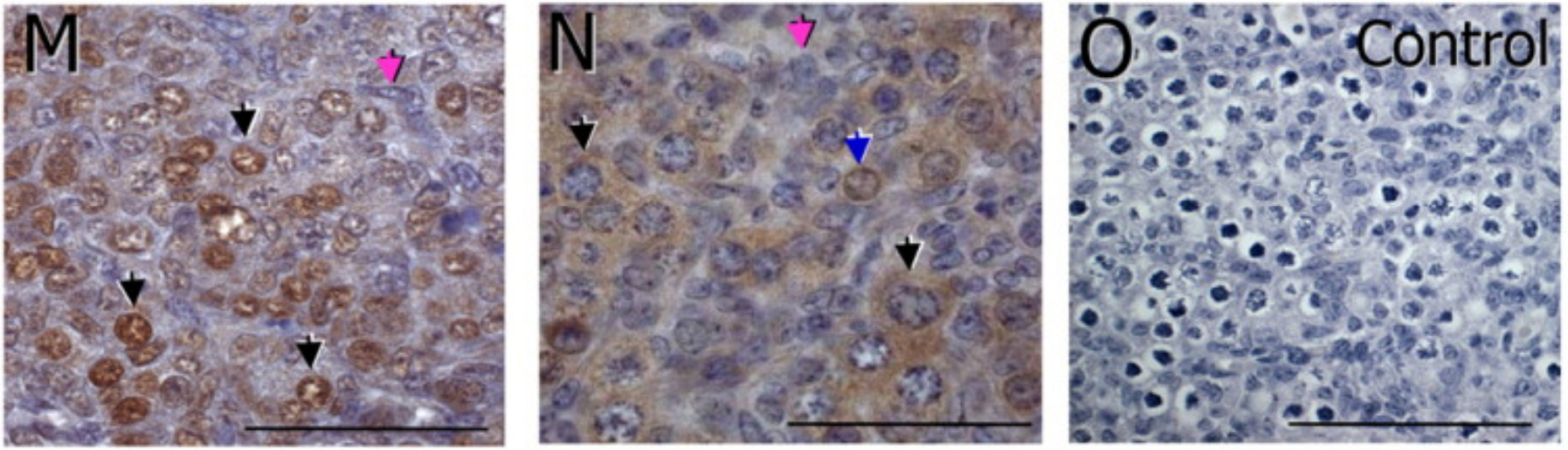


SMAD6 expressed exclusively by somatic cells

SMAD6; 14wk fetal ovary

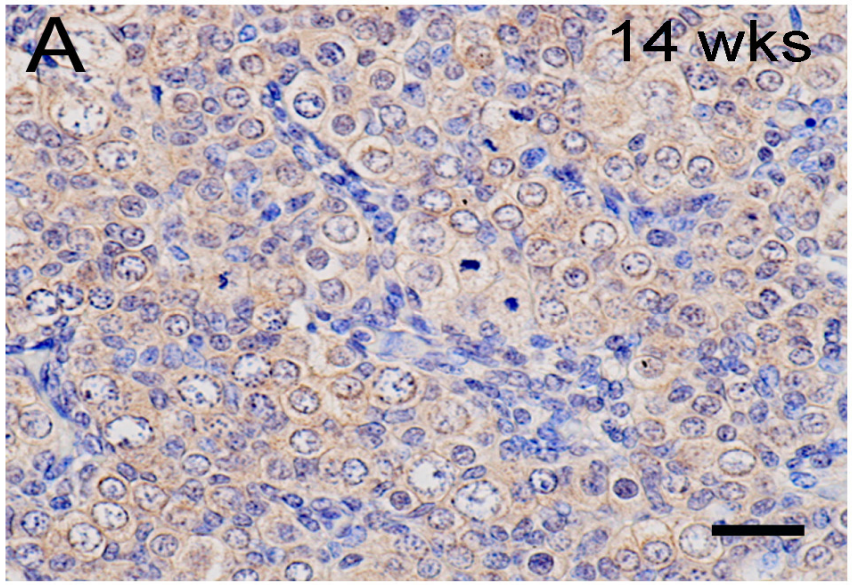
RanBP5 / Importinβ3 relocalises at meiosis in the fetal mouse ovary

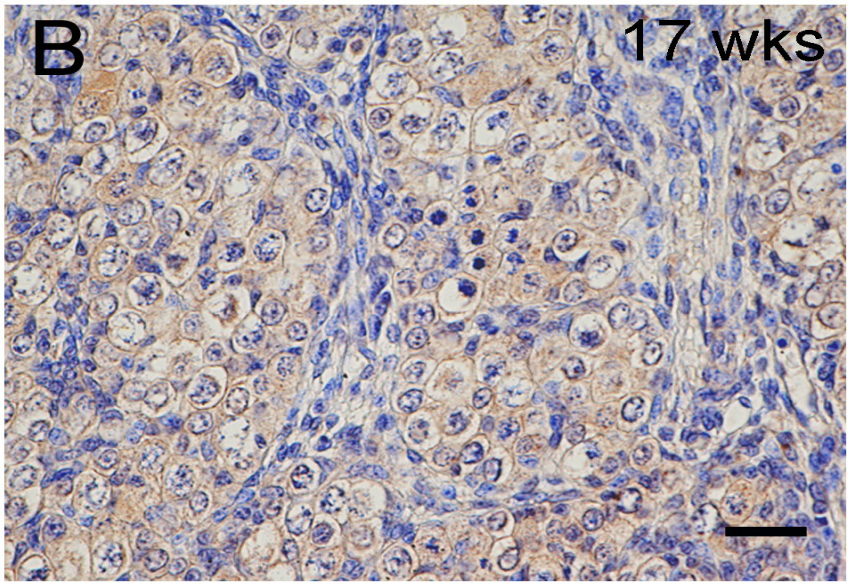
E13.5 E14.5

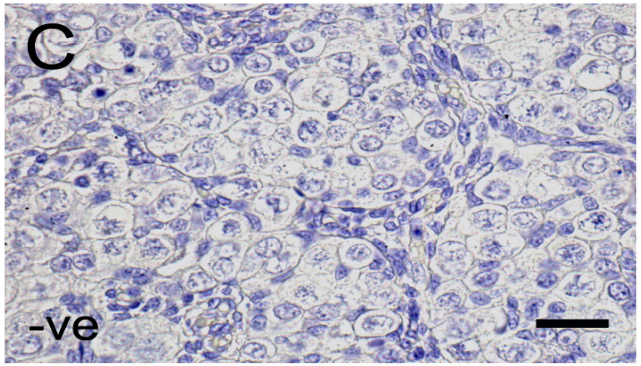


Hogarth *et al.* (2007) Dev Dyn 236:2311

Is BMP4 an autocrine factor?







Antibody specificity?

Typical cross-reaction of 5-10% with BMP2, and BMPRs

K Hogg

Effects of dorsomorphin on gonadal development

62d uncultured

DMSO

10uM Dorsomorpin

DMSO

10uM Dorsomorphin