In vitro follicle growth to investigate follicular paracrine interactions –

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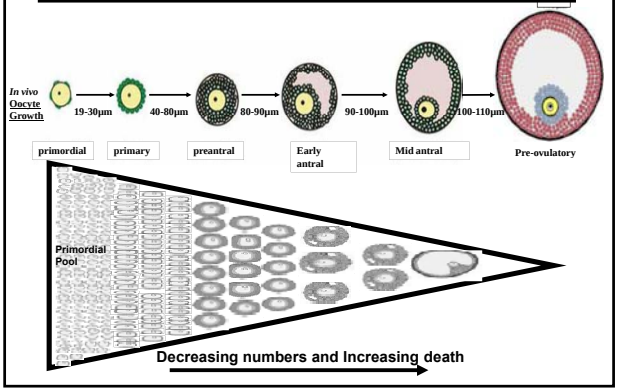
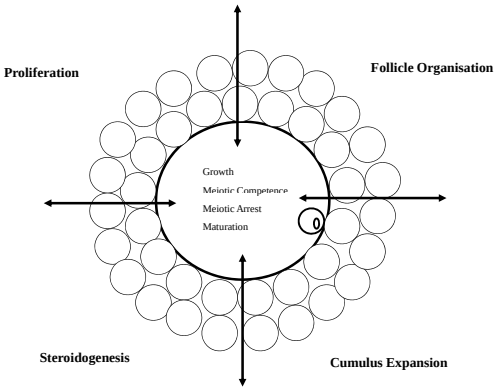
Please Note: These slides may not represent the final content of the presentation

Oocyte/Follicle Development



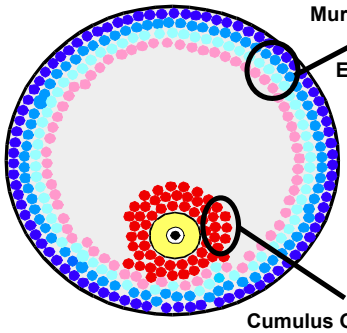
Why culture follicles?

- **Define the fundamental mechanisms of oocyte development (basic science)**
- **Clinical applications: Fertility preservation – restore fertility**
- **Agriculture**
- **Genetic modification**
- **Endangered species**
- **Germ line preservation**
- **Toxicity testing**

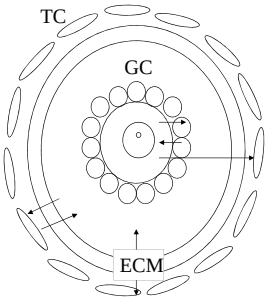


Culture of Preantral Follicles: Rodent Models

- Whole preantral follicles
  - Physiological model
  - Useful for study of mechanisms of oocyte/follicle development
  - Require manual dissection
- Oocyte-granulosa cell complexes
  - Maintain oocyte-granulosa cell interaction
  - Useful for production of mature oocytes
  - Easily isolated using enzymes (e.g. collagenase)



Cell-Cell-Matrix Interactions

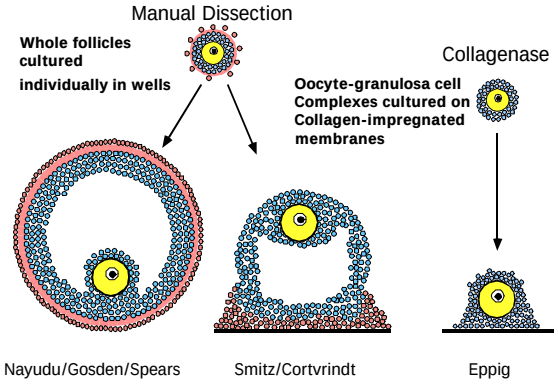


Oocyte-follicle interaction
Oocyte growth
Cumulus expansion
GC proliferation/differentiation
Steroidogenesis
Theca formation

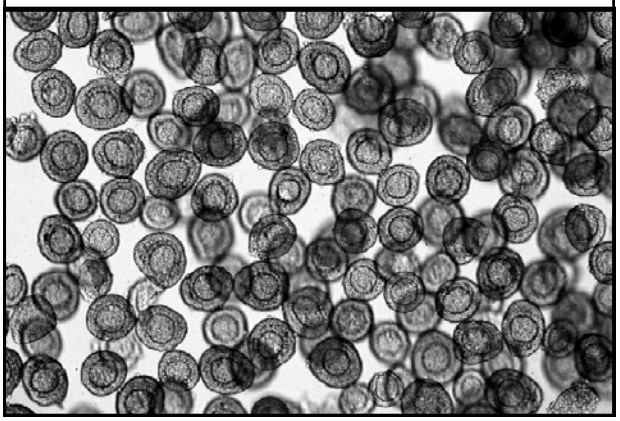
Granulosa-theca interaction
Proliferation
Steroidogenesis

ECM-granulosa/theca interaction
Morphology
Proliferation
Steroidogenesis
Survival

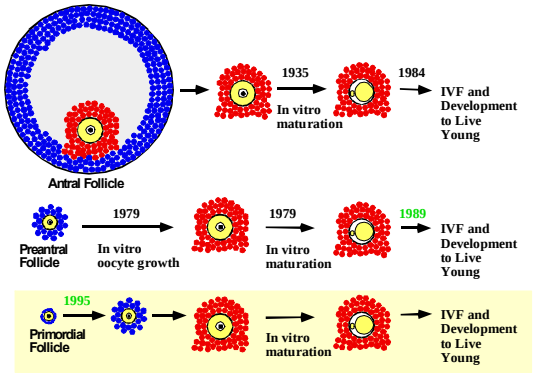
Culture Systems for Mouse Preantral Follicles/Oocytes



Oocyte-granulosa cell complexes isolated from 12 day old mice



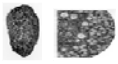
Mouse Follicle Culture Systems



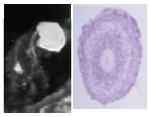
Slide from John Eppig

Culture systems that support development of follicles from domestic species (cattle and sheep)

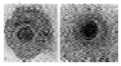
DEVELOPMENT OF CULTURE SYSTEMS



Cortical Strip Culture: Primordial Initiation

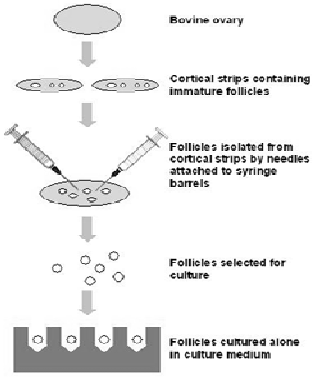


Isolated Follicles: Preantral -antral transition



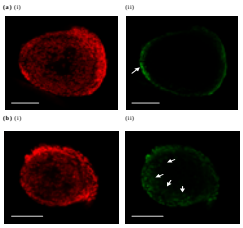
Antral Follicles: Oocyte Competence

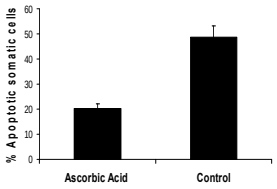
Preantral Follicle Culture



Isolated Bovine Preantral Follicles

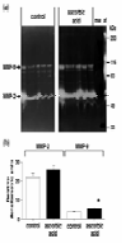
Development of a serum free culture system





Thomas F.H, Leask,R., Sršen,V., Riley,S.C., Spears N., Telfer E.E. (2001) Ascorbic Acid Maintains Health and Morphology of Bovine Preantral Follicles During Long Term Culture. Reproduction: 122, 487-495.

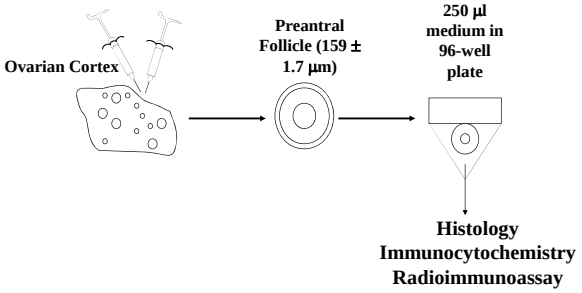
Production of MMP-9 is associated with health and antral formation



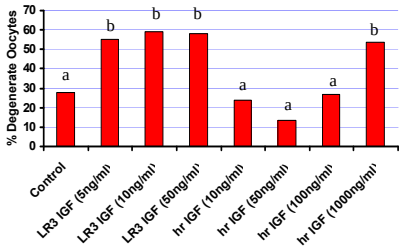
	MMP-2	MMP-9	TIMP-1	TIMP-2
Healthy	97%	53%	100%	100%
Degenerate	70%	0%	29%	29%
Antrum	100%	95%	100%	100%
No antrum	47%	47%	43%	43%

McCaffery, FH, Leask, R., Riley, S.C.,Telfer,E.E. (2000)
Culture of bovine preantral follicles in a serum free system: markers for assessment of growth and development. Biology of Reproduction 63, 267-273

Culture System: Bovine and Ovine follicles

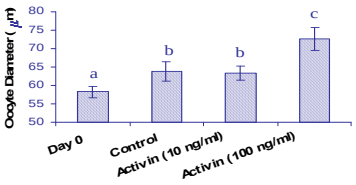


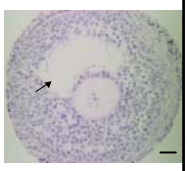
Effect of IGF-1 exposure on oocyte degeneration



Thomas, Campbell, Armstrong & Telfer.(2007) Reproduction (In press)

Effect of activin on oocyte growth *in vitro*

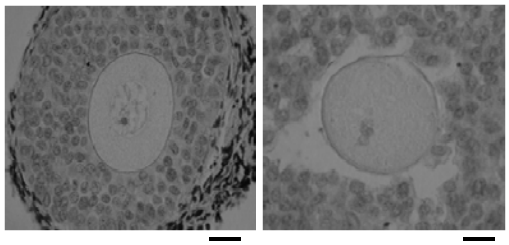




100ng/ml activin

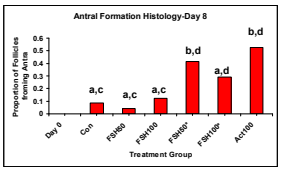
Thomas, Armstrong, Telfer (2003) Activin promotes oocyte development in ovine preantral follicles in vitro. ***Reproductive Biology and Endocrinology***. **1**: 76-81

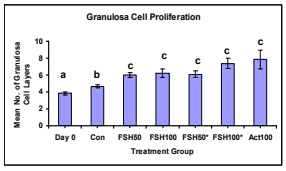
Oocyte Morphology

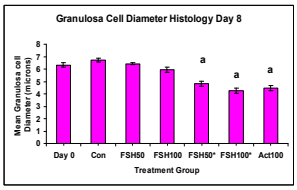


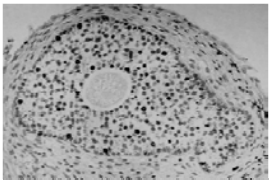
20 μm 20 μm

Activin *v* FSH & Activin



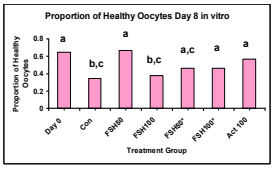


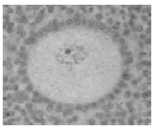




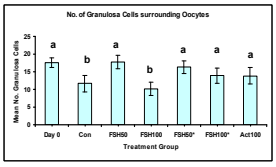
Activin & 50ng/ml – Ki67, Day 8

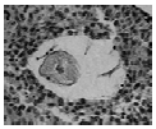
Activin *v* FSH & Activin II





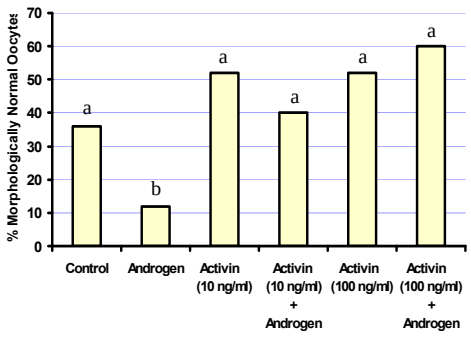
FSH50ng/ml





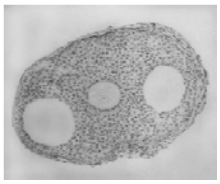
Control

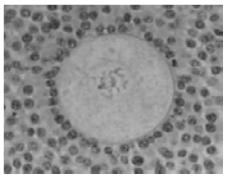
Oocyte Morphology



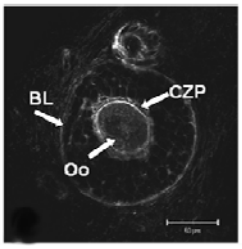
Activin/FSH combination affects

- Follicle Growth
- Antral cavity formation
- Oocyte Development

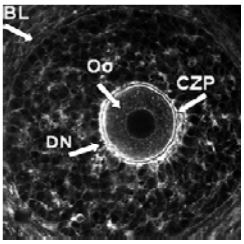




Actin Distribution & Density

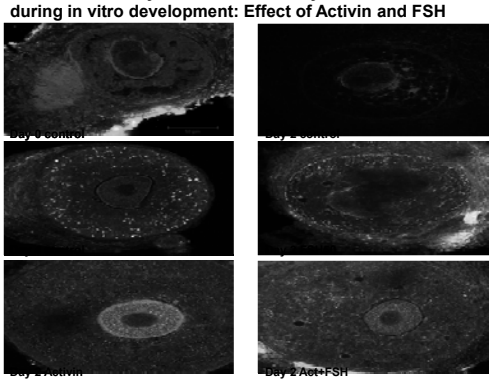


Control



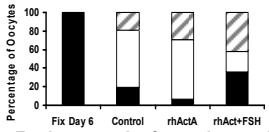
rhAct A

Stabilisation of Cytoskeleton and oocyte-somatic cells during in vitro development: Effect of Activin and FSH



McLaughlin et al., 2010 MHR

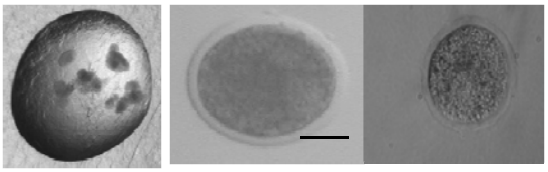
Growth of bovine IVG oocytes 6 days step 1 followed by 12 days step 2



Mean oocyte diameter

- ≤ 50
- 50-80
- 80-100

Further growth of complexes within alginate (Step 3) before IVM



Oocytes of up to 108 microns.

References

- **References:**
- McCaffery, FH, Leask, R., Riley, S.C., Telfer, EE. (2000) Culture of bovine preantral follicles in a serum free system: markers for assessment of growth and development. **Biology of Reproduction. 63**, 267-273
- Thomas F.H, Leask,R., Sršen,V., Riley,S.C., Spears, N., Telfer, EE. (2001) Ascorbic Acid Maintains Health and Morphology of Bovine Preantral Follicles During Long Term Culture. ***Reproduction*. 122,** 487-495.
- Thomas F.H, Walters, KA and Telfer, E.E. (2003) How to make a good oocyte: An update on in vitro models to study follicle regulation. ***Human Reproduction Update.* 9,** 1-15
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- Ding CC, Thong KJ, Krishna A, **Telfer EE**.2010 Activin A inhibits activation of human primordial follicles in vitro. **J Assist Reprod Genet**. ;27(4):141-7.
- McLaughlin M, Patrizio P, Kayisli U, Luk J, Thomson TC, Anderson RA, **Telfer EE**, Johnson J (2011) mTOR kinase inhibition results in oocyte loss characterized by empty follicles in human ovarian cortical strips cultured in vitro. **Fertil Steril**; 2011 Nov;96(5):1154-9