In vitro development of human ovarian follicles

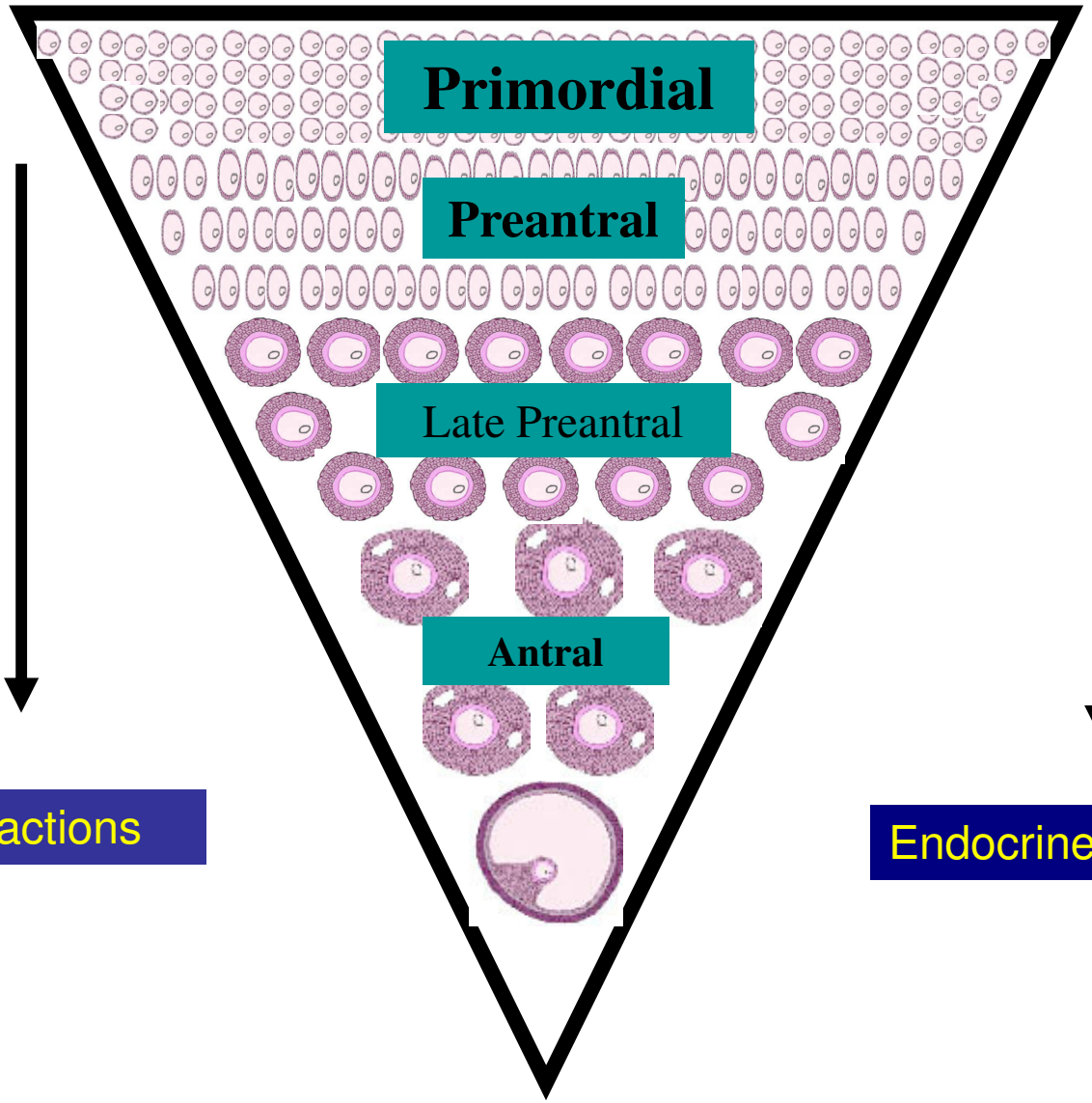
Evelyn E Telfer
Institute of Cell Biology
University of Edinburgh

Fertility preservation options

- Embryo Freezing
- Oocyte Freezing (hormonal stimulation)
- Immature oocyte freezing (IVM)

- Whole Ovary Freezing
- Ovarian Tissue Freezing (Cortical strips)

Developmental Regulation of oocyte number *in vivo*



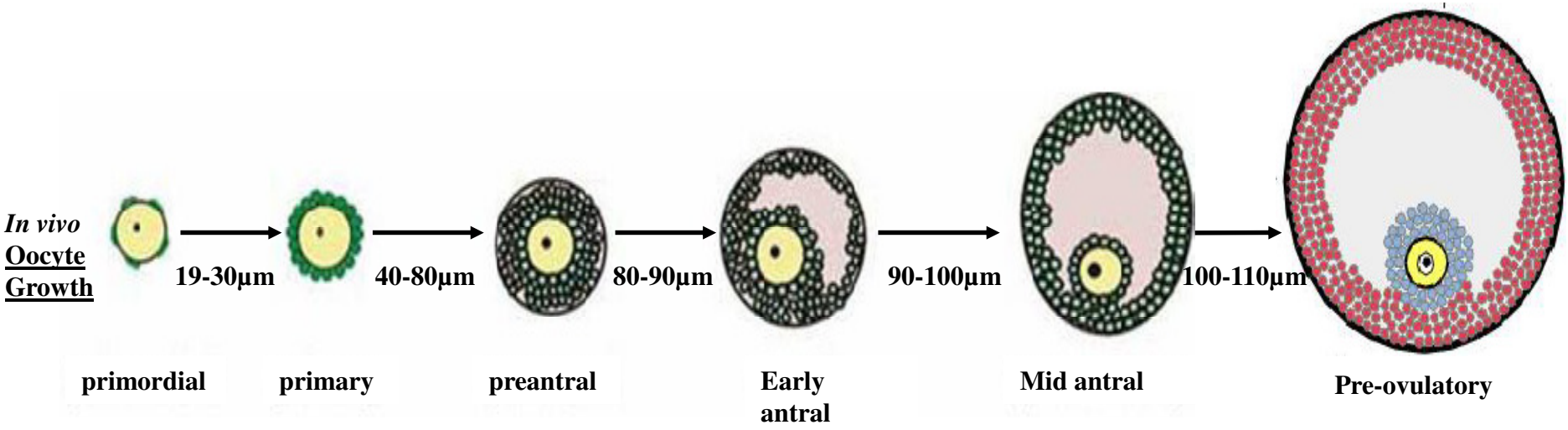
Decreasing Numbers with Development

Increasing levels of apoptosis

Follicle Interactions

Endocrine Regulation

Oocyte/Follicle Development



Growth/ Meiotic Arrest

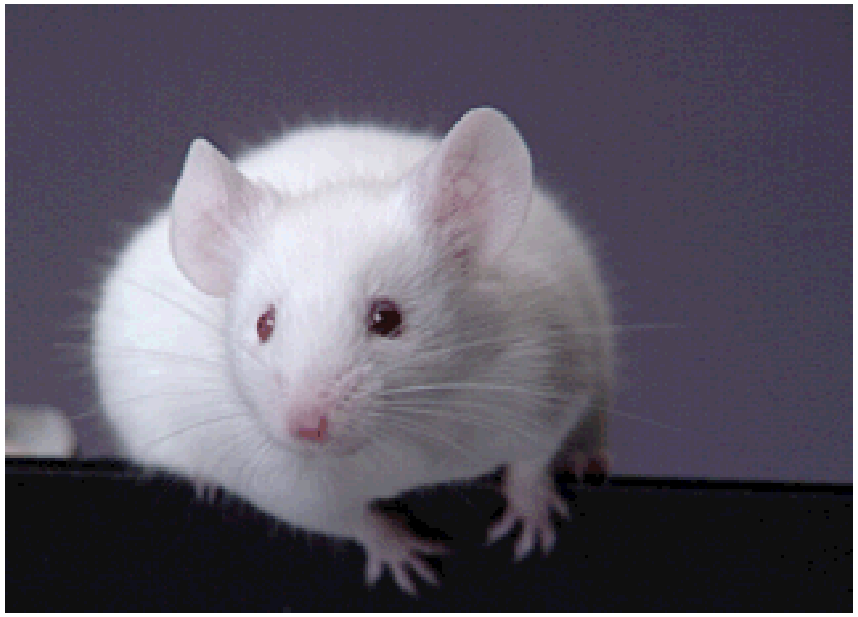
Acquisition of Meiotic Competence

Acquisition of Developmental Competence

Transcription/Transcriptional Repression

Genomic imprinting

Mice from Primordial Follicles…



Eppig & O'Brien 1996

Eggbert: First mouse born from an *in vitro* grown primordial follicle: 2 step system total of 22 days *in vitro* before IVM and IVF

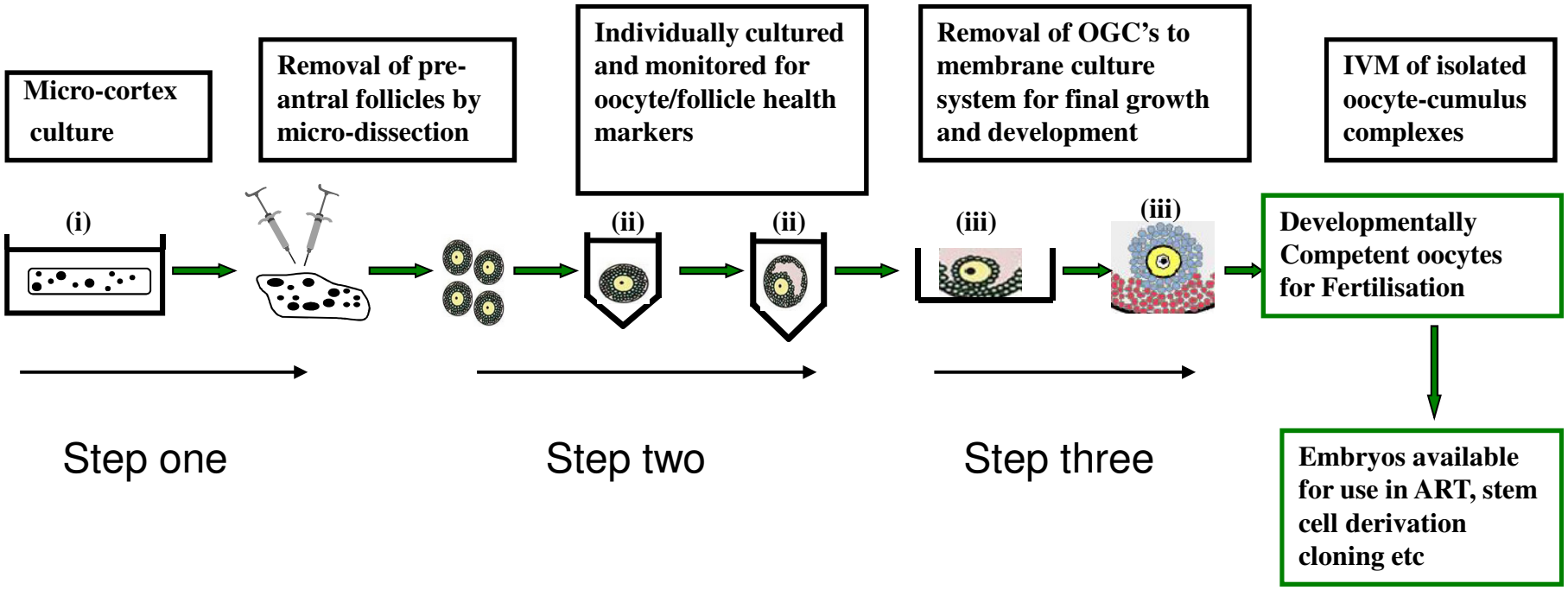
FOLLICLE CULTURE

- Promises so much
- Slow to develop in humans and domestic species
- Where are we now?

Developing a multistep culture system

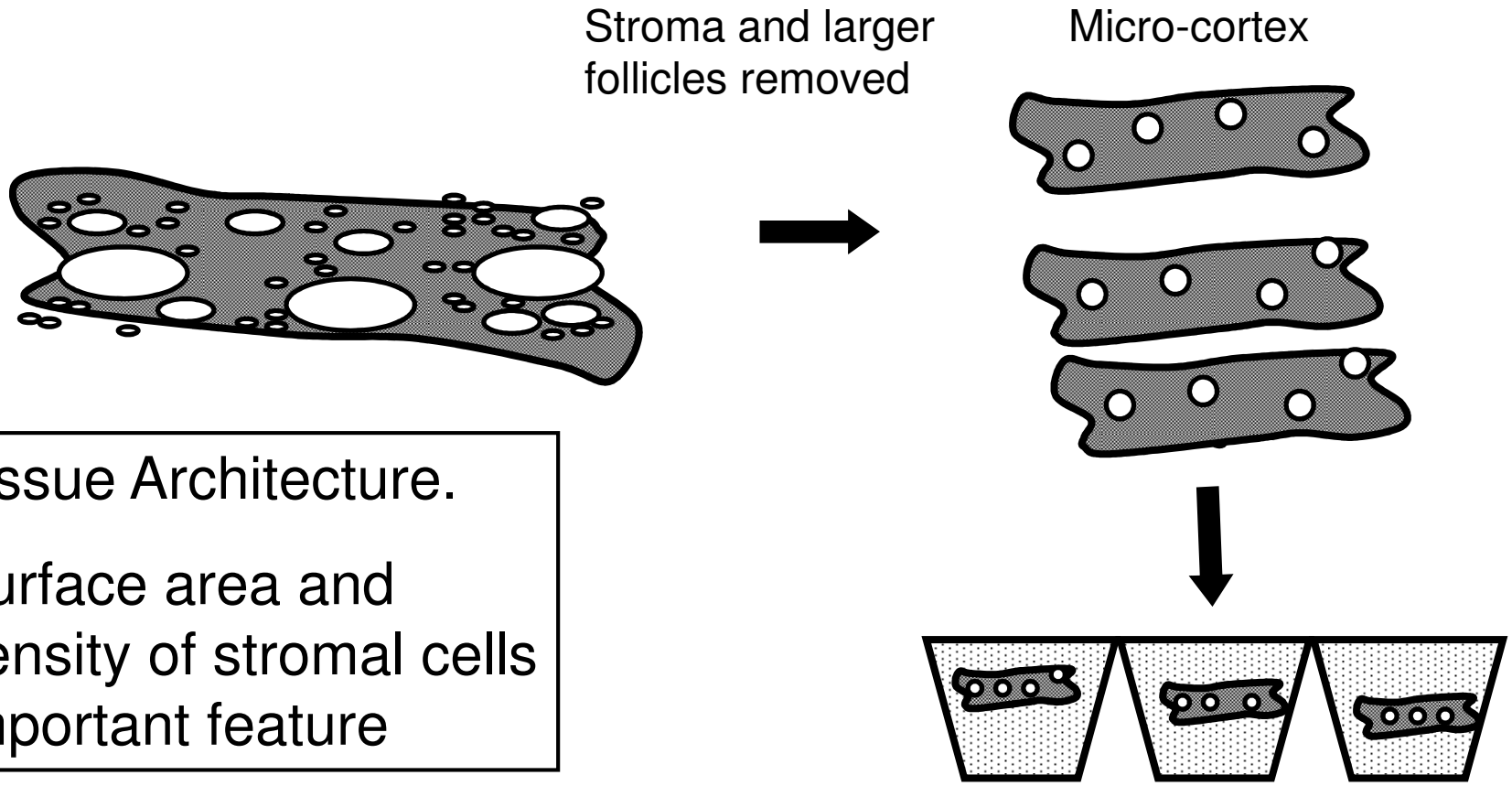
- 1) Optimising growth from primordial/primary stages
- 2) Supporting growth of isolated preantral follicles
- 3) Final stages of oocyte development
- 4) Testing function and normality

Proposed Multi-step Culture System to support development of bovine/human oocytes from primordial stages (IVG)



Step 1: Preparation of cortex

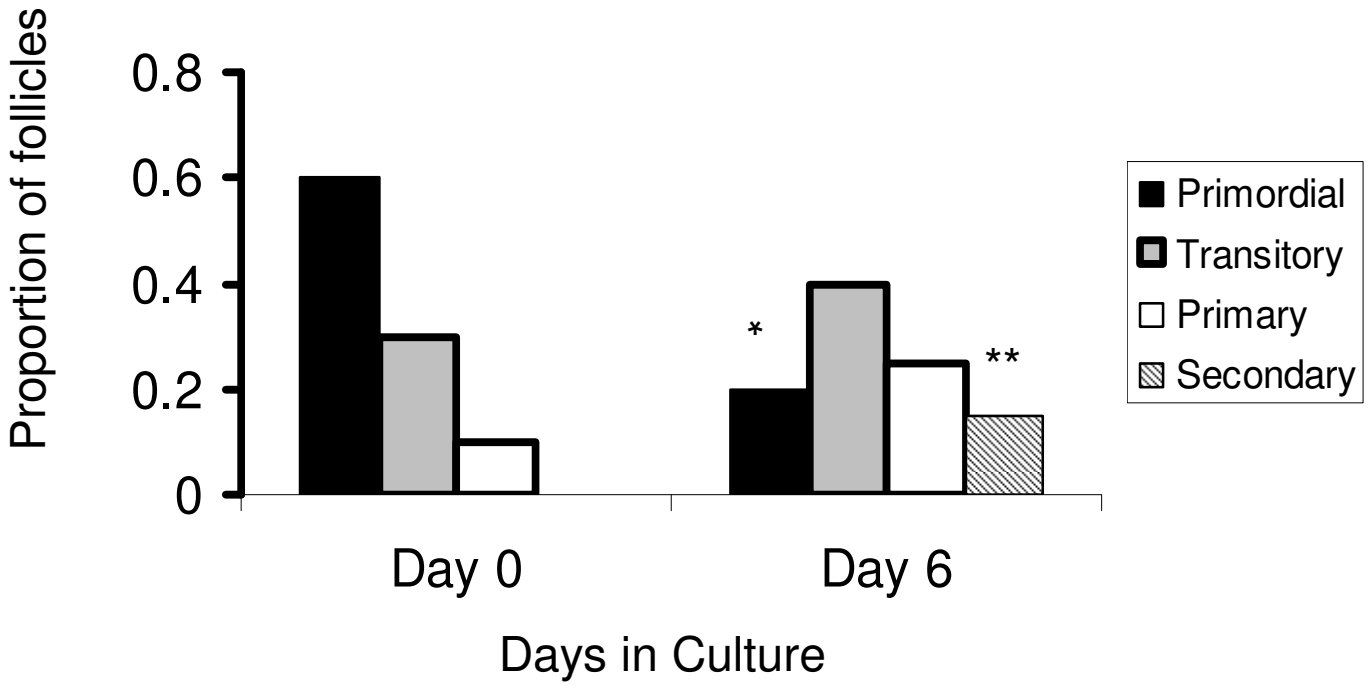
Ovarian cortical biopsies



Tissue Architecture.

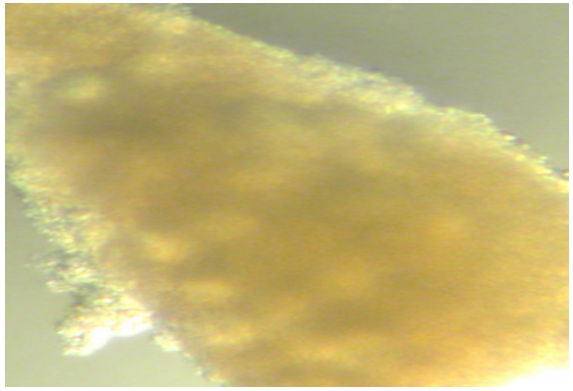
Surface area and density of stromal cells important feature

Stages of follicle development present in micro-cortex at end of culture

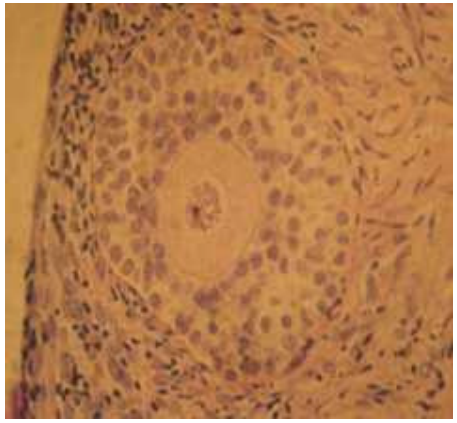


Telfer,McLaughlin,Ding & Thong (2008, Human Reproduction)

Follicle growth within micro-cortex



Cultured micro-cortex



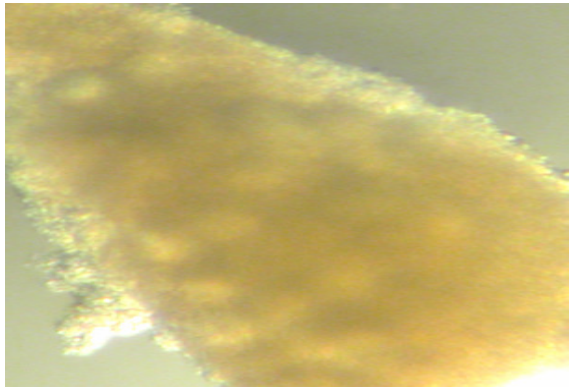
Histology follicles grown within micro-cortex

Human Follicle development *in vitro* (6 days)

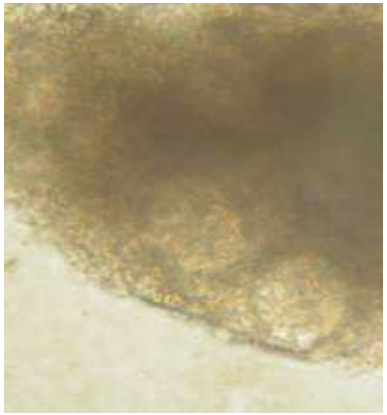
Growth within micro-cortex

- Optimal time and size to remove growing follicles from micro-cortex environment.
- In our hands: 6-8 days (depending on size)
- Leaving follicles longer results in increased death and poor quality follicles/oocytes.

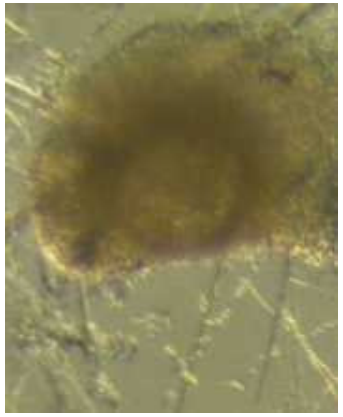
Isolating Growing Follicles



Cultured micro-cortex



Follicles before isolation



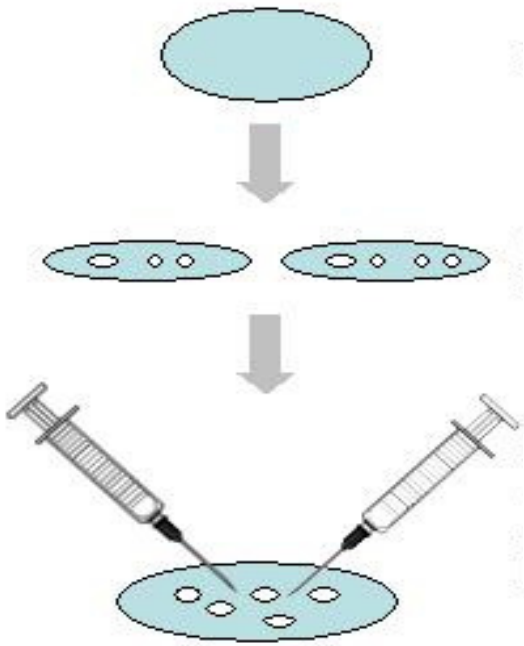
Isolated Follicle

Human Follicle development *in vitro* (6 days)

Two Step Serum Free Culture System for Human oocytes

Step one

6 days



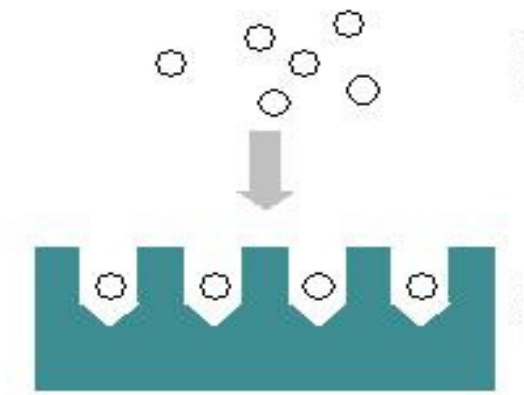
Cortical Biopsy (n=6) from women undergoing elective C section

Strips cultured for 6 days in serum free medium

Preantral follicles (66-132µm) Mean 100+/-3.4. Isolated by microdissection

Step Two

4 days

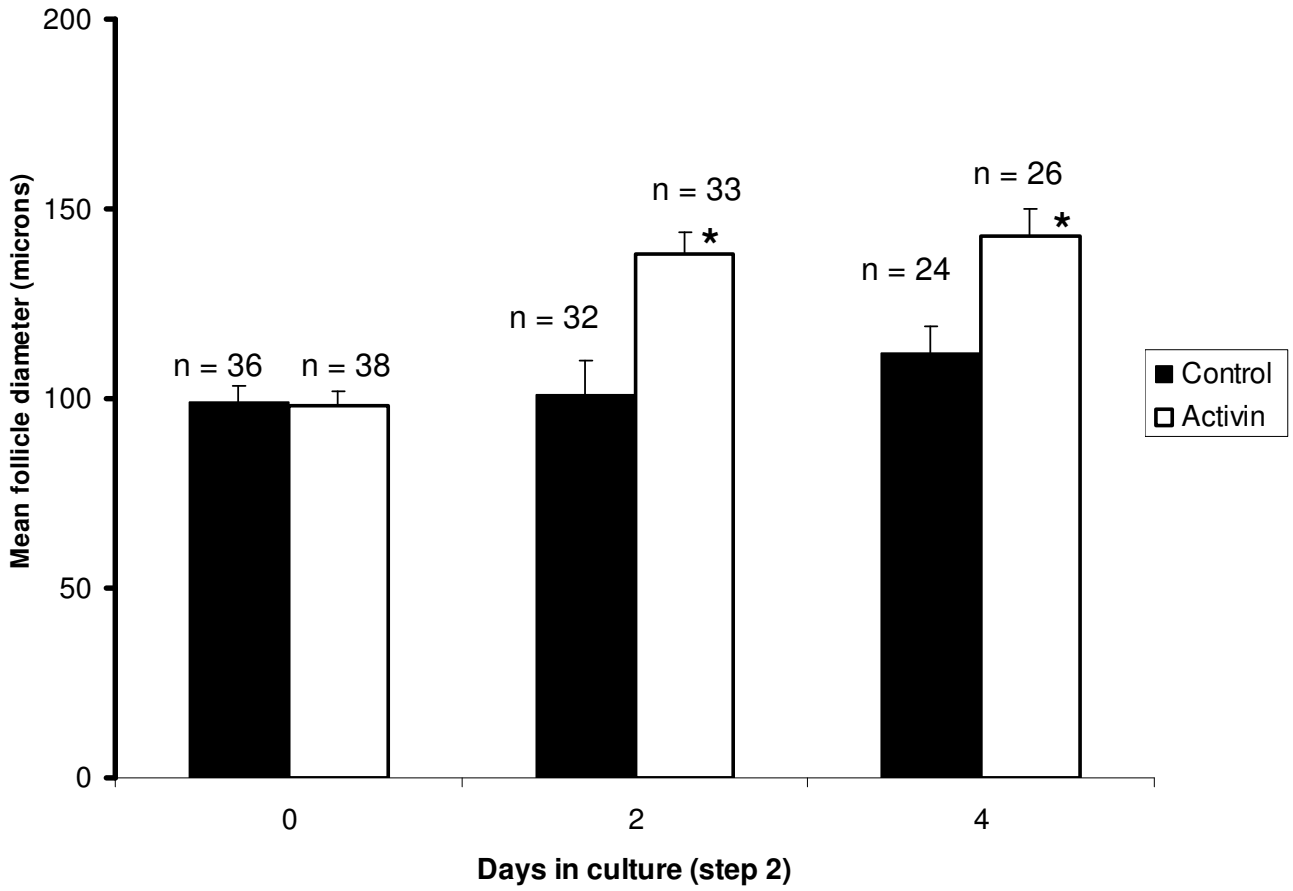


Follicles selected for culture

Follicles cultured in control medium (McCoys) (n=36) or McCoys + 100ng/ml Activin (n=38)

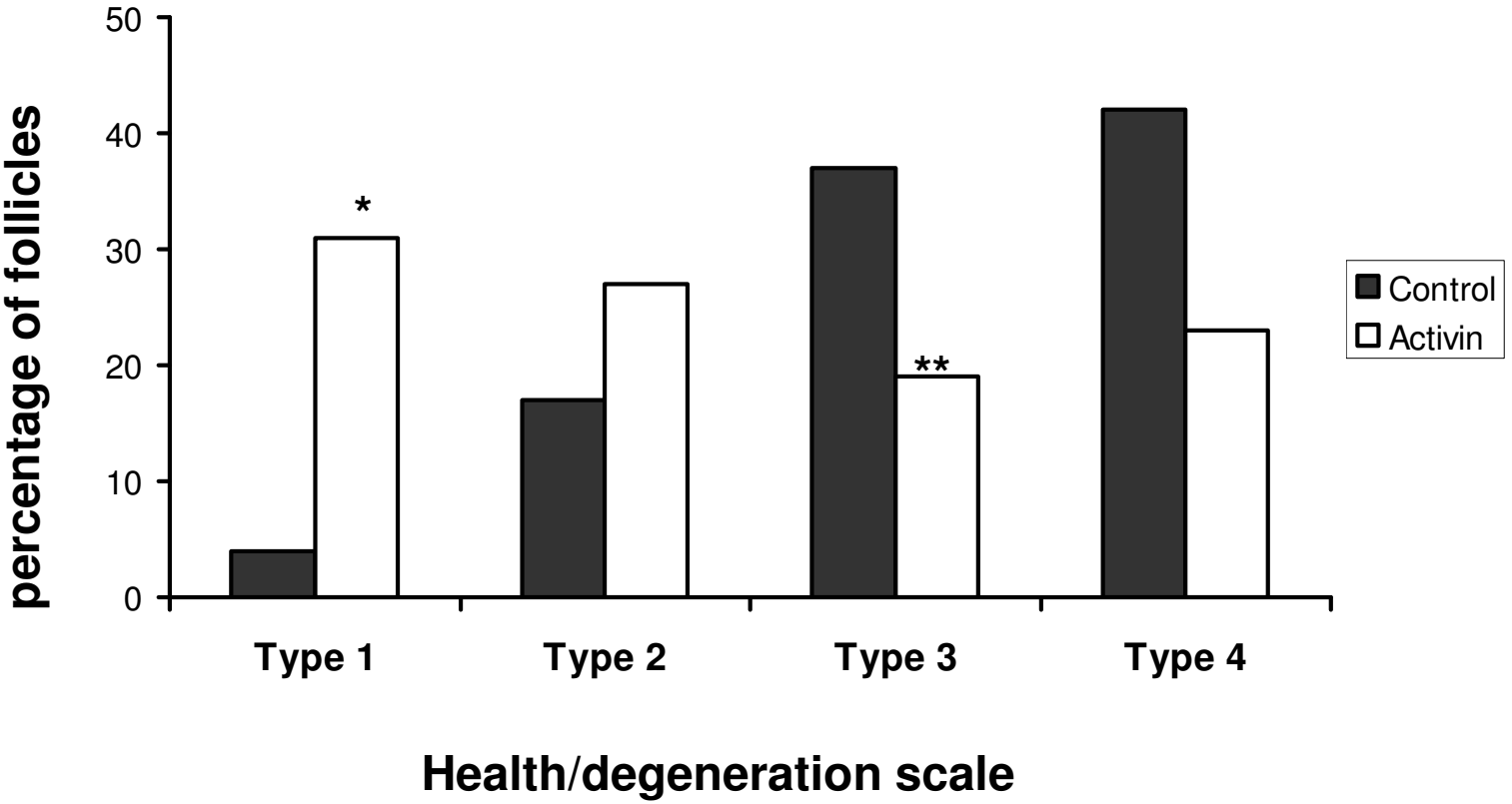
Telfer et al., 2008a

Growth of isolated human preantral follicles *in vitro*

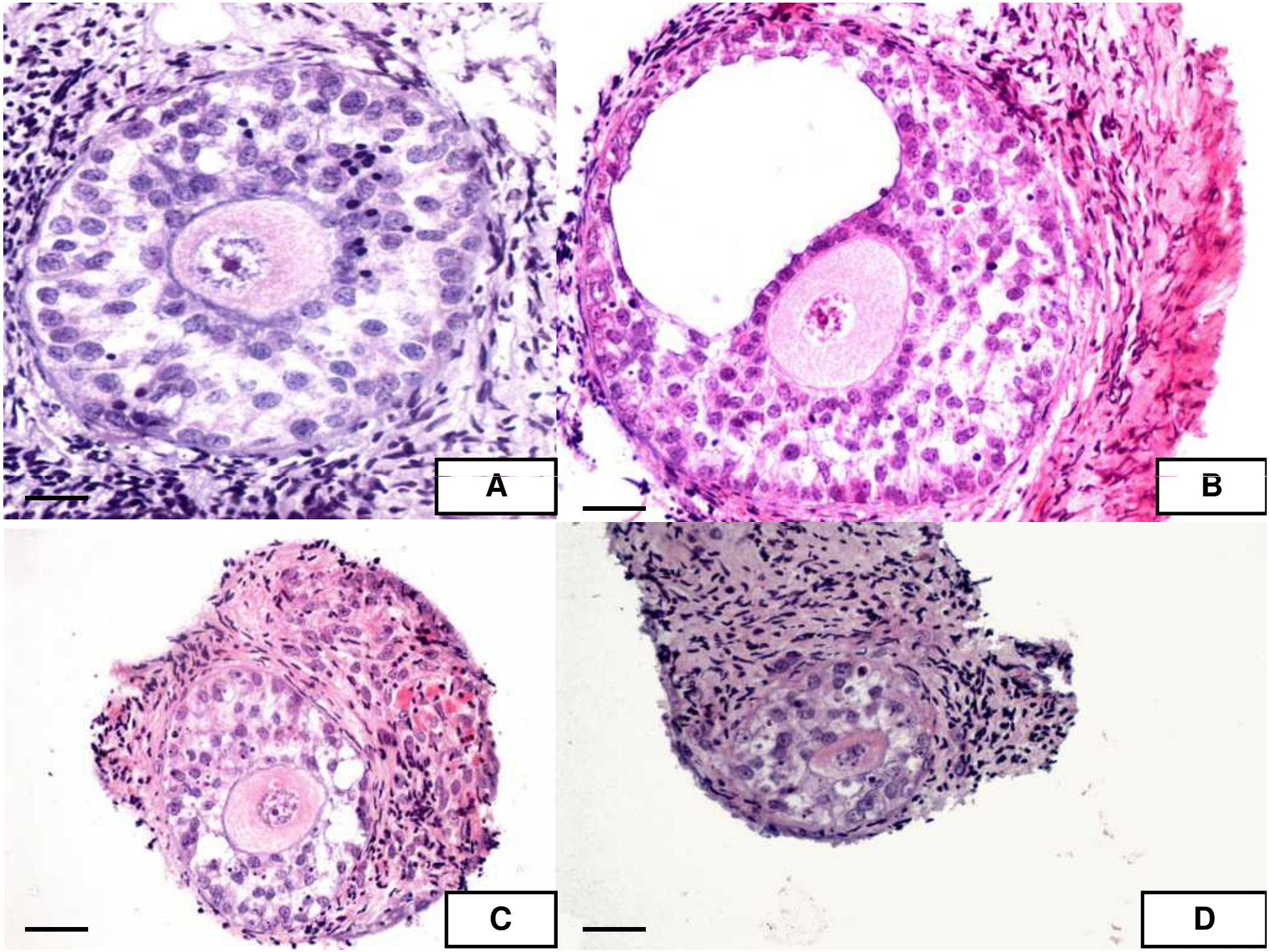


Telfer *et al.*, 2008 Human Reproduction

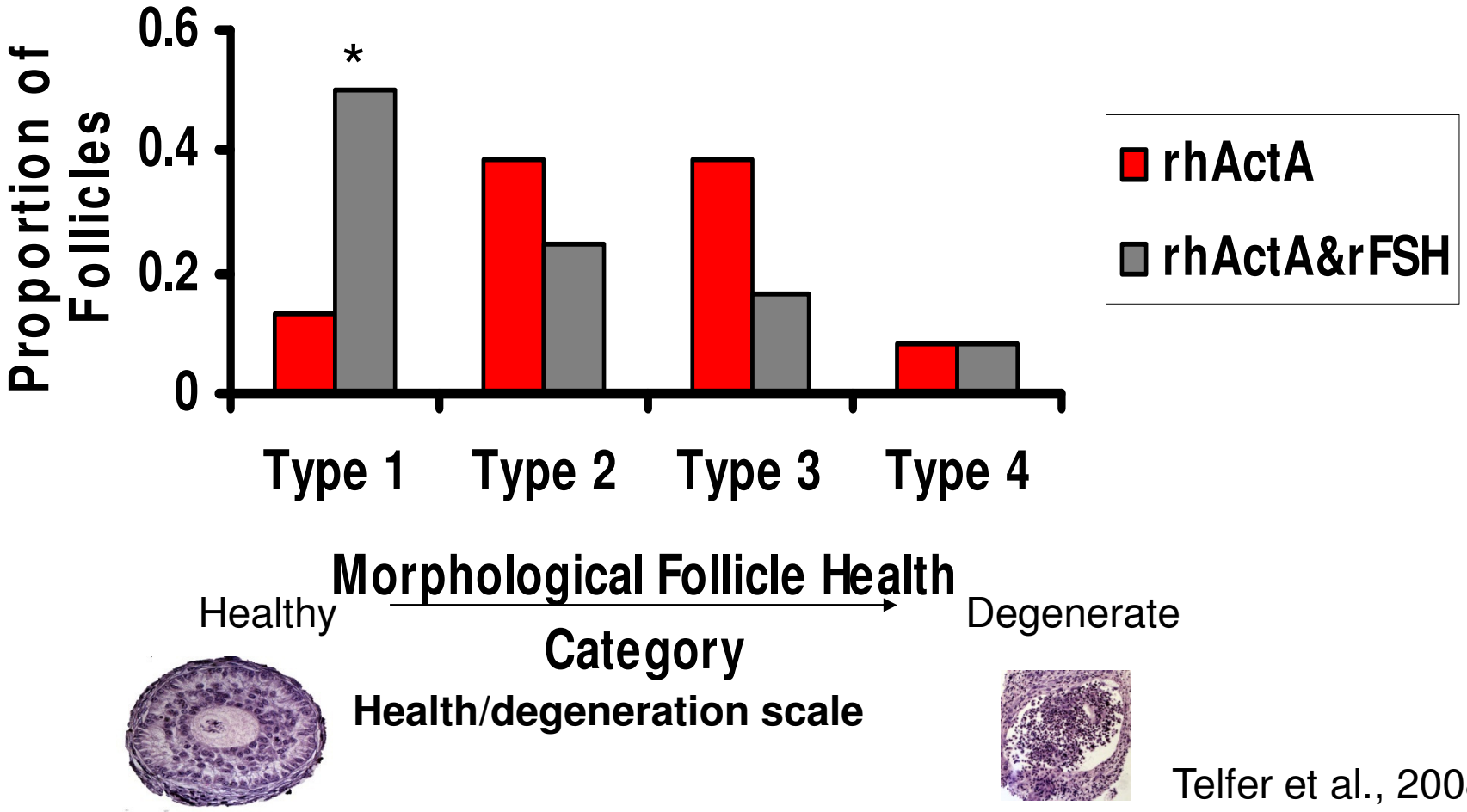
Health of *in vitro* grown human follicles after 6 days in cortical strip culture (step 1) followed by 4 days in isolated culture (step 2).



Telfer *et al*., 2008 Human Reproduction


A
B
C
D

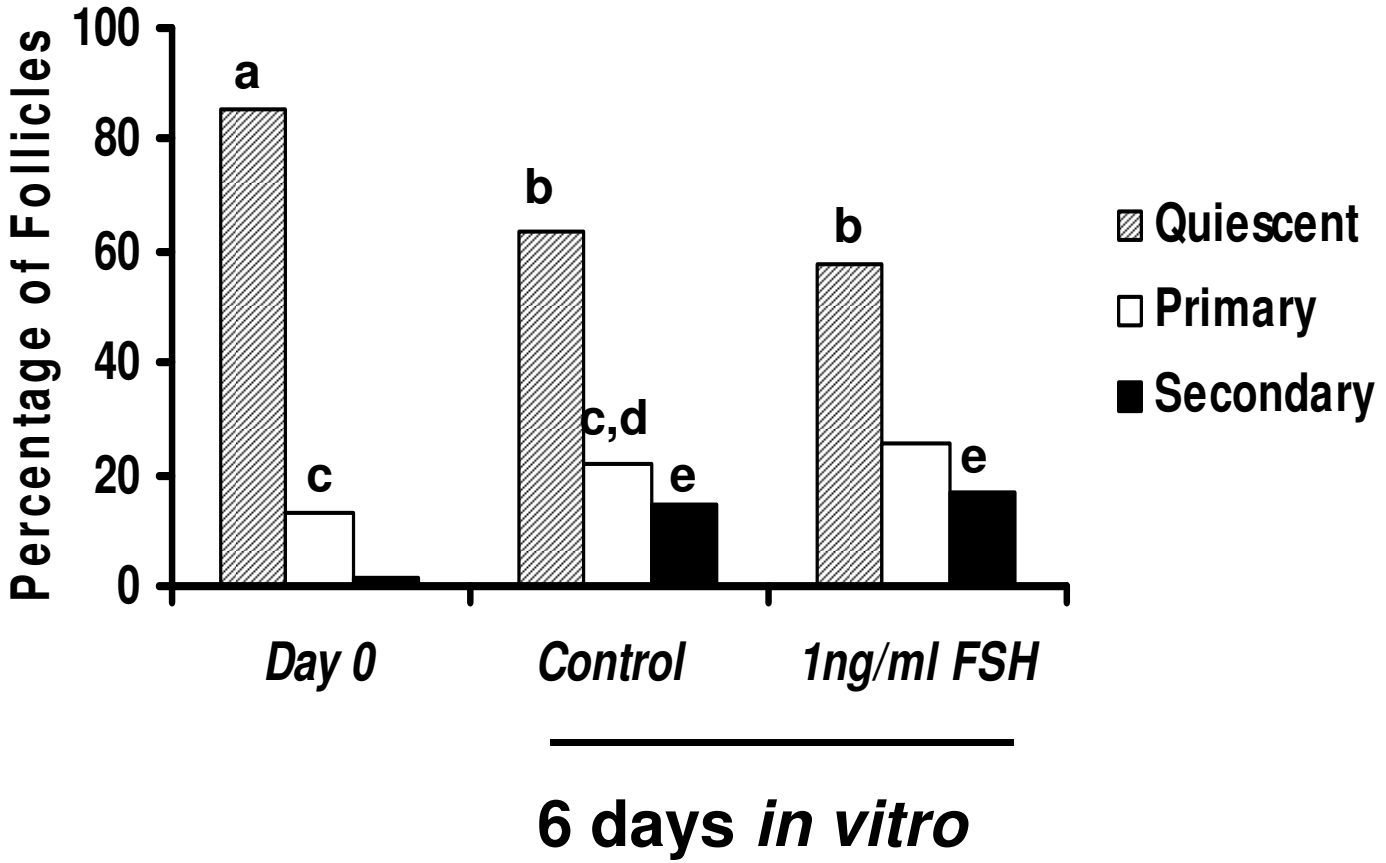
Morphological Health of *In Vitro* Grown Human Follicles: Activin versus Activin + FSH



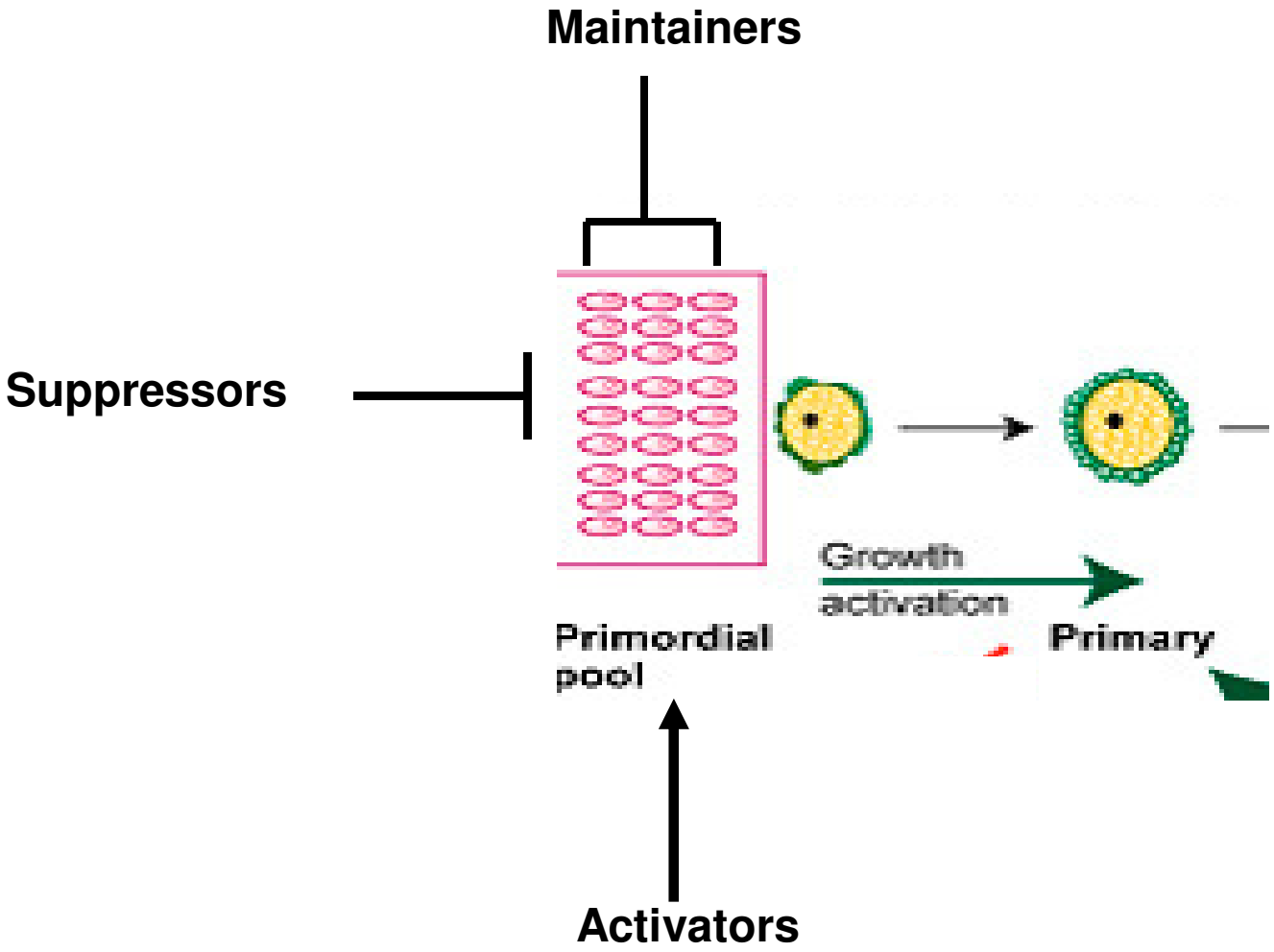
Telfer et al., 2008b

Activin and FSH have a positive effect on isolated growing follicles but what about on initiation of Primordial Follicles?

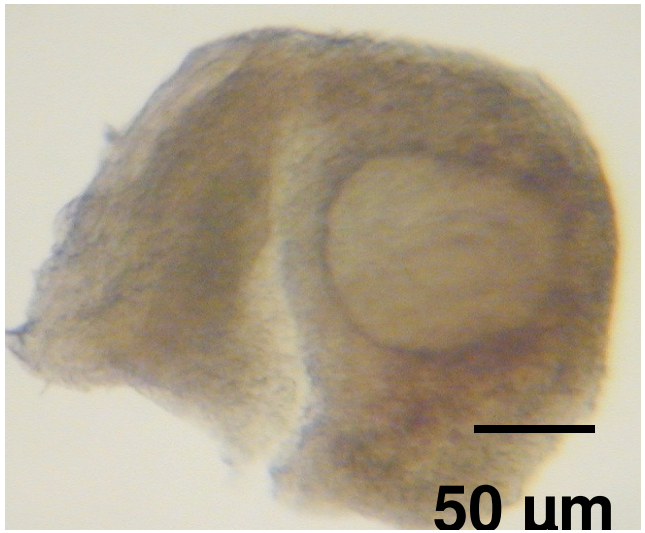
Effect of FSH on Primordial activation

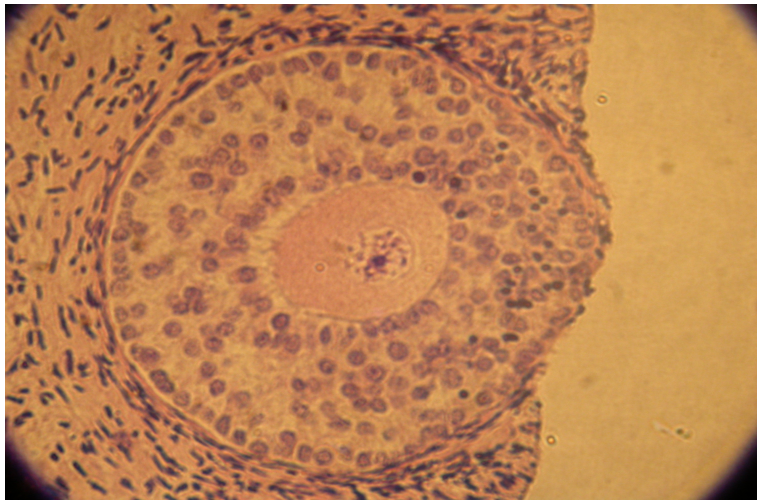


Regulating the rate of depletion of the Primordial Pool

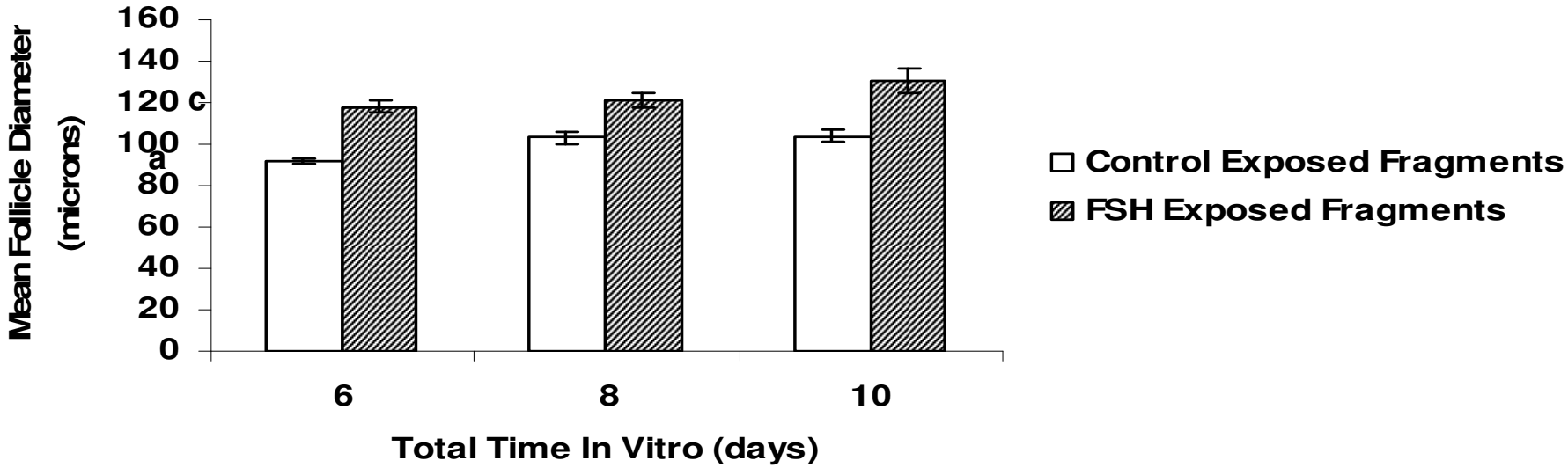


Low dose FSH increases size of growing follicles



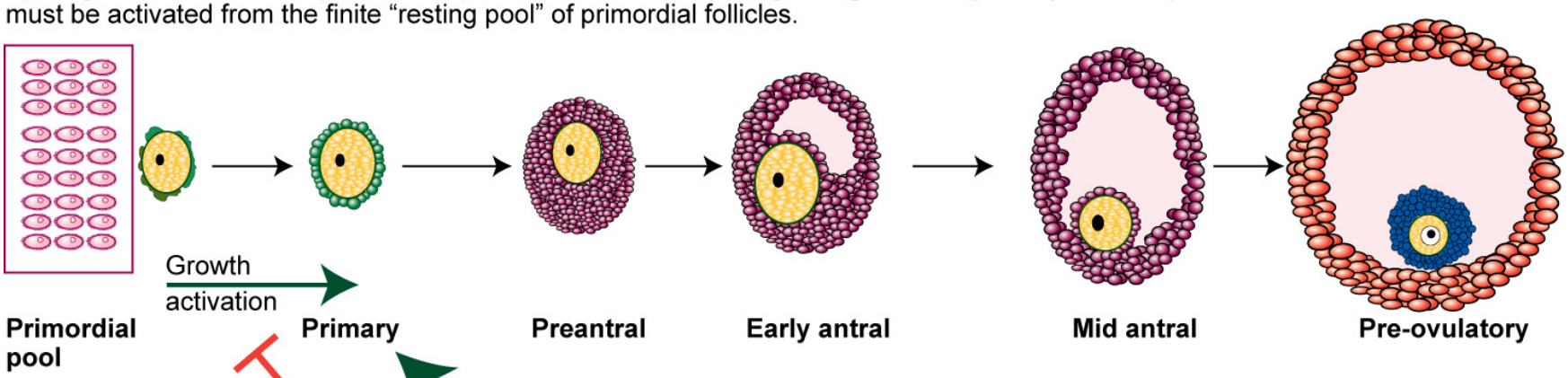


Growth of Isolated Human Secondary Follicles Dissected from Cultured Cortical Fragments

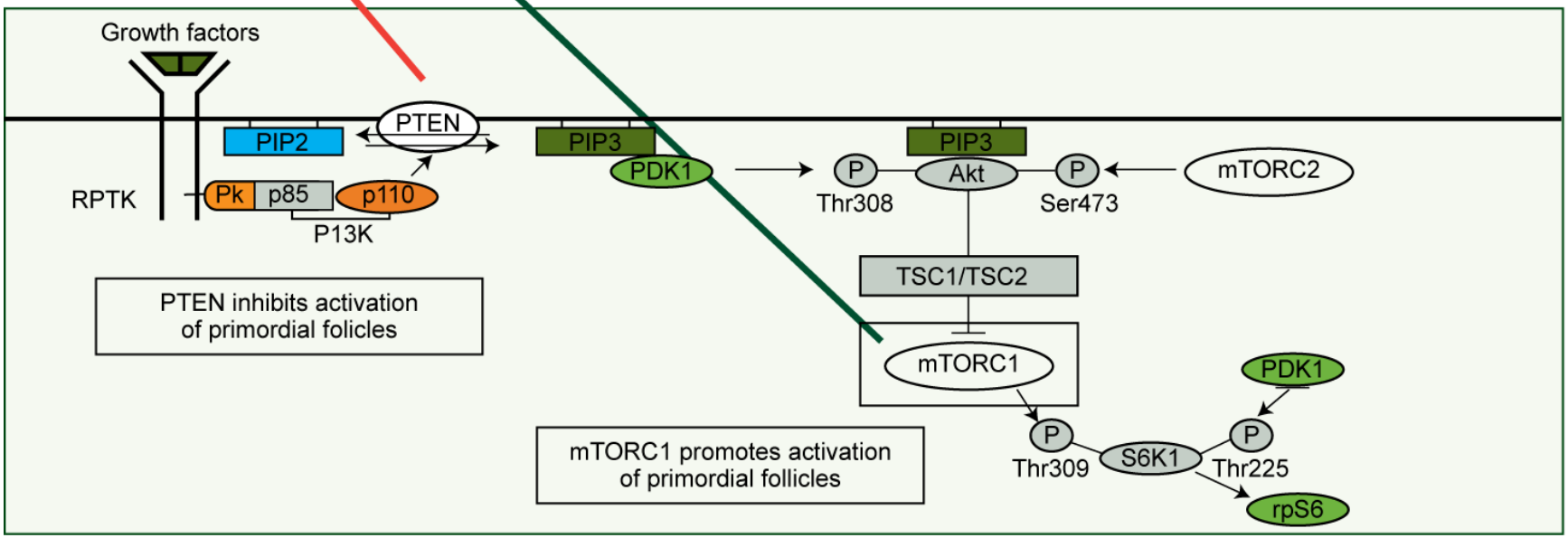


PI3K signalling regulating Activation of Primordial Follicles

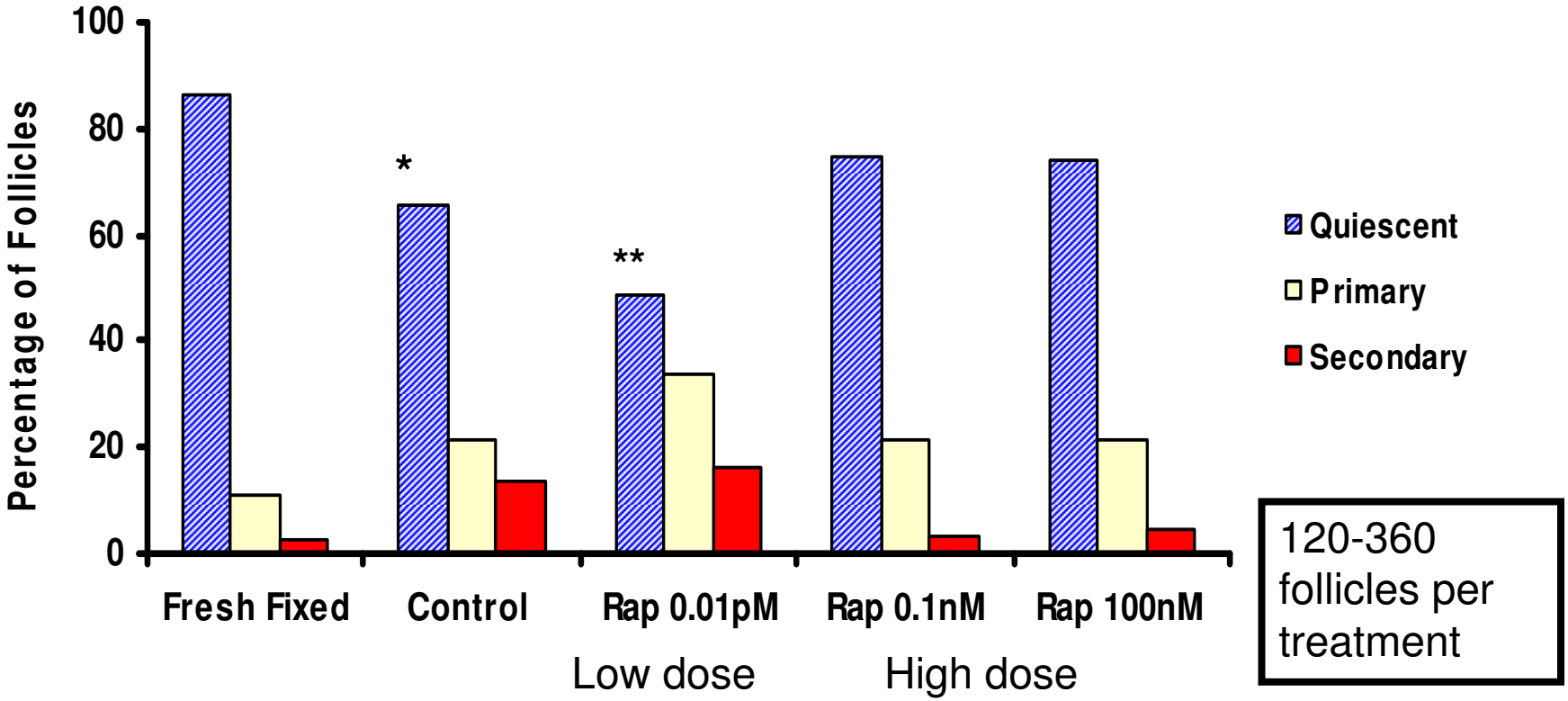
A) Stages of follicle development from primordial to ovulatory. All growing follicles (primary onwards) must be activated from the finite "resting pool" of primordial follicles.



B) P13K signalling



Inhibiting mTOR affects initiation of human primordial follicles *in vitro*



Applications of follicle/oocyte culture systems (IVG)

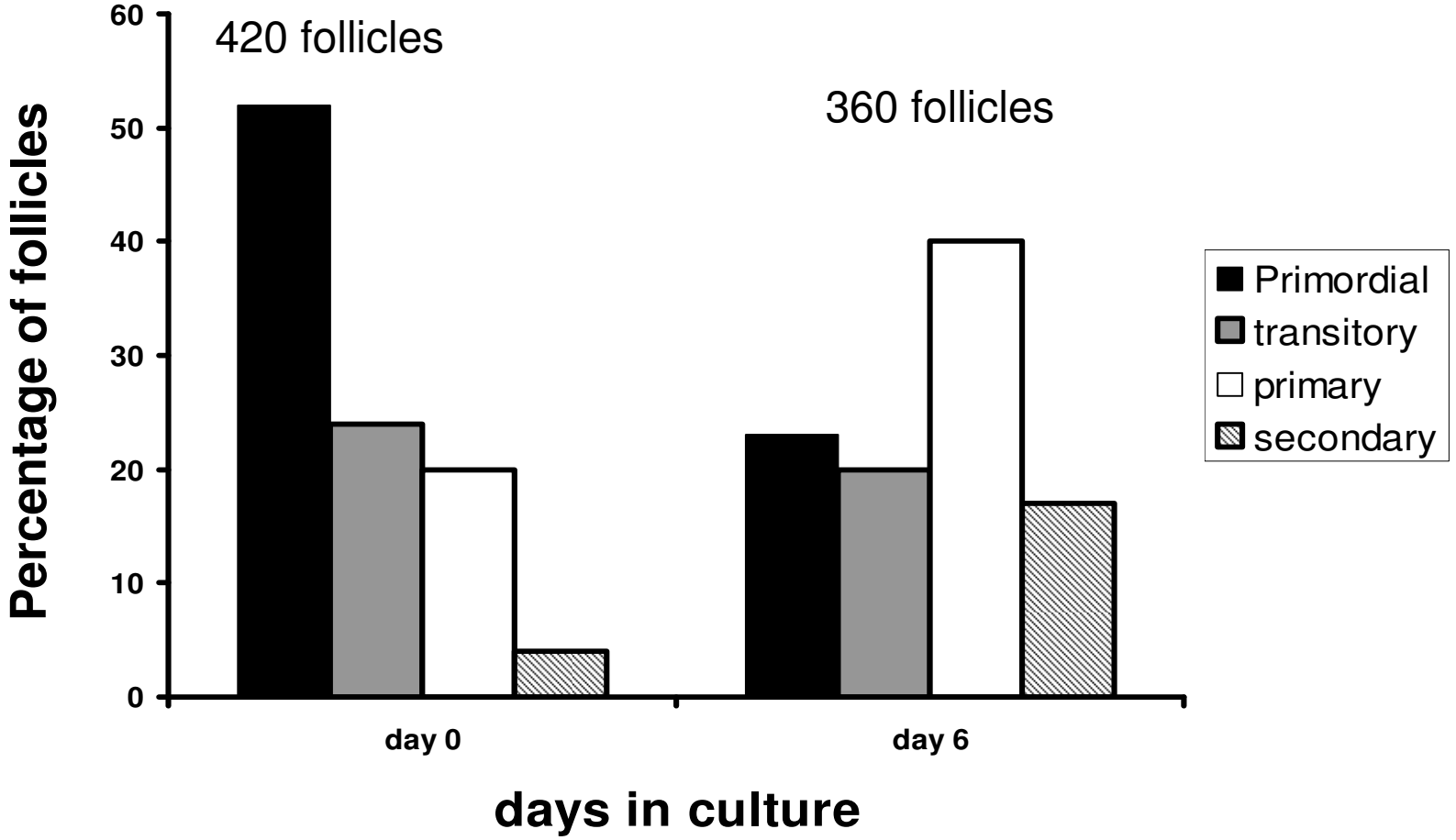
Current

- Basic research tool
- Tissue viability assessment

Potential

- Fertility preservation (frozen tissue)

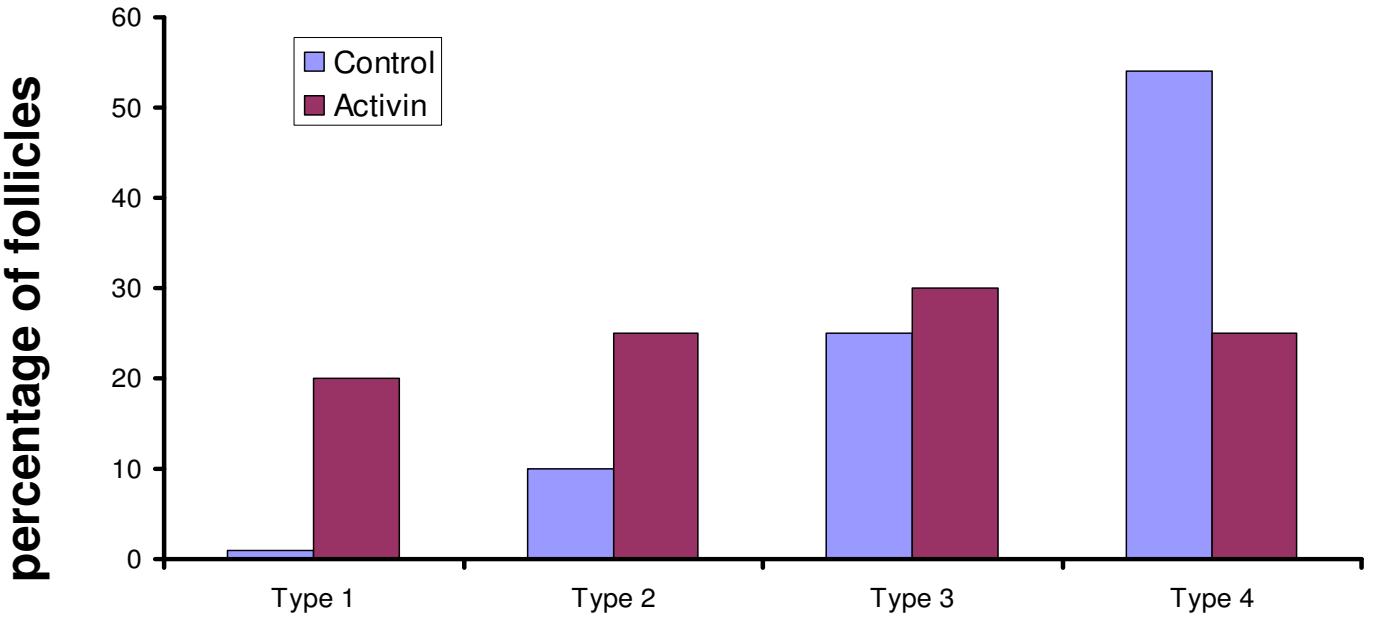
Development of follicles within cortical strips from vitrified tissue (Step one)



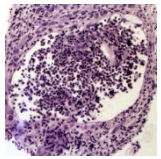
Vitrified Tissue (n=10 biopsies)

Telfer et al., 2008c

Health of *in vitro* grown human follicles after 6 days in cortical strip culture (step 1) followed by 4 days in isolated culture (step 2).



Health/degeneration scale



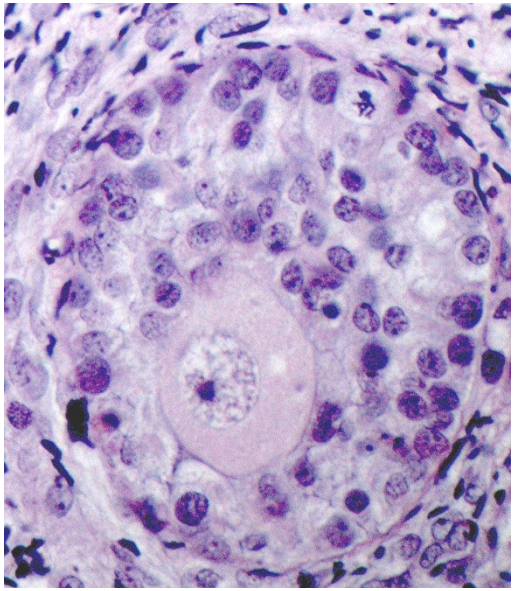
Vitrified tissue source

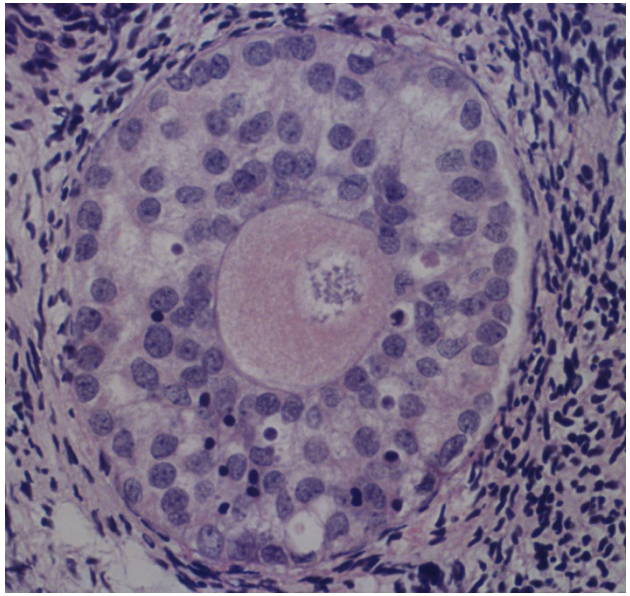
Telfer et al., 2008c

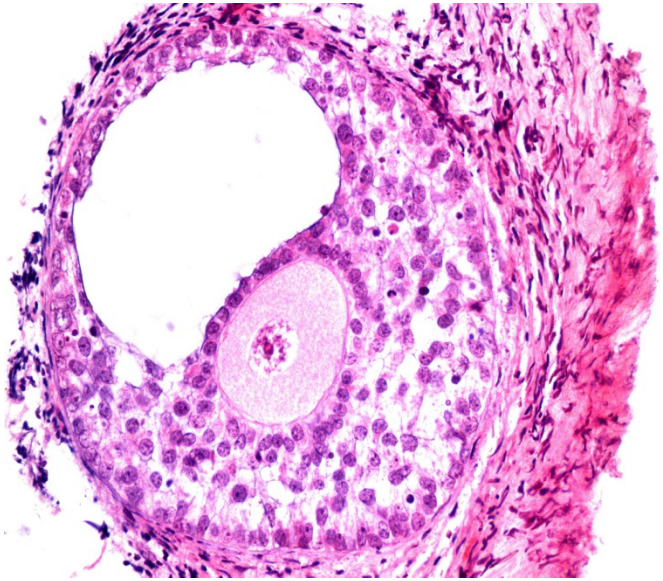
+ Activin A

Vitrified cultured

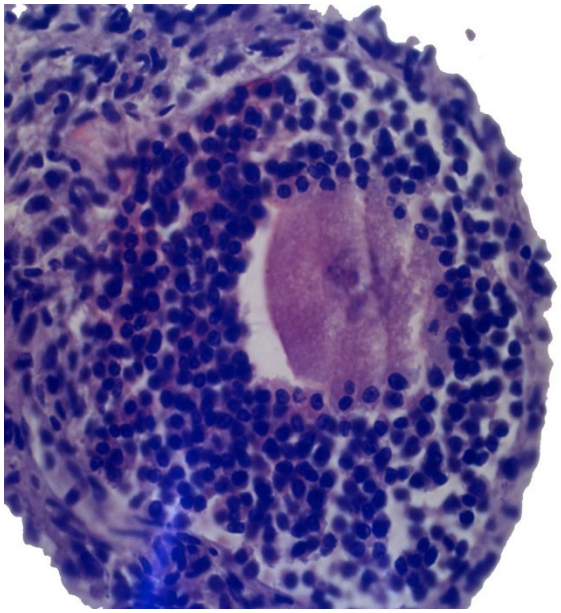
Fresh Cultured

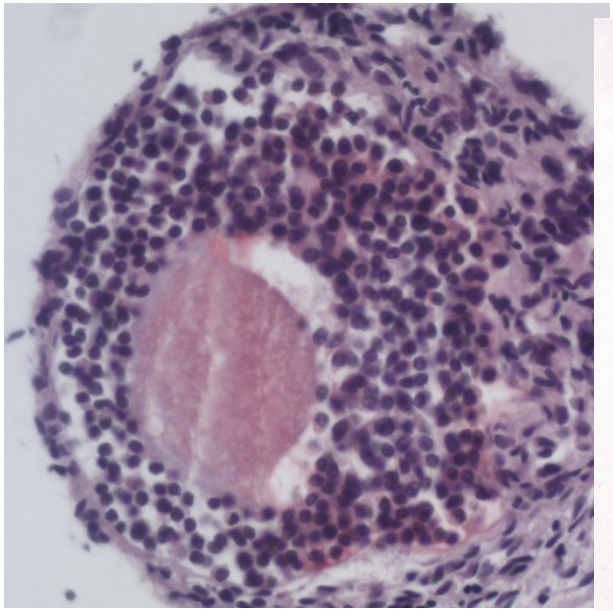


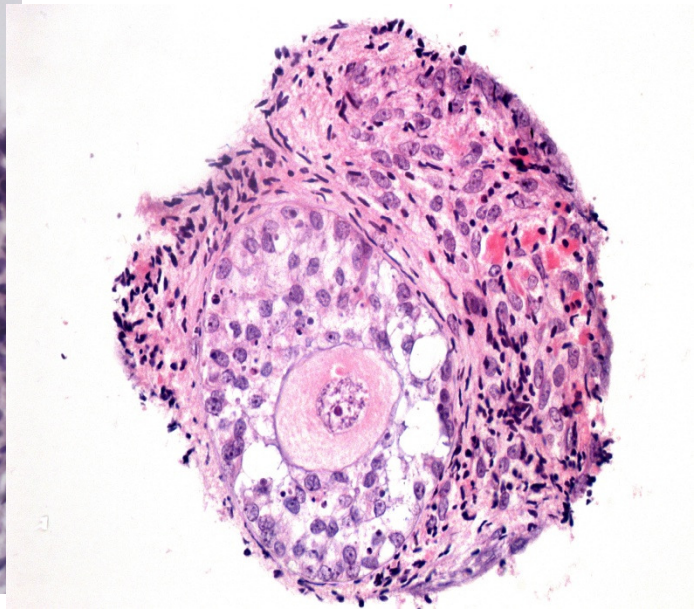




- Activin A

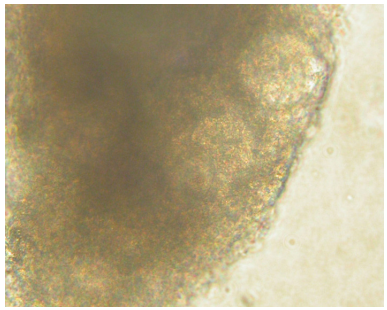


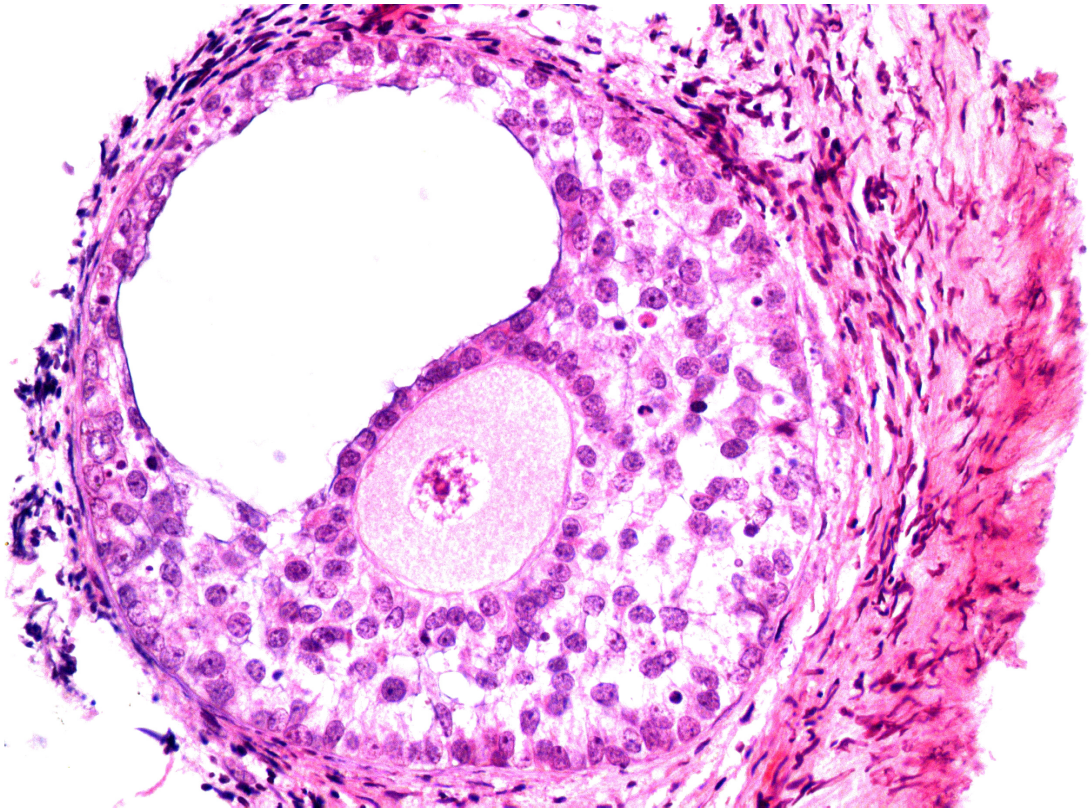




6 days strip culture + 4 days isolated culture

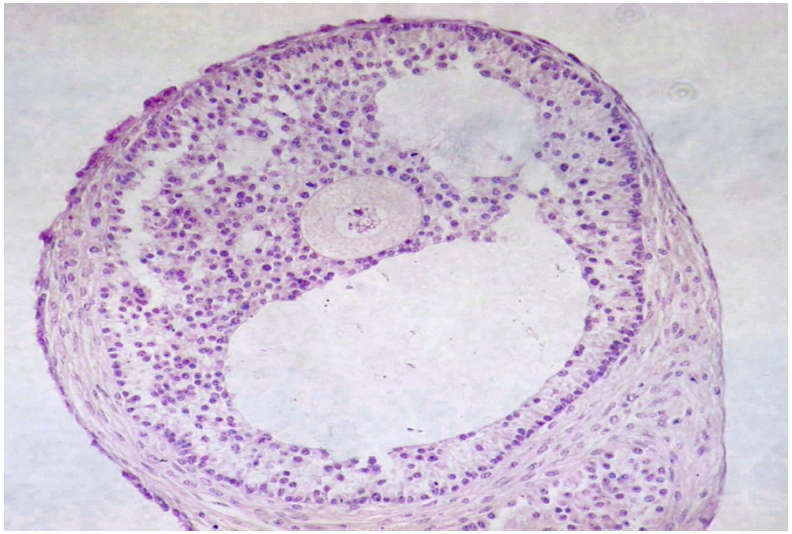
Antral development from *in vitro* grown human primordial follicles within 10 days

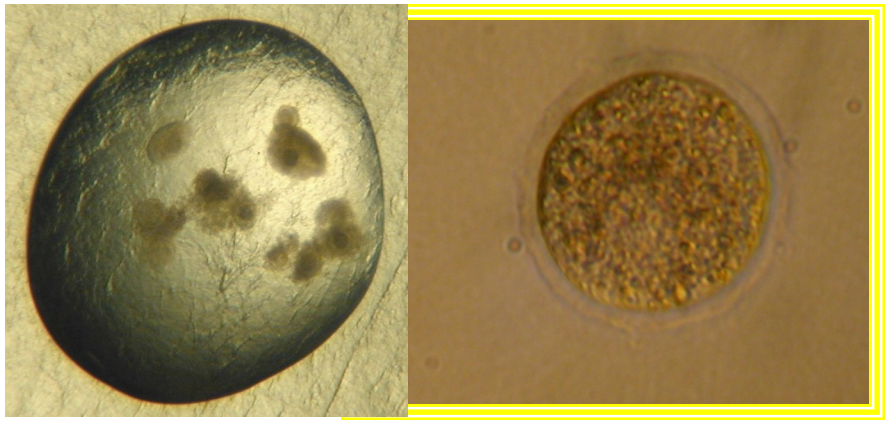




Telfer et al., 2008: A two step serum free culture system supports development of human oocytes from primordial follicles in the presence of activin. **Human Reproduction** 23: 1151-1158

Bovine Folicles cultured for 8 days from primordial (step 1) then 12 days from the preantral stage (step 2)





Oocytes of up to 108 microns produced *in vitro* ***(Met II & PB, IVM)***

Accelerated growth?
Or
Growth without brakes?….

How long does complete oocyte development take?

Growth Rate of Follicles

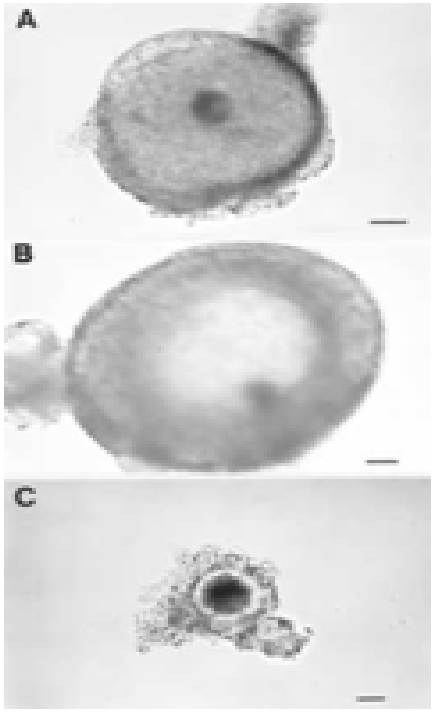
in vivo

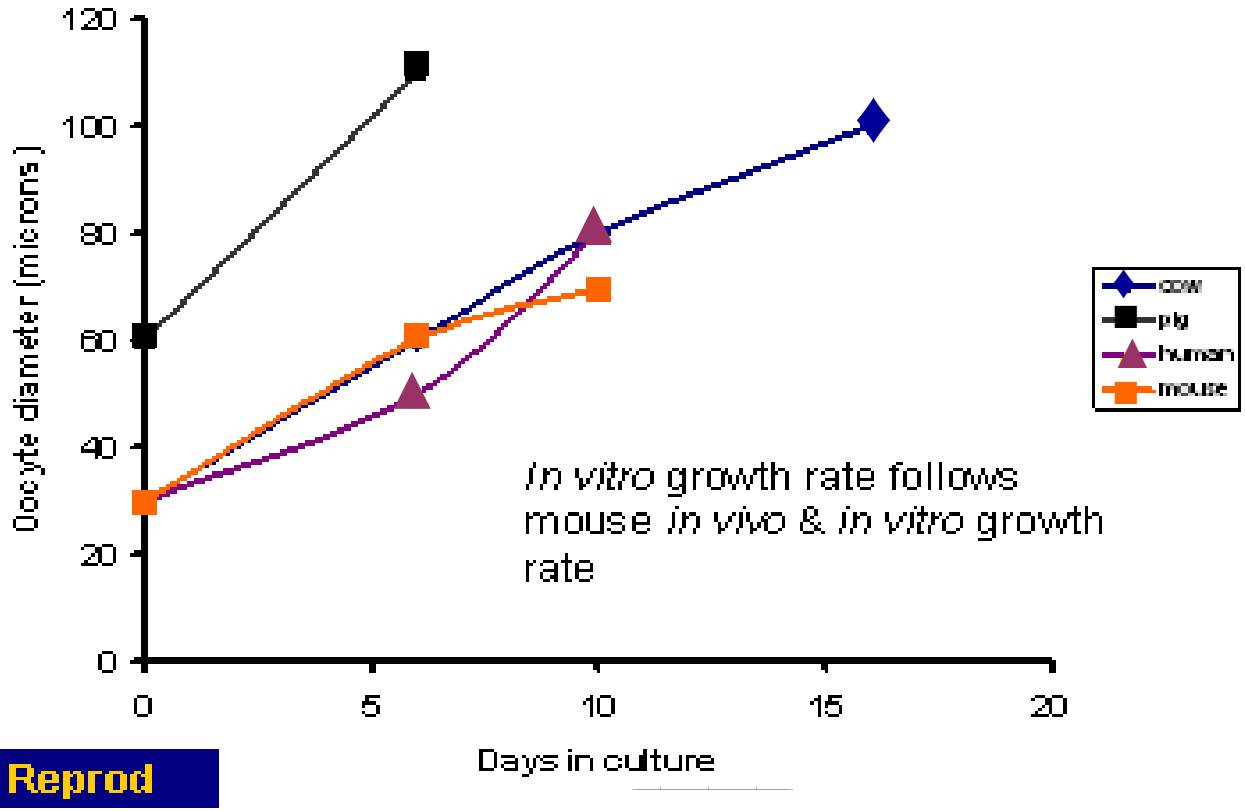
	Preantral	'Mature' Size	Time
Mouse	100-200µm	500-600µm	10-12 days
Pig	150-300µm	1.5-3mm	40-50 days
Cow	100-150µm	3.8->8.5mm	40-50 days
Woman	120-300µm	4.00-6.00mm	70-100

Does a human oocyte really need 70 days to develop or is this time frame a consequence of inhibition regulating follicle development?

Oocyte Growth rates *In Vitro*

(Brakes off)

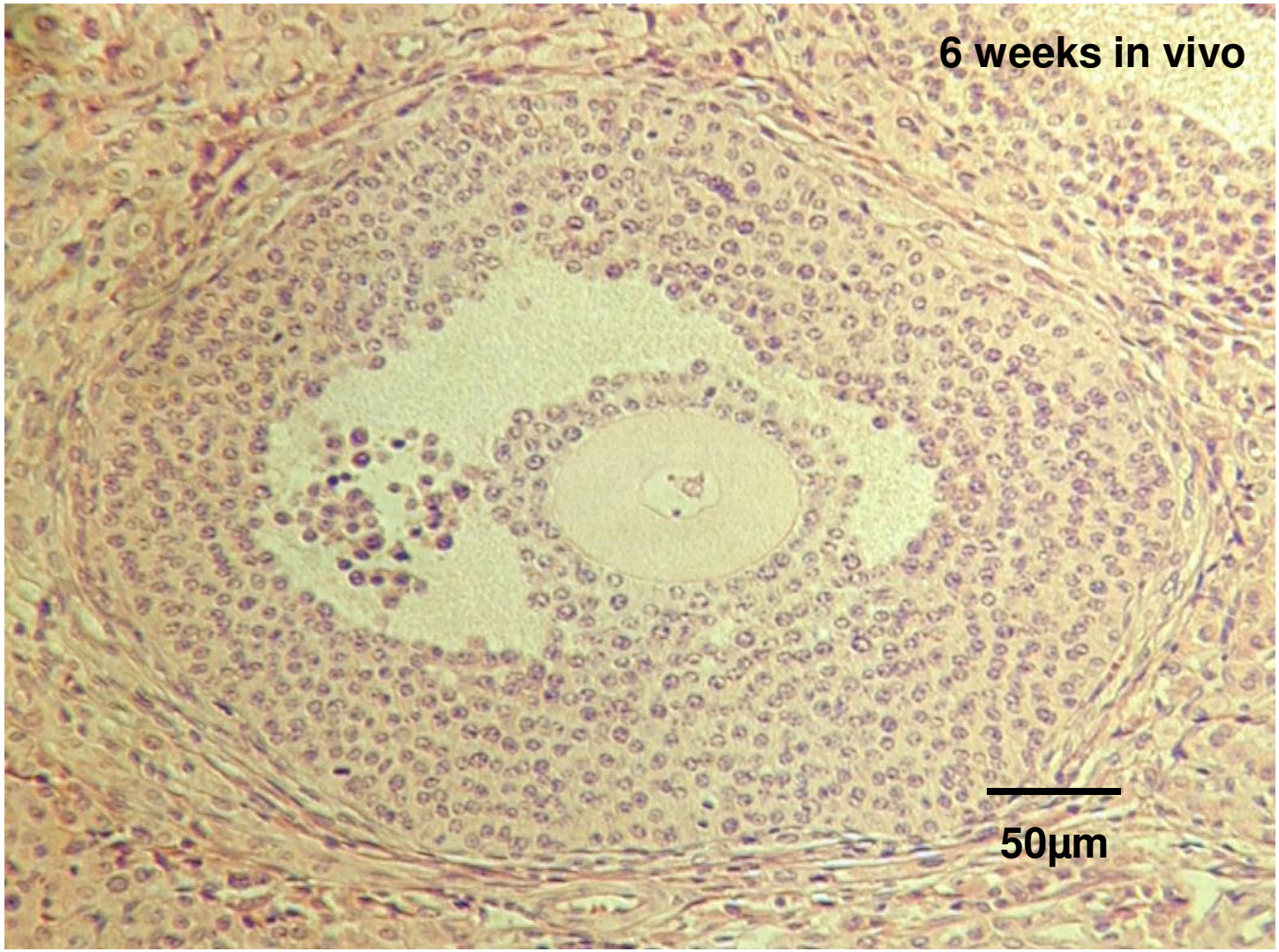




Wu et al., 2000 Biol Reprod

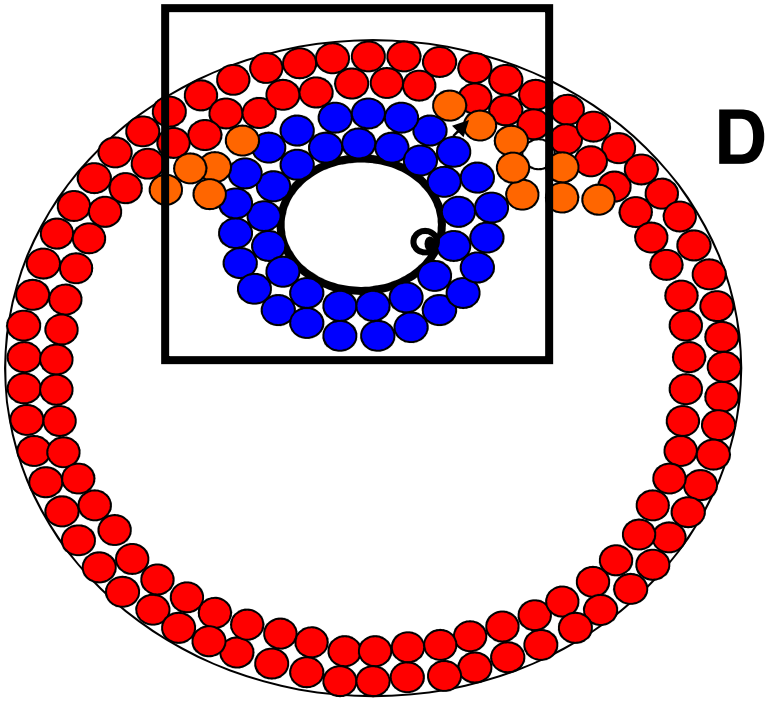
Cultured Follicles transplanted under KC of SCID mouse

Brakes back on



Collaboration with Daniel Brison and Helen Picton

Competing functions within the follicle



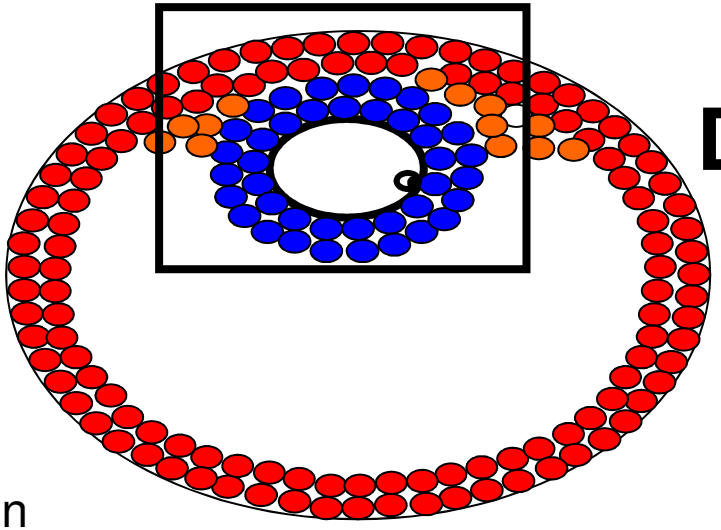
OOCYTE DEVELOPMENT

Most important feature of cumulus lineage is physical interaction with the oocyte

ENDOCRINE FUNCTION

In Vitro Growth of Oocytes

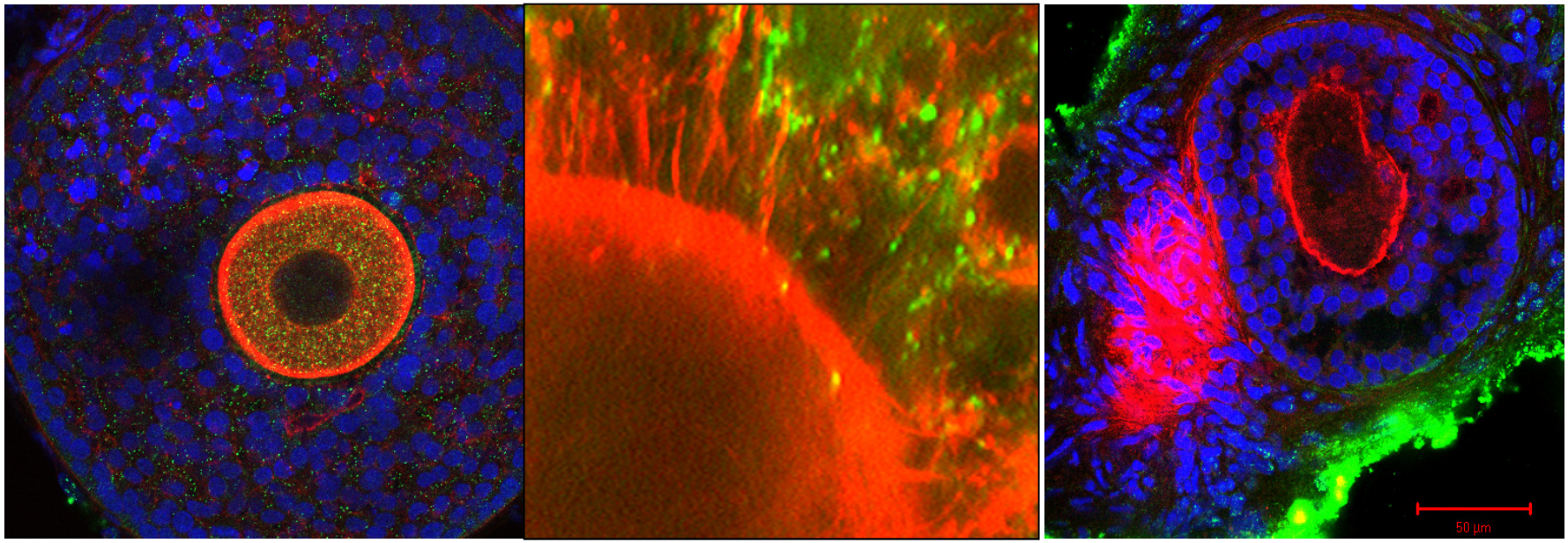
Optimising conditions to support oocyte development through co-ordinating oocyte-somatic cell interactions



OOCYTE DEVELOPMENT

Endocrine Function

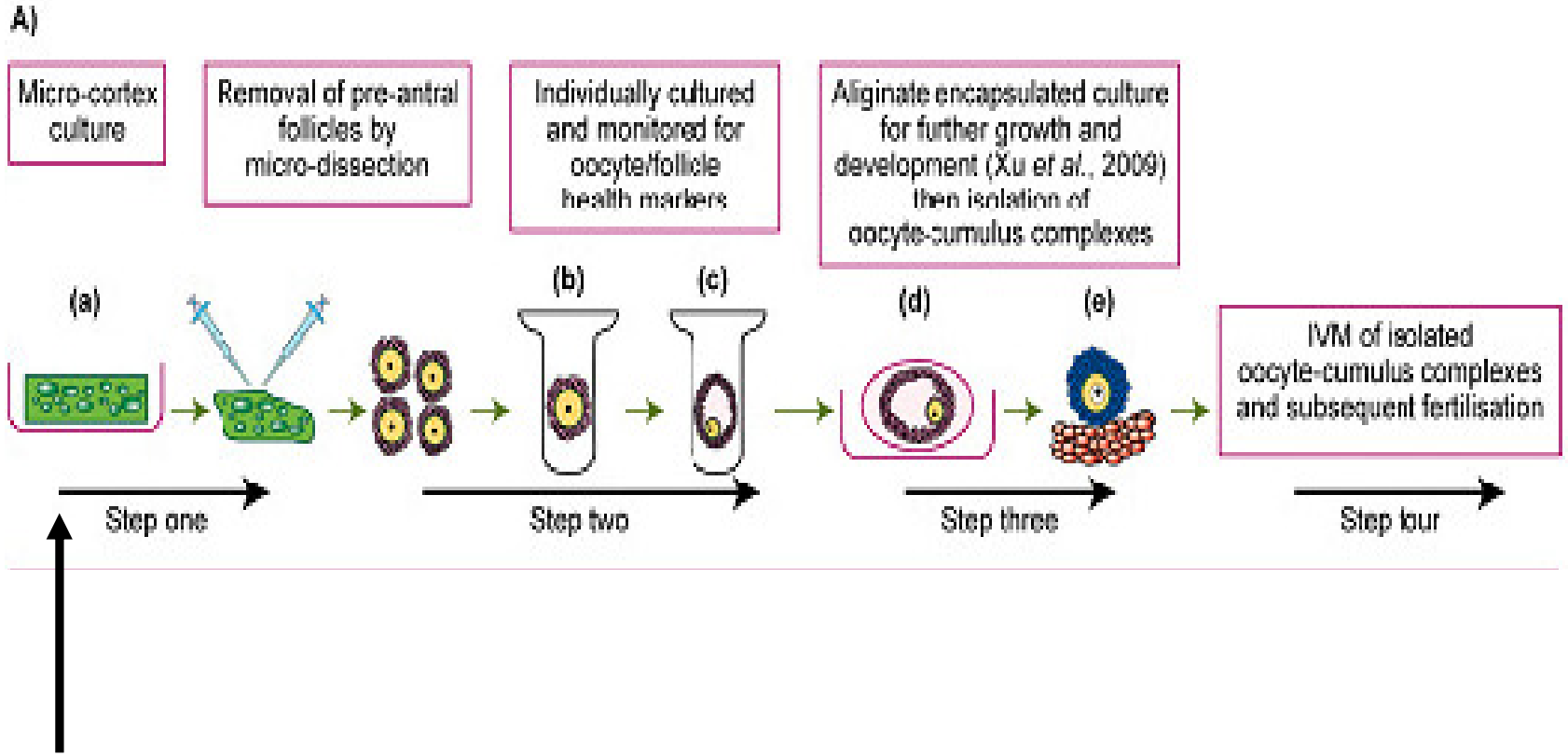
Confocal imaging of oocyte-somatic cell interface



Optimising oocyte-somatic cell communication during IVG

David Albertini KUMC

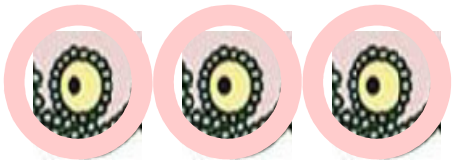
Multi-step Culture system to support human oocyte development



Activation

NEXT STEP……

Final Stages of Development



Complexes Isolated from IVG antral follicles

For further growth

IVM +IVF

Tests for "normality" Methylation etc

Acknowledgements

- Marie McLaughlin
- John Binnie
- Joyce Leyden
- Christina Ding

- Outi Hovatta (Karolinska)
- Kui Liu (Umea)

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- Suresh Kini (Edinburgh)
- David Albertini (Kansas)
- John Bromfield (Kansas)
- Joshua Johnson (Yale)

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- Richard Anderson (Edinburgh)
- Norah Spears (Edinburgh)

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- NHS Endowment fund (Human work)

John Eppig

Follicular Development