Novel systems and factors to improve oocyte quality in vitro

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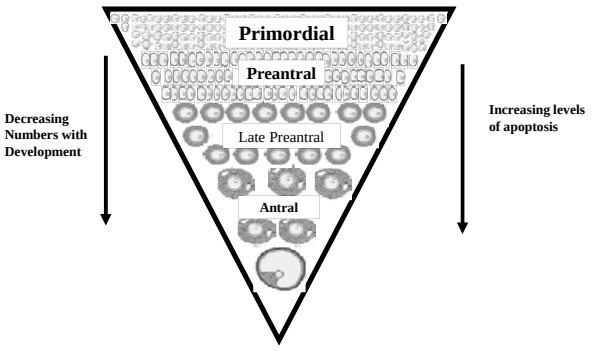
PLEASE NOTE

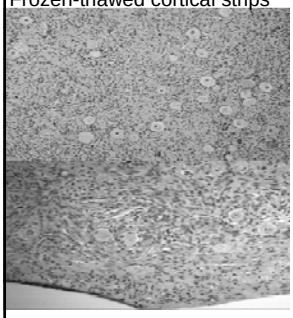
- The final presentation will have additional content.

Store of "resting" primordial follicles utilised throughout reproductive life



In vitro growth of immature oocytes

Frozen-thawed cortical strips



Rhabdomyosarcoma Pt 15yrs:thawed from slow freeze

Cortical strips contain mainly primordial follicles.

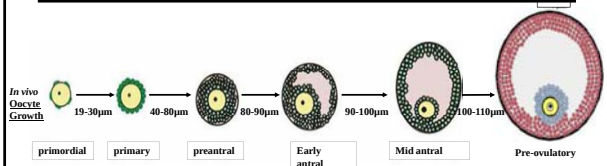
The challenge now is to develop oocytes *in vitro* from primordial stages to maturation and fertilisation.

Where are we now?

Growing Primordial Follicles in the lab (*In Vitro Growth*) IVG

- **Define the fundamental mechanisms of oocyte development (basic science)**
- **Fertility Preservation**
- **Animal Breeding**
- **Endangered species**
- **Toxicity testing**

Making a good egg is complicated



Growth/ Meiotic Arrest

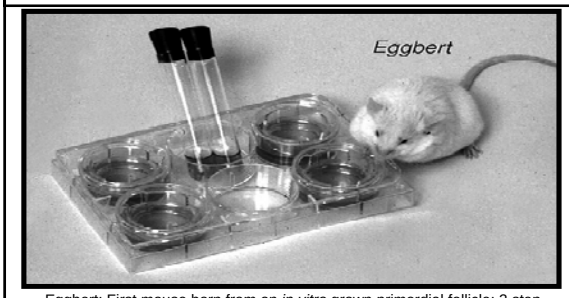
Acquisition of Meiotic Competence

Acquisition of Developmental Competence

Transcription/Transcriptional Repression

Genomic imprinting

Mice from Primordial Follicles...



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

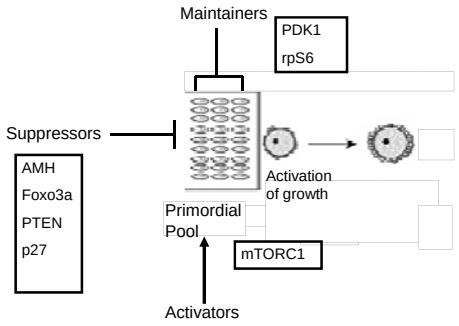
Developing a multistep culture system for human oocytes

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

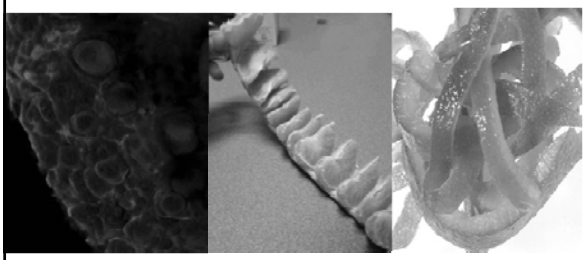
Source of Human OvarianTissue For Research

- Small strip of ovarian cortex donated after informed consent: Caesarean section/Gynaecological procedures/ Fertility Preservation
- Tissue from 3-45 years (fresh and frozen)
- Clinical collaborators Hamish Wallace and Richard Anderson

Step one: Activation of quiescent primordial follicles



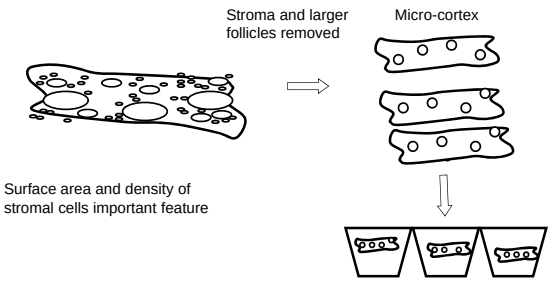
Key Step: Preparation of Tissue



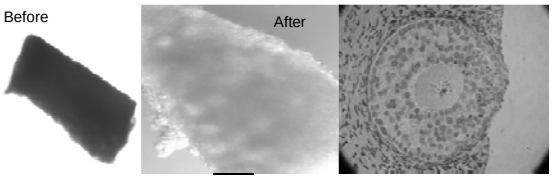
Optimum density of underlying stromal tissue.
Tissue stretched to loosen stromal cells

Step 1: Preparation of cortex

Ovarian cortical biopsies

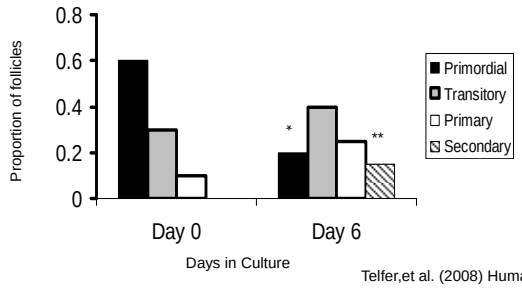


Follicle growth within micro-cortex



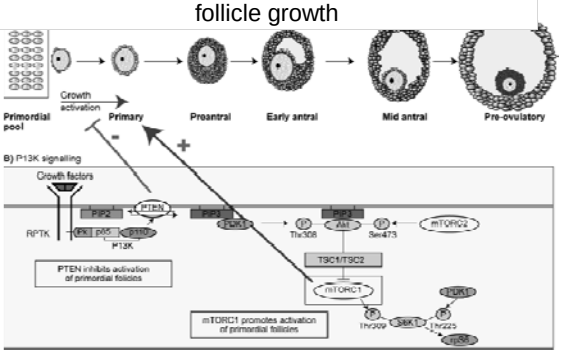
Human Follicle development *in vitro* (6 days)

Growth within micro-cortex



Telfer,et al. (2008) Human Reproduction

Pathways affecting rate of activation and early follicle growth

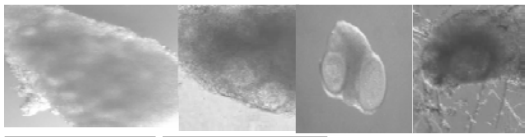


McLaughlin et al (2011) Fert Steril

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 growing follicles longer in step 1 results in increased death and poor quality follicles/oocytes.

Isolating Growing Follicles

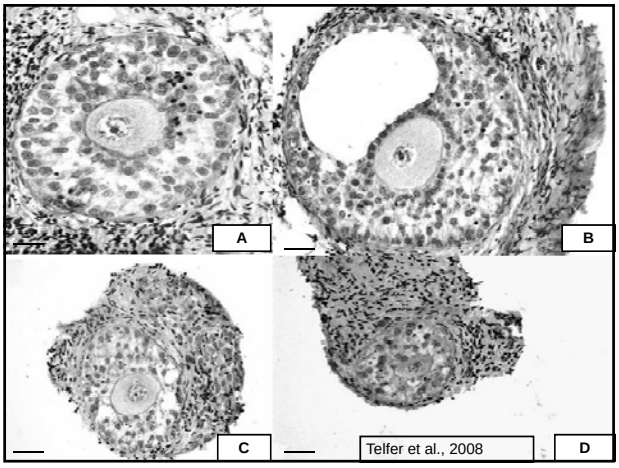


Cultured micro-cortex

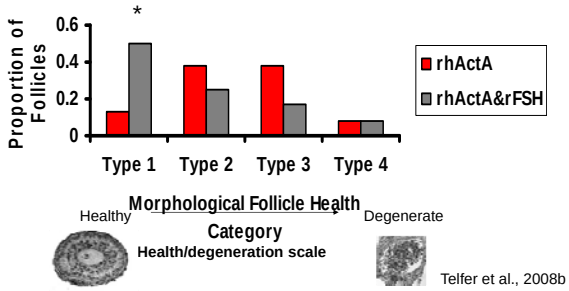
~~Follic~~les before isolation

Isolated Follicles

Human Follicle development *in vitro* (6 days)

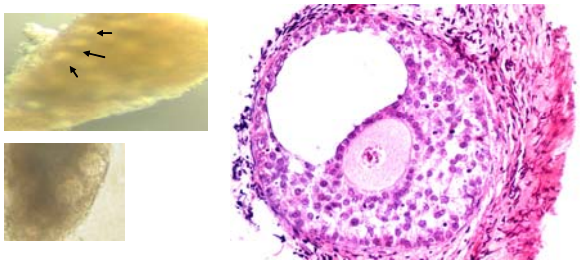


Optimising Growth Medium: Activin versus Activin + FSH



Telfer et al., 2008b

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

Applications of follicle/oocyte culture systems (IVG)

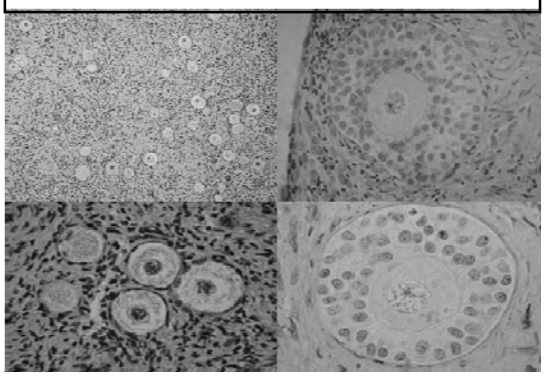
Current

- Basic research tool
- Tissue viability assessment

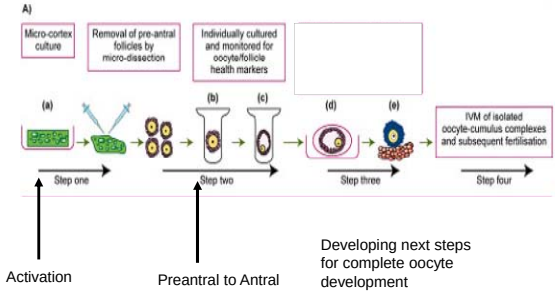
Potential

- Fertility preservation (frozen tissue)

Cryo tissue before/after step 1 of culture



Multi-step Culture system to support human oocyte development



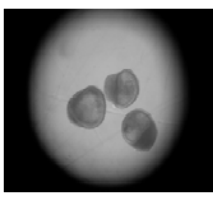
Activation

Preantral to Antral

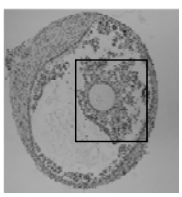
Developing next steps for complete oocyte development

Telfer et al., 2011

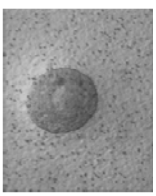
Third step in human follicle culture



In vitro Grown Follicles (after 2 steps)



Remove oocyte and surrounding cells



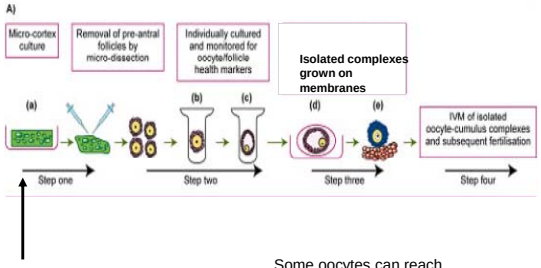
Step 3: Culture on membranes

McLaughlin, Anderson, Wallace, Albertini & Telfer (unpublished)

Preliminary results using a three step culture system

NEW DATA WILL BE PRESENTED HERE

Multi-step Culture system to support human oocyte development



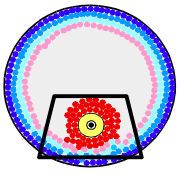
Activation

Some oocytes can reach Metaphase II

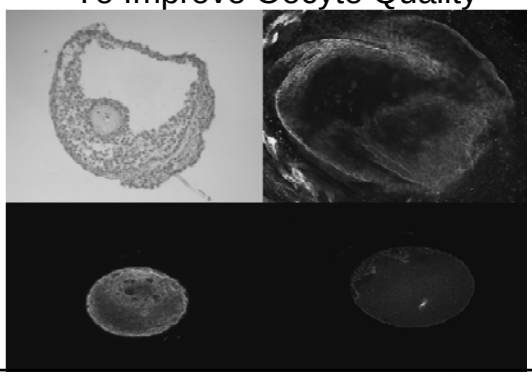
Telfer et al., 2011

Oocyte Development *In Vitro*

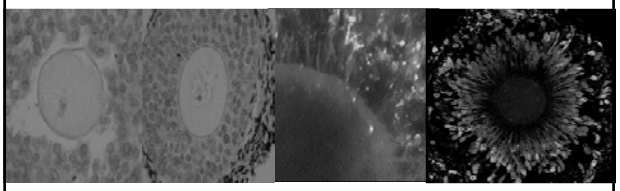
- Once an antral cavity has formed oocyte development does not depend upon development of the entire follicle
- Conditions that promote follicular development could be antagonistic to optimal oocyte development
- Good progress but much to be done.



Improving Culture Conditions To Improve Oocyte Quality



Supporting Communication Between the Oocyte and surrounding cells is key to good egg development



Our work is focusing on optimising oocyte-somatic cell communication during In Vitro Growth

Can we make new oocytes in vitro?

Combining OSCs with IVG systems…….the future

Culture techniques at the centre of Fertility Preservation Strategies

Ovarian tissue cryopreserved → **Test tissue viability by IVG prior to transplantation (density testing etc)**

Cultured in multi step system

Production of developmentally competent oocytes

Acknowledgements

Marie McLaughlin

John Binnie

Susan Spence

Joan Creiger

Hamish Wallace

Richard Anderson

David Albertini (Kansas)

Jon Tilly (Harvard,MGH)

MRC Medical Research Council

BBSRC bioscience for the future

All the patients who kindly donate their ovarian tissue and the clinical teams that support this