LEARNING OBJECTIVES

At the conclusion of this presentation, participants should be able to:
1. Define the steps required to achieve complete in vitro growth from primordial follicles (multi-step culture system)
2. Describe differences in potential of IVG for different patient groups
3. Summarise steps required before IVG could reach clinical application

DISCLOSURE

I have nothing to disclose
**In Vitro Gametogenesis/Growth (IVG)**

- Development of immature gametes to maturity entirely *in vitro* (*Primordial Follicles*)
- Formation of gametes from stem cells (ESCs, iPSCs, Germline Stem Cells)

This presentation will concentrate on growth from primordial follicles.

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**Oocyte Formation/Follicle Development**

- Growth/ Meiotic Arrest
- Acquisition of Meiotic Competence
- Acquisition of Developmental Competence
- Transcription/Transcriptional Repression
- Genomic imprinting

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**In Vitro Gametogenesis/Growth (IVG)**

- Define the fundamental mechanisms of oocyte development (basic science)
- Clinic: Fertility Preservation
- Clinic: Next generation IVF (IVG)
- Animal Production
- Endangered species
- Toxicity testing
**A Brief History of Mouse Oocyte Development In Vitro**

- 1976: In vitro oocyte growth
- 1979: In vitro maturation
- 1984: IVF and Development to Young

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**Mice from In Vitro Grown 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. Eppig & O’Brien, 1996; O’Brien et al., 2003

More recently: Complete in vitro generation of fertile oocytes from primordial germ cells Morohaku et al., 2016 and iPSCs Hikabe et al., 2016

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**Developing systems to grow human oocytes *in vitro***

- **Cortical oorion biopsy**
- **Fragmentation**
- **Isolation of follicles**
- **In vitro culture**
- **Artificial ovary**

*In some cases IVG would be the only option (prepubertal girls where only tissue is stored but cannot be transplanted)*

Tissue freezing in Edinburgh since 1996. Now Centres worldwide for Fertility Preservation
Human ovarian cortical biopsies taken for fertility preservation contain mainly primordial/unilaminar follicles. The challenge is to develop oocytes in vitro from primordial stages to maturation and fertilisation.

Frozen-thawed human ovarian cortical strips

Developing IVG systems for human oocytes: Multi-step system required
• 1) Optimising growth from primordial stages (Activation)
• 2) Supporting development of isolated growing follicles
• 3) Final stages of oocyte development
• 4) Testing function (meiotic and fertilisation potential) and normality

 Sources of Human Ovarian Tissue For Research
Small strip of ovarian cortex donated after informed consent:
• Caesarean section (Healthy women)
• Fertility Preservation (various cancers and Turners syndrome)
  Some tissue obtained after chemo treatment.
• Tissue from 15 months-45 years (fresh and cryopreserved)
• Transgender patients (whole ovaries at time of gender reassignment surgery)

Clinical Collaborators: Richard Anderson, Hamish Wallace, Neale Watson
Step 1: Micro-Cortex Culture (Follicle Activation)

Cortical biopsy cut into strips

- Micro-cortical fragments
  - Underlying stroma reduced
  - Larger follicles removed
- Free floating cultures: basic conditions serum free medium

Telfer et al., 2008 Human Reproduction 23: 1151-8

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Initiation of Primordial Follicle Growth in vitro

Regulation of Primordial Follicle Activation

Pharmacological manipulations of the PI3K pathway can alter the rate of follicle activation.

Telfer et al., 2008 Human Reproduction 23: 1151-8

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Step one: Activation and Growth of Quiescent Follicles

- Primordial Follicles activate within a loose micro-cortex
- Hippo signalling disruption ([Tissue architecture crucial](#))
- Isolated Primordial Follicles do not activate in vitro
- Optimal time & size to remove growing follicles from micro-cortex environment
- 6-8 days; ≥ 100µM mean diameter
- Prolonging Step 1 results in increased death and poor quality follicles/oocytes

Telfer et al., 2008 Human Reproduction 23: 1151-8
Step 2: Isolation of Growing Follicles

Manual dissection using needles and fine scalpel (No enzymes)
Follicles Cultured individually in V shaped wells (No Alginate).
Activin A supplementation of medium, Additional 8-10 days in vitro

Telfer et al., 2008 Human Reproduction 23: 1151-8

Antral Follicle Development from Primordial Follicles grown in vitro after Step 1 (6-8 days) and step 2 (8-10 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

Slow Growth Methods
(IVG of Human Preantral Follicles)

Almost fully grown oocytes (95 microns) obtained within alginate encapsulated follicles grown for 24 days. Woodruff Group

Step Three: Isolating Oocyte-Granulosa Cell Complexes from IVG antral follicles

In vitro Grown Follicles (after 2 steps)
Remove oocyte and surrounding cells
Step 3: Culture Oocyte-Granulosa cell complex on membranes


In Vitro Maturation (IVM) of In Vitro Grown (IVG) Oocytes

Metaphase II oocytes obtained from human IVG (19-21 days) follicles following 24h IVM

All IVG Oocytes that formed Metaphase II spindles had large polar bodies.

Approx 30% of oocytes that complete the culture process can reach Metaphase II: Epigenetic Status and Fertilisation potential?

Multi-step Culture system to support human oocyte development

1. Preparation of the tissue
2. Multinuclear follicles (b) can be formed within 4 days of culture.
3. Chorionic formation (c) occurs within 8 days of culture.

In Vitro Growth of Oocytes from Prepubertal Girls

Oocytes developed in vitro from pre-pubertal mice: What about oocytes from young girls?
### Increasing amounts of stored tissue of pre-pubertal girls for Fertility Preservation

#### Study: Patients and samples

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Age (years)</th>
<th>Biopsy State</th>
<th>Menarche</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhabdomyosarcoma</td>
<td>3.0</td>
<td>Fresh</td>
<td>Premenarche</td>
</tr>
<tr>
<td>Epidermoidoma</td>
<td>8.2</td>
<td>Fresh</td>
<td>Premenarche</td>
</tr>
<tr>
<td>Rhabdomyosarcoma</td>
<td>7.9</td>
<td>Cryopreserved</td>
<td>Premenarche</td>
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<tr>
<td>Rhabdomyosarcoma</td>
<td>10.6</td>
<td>Fresh</td>
<td>Premenarche</td>
</tr>
<tr>
<td>Ewing’s Sarcoma</td>
<td>12.0</td>
<td>Fresh</td>
<td>Post menarche</td>
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<tr>
<td>Sacral Sarcoma</td>
<td>12.3</td>
<td>Fresh</td>
<td>Premenarche (early puberty)</td>
</tr>
<tr>
<td>Acute Myeloid</td>
<td>14.4</td>
<td>Fresh</td>
<td>Premenarche (early puberty)</td>
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<tr>
<td>Leukaemia</td>
<td>14.4</td>
<td>Fresh</td>
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<tr>
<td>Hodgkin’s Disease</td>
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<tr>
<td>Rhabdomyosarcoma</td>
<td>16.0</td>
<td>Cryopreserved</td>
<td>Post menarche</td>
</tr>
</tbody>
</table>

Samples from Edinburgh Fertility preservation service (3-16)(all laparoscopic biopsies)

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The immature human ovary shows loss of abnormal follicles and increasing follicle developmental competence through childhood and adolescence.

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Follicles activate growth (step 1) in young tissue and growing follicles can be isolated for step 2.

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Tissue cultured from young girls (3-10 and 12-15).
**Conclusions**

- Follicles can initiate growth at all ages, to secondary stage
- Follicles from younger girls grow slowly, and show little oocyte growth
- Follicles from adolescent girls grow more slowly than those from adult women but show significant oocyte growth compared to younger girls
- Culture System needs to be adapted according to age and tissue origin

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**Range of ovarian tissue developed in vitro**

<table>
<thead>
<tr>
<th>Tissue Source</th>
<th>Endpoint achieved in vitro (multi-step system)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy women (end of pregnancy)</td>
<td>Metaphase II oocytes</td>
</tr>
<tr>
<td>Prepubertal girls (FP source)</td>
<td>Multilaminar stages</td>
</tr>
<tr>
<td>Turner's Patients</td>
<td>Multilaminar/early antral</td>
</tr>
<tr>
<td>Chemo treated</td>
<td>Variable depends on age and treatment</td>
</tr>
<tr>
<td>Gender Reassignment</td>
<td>Metaphase II oocytes</td>
</tr>
</tbody>
</table>

IVG: System has to be adapted for tissue type
Summary
• Multi step culture system supports human oocyte growth and development from Primordial Stages
• Optimisation of each step required
• Further testing required (epigenetic status)
• Fertilisation potential?
• A model system for human oocyte development

Next Steps
• Improving Culture Media (additives/timings)
• Developing a Bio-reactor (Catapano, Gualtieri, Talevi, Naples)
• Physical Conditions to improve polar body formation
• Compare Culture systems (FastGrow) versus SlowGrow (Picton system)

Next Steps Towards Clinical Application
• Determining health and developmental competence of IVG oocytes (sequencing, epigenome, metabolome)
• Fertilisation of IVG human oocytes: HFEA approval
• Embryo Testing
• Parallel studies on a large animal model (sheep and cow) embryo testing and transfer. Live Young
Now being used to study potential to generate new oocytes from putative germ-line stem cells: Complete In Vitro Gametogenesis.

Future: In Vitro Gametogenesis/Growth: Making new germ cells

Isolation of Oogonial Stem Cells (OSCs)

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