In vitro systems to support development of immature human oocytes

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Making an Egg is Complicated

Growth/ Meiotic Arrest
Acquisition of Meiotic Competence
Acquisition of Developmental Competence
Transcription/Transcriptional Repression
Genomic imprinting
Mice from Primordial Follicles (and now PGCs and iPSCs)


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

Cortical ovarian biopsy

Fragments
- Avascular transplantation
  - >86 Live Births

Isolated follicles
- In vitro culture
- Artificial ovary
  - Avoids transmission of malignant cells

Picton Group (Leeds), Woodruff group (Chicago), Hovatta (Stockholm), Dolmans/Donnez (Brussels)
Developing human primordial follicles from cortical strips *in vitro*

Frozen-thawed cortical strips

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.

Rhabdomyosarcoma Pt 15yrs: thawed from slow freeze
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 (meiotic and fertilisation potential) and normality
Source of Human Ovarian Tissue For Research

• Small strip of ovarian cortex donated after informed consent: Caesarean section/Gynaecological procedures/Fertility Preservation
• Tissue from 18 months-45 years (fresh and cryopreserved)
• Clinical collaborators Richard Anderson and Hamish Wallace
Step 1: Activation of primordial follicles and growth within micro-cortex

Micro-cortex: start of culture

After 6 days

Human Follicle development *in vitro* (6 days)

Telfer et al., 2008 Human Reproduction
Activation and Growth of Quiescent Follicles

- 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

Step 2: Isolating Growing Follicles

Cultured micro-cortex

Follicles before isolation

Isolated Follicles

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

Step 3: Isolating Oocyte-Granulosa Cell Complexes

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

McLaughlin, Albertini, Wallace, Anderson & Telfer (under review)
Mean Oocyte Diameter at end of Multi-step Culture

Oocyte diameter (microns)

Time on membranes

Step 3

Oocyte Development IVG/IVM
Metaphase II oocytes from human IVG follicles
Approx 30% of oocytes that complete culture process can reach Metaphase II: Epigenetic Status and Fertilisation potential?

McLaughlin, Albertini, Wallace, Anderson & Telfer (under review)
Summary

• Multi step culture system supports human oocyte growth and development
• Optimisation of each step required
• Further testing required (epigenetic status)
• Fertilisation potential?
• How does age and prior treatment affect IVG? (Fertility Preservation)
In Vitro Growth of Oocytes from Prepubertal Girls

Oocytes developed in vitro from pre-pubertal mice: What about oocytes from young girls?
## Patients and samples

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<th>Age (years)</th>
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</table>

Samples from Edinburgh Fertility preservation service (3-16) (all laparoscopic biopsies)
Morphologically abnormal oocytes (Big Fat Oocytes) found in young ovaries.

A 8 yrs

B 14 yrs

C

D

% GV Absent Follicles

Mean Follicle Diameter (microns)

Age (yrs)

Prepubertal  Pubertal  Adult

µm

25

50

8 yrs 14 yrs
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.

Tissue cultured from young girls (3-10 and 12-15)
Effect of age on In Vitro Growth (IVG) (Step 2)

Growth rate during step 2 of culture
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
In vitro growth of human follicles after Chemotherapy

Tissue from Hodgkin lymphoma patients who had previously received chemotherapy (ABVD: 8 or OEPA-COPDAC: 3) between 4 weeks and 36 months previously.

ABVD adriamycin, bleomycin, vinblastine and dacarbazine (low risk, non-sterilising)

OEPA-COPDAC vincristine, etoposide, prednisone, doxorubicin (OEPA) and cyclophosphamide, vincristine, prednisone, dacarbazine (COPDAC) (high risk, sterilising)
Non-growing follicle density is increased following adriamycin, bleomycin, vinblastine and dacarbazine (ABVD) chemotherapy in the adult human ovary

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In vitro follicle development

Distribution of Follicle Stages (%)

Healthy
Untreated
ABVD
OEPA-COPDAC

Non-growing
Primary
Secondary

Day
0
6
0
6
0
6
0
6
Health of Oocytes is reduced in OEPA-COPDAC but not ABVD exposed follicles
Tissue From ABVD treated patients appeared to have increased density of non-growing follicles and features associated with the pre-pubertal ovary (clustering and bi-nucleate oocytes).

ABVD Tissue immuno-stained for germline marker DDX4

ABVD Tissue shows clustering of follicles
Also seen in pre-pubertal tissue (Anderson et al., 2014 Human Reproduction 29: 97-106)
Similarities between pre-pubertal tissue and ABVD treated tissue

(i) 22 year old ABVD treated

(ii) 8 year old no chemo

(iii) 16 year old OEPA COPDAC treated

(iv) 23 year old healthy woman
Summary

• Human IVG systems that support development of immature oocytes to Metaphase II have been developed
• Model system to understand mechanisms of human oocyte development
• Current role is as a basic research tool
• Safety concerns with IVG human oocytes need to be addressed [Array of normality tests (Picton)]
• Still a long way to go to unscramble the human oocyte!
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