Ovarian & Immature Testicular Tissues

Jill Davies, Faranaz Auckburally, Marta Kolad, Agnes Jaciow, Emma Tomlinson
INTRODUCTION

- Ovarian tissue cryopreservation service
- Immature testicular tissue cryopreservation service
- Challenges of setting up this CLINICAL service in Oxford, UK
OXFORD SERVICE:

- OXFORD Collaboration:
  - Oxford University
  - Oxford University Hospital NHS Foundation Trust

- Clinical program
  - Patients from England, Wales, Ireland
  - Private patients

- Multidisciplinary team
  - Oxford Cell & Tissue Biobank
  - Oxford Children’s hospital
  - Nuffield Dept of Obstetrics & Gynaecology

- IVI Fertility Clinic

- Charitable funds
CLINICAL PROGRAM: WHY?

- **childhood cancer**
- **Survival 70-90% !!**

Challenges of long term survival:
- Growth and development
- Organ function
- Carcinogenesis
- Psychosocial
- Infertility and reproduction
Non Malignant conditions requiring Bone marrow transplantation

- Sickle cell disease
- Severe aplastic anemia
- Thalassemia
1. Remove ovarian cortex biopsy strips or whole ovary before high risk gonadotoxic chemo or radio therapy treatment
2. Cryopreserve Primordial follicles within outer 1-2mm of ovary ("ovarian cortex")
3. Re-implant tissue when patient is ready to start a family & in remission
4. Orthotopic transplant tissue back & wait for natural pregnancy
5. or Heterotopic transplant, remove eggs, ICSI & embryo transplant

1. When patient in remission & ready to start a family & has a partner
2. Thaw ovarian cortex strips
3. *in vitro* maturation of tissue
4. mature oocytes
5. ICSI
6. Implant embryo
IMMATURE TESTICULAR TISSUE TRANSPLANTATION
Parents offered FP after cancer diagnosis, before the start of chemotherapy.

Testicular biopsy taken from one testis

cryopreservation and storage in vapour phase liquid nitrogen.

patient receives chemotherapy.

childhood cancer survivor grows up and decides to start a family,

Spermatogonial stem cells (SSC’s) harvested from his frozen testicular biopsy, injected into rete testis using an ultrasound guided probe to restore fertility.

After his fertility is restored, he can now father biological children.

from Hermann and Orwig (Eds) "Male Germline Stem Cells: Developmental and Regenerative Potential", Springer 2011
BACKGROUND: HISTORY

- US and EU have FP in place for > 15 years
- 1st live birth following transplant of thawed ovarian tissue 2004
- 1st patient who had ovarian tissue stored in Belgium became adult & had successful pregnancy (2015)
- ITT service started later
- Now publications showing >100 live births from ovarian tissue transplantation
INTRODUCTION: OXFORD SERVICE

Oxford “Fertility Preservation” service for young cancer patients includes:

- **Ovarian tissue cryopreservation** for females age 1 – 40 years
- **Oocyte IVM & Vitrification of mature eggs**
- **Immature testicular tissue “ITT”** cryopreservation for pre pubertal boys

- Also: procurement & distribution of ovarian & testicular tissue for **research** with data (& from healthy, diseased, living, deceased patients)
OPTIONS: FEMALES UNDERGOING GONADOTOXIC TREATMENT

- Standard female FP option in UK prior to 2013:
  - Protection of ovaries,
  - ovarian transposition
  - use of gonadotropin
  - IVF

- **BUT** patients must:
  - Age = post pubertal
  - have male partner
  - be able to delay treatment for IVF
  - undergo hormonal stimulation

- Therefore, ovarian tissue cryopreservation ("OTCP") is best FP option for:
  - pre-pubertal girls
  - many young females who cannot delay treatment
Options: Males undergoing gonadotoxic treatment:

- Standard male FP option in UK prior to 2013:
  - Adults = sperm freezing
- But patients must:
  - Be age = Adult
  - Able to provide semen sample

- Therefore, immature testicular tissue ("ITT") cryopreservation is best FP option for:
  - Pre-pubertal males
  - Males who cannot provide semen sample

- Most tissue still in storage in EU/US centres (whilst boys reach adulthood)

- ITT = success in animal models only!
Oxford OCTP eligibility criteria:
- females age range 1 – 40 yrs
- undergoing high risk treatment
- receiving curative treatment

Oxford ITT eligibility criteria:-
- Pre pubertal boys
- high risk treatment
- Receiving curative treatment

Feb 2014 – UK NICE guidance
- Oncologists must offer FP for all young cancer patients
LIMITATIONS: OTCP

- Main concern with OTCP is risk of transmission of tissue with malignant cells
- Oxford collaborated with OFU and IVI Fertility Clinics
- Fluid collected from ovary in vivo and from dissection fluid
- IVF unit identified immature oocytes
- Immature oocytes -> *In-Vitro* Maturation (IVM) & Vitrified Oocytes from 2 yr old patient!
- Also: OU human genetics team metastatic cell analysis
EU & UK LEGISLATION:

- Legislation: EU tissue & Cells Directives & UK Human Tissue Act, Quality & Safety for Human Application regulations
- In UK OTCP & ITT must be in centre with HTA “Human Application” sector license
  + 2013 = 1st UK clinical ovarian FP license
  + 2015 = 1st UK immature testicular FP license
- On-site inspection every 2 yrs
- Environment: pharmaceutical manufacturing grade ‘A’ cleanroom
- Oocyte aspiration must be done in centre with HFEA license (cleanroom not required)
VALIDATION:

- complex Legal UK & EU requirements
- Legislation by HTA & HFEA changed

- Submit ‘Preparation Process Dossier’
- must use established protocol
- Oxford link = Hadassah Hebrew University Hospital
- Hadassah protocols
- Training by/in Israel

Hadassah =100% live births!
STRATEGY – OTCP RESEARCH:

- Oxford University research:
  - **OCTP research program**: led by Dr Suzannah Williams
  - Developing IVM technique
  - Association with Edinburgh led by Prof Evelyn Tefler
  - **ITT research program**: led by Dr Kevin Coward/Prof Anne Goriely
  - Collaboration with Edinburgh led by Prof Rod Mitchell
  - **OCTP/ITT- Biomedical tissue** engineering validation led by Prof Zhangfeng Cui
- **OUHFT Histopathology research**: led by Dr Sanjev Manek & Clare Verill
- **OUHFT psychological research**: led by Dr Sheila Lane
VALIDATION - HUMAN OVARIAN TISSUE

OU Biomedical Engineering team:

- Calcein AM/ethidium homodimer-1 live/dead assay

- Pre & post freeze results
  - ‘live’ follicles = green
  - ‘dead’ follicles = red
VALIDATION:

OUHFT Histopathology team:

- validation with animal tissue
- & first 10 OTCP patients
- Post thaw tissue histology (H & E stain),

Expected “normal” ovarian tissue morphology
VALIDATION:

OUHFT Histopathology team:

- Post thaw tissue histology (H & E stain),
- we found:
  - Normal morphology
  - Primordial Follicle count
  - Presence of mature follicles
Oxford University Williams lab

- In vitro culture methodology for thawed OTCP tissue
  - Follicle cell counts? Increase?
  - Primordial follicles => ? Do any develop to primary & secondary follicles?
  - Molecules secreted by growing follicles? Are hormones & growth factors secreted?
  - Genomic studies

- Tissue transplanted into nude mice
  - Ongoing clinical follow up
  - Post-thaw Tissue viability ok
  - > 24 hrs before processing?
  - Position of transplant?

Details of these studies currently submitted for publication
“NOVEL” tissue - new UK & EU legislation

mandatory Clinical follow up - data register kept by OCTB:

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<tr>
<th>OHVB ID</th>
<th>MRN</th>
<th>Last name</th>
<th>First Name</th>
<th>Initials</th>
<th>DOB</th>
<th>Age at OTCP</th>
<th>Tanner Scale - Breast (1-5)</th>
<th>Tanner Scale - Pubic Hair (1-5)</th>
<th>Referrer</th>
<th>Referral Date</th>
<th>Local Hospital</th>
<th>Diagnosis</th>
<th>Treatment received prior to OTCP</th>
<th>Prior treatment which may affect OTCP</th>
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<th>Treatment plan - Radiotherapy</th>
<th>Consentor</th>
<th>Date of Consent</th>
<th>Research Q1 - Remnants</th>
<th>Research Q2 - &gt;50 Strips</th>
<th>Research Q3 - Not Needed</th>
<th>Research Q4 - Animal R&amp;D</th>
<th>Concomitant procedures</th>
<th>Surgeon</th>
<th>OHVB Tech</th>
<th>Ward Patient</th>
<th>Date Patient admitted</th>
<th>Date patient discharged</th>
<th>How many days inpatient</th>
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<tr>
<th>Procurement Date</th>
<th>Procurement Time</th>
<th>Whole ovarian or biopsies</th>
<th>Right or Left Ovary</th>
<th>How many biopsies</th>
<th>Aspiration in Theatre by</th>
<th>Aspiration in OHVB Lab</th>
<th>Transport time</th>
<th>Processed by</th>
<th>Dissection start time</th>
<th>Cryoprotection start time</th>
<th>Cryopreservation start time</th>
<th>OTCP Storage time</th>
<th>Strips, segments and Gates in storage</th>
<th>Follide</th>
<th>Follicle description</th>
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| Ovary weight | Evidence of malignancy | AMH (pmol/L) | Total No Strips stored | Thaw date (1) | No of strips thawed (1) | Destination of strips (1) | No of strips remaining in storage | No of Immature eggs - (Theatre aspirate) | No of Immature eggs - dissection remnants | No of Mature eggs in the storage - Theatre aspirate | No of Mature eggs in the storage - dissection remnants | No of Mature eggs in Storage | Total No of Mature eggs in Storage | Pre Screening Blood Results | FSH Result (pre OTCP) | AMH Result (pre OTCP) | Mandatory Blood Results |
|---------------|-----------------------|--------------|------------------------|-----------------|-------------------------|-------------------------------|-------------------------------|-----------------------------------|---------------------------------|----------------------------------|------------------|-------------------------|----------------------|------------------------|----------------------|------------------------|
|               |                       |              |                        |                 |                         |                                |                                |                                    |                                  |                                  |                  |                        |                      |                        |                      |                        |

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<th>Transport Fluid Micro</th>
<th>Cryoprotectant Fluid Micro</th>
<th>Adverse Events</th>
<th>Notes</th>
<th>status code</th>
<th>benign / malignant</th>
<th>diagnosis code</th>
<th>referral centre</th>
<th>Pubertal status code</th>
<th>Treatment prior to OTCP code</th>
<th>Concomitant procedure code</th>
<th>Opened closed procedure code</th>
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1st patient Sep 2013 (within 1 week of Oxford gaining HTA license)
TESTICULAR TISSUE FP SERVICE

1\textsuperscript{st} patient Oct 2015 (same time as license awarded)
PROCUREMENT: OVARIAN TISSUE

- Remove ovarian cortex biopsy strips or whole ovary
- Preserve Primordial follicles in outer 1-2mm of ovary ("ovarian cortex")
- Re-implant tissue when patient = adult & in remission
PROCUREMENT: OVARIAN TISSUE

- “normal” morphology of ovarian tissue section
- Acceptable tissue quality if >5 primordial follicles per section
Ovarian - Biopsy or whole ovary
Testicular - Wedge biopsy

Surgical Consent – do not specific side (unless good reason pre-agreed)

Minimise bioburden during procurement (because no tissue terminal sterilisation)
- Aseptic technique (gloves, patient skin prep)
- Standard keyhole technique (with Burt bag, need to enlarge port incision for removal of large ovaries)
- Single port (no bag)
- Handover pre-arranged surgeon -> scrub nurse -> tissue bank technician
- Environment monitoring of theatre
Procurement - Biopsies only

Cut into ovary cortex
Cut off ovary cortex biopsy
Cortex biopsy held in laparoscopic forceps

Biopsy placed in sterile container
Biopsy taken out of body through port
Procurement - whole ovary

Isolate & inspect ovary

Cauterise ovary pedicle

Ovary detached

Bag opened & ovary dropped into sterile container

Bag brought out of body through laparoscope

Ovary placed inside bag
PROCUREMENT: IMMATURE TESTICULAR TISSUE

- to remove 1/3 testicular tissue from one testes
- Midline incision & wedge biopsy
- No reconstruction needed
Development of the testis in pre-pubertal boys with cancer after biopsy for fertility preservation

M Uijldert, A Meißner, A.A. de Melker, A.M.M. van Pelt, M.D. van de Wetering, R.R. van Rijn, M van Wely, F van der Veen, S Repping

*Human Reproduction, https://doi.org/10.1093/humrep/dex306*

**Published:** 12 October 2017  **Article history ▼**

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**Abstract**

**STUDY QUESTION**
Is testicular growth affected by a testicular biopsy intended for fertility preservation in pre-pubertal boys with cancer?

**SUMMARY ANSWER**
Testicular growth of the biopsied testis is not impeded in comparison to the non–biopsied contralateral testis up until 1 year after surgery.
Witnessing, packaging, labelling & transport to Tissue bank

Donor id & consent checked by tissue bank & with theatre staff

Single-use aseptic kit for packaging tissue

Scrub nurse passes wedge biopsy to tissue bank technician

Tissue immersed in nutrient media, labelled & double wrapped

Tissue transported quickly to tissue bank cleanroom for processing

Tissue transported in cooled (2 – 8°C) validated shipping container
Tissue is processed within a UK tissue bank (rather than an IVF/ART centre) because EU & UK regulations require processing to be within a Grade A air quality cleanroom (similar to pharmaceutical drug manufacturing).

Each tissue bank has to have a license to process tissue & must validate all techniques. Each tissue bank also has to undergo inspection every 2 years. Licenses are displayed in each tissue bank.
Transferring tissue into pharmacy manufacturing grade A cleanroom

Container with tissue transferred through hatch into cleanroom with 45 pa pressure & Grade A air quality environment

Two Class II microbiological safety cabinets prepared for processing tissue

Staff in irradiated body suit use single-use-only disposable sterile processing kit
2 technicians dissecting tissue into strips/segments
Cryoprotection of tissue segments

tissue segments are placed in cooled cryoprotectant mixture. Cryoprotectant replaces intracellular water.

After 1 hour incubation, each segment is removed & placed in a labelled cryovial with 1ml cryoprotectant.
Controlled slow cooling program designed specifically for each tissue

Load pre-cooled cryovials on cryocanes

Pressurised vapour phase nitrogen used to cool according to ITT program
Controlled slow cooling program designed specifically for each tissue

Cryovials removed from CRF at -150°C
Loading cryovials in cryocanies into controlled rate freezer (CRF)

Cooling program is designed to prevent tissue damage e.g. taking account of latent heat of crystallisation etc

Vapour phase nitrogen is pumped into chamber of CRF on demand
Seeding, cooling, removal & packaging of cryovials

To prevent damage due to super-cooling, crystallisation is induced @ -9°C by touching meniscus of each cryovial with precooled forceps.

Cryovials from each donor are packaged in sterile bag and protective box & labelled.
Transfer of tissue to vapour phase nitrogen storage refrigerators

Box containing cryovials from one patient is transferred to pre selected rack position for long term storage.

Tissue is stored in vapour phase nitrogen @ < -170°C.

ITT stored after cryoprotection and slow controlled freezing may be safely preserved below -130°C for up to 55yrs. 24 hour temperature monitoring alarm systems are used.
OTCP & ITT Tissue in vapour phase nitrogen until

- Patient becomes an adult
- In remission
- Patient attends Oxford FP clinic
- Transplant is arranged

Hadassah = 100% live births!

- Until method developed for ITT in humans, Oxford encourages patients to give 10% for research
  - OU
  - Edinburgh
  - Grow Sperm
- Total 190 OTCP patients (Jul 2017)
- takes 30 mins
- at same time as other procedures (e.g. Central venous line, Bone marrow transplant aspirate)
- usually = day cases
- No serious adverse events

- Also - 3rd party procurement centres
- 48 ITT patients
- N= increasing rapidly
- age = 5.8yrs
- 56% Benign
- Takes 15 mins
- Average no of strips = 11
- Day cases
- No serious adverse events
OXFORD TRANSPLANTATION

- 2 x OTCP patients due to have transplants in Nov 2017
- Technique = keyhole surgery
- Orthotopic - into remaining ovary
- Oxford team attended training in Israel Dec 2016
- Israeli surgeon will attend transplant in Oxford

Hadassah = 100% live births (after 1 or more transplants)

- ITT patients still too young (no attempts at transplant yet)
• 1 x OTCP patient wishes to have transplant to avoid menopause @ 13 yrs

• Benign diagnosis, so ready for transplant

• ?use injection of tissue technique

• But = New indication & methodology

• **therefore Oxford need HTA authorisation & OUHFT TAG approval**
RESULTS - OTCP PATIENT SURVEY

- ‘New service’ evaluation
- good feedback
- Patients/parents helping with advice for:
  + Consent forms
  + Information leaflets for children
  + Website
  + Publicity of service
- Online consent package?
- Concerns about being charged for IVM & storage (IVI given very generous donation – money & help)
INTRODUCTION OF PRINCIPLES OF ISO 9001:2015

Adopting a Risk Based Thinking Quality Management System Approach in a Tissue Establishment

Jill Davies, Emma Tomlinson, Faranaz Auckburally, Marta Kolad, Agnes Jaciow, Michael Sebastian & Vinod Motiani.

Oxford University Hospitals NHS Trust
Example 3. Procurement technique change

- OCTB training staff at ovarian procurement site
- Emphasis on minimising microbial contamination at all stages of processing

- Surgeons & scrub nurse take extra care with aseptic technique
- OCTB technician observing
- Tissue handover practised
- Environmental monitoring

- Opportunities for improvement suggested by anyone in MDT
- Team working enhanced
- Good feedback in service review with patients/parents

- All MDT staff engaged
- Procurement process is accepted as critical
- Microbial bioburden is carefully controlled & reduced
Example 4. Tissue Dissection Change

- Focus on End user requirements (for transplantation)
- Observation at other transplant centres
- Representatives from whole MDT involved in planning change

- Benchmarking & Literature review (innovative technique)
- Post operative patient follow up planned
- Surgeons practising transplant technique with research tissue
- Proactive Culture within MDT of continual improvement

- OCTB staff trained
- Dissection technique changed
- Tissue prepared for transplantation in different dimensions (but same thickness)
- Dimensions all recorded

- OCTB staff changed technique without any problems
- Faster technique, therefore improved tissue viability
- End users satisfied (more options for transplant)
- Patient/parent satisfied
ETHICAL ISSUES

After Patient Death do you go back to ask for research use?
Consent before it was known about Animal studies – do you go back to do you Re-consent At 18yrs?
What if patient sues parent/us for assault?
Trangender patients
Private patients (generate funds for service?)
Social freezing (females who chose to delay pregnancy)
Developing decision aid development patient/parents
3rd party procurement
Funding – no national funding yet (except for patients having sex change)
No charges to patient (cf IVF patients)
What if patient already has one child?
Should we put time limit on storage? Charge after X Years?
What do we do when we lose contact with patient?
How do we do this legally?
Should we offer service to patients >35?
Oocyte aspiration & vitrification by OCTB? Is there training for tissue bankers doing ART work?
• More patient awareness & involvement

• Extend 3rd party procurement service option (to manage increase in patients)

• Increase tissue for Research teams for:
  • Oxford
  • Edinburgh

• Gain national funding!
Thank you
Acknowledgements

Oxford Cell & Tissue Biobank Team
Emma Tomlinson, Faranaz Auckburally, Marta Kolad, Agnes Jaciow, Michael Sebastian, Vinod Motiani.
Sheila Lane
Stephen Kennedy
Kokila Lakhoo
Christian Becker
Enda McVeigh
Sanjiv Manek & Clare Verrill
Suzannah Williams (ovarian research team)
Zhangfeng Cui (tissue engineering team)
Anne Goriely/Kevin Coward (testicular research team)
HTA & HFEA