Fertility Preservation in Prepubertal Boys

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SPEG Dunkeld 2019
Children Survive Cancer

~1:500 young adults is a survivor of childhood cancer
# Gonadotoxic Cancer Treatment

<table>
<thead>
<tr>
<th><strong>LOW RISK (&lt;20%)</strong></th>
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<tbody>
<tr>
<td>• Acute lymphoblastic leukaemia</td>
<td>• Wilms Tumour</td>
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<tr>
<td>• Soft-tissue sarcoma: stage 1</td>
<td>• Germ cell tumours (no radiotherapy)</td>
</tr>
<tr>
<td>• Retinoblastoma</td>
<td>• Brain tumour (surgery, radiotherapy &lt;24Gy)</td>
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<table>
<thead>
<tr>
<th><strong>MEDIUM RISK (20-80%)</strong></th>
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<tbody>
<tr>
<td>• Acute myeloblastic leukaemia</td>
<td>• Neuroblastoma</td>
</tr>
<tr>
<td>• Hepatoblastoma</td>
<td>• Non-Hodgkins lymphoma</td>
</tr>
<tr>
<td>• Osteosarcoma</td>
<td>• Hodgkins lymphoma: alternating treatment</td>
</tr>
<tr>
<td>• Ewings sarcoma: non-metastatic</td>
<td>• Ewings sarcoma: non-metastatic</td>
</tr>
<tr>
<td>• Soft tissue sarcoma: stage II or III</td>
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<thead>
<tr>
<th><strong>HIGH RISK (&gt;80%)</strong></th>
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<tr>
<td>• Total body irradiation</td>
<td>• Hodgkins lymphoma: alkylating agents</td>
</tr>
<tr>
<td>• Localised radiotherapy (pelvic/testis)</td>
<td>• Soft tissue sarcoma: stage IV (metastatic)</td>
</tr>
<tr>
<td>• Chemotherapy for BMT</td>
<td>• Ewings sarcoma: metastatic</td>
</tr>
</tbody>
</table>

Wallace WHB et al. Lancet Oncology. 2005
Fertility Preservation for Prepubertal Boys

Fertility preservation: don’t forget the boys

Rod T Mitchell, consultant paediatric endocrinologist and Wellcome Trust fellow1, Leena Nahata, assistant professor of clinical paediatrics2, Gwendolyn P Quinn, professor3
Cancer Treatment and Prepubertal Testis

**PREPUBERTAL TESTIS**

- **Chemotherapy**
- **Sertoli Cell**
- **GERM CELL** (Incl. SSCs)
- **Peritubular Cell**

**Leydig cells**

**Blood vessel**

**SSC** – Spermatogonial Stem Cell
Approach to Fertility Preservation in Prepubertal Boys

PROTECTION
Chemo-Protective Agent

Chemotherapy

RESTORATION
Biopsy
Transplant
Approach to Fertility Preservation in Prepubertal Boys

- Biopsy
- Chemotherapy
- Transplant

RESTORATION
A Fertility Preservation Programme for Boys

Established in Edinburgh 2015

Clinical
- Long-term storage of testicular tissue (up to 55 years)

Research
- Strategies to protect or restore fertility in boys

Anderson RA* & Mitchell RT* et al. The Lancet Diabetes and Endocrinology, 2015
Criteria for Prepubertal Patient Selection

2014 ‘Edinburgh Criteria’
- Age 0-16 years
- Unable to produce a semen sample
- No testicular pathology
- High (>80%) risk of sub/infertility
  - High dose alkylating agents
  - Radiotherapy to pelvis
- Intention to cure
- Consent (parent +/- patient)

Anderson RA* & Mitchell RT* et al. The Lancet Diabetes and Endocrinology, 2015
Testicular Biopsy Procedure

Images Courtesy of AMC Center for Reproductive Medicine
Tissue Preparation and Cryopreservation

Images Courtesy of AMC Center for Reproductive Medicine
UK Programme for Fertility Preservation

Guardian, December 2018
Prepubertal Patients – UK Experience

Since 2015: n=>200   Age = 0-16y

Malignant
• Solid Tumours – Medulloblastoma, Neuroblastoma
• Haematological Malignancies – Lymphoma, Acute Myeloblastic Leukaemia

Non-Malignant (requiring haematopoietic stem cell transplant)
• Haematological - Aplastic Anaemia, Thalassaemia, Sickle Cell Anaemia, Diamond-Blackfan, Wiskott-Aldrich, Immunodeficiency
• Others – Metabolic, Genetic
Restoration of Fertility

- Biopsy
- Chemotherapy
- Transplant

RESTORATION
Fertility Restoration using Prepubertal Testis Tissue

Pre-Treatment Biopsy

Potential Clinical Options

Animal Model Proof-of-Principle

Adapted from Mitchell RT et al, Endocrine Development, 2009

Sato T et al, Nature, 2010

Honaramooz et al, Nature, 2002

Kanatsu-Shinohara et al, Hum Reprod, 2003
Transplantation of Testicular Tissue from Non-human Primates – Importance of Graft Site

- **Ectopic**
  - Marmoset Monkey Transplants

- **Intra-testicular**

<table>
<thead>
<tr>
<th>4 Months</th>
<th>9 Months</th>
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<tbody>
<tr>
<td>Recovered</td>
<td>Degenerated</td>
</tr>
<tr>
<td>54% (n=26)</td>
<td>25% (n=12)</td>
</tr>
<tr>
<td>39% (n=7)</td>
<td>39% (n=7)</td>
</tr>
<tr>
<td>50% (n=9)</td>
<td>50% (n=9)</td>
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Complete Spermatogenesis in Marmoset Monkey Testis – Intratesticular Xenografts

Pre-Graft

H+E

MAGE-A4

VIMENTIN

Xenograft (9 months)

H+E

CREM

ACROSIN

Host Testis

Graft

Human Prepubertal Intratesticular Xenografts – Role of Gonadotrophins

LH(hCG)/FSH:

• Somatic cell development/function
  - Leydig cell – testosterone/steroidogenesis
  - Sertoli cell – maturation

• Germ cell development
  - Entry into meiosis
  - Sperm production
Protection of Fertility

PROTECTION

Chemo-Protective Agent

Chemotherapy

[Diagram showing the process of protecting fertility during chemotherapy]
Effects of In-vitro Chemotherapy Exposure on Germ Cell Number in Prepubertal Mouse Testis

Mouse Testis (Pnd5) n=8

Chemotherapy

Day 0 1 2 3 4

Analyse

Culture

Germ Cells/mm²

CISPLATIN

VEH LOW MID HIGH

DOXORUBICIN

VEH LOW MID HIGH

*** *** *** ***
Effects of In-vitro Chemotherapy Exposure on Stem Cells in Prepubertal Mouse Testis

DDX4 – Spermatogonia (SG)
PLZF – Undifferentiated SG
Human Fetal Testis as a Model for Prepubertal Germ Cell Development

GONOCYTE (POUSF1) → PRE-SPERMATOGONIA (MAGE-A4) → SPERMATOGONIA (MAGE-A4)

INFANCY → CHILDHOOD/PRE-PUBERTY

‘Hanging Drop’

Jorgensen A...Mitchell RT. Cell Reports. 2018
Granulocyte Colony-Stimulating Factor (G-CSF) as a Chemo-Protectant in Adult Mouse Testis

Granulocyte colony-stimulating factor (G-CSF) promotes spermatogenic regeneration from surviving spermatogonia after high-dose alkylating chemotherapy

Travis Kotzur¹, Roberto Benavides-García¹, Jennifer Mecklenburg¹, Jamila R. Sanchez¹, Matthew Reilly² and Brian P. Hermann¹

Diagram: % of Seminiferous Tubules

- Empty
- 1° Sct
- Rnd Std
- Complete

Legend:
- Control
- Busulfan Only
- Busulfan + G-CSF
G-CSF as a Chemo-Protectant in Human Testis

G-CSF

- Clinically used to in cancer patients to mobilise stem cells:
  - Haematopoietic Stem Cell Transplant
  - Chemotherapy-induced neutropenia
Summary – Fertility Preservation in Boys

• Prepubertal boys are at risk of future infertility as a result of exposure to cancer treatment

• Testicular tissue cryopreservation is established but should form part of ethically approved clinical research studies

• Current research is focused on:
  - understanding chemotherapy effects on germ cell development and fertility
  - restoring fertility potential using cryopreserved tissues
  - identification of therapies to protect the gonad from chemotherapy
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Effect of Gonadotrophins on Sertoli Cell Function in Prepubertal Human Testis Xenografts

AMH - immature SC
Androgen Receptor - mature SC

Marsida Hutka, PhD Student (Unpublished)