Embryo cryopreservation, egg freezing, and ovarian tissue freezing before chemotherapy

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Conflict of interest

None
Why focus on fertility preservation?

- Survival rates among young cancer patients have increased significantly during recent years (Annual incidence: 50/100,000)
- Modern treatment regimes bear a high risk of gonadotoxic effects
- The cancer patients want it
- Technical developments have made fertility preservation a realistic option
Reasons for fertility preservation

- Gonadotoxic treatment regimes for cancer – chemotherapy, radiation e.g. before bone marrow transplantation
  - or
- Facing premature ovarian failure
  or
- Childbearing delayed to later in life – social reasons
Well-established methods

• Embryo cryopreservation

• Ovarian transposition
Less well-established methods
(some would say still experimental)

• Oocyte cryopreservation

• Ovarian suppression

• Ovarian tissue freezing
Chemotherapy and gonadotoxicity

Risk of inducing detrimental effects on the gonad

- The specific chemotherapeutic drug used
- Dose of chemotherapy
- Duration of chemotherapy
- Age of woman
Gonadotoxic Drugs

- **Alkylating agent**
  - Cyclophosphamide
  - Chlorambucil
  - Mustine
  - Melphalan
  - Busulfan
  - Carmustine
  - Lomustine

- **Antimetabolites**
  - Cytarabine

- **Vinca alkaloids**
  - Vinblastine

- **Others**
  - Procarbazine
  - Cisplatin

The ovaries are especially susceptible to alkylating agents.
Candidates for ovarian cryopreservation

• Cancer patients
e.g. lymphomas Hodgkin, non-Hogkin, Breast cancer, Ewing sarcoma, Wilms tumor

• But also

• Autoimmune diseases e.g. acute glomerulonephritis, Behcet’s disease, SLE
• Endometriosis
• Premature ovarian insufficiency – Turner patients
Methods
Embryo cryopreservation

Standard IVF procedure 2-3 weeks treatment before ovum pick-up
(this delay acceptable in most cancers)

But not applicable in children or very young females

Demands use of semen from husband or use of donor sperm

Pregnancy rate per transfer between 20-30%
Ovarian transposition

• In genital, intestinal, or urinary tract malignancies – often pelvic radiation

• Metaanalysis not conclusive – menstrual cycles – time to pregnancy studies lack
Experimental methods?

At least less well-established methods
Oocyte cryopreservation

Techniques improved significantly the last 5-6 years
Ovarian suppression

Suppression of folliculogenesis with gonadotropin-releasing hormone agonists (GnRH-a)

90% of follicles are primordial – therefore unresponsive to GnRH-a

Randomized trials currently underway
Ovarian tissue freezing

In Denmark more than 870 since 2000
70 autotransplantations
HALF HUMAN OVARY

Small antral follicles

Corpus luteum
Only the ovarian cortex is cryopreserved
Preparation of human ovarian tissue for cryopreservation
Freeze-thawed mouse ovary implanted under the kidney capsule of an ovariectomized mouse for 2 weeks
The Danish Model: “The woman stays – the tissue moves”
Rationale:

Transport ovarian cryopreservation

- Establish a possibility for women who may otherwise be deprived the chance of ovarian cryopreservation
- Centralize the service of this relative seldom performed procedure
- The ischemic period following transplantation reduces the follicle pool most dramatically
Difficult to perform oocyte retrieval and cryopreservation close to one another

Ovary two days after oocyte retrieval
Following ovarian stimulation the ovarian cortex is very fragile

Ovary two days after oocyte retrieval
### Diagnosis for cryopreservation of ovarian tissue in Denmark: cumulative (October 2015)

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No.</th>
<th>Diagnosis</th>
<th>No.</th>
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</thead>
<tbody>
<tr>
<td>Breast cancer</td>
<td>280</td>
<td>Invasive mole</td>
<td>5</td>
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<tr>
<td>Mb. Hodgkin, Non-Hodgkin</td>
<td>173</td>
<td>Thalassaemia</td>
<td>5</td>
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<tr>
<td>Colon-Rectum cancer</td>
<td>16</td>
<td>BRCA1-gene</td>
<td>2</td>
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<tr>
<td>Leukaemia (AML, ALL, CML)</td>
<td>61</td>
<td>Aplastic Anaemia</td>
<td>12</td>
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<tr>
<td>Ewing’s &amp; other sarcomas</td>
<td>74</td>
<td>SLE</td>
<td>6</td>
</tr>
<tr>
<td>Reproductive system, incl. Ovarian &amp; Cervical cancer</td>
<td>81</td>
<td>Sex anomalies incl. Turner syndrome</td>
<td>9</td>
</tr>
<tr>
<td>Various others</td>
<td>46</td>
<td>Other Diseases</td>
<td>38</td>
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</table>
61 deceased patients
14% of the cohort
24 children/37 women
Clinical results - transplantation

- Laparoscopy / mini-laparotomy
- Orthotopic (ovary)
- Heterotopic (sub-peritoneal on anterior abdominal wall and lateral pelvic wall)
Orthotopic transplantation of ovarian tissue
Heterotropic transplantation to a subperitoneal pocket
### Outcome and number of Danish women transplanted with frozen/thawed ovarian tissue according to diagnosis (Jan 2016)

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Women</th>
<th>Transplantation</th>
<th>Pos. hCG</th>
<th>Clin. Preg.</th>
<th>Children</th>
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<tr>
<td></td>
<td>Total</td>
<td>Transport</td>
<td></td>
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<td></td>
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<tr>
<td>Breast cancer</td>
<td>20</td>
<td>23</td>
<td>17</td>
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<tr>
<td>Mb. Hodgkin lymphoma</td>
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<td>13</td>
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<tr>
<td>Non-Hodgkin lymphoma</td>
<td>5</td>
<td>9</td>
<td>3</td>
<td>5</td>
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<tr>
<td>Cervical cancer</td>
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<td>6</td>
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<tr>
<td>Aplastic anaemia</td>
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<tr>
<td>Sarcoma incl. Ewing</td>
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<td>5</td>
<td>4</td>
<td>3</td>
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<td>Paroxystic Nocturnal</td>
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<td>0</td>
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<tr>
<td>Haemoglobinuria</td>
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<td>Ovarian cancer</td>
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<tr>
<td>Colon cancer</td>
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<tr>
<td>Anal cancer</td>
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<td></td>
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<tr>
<td>Various others *</td>
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<td>6</td>
<td>3</td>
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<tr>
<td><strong>Total</strong></td>
<td>58</td>
<td>70</td>
<td>49</td>
<td>30</td>
<td>26</td>
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</tbody>
</table>

*) Wegener's granulomatose, Mola, Morbus Behcet, Autoimmune vasculitis, HUS, Thalassaemia
Results

- All women have regained ovarian function
- Puberty induced in a 13-year-old girl
- Total 26 clinical pregnancies
  - 15 deliveries (10 IVF/5 spontaneous)
  - 9 spontaneous abortions (1. trimester)
  - 1 spontaneous abortion (2. trimester)
  - 1 legal abortion (spontaneous pregnancy)
Questions to be answered

- Longevity of ovarian grafts
- Effectivity whereby fertility is restored
- Safety of auto-transplantation
- When is a woman too old to have tissue cryopreserved
Conclusions

- Ovarian cryopreservation is now a clinical option
- Safety is still unresolved but no relapses yet
- In combination with ART, results from Denmark suggests that ovarian cryopreservation is a robust way of preserving fertility in girls and young women
Thank you for your attention