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Declared no potential conflict of interest.
Current status of heterotopic transplantation of frozen-thawed ovarian tissue

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Early Pioneers of Ovarian Transplantation

- **1895** ROBERT MORRIS: 12 ovarian transplantation in human (by 1901)
- **1906** ALEXIS CARREL: whole ovary transplant


What is Ovarian Tissue Banking for?
For

- Preservation of fertility
- Restoration of ovarian function
- Prevention of POF
- Conservation of endangered animals
- Investigational tool for follicular biology and ovarian physiology
At present, the main purpose of ovarian cryobanking is fertility preservation in cancer patients.
How to restore fertility with cryobanked ovarian tissue?
Most growing and mature follicles do not survive after freezing and thawing of ovarian tissue.

Thus, primordial and primary follicles are the main components of frozen-thawed ovarian tissue.

How to develop follicles in stored ovarian tissue to maturity?
In vitro culture
heterotopic
Current Development in Heterotopic Ovarian Tissue Transplantation
Heterotopic autotransplantation of ovarian cortical strips to the forearm

35 y female with Cx. CA fresh ovarian tissue transplantation before pelvic irradiation. Follicular growth 10 weeks after transplantation

Oktay et al., JAMA. 2001;286
30 years old, stage II Breast Cancer

- Restoration of ovarian function: 85 days
- Eight consecutive percutaneous oocyte retrieval (six after ovarian stimulation)
- Of 20 oocytes retrieved, 8 were suitable for IVF (5 IVM)
- Two fertilized (one abnormal, one 4 cell stage embryo)

Oktay et al., 2004, Lancet, 363
28 y female with HD stage IIB, Follicle growth from sub peritoneal transplant under the abdominal wall
↓ follicle aspiration x4, embryo transfer x2, 1 biochemical pregnancy
Heterotopic Autotransplantation of Cryobanked Human Ovarian Tissue

- JH: 38 y old, adenocarcinoma of cervix; 2002
- AS: 37 y old, carcinoma of cervix; 2002 & 2003
- SH: 29 y old, carcinoma of cervix; 2003 & 2004
- MS: 29 y old, Breast cancer; 2005 & 2006

Restoration of ovarian function: 100%
Duration: 3 months to 5 years
Restoration of fertility: ?

Kim SS et al., 2009, F&S, 91
Cryopreservation
Preparation of ovarian tissue:
Slow freezing of ovarian tissue

- **Freezing solution (FS):** 1.5 M DMSO + 1% human serum albumin + 0.1 M sucrose (in Leibovitz L-15 medium)
- **Thawing solution (TS):**
  - TS1- 1.0M DMSO + 0.1M sucrose
  - TS2- 0.5M DMSO + 0.1M sucrose
  - TS3- 0.1M sucrose
1) Start at 0°C and cool at 2°C/min to -7°C
2) Soak for 5 min before manual seeding
3) Seed at -7°C and hold for 5 min
4) Continue to cool at 0.3°C/min to -40°C
5) Cool at faster rate of 10°C/min to -120
6) Store at -196°C
Transplantation
Autotransplantation of frozen-thawed human ovarian tissue to the heterotopic sites

Abdomen: rectus muscle & rectus sheath
Endocrine Function
Restoration of Ovarian Function:

• **3-5 months** after transplantation, the return of ovarian function was evidenced (FSH <20 IU/L, E2 >20 pg/ml), but lasted only for 3-5 months.

• Three patients underwent second transplantation. The return of ovarian function was faster (**2-4 months**) after second transplantation.

• **Long term** ovarian function lasting for **12-60 months** has been established after second transplantation in all 3 patients.
FSH

months after transplant

Estradiol

Re-Transplantation
Weekly serum FSH/LH/E2 concentrations for 24 wk from May 2004 (8 months post-transplantation) through November 2004
Fertility
Ovarian stimulation was initiated when growing follicles were identified by UTZ or palpation: 300 IU rFSH and ganirelix 0.25mg for 3 – 7 days.

2 growing follicles in the ovarian graft (subcutaneous, pre-rectus)
Human oocytes retrieved from heterotopic grafts when the follicle size reached 14-20 mm.

First oocyte retrieval: 9 months after transplantation (2 MI oocytes)

June 2005
Second oocyte retrieval: 14 months after transplantation

- (1 MII oocytes, 2 MI oocytes)

- (6 cell & 3 cell embryos after 3 day culture)

- (2 cell & PN embryos after 2 day culture)
Is there any future for heterotopic transplantation of cryobanked ovarian tissue?
Pros

• Convenient for repeated multiple transplantations
• Non invasive procedure
• Easily accessible for oocyte retrieval without anesthesia
• Feasible for patients with severe pelvic adhesion

Cons

• IVF procedure required
• Efficacy not proven (No live birth yet)
• Suboptimal (unknown) environmental effects on follicle growth and maturation
• Possible poor quality oocytes
Issues with heterotopic ovarian transplantation

- Cryoinjury
- Ischemic injury
- Optimal heterotopic sites
- Environmental factors/ Follicle growth pattern
- Egg retrieval technique
- Efficacy
Issue 1: Cryoinjury

Apoptotic rates of primordial follicles

Fresh Slow freezing Vitrification

2D gel electroporesis images (the protein expression patterns)

protein expression changes
Vitrification, at least in theory, should eliminate ice formation and minimize cryoinjury.

Vitrification may replace slow freezing in the future as a method of ovarian tissue cryopreservation (??)
Issue 2: Ischemic Injury

Incubation period (hour)

Room Temp (n=6)

Ice (n=6)
Significant ischemic damage can be seen about 24 h after transplantation, but angiogenesis takes more than 48 h.
No treatment

3h

24h

Ascorbic acid

24h

24h
We need to develop new strategies that can protect ovarian tissue from ischemia or facilitate angiogenesis to make ovarian tissue transplantation clinically reliable and robust technology.
Issue 3: Optimal heterotopic site

• Rich in vasculature
• Easily accessible
• Stable in temperature
• Enough space to accommodate large ovarian graft and to support full follicular growth
• **Tested sites:** subcutaneous (forearm, hip, abdominal wall), rectus muscle, uterus, breast tissue, superficial fascia of pectoralis muscle, subperitoneal tissue beneath the abdominal fascia between the umbilicus and the pubic bone
Issue 4: Environmental factors/ Oocytes quality

- Temperature
- Local pressure
- Paracrine factors
- Cytokines
- Blood supplies
Issue 5: Egg retrieval

- Ovarian stimulation protocol
- Aspiration needles (size, length)
- Aspiration pressure
- When to retrieve
- IVM
Issue 6: Efficacy

- Follicle development: <50%
- Egg retrieval: 3/5 (7/8)
- Fertilization: 4/7 (2/20)
- Embryo development: 3/4 (1/2)
- Pregnancy rate: 0 (0)
Conclusions

• Ovarian tissue cryobanking is a promising strategy to preserve fertility in cancer patients.

• Stored ovarian tissue can be grafted either orthotopically or heterotopically.

• To date, there is no pregnancy with heterotopic transplantation of frozen-thawed human ovarian tissue.
Conclusions

• Clinical practicability of heterotopic transplantation is questionable at present as the environment of heterotopic sites may not be as favorable for normal follicular development.

• Nevertheless, there are many advantages of heterotopic transplantation, especially when the hostile conditions of pelvic cavity preclude orthotopic transplantation.
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Thank you!

Kansas City
<table>
<thead>
<tr>
<th>Stimulation dose (FSH/HMG) and duration(^{\ast})</th>
<th>Peak oestradiol (pmol/L)(^{\dagger})</th>
<th>Follicle size (mm)(^{\dagger})</th>
<th>Oocyte</th>
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<tbody>
<tr>
<td>1 None</td>
<td>888</td>
<td>10.9</td>
<td>GV</td>
</tr>
<tr>
<td>2 3675/2175, 13 days</td>
<td>980</td>
<td>14.2</td>
<td>FZ</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10.7</td>
<td>Degenerated</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8.9</td>
<td>GV</td>
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<tr>
<td>4 None</td>
<td>1093</td>
<td>9.1</td>
<td>FZ</td>
</tr>
<tr>
<td>5 3225/1725, 11 days</td>
<td>2257</td>
<td>14.5</td>
<td>FZ</td>
</tr>
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<td>13.1</td>
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<td>9.5</td>
<td>FZ</td>
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<tr>
<td></td>
<td></td>
<td>6.4</td>
<td>GV</td>
</tr>
<tr>
<td>6 2025/2475, 10 days</td>
<td>1868</td>
<td>12.8</td>
<td>Mature</td>
</tr>
<tr>
<td></td>
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<td>10.8</td>
<td>FZ</td>
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<tr>
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<td>8.4</td>
<td>FZ</td>
</tr>
<tr>
<td>7 2100/2400, 10 days</td>
<td>921</td>
<td>11.8</td>
<td>Mature</td>
</tr>
<tr>
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<td>8 2025/1500, 10 days</td>
<td>987</td>
<td>11</td>
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<tr>
<td></td>
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<td>9.9</td>
<td>Mature, GV, GV</td>
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Oktay et al., 2004, Lancet, 363
Ovarian function after autografting of human ovarian tissue

Callejo et al., 2001, J Clin Endocrino Metab, 86
<table>
<thead>
<tr>
<th>Identified proteins of significance by MALDI-TOF</th>
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<tbody>
<tr>
<td>Before</td>
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## Protein Functions

<table>
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<tr>
<th>Protein Type</th>
<th>Description</th>
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<tbody>
<tr>
<td>RAB4B Protein</td>
<td>A family of GTP-binding protein. Regulate vesicular trafficking</td>
</tr>
<tr>
<td>SERPINB5s Protein</td>
<td>Serine proteinase inhibitor B family. Related with inflammation and tumor cells</td>
</tr>
<tr>
<td>Lectin</td>
<td>Related cell structure (glycoprotein) and growth</td>
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<tr>
<td>Laminin receptor</td>
<td>A family of extracellular matrix proteins. Multiple biological activities (growth differentiation, angiogenesis etc.)</td>
</tr>
<tr>
<td>Actin</td>
<td>Protein related to microfilaments and fibers</td>
</tr>
<tr>
<td>Glutathione S-transferase</td>
<td>A family of catalytic enzyme. Antioxidant</td>
</tr>
<tr>
<td>CMP-NeuAc:GM3 sialytransferase</td>
<td>Inhibition of EGF-mediated receptor activity and cell proliferation by HK1-ceramide</td>
</tr>
</tbody>
</table>
Stages of folliculogenesis in the adult human ovary (Gougeon)
Autotransplantation of human ovarian tissue

• **Orthotopic**
  - Oktay K, 2000 (frozen-thawed)
  - Radford J, 2001 (frozen-thawed)
  - Donnez J, 2004 (frozen-thawed)
  - Meirow D, 2005 (frozen-thawed)
  - Silber S, 2005 (fresh, frozen-thawed)
  - Demeestere I, 2007 (frozen-thawed)
  - Piver P, 2009 (frozen-thawed)

• **Heterotopic**
  - Oktay K, 2001 (fresh)
  - Callejo J, 2001 (fresh)
  - Kim SS, 2003 (frozen-thawed)
  - Oktay K, 2004 (frozen-thawed)
  - Rosendahl M, 2006 (frozen-thawed)
  - Kim SS, 2009 (frozen-thawed)
Immunofluorescent localization of microtubules and chromatins

Kim et al., Hum Reprod, 2005