Freezing of ovarian tissue beyond fertility preservation in cancer patients

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Established and new aspects of ovarian tissue freezing

1. Fertility preservation for cancer patients
2. Rare genetic diseases – confirmed potential in children
3. Known causes of POI -- In combination with in vitro follicle activation
4. Endometriosis
5. Postponing menopause
6. Social freezing – alternative to mature oocytes
7. Anovulatory PCOS women – modern wedge resection
Normal utilization of human follicles

Follicles are present in an enormous surplus

❖ At puberty girls have available $\approx 500,000$ follicles
❖ They will ovulate a total of $\approx 480$ oocytes in total
❖ The remaining $\approx 99\%$ of the follicles will degenerate

Frozen/thawed ovarian tissue

Follicles vitality stained with Neutral Red

26 year old woman
One follicle per month secures ovarian function

The follicle is the steroid producing unit of the ovary and a single follicle – the preovulatory follicle – produces more 90% of the available oestradiol. Change marginally with the age of the woman!
Cryopreservation of Human Ovarian Tissue and restoration of Ovarian Function

Retrival of one ovary

Preparation of cortical tissue

Freezing

Thawing

Transplantation

Freezing
Women with only one ovary: Age a menopause

- **Study 1:** 24,152 Japanese women age >40 ⇒ 3.4% underwent unilateral oophorectomy ⇒ Age at menopause was reduced 1.2 year

- **Study 2:** 23,580 Norwegian women ⇒ 4.5% underwent unilateral oophorectomy ⇒ Age at menopause was reduced one year

Yasui T et al., *Maturitas.*, 2012;72:249  
Bjelland EK et al., *Hum Rep* 2014;29;835
Danish patients with rare genetic diseases who have received ovarian tissue freezing for fertility preservation

<table>
<thead>
<tr>
<th>Diagnose</th>
<th>No. patients</th>
<th>Mean age (years, range)</th>
<th>Mean no. of cortex pieces (range)</th>
<th>FSH (IU/L) N*, Mean (range)</th>
<th>LH (IU/L) N*, Mean (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thalassemia</td>
<td>11</td>
<td>10.8 (2.8-17.4)</td>
<td>14 (3-26)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>POI</td>
<td>8</td>
<td>23.3 (17.5-27.3)</td>
<td>17 (17-27)</td>
<td>2, 8.9 (8.4-9.3)</td>
<td>2, 5.4 (3.7-7.0)</td>
</tr>
<tr>
<td>Turner Syndrome</td>
<td>7</td>
<td>15.5 (8.8-22.4)</td>
<td>18 (12-29)</td>
<td>7, 8.9 (2.0-31.0)</td>
<td>7, 5.7 (1.0-17.6)</td>
</tr>
<tr>
<td>Galactosemia</td>
<td>6</td>
<td>3.8 (0.3-11.7)</td>
<td>7 (3-13)</td>
<td>1, 19.7</td>
<td>1, 0.6</td>
</tr>
<tr>
<td>Sickle cell anemia</td>
<td>3</td>
<td>11.4 (9.0-16.0)</td>
<td>23 (15-34)</td>
<td>1, 2.0</td>
<td>1, 0.3</td>
</tr>
<tr>
<td>Fanconi anemia</td>
<td>2</td>
<td>8.7 (6.9-10.5)</td>
<td>10 (9-11)</td>
<td>1, 4.2</td>
<td>NA</td>
</tr>
<tr>
<td>XXY/X0 mosaic</td>
<td>2</td>
<td>11.6 (6.5-16.6)</td>
<td>4 (3-4)</td>
<td>2, 25.2 (5.4-45.0)</td>
<td>2, 6.8 (0.3-13.2)</td>
</tr>
<tr>
<td>XY phenotypic girl</td>
<td>2</td>
<td>8.6 (1.6-15.5)</td>
<td>10 (4-15)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Osteopetrosis</td>
<td>1</td>
<td>11.0</td>
<td>22</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

Yding Andersen C et al., Reproduction, 2019; PMID: 31284266
Does ovarian tissue excised from young children work when transplanted as adults?

❖ The patient (from UAE) suffered from Thalassemia, had ovarian tissue frozen in 2001 (Leeds, UK) at the age of 9 years prior to bone marrow transplantation
❖ The tissue was brought to Denmark in 2015 and transplanted back
❖ Conceived after IVF treatment in the UK and give birth in December 2016

Matthwes S et al. Minverva 2018;70:432-435
Successful pregnancy in a woman previously suffering from β-thalassaemia following transplantation of ovarian tissue cryopreserved before puberty

Matthews SJ, Picton HM, Ernst E & Yding Andersen C

![Graph showing levels of FSH & LH (IU/L) and AMH (ng/ml) over months before or after transplantation](image)

Matthwes S et al. Minverva 2018;70:432-435
Follicular density in ovaries from girls with thalassemia compared to normal girls

Mamsen et al., 2019, submitted
Morphology of follicles in an ovary from a girl with thalassemia

Mamsen et al., 2019, submitted
IHC of follicles from normal ovaries and ovaries with thalassemia

Mamsen et al., 2019, submitted
Hormone concentrations in follicle fluids from β-thalassemia (BT) and sickle cell disease (SCD) patients (N=18) as compared to controls

<table>
<thead>
<tr>
<th></th>
<th>Estradiol (nmol/L)</th>
<th>Testosterone (nmol/L)</th>
<th>AMH (ng/mL)</th>
<th>Inhibin-A (ng/mL)</th>
<th>Inhibin-B (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean βT and SCD ±SEM</td>
<td>71 ±22</td>
<td>507 ±127</td>
<td>1133 ±170</td>
<td>5± 0.4</td>
<td>64± 0.4</td>
</tr>
<tr>
<td>Mean normal ± SEM (range)</td>
<td>71 ±26 (3-39)</td>
<td>158 ±12 (13-573)</td>
<td>754 ±67 (4-3918)</td>
<td>9 ±1 (2-43)</td>
<td>91 ±10 (2-621)</td>
</tr>
</tbody>
</table>

Mamsen et al., 2019, submitted
Two for one – both cortical tissue and mature oocytes

Collection of immature oocytes from the surplus medulla tissue
Human IVM oocytes collected from small antral follicles (0.5–3 mm in diameter)

We have in several instances been able to collect in excess of 100 oocytes from one ovary

Can this exceptional high number of immature oocytes be used as an additional source of fertility?
Patient characteristics and outcome of IVM

<table>
<thead>
<tr>
<th></th>
<th>Number of patients</th>
<th>Age (years)</th>
<th>Ovarian volume (mL)</th>
<th>Number of IVM oocytes</th>
<th>Number of oocytes matured</th>
<th>Maturation rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>25</td>
<td>28 ± 1</td>
<td>9 ± 1 (2.7–19.8)</td>
<td>36 ±5 (7-100)</td>
<td>11 ±2 (1–30)</td>
<td>31 ±3 (6–54)</td>
</tr>
</tbody>
</table>

Data are mean per patient ±SEM (range)
Fertility preservation to women with ovarian cancer, borderline ovarian cancer, recurrent dermoid cysts etc. Two for one!

❖ It is not likely that replacement of malignant ovarian tissue will take place with current technology – but perhaps in the future by creating artificial ovaries based on isolated follicles

❖ The isolated immature oocytes itself do not contain malignancy and those matured to MII can be frozen and can provide a source of fertility for this group of patients (needs to be performed)

❖ The first pregnancies from MII oocytes originating in such small follicles have already been reported
Premature ovarian insufficiency (POI)

Know genetic causes of POI warrant fertility preservation –

These years many GWAS identify new genetic conditions that predispose to POI

Option: Secure fertility and endocrine function while the tissue is still functional
Long term consequences of POI

- **Cognitive dysfunction**
  - memory / concentration problems, increased risk of dementia

- **Autoimmune and thyroid disease risk**

- **Cardiovascular system** - Impaired endothelial function, ↑ triglycerides, cholesterol and LDL

- **Bones** – osteopenia, osteoporosis, ↑ fracture risk

- **Hormones** – hypoestrogenism, infertility

- **Urogenital symptoms** – vaginal dryness, vaginal irritation and itching, sexual disorders

- **Mortality** – increased risk of premature death

Meta-analysis with three studies comparing women with bilateral oophorectomy before menopause with referent women.

Significant increased risk of cognitive decline and dementia of women with POI.

These studies suggest a sizeable neuroprotective effect of estrogen NATURALLY PRODUCED by the ovaries before age 50 years.

The neuroprotective or harmful effects of estrogen depend on age at the time of initiation of treatment.

Women who undergo bilateral oophorectomy prior to menopause, women with POI or early natural menopause should be considered for HRT until natural menopause.
Aetiopathogenesis of POI

❖ Multi-factoral background

❖ A major part is regarded as idiopathic

❖ But suspected to be genetically determined – Abnormal X chromosome (12-20%) – Turner syndrome

Autoimmune antibodies (4-30%)
Autoimmunological causes included anti-ovarian autoantibodies and autoimmune hypothyroidism (24%), adrenal insufficiency, diabetes type 1, hypoparathyroidism and pernicious anaemia
Heritability and identification of POI

Approximately 10–15% of women with POI will have a first degree relative who is also affected.

If mother or older sister experienced early menopause there is an around six-times higher risk for the woman herself.

Over half of patients see three or more clinicians before the POI diagnosis is made and in a quarter the diagnosis took more than 5 years.
Information to relatives of women with spontaneous POI who are concerned about their risk for developing POI

- Currently there is no proved predictive test to identify women that will develop POI except for the presence of a mutation known to be related to POI
- There are no established POI preventing measures,
- Fertility preservation appears as a promising option, although studies are lacking
- Their potential risk of earlier menopause should be taken into account when planning a family
Patients with imminent POI having one ovary cryopreserved in Denmark – experience up until now!

Eight patients with an age ranging from 18-27 years with normal FSH levels have received fertility preservation – all with a family history of POI – in several instances combined with OS and oocyte vitrification.
Activation of follicle growth \textit{in vitro} – reproductive aged women with poor response having difficulties conceiving.

- Ovarian cortical biopsy
- \textit{In vitro} activation of follicles
- IVF
- Autotransplantation
Endometriosis

Severe cases of endometriosis

Often these patients ends up having no ovarian function

Secure ovarian tissue for cryopreservation in time

during the first operations
Postponing menopause: LIFE EXPECTANCY and age at menopause – the gap only widens!

Life expectancy today is far beyond any historic records

Today half of all newborn girls is estimated to live more than 100 years in many western countries

More than 75 millions women in the western world are postmenopausal

In Japan and HK the average life expectancy is >87 years increasing with 3 months per year – more than 100,000 are above 100 years with 90 % being women

Many women will experience the sequelae of menopause including the risk of osteoporosis and cardiovascular disease etc.
LIFE EXPECTANCY and age at menopause – the gap only widens!
Postponing menopause
Avoid the sequelae of loss of endogenous hormones

One chip of ovarian cortex could be replaced – one at a time – providing natural menstrual cycles for a period

Her own tissue, her own menstrual cycles with natural variations in the whole armentarium of hormones including luteal phase progesterone
Endogenous estrogen exposure was positively associated with cognitive status.

Longer duration of HT use was positively associated with cognitive status.

Women who initiated HT within 5 years of menopause compared favorably with those initiating HT 6-or-more years later.
Anovulatory PCOS patients seeing infertility treatment: Modern wedge resection anno 2019

Cryopreservation of ovarian tissue

New Concept: Remove ovarian tissue – prepare cortical tissue and freeze the tissue for later use while aspirating immature oocyte for IVM
To augment the number of children born this municipality in Japan donated a total of around 700.000 Euro for freezing oocytes.
Why not store tissue instead of oocytes

Many women do not use the stored oocytes – up to 90 % –
either they were still fertile when mister “Right” appeared or
he didn’t come around.

Further, immature oocytes
may act on top of the tissue.

Anyway, the ovarian tissue
may be used for endocrine
purposes.
INDICATIONS FOR OVARIAN TISSUE CRYOPRESERVATION BEYOND FERTILITY IN CANCER PATIENTS

- POI
- INDUCTION OF PUBERTY
- SOCIAL FREEZING
- MODERN OVARIAN RESECTION
- ACHIEVING OR POSTPONING NATURAL MENOPAUSE (cHRT)
- CULTURE OF IMMATURE OOCYTES
- OVA
- OTC

Kritensen & Yding Andersen, Front. Endocrinol; PMID: 30002647
The wider perspective of the ability to cryostore functional ovarian tissue

*Except for the operation, excision of ovarian tissue appears to have no or low impact on fertility and at the age of menopause.*

*In contrast frozen/thawed tissue may:*

- Extent the period of natural ovarian activity
- May help young girls with rare genetic diseases
- Women at risk of premature ovarian insufficiency
- Women with a disposition of osteoporosis, risk of cardiovascular disease and avoid other menopausal effects
- A number of new options with malignant and benign diseases
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