

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

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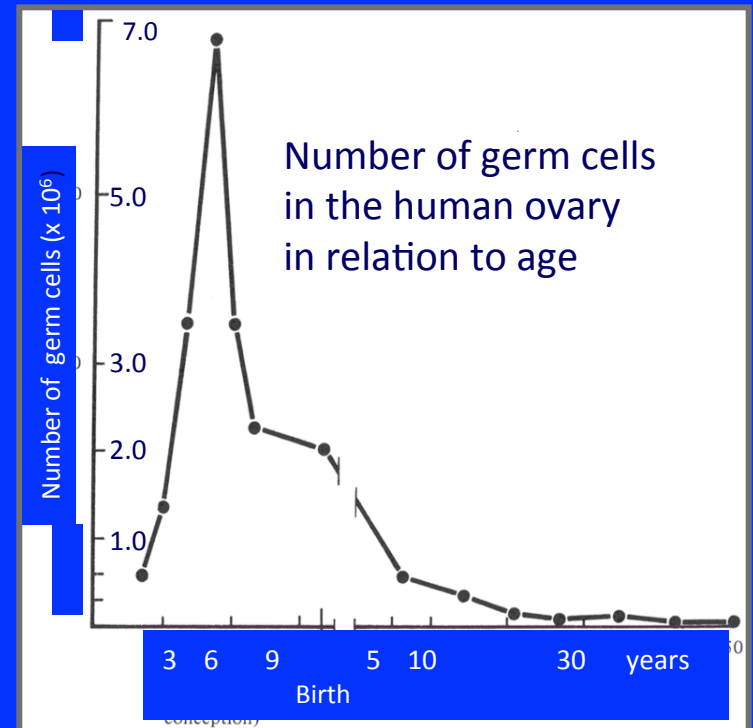
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Conflict of interest

None

# Why focus on fertility presevation?

- ❖ Survival rates among young cancer patients have increased significantly during recent years (Annual incidence: 50/100.000)
- ❖ Modern treatment regimes bears 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

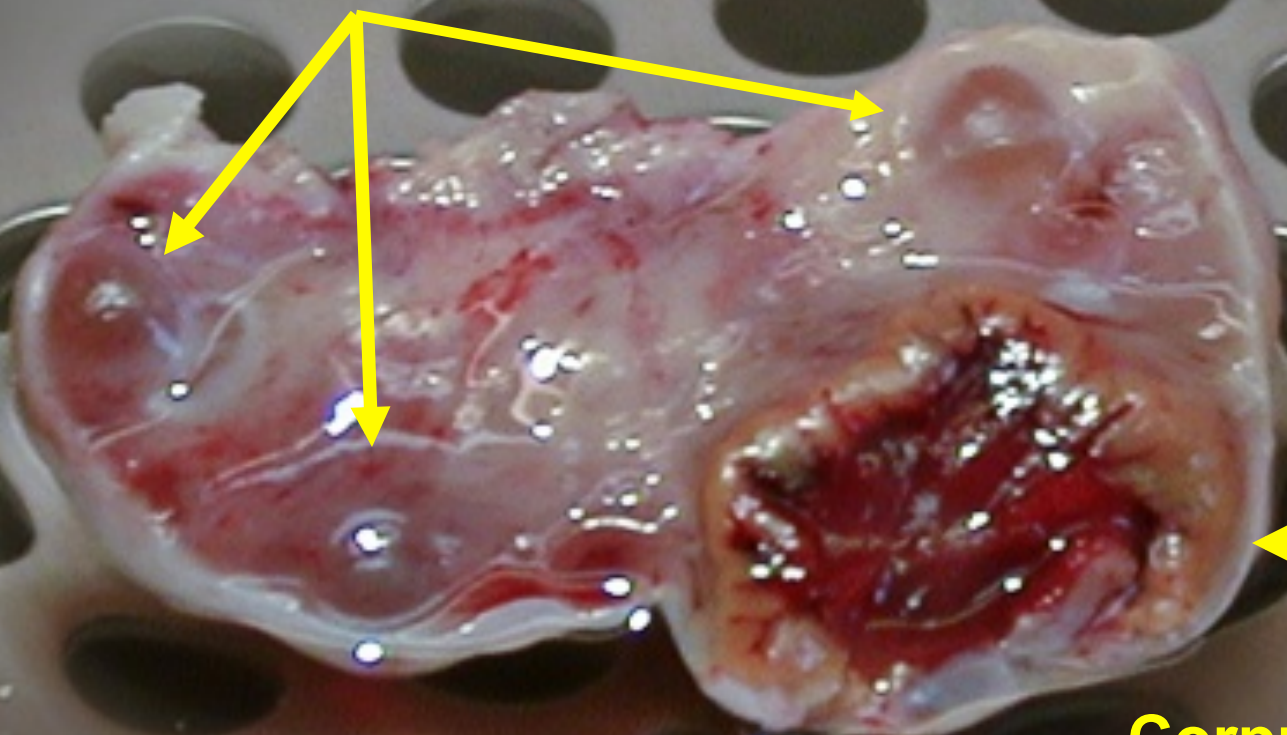
Randomized trials currently underway

# Ovarian tissue freezing

In Denmark more than 870 since 2000  
70 autotransplantations



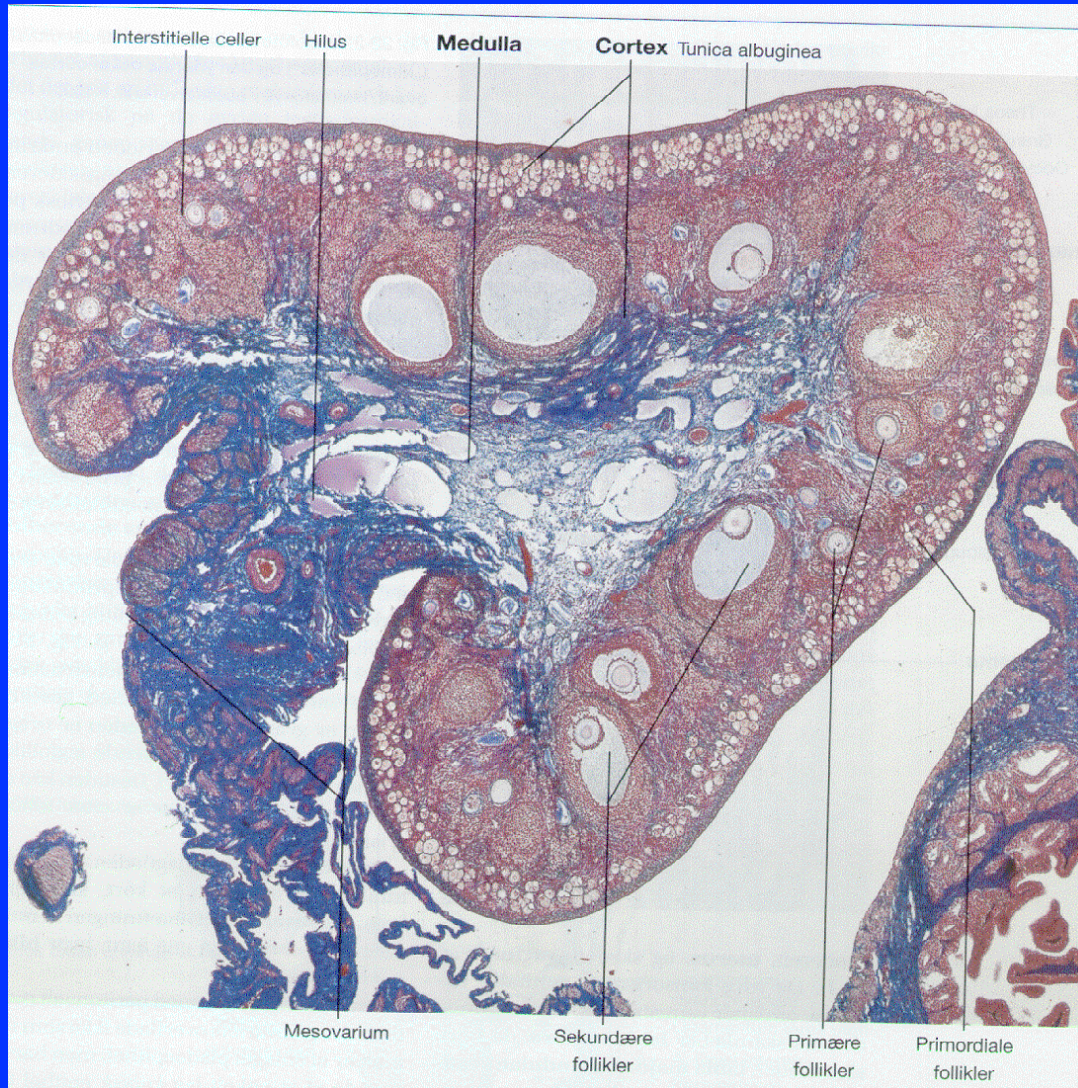
**Small antral follicles**



**Corpus luteum**

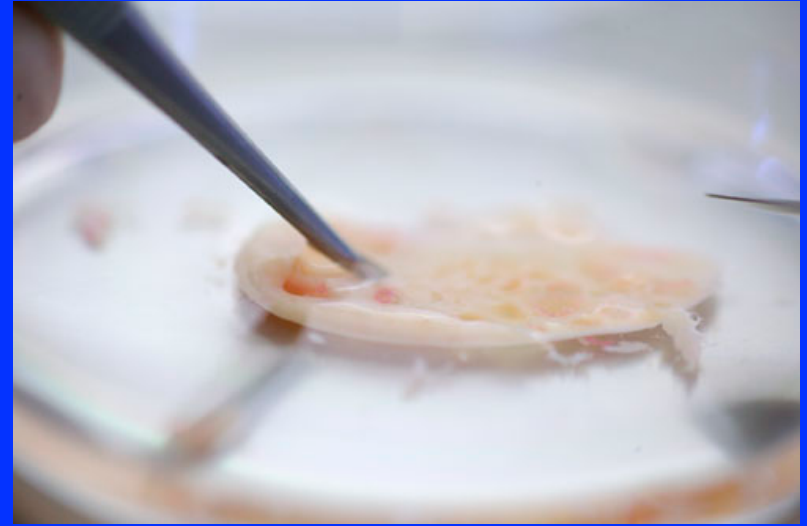
**HALF HUMAN OVARY**

# Only the ovarian cortex is cryopreserved

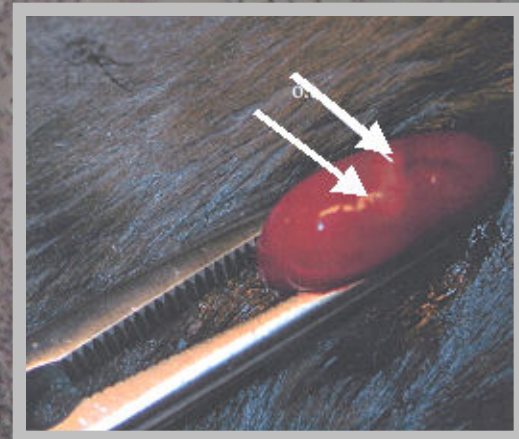
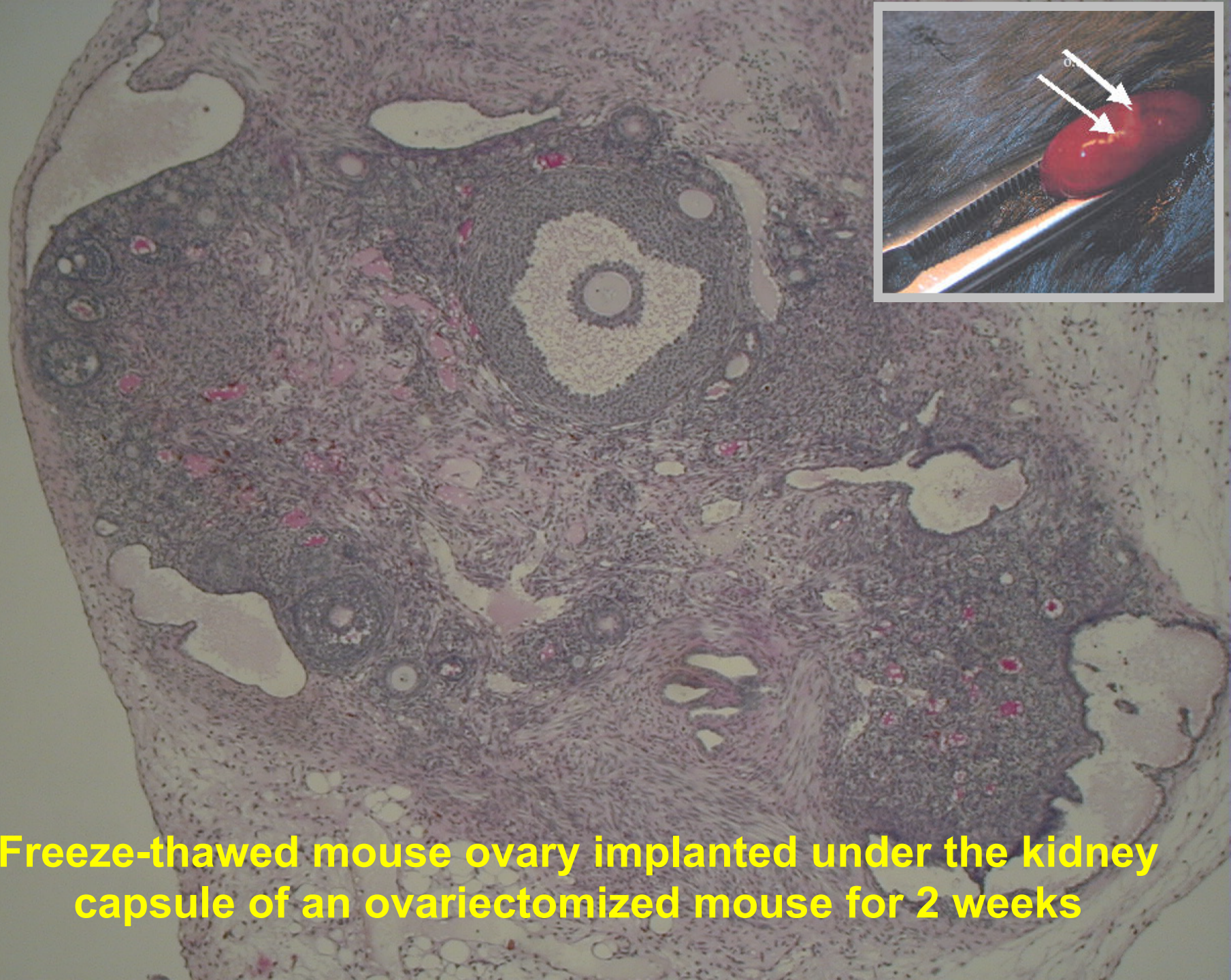




# Preparation of human ovarian tissue for cryopreservation



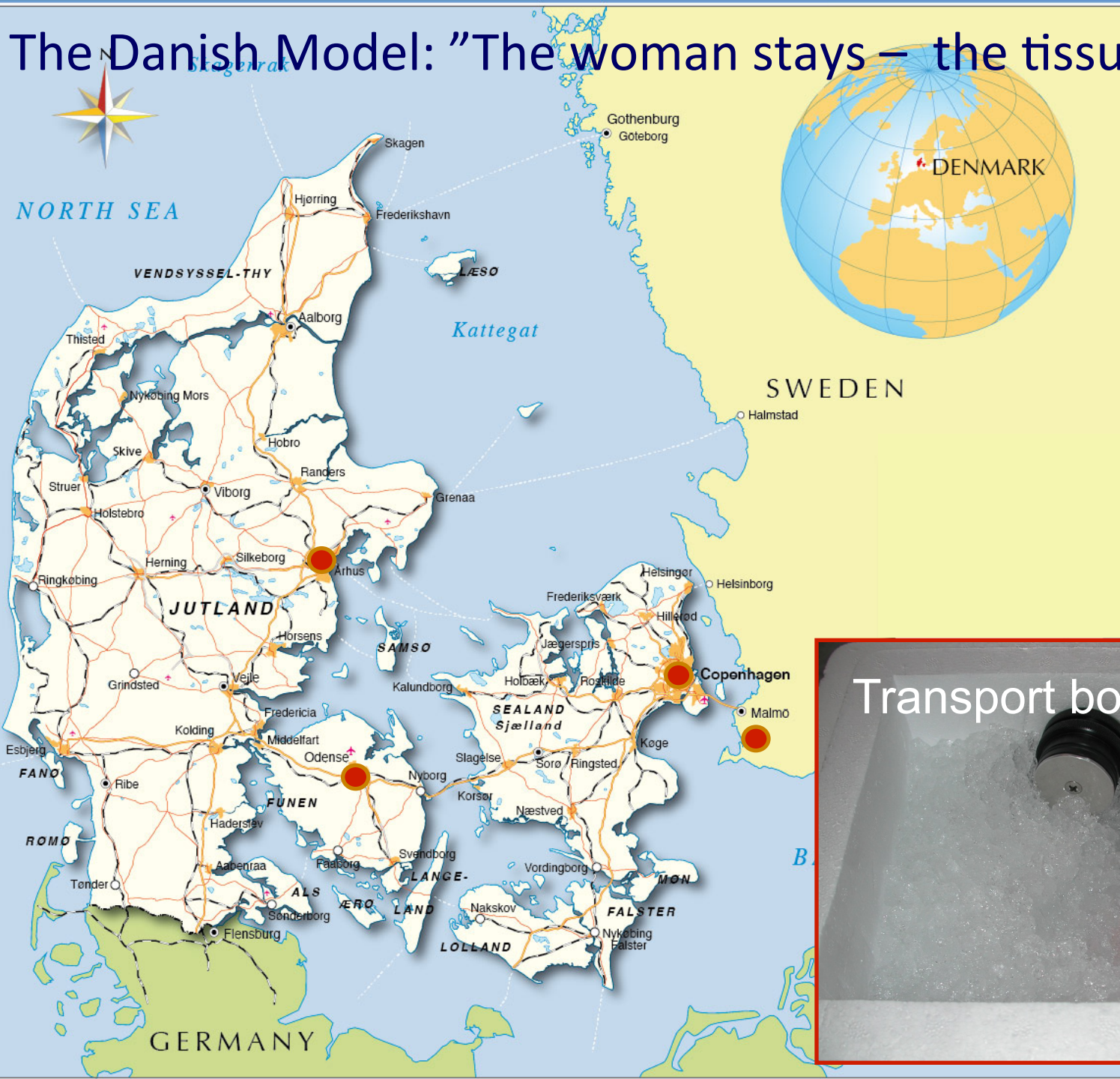




**Freeze-thawed mouse ovary implanted under the kidney capsule of an ovariectomized mouse for 2 weeks**



# The Danish Model: "The woman stays – the tissue moves"

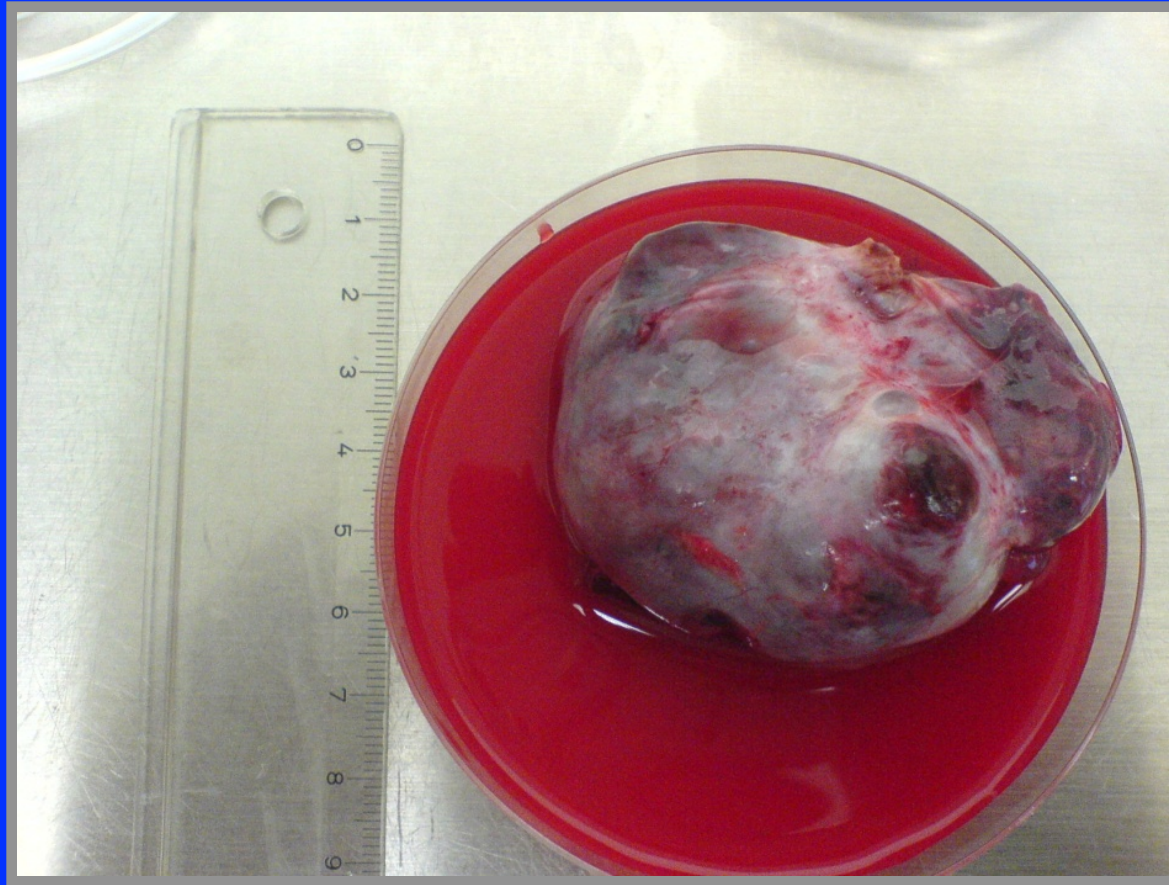


# **Rationale:**

## **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**

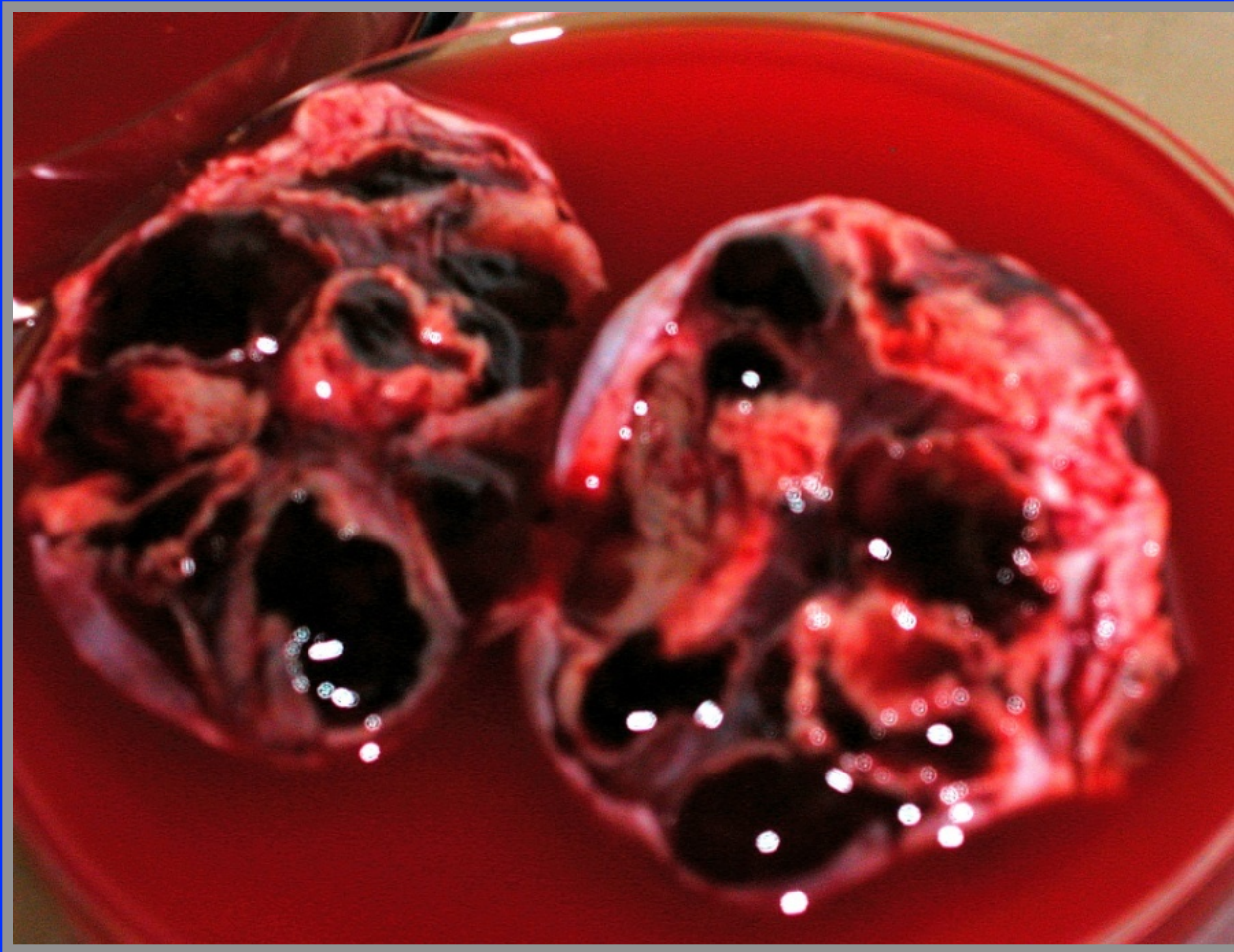
**Difficult to perform oocyte retrieval and  
cryopreservation close to one another**



**Ovary two days after oocyte retrieval**



**Following ovarian stimulation the ovarian cortex is very fragile**



**Ovary two days after oocyte retrieval**



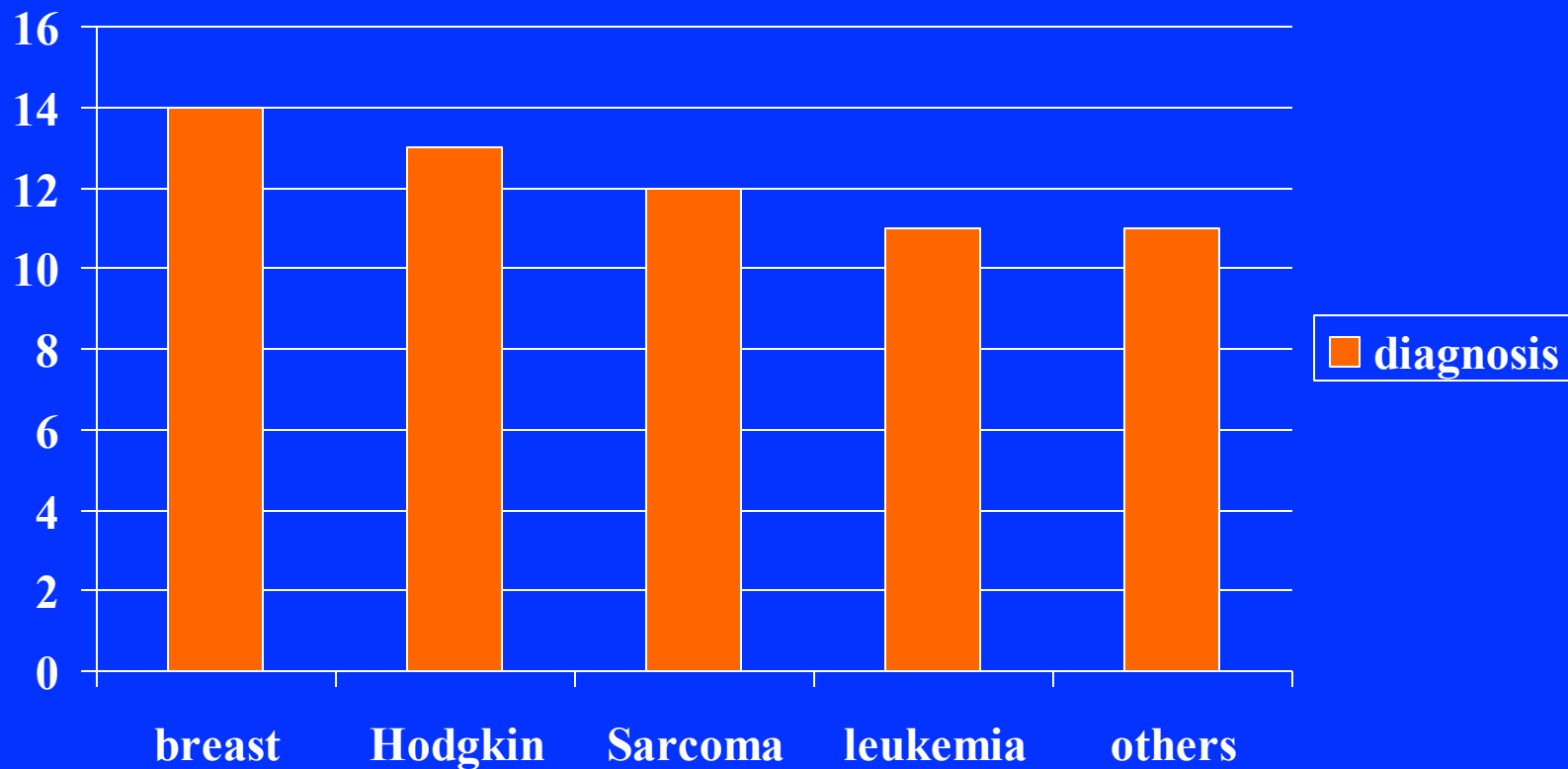
## Diagnosis for cryopreservation of ovarian tissue in Denmark: cumulative (October 2015)

Diagnosis	No.	Diagnosis	No.
Breast cancer	280	Invasive mole	5
Mb. Hodgkin, Non-Hodgkin	173	Thallasaemia	5
Colon-Rectum cancer	16	BRCA1-gene	2
Leukaemia (AML, ALL, CML)	61	Aplastic Anaemia	12
Ewing' s & other sarcomas	74	SLE	6
Reproductive system, incl. Ovarian & Cervical cancer	81	Sex anomalies incl. Turner syndrome	9
Various others	46	Other Dieaseses	38

# 61 deceased patients

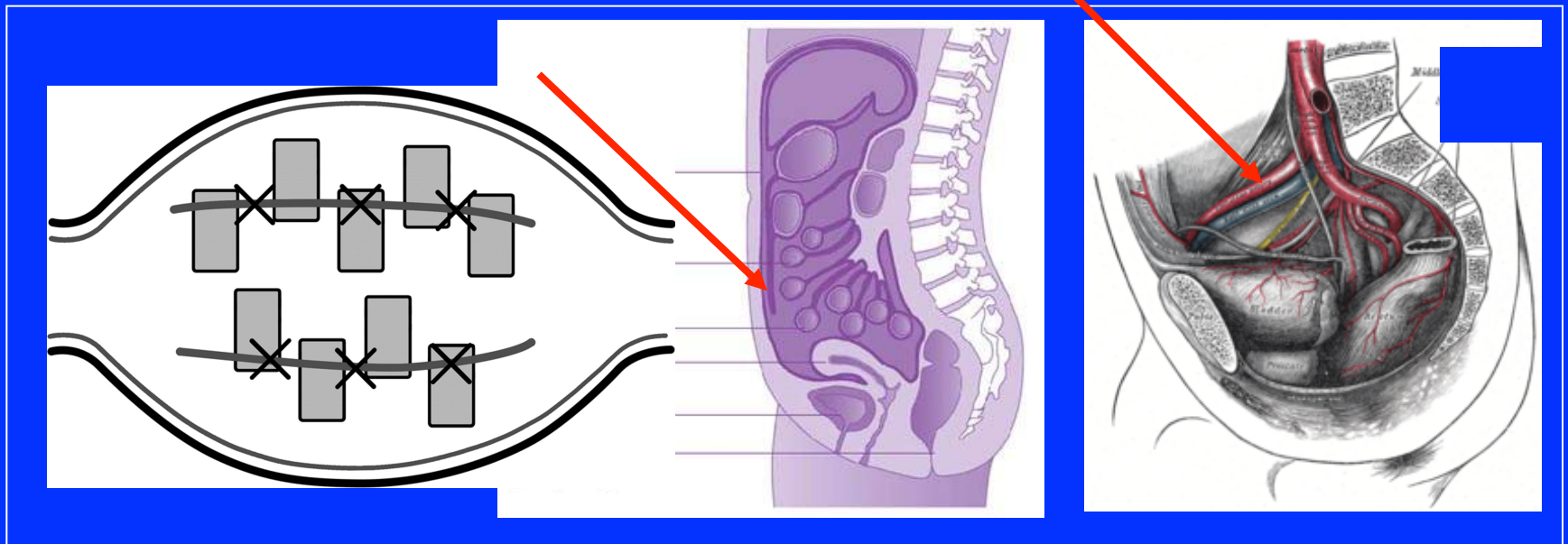
14% of the cohort

24 children/37 women

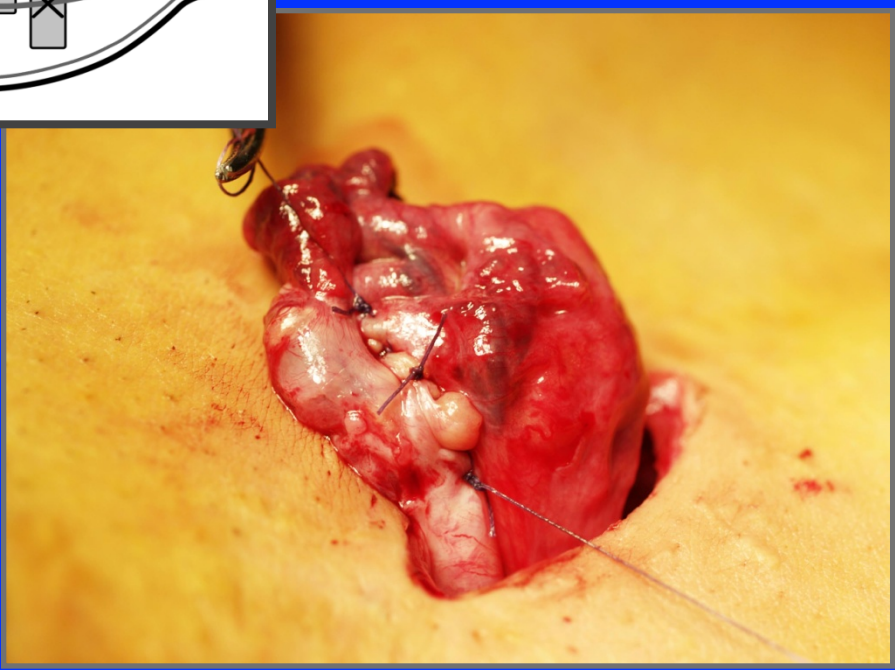
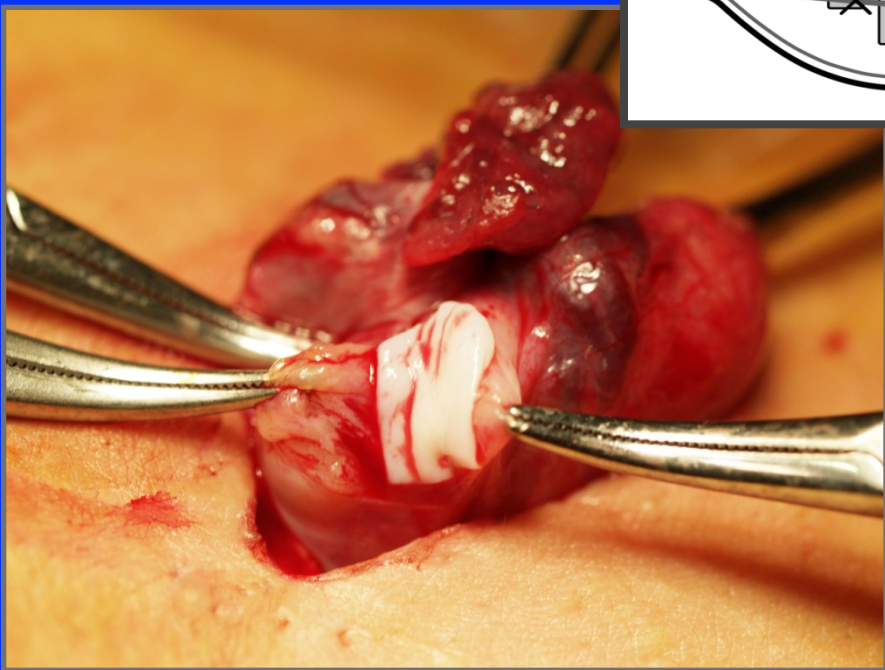
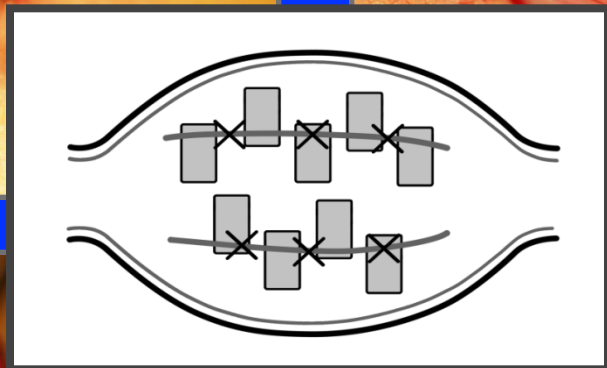
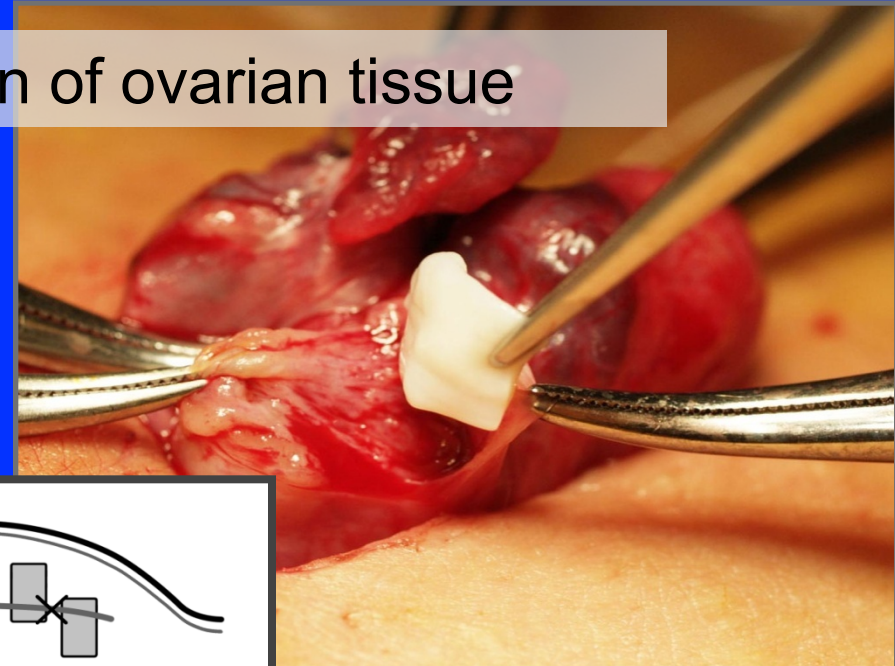
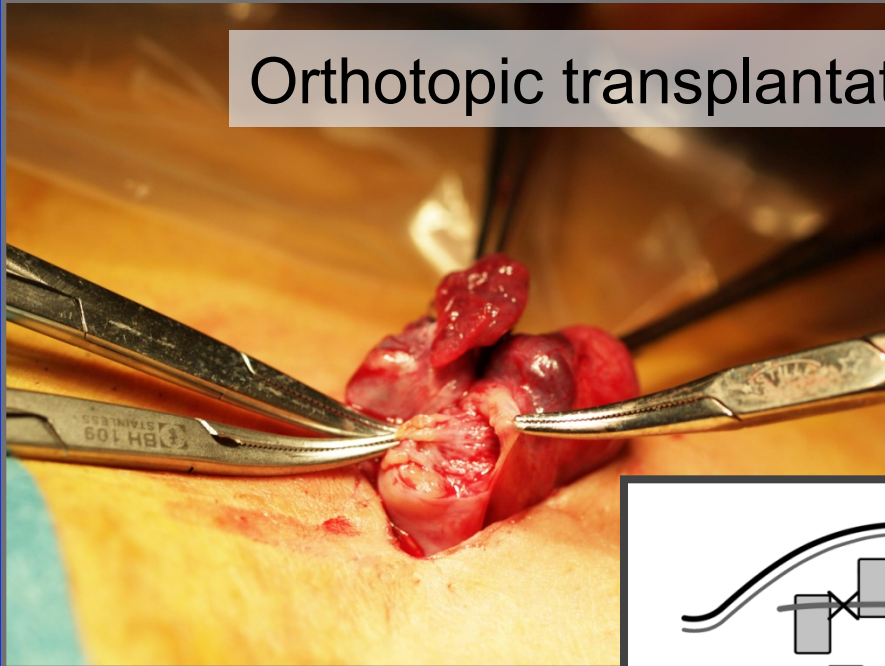


# Clinical results - transplantation

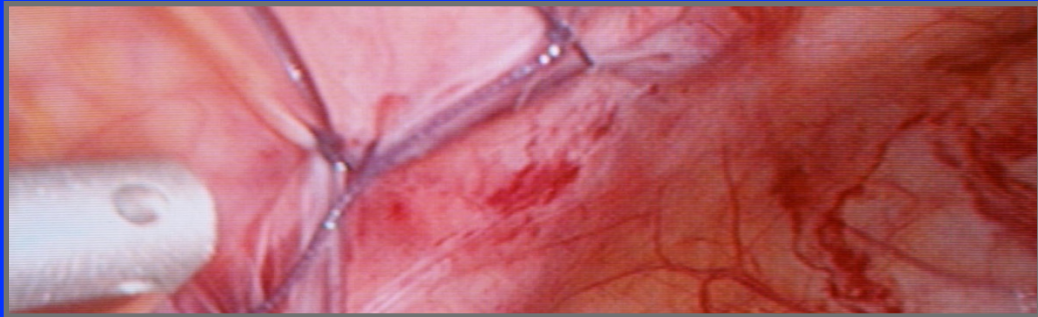
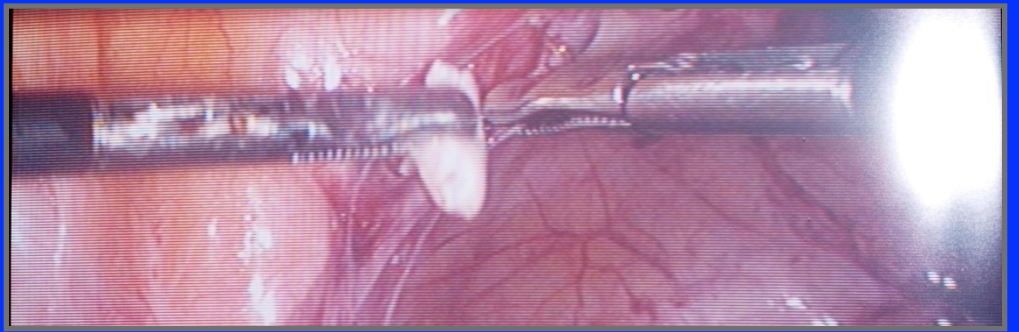
- Laparoscopy / mini-laparotomy
- Orthotopic (ovary)
- Heterotopic (sub-peritoneal on anterior abdominal wall and lateral pelvic wall)



# 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)

Diagnosis	Women	Transplantation		Pos. hCG	Clin. Preg.	Children
		Total	Transport			
Breast cancer	20	23	17	9	9	3
Mb. Hodgkin lymphoma	9	13	9	4	3	2
Non-Hodgkin lymphoma	5	9	3	5	2	1
Cervical cancer	5	6	6			
Aplastic anaemia	3	3	1	1	1	1
Sarcoma incl. Ewing	5	5	4	3	3	3
Paroxysmic Nocturnal Haemoglobinuri	2	2	0	1	1	1
Ovarian cancer	1	1	1	2	2	2
Colon cancer	1	1	1	2	2	2 x (2 <sup>nd</sup> tri. Abor.)
Anal cancer	1	1	1			
Various others *	6	6	6	3	3	2
<b>Total</b>	<b>58</b>	<b>70</b>	<b>49</b>	<b>30</b>	<b>26</b>	<b>15</b>

\*) Wegeners 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

