# The role of ovarian tissue freezing in contemporary fertility preservation


**Claus Yding Andersen, Professor, MSc, DMSc**

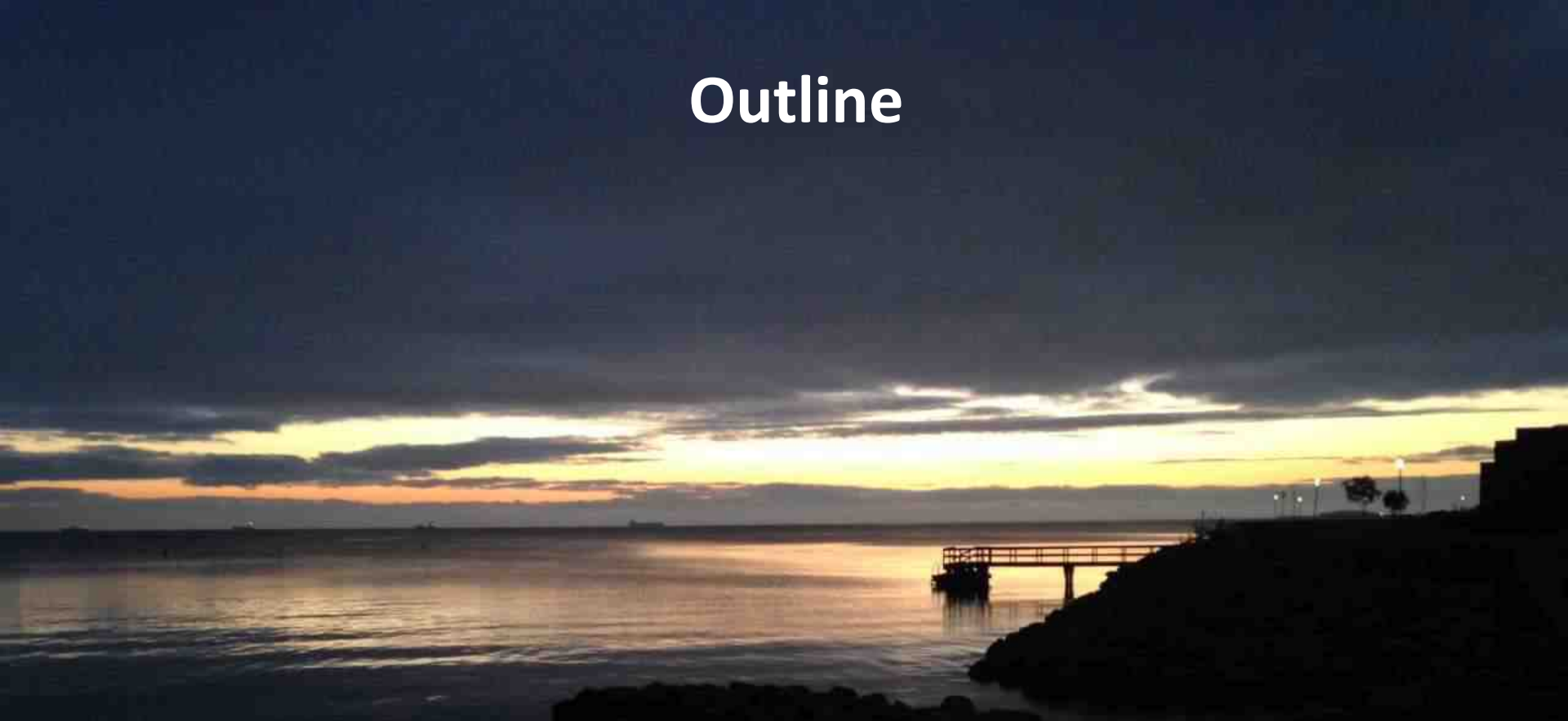
**Laboratory of Reproductive Biology**
**University Hospital Copenhagen, Copenhagen, Denmark**

**E-mail: yding@rh.dk**



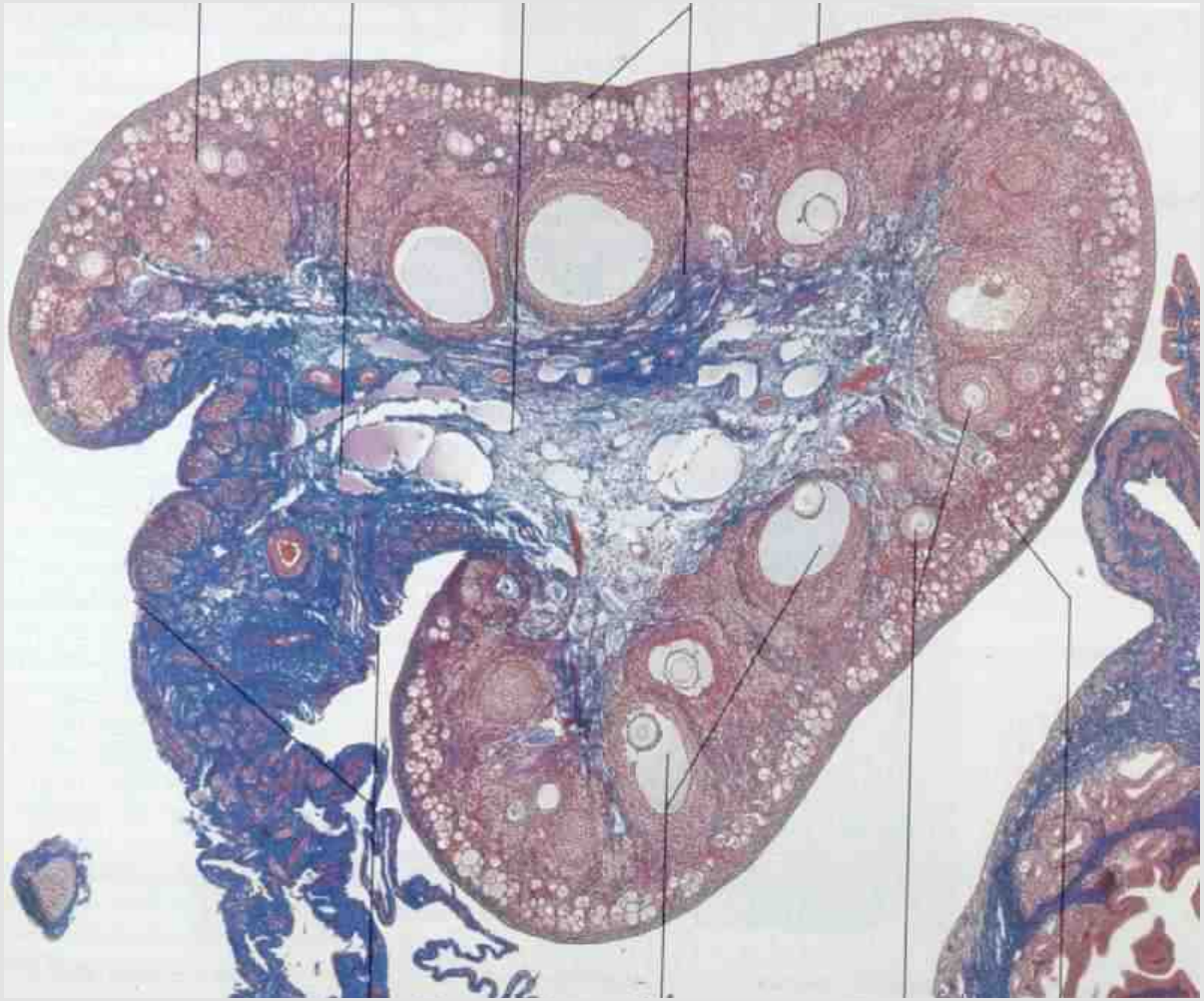
***5th Congress of Society of Reproductive Medicine and Surgery***
***Cornelia Diamond Resort Hotel, Antalya, Turkey , October 29 – November 1, 2015***

# Outline



- Transplantation of ovarian tissue for fertility preservation
- Update of results internationally and in Denmark
- How to express the pregnancy potential of the grafted tissue
- Risks of transplanting malignant cells via ovarian tissue

# Only the ovarian cortex is cryopreserved



# Preparation of human ovarian tissue for cryopreservation

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

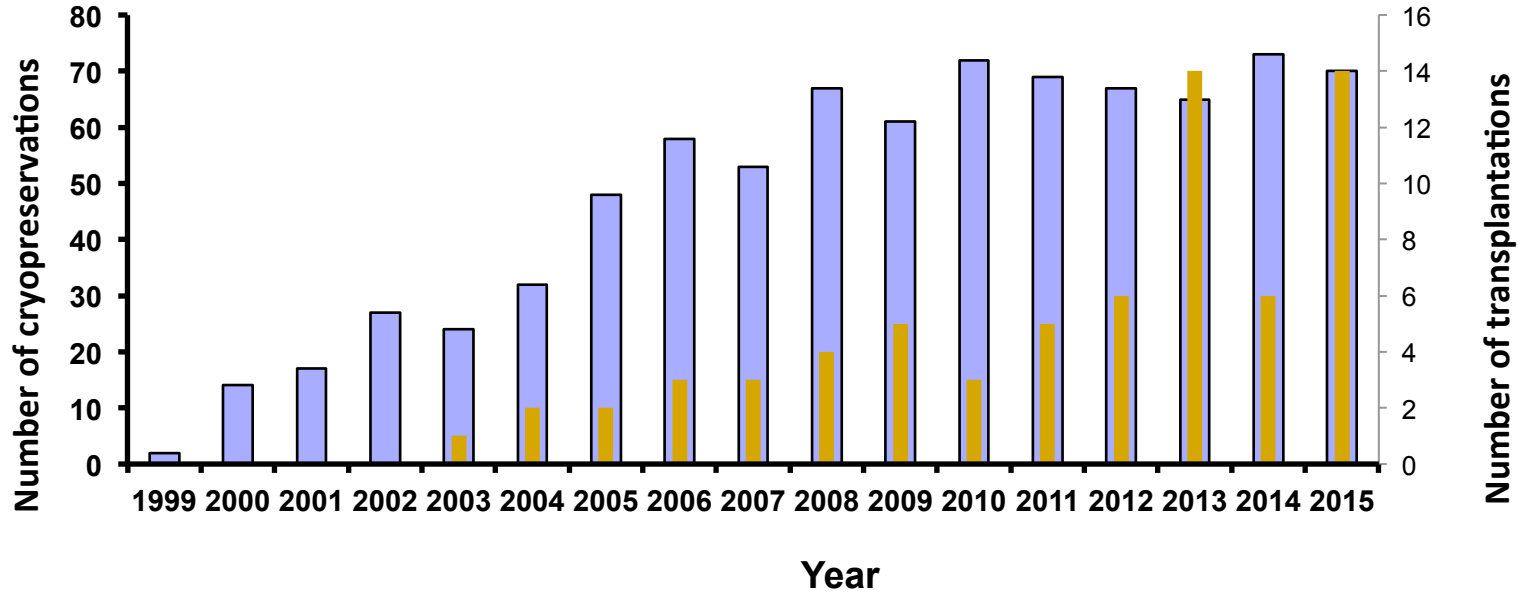
| Diagnosis | No. | Diagnosis | No. |
|---|---|---|---|
| Breast cancer | 280 | Invasive mole | 5 |
| Mb. Hodgkin, Non-Hodgkin | 173 | Thallasaemia | 5 |
| Colon-Rectum cancer | 16 | BRCA1-gene | 2 |
| Leukaemia (AML, ALL, CML) | 61 | Aplastic Anaemia | 12 |
| Ewing's & other sarcomas | 74 | SLE | 6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

# Age distribution of girls/women having ovarian tissue cryopreserved at University Hospital of Copenhagen (October 2015)

| Age (years) | 0–5 | 5–10 | 10–15 | 15–20 | 20–25 | 25–30 | 30–35 | 35-40 |
|---|---|---|---|---|---|---|---|---|
| No. pt. | 33 | 37 | 48 | 110 | 120 | 194 | 205 | 71 |
| Mean no. of cortex | 9 | 11 | 18 | 23 | 23 | 23 | 24 | 23 |
| Range | 4-18 | 3-22 | 1-37 | 11-47 | 6-43 | 2-69 | 3-56 | 10-42 |
| Mean Ovarian volume (ml) | 0,8 | 1,4 | 3,3 | 6,3 | 6,1 | 6,9 | 8,0 | 7,2 |

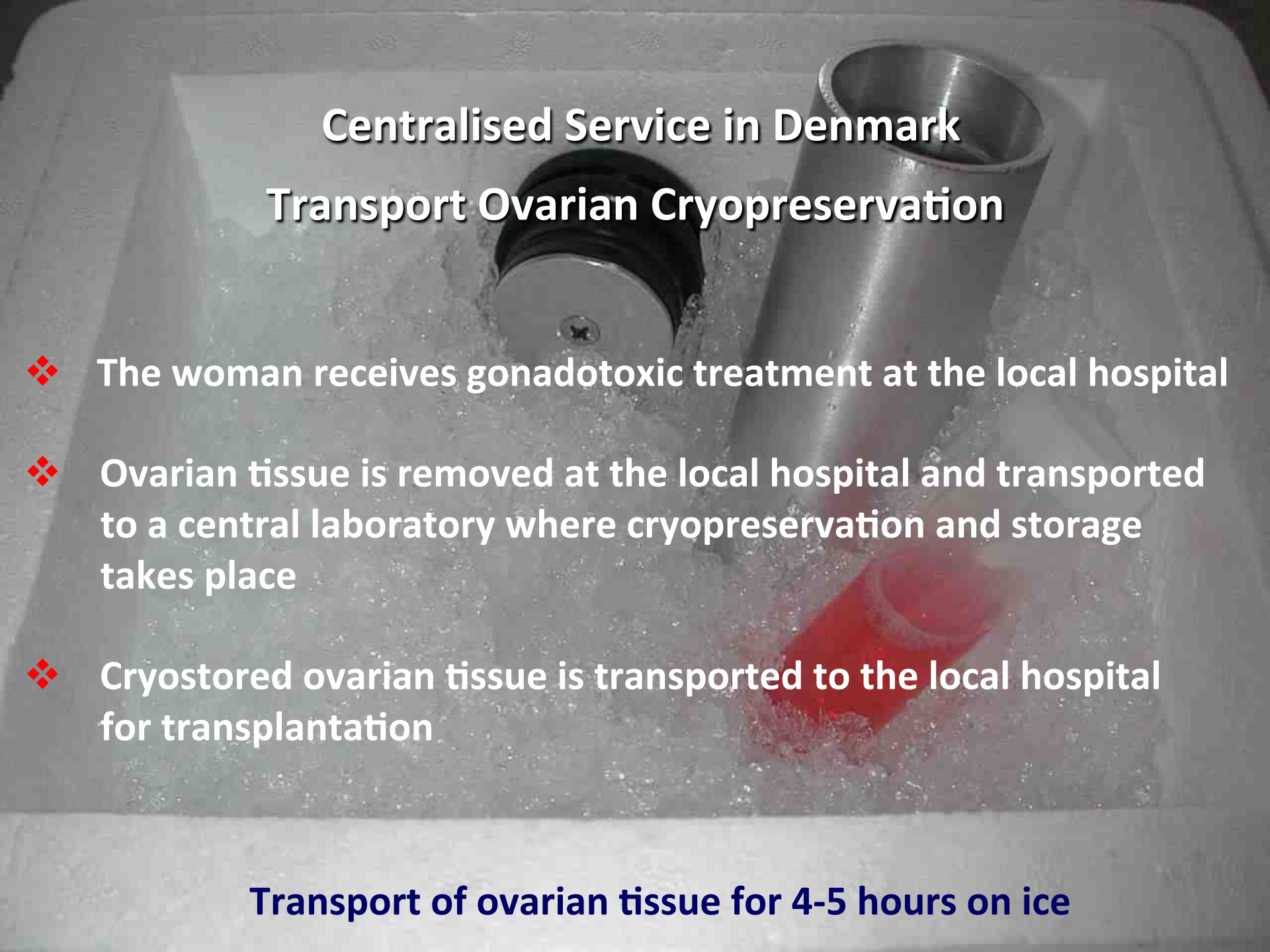
# Transplantation of frozen/thawed activity to Danish patients

## Total number of transplantations: 70




**13-14 cryopreservations per million inhabitants per year**

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



# Centralised Service in Denmark

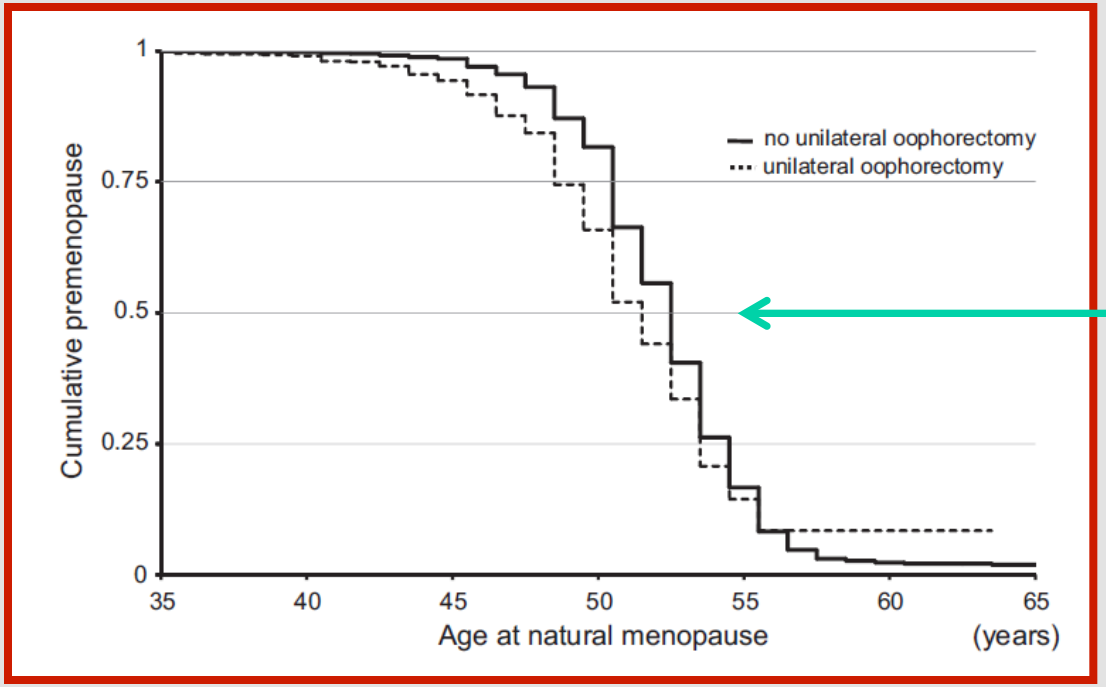
## Transport Ovarian Cryopreservation

- The woman receives gonadotoxic treatment at the local hospital
- Ovarian tissue is removed at the local hospital and transported to a central laboratory where cryopreservation and storage takes place
- Cryostored ovarian tissue is transported to the local hospital for transplantation

**Transport of ovarian tissue for 4-5 hours on ice**

# Women with only one ovary: Age a menopause

- The Japan Nurses' Health Study (epidemiological study)
- 24,152 pre- and postmenopausal (25%) women age 40+
- 3.4% had received an unilateral oophorectomy




**Difference in median age: 1.2 year (p<0.0001)**

Yasui T *et al.*, *Maturitas.*, 2012;72:249

# Fertility of women having one ovary removed for fertility preservation

- 143 women unilateral oophorectomy
  (>18 years; >24 months from excision; 78% participation)
- Mean follow-up time 58 months (24-129);
- 57 women who did not become menopausal had attempted to become pregnant – 41 (72% succeeded);
  37 natural conception – 4 ART pregnancies
  5 additional unwanted pregnancies
- 80% confirmed they wanted to use the tissue if necessary
- 31/143 (22%) were parous prior to freezing
- 84 had not yet a pregnancy wish (23% still on medication or advised against it)

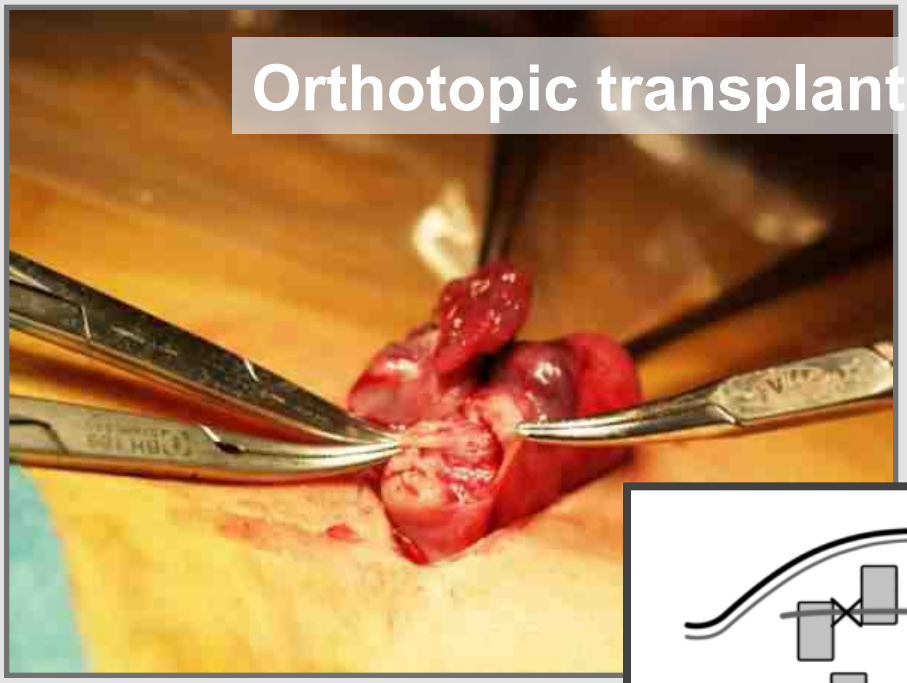
Schmidt KT *et al., RBMOnline,* 2013;26:272

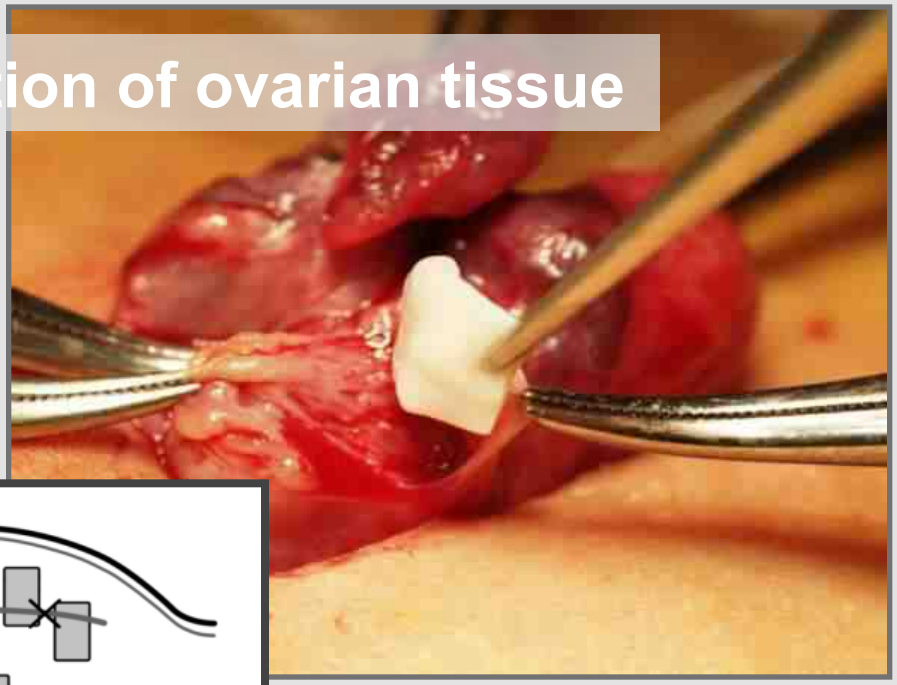
## Danish Patient being transplanted (June 2012)

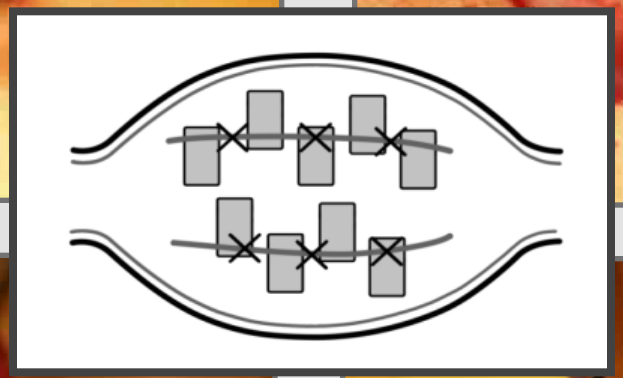
*”Having back my menstrual cycles and being a woman again was as good as having my hair back after having completed chemotherapy”*

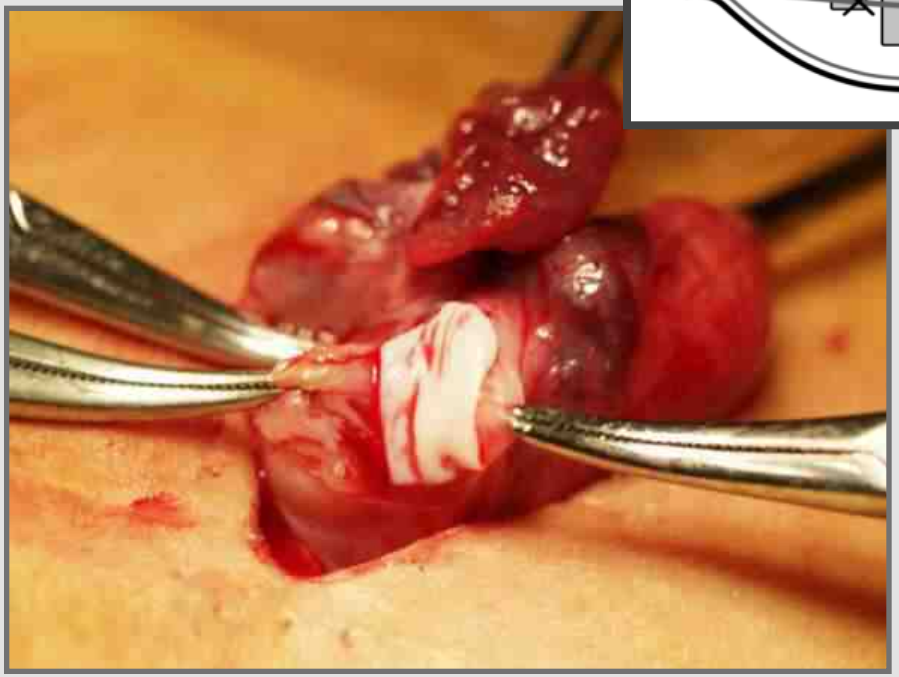
*”Many women express comfort with doctors taking action to potentially save fertility after they have being cured and help them to withstand often harsh and hefty treatment”*

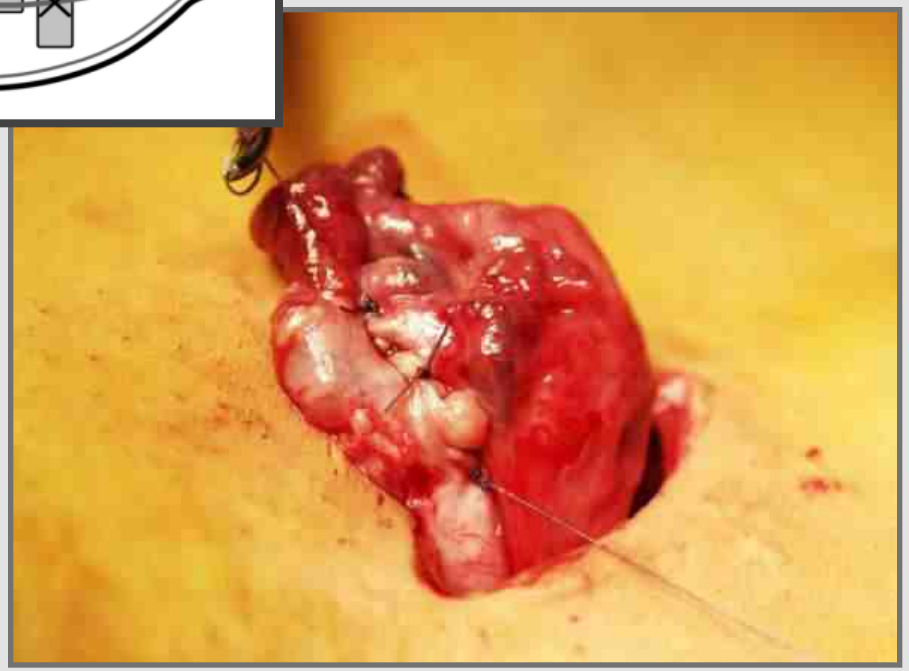
# Orthotopic transplantation of ovarian tissue

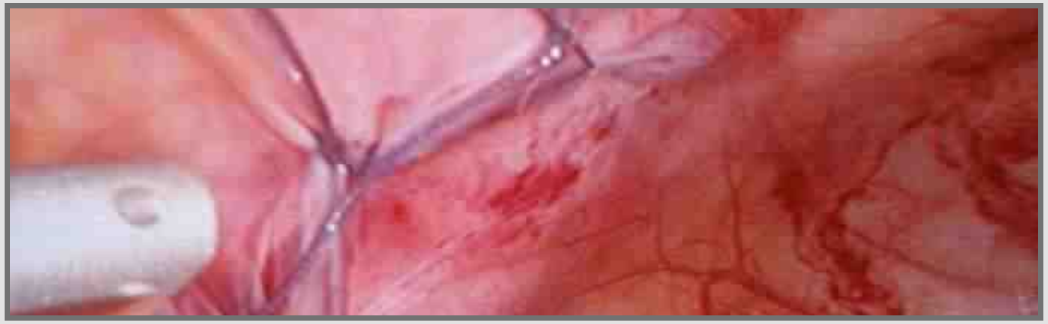






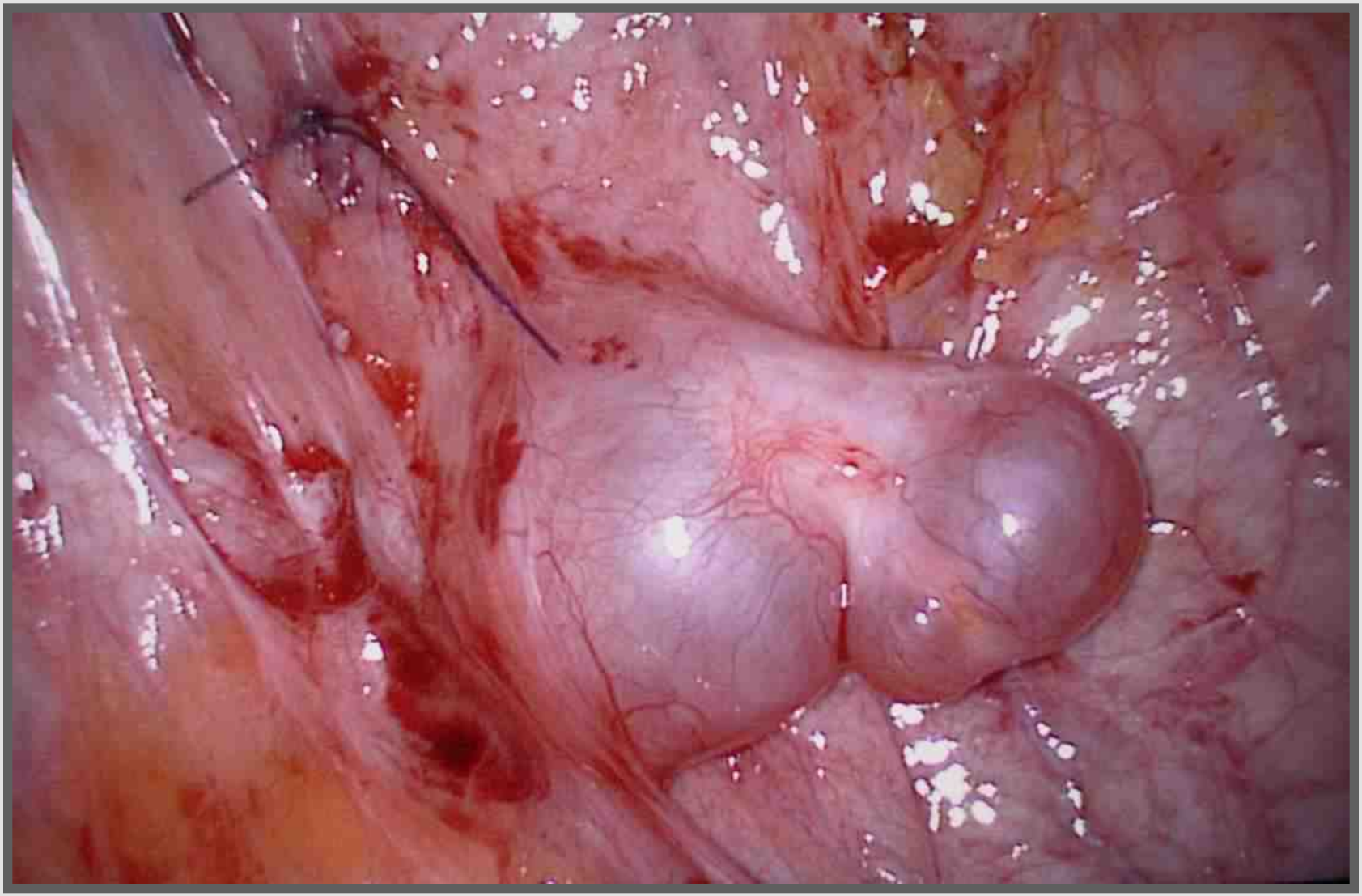






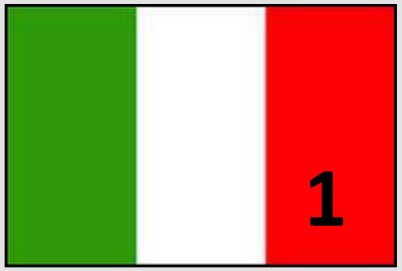
# Heterotropic transplantation to a subperitoneal pocket

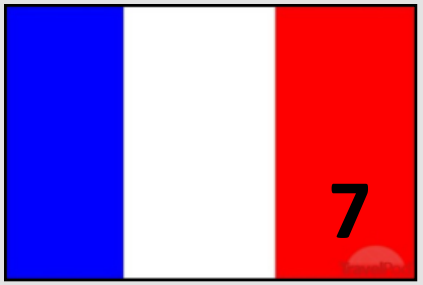
# Heterotropic implanted human ovarian tissue at a peritoneal pocket



# Children born from transplantation of frozen/thawed ovarian tissue (N=73)





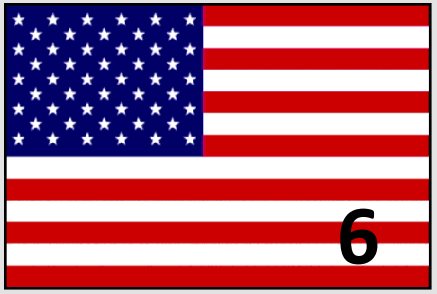


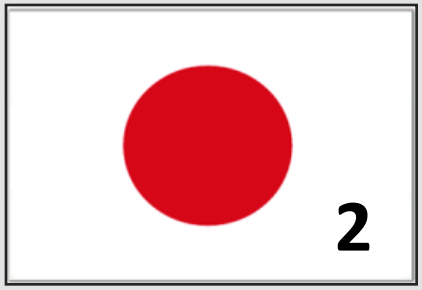
All normal babies
Weight and gestational age

Orthotopic >>heterotopic
All except for two is a result of a slow- freezing protocol

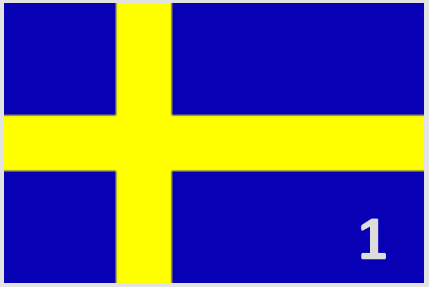






# Outcome and number of Danish women transplanted with frozen/thawed ovarian tissue according to diagnosis (Sep 2015)

| Diagnosis | Women | Transplantation | | Pos. hCG | Clin. Preg. | Children |
|---|---|---|---|---|---|---|
| | | Total | Transport | | | |
| Breast cancer | 19 | 22 | 16 | 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 |
| Paroxystic Nocturnal Haemoglubinuri | 2 | 2 | 0 | 1 | 1 | 1 |
| Ovarian cancer | 1 | 1 | 1 | 2 | 2 | 2 |
| Colon cancer | 1 | 1 | 1 | 2 | 2 | (1) + ($2^{nd}$ tri. Abor.) |
| Anal cancer | 1 | 1 | 1 | | | |
| Various others * | 6 | 6 | 6 | 3 | 3 | 1 + (1) |
| Total | 57 | 69 | 48 | 30 | 26 | 14 (2) |

*) Wegeners granulomatose, Mola, Morbus Behcet, Autoimmune vasculitis, HUS, Thalassaemia

# Success and challenges in fertility preservation after ovarian tissue grafting

**It is basically impossible to calculate a precise pregnancy rate for transplantation of ovarian tissue**

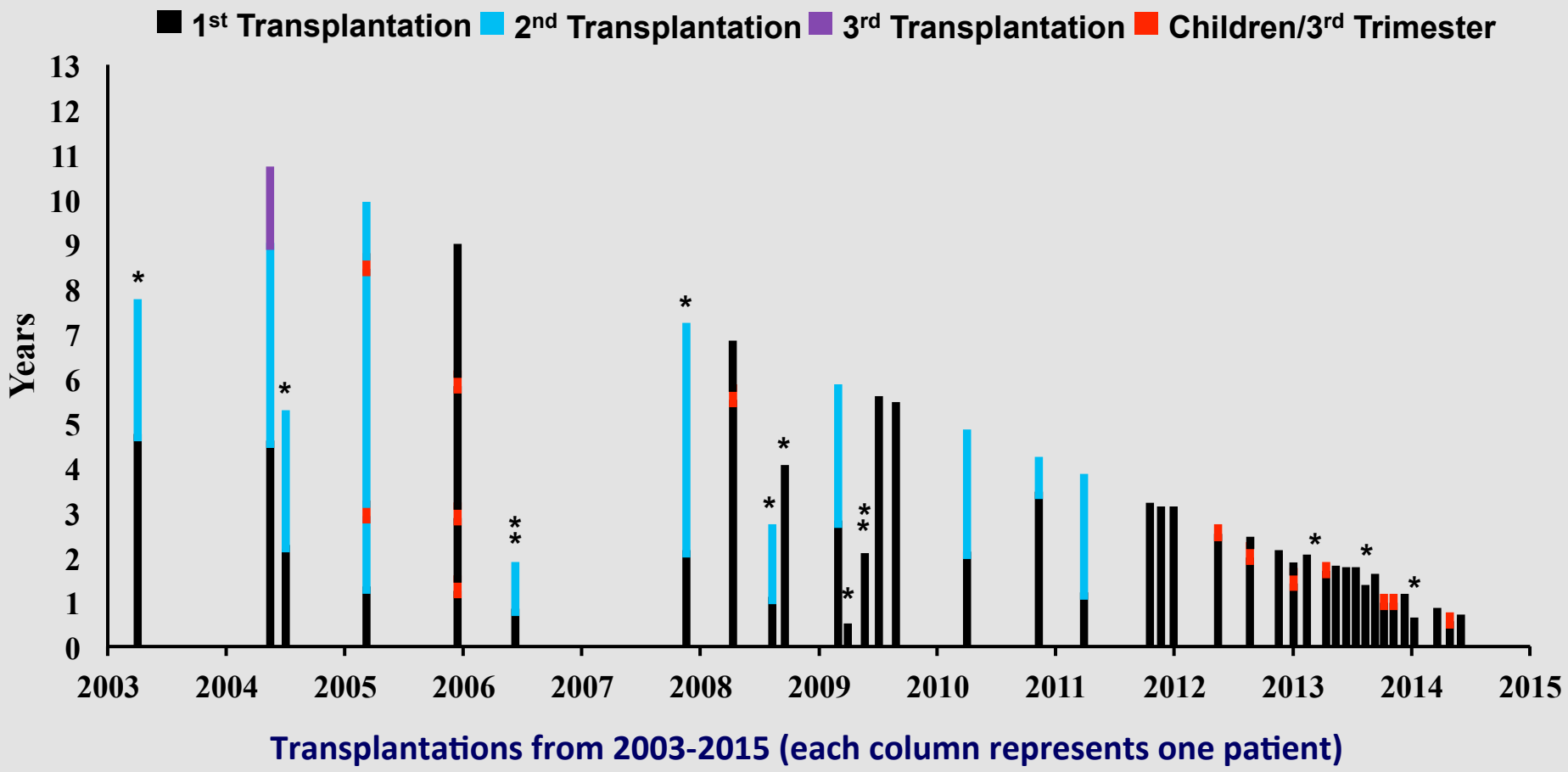
**The pregnancy rate is constantly increasing as the tissue remains active in a number of women**

**We need to wait until a large cohort of patients have had all their tissue transplanted and it has stopped functioning**

Yding Andersen C, *Lancet* 2015;**385**:1947

# Longevity of transplanted ovarian tissue and children/3rd trimester pregnancies from Danish patients

One asterisk (*) depicts patients were the graft(s) has stopped working
Two asterisks depict deceased patients




Klüver-Jensen *A et al. Hum Rep*. (in press)

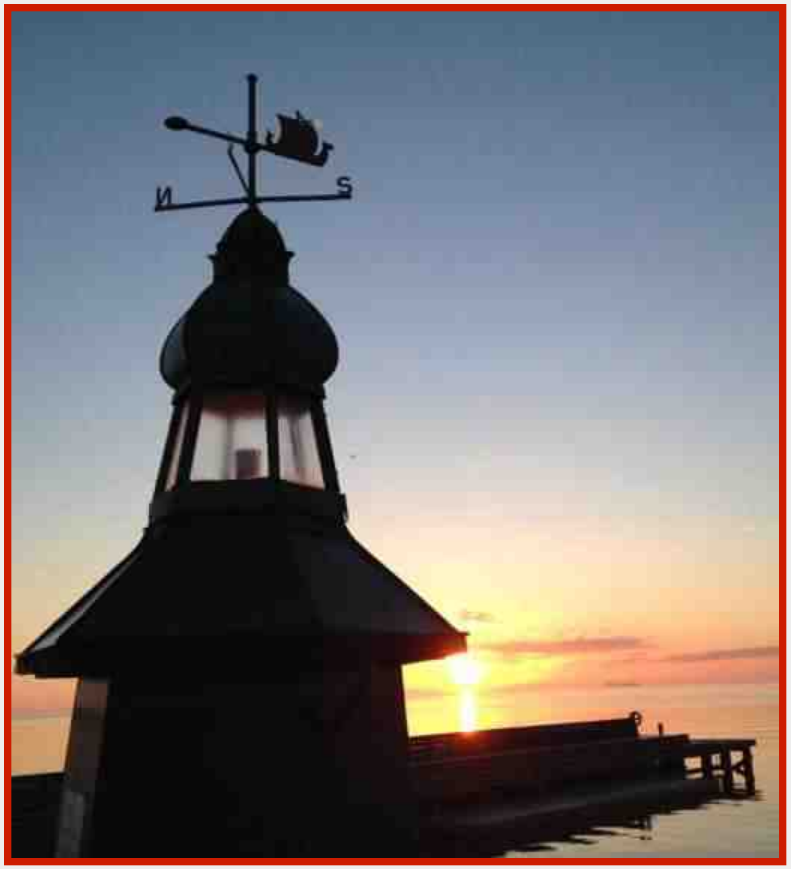
## FOLLOW-UP – CONCLUSIONS

- Patients appreciate fertility preservation and find comfort and strength in knowing that they may become mother in the future
- The tissue provides fertility with good efficacy but the true potential needs to await larger series of women where the tissue have stopped working
- Improving transplantation efficacy is important
- The longevity is surprising long in terms of menstrual cycles - often many years
- Women that do not enter menopause as a consequence of cancer treatment remain fertile and have children

# Risk of transplanting malignancy

**What is the risk that the cryopreserved ovarian tissue harbor cells from the original cancer?**

**Does the transplanted tissue itself result in cancer?**



***The true answer:***

**We are not quite sure yet, but clinical experience is now becoming stronger. The risk is low but in some cancers for instance leukemia extra considerations are required**

# Malignant cells are localised to the ovary in end stage cancer disease 5571 cases in total (%, actual numbers)

**(National autopsy files collected by the Japanese Society for Pathology)**

| Diagnosis | Age groups (years) | | | |
|---|---|---|---|---|
| | <10 | >10-20 | >20-30 | >30-39 |
| Leukaemia | 7.9 (31/392) | 10.2 (52/511) | 7.8 (34/438) | 7.9 (54/686) |
| Lymphoma | 10.5 (8/76) | 10.7 (15/140) | 13.9 (27/194) | 14.7 (48/326) |
| Pulmonary carcinoma | 0 (0/11) | 21.4 (3/14) | 21.0 (13/62) | 24.8 (73/294) |
| Gastric carcinoma | 0 (0/1) | 78.3 (18/23) | 60.4 (125/207) | 54.2 (468/864) |
| Uterine cancer | 0 (0/1) | 0 (0/3) | 12.8 (10/78) | 13.3 (46/346) |
| Breast cancer | 0 (0/0) | 0 (0/3) | 19.4 (14/72) | 25.0 (143/573) |
| Colon carcinoma | 0 (0/0) | 16.7 (2/12) | 31.1 (14/45) | 26.1 (52/199) |

Kyono K *et al.*. *Fertil. Steril*. 2010;**93**:2429

# Evaluating safety of transplanting ovarian tissue

- In *vitro* tests
  - histology, IHC, Q-PCR, immundeficient mice
- Transplanting tissue to women provides the real picture
  - minimal infectious dose - number of malignant cells,
  - sensitivity of employed methods – too sensitive or too insensitive
  - effect of the cryopreservation – wash out – cancer cells
  - the pieces of tissue used for grafting remain unchecked
  - amount of tissue – on average ten pieces
  - the type of disease and stage of progression at the time of tissue collection

# Data collection and references for Transplantation of frozen/thawed ovarian tissue to patients with a malignant diagnosis (Worldwide June 2015)

| | Data collection and Reference |
|---|---|
|  | Sherman Silber, RBMOnline 2015; Kim SS, JARG 2012 |
|  | Personal communication with Michael von Wolff, data including year 2014 |
|  | Dror Meirow et al., Abstract ESHRE 2012; Shapira M et al., Acta Haematol, 2014, personal communication |
|  | Kate Stern, Personal communication; Burmeister L & Kovacs G et al., Med. J. Aus. 2013 |
|  | J. Donnez et al., JCEM 2012; J. Donnez et al., Fertil Steril, 2013; personal communication |
|  | J. Donnez et al., Fertil Steril, 2013 (IVI-Pellecier) |
|  | Kenny Rodriques-Wallgreen, Stockholm, Personal communication; Margareta Kitlinsky, Malmø, Personal communication |
|  | Isabelle Demeestere et al., Hum. Rep. 2006, Imbert R et al., Hum. Rep. 2014 |
| UK, Norway | Tom Tanbo et al., 2015, Acta Obstet Gynaecol Scandinavia, (in press); Radford et al., Lancet 2001 |
|  | Claus Yding Andersen, Personal communication |

**In addition centres have reported single cases with non-malignant diseases**

# Transplantation of frozen/thawed ovarian tissue (Worldwide September 2015)

| | Breast cancer | Mb. Hodgkin | Non-Hodgkin | Ewing & other Sarcoma | Brain tumour | Colon, rectal & Anal cancer | Ova-rian cancer | Cervi-cal cancer | CML, AML, ALL | Lym-pho-ma | Haemato-logical malignancies | Malig-nant without haemato-logical | Non-malig nant | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
|  | 2 | 3 | | 1 | 1 | | | 3 | 1 | | | | 5 | 16 |
|  | 16 | 16 | 3 | | | 4 | 3 | | | 1 | | 2 | 2 | 47 |
|  | 3 | 8 | 4 | 2 | | | | | 2 | | | | | 19 |
|  | 2 | 2 | 3 | 1 | | | 1 | 5 | | | | | 7 | 21 |
|  | 1 | 5 | 2 | | 1 | | | 1 | 1 | | | 2 | 6 | 19 |
|  | | | | | | | | | | | 8 | 13 | 1 | 22 |
|  | 2 | 2 | | 3 | | | | 1 | | | | | 2 | 10 |
|  | 1 | 2 | 1 | | | 1 | | | | | | | 1 | 6 |
| UK, Norway | | 2 | | | | | | | | 1 | | | | 3 |
|  | 19 | 9 | 5 | 5 | | 2 | 1 | 5 | | | | | 11 | 57 |
| **Total** | **46** | **49** | **18** | **12** | **2** | **7** | **5** | **15** | **4** | **2** | **8** | **17** | **35** | **220** |

# Relapse following transplanting frozen/thawed ovarian tissue

## worldwide results (June 2015)

| Diagnosis | Women |
|---|---|
| Mammae cancer | 2 |
| Cervical cancer | 2 |
| Granulosa cell tumor | 1 |
| Ewing sarcoma | 1 |

**Relapse is expected in this group of patients**

# Safety of transplanting frozen/thawed ovarian tissue in Danish patients with a previous malignant diagnosis (June 2015)

**Relapse in women transplanted with ovarian tissue:**
**3 of 41 (7%)**

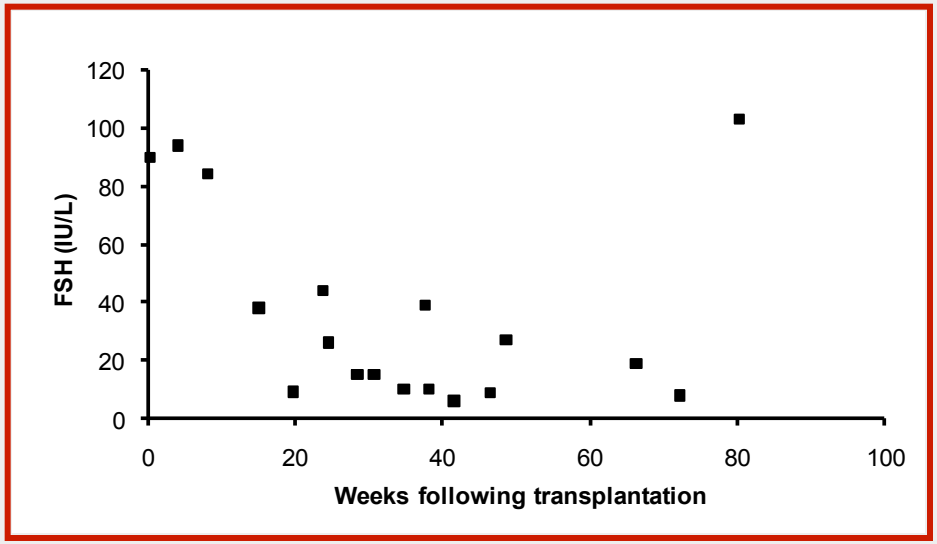
**Women with ovarian tissue retrieved who died within a 2 year period following cryopreservation**

**48 dead out of 691 (7%)**

Klüver-Jensen *A et al. Hum Rep*. (in press)

# Stimulation of puberty in a girl with chemo - and radiation therapy induced ovarian failure by transplantation part of her frozen/thawed ovary

- 9 year old girl with Ewing's sarcoma 2004
- One ovary (10 pieces of cortex) frozen
- High density of follicles in the frozen cortex
- EURO EWING-99 protocol plus 41 Gy irradiation
- Year 2009 at the age of 13,4 years no signs of puberty and high levels of FSH




- June 22nd 2009 two pieces of cortex were implanted in the remaining ovary at Aarhus University Hospital

Ernst E *et al.*, *Eur. J. Cancer* 2013;**49**: 911

# Induction of puberty

- Puberty is induced by endogenous hormones
- In early 2013 this patient experience relapse in the thorax
- She undergoes intense treatment, but without a successful result and she dies in January 2014
- The parents grants permission to evaluate the remaining eight pieces of ovarian cortex for the presence of the Ewing sarcoma marker EWS/FLI translocation

# Concentration of mRNA and presence of the Ewing sarcoma specific EWS/FLI translocation in eight ovarian biopsies

| Sample/Tube | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| 2 | 258 ng/ul | 316 ng/ul | 329 ng/ul | 154 ng/ul | 436 ng/ul | 378 ng/ul |
| 3 | 522 ng/ul | 203 ng/ul | 384 ng/ul | 319 ng/ul | 434 ng/ul | 300 ng/ul |
| 4 | 43 ng/ul | 71 ng/ul | 279 ng/ul | 424 ng/ul | 182 ng/ul | 40 ng/ul |
| 6 | < 5 ng/ul | 25,5 ng/ul | 10 ng/ul | 172 ng/ul | 76 ng/ul | 11 ng/ul |
| 7 | < 5 ng/ul | 67,2 ng/ul | < 5 ng/ul | 6,8 ng/ul | 16,0 ng/ul | 6,0 ng/ul |
| 8 | 15,6 ng/ul | 13,2 ng/ul | < 5 ng/ul | < 5 ng/ul | < 5 ng/ul | < 5 ng/ul |
| 9 | 119 ng/ul | 72,4 ng/ul | 143 ng/ul | 109 ng/ul | 58,8 ng/ul | 138 ng/ul |
| 10 | 49,2 ng/ul | 111 ng/ul | 140 ng/ul | 212 ng/ul | 124 ng/ul | 79,6 ng/ul |

**Each biopsy was divided into six pieces and mRNA extracted manually**

**Except from four different tubes deriving from different samples all proved to be negative for the EWS/FLI translocation but positive for the control gene**

Yding Andersen *et al., JARG*, 2014;**31**:1567

# The real life experience – Safety of transplanting ovarian tissue in Denmark

- No relapses related to transplanting ovarian tissue
- Two patients have had tissue transplanted for more than 10 years
- Fifteen patients have had tissue transplanted for more than 5 years
- No women have had cancers related to the ovary

**Reassuring results that does not suggest a major problem**

# Transplantation of Danish patient with stage one 1C ovarian cancer on the contraleteral ovary

- Ovarian tissue of the contralateral ovary excised Nov. 2003, aged 23,5 years
- 8 years later, Dec. 2011 she returns to for transplantation, 80% transplanted
- The gynaecological oncologists gave green light
- One piece of ovarian cortex was transplanted to an immun-deficient mouse for 20 weeks. Cheked by the original pathologist
- Becomes pregnant but have spontaneous abortion in week 8
- Becomes pregnant again and have delivered twins in May 2015
- Tissue removed August 2015

# Causion with cancer originating in the ovary

Human Reproduction, Vol.29, No.8 pp. 1828–1834, 2014

human reproduction

LETTERS TO THE EDITOR

## Delivery of twins following heterotopic grafting of frozen-thawed ovarian tissue

C.J. Stern, D. Gook, L.G. Hale, F. Agresta, J. Oldham, G. Rozen* and T. Jobling
*Reproductive Services, Melbourne IVF and Royal Women's Hospital, Parkville, VIC, Australia*

*Correspondence address. 20 Flemington Road, Parkville, VIC 3052, Australia. Tel: +61383453200; E-mail: genia.rozen@mivf.com.au

doi:10.1093/humrep/deu119
Advanced Access publication on May 28, 2014

Sir,

We have recently published a case report in your journal (Stern *et al.*, 2013) describing the first reported clinical pregnancy following heterotopic grafting of cryopreserved ovarian tissue in a woman after a bilateral


Real life experience with replacement of ovarian tissue suggests
that the procedure is safe at least with early stage cancer.
Though a few clouds remain

# Conclusions

- Centralized service for the laboratory part of fertility preservation is attractive also for the referral centers
- Transport of fresh tissue for at least 4-5 hours is acceptable
- Ovarian tissue do provide fertility, its efficacy is increasing as long as the tissue remains active but the overall efficacy cannot be calculated at present
- Safety data from real clinical situations is now accumulating and is reassuring