

# Outcomes of transplantations of cryopreserved ovarian tissue to 41 women in Denmark

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**STUDY QUESTION:** What are the results of transplanting cryopreserved ovarian tissue?

**SUMMARY ANSWER:** The transplanted ovarian tissue can last up to 10 years, with no relapses following the 53 transplantations, and the chance of a successful pregnancy is currently around one in three for those with a pregnancy-wish.

**WHAT IS KNOWN ALREADY:** Cryopreservation of ovarian tissue is now gaining ground as a valid method for fertility preservation. More than 36 children worldwide have now been born following this procedure.

**STUDY DESIGN, SIZE, DURATION:** This is a retrospective cohort study of 41 women who had thawed ovarian tissue transplanted 53 times over a period of 10 years, including 1 patient who was lost to follow-up.

**PARTICIPANTS/MATERIALS, SETTING, METHODS:** The 41 Danish women, who had in total 53 transplantations, were followed for ovarian function and fertility outcome. Safety was assessed by monitoring relapse in cancer survivors.

**MAIN RESULTS, AND THE ROLE OF CHANCE:** Among 32 women with a pregnancy-wish, 10 (31%) had a child/children (14 children in total); this included 1 woman with a third trimester on-going pregnancy. In addition, two legal abortions and one second trimester miscarriage occurred. A total of 24 clinical pregnancies were established in the 32 women with a pregnancy-wish. The tissue remained functional for close to 10 years in some cases and lasted only a short period in others. Three relapses occurred but were unlikely to be due to the transplanted tissue.

**LIMITATIONS, REASONS FOR CAUTION:** Self-report through questionnaires with only in-one hospital formalised follow-up of transplanted patients could result in unreported miscarriages. The longevity of the tissue may vary by few months compared with those reported because some patients simply could not remember the date when the tissue became non-functional.

**WIDER IMPLICATIONS OF THE FINDINGS:** Cryopreservation of ovarian tissue is likely to become integrated into the treatment of young women, with cancer, who run a risk of losing their fertility. The full functional lifespan of grafts is still being evaluated, because many of the transplanted women have continued to maintain ovarian activity. Some of our first cases have had tissue functioning for ~10 years.

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**Key words:** ovarian transplantation / fertility preservation / reproductive outcome / ovarian graft longevity / transplantation safety

## Introduction

The chances of surviving a cancer disease are considerably better today than just a few decades ago (Tonorezos *et al.*, 2015). Many young patients

can now have a realistic hope of living a normal life following recovery (Siegel *et al.*, 2015). This has created an awareness of quality-of-life aspects after cancer treatment, with fertility being an important issue for many girls and young women (Lamar and DeCherney, 2009; Loren

*et al.*, 2013b). Modern effective treatment regimens often pose a considerable risk of causing infertility, either directly or later on as premature ovarian insufficiency (POI). Therefore, fertility preservation is increasingly becoming an integral part of treating young cancer patients. Freezing of ovarian tissue can be performed on short notice and is often the preferred fertility preservation technique due to an urgent need to commence treatment. Cryopreservation of ovarian tissue includes surgical removal and freezing of tissue prior to commencing treatment. If the cancer treatment renders the patient infertile, transplantation of cryopreserved ovarian tissue may restore fertility (Andersen *et al.*, 2012). However, because fertility preservation is still in its early days and counselling may not be offered to 30–50% of young women diagnosed with cancer, a greater awareness of these new possibilities is still required (Gwede *et al.*, 2012; Goodwin *et al.*, 2007; Anderson *et al.*, 2008; Quinn *et al.*, 2009; Loren *et al.*, 2013a; Conroy and Swinglehurst, 2014).

The programme of ovarian cryopreservation started in the year 2000 in Denmark following governmental approval, which made the treatment free of costs for patients. Since then, almost 800 patients have had tissue frozen, with a steady annual activity of ~13–14 cases per million inhabitants per year. Patients are counselled and tissue can be excised at four different centres, three in Denmark and, more recently, one centre in southern Sweden. The tissue is transported (for up to 5 h) to one central laboratory, where preparation and storage of tissue takes place. If the patient requests transplantation, tissue is transported to the local hospital. This model has been termed the Danish model ('the woman stays—the tissue moves') and has now been introduced in different countries around the world.

Our programme has performed 53 transplantations to 41 patients during the last 10 years and here we report our collective experience from this 1 centre.

## Materials and Methods

### Patients

From 2003 until June 2014, a total of 41 patients had received a total of 53 transplantations of frozen/thawed ovarian tissue. The mean age was 29.8 years at the time of cryopreservation and the diagnoses are listed in Table 1. All patients who had frozen/thawed ovarian tissue transplanted more than 6 months prior to 1 January 2015 have been included. All but two women had one entire ovary cryopreserved. No gross pathology near or on the ovaries was observed during removal. Information of the first 18 patients has previously been published (Greve *et al.*, 2012b), but the follow-up period is now extended by 3 years.

### The Danish programme for cryopreservation of ovarian tissue

The programme was initiated in 1999. The three centres in Denmark and one centre in southern Sweden offer the initial counselling of patients and perform the harvesting of the ovarian tissue. No complications or sequelae have been reported to our centre from any of the hospitals in connection with excision of the tissue.

In this retrospective cohort study, only patients from the three Danish centres are evaluated, since the southern Sweden has only recently joined our programme.

Preparation of tissue for cryopreservation and storage is centralized at one centre and performed as previously described (Rosendahl *et al.*, 2011a). Tissue excised at the hospital where the processing laboratory is located is

transported in 37°C medium, whereas tissue from other hospitals is cooled in medium placed on crushed ice. Tissue preparation in ice-cold saline is then performed up to 5 h after excision (Schmidt *et al.*, 2003b; Rosendahl *et al.*, 2008).

The transplantations are currently performed at two of the three Danish hospitals, at Copenhagen University Hospital (Rigshospitalet, Copenhagen) ( $n = 16$ ) and at Århus University Hospital (Århus) ( $n = 37$ ). At Århus University Hospital, only one surgeon performs the transplantations, whereas a team of two or three surgeons have performed the transplantations at Rigshospitalet.

The Public Healthcare System in Denmark covers all Danish citizens and is paid through taxes and provides free of charge counselling, excision of tissue, transport, preparation and storage of the cryopreserved tissue, transplantation (more than once) and, if necessary, subsequent IVF treatment.

### Guidelines for retrieving ovarian tissue

The clinical guidelines for offering cryopreservation of ovarian tissue are as follows: a risk of POI exceeding ~50%; age below 35 years (flexible according to AMH level and biological age); a higher than 50% chance of 5-year survival (flexible according to treatment); no disseminated disease; and no contradictions against operation or anaesthesia.

Each patient is counselled by a consultant specialised in fertility preservation at each hospital, who makes an individual evaluation and, in collaboration with patient, decides the most appropriate treatment.

### Hormone measurements

The LH and FSH levels were measured at the Department of Clinical Biochemistry at the local hospital according to their standard procedures.

### Follicle density prior to freezing

The follicular density was calculated based on histological sections from a sample of ovarian cortex from each individual patient (Tryde Schmidt *et al.*, 2004). Only data on 22 patients were available due to the lack of cortical biopsies collected ( $n = 10$ ) and the exclusion of patients with no pregnancy-wish ( $n = 7$ , including 1 prepubertal girl) and patients who pursued surrogacy pregnancy ( $n = 2$ ).

### Statistics

The statistical analysis included student *t*-test that was performed in order to compare means between two groups of patients. A *P* value of <0.05 was accepted as statistically significant.

### Ethical approval

The Ethical Committee of Copenhagen and Frederiksberg approved the project (H-2-2011-044) and the Minister of Health also approved the procedure. Data were collected from patient records, and the collection and storage of data was approved by the Minister of Health (J.no.: 30-1372) and by the Danish Data Protection Agency.

## Results

### Diagnosis and age of women undergoing ovarian transplantation

Fifty-three transplantations were performed in 41 patients. Eleven women had a second and one woman had a third transplantation performed (Table 1). The mean age at freezing was 29.8 years whereas the first grafting was performed at the mean age of 32.9 years. The

**Table 1** Diagnosis and age of 41 women undergoing transplantation with frozen/thawed ovarian tissue in Denmark.

Diagnosis	No. of women		Age (years) (mean; range)			
	1st/2nd/3rd transplantation	Tissue transported on ice prior to freezing	Cryopreservation	1st transplant	2nd transplant	3rd transplant
Breast cancer	12/3	9	33.9 (26.0–38.7)	36.5 (28.7–43.2)	38.0 (36.7–39.4)	
Mb. Hodgkin	5/4	4	29.4 (25.6–34.1)	32.0 (28.0–37.3)	32.3 (29.4–36.7)	
Non-Hodgkin	5/3/1	1	31.1 (25.9–35.1)	33.8 (29.6–37.3)	37.6 (35.4–39.1)	39.8
Cervical cancer	3/1	3	25.8 (21.2–30.7)	29.1 (24.3–32.2)		
Aplastic anaemia	2	–	29.3 (26.2–32.3)	33.1 (31.3–35.0)		
Ewing sarcoma	2	2	18.3 (9.5–27.1)	21.3 (13.8–28.8)		
Paroxysmal nocturnal haemoglobinuria	2	–	22.2 (19.0–25.4)	25.1 (21.7–28.5)		
Sarcoma	2	2	35.7 (33.5–37.8)	37.8 (35.9–39.6)		
Haemolytic uraemic syndrome	1	1	33.3	38.5		
Ovarian cancer	1	1	23.5	31.9		
Colon cancer	1	1	26.1	28.8		
Anal cancer	1	1	37.0	38.1		
Various others <sup>a</sup>	4	4	26.6 (23.1–30.0)	31.0 (27.9–33.0)		
Total	53 (41/11/1)	29	29.8 (9.5–38.7)	32.9 (13.8–43.2)	35.4 (29.4–39.4)	39.8

<sup>a</sup>Autoimmune Small-Vessel Vasculitis; Morbus Behcet; Choriocarcinoma; Wegener's Granulomatosis.

mean ages at the second and third transplantations were 35.4 and 39.8 years (Table 1).

The original diagnoses are summarised in Table 1; for example, 12 women diagnosed with breast cancer had tissue transplanted, 3 of whom had a second transplantation. Nine of these women had tissue transported prior to freezing.

### Amount of tissue transplanted and transplantation sites

On average, 45% (i.e. 9.5 pieces of cortical tissue) of one ovary was transplanted the first time with 36% transplanted the second time. Over time, the amount of reimplanted tissue has differed. In the beginning, fewer pieces of cortex were transplanted to women who had a pregnancy-wish compared with today. This change was motivated by the constant low levels of AMH experienced after transplantation (Greve et al., 2012b), so we wanted to augment the follicle pool as much as possible. In women who want to avoid a menopausal state, but do not want to conceive, a lower number is usually transplanted in order to stretch the pool of follicles for as long as possible.

Fifteen patients had ovarian tissue transplanted only to the remaining ovary, 14 had tissue transplanted to the remaining ovary and into a peritoneal pocket, 5 had tissue transplanted into a peritoneal pocket only, 3 had tissue transplanted to the remaining ovary and peritoneal pockets corresponding to the abdominal wall, and 3 had tissue transplanted to all three transplantation sites. One patient only received tissue into the abdominal wall (Supplementary data, Table S1) (Schmidt et al., 2011; Macklon et al., 2014).

After transplantation, the patients were usually been in regular contact with the fertility clinic for evaluation of function and fertility. Only in one

centre did patients follow a formalised follow-up schedule and therefore a questionnaire was sent to patients in January 2015. All patients in the cohort also had information collected from their patient records.

### Fertility outcome of patients with a pregnancy-wish

Six women had tissue transplanted to avoid menopausal symptoms only and did not want to become pregnant. Two women had originally a hysterectomy performed and required a surrogacy mother. The diagnoses of these women were breast cancer ( $n = 1$ ), haemolytic uraemic syndrome ( $n = 1$ ), Hodgkin's disease ( $n = 1$ ), cervical cancer ( $n = 3$ ) (one of these is trying surrogacy) and soft tissue sarcoma ( $n = 2$ ) (one of these patients had a sarcoma in her uterus and is also trying surrogacy). One Ewing sarcoma patient was a prepubertal girl, in whom tissue was transplanted to induce puberty (Ernst et al., 2013a; Yding Andersen et al., 2014). The remaining 32 patients expressed a pregnancy-wish (Table II). A total of 42 transplantations have been performed on these 32 women resulting, so far, in 21 women obtaining at least 1 positive pregnancy test (63%). A total of 28 positive hCG tests have been recorded so far and 24 of these have developed to clinical pregnancies with fetal heart beats at Week 7 (Table II), resulting in 13 healthy children (11 singleton pregnancies and 1 twin pregnancy) plus one ongoing singleton third trimester pregnancy. Three abortions occurred, including one miscarriage in gestational week 19 due to premature preterm rupture of membranes (PPROM) and two legal abortions: one because the woman was separating from her partner (Greve et al., 2010) and one because the woman experienced relapse of her breast cancer (Ernst et al., 2013b).

It is well known that radiation to a field that includes the uterus is associated with a range of adverse reproductive outcomes, including

**Table II** The current reproductive outcome of women having frozen/thawed ovarian tissue transplanted in Denmark (January 2015; data represent women who had a pregnancy-wish at transplantation).

Diagnosis	Number of:		Number of pregnancies				
	Women	Transplantations	NC	IVF	Pos. hCG	Clinical	Children
Breast cancer	11	13	6	3	9	9	3
Mb. Hodgkin	4	8	–	4	4	3	2
Non-Hodgkin	5	9	1	4	5	2	1 ongoing singleton <sup>a</sup>
Aplastic anaemia	2	2	1	–	1	1	1
Ewing sarcoma	1	1	2	1	3	3	3
Paroxysmal nocturnal haemoglobinuria	2	2	1	–	1	1	1
Ovarian cancer	1	1	–	2	2	2	2
Colon cancer	1	1	–	1	1	1	1 miscarriage <sup>b</sup>
Anal cancer	1	1	–	–	–	–	–
Various others <sup>c</sup>	4	4	2	–	2	2	1
Total	32	42	13	15	28	24	13 (+1 ongoing)

NC, natural conceived; IVF, *in vitro* fertilization.

<sup>a</sup>Ongoing third-trimester pregnancy.

<sup>b</sup>Second-trimester miscarriage caused by PPRM.

<sup>c</sup>Autoimmune Small-Vessel Vasculitis; Morbus Behcet; Choriocarcinoma; Wegener's Granulomatosis.

miscarriage, premature delivery and stillbirth (Hawkins and Smith, 1989; Signorello *et al.*, 2006, 2010; Reulen *et al.*, 2009; Sudour *et al.*, 2010). The second trimester miscarriage, due to PPRM in gestational week 19, occurred to a former colon cancer patient who had received radiation to her pelvis. The remaining seven clinical pregnancies resulted in spontaneous abortions in the first trimester.

Five of the 32 women had low but not absent ovarian function at the time of transplantation. All five of these women have succeeded in becoming pregnant. One had a clinical pregnancy, three has given birth and one has an ongoing pregnancy. It is unknown whether the oocyte that resulted in a pregnancy originated in the *in situ* positioned tissue or in the transplanted tissue. All five women had low ovarian reserve before transplantation and had been unsuccessful in becoming pregnant despite several attempts. Transplantation was offered to boost their fertility, and all five women became pregnant within the first year after transplantation.

Ten women have conceived 13 children plus there is one ongoing singleton pregnancy; 8 were conceived naturally and 6 were with the assistance of IVF. Thus, the current rate of women having a child/children in our setting is 31% (10/32).

### Pregnant versus non-pregnant women

On average, 55% of one ovary (i.e. 13.5 pieces of ovarian tissue) was transplanted to women who successfully conceived, which was similar to those who failed (53% of one ovary, i.e. 11.8 pieces of ovarian tissue) (Table III).

There was no significant difference in the age at the time of cryopreservation for women who subsequently did or did not conceive (29.7 versus 30.8 years of age) or at the time of the first transplantation (32.5 versus 34.4 years of age) (Table III).

The follicular density was also not significantly different in cortical tissue from women who did ( $36 \pm 16$  follicles/mm<sup>3</sup> (mean  $\pm$  SEM),  $n = 14$ ) or did not conceive ( $22 \pm 9$  follicles/mm<sup>3</sup>,  $n = 7$ ) (Table III).

### Safety: risk of grafting malignant cells

Three of the 41 transplanted women experienced relapse after transplantation of frozen/thawed tissue. Two relapses occurred locally in the breast of former breast cancer patients. The relapse of the Ewing's sarcoma patient occurred in the hemithorax. All relapses appeared to be unrelated to the transplantation of ovarian tissue (Greve *et al.*, 2010; Rosendahl *et al.*, 2011a; Ernst *et al.*, 2013b). Two of the three patients with relapse are deceased (see Supplementary data Table SI). The relapse rate on the transplanted patients was 7% (3 of 41 patients).

Data on the relapse rate of women receiving fertility preservation have currently not been recorded. However, data on patients from this cohort are available. In order only to include patients who experienced a relapse, we have included patients who died >2 years after the date of cryopreservation.

We are aware that some patients with a relapse are not deceased whereas others may, on the other hand, have died of reasons unrelated to their cancer. With this definition, there were 48 deaths among 691 patients when excluding those who had transplantation. The relapse rate of this cohort of patients is 7% (48 of 691 patients) giving a figure similar to that of the transplanted patients.

Altogether, for three patients, it has been >10 years since they had the tissue transplanted; for six patients, it has been >8 years and for 15 patients, it has been >5 years, whereas the remaining patients have had tissue transplanted for between half a year and 5 years.

None of the patients have experienced development of ovarian malignancies in the transplanted tissue.

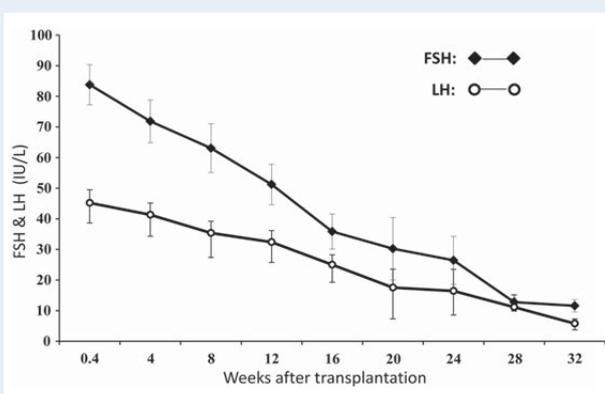
### Hormone levels and longevity

The majority of patients were followed with regular visits after transplantation. Blood samples were taken approximately once a month; ultrasound examination was not systematically performed simultaneously. Levels of FSH and LH showed a gradual decline (Fig. 1), with a return

**Table III** Parameters with a possible effect on pregnancy outcome (Mean  $\pm$  SEM).

Parameter	Pregnant	Non-pregnant	Statistics
Amount of transplanted tissue from one ovary (per cent)	55 $\pm$ 4	53 $\pm$ 6	NS
Age of the patient at cryopreservation (years)	29.7 $\pm$ 1.1	30.8 $\pm$ 1.5	NS
Age of the patient at first transplantation (years)	32.5 $\pm$ 1	34.4 $\pm$ 1.4	NS
Follicular density (follicles/mm <sup>3</sup> cortical tissue)	36 $\pm$ 16	22 $\pm$ 9	NS

NS, non-significant.

**Figure 1** Hormone levels of FSH and LH in all patients without ovarian function from before transplantation and through the following weeks.

to premenopausal levels 4–5 months after transplantation leading to cessation of menopausal symptoms and renewed menstrual cycles. The prepubertal girl, who was 13.8 years at the time of transplantation, succeeded in having puberty induced (Ernst et al., 2013a).

Furthermore, five women were not amenorrhic at the time of transplantation but experienced low ovarian reserve, with a low AFC of  $\sim$ 1, and had difficulties in conceiving. These five women had tissue transplanted to augment the follicle pool and thus increase their chances of a pregnancy.

The longevity of the tissue after the first, second and, for one patient, third transplantation is illustrated in Fig. 2. The tissue is still functioning in many of these patients. In some patients, the tissue from the first transplantation was still functioning even though they had a second transplantation to boost fertility.

The functional life span of the grafts has been >10 years for two patients, >7 years in another three patients, >4 years in another seven patients, between 2 and 4 years in 15 patients, between 1 and 2 years in seven patients, and less than a year in four patients. In two

patients, one who only had three pieces of cortical tissue cryopreserved and one who experienced a relapse of breast cancer, the functional duration of the tissue was <1 year. One woman who was 37 years at the time of cryopreservation did not regain endocrine function.

## Discussion

To our knowledge, this is the largest series of transplantations of frozen/thawed ovarian tissue performed worldwide. Our data demonstrate that the grafted tissue robustly restores ovarian function. The tissue provides fertility with good efficacy, and our programme has resulted in 13 children plus one ongoing singleton pregnancy) so far, showing that at present the pregnancy rate is  $\sim$ 30% among women with a pregnancy-wish. However, the true efficacy requires longer observation time and remains to be determined. Equally important is the safety of transplanting tissue harvested when the woman had cancer. Currently, none of the patients have experienced relapse as a result of having tissue transplanted. The longevity of transplanted tissue is variable, but many patients have so far experienced several years of ovarian function. Additionally, our programme has clearly demonstrated that transport of ovarian tissue for several hours before freezing is feasible and allows for an effective centralised service.

Collectively, cryopreservation of ovarian tissue is becoming an established method for fertility preservation and is likely to become an integrated part of cancer treatment in young patients.

Unlike IVF treatment, where a pregnancy test determines the outcome after 2 weeks, the outcomes of replacing ovarian tissue take a long time to be determined. If the tissue is grafted into the remaining *in situ* ovary, natural fertility is possible with chances of conception as long as the tissue remains active. Therefore, the success rates increase over time. Furthermore, cancer survivors are different from infertility patients, who often have failed to conceive for a long time and now turn to the medical profession for help, whereas cancer survivors often have a different perspective. They have recently recovered from a potentially deadly disease and often they want to test the possibility of having children naturally and may only later on want to exploit traditional infertility treatment. The difficulty in calculating an exact efficacy rate for fertility comes from the fact that ovarian grafting recreates an organ function including an endocrine function rather than just fertility. Several patients basically want to be normal young women, avoid menopausal symptoms and have menstrual cycles and may only consider conceiving at a later stage. Initially, they may not have a partner or may have been advised against pregnancy. Indeed, our programme included three women with cervical cancer; two of whom underwent hysterectomy. One of these hysterectomised women was attempting to have a child via surrogacy. This aspect obviously further impedes calculating an exact efficacy rate. Moreover, there were two legal abortions in this cohort, which adds to the complexity of calculating a precise efficiency. In addition, five patients had low but not absent ovarian function at the time of transplantation. Thus, it is unknown whether they conceived from the transplanted tissue or from endogenously developed follicles.

All of this makes it impossible to provide an exact pregnancy rate, in contrast with traditional infertility treatment, and one cannot compare the two.

Even though The Danish Cryopreservation Program does not have a formalised follow-up programme, the functional duration of the grafts is accurate within a few months. A formalised programme would have



## Supplementary data

Supplementary data are available at <http://humrep.oxfordjournals.org/>.

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## Authors' roles

A.K.J. contributed to the study design, figures, data collection, data analysis, and the writing and revision of the report. S.G.K. contributed to the data analysis, figures, and the writing and the revision of the report. K.T.M. contributed to the data collection and revision of the report. J.V.J. contributed to the data collection and the revision of the report. J.F. contributed to the data collection and revision of the report. E.E. contributed to the data collection and revision of the report. C.Y.A. contributed to the study design, figures, data analysis, and the writing and revision of the report.

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## Conflict of interest

None declared.

## References

- Anderson RA, Weddell A, Spoudeas HA, Douglas C, Shalet SM, Levitt G, Wallace WHB. Do doctors discuss fertility issues before they treat young patients with cancer? *Hum Reprod* 2008;**23**:2246–2251.
- Andersen CY, Kristensen SG, Greve T, Schmidt KT. Cryopreservation of ovarian tissue for fertility preservation in young female oncological patients. *Futur Oncol* 2012;**8**:595–608.
- Bastings L, Beerendonk CC, Westphal JR, Massuger LF, Kaal SE, van Leeuwen FE, Braat DD, Peek R. Autotransplantation of cryopreserved ovarian tissue in cancer survivors and the risk of reintroducing malignancy: a systematic review. *Hum Reprod Updat* 2013;**19**:483–506.
- Corney RH, Swinglehurst AJ. Young childless women with breast cancer in the UK: a qualitative study of their fertility-related experiences, options, and the information given by health professionals. *Psychooncology* 2014;**23**:20–26.
- Dolmans M-M, Marinescu C, Saussoy P, Van Langendonck A, Amorim C, Donnez J. Reimplantation of cryopreserved ovarian tissue from patients with acute lymphoblastic leukemia is potentially unsafe. *Blood* 2010;**116**:2908–2914.
- Dolmans MM, Luyckx V, Donnez J, Andersen CY, Greve T. Risk of transferring malignant cells with transplanted frozen-thawed ovarian tissue. *Fertil Steril* 2013;**99**:1514–1522.
- Donnez J, Dolmans MM. Fertility preservation in women. *Nat Rev Endocrinol* 2013;**9**:735–749.
- Ernst E, Kjaersgaard M, Birkebaek NH, Clausen N, Andersen CY. Case report: stimulation of puberty in a girl with chemo- and radiation therapy induced ovarian failure by transplantation of a small part of her frozen/thawed ovarian tissue. *Eur J Cancer* 2013a;**49**:911–914.
- Ernst EH, Offersen BV, Andersen CY, Ernst E. Legal termination of a pregnancy resulting from transplanted cryopreserved ovarian tissue due to cancer recurrence. *J Assist Reprod Genet* 2013b;**30**:975–978.
- Goodwin T, Elizabeth Oosterhuis B, Kiernan M, Hudson MM, Dahl GV. Attitudes and practices of pediatric oncology providers regarding fertility issues. *Pediatr Blood Cancer* 2007;**48**:80–85.
- Greve T, Ernst E, Markholt S, Schmidt KT, Andersen CY. Legal termination of a pregnancy resulting from transplanted cryopreserved ovarian tissue. *Acta Obs Gynecol Scand* 2010;**89**:1589–1591.
- Greve T, Clasen-Linde E, Andersen MT, Andersen MK, Sorensen SD, Rosendahl M, Ralfkiaer E, Andersen CY. Cryopreserved ovarian cortex from patients with leukemia in complete remission contains no apparent viable malignant cells. *Blood* 2012a;**120**:4311–4316.
- Greve T, Schmidt KT, Kristensen SG, Ernst E, Andersen CY. Evaluation of the ovarian reserve in women transplanted with frozen and thawed ovarian cortical tissue. *Fertil Steril* 2012b;**97**:1394–1398.e1.
- Greve T, Wielenga VT, Grauslund M, Sorensen N, Christiansen DB, Rosendahl M, Yding Andersen C. Ovarian tissue cryopreserved for fertility preservation from patients with Ewing or other sarcomas appear to have no tumour cell contamination. *Eur J Cancer* 2013;**49**:1932–1938.
- Gwede CK, Vadaparampil ST, Hoffe S, Quinn GP. The role of radiation oncologists and discussion of fertility preservation in young cancer patients. *Pract Radiat Oncol* 2012;**2**:242–247.
- Hawkins MM, Smith RA. Pregnancy outcomes in childhood cancer survivors: probable effects of abdominal irradiation. *Int J Cancer* 1989;**43**:399–402.
- Lamar CA, DeCherney AH. Fertility preservation: state of the science and future research directions. *Fertil Steril* 2009;**91**:316–319.
- Loren AW, Brazauskas R, Chow EJ, Gilleece M, Halter J, Jacobsohn DA, Joshi S, Pidala J, Quinn GP, Wang Z et al. Physician perceptions and practice patterns regarding fertility preservation in hematopoietic cell transplant recipients. *Bone Marrow Transplant* 2013a;**48**:1091–1097.
- Loren AW, Mangu PB, Beck LN, Brennan L, Magdalinski AJ, Partridge AH, Quinn G, Wallace WH, Oktay K. Fertility preservation for patients with cancer: American Society of Clinical Oncology clinical practice guideline update. *J Clin Oncol* 2013b;**31**:2500–2510.
- Macklon KT, Jensen AK, Loft A, Ernst E, Andersen CY. Treatment history and outcome of 24 deliveries worldwide after autotransplantation of cryopreserved ovarian tissue, including two new Danish deliveries years after autotransplantation. *J Assist Reprod Genet* 2014;**31**:1557–1564.
- Quinn GP, Vadaparampil ST, Lee J-H, Jacobsen PB, Bepler G, Lancaster J, Keefe DL, Albrecht TL. Physician referral for fertility preservation in oncology patients: a national study of practice behaviors. *J Clin Oncol* 2009;**27**:5952–5957.
- Reulen RC, Zeegers MP, Wallace WHB, Frobisher C, Taylor AJ, Lancashire ER, Winter DL, Hawkins MM. Pregnancy outcomes among adult survivors of childhood cancer in the British Childhood Cancer Survivor Study. *Cancer Epidemiol Biomarkers Prev* 2009;**18**:2239–2247.
- Rosendahl M, Yding Andersen C, Ernst E, Westergaard LG, Rasmussen PE, Loft A, Nyboe Andersen A. Ovarian function after removal of an entire ovary for cryopreservation of pieces of cortex prior to gonadotoxic treatment: a follow up study. *Hum Reprod* 2008;**23**:2475–2483.
- Rosendahl M, Andersen MT, Ralfkiaer E, Kjeldsen L, Andersen MK, Andersen CY. Evidence of residual disease in cryopreserved ovarian cortex from female patients with leukemia. *Fertil Steril* 2010;**94**:2186–2190.
- Rosendahl M, Schmidt KT, Ernst E, Rasmussen PE, Loft A, Byskov AG, Andersen AN, Andersen CY. Cryopreservation of ovarian tissue for a decade in Denmark: a view of the technique. *Reprod Biomed Online* 2011a;**22**:162–171.

- Rosendahl M, Timmermans Wielenga V, Nedergaard L, Kristensen SG, Ernst E, Rasmussen PE, Anderson M, Schmidt KT, Andersen CY. Cryopreservation of ovarian tissue for fertility preservation: no evidence of malignant cell contamination in ovarian tissue from patients with breast cancer. *Fertil Steril* 2011b;**95**:2158–2161.
- Rosendahl M, Greve T, Andersen CY. The safety of transplanting cryopreserved ovarian tissue in cancer patients: a review of the literature. *J Assist Reprod Genet* 2013;**30**:11–24.
- Schmidt KLT, Byskov AG, Nyboe Andersen A, Müller J, Yding Andersen C. Density and distribution of primordial follicles in single pieces of cortex from 21 patients and in individual pieces of cortex from three entire human ovaries. *Hum Reprod* 2003a;**18**:1158–1164.
- Schmidt KLT, Ernst E, Byskov AG, Nyboe Andersen A, Yding Andersen C. Survival of primordial follicles following prolonged transportation of ovarian tissue prior to cryopreservation. *Hum Reprod* 2003b;**18**:2654–2659.
- Schmidt KT, Rosendahl M, Ernst E, Loft A, Andersen AN, Dueholm M, Ottosen C, Andersen CY. Autotransplantation of cryopreserved ovarian tissue in 12 women with chemotherapy-induced premature ovarian failure: the Danish experience. *Fertil Steril* 2011;**95**:695–701.
- Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. *CA Cancer J Clin* 2015;**65**:5–29.
- Signorello LB, Cohen SS, Bosetti C, Stovall M, Kasper CE, Weathers RE, Whitton JA, Green DM, Donaldson SS, Mertens AC et al. Female survivors of childhood cancer: preterm birth and low birth weight among their children. *J Natl Cancer Inst* 2006;**98**:1453–1461.
- Signorello LB, Mulvihill JJ, Green DM, Munro HM, Stovall M, Weathers RE, Mertens AC, Whitton JA, Robison LL, Boice JD. Stillbirth and neonatal death in relation to radiation exposure before conception: a retrospective cohort study. *Lancet* 2010;**376**:624–630.
- Sudour H, Chastagner P, Claude L, Desandes E, Klein M, Carrie C, Bernier V. Fertility and pregnancy outcome after abdominal irradiation that included or excluded the pelvis in childhood tumor survivors. *Int J Radiat Oncol Biol Phys* 2010;**76**:867–873.
- Tonorezos ES, Hudson MM, Edgar AB, Kremer LC, Sklar CA, Wallace WHB, Oeffinger KC. Screening and management of adverse endocrine outcomes in adult survivors of childhood and adolescent cancer. *Lancet Diabetes Endocrinol* 2015;**3**:545–555.
- Tryde Schmidt KL, Yding Andersen C, Starup J, Loft A, Byskov AG, Nyboe Andersen A. Orthotopic autotransplantation of cryopreserved ovarian tissue to a woman cured of cancer - follicular growth, steroid production and oocyte retrieval. *Reprod Biomed Online* 2004;**8**:448–453.
- Yding Andersen C, Ernst E, Bærentzen S, Birkebæk NH, Clausen N. No malignancy detected in surplus ovarian tissue from a former Ewing sarcoma patient who experienced relapse four years after being grafted with frozen/thawed ovarian tissue. *J Assist Reprod Genet* 2014;**31**:1567–1568.