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

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Submitted on May 13, 2015; resubmitted on August 5, 2015; accepted on August 24, 2015

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...
up period is now extended by 3 years.

Tissue excised at the hospital where the processing laboratory is located is of the tissue. The programme for 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; Corney 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 I. 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 Aarhus University Hospital (Aarhus) \((n = 37)\). At Aarhus 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 I). The mean age at freezing was 29.8 years whereas the first grafting was performed at the mean age of 32.9 years. The
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; 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.

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

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. of women</th>
<th>Age (years) (mean; range)</th>
<th>Calculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast cancer</td>
<td>12/3</td>
<td>33.9 (26.0–38.7)</td>
<td>36.5 (28.7–43.2)</td>
</tr>
<tr>
<td>Mb. Hodgkin</td>
<td>5/4</td>
<td>29.4 (25.6–34.1)</td>
<td>32.0 (28.0–37.3)</td>
</tr>
<tr>
<td>Non-Hodgkin</td>
<td>5/3/1</td>
<td>31.1 (25.9–35.1)</td>
<td>33.8 (29.6–37.3)</td>
</tr>
<tr>
<td>Cervical cancer</td>
<td>3/1</td>
<td>25.8 (21.2–30.7)</td>
<td>29.1 (24.3–32.2)</td>
</tr>
<tr>
<td>Aplastic anaemia</td>
<td>2</td>
<td>29.3 (26.2–32.3)</td>
<td>33.1 (31.3–35.0)</td>
</tr>
<tr>
<td>Ewing sarcoma</td>
<td>2</td>
<td>18.3 (9.5–27.1)</td>
<td>21.3 (13.8–28.8)</td>
</tr>
<tr>
<td>Paroxysmal nocturnal</td>
<td></td>
<td>22.2 (19.0–25.4)</td>
<td>25.1 (21.7–28.5)</td>
</tr>
<tr>
<td>haemoglobinuria</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sarcoma</td>
<td>2</td>
<td>35.7 (33.5–37.8)</td>
<td>37.8 (35.9–39.6)</td>
</tr>
<tr>
<td>Haemolytic uraemic</td>
<td>1</td>
<td>33.3</td>
<td>38.5</td>
</tr>
<tr>
<td>syndrome</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ovarian cancer</td>
<td>1</td>
<td>23.5</td>
<td>31.9</td>
</tr>
<tr>
<td>Colon cancer</td>
<td>1</td>
<td>26.1</td>
<td>28.8</td>
</tr>
<tr>
<td>Anal cancer</td>
<td>1</td>
<td>37.0</td>
<td>38.1</td>
</tr>
<tr>
<td>Various others*</td>
<td>4</td>
<td>26.6 (23.1–30.0)</td>
<td>31.0 (27.9–33.0)</td>
</tr>
<tr>
<td>Total</td>
<td>53 (41/11/1)</td>
<td>29.8 (9.3–38.7)</td>
<td>32.9 (13.8–43.2)</td>
</tr>
</tbody>
</table>

*Autoimmune Small-Vessel Vasculitis; Morbus Behcet; Choriocarcinoma; Wegener’s Granulomatosis.

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...
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 PPROM 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 ± 16 follicles/mm² (mean ± SEM), n = 14) or did not conceive (22 ± 9 follicles/mm², 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

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**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).

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Number of Women</th>
<th>Number of Transplantations</th>
<th>Number of pregnancies</th>
<th>Pos. hCG</th>
<th>Clinical</th>
<th>Children</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast cancer</td>
<td>11</td>
<td>13</td>
<td>6</td>
<td>3</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>Mb. Hodgkin</td>
<td>4</td>
<td>8</td>
<td>−</td>
<td>4</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Non-Hodgkin</td>
<td>5</td>
<td>9</td>
<td>1</td>
<td>4</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Aplastic anaemia</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>−</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Ewing sarcoma</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Paroxysmal nocturnal haemoglobinuria</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>−</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Ovarian cancer</td>
<td>1</td>
<td>1</td>
<td>−</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Colon cancer</td>
<td>1</td>
<td>1</td>
<td>−</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Anal cancer</td>
<td>1</td>
<td>1</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Various othersc</td>
<td>4</td>
<td>4</td>
<td>2</td>
<td>−</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>32</td>
<td>42</td>
<td>13</td>
<td>15</td>
<td>28</td>
<td>24</td>
</tr>
</tbody>
</table>

NC, natural conceived; IVF, in vitro fertilization.
aOngoing third-trimester pregnancy.
bSecond-trimester miscarriage caused by PPROM.
cAutoimmune Small-Vessel Vasculitis; Morbus Behcet; Choriocarcinoma; Wegener’s Granulomatosis.
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 amenorrhoeic at the time of transplantation but experienced low ovarian reserve, with a low AFC of ≏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.

### Table III Parameters with a possible effect on pregnancy outcome (Mean ± SEM).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pregnant</th>
<th>Non-pregnant</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amount of transplanted tissue from one ovary (percent)</td>
<td>55 ± 4</td>
<td>53 ± 6</td>
<td>NS</td>
</tr>
<tr>
<td>Age of the patient at cryopreservation (years)</td>
<td>29.7 ± 1.1</td>
<td>30.8 ± 1.5</td>
<td>NS</td>
</tr>
<tr>
<td>Age of the patient at first transplantation (years)</td>
<td>32.5 ± 1</td>
<td>34.4 ± 1.4</td>
<td>NS</td>
</tr>
<tr>
<td>Follicular density (follicles/mm³ cortical tissue)</td>
<td>36 ± 16</td>
<td>22 ± 9</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS, non-significant.

### 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 ≏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
improved data but many of the women live far from the hospital where
the transplantation(s) were performed and are reluctant to spend the
time being followed up.

The frequency of failed clinical pregnancies (8 of 24) is higher than
expected and is likely to reflect that pregnancy in cancer survivors is
more difficult than that in healthy women and emphasizes that these
pregnancies should be considered obstetrical high risk pregnancies and
thus require appropriate monitoring.

Neither age at cryopreservation, age at first transplantation, the
amount of tissue grafted or the follicular density of the grafted tissue
proved to predict successful conception. This may reflect the fact that
the numbers are still relatively small but may also indicate that cancer sur-
vivors are a heterogeneous group of patients in whom more factors than
normal influence the outcome.

It is reassuring that the three relapses observed are most likely unre-
related to the transplantation of ovarian tissue. Two breast cancer patients
had relapses locally to the breast, whereas 80% of the tissue from the
Ewing's sarcoma patient revealed no sign of contamination (Rosendahl
et al., 2011a; Ernst et al., 2013b; Yding Andersen et al., 2014). There is
currently no method available to detect malignant cell contamination
to the ovarian tissue with certainty. A number of methods have been
used (grafting to immunodeficient mice (Dolmans et al., 2010; Greve et al.,
2012a, 2013), molecular biological methods and immuno-
histochemistry (Rosendahl et al., 2010, 2011b, 2013; Bastings et al.,
2013; Donnez and Dolmans, 2013)) to assure safety, but none of
these methods are completely effective, making the present empirical
data more important. There does not appear to be malignant cells present
in numbers that can cause relapse. The collective experience
worldwide supports our results on safety in most types of cancer, but
follow-up on transplanted patients is of upmost importance. We have
not yet performed transplants in patients who have suffered from leukae-
mia since the ovarian tissue may harbour malignant cells in this group of
patients (Rosendahl et al., 2011b). However, if the tissue has been col-
lected after the first series of chemotherapy when the patient is in full
remission, and if there is an available molecular marker that proves to
be negative in a tissue sample, transplantation may be considered
(Dolmans et al., 2010; Greve et al., 2012a).

It is also reassuring that the relapse rate with or without transplanted
tissue in our cohort is similar, although the frequency in the non-
transplanted cohort is only an estimate. It appears that the transplant-
ing ovarian tissue on patients following our selection criteria is safe.
Obviously, we have not yet transplanted tissue to former patients
who have recovered from leukaemia and this group of patients still is
a challenge.

The functional duration of the transplanted tissue is variable. On
one hand, two women have now experienced ovarian activity for > 10
years with the tissue still being functional and several women
have had active tissue for > 5 years. On the other hand, a few women
experienced activity of less than a year. We have been unable to pinpoint
specific parameters of importance for the functional duration of
the tissue. One reason for the poor prediction is that the number of
follicles transplanted is unknown. The follicular density in individual
pieces of cortex may vary with more than three orders of magnitude
(Schmidt et al., 2003a), making it impossible to know how many
follicles are actually present in the grafted pieces. However, the tissue
remains active in the majority of women and it is necessary to wait for
menopause to appear in order to provide more accurate estimates on
longevity.

It is noticeable that two women became pregnant > 5 years after
transplantation, showing that the tissue actually maintains fertility even
after prolonged periods of time and justifies our policy of retrieving
one entire ovary instead of only part of one ovary.

In conclusion, freezing ovarian tissue is now gaining ground as a valid
method for fertility restoration and for providing ovarian activity with
cycling levels of sex hormones for several years. Out of the 32 women
with a pregnancy-wish, 10 have so far managed to have their own chil-
dren. The level of safety appears to be high, with no relapse due to trans-
plantation of ovarian tissue recorded to date.
Supplementary data

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

Acknowledgements

Bettina Abildgaard Skjellerup, secretary at the Fertility Clinic at the University Hospital Aarhus, is thanked for helping in collecting data.

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 the revision of the report. C.Y.A. contributed to the study design, figures, data analysis, and the writing and revision of the report.

Funding

The Child Cancer Foundation in Denmark (2012–26) and the EU inter-regional project ReproHigh are thanked for having funded this study. They had no role in the study design, collection and analysis of data, data interpretation or writing of the report.

Conflict of interest

None declared.

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