Ovarian function after removal of an entire ovary for cryopreservation of pieces of cortex prior to gonadotoxic treatment: a follow-up study

Mikkel Rosendahl1,2,5, Claus Yding Andersen1, Erik Ernst3, Lars G. Westergaard4, Per Emil Rasmussen4, Anne Loft2 and Anders Nyboe Andersen2

1Laboratory of Reproductive Biology, Copenhagen University Hospital, Rigshospitalet, Denmark; 2The Fertility Clinic, Copenhagen University Hospital, Rigshospitalet, Denmark; 3Reproductive Laboratory, Skejby University Hospital, Aarhus, Denmark; 4The Fertility Clinic, Odense University Hospital, Denmark
5Correspondence address: E-mail: mikkel.rosendahl@rh.regionh.dk

BACKGROUND: Ovarian function was studied in Danish patients who had ovarian tissue cryopreserved, and the patients’ experiences with the procedure were investigated. METHODS: There were 92 women who had an entire ovary removed for cryopreservation 18–75 months earlier. Reasons included: breast cancer (n = 31; 34%), Hodgkin’s lymphoma (n = 23; 25%), bone marrow transplantation (BMT) (n = 19; 21%) and others (n = 19; 21%). Patients completed a questionnaire, and transvaginal ultrasonic antral follicle count and serum analysis for follicle stimulating hormone (FSH) and anti-Müllerian hormone (AMH) were performed in 73 women. RESULTS: In total, 11% of the BMT patients had normal ovarian function. Hodgkin’s patients who only received ABVD (doxorubicin, bleomycin, vinblastine and dacarbazine) (n = 12) and 60% of the breast cancer patients showed little evidence of ovarian damage. Regular menstruation was shown to be a good indicator of ovarian function. The cryopreservation procedure rarely complicated cancer treatment (5%) and 84% felt comforted because they had potentially secured their fertility. CONCLUSIONS: Cryopreservation of ovarian tissue should be considered in young female patients with Hodgkin’s lymphoma receiving more aggressive treatment than ABVD and in patients scheduled for BMT. The recommendation for breast cancer patient should be individualized. The cryopreservation process did not delay cancer treatment.

Keywords: fertility preservation; cryopreservation; ovarian reserve; cancer; AMH

Introduction
Survival rates for many types of cancer have improved over the past decades (McVie, 1999). Consequently, in future, more women will face the risk of treatment-induced infertility and with the prospect of living beyond cancer, preservation of fertility becomes of priority for both patients and physicians (Lee et al., 2006).

Cryopreservation of embryos and oocytes may preserve fertility in women with certain types of cancer. However, the process of obtaining embryos and oocytes is often time-consuming at a time when immediate start of therapy is vital. Further, for certain cancers, i.e. estrogen-sensitive breast cancer, the significance of ovarian stimulation with increased levels of serum estradiol remains unclear (Oktay et al., 2003).

Cryopreservation of ovarian cortex prior to gonadotoxic treatment is now evolving as a promising way to rescue ovarian function, and autotransplantation of the frozen/thawed ovarian tissue has now successfully re-established ovarian function in a number of women, resulting in regular menses and normalization of gonadotrophin levels. The success of the procedure has been further established by the birth of five children following autotransplantation, including two children from our programme (Donnez et al., 2004; Meirow et al., 2005; Demeesteere et al., 2007; Yding Andersen et al., 2008) Globally, more than a thousand women are likely to have had ovarian tissue cryopreserved and an even higher number of premenopausal women have received cancer therapy. However, little is known about the dual impact of the combination of removal of ovarian tissue and the gonadotoxic treatment on the residual ovarian function.

The three aims of present study were: (i) to evaluate the frequency of impaired ovarian function in a group of women who had an ovary removed for cryopreservation of pieces of cortex prior to gonadotoxic therapy, (ii) to investigate the patients’ experiences and opinions towards ovarian tissue cryopreservation and the operation and (iii) to establish the frequency of
surgical complications and whether the programme had delayed or otherwise affected the patients’ treatments.

Materials and Methods

Participants
In this study, we included women over the age of 18, who had one intact ovary, as the other ovary had been removed for cryopreservation of pieces of ovarian cortex at our laboratory more than 18 months earlier, prior to administration of chemo- and/or radiation therapy. From 1999 to November 2007, 250 patients had ovarian tissue cryopreserved at our laboratory, and 119 of the patients fulfilled the above-mentioned criteria. Eighteen patients had died and one had immigrated. Of the remaining 100 patients, 92 returned a completed questionnaire and were invited to a physical examination. There were 14 patients excluded from physical examination due to ongoing pregnancy (7) or treatment for relapse of disease (7). Five patients failed to show up. In total, 73 of the 100 patients also completed the physical examination.

According to diagnosis and treatment, patients were grouped into four categories: (i) breast cancer ($n = 31$, 34%), (ii) Hodgkin’s lymphoma ($n = 23$, 25%), (iii) bone marrow transplantation (BMT) ($n = 19$, 21%), including patients who had BMT for leukaemia ($n = 14$), haemopoietic stem cell disorders ($n = 4$) and sarcoma ($n = 1$) and (iv) others ($n = 19$, 21%): sarcoma/dermoid tumour (5), non-Hodgkin’s lymphoma (3), gynaecological disease (mola, teratoma and cervical cancer) (5), autoimmune disease (2), pineoblastoma (1), rhino-pharynx- (1), colon- (1) and skin cancer (1).

Mean age (range) of the patients at time of diagnosis and follow-up was 25.4 years (9–37) and 28.7 years (18–41), respectively. There were 10 patients under the age of 18 at the time of ovarian tissue cryopreservation and a parent or legal guardian signed informed consent.

The mean time from the cryopreservation procedure to follow-up was 34 months (range 18–75 months). An age-matched group of 38 women with cancer served as reference. All these women had two ovaries and had not yet started treatment for the following diagnoses: breast cancer (15), Hodgkin’s lymphoma (10), non-Hodgkin’s lymphoma (5), sarcoma (3), autoimmune disease (2), leukaemia, mesenchymal liver cancer or cervical cancer (3). Detailed information about the patient groups, the reference group and the two largest reference subgroups (breast cancer and Hodgkin’s) are outlined in Table I.

Ethical approval
The study was undertaken with the informed consent of the patients. The Danish ovarian tissue cryopreservation programme was approved by the Danish Ministry of Health and by the Biomedical Research Ethical Committee System.

Questionnaire
The questionnaire consisted of 39 questions covering six topics: social and economic conditions (9), disease and treatment (7), menstrual pattern, menopausal symptoms and pregnancy (9), information about cryopreservation (3), opinions about, and complications from, the operation (6) and opinions about cryopreservation (5).

With the patients consent, the treating departments were contacted and all available data on chemo- and radiation therapy were obtained.

Physical examination
Transvaginal ultrasound examination and blood samples were performed on cycle Day 2–5 in patients with regular menstruation (21–35 days). Patients with irregular or absent bleeding were examined at a random cycle day. Patients using hormone replacement therapy (HRT) or oral contraceptives (OC) were examined after a period of more than four weeks without exogenous hormones. Three different observers performed the ultrasound examination.

Ovarian volume was calculated using the longest longitudinal ($d_1$), antero-posterior ($d_2$) and transversal diameters ($d_3$) in the prolate ellipsoid formula:

$$\text{Volume (cm}^3\text{)} = \frac{d_1 \times d_2 \times d_3 \times \pi}{6}.$$

Antral follicles were counted (AFC) and grouped according to diameter ($<5$; $5–8$; $8–10$ and $>10$ mm). Follicular structures larger than 20 mm were defined as ovarian cysts.

Power Doppler was used by one observer to evaluate ovarian stromal blood flow. A semi-quantitative score was allocated to the ovary according to the number and area of the power Doppler signals. Pulse repetition frequency was regulated individually to prevent noise. Score 1 (low flow): a few and scanty signals, suggesting a poor vascularization. Score 3 (good flow): several pronounced power Doppler signals. Score of 2 (moderate flow): intermediary signals (Popovic-Todorovic et al., 2003).

Hormone measurements
Serum levels of estrogen and follicle stimulating hormone (FSH) were analysed using fresh serum and done by the hospitals departments of clinical biochemistry.

Serum levels of anti-Müllerian hormone (AMH) and inhibin-A and -B were analysed with specific ELISA kits according to manufacturer’s instructions. AMH (DSL-10-14 400; Diagnostic System Laboratories Inc., Webster, TX, USA), detection limit: 0.05 ng/ml. Inhibin-A and -B: product codes MCA950ZZ and MCA1312KZZ, Oxford Bio-innovation Ltd, Oxfordshire, England, UK, detection limits: inhibin-A: $<12$ pg/ml, inhibin-B $<20$ pg/ml. All AMH analyses were performed on frozen serum in one laboratory. Inhibins were analysed on fresh serum in one laboratory. All inter- and intra-assay variations were $<10\%$.

Statistics
Statistical analysis was performed using SPSS 13.0 and 15.0 Chicago, IL, USA.

Statistical significance was $P < 0.05$. When fulfilling requirements for normal distribution and homogeneity of variances, data were analysed using parametric analyses (one-way ANOVA, independent samples t-test). Otherwise non-parametric analyses were used (Kruskal–Wallis, Mann–Whitney). In table analysis, chi-square test was used. When the expected count/cell was $<5$, Fisher’s exact test was used.

Binary logistic regression was performed with manual, forward elimination of covariates.

In calculations of mean values for estradiol, inhibin-A and -B, ‘undetectable’ was denoted as one point under the detection limits (0.03 nmol/l, 11, 19 pg/ml and 0.04 ng/ml, respectively) in order to avoid underestimation of the values.

For analysis of questionnaires, missing answers were not included in calculations.

Results

Chemotheraphy and radiation therapy

Breast cancer
The 31 breast cancer patients had received cyclophosphamide, epirubicin and fluorouracil (CEF) ($n = 31$), CEF and docetaxel ($n = 6$) or CEF, docetaxel and gemcitabine ($n = 1$).
### Table I. Patient characteristics and markers of ovarian function.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>All</th>
<th>Breast cancer</th>
<th>Hodgkin’s</th>
<th>BMT*</th>
<th>Other</th>
<th>Reference group*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients/diagnose, n and (%)</td>
<td>92</td>
<td>31 (34%)</td>
<td>23 (25%)</td>
<td>19 (21%)</td>
<td>19 (21%)</td>
<td>5 (5%)</td>
</tr>
<tr>
<td>Age at diagnosis, mean years ± 2SEM</td>
<td>25.4 ± 1.3</td>
<td>30.1 ± 1.5</td>
<td>24.6 ± 2.3</td>
<td>19.4 ± 2.6</td>
<td>24.6 ± 2.4</td>
<td>25.6 ± 2.4</td>
</tr>
<tr>
<td>Age at follow up, mean years ± 2SEM</td>
<td>28.7 ± 1.2</td>
<td>33.0 ± 1.5</td>
<td>27.6 ± 2.4</td>
<td>24.4 ± 2.1</td>
<td>27.2 ± 2.3</td>
<td>28.2 ± 2.8</td>
</tr>
</tbody>
</table>

**Follow-up time**

| All groups (mean months ± 2SEM) | 34.5 ± 3.4 | 33.5 ± 6.6 | 32.6 ± 4.8 | 40.8 ± 8.4 | 32 ± 7.2 | 29.4 ± 5.2 | 38 ± 17 | NS* |
| 18–35 months by diagnosis, % horizontally | | 100 (n = 62) | 36 | 27 | 13 | 24 | — | — |
| 36–59 months by diagnosis, % horizontally | | 100 (n = 26) | 26 | 32 | 37 | 5 | — | — |
| ≥60 months by diagnosis, % horizontally | | 100 (n = 11) | 11 | 36 | — | 27 | — | — |

**Menstrual pattern**

| Regular bleeding, % | 47 | 60 | 60 | 11 | 50 | 40 | 60 | P = 0.004* |
| ≥1 menopausal symptoms/using HRT, % | 58 | 53 | 50 | 90 | 41 | 20 | 40 | P = 0.016* |

**Ovarian parameters (n = 73)**

| Antral follicle count (mean ± 2SEM) | 3.6 ± 1.1 | 4.0 ± 1.40 | 5.5 ± 3.2 | 0.5 ± 0.6 | 5.0 ± 2.3 | 4.33 ± 1.7 | 6 ± 6.9 | P < 0.001* |
| Ovarian volume, cm³ (mean ± 2SEM) | 7.8 ± 2.4 | 14.4 ± 6.0 | 6.2 ± 3.7 | 2.2 ± 1.1 | 4.8 ± 1.8 | 4.7 ± 1.7 | 4 ± 2.8 | P < 0.001* |
| Low stromal power Doppler, % of patients | 65 | 44 | 58 | 93 | 70 | 1/1 | 2/4 | — |
| Patients with lcyst>20 mm, N | 7 | 2 | 3 | 1 | 1 | — | — | — |
| Patients with ≥2 cyst>20 mm, N | 6 | 5 | 2 | — | — | — | — | — |

**Endocrinology**

| AMH ng/ml (mean ± 2SEM) (n = 70) | 0.29 ± 0.11 | 0.27 ± 0.12 | 0.54 ± 0.42 | 0.09 ± 0.10 | 0.32 ± 0.21 | 0.23 ± 0.25 | 0.54 ± 0.6 | P = 0.006* |
| Inhibin-A pg/ml (mean ± 2SEM) (n = 50) | 19.7 ± 6.6 | 28.4 ± 15.2 | 14.9 ± 6.6 | 11.8 ± 1.6 | 25.5 ± 22.7 | All <12 | All <12 | NS (P = 0.07)* |
| Inhibin-B pg/ml (mean ± 2SEM) (n = 50) | 31.7 ± 6.6 | 45.2 ± 19.0 | 28.6 ± 15.2 | 22.3 ± 5.6 | 32.7 ± 20.2 | All <20 | All <20 | NS (P = 0.07)* |
| Estradiol nmol/l (mean ± 2SEM) (n = 73) | 0.51 ± 0.20 | 0.49 ± 0.46 | 0.21 ± 0.08 | 0.21 ± 0.07 | 0.63 ± 0.70 | 4.86 ± 9.3 | 0.20 ± 0.21 | P = 0.025* |
| FSH IU/l (mean ± 2SEM) (n = 73) | 26.8 ± 6.6 | 14.7 ± 5.1 | 21.0 ± 11.0 | 46.2 ± 16.2 | 27.2 ± 15.2 | 28.7 ± 30.4 | 27.9 ± 36.6 | P = 0.04* |
| FSH>15 IU/l, %/group | 47 | 33 | 38 | 83 | 40 | 20 | 25 | P = 0.008* |

*Leukaemia, aplastic anaemia, paroxysmal nocturnal haemoglobinuria, and others who received bone marrow transplantation; **premenopausal women with cancer and two ovaries, prior to chemotherapy (n = 38), ³Chi-square, ⁴Kruskal–Wallis test.
Radiation therapy was administered to 23 patients, none of them below the diaphragm.

**Hodgkin’s lymphoma**

Of the 23 patients, 11 had received doxorubicin, bleomycin, vinblastine, dacarbazine only (ABVD) and one had received only ABV. The remaining 11 patients received one or more of the following regimens, either alone or after treatment with ABVD: bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, prednisolone (BEACOPP), cyclophosphamide, vincristine, procarbazine, prednisolone (COPP), carmustine, etoposide, cytosine and melphalan (BEAM) or methyl-GAG, ifosfamide, methotrexate, etoposide (MIME). Four patients had autologous BMT. There were 18 patients (83%) who had received radiation above the diaphragm and one had received total body irradiation (TBI).

**Bone marrow transplantation**

Of the 19 cases of BMT, 13 included both TBI and cyclophosphamide. Three patients had received cyclophosphamide but not TBI. One patient received TBI but no cyclophosphamide and two patients had BMT performed with neither cyclophosphamide nor TBI.

**Others**

The last group had received 14 different chemotherapeutic agents; two patients had received cyclophosphamide. Ten patients had received radiation therapy and in three cases, to areas potentially involving the ovary.

Of all 92 patients, 57 (62%) had received cyclophosphamide.

**Pregnancies**

After termination of chemotherapy, 25 of the women desired pregnancy. Four women received autotransplantation of ovarian tissue. One had a biochemical pregnancy (Rosendahl et al., 2006); one had a blighted ovum that was terminated in week 7 of gestation. Two women conceived and gave birth to two healthy children (Yding Andersen et al. 2008). One of the women subsequently conceived spontaneously and is currently pregnant.

Another 15 women also conceived, and one woman conceived twice. Four healthy children had been born and seven women had ongoing pregnancies at the time of inclusion. One patient, who experienced severe hot flushes, mood changes and vaginal dryness and who had been amenorrhoeic for several months got pregnant unexpectedly and due to personal reasons had to terminate the pregnancy. The results and bleeding regularity of the individual patients who experienced pregnancy are shown in Table II.

**Ovarian parameters**

Mean ovarian volume was $7.8 \pm 2.4 \text{ cm}^3$ (mean $\pm$ 2SEM), but varied between the groups. Breast cancer patients had the largest volume at $14.4 \pm 6.0 \text{ cm}^3$ and BMT patients had the smallest volume ($2.2 \pm 1.1 \text{ cm}^3$) ($P < 0.001$).

Mean AFC ($<20 \text{ mm}$) was $3.6 \pm 1.1$ (mean $\pm$ 2SEM). BMT patients had the lowest count at $0.5 \pm 0.6$ and Hodgkin’s patients the highest at $5.5 \pm 3.2$ ($P < 0.001$).

Ovarian stromal Doppler flow was available in 52 (71%) patients and was graded as low in two-thirds of the cases. Again the BMT group was noticeable as 93% of the patients had low flow, whereas only 44% of breast cancer patients had low flow ($P < 0.001$) (Table I).

**Endocrinology**

Hormone measurements for each of the patient groups are presented in Table I. Estradiol was highest in breast cancer patients (0.94 nmol/l) (1.14 nmol/l in patients without anti-estrogens) and lowest in the BMT and Hodgkin’s groups (0.21 nmol/l).

AMH was measured in 70 (96%) patients and was undetectable ($<0.05 \text{ ng/ml}$) in 36 cases (51%); breast cancer: 7/23 (30%), Hodgkins: 6/14 (43%), BMT: 16/17 (94%) and others: 7/15 (47%).

Inhibin-B was measured in 52 patients and was undetectable in 37 (71%). In the BMT group, 1/15 (7%) patients had detectable inhibin-B levels in contrast to 8/15 (53%) patients of breast cancer patients.

Mean FSH level for all patients was 27 IU/l and 47% of the patients had FSH levels $\geqslant 15$ IU/l. BMT patients had the highest mean FSH levels at 46.2 IU/l, whereas the mean for breast cancer patients was only slightly elevated at 14.7 IU/l (12.6 IU/l without anti-estrogens).

There was no obvious relationship between FSH and age between or within the groups.

Binary logistic regression showed that treatment with cyclophosphamide was predictive of an FSH value $>15$ IU/l with an odds ratio (OR) of 3.0 (95% CI 1.1: 8.1) Non-significant covariates were smoking, age, age at diagnosis, BMT and radiation to a field involving the ovary.

Patients with Hodgkin’s lymphoma, treated with ABVD alone, had significantly lower serum FSH levels (7.4 $\pm$ 4.9 versus 36.4 $\pm$ 17.9 IU/l; mean $\pm$ 2SEM) and higher AFC (8.8 $\pm$ 4.4 versus 0.7 $\pm$ 0.9) and AMH levels (1.02 $\pm$ 0.7 versus 0.05 $\pm$ 0.02 ng/ml) than patients treated with COPP, BEACOPP, MIME or BEAM either alone or following ABVD ($P < 0.04$). Compared with patients treated with ABVD alone, patients treated with COPP, BEACOPP, MIME or BEAM further had significantly increased risk of FSH levels $>15$ [OR 13; 1.07;166; (95% CI)].

Of the 31 breast cancer patients, 19 (61%) used anti-estrogenic treatment (i.e. tamoxifen). There was no significant difference between the two groups in levels of estradiol, AMH, inhibin-B or FSH, nor in AFC, ovarian volume or bleeding regularity.

In the Others group, two subgroups (gynaecological cancer and sarcoma/dermoid tumour) represented half the patients. Their individual values are shown in Table I. Three patients had radiation to a field potentially involving the ovaries. Only two of them (skin cancer/cervical cancer) had FSH levels $>12$ IU/l (mean 63 IU/l) and undetectable serum AMH levels.
**Menstrual pattern**

Of all the patients, 47% reported to have regular menstrual cycles at the time of follow-up (Table III). Absence of bleeding or the use of HRT was reported by 33% of the patients and the remaining 20% of the patients had irregular bleeding. However, there were great variations between the four groups, as 60% of Hodgkin's and breast cancer patients, 50% of other patients and only 11% of patients receiving BMT patients reported regular monthly bleeding.

Accordingly, 58% of the patients reported one or more menopausal symptoms (hot flushes, vaginal dryness/irritation and mood changes) or used HRT to prevent menopausal symptoms.

Serum levels of estradiol, AMH, inhibin-B and AFC were significantly higher and FSH was significantly lower in patients with regular bleeding, whereas low stromal Doppler flow was predictive of the absence of regular bleeding, OR 6.3 (95% CI: 1.6; 24.4).

Variations of the individual parameters within the groups in relation to menstrual regularity are shown in Fig. 1.

**Questionnaire**

Data from the questionnaires are summarized in Table IV.

In one case, the laparoscopy was converted to laparotomy. Of the patients, 78% were discharged either on the day of ovarian tissue cryopreservation or the day after. Two of the eight patients, who were discharged more than 2 days after the operation, had other surgery performed at the same time. Three patients were re-operated: two for cutaneous infections that needed shifting and one for a bladder lesion that occurred during laparoscopy. In two of these cases, patients reported that onset of chemotherapy was delayed due to the cryopreservation procedure; however, all three patients who were re-operated on stated that they were glad they had ovarian tissue removed for cryopreservation.

**Table II.** Pregnanacies after cryopreservation of ovarian cortex.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Bleeding</th>
<th>Menopausal symptoms</th>
<th>Pregnancies</th>
<th>Abortions spontaneous/provoked</th>
<th>Birth/ongoing pregnancy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast cancer</td>
<td>Reg</td>
<td>—</td>
<td>2</td>
<td>—</td>
<td>1/1</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>Irr</td>
<td>—</td>
<td>1</td>
<td>1/1</td>
<td>—</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>Reg</td>
<td>1</td>
<td>1</td>
<td>—</td>
<td>—/1</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>Reg</td>
<td>1</td>
<td>1</td>
<td>—</td>
<td>—/1</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>Reg</td>
<td>1</td>
<td>1</td>
<td>—</td>
<td>—/1</td>
</tr>
<tr>
<td>Hodgkin’s lymphoma*</td>
<td>Reg</td>
<td>1</td>
<td>1</td>
<td>1**/1</td>
<td>—</td>
</tr>
<tr>
<td>Hodgkin’s lymphoma*</td>
<td>Reg</td>
<td>1</td>
<td>1</td>
<td>1**/1</td>
<td>—</td>
</tr>
<tr>
<td>Hodgkin’s lymphoma*</td>
<td>Reg</td>
<td>1</td>
<td>1</td>
<td>—</td>
<td>—/1</td>
</tr>
<tr>
<td>Hodgkin’s lymphoma*</td>
<td>Reg</td>
<td>1</td>
<td>1</td>
<td>—</td>
<td>—/1</td>
</tr>
<tr>
<td>Hodgkin’s lymphoma*</td>
<td>Reg</td>
<td>1</td>
<td>1</td>
<td>—</td>
<td>—/1</td>
</tr>
<tr>
<td>Non-Hodgkin Lymphoma</td>
<td>Reg</td>
<td>1</td>
<td>1</td>
<td>—</td>
<td>—/1</td>
</tr>
<tr>
<td>Pineoblastoma</td>
<td>F, M</td>
<td>1</td>
<td>1</td>
<td>1/1</td>
<td>—</td>
</tr>
<tr>
<td>Sarcoma*</td>
<td>Reg</td>
<td>2</td>
<td>1</td>
<td>1/1</td>
<td>—</td>
</tr>
<tr>
<td>Rhinopharynx cancer</td>
<td>Reg</td>
<td>1</td>
<td>1</td>
<td>1/1</td>
<td>—</td>
</tr>
<tr>
<td>Aplastic anaemia</td>
<td>None</td>
<td>F, M, D</td>
<td>1</td>
<td>—/1</td>
<td>—</td>
</tr>
<tr>
<td>Total</td>
<td>19</td>
<td>4/1</td>
<td>6/8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Pregnancies after autotransplantation of ovarian tissue. Reg, regular bleeding 21< days < 35. Irr, irregular bleeding; F, hot flushes; M, mood changes; D, vaginal dryness. **Blighted ovum, ***biochemical pregnancy.

**Table III.** Ovarian markers in relation to bleeding regularity.

<table>
<thead>
<tr>
<th>Distribution according to menstruation</th>
<th>Regular bleeding 47%</th>
<th>Irregular or no bleeding 53%</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Follow-up group</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18–35 months, % (n = 62)</td>
<td>46</td>
<td>54</td>
<td>NS</td>
</tr>
<tr>
<td>36–59 months, % (n = 19)</td>
<td>39</td>
<td>61</td>
<td></td>
</tr>
<tr>
<td>≥ 60 months, % (n = 11)</td>
<td>55</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td><strong>Endocrinology (mean ± 2SEM)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AMH ng/ml (n = 70)</td>
<td>0.54 ± 0.22</td>
<td>0.15 ± 0.11</td>
<td>P &lt; 0.001 *</td>
</tr>
<tr>
<td>Inhibin-α pg/ml (n = 50)</td>
<td>32.8 ± 21.0</td>
<td>15.1 ± 4.3</td>
<td>NS (P = 0.08)*</td>
</tr>
<tr>
<td>Inhibin-B pg/ml (n = 50)</td>
<td>54.5 ± 21.3</td>
<td>23.7 ± 5.0</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Estradiol nmol/l (n = 73)</td>
<td>0.52 ± 0.23</td>
<td>0.51 ± 0.32</td>
<td>P = 0.03*</td>
</tr>
<tr>
<td>FSH IU/L (n = 73)</td>
<td>7.86 ± 1.9</td>
<td>39.2 ± 9.0</td>
<td>P &lt; 0.001*</td>
</tr>
<tr>
<td><strong>Ovarian parameters (n = 73)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antral follicle count (mean ± 2SEM)</td>
<td>6.25 ± 2.0</td>
<td>2.18 ± 1.00</td>
<td>P &lt; 0.001*</td>
</tr>
<tr>
<td>Ovarian volume cm³ (mean ± 2SEM)</td>
<td>8.54 ± 3.4</td>
<td>7.34 ± 3.5</td>
<td>NS (P = 0.054)*</td>
</tr>
<tr>
<td>Ovarian cysts 2 cysts &gt; 20 mm, N</td>
<td>4</td>
<td>9</td>
<td>NS*</td>
</tr>
<tr>
<td>Low stromal power Doppler</td>
<td>15%</td>
<td>85%</td>
<td>OR 6.3 (1.6; 24.4 95% CI)</td>
</tr>
</tbody>
</table>

Overall, 91% stated that they were pleased that they had the procedure performed.

**Age groups**
In this study, 10 of the patients were under the age of 18 at the time of diagnosis. Individual values of these patients are found in Table V.

In the questionnaire, all 10 patients replied that they were glad that the procedure had been done.

**Discussion**
In this study, we obtained three central results.

1. Overall, approximately half of the women showed evidence of a treatment-induced diminished ovarian reserve. As expected, patients in the BMT group were most severely affected, whereas breast cancer patients less frequently experienced ovarian failure. For Hodgkin’s patients, the degree of ovarian damage was determined by the treatment.

2. Ovarian reserve markers such as AMH, FSH, inhibin-B and AFC correlated well with the reported menstrual patterns.

3. The vast majority of the patients were satisfied with the given information and the procedure itself. They had few complications and the cryopreservation procedure did not affect their cancer treatment.
would had regular bleeding after therapy, making individual counselling difficult in future patients. In a recent study of premenopausal women with breast cancer, low levels of AMH and inhibin-B prior to chemotherapy were predictive of chemotherapy-related amenorrhoea (Anders et al., 2008) and in future, such markers may well play an important role in the individual counselling of breast cancer patients considering fertility preservation.

Breast cancer patients had the lowest mean serum level of FSH, the highest serum levels of estradiol and inhibin-B and the largest ovarian volume. However, the high levels of estradiol and the large ovarian volume did not correlate with the low level of AMH and the AFC in these patients. These inconsistencies may be explained by a higher number of hormone producing cysts present in the ovaries of these patients, resulting in a negative feedback on pituitary FSH release, which as a result may camouflage a less favourable ovarian function (Table I). In agreement with this, in a recent study, where 595 premenopausal women received chemotherapy for breast cancer, the proportion of women with regular bleeding 15 months after diagnosis was 55%, but gradually decreased to 35% 5 years after diagnosis (Petrek et al., 2006). This effect was particularly pronounced in the group receiving a cyclophosphamide and fluorouracil containing regimen (CMF), which is very similar to the CEF treatment, received by all patients in our study. In the present study, only four breast cancer patients were seen after 5 years, which could explain why apparently intact ovarian function was seen in half the patients.

In Hodgkin’s patients, ovarian function was related to the chemotherapy regimen. Patients receiving other regimens than ABVD, either alone or supplementary to ABVD, had strongly reduced ovarian reserve in contrast to the group that had received ABVD alone in which most patients had almost unaffected ovarian reserve. The lesser gonadotoxic effect of ABVD compared with regimens containing alkylating drugs confirms findings from other studies (Franchi-Rezgui et al., 2003), and is further supported by an only marginally reduced fertility rate after two to four series of ABVD.

As expected, the ovarian function of patients receiving BMT was most severely affected and only 11% of the patients subsequently showed sign of ovarian activity. In our study, all but two BMT patients had received TBI and/or cyclophosphamide, and our results confirm the known severely gonadotoxic effect of cyclophosphamide (Meirow and Nugent, 2001). This group had the longest mean follow-up time, which could potentially affect ovarian function; however, they were also the youngest group, indicating that the true reason for the ovarian impairment was indeed the gonadotoxic treatment.

Thus, patients receiving BMT with expected long-term survival should be considered for ovarian cryopreservation or other fertility preservation measures.

Of the four groups, breast cancer patients had the highest chance of maintaining regular menstruation after cancer treatment; however, based on age, age at diagnosis, smoking or treatment, we were unable to identify which of the patients...
(Aisner et al., 1993; Hodgson et al., 2006). Moreover, two of the presented patients who had ovarian tissue cryopreserved prior to BMT had previously received four and six series of ABVD, respectively. Both patients received autotransplantation of ovarian tissue in our programme and both patients conceived: one had a clinical pregnancy and the other gave birth to a healthy boy (Yding Andersen et al., 2008).

Nevertheless, ABVD is not entirely without gonadotoxic effect as demonstrated by the AFC, AMH and FSH values in the ABVD-only group. Even though these markers were less affected than in the other treatment group, they are clearly below the reference group (Table I). Even so, acknowledging the milder gonadotoxic effect of ABVD, the indications for cryopreservation of ovarian tissue should carefully be considered in patients receiving no more than two to four series of ABVD.

AFC, AMH and FSH are often used to assess ovarian reserve and to predict outcome of assisted reproductive techniques in women with two ovaries (Popovic-Todorovic et al., 2003; Macklon and Fauser, 2005; Overlie et al., 2005; Broekmans et al., 2006), but little is known about the normal levels of these markers in women with only one ovary. Women with a single ovary may have slightly, elevated serum FSH levels (Cooper and Thorp, 1999), and animal studies have shown increased serum FSH levels, an increased number of antral follicles in the remaining ovary but a decrease in the total number of antral follicles per animal, compensatory hypertrophy in the remaining ovary and menstrual irregularity after unilateral oophorectomy (Butcher, 1985; Martin et al., 1986; Meredith et al., 1992). However, women with one ovary appear to have an unreduced fertility potential, be it naturally or via IVF (Lass, 1999). In the present study, we observed that the ovarian reserve markers correlated with each other as would be expected in women with two ovaries and we conclude that in women with a single ovary, these markers reflect the ovarian reserve equally well.

The patients’ information on bleeding regularity correlated very well with the serum levels of AMH, FSH, with AFC in a single ovary and power Doppler flow and hence, simply asking the patient whether she has regular bleeding, without the use of HRT, is a rough but rapid, inexpensive and non-invasive tool with high sensitivity to evaluate ovarian function after therapy.

The patients in this study were overall satisfied with the information and the course of the procedure. Even the 10 girls who were under the age of 18 at the time of ovarian tissue cryopreservation replied that they were glad the procedure had been done, despite their young age at the time of the procedure and that the decision had been made by a parent or legal guardian. Considering the patient material and the experimental nature of the treatment, the surgical complication rate was low and only 3% of the patients stated that the procedure had complicated the course of their treatment. These are all important points, as the cryopreservation procedure should be as swift and gentle as possible and not delay or otherwise disturb cancer treatment. Further, these findings may serve as advice for patients, or parents to young patients, for whom ovarian cryopreservation is indicated from a medical perspective, but where the patients are concerned about a long and painful hospitalization.

In conclusion, this study has confirmed that the risk of ovarian damage depends on the treatment regimen, but is also influenced by individual factors. Further, regular menstruation more than 18 months after cryopreservation of ovarian tissue indicates a good residual ovarian function.

Finally, the entire process of cryopreservation is well tolerated by the patients, both physically and emotionally, and does not interfere considerably with the treatment plan.

Funding

Financial support from the Danish Cancer Foundation is gratefully acknowledged.

References


Cooper GS, Thorp JM, Jr. FSH levels in relation to hysterectomy and to unilateral oophorectomy. Obstet Gynecol 1999;94:969–972.


Meredith S, Dudenhoefler G, Butter RL, Lerner SP, Walls T. Unilateral ovariectomy increases loss of primordial follicles and is associated with


Submitted on April 10, 2008; resubmitted on May 26, 2008; accepted on June 3, 2008