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ARTICLE

A 10-year follow up of reproductive function in women treated for childhood cancer

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Abstract Previously, this study group found that female childhood cancer survivors could be at risk of early cessation of fertility. The aim of the present study was to evaluate reproductive function in the same group of survivors 10 years after the initial study. Of the original cohort of 100, 71 were re-examined. Thirty-six survivors reported regular menstrual cycles. When they were compared with 210 controls, they differed significantly in antral follicle count (AFC) (median 15 versus 18, $P = 0.047$) but not in anti-Müllerian hormone (AMH) (median 13.0 versus 17.8 pmol/l). Survivors cured with minimal gonadotoxic treatment had significantly higher AMH and AFC compared with survivors cured with either potentially gonadotoxic treatment or treatment including alkylating chemotherapy and ovarian irradiation (20.0, 5.8 and <3 pmol/l, $P < 0.001$; and 15, 9 and 2, $P = 0.03$, respectively). Thirty-eight survivors had achieved at least one live birth. Complicated second-trimester abortions ($n = 4$) were observed primarily in survivors cured with radiotherapy affecting pelvic organs. In conclusion, childhood cancer survivors have signs of diminished ovarian reserve. However, if the ovarian function is preserved in the early to mid-twenties, it is likely to persist until the mid-thirties, giving a good chance of childbearing.

KEYWORDS: chemotherapy, childhood malignancy, radiotherapy, reproductive function

Introduction

Advances in antineoplastic treatment during recent decades have improved the prognosis after childhood cancer, and the long-term survival rate is now above 70% (Kaatsch, 2010). Accordingly, the majority of survivors will reach reproductive age (Curry et al., 2006; Gatta et al., 2005; Ries

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et al., 2006). Chemotherapy and radiotherapy is known to have an impact on reproductive function and some long-term survivors may develop ovarian failure, reduced fertility and premature menopause (Chemaitilly et al., 2006; Green et al., 2009b; Lantinga et al., 2006; Sklar et al., 2006).

Exposure to ovarian irradiation and alkylating agents has been identified as the most important independent risk factor of gonadal damage (Lie et al., 2009; van Beek et al., 2007). The pregnancy rate of survivors is low compared with healthy controls, but many survivors do conceive (Dama et al., 2009; Green et al., 2009a; Madanat et al., 2008; Reulen et al., 2009), and a large majority of pregnancies result in live births with no increased risk of congenital malformations or cancer in the offspring (Chiarelli et al., 2000; Green et al., 2002; Lie et al., 2010; Madanat-Harjuoja et al., 2010; Sankila et al., 1998; Winther et al., 2004, 2009).

In a population-based study, this study group previously indicated that among 100 childhood cancer survivors, the majority had both endocrine and sonographic signs of compromised ovarian function (Larsen et al., 2003). A total of 17% of the cohort developed ovarian failure, most likely because of their cancer treatment. The key finding, however, was that even though 70% of the survivors had regular menstrual cycles and serum FSH concentrations within the normal range, they showed signs of a reduced ovarian reserve with lower antral follicle counts, lower inhibin B concentrations and increased oestradiol concentrations in the early follicular phase compared with age-matched controls. Therefore, it was postulated that the presence of a spontaneous menstrual cycle after cancer treatment did not reflect a fully preserved ovarian function and that these women might have a shortened fertile lifespan and could enter menopause prematurely.

The aim of the present study was to re-examine the ovarian function in the cohort of adult childhood cancer survivors 10 years after the initial study using the same markers of ovarian reserve and, in addition, a relatively new marker, anti-Müllerian hormone (AMH). Further, this study also aimed to record fertility in terms of pregnancies and deliveries in the cohort of female childhood cancer survivors.

Materials and methods

Patients

In 2000, the original cohort of 100 female childhood cancer survivors was identified from the Danish Childhood Cancer Registry. These survivors met the following inclusion criteria: (i) diagnosed between January 1970 and December 1996; (ii) less than 15 years old at the time of diagnosis; (iii) treated with radiotherapy and/or chemotherapy; (iv) off treatment for at least 1 year; (v) in complete remission; and (vi) at least 18 years old at study inclusion (Larsen et al., 2003).

At the follow up in 2010, the cohort of 100 survivors was reduced to 93 eligible participants, as three survivors had emigrated and four had died. Of the 93, four declined participation and 17 did not respond. One participant from the original study was not contacted since she had expressed serious concerns at her earlier study participation. Thus, 71 (76%) participated in the present study.

In 2010, the childhood malignancies included acute lymphoblastic leukaemia ($n = 35$), acute myeloid leukaemia ($n = 2$), chronic myeloid leukaemia ($n = 1$), non-Hodgkin's lymphoma ($n = 3$), Hodgkin's lymphoma ($n = 6$), Wilm's tumour ($n = 13$), neuroblastoma ($n = 6$), Ewing's sarcoma ($n = 2$), soft-tissue sarcoma ($n = 1$) and malignant teratoma ($n = 2$). Female survivors with brain tumours were not included in the original study and are as such not represented in this follow-up study.

Interview, sonography and endocrinology

Survivors with regular menstrual cycles were examined in the early follicular phase of a menstrual cycle, i.e. cycle days 2–5. Survivors using oral contraceptives or oestrogen–progesterone hormonal replacement therapy (HRT) were examined during withdrawal bleeding on cycle days 2–5.

As part of the examination, height and weight was recorded and a full reproductive history was taken. Data on diagnosis and treatment were retrieved from the original study (Larsen et al., 2003). For each survivor participating in the present study, the total dose of any chemotherapeutic agent was obtained and, further, a potential irradiation field was noted from original files and X-rays and the total irradiation dose was recorded.

Ovaries were examined by transvaginal sonography with a 4–9 MHz probe (Endovaginal Endfire Transducer Type 8806) using a Pro Focus ultrasound scanner (Type 2202, Class 1 Type B) from B-K Medical, Herlev, Denmark. The length and height of the ovaries was measured in the sagittal plane and the width in the transverse plane after rotating the transducer 90°. Ovarian volume was calculated using the following formula $d1 \times d2 \times d3 \times \pi/6$, where d1, d2, and d3 represent the three maximal longitudinal, anteroposterior and transverse diameters. The ovarian volume was recorded as the mean volume of the two ovaries. The number of small antral follicles with a size of 2–10 mm was counted for each ovary and the antral follicle count (AFC) was recorded as the number of follicles in both ovaries. If only one ovary was identified, the volume and the AFC of that ovary was recorded. The same fertility specialist (ECL) performed all ultrasound scans both in 2000 and 2010.

Endocrine profiles were assessed through blood samples collected on the day of the sonography and analysed for basal concentrations of FSH, LH, oestradiol, inhibin B and AMH. FSH, LH and oestradiol concentrations were measured in the department of Clinical Biochemistry, Rigshospitalet. The FSH, LH and oestradiol analyses are based on electrochemiluminescence immunoassays using the Roche Elecsys kits (Roche Diagnostics Corp., Indianapolis, USA). The inhibin B gen II assay (DSL Beckmann) was used for the Inhibin B analysis. AMH serum samples were analysed by an ultrasensitive sandwich enzyme immunometric assay using the EIA AMH/MIS kit (Immunotech A16507; Beckman Coulter, Marseille, France). According to the manufacturer, the intra- and inter-assay coefficients of variation were $\leq$12.3% and $\leq$14.2%, respectively, and the analytical sensitivity, defined as the lowest AMH concentration from the

zero calibrator, was 0.7 pmol/l. The functional sensitivity, defined as the lowest concentration that gives a day-to-day coefficient of variation ≤25%, was estimated to be 3 pmol/l. As compared with the analytical sensitivity, the functional sensitivity is a better indicator and, therefore, the lower detection limit of AMH was set at 3 pmol/l. The AMH concentration was measured with a spectrometer (Power-Wave XS; Bio-Tek Instruments) at 450 nm by interpolation from a calibrator curve. For all samples, AMH concentration measurements were performed twice, and if the intra-sample coefficient of variance exceeded 15%, the samples were re-analysed. AMH concentrations of controls were all measured with the same kit and in the same laboratory and the lower detection limit was 0.7 pmol/l.

Controls

The control group consisted of healthy women aged 20–40 years participating in a PhD study regarding ovarian function in the background population. For each comparative analysis, the highest possible number of age-matched controls with two ovaries and regular menstrual cycles was randomly selected, resulting in at least six controls per case for each analysis.

Data analysis

Results are presented as medians and ranges. The chi-squared test for independence and Fisher's Exact test were used to explore the relationship between categorical variables when comparing participants with non-participants. The Mann–Whitney *U*-test was used to test for differences between cases and controls on continuous measures. For subgroup analysis on more than two groups, the Kruskal–Wallis test was used to test for differences on continuous measures. The Wilcoxon signed rank test was used to test for differences between repeated measures. Spearman's rank order correlation was used to test for a correlation between AMH concentrations and AFC.

The control group was created through a random selection of controls of the same age as cases. Cases were divided into age groups covering intervals of 1–2 years, and a computer was used to randomly match controls with cases of the same age group. The highest possible number of controls was always selected for each analysis and always the same ratio of controls per case. Three survivors (one with regular spontaneous menstrual cycles and two using oral contraceptives) were too old to be matched with controls and were therefore excluded from the comparative analyses. All controls matched cases on cycle characteristics. A regular cycle was defined as a cycle length equal to or less than 35 days.

A *P*-value <0.05 was considered statistically significant. Statistical analyses were performed with the Statistical Package for Social Sciences version 19.0 (SPSS, Chicago, IL, USA).

Ethics

All participants gave their written informed consent. The study was approved by the regional ethical committee of the Capital Region, Denmark (project ID: H-3-2010-029, approved 11 May 2010) and was conducted in accordance with the Helsinki II declaration.

Results

Figure 1 summarizes the cycle characteristics in the 71 childhood cancer survivors both at study inclusion in 2000 and in 2010. Ten years ago, 53 out of the participating 71 survivors had regular menstrual cycles, eight used oral contraceptives and 10 used HRT because of treatment-induced premature ovarian insufficiency. In the latter group at study inclusion, the ovarian capacity was unchanged and therefore nine women still used HRT in 2010, and one woman was pregnant after oocyte donation. Out of eight women using the contraceptive pill in year 2000, one had continued this treatment, one survivor had developed oligomenorrhoea whereas six had regained ovarian cyclicity after discontinuation of the contraceptive pill. Finally, out of 53 survivors with regular menstrual cycles in 2000, 10 now used oral contraceptives, five were pregnant, five had oligomenorrhoea (i.e. cycle length >35 days) and three had developed amenorrhoea due to premature ovarian insufficiency and were using HRT. A total of 30 survivors still reported regular menstrual cycles. When analysing the paired data of these 30 survivors who had regular cycles in both 2000 and 2010, the AFC and oestradiol and FSH concentrations were unchanged after the 10-year follow-up period (**Table 1**).

Table 2 shows clinical data and endocrine profiles of the 71 survivors examined in 2010. Survivors who had entered menopause during the follow-up period ($n = 3$) or were on HRT ($n = 9$) had elevated FSH concentrations (median 35.0 IU/l), an AMH concentration below 3 pmol/l and small and follicle-depleted ovaries, confirming premature ovarian insufficiency. Survivors who had developed oligomenorrhoea during

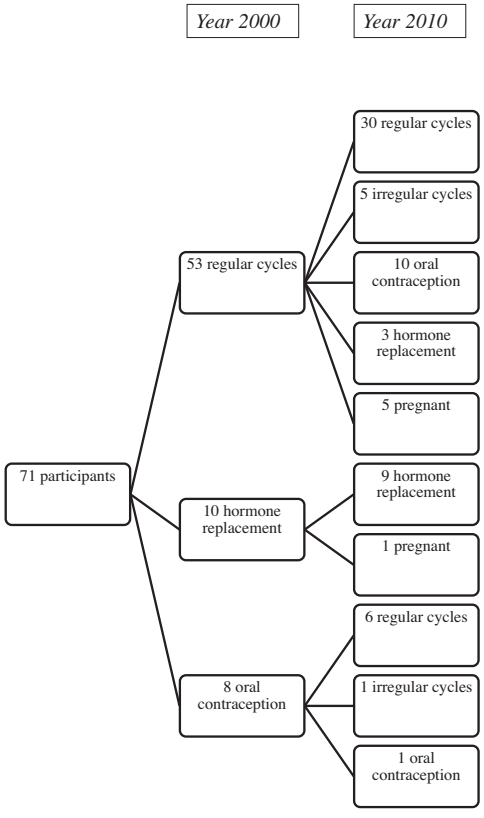

Figure 1 Flowchart of menstrual cycle characteristics in the 71 participating survivors in year 2000 and 2010.

Table 1 Ovarian reserve parameters in 30 survivors with regular menstrual cycles in 2000 and 2010.

	2000	*2010*
FSH (IU/l)	6.7 (4.1–15.1)	6.9 (2.2–21.6)
Oestradiol (nmol/l)	0.11 (0.05–0.41)	0.16 (0.05–0.43)
AFC (2–10 mm)	15 (5–27)	15 (3–34)

Values are median (range). Wilcoxon rank test. There were no statistically significant differences between the two groups.
AFC = antral follicle count (both ovaries).

the follow-up period ($n = 6$) had high AMH concentrations (median 38.0 pmol/l), and a high AFC, suggesting some degree of polycystic ovarian syndrome. AMH concentrations and AFC were highly correlated ($r = 0.83$; $P < 0.001$) (**Figure 2**).

In **Table 3**, all survivors with regular menstrual cycles in 2010 ($n = 35$) are compared with a cycle- and age-matched control group ($n = 210$). The two groups are comparable with regard to age and body mass index. With regard to ovarian reserve parameters, the survivors had a significantly lower AFC than the control group (median 15.0 versus 18.0; $P = 0.047$). There was no statistical difference in AMH concentrations, although there was a tendency towards lower concentrations of AMH in the survivors compared with controls (median 13.0 versus 17.8 pmol/l). The study showed no significant difference in ovarian reserve parameters when survivors using oral contraceptives ($n = 11$) were compared with the eligible age-matched controls also using oral contraceptives ($n = 54$) (data not shown).

Ovarian reserve in relation to treatment characteristics

Table 4 demonstrates the ovarian reserve parameters in relation to treatment intensity. Group 1 (minimal gonadotoxic treatment) represents survivors who were cured with non-alkylating chemotherapy and in seven cases additionally with radiotherapy not including the ovaries. Group 2 (potentially gonadotoxic treatment) includes survivors treated with alkylating chemotherapy and in four cases additionally with radiotheraphy not including the ovaries. Group 3 (maximum gonadotoxic treatment) comprises nine survivors who received both alkylating chemotherapy and ovarian irradiation. Both AMH concentration and AFC differed significantly between the three groups ($P < 0.001$ and $P = 0.003$, respectively). Furthermore, when performing subgroup analyses, the AMH concentration was significantly higher in survivors from group 1 when compared with survivors from both group 2 and group 3 ($P = 0.001$ and $P < 0.001$, respectively) and survivors from group 2 had a significantly higher AMH concentration than survivors from group 3 ($P = 0.007$) (**Figure 3**).

Fertility and pregnancy outcome in relation to treatment intensity

Out of the participating 71 survivors, 46 (65%) had been pregnant and had achieved a total of 85 pregnancies, of which 18 pregnancies were achieved after fertility treatment: insemination with husband's semen ($n = 8$), insemination with donor semen ($n = 2$), oocyte donation ($n = 5$), IVF ($n = 1$) and intracytoplasmic sperm injection ($n = 2$). Thirty-eight women had achieved at least one live birth. The total number of live births by these 38 survivors was 59 and thus the live birth rate per pregnancy was 69% (59/85) and the number of children per woman of the entire cohort was 0.8 (59/71). The median age of the 38 women who had given birth was 35.1 years (range 28.4–48.6 years) while the median age of the 33 women who had not achieved a live birth was 34.9 years (range 27.5–53.6 years). Of the 33 childless survivors, 12 reported that their childlessness was involuntary.

In group 1 ($n = 36$), 19 women had achieved 32 live births. As regards group 2 ($n = 26$) and group 3 ($n = 9$), there were 26 live births by 18 survivors and one live birth by one survivor, respectively. Three survivors from group 1 had experienced four uncomplicated first-trimester abortions. In group 2, four survivors had experienced five spontaneous abortions, of which two were second-trimester abortions complicated by severe uterine bleeding. Among the nine

Table 2 Clinical data, endocrine profile and sonographic data at 10-year follow up according to cycle type.

	Regular cycles (n = 36)	*Oligo- or amenorrhoea (n = 6)*	*Oral contraceptives (n = 11)*	*Menopause, HRT (n = 12)*	*Pregnant (n = 6)*
Age at inclusion (years)	35.2 (27.8–46.6)	32.3 (27.5–37.6)	34.9 (28.3–45.2)	37.9 (29.6–53.6)	31.5 (28.7–38.3)
BMI (kg/m^2)	22.3 (16.2–36.3)	21.9 (20.6–29.7)	23.8 (20.0–31.9)	22.9 (18.1–31.4)	23.7 (18.4–35.2)
Endocrine profile					
AMH (pmol/l)	13.0 (<3.0–71.0)	38.0 (30.0–60.0)	18.0 (<3.0–33.0)	<3	3.5 (<3–20.0)
FSH (IU/l)	6.6 (2.2–21.6)	5.4 (1.7–7.0)	6.0 (0.2–18.3)	35.0 (4.4–52.6)	0.2 (0.2–78.9)
Inhibin B (pg/ml)	57 (10–213)	69 (8–157)	34 (3–108)	<3	4 (3–19)
LH (IU/l)	6.0 (2.5–17.5)	5.3 (3.2–9.7)	4.0 (0.1–10.0)	4.0 (0.1–10.0)	0.1 (0.1–0.2)
Oestradiol (nmol/l)	0.2 (0.1–0.8)	0.2 (0.1–0.6)	0.1 (0.0–0.3)	–	8.5 (0.2–78.9)
Sonography					
AFC (2–10 mm)	15 (3–34)	23 (7–40)	13 (2–23)	0 (0–1)[a]	–
Ovarian volume (ml)	6.1 (2.5–12.3)	10.6 (5.6–15.9)	4.0 (1.9–10.2)	1.7 (0.3–3.0)[a]	–

Values are median (range).
AFC = antral follicle count (both ovaries); AMH = anti-Müllerian hormone; BMI = body mass index.
[a]Data from seven survivors as in five survivors the ovaries could not be detected using ultrasound.

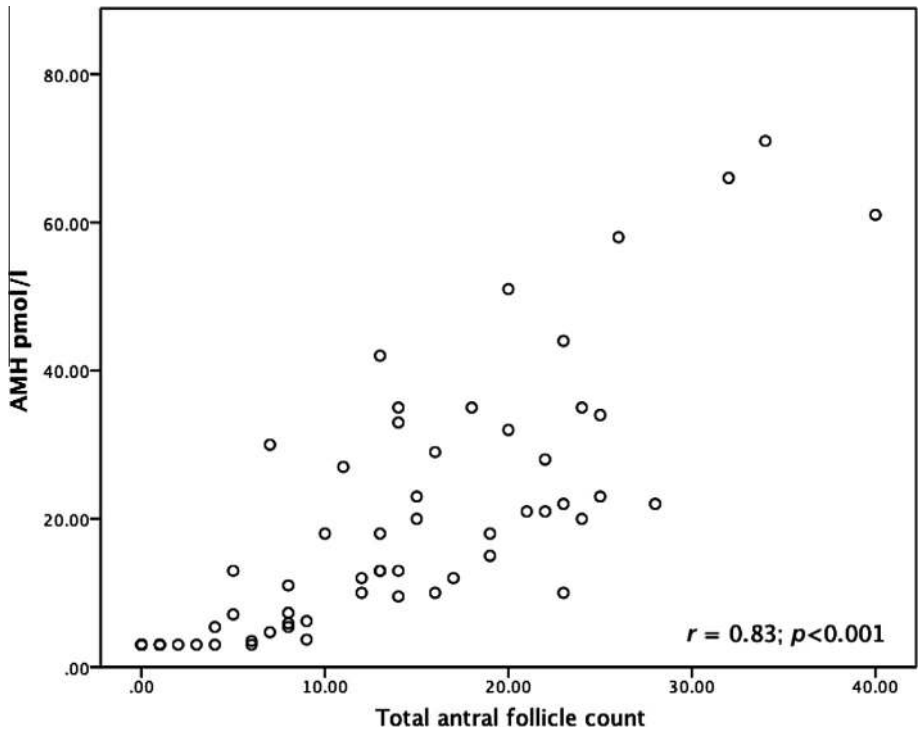

Figure 2 Correlation between anti-Müllerian hormone (AMH) and antral follicle count (2—10 mm) in both ovaries in all 71 participating survivors.

Table 3 Clinical data, endocrine profile and sonographic data in survivors with regular menstrual cycles at study inclusion in 2010 compared with controls.

	Regular cycles (n = *35*)	*Controls* (n = *210*)	P-*value*
Age at inclusion (year)	34.9 (27.8—42.4)	34.6 (27.2—41.5)	NS
BMI (kg/m^2)	22.3 (16.2—36.3)	22.2 (15.8—42.4)	NS
AMH (pmol/l)	13.0 (<3—71)	17.8 (0.9—126.3)	NS
FSH (IU/l)	6.6 (2.2—21.6)	6.7 (2.2—15.5)	NS
Oestradiol (nmol/l)	0.2 (0.1—0.8)	0.2 (0—2.1)	NS
AFC (2—10 mm)	15 (3—34)	18 (1—80)	0.047

Values are median (range). Mann—Whitney U-test. One of the thirty-six survivors with regular cycles could not be matched due to age (>43 years).
AFC = antral follicle count (both ovaries); AMH = anti-Müllerian hormone; BMI = body mass index.

survivors in group 3, only four pregnancies had been achieved: one pregnancy with a donor egg, although prematurely, and three achieved with own eggs, resulting in one stillbirth and two second-trimester abortions also complicated by severe uterine bleeding.

Discussion

Ten years ago, this study group assessed ovarian reserve in a cohort of 100 long-term childhood cancer survivors. The majority of the survivors had intact ovarian function in their mid-twenties, although discrete endocrine and sonographic signs of a diminished ovarian reserve. As far as is known, this is the first 10-year re-examination follow up of female childhood cancer survivors with regard to ovarian function. The main findings are that survivors with regular menstrual cycles in their mid-twenties have a high chance of preserved ovarian function at least until their mid-thirties. Further, more than half of the survivors had achieved at least one live birth. However, the significantly lower AFC in the survivors when compared with an age-matched control group may imply that the survivors have a shortened reproductive span. Even though eight out of 53 survivors (15%) had lost ovarian cyclicity during the follow-up period, only in 3 survivors (6%) was this caused by premature ovarian insufficiency.

The AFC and FSH concentrations did not change during the follow up in survivors who had regular menstrual cycles both in the previous and the present study. It is well described that alterations in of FSH concentrations normally appear rather late in the menopausal transition. In contrast, the AFC decreases with each decade of life. This study could not demonstrate a decline, most certainly because of the small number of participants (n = 30). Further, the ultrasound equipment used in 2000 did not have as high a resolution as the equipment used in 2010, implying that not all

Table 4 Ovarian reserve in survivors included in 2010 according to different treatment groups.

	Group 1: minimal gonadotoxic treatment (n = 36)	*Group 2: potentially gonadotoxic treatment (n = 26)*	*Group 3: gonadotoxic treatment (n = 9)*	*P-value*
Age at inclusion (years)	35.4 (27.5–45.2)	34.9 (27.8–53.6)	33.3 (29.6–42.4)	NS
AMH (pmol/l)	20.0 (<3–66.0)	5.8 (<3–71.0)	<3 (<3–4.7)	<0.001
AFC (2–10 mm)	15 (0–40)	9 (0–34)	2 (0–7)	0.003

Values are median (range). Kruskal–Wallis test.
Group 1 = non-alkylating chemotherapy and ovaries not in radiation field in seven cases also treated with radiotherapy; cytotoxic agents asparaginase, cytaribine, dactinomycin, daunorubicin, doxorubicin, 6-mercaptopurine, methotrexate, prednisone, vincristine.
Group 2 = chemotherapy including alkylating agents and ovaries not in radiation field in four cases also treated with radiotherapy; cytotoxic agents camustine, chlorambucil, cisplatin, cyclophosphamide, dacarbazine, ifosfamide, mustagene, procarbazine, asparaginase, bleomycin, cytaribine, dactinomycin, doxorubicin, 6-mercaptopurine, methotrexate, prednisone, thioguanine, vepeside, vinblastine, vincristine, VM-26.
Group 3 = chemotherapy including alkylating agents and radiotherapy with ovaries in radiation field (pelvic irradiation and total body irradiation); cytotoxic agents asparaginase, busulfan, cisplatin, cyclophosphamide, cytaribine, dacarbazine, dactinomycin, doxorubicin, dounorubicin, 6-mercaptopurine, methotrexate, prednisone, thioguanine, vincristine, VM-26.
AFC = antral follicle count (both ovaries); AMH = anti-Müllerian hormone.

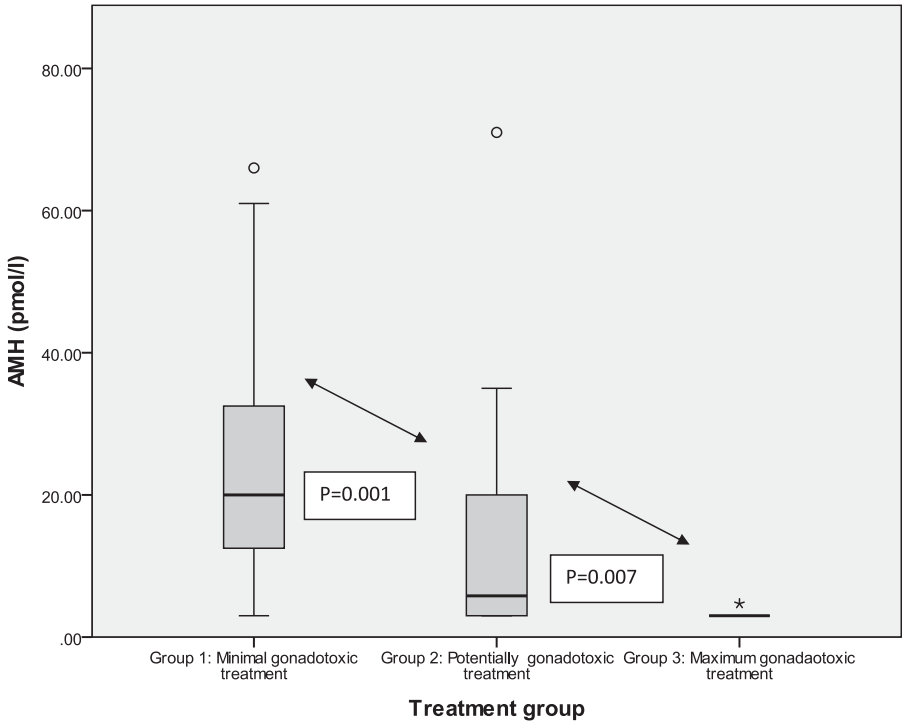

Figure 3 AMH concentrations in 71 female childhood cancer survivors according to treatment intensity. Group 1 = non-alkylating chemotherapy and ovaries not in radiation field; Group 2 = chemotherapy including alkylating agents and ovaries not in radiation field; Group 3 = gonadotoxic chemotherapy including alkylating agents and ovaries in radiation field.

small follicles were visualized and counted in the previous study.

Although, clinical studies are few and have small sample sizes, several register- and questionnaire-based studies have described ovarian function in childhood cancer survivors. A large retrospective questionnaire study by Sklar et al. (2006) found the non-surgical incidence of premature menopause to be 8% among 2819 childhood cancer survivors. The median age of the survivors was 29 years (range 18–50 years) and all had developed spontaneous menstrual cycles for at least 5 years following the time of diagnosis. Data was self-reported and menopause was defined as the absence of a menstrual period for 6 months or more, excluding pregnancy and hormonal therapy. However, it is not stated whether or not they excluded possible oligo- or amenorrhoea. It is, nevertheless, evident that the cumulative incidences of premature menopause found in the present study and Sklar et al. (2006) should be considered as interim, as there is a risk that more survivors of both cohorts with time will reach menopause prematurely, considering the relatively young age of both cohorts.

The type of antineoplastic treatment is widely accepted to be the most important independent risk factor of ovarian damage (Chemaitilly et al., 2006; Lie et al., 2009). In

accordance with these studies, the present study found that survivors treated with ovarian irradiation had a seriously diminished ovarian reserve and substantial difficulties in conceiving and that survivors treated with alkylating agents had a lower ovarian reserve than survivors solely treated with non-alkylating agents.

The degree of cycle variation of AMH concentration has been debated intensively. However, apart from a rise during the follicular phase in women with high AMH concentrations, the cycle variations are generally believed to be limited (Hehenkamp et al., 2006; Sowers et al., 2010; Tsepelidis et al., 2007). All serum AMH concentrations in this study were taken in the early follicular phase in both survivors and controls. Even though the median AMH concentration of the survivors was 4.8 pmol lower than that of controls, this study could not demonstrate a statistically significant difference. There was a significant difference in AFC between survivors and controls. It must be emphasized that AMH concentrations were found to be highly correlated to AFC, supporting the accuracy of the ultrasound examination. Bath et al. (2003) compared the endocrine profile of 10 childhood cancer survivors with regular cycles to healthy controls ($n = 11$) and found significantly lower AMH concentrations in the survivors. A larger study by Lie et al. (2009), on the contrary, showed no significant difference in the AMH concentrations of childhood cancer survivors ($n = 185$) when compared with healthy controls ($n = 42$); even so, 27% of the survivors had AMH concentrations below the 10th percentile of the concentration in the healthy controls, and subgroup analysis revealed that survivors treated with alkylating chemotherapy or abdominal irradiation had significantly lower AMH concentrations compared with controls. As such, the present results are in accordance with the study by Lie et al. (2009).

The great variation in AMH concentrations among normal women is in accordance with a recent study (La Marca et al., 2010) including 277 women with regular cycles and is also in accordance with data from the cross-sectional study of the 863 normal women providing the control population for this study (Bentzen et al., 2013). In contrast to other studies (Streuli et al., 2008; van Beek et al., 2007), the present study found AMH to be significantly decreased in users of oral contraceptives and, therefore, this factor was controlled for in all analyses. The possible influence of oral contraceptives on AMH concentrations has also been shown by van den Berg et al. (2010) and Bentzen et al. (2012).

In this cohort, the majority of survivors treated with pelvic or total body irradiation had experienced adverse pregnancy outcomes regardless of whether the conceptions had been achieved with own or donated eggs. Contrary to the ovaries, the uterus is more vulnerable to radiotherapy at younger ages. Thus, adult uterine volume is reduced in childhood cancer survivors treated with uterine irradiation and, indeed, uterine size is positively correlated to age at the time of uterine irradiation. Further, the endometrial response to sex steroid replacement therapy is insignificant or limited among survivors treated with uterine irradiation (Bath et al., 1999; Larsen et al., 2004). It is important to remember that survivors might not only face the difficulties of conception following antineoplastic treatment, but they may also experience complications during pregnancy and post partum, as survivors receiving abdominal radiotherapy also have an increased risk of preterm delivery and post-partum haemorrhage (Lie et al., 2010; Mueller et al., 2009; Sudour et al., 2010). Therefore, offering assisted reproduction treatment to survivors treated with abdominal irradiation should be considered very carefully.

Among the 71 participants in this study, 65% had been pregnant with a live birth rate of 69%. This proportion is very similar to a large British study analysing 4113 singleton pregnancies among female childhood cancer survivors which found a live birth rate of 73% (Reulen et al., 2009). Further, in a study by Green et al. (2002), a live birth rate of 63% was found.

Multiple studies have described the pregnancy rate of survivors as reduced (Dama et al., 2009; Green et al., 2009a; Madanat et al., 2008). So far, in the present study, the number of children per survivor is 0.8. As in the rest of Europe (ESHRE Capri Workshop Group, 2010), the age at first childbirth in Denmark is increasing. In 2010, the average number of children per woman in Denmark was 1.9 and the average age at first childbirth was 29.1 years (Statistics Denmark, 2011). In 2010, 46% of the survivors were childless and their median age was 34.9 years. For comparison, in 2009, only 19.4% of Danish women aged 36 were childless (Statistics Denmark, 2011). However, this study was not able to distinguish between psychological or somatic factors related to not having a child, so the impact of psychological factors following childhood cancer on the survivors' desire to have children cannot be estimated.

Although this study shows positive results regarding the ovarian function in the far majority of the female childhood cancer survivors, it also confirms the gonadotoxic effect of antineoplastic treatment. The next step is to implement this knowledge into clinical practice. In light of this study's findings, all cancer survivors should early in adult life be offered a fertility awareness consultation, including measurement of AMH and AFC to identify survivors at risk of entering menopause prematurely. Survivors identified with AFC or AMH below the lower limits of a representative normal population could either be advised to plan pregnancy in near future or be offered oocyte vitrification in order to preserve fertility. Oocyte vitrification is also a possibility before gonadotoxic treatment in girls and adolescents who have reached menarche. Finally, children scheduled to receive treatment with a high risk of inducing premature ovarian insufficiency should be offered cryopreservation of ovarian tissue prior to treatment to preserve both endogenous hormone production and fertility (Schmidt et al., 2010)

To conclude, the majority of survivors who had an apparently normal ovarian function in their mid-twenties have conceived and delivered and the ovarian reserve seems almost unchanged during the 10-year follow-up period. Thus, if the ovarian function in childhood cancer survivors is preserved in the early- to mid-twenties, it is likely to persist until the mid-thirties, with a good chance of successful childbearing. It must be emphasized, however, that the data on fertility outcome is still preliminary. Further, future follow-up studies should clarify whether the average age of onset of menopause is reduced, since AFC among survivors with regular menstrual cycles was significantly lower than in age-matched control subjects.

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