
Fertility in Female Childhood Cancer Survivors

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Abstract

Advances in childhood cancer treatment over the past decades have significantly improved survival, resulting in a rapidly enlarging group of childhood cancer survivors. There is much concern, however, about the effects of treatment on reproductive potential. In women there is evidence that both chemotherapy and radiotherapy may have an adverse effect on ovarian function, ovarian reserve and uterine function, clinically leading to sub-fertility, infertility, premature menopause and/or adverse pregnancy outcomes. Here we will first address normal female fertility and methods to detect decreased fertility. Hence we will focus on direct effects as well as late fertility-related adverse effects caused by chemotherapy and radiotherapy, and we will conclude with a summary of current options for fertility preservation in female childhood cancer survivors.

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Normal Female Fertility

Many factors influence whether a woman can produce offspring. A tightly interwoven system, the hypothalamic-pituitary-ovarian axis, is responsible for oocyte maturation, ovulation and proliferation of the uterine lining. This axis is also involved in the onset of puberty and the development of secondary sex characteristics. A successful pregnancy, however, not only requires a fully functional hypothalamic-pituitary-ovarian axis but also a uterus, which is receptive to implantation and is capable of growing with the developing fetus to term.

At birth, the hypothalamic-pituitary axis is active for a very brief period, after which it remains quiescent until puberty begins. At puberty pulsatile bursts of gonadotropin-releasing hormone (GnRH) are produced by the hypothalamus, which

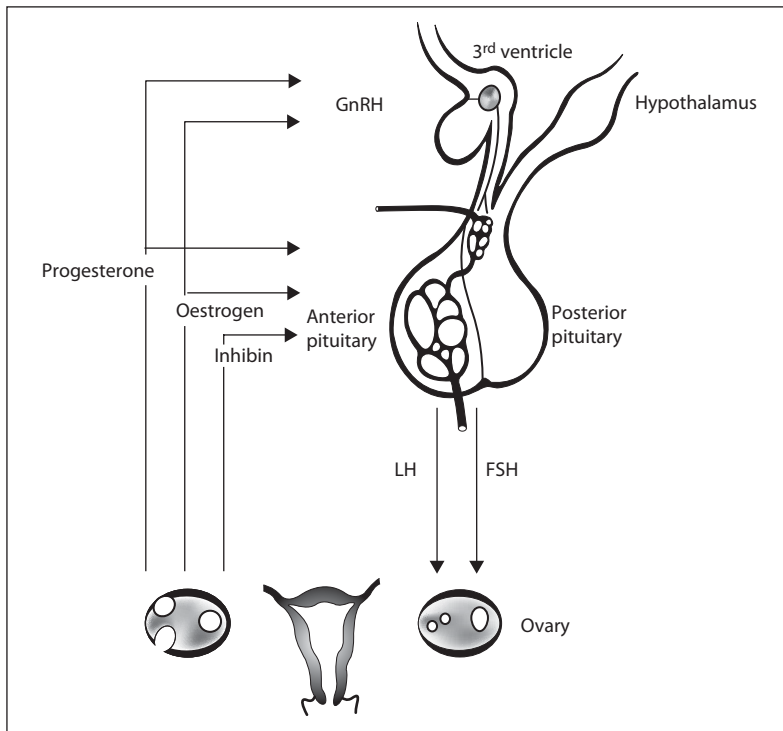


Fig. 1. The hypothalamic-pituitary-ovarian axis.

stimulate the pituitary to release follicle-stimulating hormone (FSH) and luteinising hormone (LH). These hormones, in turn, facilitate oocyte maturation in the ovaries, which results in the production of hormones involved in the development of secondary sex characteristics. On average, puberty in girls commences at the age of 11 years (standard deviation 1 year) and should progress from one Tanner stage to the next every 6–9 months. If not, one should be vigilant for gonadal damage.

In contrast to men, the number of primordial follicles in the ovaries of females is set at birth at approximately 1 million, although in recent years this view has been challenged [1]. At menarche, approximately 400,000 follicles remain, but only 300–400 will undergo further maturation in an ovulatory cycle. The ovaries are regulated by the hypothalamus and the pituitary. Their function includes oocyte maturation and ovulation, and the production of hormones.

Each month, under the influence of FSH released by the pituitary, a number of resting follicles mature to antral follicles. These maturing antral follicles produce hormones which respectively reduce FSH secretion by inhibition of the hypothalamus and the pituitary via negative feedback (inhibin B), and promote further follicle growth and the development of the endometrium (oestradiol; fig. 1).

The dominant follicle is selected from this cohort of maturing antral follicles and an LH surge, delivered by the pituitary, results in the release of the ovum from the dominant follicle into the fallopian tube. Following ovulation, the 'remains' of the dominant follicle, now called the corpus luteum, produces large amounts of progesterone involved in the development and maintenance of the uterine lining. When conception, and thus implantation, fails to occur the endometrium is shed and the menstrual cycle starts again. The number of follicles thus gradually reduces with age until approximately 1,000 follicles are left. A woman has then reached menopause at an average age of 51 years. This gradual decline of primordial follicles with age is illustrated by a model from Faddy and Gosden [2].

Any 'insult' to this tightly interwoven system may adversely affect fertility. Damage to the hypothalamic-pituitary axis may lead clinically to a delayed or arrested puberty, resulting in primary amenorrhoea if damage occurs before puberty, and secondary amenorrhoea with damage during or after puberty. Damage to the ovaries, resulting in a reduction or depletion in the primordial follicles, may lead to infertility, reduced fertility or a premature menopause with subsequent risks of menopause-related conditions. Adverse effects of treatment to the uterus may result in low birth weight babies, spontaneous abortions or miscarriages due to the inability of the uterus to carry a fetus to term.

Childhood cancer and its treatment may lead to any of the fertility defects described above [3, 4]. In general, females seem less sensitive to the adverse effects of chemotherapy than males. Studies have shown that reproductive function can usually be normal after treatment of childhood cancer. However, poor ovarian function, infertility and damage to the uterus have also been described following childhood cancer treatment [3, 4]. In addition, seemingly normal ovarian function, assessed by the resumption of regular menses after therapy, normal hormone levels, and even pregnancy, does not mean that the ovaries escaped damage. Treatment may have accelerated the decline of the non-renewable pool of primordial follicles in the ovaries, reducing fertile lifespan and resulting in premature menopause [5]. On the other hand, the absence of regular menstrual cycles does not necessarily imply infertility [3]. Mechanisms underlying recovery of ovarian function are unclear and there are no indicators that allow the identification of women who recover ovarian function, other than the fact that it is more likely to occur in younger women [6].

Detection of Decreased Fertility

General Aspects of Female Fertility Evaluation

Normal fertility requires: (1) adequate development of ovarian follicles that contain an oocyte from which preferably only one becomes dominant in readiness for

ovulation; (2) ovulation; (3) normal transportation of the gametes; (4) fertilisation of the oocyte by one sperm; (5) implantation of the resulting conception, and (6) support of the conceptus such that an ongoing pregnancy results.

This highly complex constellation demands many properly functioning body features both anatomically and physiologically.

During routine evaluation of an infertile couple a number (but not all) of these functions are investigated. Briefly: (1) ovarian competence is studied; (2) anatomical conditions are evaluated that are needed to allow the gametes (oocyte and sperm) to encounter and an embryo to nidate, and (3) verification that ovulation took place and that adequate sperm is available. Moreover sexual behaviour, general health and environmental aspects need evaluation. In the context of this chapter we will briefly summarise some general features of the natural decline of fertility and practical ways by which the various sub-fertility aspects are addressed in the female [for complete reviews see, 7, 8].

Natural Decline of Female Fertility and Ovarian Reserve Testing

From studies on natural populations in which no consistent methods of birth control are applied, it has been shown that natural fertility starts to decline after the age of 30, accelerates in the mid-30s and will lead to sterility at a mean age of 41. The reduction in female fertility can also be seen in contemporary population studies. The chance of not conceiving a first child within 1 year increases from less than 5% in women in their early 20s to approximately 30% or over in the age group of 35 years and older. So although the majority of women of older age will obtain the desired pregnancy within a 1-year period, the chance of becoming sub-fertile increases approximately 6-fold in comparison with very young women. This age-related decline in fertility is the result of a progressive decrease in quality and number of oocytes from follicles left in the ovaries (ovarian reserve).

Women starting an infertility workup will undergo extensive testing. An accurate measure of the quantitative ovarian reserve would theoretically involve the counting of all follicles present in both ovaries, as was done in post-mortem studies. For obvious reasons, in ovarian reserve testing, the true size of the follicle pool has not been used as the benchmark for evaluation. Aside from its invasiveness and the potential complications of the procedure, the taking of an ovarian biopsy cannot be considered a reliable way to determine ovarian competence in an individual patient in either a quantitative or qualitative sense.

Over the past two decades a number of less invasive estimates have been proposed to predict the competence of the ovaries. Static estimates are calendar age, basal early follicular phase levels of hormones such as FSH, E_2 and inhibin B, anti-mullerian hormone and ultrasonic appearance of the ovary in terms of numbers of

antral follicles (antral follicle count: AFC) and the volume of the ovary. Dynamic estimates are the clomiphene challenge test (CCT), the GnRH agonist stimulation test and the Exogenous FSH Ovarian Reserve Test (EFORT).

Of all these, the measurement of early follicular FSH is probably most widely used in the diagnostic workup of a couple with infertility. A raised basal FSH is associated with a decreased success rate in assisted reproduction. The number of antral follicles and ovarian volumes correlate well with female calendar ageing and can predict rather well the number of follicles that can be obtained in assisted reproductive technologies (ART). In addition, several recent studies indicate that anti-mullerian hormone could be a potential candidate for the assessment of ovarian reserve as it correlates with AFC, calendar age and ART yield. It is produced by pre-antral follicles and, as such, is a relatively stable measure of follicle numbers as a consequence of the limited inter-cyclic variability.

With regard to dynamic hormonal tests, we showed in a recent prospective study in in vitro fertilisation (IVF) patients that the best predictor of a poor response to IVF was the CCT (ROC-AUC = 0.88) while the E_2 increment in the EFORT had an ROC-AUC of 0.75 and the inhibin B-increment had an ROC-AUC of 0.86. For high response to IVF, univariate logistic regression showed that the best predictor is the inhibin B-increment in the EFORT (ROC-AUC = 0.92). The E_2 increment in the EFORT had an ROC-AUC of 0.83 which made us conclude that the EFORT is not a better predictor to identify poor responders than the CCT and that the inhibin B increment in the EFORT will best predict a high response. Others showed that GAST is a good predictor of ovarian response in ART in comparison to basal FSH and CCT.

Thus, currently available ovarian reserve tests are not sensitive and specific enough to justify general application and the ideal test has yet to be identified. Such a test in the standard diagnostic work up would potentially identify all patients with a chance of becoming pregnant and should identify poor and high responders such that it would enable us to determine for each individual patient the optimal ART stimulation scenario.

Detection of Ovulation

Regular menstrual cyclicity is usually indicative of ovulation. But ovulation needs to be confirmed. In clinical practise, detection of ovulation is based on tracing the acute changes (1) resulting from the shifting endocrine environment (detection of a rise in LH in a daily urine sample, registration of a shift in daily registered basal body temperature or the measuring of progesterone in a timed single serum sample), and (2) in anatomical appearance (disappearance/collapse of the dominant follicle and occurrence of a corpus luteum detected by means of a timed ultrasound scan).

Oligomenorrhoea and amenorrhoea are conditions in which the growth of follicles and ovulation are disturbed. These conditions contribute to about 20% of all cases of sub-fertility. According to the World Health Organisation (WHO) classification these disorders can be divided into 3 groups: WHO I, WHO II and WHO III. This classification is based on the levels of gonadotropic hormones, LH and FSH, found during laboratory testing as is routinely applied in these patients.

Class WHO I is characterised by very low levels of LH and FSH indicating disorders at the level of the brain (insufficient brain-derived GnRH) or a damaged pituitary. In WHO II patients normal LH and FSH levels are found. This class represents most (>80%) of all oligomenorrhoeic women. The most frequent disorder responsible for this is polycystic ovary syndrome which is further characterised by many small cysts in the ovaries (>12 in each) observed by ultrasound and/or signs of hyper-androgenism such as high levels of serum androgens and/or hirsutism/severe acne.

Finally WHO III patients have elevated levels of gonadotropins. This condition is present in the case of absent ovarian function which is analogous to the physiologically post-menopausal state. Indeed often these patients also report other typical menopausal complaints such as hot flushes. This latter group consists of approximately 5% of women with ovulatory disorders. However, among cancer survivors this is the most frequently encountered type of cycle disturbance.

Evaluation of the Female Reproductive Tract in Sub-Fertility

Uterus

Congenital and acquired uterine anomalies such as uterus bicornis/unicornis, septae, polyps, myomata, adhesions and adenomyosis in relation to infertility are rare and their relationship to possible sub-fertility is difficult to prove. This is even more difficult if interventions based on observed abnormalities are used in the absence of adequate published trials. Endoscopic hysteroscopy and laparoscopy, radiographic hysterosalpingography and abdominal/vaginal ultrasound are all useful techniques to determine these uterine anomalies. Reduced uterine volume and decreased elasticity of uterine musculature, which can be found in female childhood cancer survivors treated with abdominal irradiation, can be visualised by transvaginal ultrasound [9].

Assessment of Tubal Function

Tubal dysfunction with a negative impact on fertility due to disrupted transportation of the ovulated oocyte is a very important cause of sub-fertility and contributes to about 15%.

Cornerstones for evaluation of tubal function are: (1) radiographic hysterosalpingography, which visualises interior conditions and patency of the uterus and the fallopian tubes, and (2) diagnostic laparoscopy, which enables evaluation of intra-abdominal conditions that may affect fertility such as adhesions and endometriosis. Both tests are reliable, with good sensitivity and specificity profiles, so that their routine application in clinical practice is justified.

Post-Coital Test

While the fallopian tubes are important for transportation of the oocyte (and the early embryo), the cervix, with its abundantly available mucus around time of ovulation, is crucial for sperm transportation. Cervical function in this context is tested routinely with the post-coital test. Cervical disorders contribute to about 5% of sub-fertility. A well-timed post-coital test contributes significantly to models that predict the chance of pregnancy. Nevertheless its routine use in the investigation of a sub-fertile couple is questioned [8].

Effects of Cancer Treatment on Female Fertility

Decreased fertility and ovarian failure have been reported in female survivors of childhood cancer. Relative fertility rates vary according to the primary diagnosis and are linked to gonadotoxic effects of treatment [10]. It has been estimated that the biological ovarian age in childhood cancer survivors is approximately 10 years ahead of their chronological age [5].

Fertility-related adverse effects of treatment may be mediated through the hypothalamic-pituitary axis, the ovary or the uterus. Radiotherapy is known to act on all three of these systems; however, direct effects of chemotherapy on the hypothalamic-pituitary axis and the uterus have not been described.

Effect of Radiotherapy on Hypothalamic-Pituitary Axis

The hypothalamic-pituitary axis directly affects the functions of the thyroid gland, the adrenal gland and the ovaries, and can be considered the co-ordinating centre of the endocrine system. As a consequence, radiation to the hypothalamic-pituitary axis as part of treatment for childhood cancer carries a risk of inducing endocrine adverse effects. The radiation-induced damage to this axis depends on the total dose, fraction size, number of fractions and the duration [11].

Cranial (spinal) irradiation is used alone or in combination with surgery and/or chemotherapy for brain tumours and acute lymphoblastic leukaemia with central nervous system involvement, and has a damaging effect on the hypothalamic-pituitary

axis. As a consequence, high-dose (>24 Gy) cranial radiotherapy is associated with a risk of delayed puberty or secondary amenorrhoea in girls, whereas lower doses, paradoxically, may result in early puberty or precocious puberty [12]. Furthermore, a recent study demonstrated that females exposed to cranial or craniospinal radiotherapy are at risk of abnormal timing of menarche [13].

Hypothalamic-pituitary dysfunction secondary to radiation is progressive over time, as there is an increase in the frequency and severity of hormonal deficits with a longer time interval after radiotherapy [11]. Several studies have investigated the late effects (i.e. effects that may manifest years after completion of cancer treatment) of radiation to the hypothalamic-pituitary axis. One study showed that the majority (64%) of girls who had received craniospinal irradiation without chemotherapy developed ovarian damage as determined by elevated gonadotropins [14]. In addition, Bath et al. [15] demonstrated that young female survivors exposed to low dose (18–24 Gy) cranial irradiation showed decreased LH secretion, an attenuated LH surge, and shorter luteal phases. Since these parameters have been associated with reduced fertility and adverse pregnancy outcomes [16, 17], monitoring this group of female survivors at regular intervals after the completion of treatment is a matter of utmost importance.

Effect of Radiotherapy on the Uterus

Uterine characteristics that may be affected by radiotherapy are: volume (growth); vascularisation, and endometrial thickness. The degree of uterine damage depends on the total radiation dose and the site of irradiation. The extent of uterine damage due to childhood radiotherapy is influenced by age. At puberty, uterine shape alters from a tubular to a pear-shaped organ with an increase in volume. Therefore, the pre-pubertal uterus is more sensitive to radiation-induced damage as uterine development is not completed before the onset of puberty.

A number of studies have investigated the direct adverse effects of irradiation on the uterus. Whole abdominal-pelvic irradiation (20–30 Gy) has been reported to result in impaired uterine development and reduced volume and vascularisation [18]. Although treatment with total body irradiation (TBI) and bone marrow transplantation involves exposure to lower doses of radiotherapy than those during abdominal irradiation, it has been demonstrated that survivors after such treatments remain at high risk of reduced uterine volume, impaired blood flow and absent endometrium [19, 20]. These abnormal uterine characteristics have been associated with adverse pregnancy outcomes such as preterm delivery and low birth weight in female childhood cancer survivors [9, 21].

Hormone replacement therapy (HRT) can improve uterine size, endometrial thickness and uterine vascularisation in female survivors [19, 22]. However, the



Fig. 2. Correlation between uterine volume in the 3rd month of physiological sex steroid replacement and age at irradiation ($p < 0.05$). Reprinted from Bath et al. [19], with permission from Blackwell Publishing Ltd.

appropriate dose of sex steroids is as yet unknown [20]. Furthermore, not all females may benefit to the same extent from HRT, as patients treated pre-pubertally show a significantly smaller increase in uterine volume than patients who have been irradiated after puberty. Indeed, final uterine volume after HRT showed a significant correlation with age at irradiation (fig. 2) [19].

Furthermore, high-dose radiotherapy (>30 Gy) delivered at abdominal or pelvic sites, may result in irreversible uterine damage which cannot be overcome by sex steroid replacement therapy [22, 23]. This finding is supported by the study of Larsen et al. [23] which demonstrated that in females with an apparent preserved ovarian function, with endogenous hormone production during puberty, uterine sizes can still be very small.

Effect of Treatment on the Ovaries

Treatment-induced ovarian damage may cause acute amenorrhoea during or shortly after treatment, which may be permanent or transient. Women who retain apparently normal ovarian function after treatment or regain normal ovarian function after a period of amenorrhoea (which can last months or years) still may face problems when trying to become pregnant and/or may experience premature menopause later in life (fig. 3) [6].

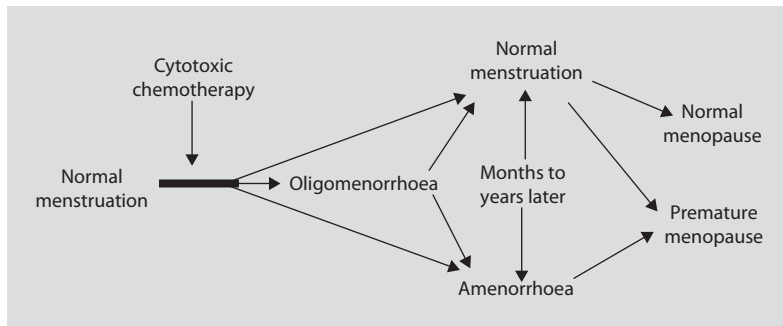


Fig. 3. The impact of chemotherapy on the menstrual cycle. Reprinted from Howell et al. [6], with permission from Elsevier.

Because many definitions of decreased fertility are used, many outcomes have been studied in childhood cancer survivors. In general, two forms of premature ovarian failure can be distinguished [24]. When ovarian failure occurs shortly after completion of therapy, it is classified as acute ovarian failure. Researchers use several cut-off points to determine acute ovarian failure rates, such as 6 or 12 months after completion of therapy, with a maximum of 5 years after cancer diagnosis.

Patients who remain (or recover) normal ovarian function during the first 5 years, may still face the risk of developing premature ovarian failure subsequently. Any occurrence of ovarian failure before age 40 is classified as premature menopause, and this may occur after the first 5 years following cancer diagnosis.

Effect of Age at Time of Treatment

Since ovarian reserve decreases with age, similar amounts of chemotherapy and/or radiotherapy may have more direct gonadotoxic effects in older compared to younger women. Taking acute ovarian failure as a measure of decreased fertility, secondary amenorrhoea rates in post-pubertal girls are higher compared to primary amenorrhoea rates in pre-pubertal girls [25]. The age effect on acute ovarian failure is also reflected in the fact that ovarian function recovery rates after bone marrow transplantation in older women are lower than in young women [26].

When premature menopause rates related to gonadotoxic treatment are compared between post- and pre-pubertal girls, however, differences are not as distinct and can even become statistically insignificant [27]. A similar age effect is seen in a cohort of 518 female survivors of Hodgkin's lymphoma treated with chemotherapy and/or supradiaphragmal radiotherapy before the age of 40 in a study by De Bruin et al. [28]: older women experience premature menopause relatively shortly after treatment, but at age 40 the cumulative incidence of premature menopause

does not differ according to age. This phenomenon was also described by Haukvik et al. [29].

Regardless of the effect of decreased ovarian reserve, it has been hypothesised that post-pubertal girls may also experience more gonadotoxicity compared to pre-pubertal girls [30].

Effect of Radiotherapy

Irradiation can cause damage to immature oocytes and hasten the natural decline of the primordial follicles in the ovaries. The degree and persistence of radiation-induced damage to the ovaries depends on the age of the individual at the time of treatment, the field of radiation, the total irradiation dose, and the dose per fraction. The ovaries of younger females are more resistant to damage from irradiation. In addition, the ovaries appear to be susceptible to damage from irradiation in a dose-dependent manner [31]. Exposure to high doses of radiotherapy can cause sterility with total depletion of the primordial follicle reserve, whereas lower doses cause only partial depletion of the primordial follicle reserve, which leads to premature ovarian failure. Furthermore, it has recently been calculated that the irradiation dose required to kill 50% of the oocytes, i.e. median lethal dose, is <2 Gy [32].

Females exposed to abdominal-pelvic irradiation appear to be at highest risk of developing acute ovarian failure [25]. Radiation doses in the range of 10–30 Gy have been found to cause acute ovarian failure. However, smaller doses of radiotherapy to the ovaries are also capable of inducing this phenomenon [25]. Furthermore, conditioning regimens given before bone marrow transplantation, which includes TBI, induce acute ovarian failure at a very high rate [24, 33].

With respect to premature menopause, radiation to the ovary is associated with the greatest risk of premature menopause [5, 27, 31]. In addition, TBI has also been identified as a severe risk factor for developing premature menopause. In patients older than 10 years, TBI caused premature ovarian failure in over 90% of the patients; in patients younger than 10 years, the ovaries appear more resistant to damage although premature menopause is also frequent in this group [12].

Effect of Chemotherapy

Chemotherapy plays an important role in the treatment of patients with childhood cancer. Alkylating agents are commonly used for childhood sarcomas, leukaemia and lymphomas [34].

Although the pathophysiological mechanisms underlying chemotherapy-induced ovarian failure are not fully understood, they are thought to be related to the cytotoxic actions of the drugs. These can be manifested on the ovaries through impairment of follicular maturation and/or depletion of primordial follicles.

Alkylating agents are the most common chemotherapeutic agents associated with gonadal damage. These agents are not cell cycle-specific and thus do not require cell proliferation for their cytotoxic action. It is believed that they act on undeveloped oocytes and possibly on the pre-granulosa cells of the primordial follicles [30]. By cross-linking DNA and introducing single-strand DNA breaks, alkylating agents destroy cells in a dose-dependent fashion [34]. Cell cycle-specific agents, like anti-metabolites, cause much less gonadotoxicity as their major effect is only on growing and dividing cells affecting ovarian follicle growth and maturation [35].

Treatment with alkylating agents has been associated in many studies among childhood cancer survivors with statistically significant risks for ovarian failure. Both acute effects [25] and effects on (premature) menopause [10, 27, 31] have been described. The highest risks were found in girls treated with the highest cumulative doses of alkylating agents. Studying gonadotoxic effects of individual drugs can be difficult, because many patients receive combination regimens in which several drugs are administered concomitantly. Chemaitilly et al. [25] found a clearly increased risk for treatment with procarbazine and cyclophosphamide. Procarbazine and cyclophosphamide were also found to be associated with significantly increased risks of premature menopause in the cohort of female Hodgkin's survivors described by De Bruin et al. [28]. The risk of procarbazine was dose-dependent [28]. The high risks associated with treatment with busulphan, CCNU and chlorambucil in the study by Chemaitilly et al. [25] were based on very small numbers of exposed patients. Busulphan, however, has also been associated with ovarian failure in several studies including girls that underwent stem cell transplantation [36–38]. Mechlorethamine (nitrogen mustard) is often combined with procarbazine in chemotherapy combination regimens, but seems not to be associated with an independent risk of ovarian failure itself [25, 28].

As previously discussed, acute gonadotoxicity of treatment can differ according to age. Chemaitilly et al. [25] found that treatment with procarbazine is associated with acute ovarian failure regardless of age, whereas treatment with cyclophosphamide only resulted in acute ovarian failure in post-pubertal girls. This is in line with the findings of Sanders et al. [37], who described virtually normal pubertal development among girls treated only with cyclophosphamide in preparation for stem cell transplantation. The fact that the effect of procarbazine is seen in pre-pubertal girls, whereas the effect of cyclophosphamide is not, suggests that procarbazine is more gonadotoxic (in the doses used to treat childhood cancers) than cyclophosphamide. This is in line with the findings from De Bruin et al. [28].

No evidence regarding harmful gonadotoxic effects of non-alkylating chemotherapeutic agents was identified in large studies of childhood cancer survivors with regard to acute ovarian failure [25] and premature menopause [10, 27, 31].

Girls treated with non-alkylating chemotherapy, however, were found to have significantly smaller ovaries and fewer antral follicles compared with controls [5].

Studies that include post-pubertal girls, adolescent and young adult female cancer survivors, and therefore represent a population with a lower baseline ovarian reserve, identified several women experiencing ovarian failure after exposure to non-alkylating agents. However, no significantly increased risks associated with these drugs were identified [28, 29, 39]. The gonadotoxic potential of non-alkylating chemotherapy is therefore regarded to be minimal in girls treated for childhood cancer.

Fertility-Related Late Effects

Treatment for childhood cancer can cause decreased fertility. The decrease itself, however, can cause health-related adverse effects in survivors.

Pregnancy Outcomes

Many studies on pregnancy outcomes of female childhood cancer survivors have been published and reviewed by Green [40]. Two studies on this subject have been performed within the Childhood Cancer Survivor Study [41, 42]. They were large enough to study pregnancy outcomes in relation to treatment. Green et al. [41] studied 4,029 pregnancies in 1,915 female childhood cancer survivors, and found a significantly decreased chance of live birth and an increased risk for miscarriage in most age groups and primary diagnosis strata. Although live births were significantly decreased and miscarriages increased in all broadly defined treatment categories, no differences between the categories were observed. Cranial irradiation was found to be associated with significantly increased risks of miscarriage, especially for miscarriage at 12 or more weeks of gestation. Non-significantly increased rates of miscarriage were found for women whose ovaries were in or near the radiation therapy field. The rate of live births was not lower and the rate of stillbirths was not higher for the patients treated with any particular chemotherapeutic agent. There were also no dose response-related risks for live births or miscarriages identified for any chemotherapeutic agent.

A subgroup of 2,201 singleton live births in 1,264 women was studied by Signorello et al. [42] in more depth with regard to the treatment effects on preterm birth, low birth weight and small gestational age. They found that the children of female childhood cancer survivors were twice as likely to be born preterm as the children of their siblings. The children also had a significantly lower birth weight, but this was attributed to their birth at earlier gestational age. Preterm birth, low

birth weight and small gestational age were all significantly associated with radiotherapy to the uterus, but not associated with radiotherapy to the ovaries or the pituitary. The effect of radiotherapy to the uterus was not significantly different for mothers irradiated as pre-pubertal girls compared to post-pubertally irradiated women. Cumulative doses of alkylating chemotherapy showed a non-significant dose-response trend towards more preterm birth among women treated with higher doses. No association with low birth weight or small gestational age of the children was observed.

Although concerns have been raised that potentially mutagenic chemotherapy and radiotherapy may cause germ-line mutations and pose an increased risk of genetic abnormalities in the children born to survivors of cancer, no such evidence has been provided by several large studies on this subject [21, 43].

Premature Menopause and Its Consequences

As discussed above, treatment of childhood cancer can cause premature menopause. Because this permanent cessation of menses can occur not only shortly after treatment, but also later in life at any age before 40 years of age, it can be regarded as a late effect of treatment. The cumulative incidence of premature menopause, occurring 5 years following diagnosis is estimated to be 15% at age 40 [31]. Together with about 6% of women experiencing acute ovarian failure within the first 5 years [25], a substantial proportion of female childhood cancer survivors develop premature menopause. Post-menopausal symptoms include hot flushes, psychosomatic complaints, and sexual dysfunction [44, 45]. Long-term absence of oestrogens can result in increased cholesterol levels, and premature menopause is associated with an increased risk of ischaemic heart disease [45, 46]. In addition, oestrogen is essential in preserving bone mineral density, and premature menopause is associated with osteoporosis [44, 45].

As all these symptoms may have a negative impact on the quality of life and physical well-being of childhood cancer survivors, HRT is often applied in post-menopausal survivors [44, 45]. The use of HRT is regarded as safe in most cancer survivors. With the exception of meningioma, breast and endometrial cancer, there is no biological evidence that HRT may increase recurrence risk [47]. HRT can decrease the risk of osteoporosis [44, 45]. In addition, HRT is found to reduce the increased risk of ischaemic heart disease in women with early menopause [46]. This result is likely to be applicable to female childhood cancer survivors with therapy-induced premature menopause.

The use of HRT, however, is associated with an increased risk of developing hormone-related tumours [47]. Female childhood cancer survivors treated with irradiation to the breast at a young age experience an increased risk for subsequent

breast cancer later in life [48]. These are mainly women successfully treated for Hodgkin's lymphoma. Gonadotoxic therapy is found to be associated with a decrease in this increased risk of second breast cancer in adolescent and young adult Hodgkin's survivors [49], but not in childhood cancer survivors [48]. In a subgroup of 185 five-year Hodgkin's survivors treated before age 21 from the Dutch Hodgkin's late effects cohort [28], however, we found that gonadotoxic therapy does lower the risk of breast cancer in these women irradiated to the breast area, but only when menopause is induced relatively shortly after treatment [unpublished data]. In particular, women with <10 years of intact ovarian function after irradiation to the breast, experienced a >10-fold significantly lower risk of subsequent breast cancer compared to those with >20 years of intact ovarian function (HR 0.09, 95% CI 0.01–0.8). Theoretically it is possible that the beneficial effects of premature menopause on future breast cancer risk in women who received chest irradiation at a young age may be masked by HRT treatment.

Although long-term HRT has a beneficial effect on women's bones, and this beneficial effect is often offset by an increased risk of venous thrombo-embolic disease, breast cancer, stroke, cognitive dysfunction and coronary artery disease [45], the risk-benefit balance for HRT treatment in female childhood cancer survivors has not yet been fully evaluated.

Psychosocial Effects of Fertility Issues

Research on female fertility following cancer treatment during childhood mainly involves the physical effects of cancer and its treatment on reproductive function and ovarian reserve. Little is known about the psychosocial consequences of sub- or infertility or the impact of a history of cancer on the decision of childhood cancer survivors to have children of their own. Available literature suggests nevertheless that having children is important for young cancer survivors [50]. However, many female childhood cancer survivors have little or no knowledge about their fertility status. Zebrack et al. [51] found that 64% of female childhood cancer survivors had no knowledge whatsoever about their fertility status and those who did knew because of a previous or ongoing pregnancy. In addition, many young cancer survivors do not recall ever having talked about the possible impact their former treatment may have on their reproductive capacity [50, 51]. Some do possess or recall information about infertility risks but this information may be inaccurate or dated.

Due to this lack of knowledge, infertility, but also pregnancies, often come as a surprise to many young female cancer survivors. In case of infertility, this inevitably causes significant emotional distress over the loss of a dream to have a child [52]. It is, however, unknown whether the psychological stress of infertility

is greater in childhood cancer survivors compared to infertile couples without the history of cancer. One can hypothesise that the burden of infertility may add to the burden of having had cancer causing greater distress in infertile childhood cancer survivors compared to other infertile couples. On the other hand worries about infertility could be more relative in view of the fact that one has survived cancer. There are no studies that have addressed these issues. It has been documented, however, that childhood cancer survivors do worry about their reproductive capacity and/or the health of their offspring, and that females worry more than males [51–53]. The high response rate (85%) to a pilot study in the VU University Medical Centre Amsterdam on reproductive function and ovarian reserve illustrates the need for information regarding this issue amongst female childhood cancer survivors. In addition, Langeveld et al. [53] found that 43% of childhood cancer survivors expressed concerns about the health of their future children, and a similar percentage was reported by Zebrack et al. [51]. This is despite the fact that evidence suggests that children of childhood cancer survivors are not at higher risk of congenital anomalies compared to children of parents without a history of cancer [21, 43]. Appropriate scientific information does not yet sufficiently reach childhood cancer survivors via healthcare professionals although this counselling could possibly reduce fertility-related anxieties.

In addition to worries about the health of their offspring, some childhood cancer survivors have concerns about their own health or their ability to be a good parent [50, 52]. The majority of younger cancer survivors, however, see their cancer experience as potentially making them better parents despite these concerns [50, 51]. Schover et al. [50] reported that 80% of young cancer survivors felt they were or would be good parents in the future. Family life and spending time with family appeared to be very important for cancer survivors and these feelings were specifically attributed to having had cancer [51].

The impact of having had cancer on the decision of childhood cancer survivors to have children of their own seems to be relatively small. Sixteen percent of younger cancer survivors felt a decreased wish to have children due to the impact of cancer. Seventy-one percent did not change their wish and 13% felt an increased wish for children [50]. Only a small percentage of childhood cancer survivors decided to forego having children, but this is not always related to their history of cancer [51].

Even if reproductive function seems to be unaffected by previous cancer treatment and the female survivor, despite her anxieties, does wish to have children, it is important that she is able to engage in an intimate relationship. Studies have suggested that peer relationships, close friendships, self-concept and social competence in non-CNS cancer survivors is relatively similar 2 years after treatment [54]. However, several long-term studies in childhood cancer survivors have shown that the history of cancer has a negative impact on intimate relationships

and marriage rates [53–55]. Sharing one's history of cancer with a new partner is particularly relevant for the young adult survivor population and a possible perceived loss of the opportunity to be a parent may be devastating to self-esteem and potentially damaging to marital or other relationships [51]. It has also been reported that childhood cancer survivors are less likely to be sexually active and that they appear to be less satisfied with their interpersonal relationships and sex life [54]. Van Dijk et al. [56] have shown that psychosexual problems are frequent in survivors of childhood cancer. Twenty percent of childhood cancer survivors felt limitations in their sexual life related to the former cancer and the achievement of several psychosexual milestones was delayed [56].

It can be concluded that as the number of female childhood cancer survivors increases, knowledge of the reproductive health status after treatment is becoming more important. Fertility-related concerns are a major source of distress in many young female cancer survivors. Adequate counselling by healthcare professionals is required as is the sharing of available knowledge in order to reduce these fertility-related anxieties.

Options for Fertility Preservation

As described in the previous paragraphs cancer and its treatment may adversely affect fertility and fertility-related issues have been shown to be a source of psychosocial distress in childhood cancer survivors [50, 52]. Information regarding possible treatment-related infertility and available methods to preserve reproductive function is, therefore, essential. However, evidence suggests that the possibility of treatment-related infertility is often not adequately addressed with the patient and/or their parents (in case of a minor) by many (paediatric) oncologists. This may partly be due to lack of knowledge. A study by Goodwin et al. [57] reported that although 90.7% of healthcare providers were aware of the adverse effects of some treatment regimes on fertility, only half were aware of gender differences in gonadotoxicity. In addition, only 53.3% had knowledge of current research and technologies in fertility preservation.

The number of established methods to preserve fertility in female cancer patients is limited, especially in pre-pubertal girls. Several options are available for females but none are as reliable or easy as sperm banking in males and most are still used in an experimental context only. The options available for females are mostly invasive and/or require drug administration. Methods to preserve fertility in females include freezing (embryo cryopreservation, oocyte cryopreservation, ovarian tissue cryopreservation), surgery (ovarian transposition) and/or drug administration (ovarian suppression). Each method has advantages and disadvantages and whether or not an option is suitable for a patient depends on age,

diagnosis, type of treatment, time available, the potential that cancer has spread to the ovaries, and the presence of a partner [58]. The different methods of fertility preservation will be briefly described below.

Embryo Cryopreservation

The only currently established method for fertility preservation in females with cancer is the cryopreservation of embryos, a technique routinely used in IVF centres [59]. The human embryo is very resistant to damage caused by cryopreservation. Embryo survival rates after thawing range from 35 to 90% while cumulative pregnancy rates of more than 60% have been described [60, 61].

Despite the fact that this technique has good success rates and is already used in young cancer patients, it also has several disadvantages. The procedure requires ovarian stimulation, oocyte retrieval and IVF. This process takes time (2–6 weeks), which may cause an unacceptable delay in the onset of treatment. In addition, ovarian stimulation is contraindicated in patients with an oestrogen-sensitive tumour, such as breast cancer. Another pitfall of this method is that a partner is required or the female involved must be willing to use donor sperm for fertilisation. Finally, embryo cryopreservation is not an option for pre-pubertal girls with cancer [60, 62].

Cryopreservation of Oocytes

As opposed to embryo cryopreservation, cryopreservation of oocytes (mature and immature) does not require a partner and there may be fewer ethical issues involved [59, 62]. However, compared to embryos, oocytes are much more vulnerable to the freeze-thaw process and the rate of success also depends on the total number of retrieved oocytes [63, 64]. Since the first successful pregnancy in 1986 [65], more than 100 babies have developed from frozen-thawed mature oocytes. However, compared to embryo cryopreservation, pregnancy rates are dramatically low. Sonmezer and Oktay [64] studied data from 21 clinical studies and reported a mean pregnancy rate per thawed oocyte of 1.52%. Even the latest studies show that the overall effectiveness of this technique is very low (<2%/thawed oocyte), despite the fact that the introduction of intra-cytoplasmic sperm injection and recent improvements in the freezing-thawing technique have resulted in somewhat higher survival rates per frozen-thawed oocyte [62, 63, 66].

An additional disadvantage of this technique is that it, like embryo cryopreservation, requires hormonal stimulation of the ovaries which is contraindicated in

some types of cancer or can cause a delay in the start of treatment. And finally, this method cannot be used in pre-pubertal girls.

For cryopreservation of oocytes to become a routine clinical procedure research should primarily focus on improving the success rate of the freeze-thaw process in addition to the evaluation of long-term health effects on the offspring born to date from frozen-thawed oocytes.

Cryopreservation of Ovarian Tissue

Until 1999, cryopreservation and autotransplantation of ovarian tissue was performed in animals only. Currently, the technique is still experimental but has led to at least 5 live births in humans [67, 68]. Although there are difficulties, this technique is potentially promising. It is the only option available for females with cancer in whom treatment cannot be delayed. It requires neither ovarian stimulation for the collection of oocytes nor a partner or sperm donor to its success.

Ovarian tissue (either cortical strips or an entire ovary) is collected laparoscopically under general anaesthesia and retrieved fragments of the ovarian cortex are subsequently frozen under strict conditions [62, 63]. Cortical strips contain vast amounts of primordial follicles and their number depends on the age of the patient [33]. Primordial follicles are much more resistant to the freeze-thawing process than mature oocytes and thus survival rates are high [33, 62]. Thawed tissue may subsequently be implanted in the patient, either orthotopically or heterotopically. Many follicles are lost due to the initial tissue ischaemia following transplantation and the survival rate after transplantation is estimated to be approximately 70% [69]. For women over 40 years of age, the benefit of this technique may therefore be limited, since the follicle yield may be too low for it to ever become a success [58]. Despite this follicle loss, recovery of the endocrine and gametogenic function of the ovary has been reported after both orthotopic and heterotopic autotransplantation of ovarian tissue, but live births have been established after orthotopic transplantation only [58, 62, 66–68].

A number of disadvantages with this technique are also recognised. Firstly, the risk of cancer cell reintroduction into the patient must not be ignored, although this risk seems to be relatively small and primarily restricted to blood-borne cancers such as leukaemia and lymphoma [66, 70]. Secondly, both laparoscopy and the general anaesthesia under which this intervention is performed have recognised morbidity rates. And finally, one must be vigilant for the transmission of viral diseases and the contamination of storage facilities [33].

Apart from women in whom treatment cannot be delayed, this method is also the only method available for pre-pubertal girls. In a recent study of 49 pre-pubertal girls due to receive potentially sterilising cancer therapy, Poirot et al. [71]

reported ovarian tissue cryopreservation to be highly feasible, safe, and acceptable to most patients and/or their families. In addition, it did not delay treatment. They concluded that cryopreservation of ovarian tissue could be systematically offered even to pre-pubertal girls at risk of sterility due to cancer treatment. These findings are in line with guidelines suggested by Weintraub et al. [72].

In conclusion, although ovarian tissue cryopreservation appears to be a safe, easy and relatively inexpensive procedure to potentially preserve female fertility, even in pre-pubertal girls, the efficacy and reliability of this method as well as the crucial issues of tissue ischaemia and cancer cell reintroduction need to be addressed in future research.

Ovarian Suppression with GnRH Analogues or Antagonists

Suppressing ovarian function by inhibition of the pituitary through the use of GnRH analogues in order to ultimately preserve fertility is a controversial method of fertility preservation, and studies in humans are limited. Some small studies render a positive effect while others show no benefit [58, 66]. In a recent study Blumenfeld et al. [70] assessed the gonadotoxic effect of chemotherapy with or without concomitant treatment with a GnRH agonist. It is the largest prospective study to date evaluating long-term ovarian function (follow-up between 2 and 15 years) in 111 female Hodgkin's lymphoma patients. Sixty-five patients received GnRH agonist treatment next to their CT treatment, 96.9% of whom resumed ovulation and regular menses compared to 3% of the controls. The number of spontaneous pregnancies were, however, not significantly different between the 2 groups.

These data suggest that ovarian damage in female patients treated for Hodgkin's lymphoma may be significantly reduced by co-treatment with GnRH agonists [70]. Therefore, GnRH co-treatment should be considered in addition to other methods of fertility preservation in women receiving gonadotoxic treatment. Combining the various modalities for a specific patient may increase the odds for preservation of future fertility [70]. GnRH analogues do not, however, protect primordial follicles from radiation damage [62].

The protective role of GnRH antagonists has been studied in animals only and data are not very promising [70].

Ovarian Transposition (Oophoropexy)

Radiotherapy may have devastating effects on the primordial follicle pool in the ovaries resulting in infertility and premature ovarian failure. An established

method to physically preserve fertility from radiotherapy to the pelvic area is oophoropexy: moving the ovaries as far out of the radiation field as possible.

Nowadays, transpositioning of the ovaries is mainly performed by laparoscopy. It is a safe, simple and effective technique which causes no delay in treatment and less adhesions compared to the previously used technique (laparotomy) [62]. The dose of radiotherapy to the ovary is reduced to 5–10% of the dose the ovary would have received *in situ*. Rates of ovarian preservation and the ability to conceive vary between 16–90% [66] and depend on the degree of scatter radiation, the presence of vascular compromise, the age of the patient, the dose of radiation, whether the ovaries are shielded, and whether concomitant chemotherapy is given [61].

The technique also has several disadvantages. Firstly, the procedure is not always reliable since the ovaries may migrate back to its original position and complications may occur [62]. Secondly, the method is only of value to patients receiving radiotherapy to the pelvic area. It does not protect the ovaries against the gonadotoxic effects of chemotherapy. Finally, even if the procedure is successful in preserving ovarian function, fertility may be compromised if the uterus remains in the radiation field.

In conclusion, although embryo cryopreservation is still the only established method for fertility preservation in female cancer patients, other experimental techniques show promising results. The prospects for ovarian tissue cryopreservation with subsequent autotransplantation are exciting and the technique has been shown not only to be feasible and safe in adult females of reproductive age but also in pre-pubertal girls. In addition, combining options of fertility preservation in order to increase a woman's chance of becoming a mother should not be overlooked.

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