

Fertility preservation for girls and young women with cancer: what are the remaining challenges?

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The excellent and balanced article by Jadoul *et al.*, in this issue of HRU, reviews the available evidence for fertility preservation in girls and young women at risk of a premature menopause. Importantly, they also present their own experience of ovarian cryopreservation in 58 cases all under 16 years old. To date there have been at least 10 pregnancies worldwide after othotopic reimplantation of frozen–thawed ovarian cortex. The success rate is unclear as the denominator (the number of women in whom frozen–thawed ovarian tissue has been reimplanted) is unknown. There have been no pregnancies reported following the reimplantation of ovarian tissue harvested pre-pubertally, but with the accepted age-related decline from birth in the number of non-growing follicles, young children are potentially ideal candidates for this procedure (Wallace and Kelsey, 2010).

Jadoul *et al.* demonstrate that it is safe and feasible to collect ovarian tissue for freezing, laparoscopically under a general anaesthetic, without complications and without delaying cancer chemotherapy. However, their case series clearly demonstrates the difficulty of giving an accurate prognosis for fertility before treatment starts, for this may change as the disease and therapeutic requirements evolve. Patients classified initially as low risk for a premature menopause (Wallace *et al.*, 2005a) may become high risk later if they relapse.

Acute lymphoblastic leukaemia is the commonest childhood malignancy with around 80% of patients becoming long-term survivors. First-line treatment of these patients is associated with an excellent prognosis for future fertility. Not only will the survivors of first-line therapy be able to have their own children naturally, but their offspring also are not at increased risk of congenital abnormalities or cancer in childhood. However, those patients who relapse after first-line treatment may require conditioning treatment with total body irradiation (TBI) and myeloablative chemotherapy, and a bone marrow transplant from an HLA-matched donor. TBI is likely to be sterilizing (Wallace *et al.*, 2005b), and in children will affect uterine development (Critchley *et al.*, 2005).

The uterus is at significant risk of damage following abdominal, pelvic or total body irradiation, in a dose- and age-dependent manner. The clinical consequences are increased risk of miscarriage and premature delivery (Sanders *et al.*, 1996). In the case of a young patient with a

pelvic sarcoma, who will require alkylating agent-based chemotherapy and radiation to the pelvis, the likelihood of a premature menopause is high (Wallace *et al.* 2005b) and, clearly, ovarian cryopreservation should be considered. However, radiation-induced damage to the uterus and surrounding structures may impair the ability of the uterus to carry a pregnancy to term (Critchley and Wallace, 2005). It remains important that these issues are discussed before treatment starts, as it is not recommended to attempt harvesting of ovarian tissue once treatment has begun (Lee *et al.*, 2006; Anderson *et al.*, 2008a). The option of harvesting ovarian tissue or freezing harvested eggs remains if the patient relapses and is due to receive potentially sterilizing chemo-radiotherapy, there is sufficient time, and the patient's health and general condition are satisfactory.

With advances in *in vitro* growth and maturation of non-growing follicles (Telfer *et al.*, 2008), it remains entirely possible that, in the not-too-distant future, reimplantation of ovarian tissue will not be necessary and fertilization of cryopreserved mature oocytes will be possible *in vitro*. The advantage of avoiding reimplantation is the avoidance of the risk of recrudescence of the original cancer, which may have been present in the stored tissue and survived cryopreservation (Rosendahl *et al.* 2010). It is imperative that harvested ovarian tissue should be examined pathologically to exclude the very real possibility of contamination of the material by malignant cells from the original cancer. This is a particular concern for haematological cancers, but is a potential concern for all cancers (Abir *et al.*, 2010). It is important to be aware that reimplantation of ovarian cortical tissue is a separate procedure at a time distant from the treatment of the original cancer. Consent for harvesting ovarian tissue from children often will have been obtained from their parents, whereas informed consent for reimplantation can be obtained from the patients at a much later date when they are competent to assess the complex issues themselves.

Should a whole ovary be removed, as advocated by Andersen *et al.*, (2008), or are cortical strips sufficient as is the majority practice (Anderson *et al.*, 2008a; Jadoul *et al.*, 2010)? The most important consideration is *primum non nocere* (first do no harm). If it remains difficult to predict which patients are at high risk of an early menopause, then conservative surgery seems sensible. However, laparoscopic

ovariectomy is a standard gynaecological operation that can be performed in peripheral centres, and Andersen *et al.* (2008) have shown that the ovary can be transported on ice to the specialized centre where it can be prepared and frozen 4–5 h after excision. There have been live births reported from reimplantation of both cortical strips and sections prepared from whole ovaries. Importantly, the preparation of the material before slow freezing appears crucial. Thin (approx 1 mm) strips of the cortex are ideal and the life of the reimplanted graft may be related to the thinness of the strips at the time of reimplantation.

It is our practice (Anderson *et al.* 2008a) to obtain informed consent before collection from the patient or guardian for disposal of the ovarian tissue if it is no longer required or the patient dies. In the event of the patient's death the material is disposed of or, if consent has been obtained, may be used for ethically approved research studies. Separately, we ask if an additional small amount of ovarian tissue can be taken at the time of collection for research studies. Our practice has been approved by the local institutional review board (IRB), and we emphasize in the patient information sheet that the procedure is voluntary and experimental, and not part of routine practice.

An intriguing question remains: should ovarian tissue that has been harvested and frozen be reimplanted to provide hormone replacement therapy (Bedaiwy MA *et al.*, 2008) or even pubertal induction in the young patient with premature ovarian failure? Silber *et al.* (2010) have shown, from studies in identical twins, that ovarian grafts will survive for at least 5 years. Indeed, several groups have reimplanted ovarian tissue once the initial graft has failed (Silber *et al.*, 2008). To our knowledge reimplantation of frozen–thawed ovarian tissue has not yet been reported in the context of the management of pubertal induction, but many groups have advocated repeated grafting for young women with premature ovarian failure for sex steroid replacement. We have always taken the view that this precious tissue should only be reimplanted if fertility is requested. Pubertal induction using hormonal treatment is well established, but there remain many questions about which sex steroid replacement regimen is appropriate for the young woman who may face many years of sex steroid therapy.

Accepting the unpredictability of the planned treatment insult, can we predict ovarian reserve for the individual patient with cancer? Clearly there is a very wide range in the number of non-growing follicles present in the individual woman. It is hypothesized that the number present determines the age at menopause when less than 1000 non-growing follicles remain (Wallace and Kelsey, 2010). Anti-Müllerian hormone (AMH) is best characterized as a product of the foetal Sertoli cells, causing regression of the Müllerian structures in the male. It is now clear that AMH is an important product of the adult ovary, produced by the granulosa cells of small growing follicles. There is good evidence that AMH declines with age and shows little variation across and between menstrual cycles. AMH is thus the best currently available marker of the number of small-growing follicles in the ovary. There is increasing information on the ability of AMH to detect chemotherapy-induced loss of ovarian reserve in survivors of cancer in childhood (Bath *et al.*, 2003); and limited data from prospective studies illustrate its ability to reflect acute gonadotoxicity (Anderson *et al.*, 2006). There is a need for more research on markers of ovarian reserve to improve our assessment of the individual patient before the onset of potentially gonadotoxic treatment. Patients with

a low ovarian reserve who are at risk of an early menopause will be at increased risk of premature ovarian failure and a reduced reproductive window, and therefore are strong candidates for consideration of fertility preservation.

The ethical considerations for children are different and more challenging from those involving adults, who are assumed to be competent to provide informed consent for an experimental procedure. Therapeutic and experimental interventions in children can only be ethical if they can be considered to be therapeutic and in the best interests of the minor. For children with cancer, ovarian tissue cryopreservation is the only realistic available option, and this will involve a laparoscopic procedure under a general anaesthetic. Probably the most important consideration when considering fertility preservation is the likelihood of a premature menopause and a very significantly reduced or absent fertility window in the individual patient. We have already seen that it is not easy to predict how the illness will evolve and what treatment exposure the young patient will receive, and therefore the absolute treatment-associated risk for early or immediate loss of ovarian function (Anderson *et al.* 2008a; Jadoul *et al.*, 2010). Our ability to assess ovarian reserve in the young patient is limited by lack of data on normal AMH values in children and uncertainty about the value of AMH in predicting ovarian reserve for the individual patient. So many uncertainties remain and add to the complexity of the consultation before the young patient with cancer begins treatment. Nevertheless, there is a definite and unquantifiable benefit of discussing issues of fertility and fertility preservation before cancer treatment starts, which include the recognition by the patient and family that long-term survival, while not guaranteed, is the expectation of the treating clinicians (Anderson *et al.*, 2008b).

The development of new experimental techniques is common in medicine. Initially enthusiasm is high and in time new techniques will find their correct place in society. However, in the field of fertility preservation there is a dearth of well-designed studies to fully evaluate exciting new techniques. There may be good reason for this. It is not likely to be feasible or indeed ethical to perform a randomized study in a well-characterized group of young women to test laparoscopic collection of ovarian cortex versus either dummy laparoscopy or indeed no intervention. It is highly unlikely that IRBs would pass such a study, or indeed that such a randomized study would be able to recruit sufficient patients. Nevertheless, when there is uncertainty about a new experimental procedure, it is important for it to be evaluated in IRB-approved clinical trials. The ASCO guideline (Lee *et al.*, 2006) recommends that ovarian cryopreservation and transplantation procedures should only be performed in centres with the necessary expertise under IRB-approved protocols that include follow-up for recurrent cancer. In Europe and North America we need to develop and accredit a small number of research-based ovarian tissue storage facilities to provide equity of access to techniques that aim to preserve fertility. All procedures should be documented and the data pooled so that we can provide a true and accurate description of the success of ovarian cryopreservation for young women at risk of a premature menopause.

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