Indications for cryopreservation of ovarian tissue

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For patients who are planning to have chemotherapy, radiotherapy or to undergo bilateral oophorectomy, loss of ovarian function will result in premature ovarian menopause and loss of fertility. For these women, although there is no successful method for the cryopreservation of human oocytes, ovarian tissue cryobanking is proposed with a view to its autotransplantation at a later date or the isolation and in-vitro maturation of oocytes. Embryo preservation is indeed not an option for single women and even for married women because delaying treatment for at least 2 months of in-vitro fertilization cycles is inappropriate and life-threatening. Following the success of animal experiments, there have been reports of ovarian cryopreservation for women having to receive chemotherapy and/or radiotherapy. We present four case reports of ovarian tissue cryobanking and review the consequences of chemotherapy and radiotherapy on gonadal function, as well as the indications for freezing ovarian tissue.

Key words: chemotherapy/cryopreservation/follicles/ovarian tissue/radiotherapy

Introduction

Advances in the diagnosis and treatment of childhood, adolescent and adult cancer have greatly enhanced the life expectancy of premenopausal women with cancer. As a result, there is a growing population of adolescent and adult long-term survivors of childhood cancer (Byrne et al., 1987). On the other hand, the current trend in many populations to delay pregnancy until a later age may increase the proportion of women with cancer not able to bear children. Thus, practitioners are dealing increasingly with the fertility outcome in the management of such cases. For this purpose, all procedures described for radiotherapy (Nahhas et al., 1971; Lushbaugh and Casaren, 1976; Hodel et al., 1982; Leporrier et al., 1987; Haie-Meder et al., 1993), chemotherapy (Viviani et al., 1985; Byrne, 1989; Gradi-
shar and Schilsky, 1989; Nicholson and Byrne, 1993; Müller et al., 1996) and surgery (Kicks and Pizer, 1992: Poynor et al., 1995; Link et al., 1996; Morris, 1996) are aimed at preserving gonadal function. For the majority of women, ovarian damage caused by radiotherapy and/or chemotherapy will result in premature menopause with its troublesome symptoms and loss of sexual interest and, in the longer term, an enhanced risk of osteoporosis and arterial vascular disease. Adequate hormone replacement therapy is a relatively easily satisfied therapy to decrease the severity of symptoms. The restoration of fertility is more difficult, and at present lies with embryo cryopreservation prior to treatment.

Sperm or oocyte cryopreservation thus has important applications in the field of oncological medicine. By inducing a blockage of the cell biological rhythm, cryopreservation has already been widely applied for fertilized oocytes at pronucleate or embryo stage. This process, widely used

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in in-vitro fertilization (IVF) programmes, is also applied for sperm cryopreservation. Indeed, in men, gamete autoconservation is successfully used in various applications: (i) preservation of reproductive potential before chemical, radiological or surgical cancer therapy which could render an individual sterile, (ii) fertility insurance before vasectomy and (iii) convenient cryobanking before infertility treatment of the partner. 

In women, gamete autoconservation is still challenging practitioners working in the fields of infertility, endocrinology, biology and paediatrics. It is therefore surprising that, in contrast to the great advances in the field of gamete cryobiology one might expect, there is still no successful method for the cryopreservation of human oocytes. Although the first pregnancy consequent upon the fertilization of a frozen–thawed oocyte was reported in 1986 (Chen) and the first live birth was reported in 1987 (Van Vem et al.), there have been only a few further pregnancies resulting from the fertilization of cryopreserved oocytes (Hwang et al., 1997; Porcu et al., 1997). The reason for the poor results of oocyte cryopreservation lies in the complexity of the oocyte itself and because of a complete lack of understanding of various biophysical changes during this process (Bernard and Fuller, 1996). The ovulating ovum is arrested in the second metaphase of meiosis, with the chromosomes attached to the delicate spindle of microtubules. The processes of cell cooling and thawing, together with exposure to cryoprotectant, affect all these cellular functions. The microtubules of the nuclear spindle depolymerize on cooling and exposure to the cryopreservation solution. Although repolymerization occurs on thawing, there is disruption of the normal process of chromatid separation, resulting in a high frequency of aneuploidy (Pickering et al., 1990). Microfilaments within the oocytes are responsible for spindle rotation, polar body extrusion and migration of the pronuclei to the centre of the oocyte prior to fusion. Disruption of these filaments will lead to failure of nuclear fusion and arrest of development of the embryo. Recently, following the success of animal experiments (Gosden et al., 1994a; Newton et al., 1996), reports have been made about ovarian tissue cryopreservation for such patients (Bahadur and Steele, 1996; Gosden, 1996; Law, 1996). Storage of ovarian tissue may provide a means of restoring long-term fertility in patients undergoing treatment that may irreversibly damage the oocyte population. Also, tissue obtained before any systemic or local treatment may provide more security with regard to genetic abnormalities for future offspring (Byrne et al., 1987; Green et al., 1989; Mulvihill, 1990; Aisner et al., 1993). There are a number of possible uses for banking ovarian tissue. Firstly, it could be done with a view to autotransplantation at a later date. Alternatively, it could be a means of storing oocytes prior to their isolation and maturation in vitro. However, there are still considerable technical and biological hurdles to overcome. Recent studies demonstrate good rates of follicular survival and normal morphology after cryopreservation, but there are many unanswerable questions about the best way for their future use. Animal experiments have shown that natural fertility can be restored by orthotopic autografting (Gosden et al., 1994a,b). However, the relevance of such procedures in humans is still questionable and, in the case of cancer, one should rule out the risk of grafting malignant cells which could cause the former disease to reappear (Shaw et al., 1996).

Others may opt to go through IVF prior to receiving treatment, producing embryos to be frozen for future use. But for women with cancer, raising hormone levels or delaying treatment for at least 2 months of IVF cycles to ensure that enough ova are collected may be inappropriate or life-threatening.

Ovarian and oocyte freezing may thus represent a real possibility. But, in such conditions, many ethical, medical and social considerations are highlighted. Is pregnancy authorized after cancer treatment? What are the risks for the mother and baby? What is the possible genetic transmission in such procreation? What is the psychology of pregnant individuals after such treatments?

The following four case reports illustrate that the natural evolution of cancer is unknown and unpredictable. Although cryopreserving ovarian tissue is untested, we consider that ovarian tissue freezing should be performed for cancer patients after informed counselling and consent from the patient. On the other hand, viability tests should be carried out on donated ovarian tissue where the donor has completed her family. We are now performing these tests in our department, having received approval from the Catholic University of Louvain Ethical Committee.

Case report 1

A 22 year old single woman with a rectal adenocarcinoma of 6 cm in size had to undergo a sigmoido-rectal resection.

In order to decrease the tumour size and increase the chance of partial resection, preoperative radiotherapy of up to 50 Gy was proposed. Ovarian transposition was proposed to preserve ovarian function. Knowing that a dose of 30 Gy provokes premature menopause in 60% of cases even in young women under 26 years of age (Shalet et al., 1976; Wallace et al., 1989) and that pelvic irradiation provokes an irradiation of 5–10% of the ovary, even if transposed outside the irradiation area, we discussed, with the
patient, the possibility of transposing the left ovary and removing the right ovary in order to freeze ovarian tissue for oocyte cryobanking. Counselling covered the issue of freezing methods and future technologies as well as the ethical considerations. Laparotomy was carried out and after left ovarian transposition, the right ovary was surgically removed and washed with saline; the outer 2–4 mm layer was dissected into cubes and frozen according to the protocol described by Newton et al. (1996). Histology of sections from a cube revealed numerous oocytes. Two weeks later, radiotherapy of the pelvis was initiated and surgery was planned to take place at the end of radiotherapy. The day before surgery, computed tomography (CT) confirmed a decrease in size of the rectal tumour up to 4 cm (Figure 1Left) and normal follicular development was seen on the transposed ovary (Figure 1Right). Menses occurred every 4 weeks. Ovulation was confirmed by progesterone >10 ng/ml during the luteal phase. During surgery, total proctectomy was carried out because of the lateral extension of the tumour and the impossibility of performing a colo-rectal anastomosis ‘in sano’. Lymph node involvement was histologically proved by histology and chemotherapy was initiated because of the local extension, the lymph node involvement and the young age. In this case, although chemotherapy was not initially proposed, it must always be considered as a possibility after surgery which could impair gonadal function even in a transposed ovary. We believe that the left ovary must be transposed and the right ovary must be removed in all cases of radiotherapy possibly associated with chemotherapy for pelvic tumours.

Case report 2

In a 25 year old married woman, Hodgkin’s disease (stage IV) was diagnosed and polychemotherapy [MOPP: methotrexate (Ledertrexate; Wyeth–Lederle, Louvain-La-Neuve, Belgium), vincristine (Oncovin; Lilly, Brussels, Belgium), procabazid (Natulan; Roche, Brussels, Belgium), prednisone (Prednisone neomycin; Asta-Medica, Brussels, Belgium; and ABV: Adriamycin (Adriamycin; Pharmacia UpJohn, Puurs, Brussels, Belgium), bleomycin (Bleomycin; Glaxo–Wellcome, Brussels, Belgium), vincristine (Velbe; Lilly, Brussels, Belgium)] followed by radiotherapy was proposed. The patient was 9 weeks pregnant and after counselling and approval from the Ethical Committee of the Catholic University of Louvain, termination of pregnancy was proposed before initiating chemotherapy. The patient requested an IVF attempt with the goal of embryo freezing. Unfortunately, this request could not be accepted because of the necessity to start chemotherapy as soon as possible. The patient then requested ovarian tissue freezing, knowing through media exposure that future technologies would become available to mature oocytes in vitro. In her case, the association of chemotherapy and radiotherapy would provoke non-reversible ovarian damage. Future technologies for the use of frozen ovarian tissue, issues concerning the viability and maturation of frozen oocytes and ethical considerations were discussed. The patient’s request to cryopreserve ovarian tissue was accepted after informed counselling and consent from the patient and approval from the Ethical Committee. A
laparoscopy was carried out and an ovarian biopsy 1.5 cm long and 3 mm wide was performed with the Palmer forceps (Storz, Tuttlingen, Germany). The ovarian tissue was placed on a Petri dish and blood was washed off with saline. It was then dissected into cubes of 1 mm in size. The freezing procedure was carried out according to the method described by Newton et al. (1996). A small piece (<1 mm in size) was histologically analysed and demonstrated the presence of follicles. Although some authors believe that ovarian tissue preservation should only be considered when the necessary surgery gives access to the ovaries without the need for additional invasive procedures, we considered that a laparoscopic procedure should be proposed to this patient when all hope of pregnancy was destroyed by the diagnosis and therapy of Hodgkin’s disease.

Case report 3

A 28 year old married, well-informed woman, with an ovarian tumour of the right ovary (Figure 2) underwent a laparoscopic procedure. The vaginal echography had revealed intracystic vegetations (Figure 2Left). Small papillary vegetations and intracystic papillary vegetations were visible and a biopsy was taken for immediate frozen analysis which revealed the presence of a serous borderline tumour. A laparoscopic salpingo-oophorectomy was carried out as well as a contralateral biopsy and peritoneal biopsies. Histology confirmed the diagnosis of a borderline tumour of the right ovary and the absence of atypical cells in the peritoneum and contralateral ovary. Three months later, a vaginal echography demonstrated the presence of a 1.2 cm cyst with intracystic papillary vegetations in the left ovary (Figure 2Right). The patient was informed of the possibility of a left ovarian borderline tumour and was encouraged, after informed consent, either to undergo an IVF attempt in order to freeze embryos or to start a pregnancy as soon as possible. In order to confirm the diagnosis of an intraovarian tumour, gonadotrophin-releasing hormone agonist (GnRHa) was given for 3 months to stop any follicles. Nevertheless, all the removed pieces of ovarian cortex were sent for freezing according to the protocol. A specimen for histology revealed the presence of very few oocytes. The patient was encouraged to initiate a second pregnancy as soon as possible because of the risk of a quick recurrence of a borderline tumour on the remnant ovary (1.2 cm in size). It must be emphasized that the patient was informed of all the risks inherent in the incomplete surgical procedure, the genetic predisposition and transmissibility of cancer. This case demonstrates that a quick recurrence of borderline ovarian tumours of the contralateral ovary can occur even if a biopsy shows the absence of pathology 3 months before.

Case report 4

A 19 year old single woman, with a right ovarian cystic tumour of 12 cm in size, underwent a laparoscopic salpingo-oophorectomy. The ovary was removed in a lapsac (Cook, Antwerpen, Belgium) to avoid spillage. The contralateral ovary looked normal. Histological examination revealed the presence of a serous borderline tumour and it was decided to perform a second-look laparoscopy 6 months later to assess the absence of peritoneal lesions and contralateral ovarian lesions. At laparoscopy, the peritoneum was normal as well as the left ovary from which a biopsy was taken, revealing normal ovarian tissue with oocytes. Four months later, the patient came back for a vaginal echography and, surprisingly, an ovarian cystic tumour of 9 cm in size was discovered (Figure 3Left and Right). A laparoscopic salpingo-oophorectomy was proposed and after informed consent concerning the immediate issues of freezing methods and future technologies, it was carried out. The ovary was removed from the peritoneal cavity in a lapsac (Cook) and placed in a Petri dish. Unfortunately, the ovarian cortex was very thin. Wide but thin layers of ovary were removed for freezing according to the protocol. Two specimens were sent for routine histological examination which failed to reveal the presence of follicles. Nevertheless, all the removed pieces of ovarian cortex were sent for freezing. Histology of the tumour...
revealed the presence of a borderline tumour. This case, as well as case report 3, highlighted the possibility of the rapid occurrence of a borderline tumour of the contralateral ovary even if a biopsy had previously demonstrated the absence of any suspected lesions. In this case, the rapid occurrence and growth of the ovarian tumour led to an overdue removal of ovarian cortex with almost no oocytes. In these four case studies, the indications and patients’ desire for cryopreservation of ovarian tissue is clear. Case reports 3 and 4 strongly suggest removing a large ovarian cortex biopsy (1 cm long, 4 mm wide) at the time of first-look surgery, that is at the time of the initial surgery for a unilateral ovarian borderline tumour.

In the following sections the current clinical applications of female gamete cryopreservation are discussed, together with the place and perspectives of ovarian tissue cryobanking.

**Gonadal function after radiotherapy and/or chemotherapy**

*Is the gonadal function of young adults impaired after therapy for malignancies during childhood or adolescence?*

In a study on proven fertility (i.e. pregnancy rates) in adult LTS (long-term survivors) of childhood cancer, Byrne *et al.* (1987) reported that men were only 76% as fertile as...
male control subjects but female LTS had approximately the same fertility rates as female control subjects who had not had cancer. In a study on potential fertility, they showed that only male LTS developed gonadal dysfunction, and that female LTS had a normal menstrual history, endocrine function, gynaecological status and ultrasonography of the gonads. However, the authors themselves suggested that the conclusions on proven fertility of their LTS in terms of pregnancy rates were too early. Others reported that menopause occurred earlier in adult women LTS than in controls (Sklar et al., 1990; Byrne et al., 1992).

Chemotherapy, radiotherapy and the pubertal stage at initiation of therapy are postulated risk factors for gonadal dysfunction as diagnosed by elevated serum gonadotrophins (Byrne et al., 1987; Haie-Meder et al., 1993; Müller et al., 1996).

Autopsy studies on the testicular (Matus-Ridley et al., 1985) and ovarian (Nicosia et al., 1985) histology of pre-, and postpubertal patients who had been treated with chemotherapy for various malignancies showed that the gonadal histology was altered regardless of the pubertal stage, sex and diagnosis.

Radiotherapy and ovarian function: risk factors

Irradiation, which is required as treatment for different types of cancer, exposes the patient to the risk of castration. Several procedures have been proposed (Nahhas et al., 1971; Lushbaugh and Casaren, 1976; Hodel et al., 1982; Leporrier et al., 1987) to preserve all the ovarian functions when radiotherapy is needed in the pelvis. Haie-Meder et al. reported in 1993 the outcome of ovarian preservation after lateral transposition in young women requiring radiotherapy with or without chemotherapy. In their study, the preservation of ovarian function was assessed at the onset of puberty among impubescent girls, during menstruation among patients with uterus preservation, and during the measurement of FSH and LH, oestradiol and progesterone concentrations in patients having undergone hysterectomy. They found that the predictive factors of ovarian function preservation were the age, the irradiation fields and dose, and the association to chemotherapy.

Age and hormonal status at diagnosis

Age by itself is a predictive factor of ovarian function. The physiological decrease in the number of primordial follicles during life makes the ovaries more sensitive to any aggressive treatment, such as chemotherapy and/or radiotherapy. In patients treated for Hodgkin’s disease, Schilsky (1981) reported a significantly shorter time from diagnosis to amenorrhoea in patients >25 years of age. Gradishar and Schilsky (1988) suggested that patients <25 years of age would not experience any significant therapy-related dysfunction during 5–10 years following completion of chemotherapy.

From the literature published to date, it is impossible to determine whether the prepubertal ovary is less susceptible to the effects of irradiation than the adult ovary. An alternative explanation for an apparently increased resistance to damage of a girl’s ovary may simply be a reflection of the earlier age, and larger number of oocytes within the ovary, rather than any effect of puberty. Furthermore, in addition to direct gonadal damage, irradiation may also affect the uterus, thus decreasing the chance of successfully carrying a pregnancy to full-term.

Irradiation fields and doses

Ovarian preservation according to the type of irradiation is known by measurements on the phantom (Haie-Meder et al., 1993). There is a significant difference in ovarian preservation according to the irradiation fields. Pelvic irradiation and inverted irradiation carry the highest probability for ovarian failure.

The dose to the less irradiated ovary

Women who received a dose ≤ 5 Gy ovarian irradiation had a higher probability of ovarian function preservation than patients who received > 5 Gy (Haie-Meder et al., 1993). In addition to dose, age has an impact on ovarian function preservation. Lushbaugh and Casaren (1976) suggested that the total dose inducing menopause was 6 Gy in women ≥ 40 years of age, while it could reach 20 Gy in girls. In the study by Haie-Meder et al. (1993), all the impubescent girls became pubescent, while the same range of doses caused menopause in 32% of the patients > 25 years of age. The dose to the ovaries, however, was the most important predictive factor of ovarian function preservation (≤ 5 versus > 5 Gy).

Association with chemotherapy

The most frequent chemotherapy combination was MOPP. When associated to radiotherapy, the incidence of ovarian function failure was significantly higher than in patients who had other or no chemotherapy (Haie-Meder et al., 1993; Apperley and Reddy, 1995).

Ovarian toxicity of combined chemotherapy regimens has been widely reported especially in women receiving MOPP for Hodgkin’s disease. MOPP produced ovarian dysfunction in 40–50% of women (Lushbaugh and Casaren, 1976; Shalet, 1980; Viviani et al., 1985). The incidence of ovarian failure reached 34% in women treated with the MOPP regimen. Haie-Meder et al. (1993) con-
sidered MOPP as one of the predictive factors of ovarian function suppression.

Girls treated for Hodgkin’s disease appeared less susceptible to ovarian dysfunction than usually occurring only in girls who had received both chemotherapy and pelvic irradiation.

In contrast to prepubertal girls treated by chemotherapy, the use of TBI (total body irradiation) for bone marrow transplantation (BMT) inflicts considerable damage. In the study of Sanders et al. (1986), 35 prepubertal girls of median age 8 years received TBI for transplants for leukaemia in Seattle. At the time of follow-up, 16 were aged 12–18 years and were progressing through puberty. Six had achieved menarche but 10 experienced delayed puberty. All were below the 50th percentile for menarche and breast and pubic hair development, and most were below the 3rd percentile. Leiper et al. (1987) also observed primary ovarian failure in three to five patients treated with TBI prepubertally.

Radiotherapy after ovarian transposition: is ovarian function and fertility preserved?

Byrne et al. (1987) showed very little reduction in women’s fertility after treatment for cancer (relative fertility: 0.93). Fertility was depressed by 25%, however, when infra-diaphragmatic radiotherapy was performed. Haie-Meder et al. (1993) found that the measured dose to the transposed ovaries was 10% of the delivered dose when the ovaries were located under the shielding blocks and 4.4% when the ovaries were outside the irradiation field. Nevertheless, the results in terms of pregnancy outcome must be evaluated with caution. In the series of Haie-Meder et al. (1993), the pregnancy outcome was 19% although ovarian function was apparently preserved. This rate was significantly lower than the rate in the general population (Haie-Meder et al., 1993). Interpretation is difficult because this low rate can also reflect a patient’s personal choice, or an effect of radiotherapy on the uterus itself which could affect implantation.

Ovarian transposition appears to be a good method to protect ovaries from the risk of castration. This risk of castration was lowest in women <25 years of age, with a radiation dose to the ovaries of <5 Gy and without MOPP chemotherapy. As the total radiation dose is the most important prognostic factor, special attention must be paid by the radiotherapist to try to leave the ovaries outside the shielding blocks of the radiation fields as this decreases the dose by 40–50%.

Combination chemotherapy and ovarian damage

In 1971, Miller et al. reported the autopsy findings in a 13 year old girl who had died of infectious complications following long-term (29 months) administration of 50–100 mg cyclophosphamide daily for juvenile rheumatoid arthritis. The ovaries were completely devoid of oogonia and developing follicles. Although the toxic effects of daily cyclophosphamide on ovarian function in adult women were well recognized, subsequent reports of such treatment in girls suggested that the prepubertal ovary was relatively resistant to therapy. Similarly, reports of girls treated for ALL (acute lymphoid leukaemia) suggested that those diagnosed pre-pubertally did not experience any problems with achieving menarche, whereas those treated during and after puberty were at higher risk of developing abnormal gonadotrophins and amenorrhoea.

Light microscopic studies revealed a marked reduction in the number of primordial and primary follicles in patients with Hodgkin’s disease who had undergone chemotherapy (Sobrinho et al., 1971; Chapman et al., 1979; Chapman and Sutcliffe, 1981). According to Chapman and Sutcliffe (1987) and Blumenfeld et al. (1996), some protection is conferred by giving them contraceptive pills or GnRHa therapy prior to chemotherapy. Indeed, the number of primordial and primary follicles in ovarian biopsies is then higher than in ovaries of patients who do not receive contraceptives (Chapman and Sutcliffe, 1981). In women receiving three different multi-drug chemotherapeutic protocols: MOPP, ABVD, and MOPP plus ABVD, Familiari et al. (1993) demonstrated that the administration of medroxyprogesterone acetate (MPA) to patients with Hodgkin’s disease protects the ovary against an acute effect of chemotherapy. Nevertheless, chronic ovarian damage (atroia) was found to be progressive despite this pharmacological protection. MPA protected follicles only from the acute, toxic effects of chemotherapy, which dramatically reduced their number and led to sterility. However, the quality of follicles was still impaired, and many underwent atresia, resulting in a shortened fertility period.

The study of Familiari et al. (1993) suggested that gonadotrophins may play a role by modulating the local ovarian factors that promote atresia in small follicles. Apart from a small reduction in the number of follicles after chemotherapy and associated hormonal protection, atretic features were also frequently found in the pool of surviving follicles, thus suggesting an additional, chronic effect of chemotherapy on the ovary. Atretic degeneration is a physiological process by which the follicle loses its integrity. Approximately 400 000 oocytes are present in the human ovary at the onset of puberty, but only ~400 are
likely to be ovulated and the remainder are eliminated (Centola, 1983). The percentage of atretic follicles present at all stages of development during the reproductive period is rather constant, reaching 99.9% in humans. The integrity of the oocyte is strictly dependent on its closely associated follicular cells, and particularly on the correct morphodynamic activity of the entire granulosa layer. When oocytes reach the diplotene stage in fetal life, they must become enclosed in a follicle, otherwise they invariably degenerate (Byskov and Hoyer, 1988). Since the normal activity of a follicle requires that its cells maintain their structural integrity, the observations of Familiari et al. (1993) on slightly altered cells in a greater number of primordial follicles indicate that factors produced by these cells are modified by chemotherapy drugs, which lead to a subsequent increase in the rate of atresia.

As seen by electron microscopy, the majority of primordial follicles from completely untreated or MPA-treated patients were morphologically comparable to healthy follicles (Dvorak and Tesarik, 1980; Stankova et al., 1985; Familiari et al., 1993). In patients who had been subjected to chemotherapy after MPA treatment, the number of follicles was slightly reduced when compared to the total numbers or to the marked loss observed in patients without hormonal protection (Sobrinho et al., 1971; Chapman et al., 1979). On the other hand, in these patients an increased frequency of the cellular features typical of early atresia was observed in both oocytes and their companion follicle cells. This occurred after four or five menses following completion of the chemotherapeutic cycle. Thus, chemotherapy seems to produce not only acute damage, leading to a reduction in the number of follicles, but also chronic damage to the quality of follicles which easily undergo atresia. Furthermore, the latter damage is not inhibited by treatment with MPA.

Marcello et al. (1990) observed a reduced number of primordial follicles which were otherwise normal in the ovaries of children who had chemotherapy for leukaemia. The discrepancy between the Marcello et al. (1990) and the Familiari et al. (1993) studies can be explained by the age of the patients. Marcello et al. (1990) studied many young patients (average age 8 years), whereas the Familiari study concerned adult women with an average age of 26.9 years. Chemotherapy causes more damage to the pool of primordial follicles belonging to adult ovaries.

**Indications for ovarian tissue cryopreservation**

We were surprised in the gynaecological clinic to find that many survivors of childhood or adolescent cancer answered ‘no’ when asked if a doctor had told them about their ovarian function and that they had cancer. They need to be informed of the long-term consequences of cancer and its therapy, even if they do not ask and even if not all treatments cause infertility.

Although most patients and their families will not refuse life-saving treatment because of possible future infertility, knowledge of health risks can allow survivors to make conscious choices regarding health behaviours.

The option of sperm banking before receiving potentially fertility-ending therapy exists. Not all apparently infertile patients remain unable to have children indefinitely. Indeed, some patients recover fertility years after treatment has ceased (Shalet et al., 1985; Green et al., 1991; Swerdlow et al., 1996) and use of birth-control methods probably is recommended if the patient does not desire pregnancy, even if fertility seems unlikely. Moreover, the use of the oral contraceptive pill gives the advantages of hormone replacement therapy and preserves ovarian function to a certain extent if the ovarian follicle reserve has been impaired.

Cryopreservation of ovarian tissue should thus be seriously considered for any patient undergoing treatment likely to impair future fertility. Treatment should be indicated for pelvic, extra-pelvic and/or systemic diseases. The age of the patient should also be taken into consideration, because the contents of the ovary are not the same in prepubertal and postpubertal women. Because a decline in fertility is now well documented after the age of 38 years, the procedure should probably be limited to patients below this limit. On the other hand, whatever the disease, when surgery can preserve the genital tract, irradiation and/or chemotherapy seem less harmful to the gonads in prepuberty than in postpubertal women (Haie-Meder et al., 1993; Sanders et al., 1996).

**Treatment for pelvic diseases (Table I)**

Two groups of patients can be distinguished in this category: women with or without gynaecological malignancy. In the case of non-gynaecological malignancy, surgery spares the female genital tract when there is no metastasis. In such cases, the fertility outcome is conditioned by the adjuvant therapy, i.e. local radiotherapy and/or chemotherapy. For chemotherapy, the rules are the same as those which will be discussed later in the section concerning systemic diseases. For radiotherapy, it has already been stated that a dose of 5–20 Gy administered to the ovary is sufficient to completely impair gonadal function (Lusbaugh and Casaren, 1976; Mulvihill et al., 1987; Haie-Meder et al., 1993), whatever the age of the patient. Even if ovarian transposition has been performed, this cannot be
considered completely safe because patients receive infra-diaphragmatic irradiation (Baker, 1971) and in many cases there is an association between radio- and chemotherapy (Baker, 1971; Hodel et al., 1982). Such pathologies represented 8.1% of cases in the series of Haie-Medder et al. (1993) with an occurrence of pelvic sarcoma in 2.1% of cases and pelvic sacroblastoma, rhabdomyosarcoma and teratoma in 6% of cases. Tumours of the large intestine and sacrum should also be considered in this group.

Table I. Indications for cryopreservation of ovarian tissue in cases of malignant disease

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Fertility is essentially impaired by radio- and/or chemotherapy.

In the case of gynaecological malignancies, a conservative fertility approach is only valuable if the uterus can be spared during surgery. This includes cases of early cervical carcinoma (Schneider et al., 1996), early vaginal carcinoma (Kicks and Pizer, 1992; Haie-Meder et al., 1993), early vulvar carcinoma (Kicks and Pizer, 1992; Haie-Meder et al., 1993) ovarian tumours of low malignancy (Link et al., 1996) and some selected cases of unilateral ovarian carcinoma, stage IA1 (Kleine, 1996). Apart from a possible conservative surgical approach in these patients, the issue of such treatment alone is still controversial, and all the results were obtained on the basis of retrospective studies and/or case reports. The use of ovarian tissue cryobanking offers two advantages: the possibility of removing both ovaries for more accurate management and the possibility of administering pelvic radiation in selected cases for the completion of treatment. These are possible indications but the practitioner should be aware of the effect of radiotherapy on the uterus, and also that such patients may not be good candidates for ovarian tissue grafting. Future experiments should enable us to answer questions about the relevance of replacing residual malignant cells through grafted tissue in such cases.

**Treatment for extra-pelvic diseases**

These are essentially cases of bone (osteosarcoma, Ewing sarcoma), thyroid, kidney and breast cancers and also cases of melanoma, neuroblastoma and bowel malignancies. In such cases, surgery does not involve the genital tract and effects on fertility are essentially caused by radiotherapy and/or chemotherapy. Radiotherapy is essentially a supra-diaphragmatic application but its consequences on the gonads could be deleterious in 10% of cases (Haie-Meder et al., 1993) with this increasing to 25% if chemotherapy is performed in addition to radiotherapy. It also seems that these patients may present a menopausal status earlier than non-treated women (Byrne et al., 1992). In cases of Wilms' tumours, genital malformations should be excluded before ovarian tissue cryopreservation, as in this group of women there is an increased risk of such anomalies (Nicholson et al., 1996). For breast cancer, the highest risk is induced by chemotherapy agents and this will be discussed below. In exceptional cases, where castration is indicated, ovarian tissue cryopreservation seems a good alternative for possible future fertility.

**Treatment for systemic diseases**

This concerns many cancers, mostly arising during adolescence or childhood, including Hodgkin’s disease, non-Hodgkin’s lymphoma, leukaemia, medulloblastoma and other systemic malignant conditions. For all such conditions, treatment consists of an association of radiotherapy and/or chemotherapy, with an increased use of marrow transplantation.

For radiotherapy, supra- or infra-diaphragmatic irradiation or a combination of both is used. In cases of supra-diaphragmatic irradiation, the risk of impairment of gonadal function is ~10%, and with infra-diaphragmatic irradiation this risk increases to 35% (Haie-Meder et al., 1993). These percentages are dependent on the total dose
and distribution of the dose administered. The association of irradiation with chemotherapy significantly increases the risk (Byrne et al., 1992). For chemotherapy, the highest risk seems to be linked to the use of alkylating agents and seems to be dose and age dependent (Chapman et al., 1979; Horning et al., 1981; Whitehead et al., 1983; Nicholson et al., 1996).

Complete amenorrhoea was reported after a dose of 5 g of cyclophosphamide in women >40 years of age, and after doses of 9 and 20 g respectively in women of 30–40 and 20–30 years of age (Shalet, 1980). An association of many chemotherapeutic agents increases the gonadal toxicity.

Conclusions

Unfortunately, even the successful development of embryo cryopreservation will be unable to restore fertility to three groups of patients, i.e. premenarchal girls, women without male partner and patients for whom chemotherapy cannot be delayed. Ovarian cryopreservation may overcome these problems. In particular, it will involve the freezing of immature oocytes, specifically primordial follicles, which have not entered the second meiotic metaphase with spindle development, and are therefore less susceptible to the toxic effects of cryopreservation. Recently, Gosden et al. (1994b) achieved considerable success in the fertilization of frozen–thawed ovarian tissue from mice and sheep. They were also able to demonstrate the presence of normal primordial follicles in thawed cat or sheep ovarian biopsies transplanted under the renal capsule of SCID mice (Gosden et al., 1994a). There may be many potential indications for ovarian tissue cryopreservation. Indeed, careful evaluation of all parameters, such as the type of disease, the survival prognosis, the age, the dose and type of treatment, should be carried out before candidate selection for such procedures. On the other hand, respecting the code of good practice, all patients who may be infertile have the right to receive proper consideration of their interests for future possibilities. The selection of cases should be carried out on the basis of a multidisciplinary staff discussion including oncologists, gynaecologists, biologists, psychologists and paediatricians. Informed counselling and consent from the patient should be obtained. It should, however, be emphasized that cryopreservation of ovarian tissue is as yet untested and viability tests should be performed before any clinical applications are carried out. Thus, great effort is still required from researchers in order to reach the ultimate goal after ovarian tissue cryobanking and the restoration of fertility with a view to obtaining healthy babies. Future experiments should be carried out to determine: (i) the potential for each type of cancer to disseminate malignant cells into the ovarian cortex; (ii) the mechanisms of revascularization of slices of ovarian cortex after freezing; (iii) the type and degree of injury to human oocytes within the ovarian tissue according to the protocol of cryopreservation and the cryoprotectant used; (iv) the type of culture and incubation needed for in-vitro maturation of primordial follicles; (v) the quality of oocytes after in-vitro maturation of follicles which may be long term.

Advice to the patient and clinician

When fertile survivors of cancer consider pregnancy, they may experience considerable anxiety generated by the fear of having an unhealthy child or by the fear that their health may not be good enough to complete a pregnancy. Four-fold more adverse pregnancy outcomes (defined as miscarriage, preterm delivery, or infants with birth defects) were observed in survivors of Wilms’ tumours (Mulvihill et al., 1987). Excess rates of perinatal and neonatal mortality and low birth weight (Sanders et al., 1996) were found in women treated with abdominal radiation. These studies suggest that radiation-induced damage to the uterus may impair adequate expansion, possibly leading to early delivery. Thus, women who have had previous abdominal or pelvic radiation therapy warrant special surveillance during pregnancy. Cardiac evaluation or pulmonary function testing before pregnancy is indicated in women who received therapy known to induce pulmonary fibrosis or pulmonary radiation (Davis and Brown, 1988; Collis, 1991). Women survivors of cancer, whose therapy impairs gonadal function because of the increased risk of premature menopause (Byrne, 1992), should be encouraged to have children sooner and women must also have the knowledge that allows them to make informed choices about their fertility and their possibility of pregnancy. Infertility programmes that offer IVF provide many infertile couples the opportunity to bear children (Edwards et al., 1991). These technologies may also offer hope to survivors of cancer who have no other prospects of pregnancy. Ovarian tissue freezing for cancer patients should be performed only after informed counselling and consent from the patient. It should be emphasized that cryopreserving ovarian tissue is untested and is likely to be increasingly undertaken. In our department, viability tests are presently being carried out on donated ovarian tissue where the donor has completed her family. Although there are relatively few clinical situations where young women are advised to have surgical treatments which on their own or because of subsequent chemotherapy or radiotherapy will lead to inevitable infertility, everything has to be done to preserve ovarian function as far as possible in order to allow young LTS women to become pregnant.
The four case reports described here demonstrate that the evolution and outcome of cancer is often unpredictable and that an association of chemo-radiotherapy is sometimes required after surgery, even if the presurgery diagnosis had not considered this therapeutic possibility. It is therefore recommended that a large laparoscopic ovarian biopsy be taken (at least from one ovary) before any therapy which could seriously impair ovarian function, even with ovarian transplantation.

References


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