Cancer is not uncommon in children. The reproductive system is an important site for late effects of cancer treatment, and normal pubertal development depends on an undamaged hypothalamic-pituitary-gonadal axis. Fertility compromise can occur due to chemotherapy, radiotherapy to the hypothalamic-pituitary-gonadal axis, or surgery. Cryopreservation techniques of germ cells are improving and may offer hope for fertility preservation.

**Table 1. Incidence and survival figures for common childhood malignancies**

<table>
<thead>
<tr>
<th>Malignancy</th>
<th>Incidence/100 000/year</th>
<th>Cure rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALL/non-Hodgkin's lymphoma</td>
<td>5.0 - 6.0</td>
<td>78 - 80</td>
</tr>
<tr>
<td>Hodgkin's lymphoma</td>
<td>0.4</td>
<td>&gt;90</td>
</tr>
<tr>
<td>Brain tumours</td>
<td>4.0</td>
<td>Depends on type</td>
</tr>
<tr>
<td>Wilms' tumours</td>
<td>0.9</td>
<td>80</td>
</tr>
</tbody>
</table>

ALL = acute lymphoblastic leukaemia.
Mechanisms of damage to ovarian function

The pharmacological action of chemotherapy is mainly aimed at disrupting the process of DNA synthesis and cell replication. In general the alkylating agents interact with DNA, preventing replication and/or transcription. Anti-tumour antibiotics such as actinomycin D work on the same principle. Other agents may damage the structure of DNA directly, while adriamycin acts by disrupting the process of DNA synthesis and cell replication. Anti-tumour antibiotics such as the taxanes disrupt the function of tubulin, which is critical in the normal mitotic process.

Specific chemotherapy agents, particularly the alkylating ones such as cyclophosphamide and chlorambucil, may cause permanent DNA damage in ovarian follicles. Other chemotherapy agents are less harmful, and include 5-fluorouracil, methotrexate, etoposide and adriamycin. There are various mechanisms of damage to the ovaries. The damage may be directly to the primordial follicles with death of follicular cells. Human and animal studies have shown that chemotherapy can damage ovarian pre-granulosa cells, with increased apoptosis during oocyte and follicle loss. Vascular effects associated with antineoplastic agents have been reported, and recognised mechanisms for such toxicity include drug-induced endovascular damage.

In a descriptive study Meirow and co-workers studied the histological features of ovarian tissue from 17 women exposed to chemotherapy and compared them with 18 patients who were not exposed. The pathologists were blinded for patient characteristics. They found injury to blood vessels and focal fibrosis of the ovarian cortex in ovaries of patients previously exposed to chemotherapy. Blood supply for the ovarian cortex is by an end-artery system and the cortex is a fairly poorly oxygenised tissue. After chemotherapy there is prominent thickening and narrowing of the vessels, and neo-vascularisation with abnormal blood vessels to the ovarian cortex is seen on microscopy. There is also cortical fibrosis. Direct damage to the follicles can also be seen after chemotherapy.

Mature follicles are more vulnerable to chemotherapy damage. Certain endocrine mechanisms may play a role in the damage to larger follicles. Anti-Müllerian hormone (AMH) is mainly secreted by growing follicles and AMH levels drop significantly during therapy. A drop in AMH may cause raised recruitment and make more follicles vulnerable to damage due to chemotherapy.

AMH may be used as a marker of ovarian reserve. Serum AMH levels can be measured to assess sub-clinical ovarian damage in patients treated with chemotherapy. A possible mechanism to protect ovarian function may be to administer anti-Müllerian hormone during treatment with chemotherapy to reduce recruitment of follicles.

Age at treatment

One of the most important clinical factors that influence the risk for permanent ovarian damage is age at treatment. The risk for ovarian failure increases with age (Table 3). This is because the number of primordial follicles is far higher at a younger age.

Amenorrhoea due to chemotherapy is more common in women who were over the age of 30 years at the time of treatment (50% of all malignant women exposed to chemotherapy who reached menopause due to chemotherapy were 30 years of age or younger).

**Table 2. The relative frequencies (%) of cancers in adolescents and young adults aged 15 - 29 years**

<table>
<thead>
<tr>
<th>Age (yrs)</th>
<th>Lymphoma</th>
<th>Leukaemia</th>
<th>Central nervous system</th>
<th>Endocrine system</th>
<th>Skin</th>
<th>Male genital</th>
<th>Female genital</th>
<th>Bone and joint</th>
<th>Soft tissue</th>
<th>Digestive system</th>
<th>Oropharynx</th>
<th>Respiratory system</th>
<th>Urinary system</th>
<th>Breast</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 - 19</td>
<td>26</td>
<td>12</td>
<td>10</td>
<td>9</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>5</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>20 - 24</td>
<td>22</td>
<td>7</td>
<td>7</td>
<td>12</td>
<td>14</td>
<td>13</td>
<td>8</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>25 - 29</td>
<td>16</td>
<td>4</td>
<td>5</td>
<td>11</td>
<td>18</td>
<td>11</td>
<td>12</td>
<td>1</td>
<td>2</td>
<td>5</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

**Table 3. The effect of age on the rate of premature ovarian failure after cyclophosphamide chemotherapy**

<table>
<thead>
<tr>
<th>Age (yrs)</th>
<th>Premature ovarian failure (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;20</td>
<td>13</td>
</tr>
<tr>
<td>20 - 30</td>
<td>50</td>
</tr>
<tr>
<td>&gt;30</td>
<td>100</td>
</tr>
</tbody>
</table>
- 89%) compared with younger women, where normal menses was preserved in 48 - 100% of cases.\textsuperscript{20-28} Chemotherapy-related amenorrhea may be transient. However, if the condition is present for more than 1 year after treatment, less than 11% of women over the age of 40 years and only 12 - 15% of women younger than 40 will experience a return to menses.\textsuperscript{26}

**Treatment for young people with cancer**

The most common forms of malignancies affecting young people and the treatment for these malignancies are summarised in Table 4. It is clear that multi-agent chemotherapy regimens and radiation may contribute to reproductive failure. It is often difficult to determine the individual effect of specific therapies on fertility outcome.

**Haematological malignancies**

There are a significant number of reports in the literature about the effects of chemotherapy on fertility and hormonal function after treatment for haematological malignancies in younger women. Treatment for Hodgkin's lymphoma with MVPP (mechlorethamine, vinblastine, procarbazine and prednisolone), MOPP (mechlorethamine, vincristine, procarbazine and prednisolone) or CHVP (chlorambucil, vinblastine, procarbazine and prednisolone) resulted in permanent ovarian failure in 19 - 63% of cases.\textsuperscript{25-27} Treatment for acute lymphoblastic leukaemia (ALL), however, had less long-term risk for permanent amenorrhea.\textsuperscript{23,24} Conditioning with chemotherapy before bone marrow transplantation is usually associated with transient amenorrhea. Cyclophosphamide doses of 200 mg/kg caused amenorrhea in all women on treatment, but all recovered normal ovarian function after bone marrow transplantation.\textsuperscript{23} Doses higher than 200 mg/kg may cause premature ovarian failure.\textsuperscript{24} Multi-agent combination chemotherapy regimens may have synergistic toxicity, and the specific contribution of each agent is often difficult to determine.

**Breast cancer**

In the USA breast cancer is the most common cancer in women of reproductive age (<40 years of age), and approximately 13% of all breast cancer diagnoses are made in women younger than 45.\textsuperscript{25} Alkylating agents (e.g. cyclophosphamide) are often included in the treatment plans for breast cancer. The higher the cumulative dose of cyclophosphamide, the higher the risk of premature menopause. In cases treated with CMF (cyclophosphamide, methotrexate and 5-fluorouracil) the incidence of amenorrhea was 61% in patients younger than 40 and 95% in patients older than 40 years.\textsuperscript{26} A slightly higher incidence of amenorrhea was found with a regimen containing FEC (fluorouracil, epirubicin and cyclophosphamide) compared with CMF (51% v. 42.6%).\textsuperscript{26} Anthracycline-based regimens had a lower incidence of amenorrhea.\textsuperscript{15} There is very little evidence with regard to taxanes and the risk of subsequent amenorrhea, but there does not appear to be an increased overall risk when it is added to chemotherapy regimens.\textsuperscript{27}  

**Pregnancy after chemotherapy**

Maltaris et al. summarised the obstetric outcome in patients with previous epithelial ovarian carcinoma after receiving fertility-preserving treatment.\textsuperscript{27} Not all of the patients received chemotherapy. A total of eight studies were included in the review, and 113 pregnancies were described out of the total of 282 patients. The number of term deliveries was 87. In this group the number of reported relapses of ovarian carcinoma was 33 and that of disease-related deaths 16.

In a study reported by Newlands et al., cyclophosphamide therapy for choriocarcinoma was associated with a reduction in the fertility rate when compared with treatment with methotrexate only;\textsuperscript{29} 79% of the total number of patients desiring pregnancy had at least one live birth after cyclophosphamide, compared with 86% in those who received methotrexate only.

**Radiotherapy damage to hormone production and fertility in women**

The extent of radiotherapy damage to ovarian function and reproduction is determined by the total dose of radiation, the fractionation schedule, and the age of the patient at the time of treatment.\textsuperscript{13,29} The human oocyte is exquisitely sensitive to the damaging effects of radiation, and the estimated median lethal dose (LD\textsubscript{50}) is less than 4 Gy.\textsuperscript{13} A descriptive study by Wallace et al. found that 37 of 38 females developed ovarian failure after whole-abdominal irradiation in childhood of 20 - 30 Gy; 71% had primary amenorrhea, i.e. never had normal pubertal development, and premature menopause occurred in the rest at a median age of 23.5 years.\textsuperscript{30} Total-body irradiation (TBI) is sometimes used alone or in combination with cyclophosphamide as conditioning for bone marrow transplantation. This treatment is often associated with infertility, and only a small number of patients (9 out of 144) had normal ovarian function after TBI at a dose of 9 - 16 Gy combined with cyclophosphamide 120 mg/kg before bone marrow transplantation. The effect of age at treatment was also demonstrated in this study, with a greater probability of recovery of ovarian function observed in younger girls.\textsuperscript{33}

The uterus may be damaged by radiotherapy, and reduced uterine volume and decreased elasticity of the myometrium can be found in girls who received pelvic or abdominal irradiation or TBI.

| Table 4. Treatment of childhood cancers\textsuperscript{44} |
|-----------------------------|-----------------|-----------------|
| **Chemotherapy** | **Cranial radiation therapy** | **Gonadal radiation therapy** |
| Acute lymphoblastic leukaemia | + | ± | ± |
| Non-Hodgkin's lymphoma | + | ± | ± |
| Hodgkin's lymphoma | + | - | ± |
| Brain tumours | ± | ± | ± |
| Wilms' tumour | + | - | ± |
before puberty.\textsuperscript{43} Even though successful pregnancies following radiotherapy have been reported, there is an increased incidence of miscarriage, intra-uterine growth restriction and premature delivery.\textsuperscript{31} It is difficult to measure uterine damage after exposure to radiotherapy, but endometrial sampling may help in the assessment of endometrial function. Exact prediction of eventual reproductive outcome, however, is very difficult.

It is clear from Table 5 that the risk of premature ovarian failure is higher if the patient receives treatment at an older age.

<table>
<thead>
<tr>
<th>Dose (Gy)</th>
<th>Ovary</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.6</td>
<td>No deleterious effect</td>
</tr>
<tr>
<td>1.5</td>
<td>No effect in &lt;40 yrs</td>
</tr>
<tr>
<td></td>
<td>Some risk of POF in &gt;40 yrs</td>
</tr>
<tr>
<td>5</td>
<td>60% sterile &lt;40 yrs</td>
</tr>
<tr>
<td></td>
<td>100% sterile &gt;40 yrs</td>
</tr>
<tr>
<td>8</td>
<td>70% sterile &lt;40 yrs</td>
</tr>
<tr>
<td></td>
<td>100% sterile &gt;40 yrs</td>
</tr>
<tr>
<td>&gt;8</td>
<td>100% sterile</td>
</tr>
</tbody>
</table>

POF = premature ovarian failure.

Ovarian trans-position outside the field of radiotherapy may reduce the dose to the ovary. Howell and Shalet described how lateral transposition of the ovaries to the para-colic gutters may reduce the radiotherapy dose by up to 95%.\textsuperscript{52} This may protect the sensitive follicles from direct dose-related damage. Other reports, however, were less optimistic and found that ovarian transposition may compromise blood supply, and there was mixed success with this technique due to scattered radiation and vascular compromise.\textsuperscript{43} Ovarian transposition may have a place in cases where the pelvic dose of radiotherapy is not high enough to be damaging to the other organs in the reproductive tract.

Cranial irradiation may cause hypo-pituitarism in doses over 30 Gy.\textsuperscript{43,44} Up to 60% of patients experienced a gonadotropin deficiency 4 years after treatment with cranial irradiation.\textsuperscript{45} Effects on other pituitary hormones such as growth hormones have also been reported.\textsuperscript{46} In a report by Nygaard et al., a cranial radiation dose of between 18 and 24 Gy was identified as a possible risk factor for a significantly lower first birth rate compared with women without any radiation.\textsuperscript{47} The presence of a regular menstrual cycle may not be an adequate indication of hypothalamic-pituitary function, and sub-fertile women who received cranial radiation need careful hormonal assessment.

Chemotherapy and testicular function

In men endocrine and exocrine functions of the gonads are separated. The average age of spermarche is 13.4 years. The alkylating agents are gonadotoxic, with procarbazine particularly harmful. This is a very useful drug used in the treatment of Hodgkin’s lymphoma, where repeated courses of alkylating agents are often needed. The Sertoli and germ cells are more chemo-sensitive than the Leydig cells; a patient with normal testosterone production may therefore have azoospermia.\textsuperscript{48} Azoospermia is likely if the volumes of the post-pubertal testes are less than 10 ml each as measured by the Prader orchidometer.

It is clear that in multi-agent chemotherapy regimens there may be synergistic toxicity of individual agents, and it is often very difficult to determine the specific contribution of each agent. Certain agents have been identified as particularly gonadotoxic to the testes, including the alkylating agents procarbazine, cisplatinum and vinblastine.\textsuperscript{46,48} Cyclophosphamide may cause azoospermia in up to 13% and oligozoospermia in 30% of patients treated with a total dose of 560 - 840 mg/kg.\textsuperscript{49} Ifosfamide is sometimes used for the treatment of sarcomas, and a dose of between 84 and 126 mg/m\textsuperscript{2} was associated with impaired spermatogenesis.\textsuperscript{50} Newer combinations such as the ABVD combination (adriamycin, bleomycin, vinblastine and decarbazine) have been shown to be less gonadotoxic, with full testicular recovery after 18 months of treatment in nearly all patients.\textsuperscript{26} The testicular seminiferous epithelium that is responsible for spermatogenesis is more sensitive to the effects of chemotherapy; however, the Leydig cells are more resistant to damage and in certain cases, although secondary sexual characteristics may develop normally, there may be severe impairment of sperm production.\textsuperscript{49,51} In higher cumulative doses Leydig cells may also be damaged,\textsuperscript{52} but this rarely occurs in clinical practice.

Radiotherapy and testicular function

Radiotherapy may damage the hypothalamic-pituitary axis if the dose is more than 30 Gy to the cranial region. Radiotherapy may also damage the testes; the damage may be reversible if the dose is between 20 and 200 cGy, but irreversible azoospermia will develop over 400 cGy. Low production of testosterone will only occur when the dose goes above 1 500 cGy.

Pubertal development is usually normal after treatment with TBI in preparation for bone marrow transplantation.\textsuperscript{48} It was found that these boys had slightly higher levels of follicle-stimulating hormone and that mean testicular volume was lower than normal at an average of 10.5 ml. Luteinising hormone (LH) was elevated, which may indicate subtle hormonal dysfunction of the Leydig cells.\textsuperscript{53} Other reported studies found no change in LH levels after preparation for bone marrow transplantation.\textsuperscript{51,52}

Conclusion

‘First do no harm’ is an important precept of Hippocrates (around 460 - 377 BC). Non-maleficence, which is the ethical principle that flows from this, is fundamental to medical ethics. Beneficence refers to actions that promote the wellbeing of others. In the management of serious disease such as cancer there is often a conflict between these two moral imperatives. The obligation not to harm others is usually more stringent than the obligation to help others. In an effort to cure cancer (an act of beneficence) the treatment itself may at the same time also cause significant harm. Many treatments have serious, harmful late effects including an effect on future fertility and hormone production. Every young patient deserves safe and effective oncological treatment but with the least long-
term negative effects. A multi-disciplinary team approach involving oncologist, surgeon and counsellor should include a reproductive health specialist if fertility may be compromised by cancer therapy. Clinicians should consider the long-term side-effects of treatment before mapping out a treatment plan, and use fertility-sparing techniques where appropriate.

It is important to consider collecting a semen sample before the initiation of chemotherapy. Cryopreservation of sperm is a well-known technique and has excellent outcomes. If pre-pubertal boys cannot produce a sample through masturbation, testicular biopsies may be harvested and stored before treatment is started. Gonadotoxic therapy, and ovarian tissue, testicular tissue, sperm and ova may be harvested and stored before treatment is started. Cryotherapy techniques offer real hope to boys and girls who need ovarian tissue or oocyte cryopreservation.

There are indications that, with improving technical ability, slow-freezing of oocytes is becoming more efficient with increases in survival rates of individual oocytes, better fertilisation rates and ultimately an improvement in pregnancy rates. More recently, vitrification (ultra-rapid cooling technique) of human oocytes has been described, and the success rate after vitrification seems to be improving.

Despite the experimental nature of ovarian tissue cryopreservation, ovarian tissue harvesting and banking has been offered to many patients in clinical practice over the past two decades. Case reports of successful pregnancy from cryopreserved ovarian tissue give hope that this technique may become part of clinical practice.

Cryotherapy techniques offer real hope to boys and girls who need gonadotoxic therapy, and ovarian tissue, testicular tissue, sperm and ova may be harvested and stored before treatment is started.


