

## Mini-review

# Treatment of gonadal damage in recipients of allogeneic or autologous transplantation for haematological malignancies

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### Summary:

**Management of iatrogenic gonadal reproductive failure and sexual morbidity assumes a priority, especially in young recipients of high-dose chemotherapy and stem cell transplantation (SCT). Hormone replacement treatment (HRT) is beneficial for correction of sexual symptoms and osteoporosis in both sexes, especially in females. Sperm banking is the standard technique for preservation of fertility in adult and sexually mature adolescent males. Testicular tissue cryopreservation has a place in well-selected azoospermic adults and in mentally and sexually competent adolescents. *In vitro* fertilisation using superovulation with embryo-cryopreservation (for future embryo transfer) is the most tried method in female SCT recipients with good results. In mentally and sexually competent adolescents and adults without a partner, ovarian cortical tissue cryopreservation has a place for subsequent re-implantation to orthotopic or heterotopic sites. Gonadotrophin releasing hormone (GnRH) co-treatment during chemotherapy, is a promising method for the future. Although generally reassuring, continued monitoring of the offspring of SCT survivors and follow-up of all recipients of SCT is important for return of spontaneous or induced fertility.** *Bone Marrow Transplantation* (2002) **30**, 629–635. doi:10.1038/sj.bmt.1703721

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focuses on the incidence of this problem, pathophysiology including factors promoting recovery, therapeutic options and future directions and research strategies.

### Incidence

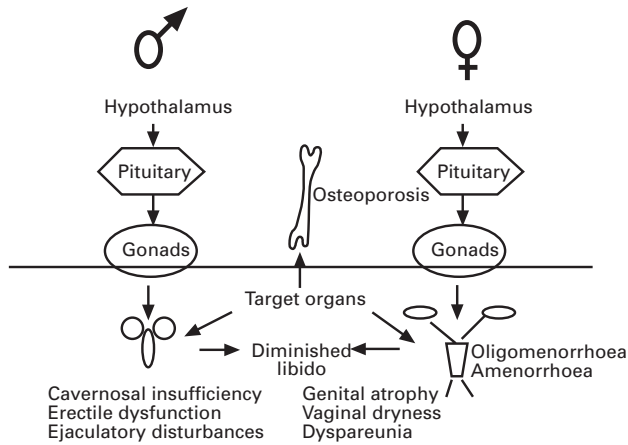
The exact incidence of SCT-induced hypogonadism is unknown. This is due to the lack of understanding of its pathophysiology, lack of standardisation of diagnostic criteria for gonadal failure or parameters of recovery, absence of a national or international registry of SCT recipients' offspring, failure of patients to report to the reproductive specialists and absence of longitudinal and follow-up data. The magnitude of the problem is likely to be very high considering the fact that the survival rate for children, adolescents and adults with haematological and other cancers has improved dramatically in recent years in both Europe<sup>1</sup> and the US.<sup>2</sup> One estimate is that they represented 1 in 900 people aged 15–44 in the US.<sup>3</sup>

### Pathophysiology

Although longitudinal data are unavailable regarding the natural history of gonadal insufficiency-premature gonadal failure syndrome (GI-PGF), cross-sectional studies have shown progressive dose-dependent ovarian follicular loss and ovarian failure following chemotherapy in human subjects.<sup>4,5</sup> Gonadal damage is an acute phenomenon and can occur as early as 72 h following the last dose of chemoradiotherapy, as evident from castrated gonadotrophin levels and structural loss of gonadal volume.<sup>6</sup> The entire reproductive endocrine (hypothalamic-pituitary-gonadal) axis,<sup>7,8</sup> including the target organs (such as endometrium and vagina in females) may all be affected<sup>9,10</sup> and contribute to adverse reproductive outcome (Figure 1).

We now appreciate that gonadal damage induced by high-dose therapy is not 'an all or a none' phenomenon. It is a syndrome ranging from a mild to moderate degree of gonadal insufficiency (GI) to severe full-blown premature gonadal failure (PGF), (ie premature menopause in females and andropause in males). Tables 1 and 2 demonstrate the pathophysiological classification of SCT-induced gonadal injury, with proposed grading for prediction of reversibility and therapeutic outcome. Although patients with severe

The adult gonad has two important functions: gametogenesis (spermatogenesis in males and oogenesis in females, respectively) and sex steroidogenesis (production of testosterone in males and oestrogen and progesterone in females, respectively). Gonadal damage induced by high-dose chemotherapy or chemo-radiotherapy and stem cell transplantation, affects both components of gonadal function, resulting in infertility, and sexual dysfunction, which may compromise quality of life of the survivors. This article



**Figure 1** Sites of damage from chemo/radiotherapy and SCT.

gonadal damage are likely to present with a more severe and permanent type of gonadal failure, this may not be true in all cases. This is further complicated by the delayed presentation of SCT-induced gonadal damage in many subjects and the lack of well-established diagnostic criteria and investigatory tools available in routine clinical practice. Even normal basal gonadotrophin and sex steroid levels do not exclude gonadal damage, as PGF is a common occurrence in SCT recipients.<sup>11</sup> This is hardly surprising as our data suggest that the pre-transplant regimen can cause diminished reserve in both male and female subjects even prior to SCT.<sup>6,11</sup> Most pre-transplant conditioning protocols for SCT include alkylating agents, irradiation or both, either of which can cause germ cell injury and damage to the Leydig cells in males and the entire follicular apparatus in females, respectively.<sup>12</sup> Multiple doses of potentially gonadotoxic antecedent therapy can cause cumulative dose-dependent damage, especially in females.<sup>13</sup> Older age, local

**Table 1** Pathophysiology of SCT induced ovarian damage and proposed grading of ovarian insufficiency (OI) and premature ovarian failure syndrome (POF) in females

	Grade I	Grade III
Age <40 years		
Biochemical	FSH and LH >10 <20 IU/l (RR 2–10) Oestradiol ≥40 <100 pmol/l (RR 80–800) Dynamic test of pituitary gonadal function will establish diminished gonadal reserve <sup>a</sup>	FSH and LH >40 IU/l (RR 2–10) Oestradiol <30 pmol/l (RR 80–800) Dynamic test as in Grade I
Biophysical	Presence of follicular activity in ovarian ultrasound scan (2 or more follicles >5 mm in diameter)	Absence of follicular activity in ovarian ultrasound scans, or follicles <3 mm in diameter
Clinical presentation	Oligomenorrhoea (<3 periods/year) or secondary amenorrhoea (absence of periods for at least 12 months) Menopausal syndrome	Secondary amenorrhoea (absence of periods for at least 12 months) Variable degree of menopausal syndrome,
Therapeutic outcome	Infertility Usually potentially reversible	Infertility Usually irreversible

Grade II = intermediate between grade I and III.

<sup>a</sup>Details of dynamic test for pituitary gonadal function are given in Ref. 6.

**Table 2** Proposed grading of testicular insufficiency (TI) and premature testicular failure syndrome (PTF) in males

	Grade I	Grade III
Age <55 years		
Biochemical	FSH and LH >10 IU/l < 20 IU/l (RR 2–10) Testosterone: Normal or <10 nmol/l (at least twice done 6 months apart) (RR 10–25 nmol/l) Testosterone/LH ratio <2.5 Dynamic test of pituitary gonadal function will establish diminished gonadal reserve <sup>a</sup> Semen analysis: persistent azoospermia (at least three semen samples done 4–6 weeks apart)	FSH and LH >20 IU/l (RR 2–10) Testosterone <10 nmol/l (at least twice done 6 months apart) Testosterone/LH ratio <2.5 Dynamic test: As in grade I Semen analysis: persistent azoospermia as in grade I
Biophysical	Testis >4 <10 ml (volume). Cavernosal arterial insufficiency may be present. Peak systolic velocity (PSV) <30 cm/s <sup>b</sup>	Testis <4 ml (volume) Cavernosal arterial insufficiency: as in grade I
Clinical presentation	Diminished libido  Erectile dysfunction	Infertility  Diminished libido Erectile dysfunction
Therapeutic outcome	Usually potentially reversible	Usually irreversible

Grade II = intermediate between grade I and III.

<sup>a</sup>Details of dynamic test for pituitary gonadal function are given in Ref. 6.

<sup>b</sup>PSV details are given in Ref. 55.

therapy (ovarian or pelvic irradiation in females and testicular radiotherapy in males) may also contribute to gonadal insult.<sup>14</sup> There are no credible data to suggest that the type of transplant has an important bearing on the severity of gonadal damage. However, total body irradiation (TBI) allografts may be more damaging to the entire reproductive axis, including the gonads and the endometrium, compared to autografts.<sup>14,15</sup> Moreover, in a recent European survey, Salooja *et al*,<sup>12</sup> has shown a higher incidence of pregnancy-related complications including poor fetomaternal outcome in allograft recipients who had TBI-based conditioning regimens. Disorders of implantation might underlie intrauterine growth retardation and preterm birth. The authors speculated that in women who received allogeneic SCT, implantation might be impaired after irradiation or graft-versus-host disease. However, there are no credible data to differentiate TBI from GVHD in this equation. However, in addition to inflicting gonadal injury, severe GVHD can cause vulvo-vaginal infection with vaginal and even cervical stenosis and disfigurement of the internal and external genitalia including the perineum, all of which may have bearing in sexual dysfunction, dyspareunia, reproductive failure and even difficult labour.<sup>10</sup>

High risk patients usually suffer severe gonadal damage, following factors being significant. (1) Age at transplant > 30 years. (2) Pubertal status: post-pubertal adults > pre-puberty. (3) Sex: males > females. (4) Local, inverted Y and pelvic radiotherapy. (5) Multiple doses of potentially gonadotoxic antecedent conventional chemotherapy, especially alkylating agents prior to SCT. (6) Inclusion of alkylating chemotherapy in the conditioning regimen for SCT. (7) Type of transplant: allogeneic transplantation based on TBI conditioning > autograft. (8) Possibly graft-versus-host disease. (9) Type of disease and tendency for gonadal infiltration (ie ALL and lymphoma).

## Diagnosis

Diagnosis of SCT-induced hypogonadism is complex, as there are no established standard criteria. Also, patients with mild damage may be severely symptomatic, whereas those with severe damage may never present to the clinician with either infertility or menopausal symptoms. We propose characterisation of GI-PGF syndrome based on clinical, biochemical and biophysical picture, which is portrayed in Tables 1 and 2. GI-PGF may have a heterogeneous picture ranging from minimal gonadal damage to full-blown PGF with total absence of gonadal activity (castrated levels of gonadotrophins and low levels of sex steroids in both sexes and persistent azoospermia in males). Males are more affected than females.<sup>16</sup> The clinical picture is variable and may not agree with the severity of biochemical or biophysical parameters. However, the high risk group is likely to sustain severe gonadal damage (grade II–III) with minimal chance of spontaneous or induced recovery compared to the chemotherapy treated subjects who are likely to sustain milder injury and probably have greater potential for reversibility. However, the distinction between the two groups may not be clear cut and there is often an overlap with the intermediate group, with an unpredictable potential for recovery or response to therapy.

## Management

In principle, the simplistic notion of management should be directed at preventing the potential damage by: (1) Reducing the damage wherever possible, by using less toxic conditioning regimens. TBI and alkylating agents should be avoided if possible. (2) Possibly preventing damage by GnRH co-treatment with conventional chemotherapy. (3) Possibly reversing damage by GnRH co-treatment with high-dose therapy. (4) Replacing lost gonadal function by substitution of mature gametes (sperm banking in males and embryo cryopreservation in females, respectively).

Therapeutic options depend on clinical presentation, age and the need for preservation of fertility. Older patients or those who have completed their family may not have fertility needs and should be managed differently from those requesting preservation of fertility.

## Management of infertility

This includes fertility counselling providing accurate information on pros and cons of planned pregnancy, contraceptive choices as well as credible data available on pregnancy complications, including fetomaternal outcome, ethical issues and fertility options available before and after SCT. The effect of pregnancy on SCT including potential risk of relapse is also discussed. This is likely to help patients in making an informed decision.

### *Fertility counselling*

Fertility counselling should start during the pre-transplant period and be continued during the post-transplant stage. Those presenting at the post-transplant stage may have sustained severe or complete PGF and the use of donor gametes or adoption may be the only option available. All information related to current and past literature must be made available to patients to facilitate counselling, and careful documentation, as well as informed consent must be obtained in all cases.

### *Pregnancy outcome*

*Feto-maternal complications:* Most research on fertility and pregnancy outcome of recipients of SCT is based on observational and descriptive data. In the last decade sporadically successful pregnancies have been reported in the literature.<sup>17–20</sup> However, most information in this area is derived from two large studies conducted in Europe<sup>12</sup> and the US.<sup>15</sup> In both series, partners of male patients had uncomplicated pregnancies and normal children, whereas female allograft recipients had a higher incidence of miscarriage, pre-term labour and low birth weight babies. In the EBMT study, pregnancy induced hypertension was significantly higher in the allograft recipients (15%) compared to 8% of the normal population. The rate in congenital malformation, developmental delay and malignant disease was not higher in the offspring of SCT recipients as compared to the control population. Miscarriage incidence was 10% which was also similar to the control population.

*Risk of structural and chromosomal congenital malformations in the offspring of SCT recipients:* SCT patients rarely become parents. However, gonadal function can recover and subsequent chromosomal damage may occur. Robbins *et al.*<sup>21</sup> has shown that chemotherapy can induce transient sex chromosomal and autosomal aneuploidy in human sperm. Monteil *et al.*<sup>22</sup> demonstrated increased aneuploid frequency in spermatozoa from a patient with Hodgkin lymphoma after chemotherapy and radiotherapy. We have also shown fludarabine-induced spermatozoal DNA damage in a patient with CLL.<sup>23</sup> Although EBMT data did not show an increased incidence of aneuploidy or congenital malformations, the number of pregnancies was too small to allow a reliable estimate of pregnancy complications. Nevertheless, pregnancy in allograft recipients who have had TBI should be treated as high risk for fetomaternal complications, including pregnancy-induced hypertension, Caesarean section, miscarriage, low birth weight singleton and multiple pregnancies. Also gonadal ageing, especially maternal, is at least theoretically, associated with an increased risk of structural as well as autosomal and sex chromosomal aneuploidy and adverse reproductive outcome.<sup>24</sup> This risk is likely to be higher in pregnancies following intracytoplasmic sperm injection (ICSI), where natural barriers to conception are lost. Hence, prenatal fetal diagnosis has a definitive place in these patients to ensure the birth of healthy offspring.<sup>25</sup>

*Risk of relapse:* Although an immunosuppressed state and, at least theoretically, pregnancy can aggravate cancer, it is unlikely to alter the natural history of the primary haematological disease. Pregnancy is therefore no longer a contraindication in SCT recipients for haematological, endometrial or even breast cancers.<sup>26</sup> Scattered case reports<sup>27,28</sup> have shown evidence of relapse of leukaemia during pregnancy. However, these patients who were at risk of relapse, might have relapsed independently of the pregnancy. Nonetheless, to ensure safety, Salooja *et al.*<sup>12</sup> recommended that patients with CML should delay pregnancy for at least 2 years after a SCT, providing that they remain Philadelphia chromosome and bcr/abl transcriptase negative. With a cautious note we would also recommend planned pregnancy in all SCT recipients as there is at least a theoretical risk of relapse during pregnancy. A pregnancy under these circumstances may compromise appropriate management of the malignancy because of fetal interest, also carrying major implications for the entire family.

*Contraception:* Spontaneous pregnancies do occur in SCT recipients and therefore all sexually active recipients of SCT should be counselled regarding contraception prior to discharge from hospital.<sup>14</sup> Barrier methods of contraception may be the best option for such patients.

## Management of infertility in males

### *Established clinical practices*

*Semen banking:* Semen banking is the gold standard for preservation of fertility in males regardless of partners.

Development of new techniques such as *in vitro* fertilisation (IVF) and intracytoplasmic sperm injection (ICSI) enables even low quality sperm to be used successfully in cancer patients, with successful pregnancies resulting.<sup>29</sup> Therefore, unless azoospermic, semen should be cryopreserved in cancer patients wherever possible.<sup>30</sup> The requirement that the patient be sexually mature and mentally competent and the need to delay anti-cancer treatment until adequate semen samples have been obtained limits the usefulness of this technique. In young boys who are Gillick competent,<sup>31</sup> it is occasionally possible to obtain sperm for cryopreservation from urine samples.<sup>32</sup>

### *Experimental strategies*

*Testicular tissue freezing:* Testicular tissue cryopreservation may have a place in well-selected adult/adolescent patients with azoospermia, or where semen samples cannot be obtained and prompt chemoradiotherapy has to be administered.<sup>32</sup> *In vitro* culture of sperm is unpredictable, but cryopreservation of testicular tissue<sup>33</sup> or isolation of germ cells from human testicular tissue, cryopreservation and autotransplantation have been reported in humans undergoing sterilising chemotherapy.<sup>34</sup> The outcome of ICSI with fresh and with frozen-thawed testicular sperm can yield successful results in obstructive azoospermia<sup>35</sup> and in cancer patients (Figure 2).

## Management of infertility in females

### *Established clinical practices*

*In vitro fertilisation (IVF) using superovulation and embryo-cryopreservation (for future embryo transfer):* This is the most tried method in female SCT recipients with promising results<sup>12</sup> despite some limitations. Requirements include presence of a functional ovary, stable partner, and the time to complete the necessary number of IVF cycles to harvest sufficient eggs before commencement of the cytotoxic regimen. This may be difficult in some patients, where prompt anti-cancer treatment should be initiated without significant delay.

### *Experimental strategies*

*Ovarian tissue cryopreservation:* Another option is to freeze ovarian cortical tissue, which contains a large reserve of eggs in the primordial follicles; this can be banked and then re-implanted to the original pedicle after treatment so that pregnancy can be achieved naturally.<sup>36</sup>

Alternatively, the tissue can be grafted to a heterotopic site, as evident from animal data<sup>37,38</sup> either as an autograft<sup>39</sup> or as a xenograft.<sup>40</sup> However, the tissue should be scrutinised for evidence of tumour contamination before it is re-implanted due to the risk of transmission of malignant cells via the ovarian grafts.<sup>41,42</sup> Mechanical isolation of human follicles and *in vitro* growth of prenatal and small pre-antral human follicles have been undertaken by some investigators,<sup>43–45</sup> but this is currently a research tool until reliable culture techniques become available.

**Oocyte freezing:** The initial report by Chen in 1986<sup>46</sup> of the first births (one singleton and one twin) after human mature oocyte cryopreservation was highly encouraging. Yang *et al*<sup>47</sup> reported cryopreserved oocyte survival and pregnancy rates similar to those of frozen embryos, with a modified oocyte-freezing regimen. However, concerns about human aneuploidy and absence of reliable freezing protocols limit its inability to translate into reproducible, clinically useful techniques.

**GnRH analogue for suppression of pituitary-gonadal axis for gonadal rescue against chemo-radiotherapeutic damage:** Human data in male and female subjects<sup>48,49</sup> suggest that prior or concomitant treatment with a GnRH analogue may be a promising approach for prevention of chemotherapy-induced ovarian failure. Our current knowledge on the ovarian action of GnRH analogues stems from experiments in rats,<sup>50,51</sup> where the uptake of tritiated thymidine was significantly reduced, indicative of reduced mitotic activity 134 days after starting GnRH analogue administration. The exact mechanism in humans remains unclear, because of the questionable presence of GnRH receptors in humans and primates.<sup>52</sup> Although more experimental in SCT patients, our recent pilot data in recipients of high-dose therapy and SCT (manuscript in preparation) suggest that the use of prior or concomitant GnRH analogues may be one of the promising ways to preserve fertility so that a greater number of follicles remain in the ovary afterwards. More prospective data and randomised controlled studies are necessary to determine the clinical potential of GnRH in fertility preservation in cancer patients (Figure 2).

We recommend that in female patients, gametogenic reserve, stability of the relationship and time available for IVF programmes and egg harvesting are important deciding factors, as are ethical, religious, cultural and other factors in deciding the best option for preservation of fertility in females.<sup>53</sup>

### Management of sexual dysfunction

Those presenting with PGF and sexual dysfunction may need hormone replacement therapy (HRT) after a definitive diagnosis is established. Male patients presenting with diminished libido and erectile dysfunction (ED) may bene-

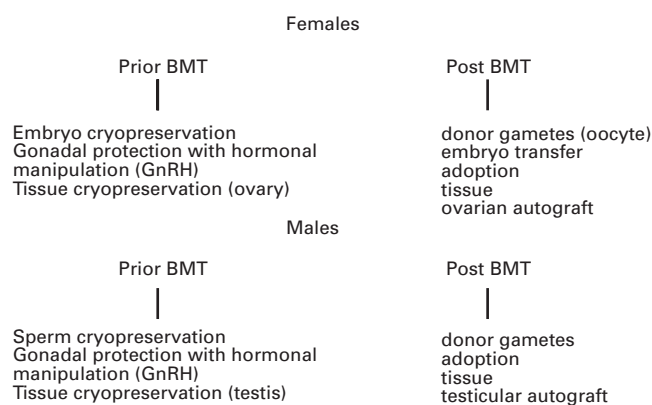
fit from combined therapy with testosterone replacement and sildenafil,<sup>54</sup> especially those with symptomatic Leydig cell insufficiency and cavernosal arteriogenic insufficiency.<sup>55</sup> Similarly, cyclical oestrogen replacement treatment (ERT) may be administered with cyclical progestogen in women with an intact uterus. HRT is not contraindicated in cancer patients, including SCT recipients.<sup>26</sup> Allograft recipients with a history of severe GVHD may have vulvovaginal and even cervical stenosis with disfigurement of the perineum, which may cause dyspareunia, poor sexuality, and low self esteem.<sup>10</sup> These patients may benefit from empathy, coupled with psychosexual counselling and cyclical ERT with progestogens.

### Follow-up

Many young men and women who have had a SCT want to know if they will be fertile, and if their children will be at greater risk of cancer or congenital malformations, and if sterilisation is likely, what alternatives exist for having genetically related offspring. Although generally reassuring, the continued monitoring of the offspring of SCT survivors and the investigation of alternative methods of conception and follow-up of all recipients of SCT will provide valid answers to many of these questions. We recommend that all SCT recipients have longitudinal follow-ups with reproductive endocrine specialists for: (1) Comprehensive testing of the hypothalamic-pituitary gonadal axis (especially in TBI-treated patients to detect asymptomatic hypopituitarism<sup>8</sup>). (2) Surveillance of gonadal function to detect spontaneous or induced recovery, of gamatogenesis. (3) All male patients on HRT should have annual prostatic specific antigen (PSA) and prostate ultrasonography. (4) All female patients on HRT should have 3-yearly mammography, and annual cervical cytology for cervical intraepithelial neoplasia (CIN), (especially those treated with TBI). (5) All patients should have periodic DEXA scans to monitor osteoporosis.

### Future research directions

Current practice for preserving fertility in patients undergoing genotoxic chemotherapy is to cryopreserve sperm with the view of using this for assisted reproductive treatment in the future. Young men with cancer frequently have disease/treatment-related oligo-asthenospermia with poor semen quality. The advent of assisted conception including ICSI has changed the picture dramatically, as successful conception is possible with very few sperm.<sup>56</sup> Although both radiotherapy and chemotherapy can induce DNA damage,<sup>25</sup> DNA repair takes place at all stages of spermatogenesis and most conceptions carrying aberrations are expected to be lost during the cleavage stage. Sperm selection techniques in ICSI do not allow assessment of genetic integrity of the spermatozoa and it is clear that mutations in the paternal genome may be passed on to children.<sup>57</sup> Therefore, there is a need for selection of spermatozoa with normal DNA to ensure the birth of healthy offspring. Bartoov *et al*<sup>58</sup> have introduced a technique of motile-sperm organelle



**Figure 2** Therapeutic options available for preservation of fertility for females and males.

**Table 3** Future directions and research strategies for preservation of fertility

## General directions

- Safe techniques of cryopreservation, storage and ethical approval by authoritative bodies
- Epidemiological surveillance of offspring of survivors of SCT after spontaneous and assisted conception

## Males

- GnRH co-treatment with chemotherapy and high-dose chemotherapy
- Screening of DNA in damaged spermatozoa
- Testicular tissue cryopreservation and *in vitro* transplantation of testicular tissue
- Testicular sperm extraction (TESE)
- Spermatid conception
- Isolation, identification and storage of stem spermatogonia-*in vitro* maturation of sperm spermatogonia

## Females

- GnRH co-treatment with chemotherapy and high-dose chemotherapy
- Oocyte freezing
- Harvesting of ovarian tissue and autologous transplant of ovarian tissue
- Prevention of follicle atresia by novel strategies

morphology examination for selection of sperm with normal nuclei to improve pregnancy rates with ICSI. Such a method may have promise in using assisted reproductive techniques (ART) in cancer patients in the future. Adequately controlled randomised controlled trials are needed to define the scope of GnRH co-treatment in the prevention of chemo-radiotherapeutic damage. Areas of future research must also include optimising freeze-thaw techniques for gonadal tissue, minimising ischaemia-perfusion injury after transplantation, primordial follicle (PMF) banking, *in vitro* culture of germ cells and detecting minimal residual disease in ovarian tissue grafts (Table 3).

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