

# Preserving fertility in young patients with lymphoma: an overview

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**Abstract:** Fertility preservation affords patients the ability to reproduce after the initial diagnosis and management of such malignancies as Hodgkin's and non-Hodgkin's lymphoma. Certain chemotherapy regimens and pelvic radiotherapy confer a high-risk of subsequent gonadal compromise in both males and females. Fortunately, early counseling and initiation of fertility-preservation strategies promptly after diagnosis enable patients to maintain hope for future reproduction. Well-established methods for fertility preservation include embryo and oocyte cryopreservation in females and sperm cryopreservation in males. These methods enable patients to utilize assisted reproductive technologies, including in vitro fertilization, at the time of desired childbearing in order to ensure genetic offspring. As most of these modalities are not feasible in some patient populations, including prepubescent patients, newer methods of fertility preservation must be created to ensure the ability to produce genetic offspring in lymphoma patients. This review provides insights into the impact of gonadotoxic treatment on ovarian and testicular function, and highlights current modalities in fertility preservation in both males and females.

**Keywords:** fertility preservation, lymphoma, ART, egg freezing

## Introduction

There will be approximately 80,000 new cases of Hodgkin's and non-Hodgkin's lymphoma in 2014, accounting for 5% of new cancer cases in the US.<sup>1</sup> Approximately 12.8% of patients diagnosed with Hodgkin's lymphoma are under the age of 20 years, and 31% are between the ages of 20 and 34 years. Conversely, only 1.6% of patients diagnosed with non-Hodgkin's lymphoma are under the age of 20 years, and 3.8% are between the ages of 20 and 34 years. Overall, it is estimated that Hodgkin's and non-Hodgkin's lymphomas comprise 8% of childhood carcinomas.<sup>2</sup> Unlike most carcinomas, surgical therapy is not the mainstay in treatment of most lymphomas, and therapeutics usually consist of chemotherapy, radiation therapy, and biologic agents, such as monoclonal antibodies, proteasome inhibitors, and epigenetic modulators.<sup>3-5</sup> Of these, depending on the aggressiveness and stage of the cancer, chemotherapy, radiation therapy, or a combination of the two is most commonly utilized in treatment of these cancers.<sup>6,7</sup>

Due to the need for gonadotoxic chemotherapy and radiotherapy, these patients are at a higher risk for premature ovarian failure (POF) or hypergonadotropic hypogonadism than people who have not received these therapies. POF occurs when all primordial follicles are destroyed prior to the age of 40 years. As a result, hypoestrogenism ensues, resulting in permanent amenorrhea and infertility despite compensatory

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elevated levels of follicle-stimulating hormone (FSH), usually  $>30$  mIU/mL.<sup>8</sup> The rate of POF in childhood cancer survivors approaches 8%, compared to 1% in the general population.<sup>9</sup>

While fertility may be impaired in all patients exposed to gonadotoxic chemotherapy and radiotherapy, some studies specifically address these risks in lymphoma patients. van der Kaaij et al reported that patients with both Hodgkin's and non-Hodgkin's lymphoma are less likely to conceive posttreatment compared to healthy controls.<sup>10</sup> Additionally, childhood cancer survivors with Hodgkin's or non-Hodgkin's lymphoma have an approximately 2-fold-higher risk of infertility compared to their healthy siblings.<sup>11</sup> Childhood cancer survivors also have worse pregnancy outcomes compared to infertile controls with assisted reproductive technology (ART), including in vitro fertilization (IVF).<sup>12</sup> Indeed, clinical pregnancy and live-birth rates with ART are approximately 70% lower in childhood cancer survivors compared to infertile controls when ART is performed after gonadotoxic therapy. The utilization of fertility-preservation options, including ART, prior to treatment may enable patients to optimize fertility outcomes and mitigate the impact of cytotoxic therapy on future childbearing. Biologic parenthood is possible for many men and women who will lose reproductive function during the treatment of lymphoma. Therefore, addressing fertility preservation with young patients diagnosed with lymphoma should be an essential aspect of their comprehensive care.

Pretreatment counseling should involve the potential impact of chemoradiation on future gonadal function and childbearing. Options for fertility preservation, associated costs, and their efficacy should be discussed as well. This discussion should occur soon after diagnosis, as some treatment options may take up to 10–12 days, possibly delaying the initiation of cancer treatment. Additionally, early consultation with a reproductive endocrinologist or fertility specialist is recommended.

Fertility preservation requires individualization. The optimal approach depends on the type of disease, chemotherapy utilized, the need for radiation therapy, and time available before initiation of treatment. Additionally the patient's sex, age, and costs should be considered.

The following review will outline basic female and male reproductive physiology. The adverse effects of gonadotoxic chemotherapy and radiotherapy on ovarian and testicular function will be addressed. Additionally, we will outline the currently available strategies for fertility preservation in both females and males afflicted with lymphoma.

## Women

### Female reproductive physiology

Oogenesis is initiated during fetal development, with a human female's peak number of oocytes occurring at approximately 20 weeks gestational age, with approximately 7 million immature primary oocytes present at the time.<sup>13</sup> This number is drastically reduced secondary to apoptosis, and only approximately 1–2 million oocytes remain at the time of birth. These oocytes are arrested in the prophase stage of meiosis I until ovulation after the onset of puberty.<sup>14</sup> These arrested immature primary oocytes are continually destroyed by atresia, and there is attrition of an additional 25% of oocytes until the initiation of puberty.

In the prepubertal state, ovarian function is suppressed due to low levels of hypothalamus-secreted gonadotropin-releasing hormone agonists (GnRH-as).<sup>15</sup> Therefore, the gonadotropins, FSH, and luteinizing hormone (LH) are not synthesized and released from the anterior pituitary in this state. In this stage of life, the ovaries are quiescent due to the lack of hormonal stimulation. Pulsatile GnRH activity during puberty results in the release of FSH and LH, and ovarian activity ensues.<sup>16</sup>

At all stages of life prior to menopause, immature oocytes are enveloped in quiescent primordial follicles. Recruitment of primordial follicles and development of the oocyte within these immature follicles is gonadotropin-independent and relies on a host of growth factors. Each cycle, a cohort of primordial follicles is recruited to grow, and after attrition only a handful will reach the gonadotropin-dependent stages of advanced growth. Only one follicle from this cohort will attain dominance and ovulate each cycle. At the time of ovulation, an oocyte will complete meiosis through metaphase II arrest until fertilization with sperm.

### Effects of chemotherapy on female fertility

Most chemotherapeutic agents work by affecting cell-cycle division.<sup>17</sup> Therefore, it can be anticipated that anticancer agents would likely affect oocytes entering a phase of maturation, as well as the growth and proliferation of the somatic cells that support the oocyte within individual ovarian follicles. This mechanism explains the immediate cessation of menses after chemotherapy initiation in postpubertal women.<sup>17</sup> This acute disruption in reproductive hormone levels may resolve upon the cessation of chemotherapy without a long-term impact on future fertility.

The impact of chemotherapy on quiescent immature follicles that have not been recruited from growth remains poorly understood. Certain chemotherapeutic agents may affect these immature follicles oocytes, due to their effects on quiescent cells. Alkylating agents, for instance, have a non-cycle-specific mechanism of action leading to single- and double-strand deoxyribonucleic acid (DNA) breaks, thereby potentially affecting both quiescent and dividing cells in the ovary (Table 1).<sup>18,19</sup> Alkylating agents may also induce cortical fibrosis and blood-vessel damage in ovarian tissue.<sup>20</sup> Cyclophosphamide and procarbazine are particularly toxic to the ovaries, and may adversely affect future fertility.<sup>21</sup> Anthracyclines, such as doxorubicin, and certain platinum agents also confer a high risk of gonadotoxicity.<sup>22</sup>

As previously mentioned, biologic therapies, such as proteasome inhibitors and monoclonal antibodies, are also commonly used for the treatment of lymphomas.<sup>3,4</sup> Unfortunately, there is a paucity of data with regard to the long-term impact of these drugs on gonadal function.

### Common regimens in Hodgkin's lymphoma

The ABVD (adriamycin, bleomycin, vinblastine, dacarbazine) regimen used in Hodgkin's lymphoma is associated with a POF rate of less than 10% in the reproductive age.<sup>7</sup> Conversely, POF rates with the BEACOPP (bleomycin, etoposide, adriamycin, cyclophosphamide, oncovin, procarbazine, prednisone) regimen exceed 50% in the reproductive age. Similarly, MOPP (mechlorethamine, oncovin, procarbazine, prednisone) regimens are associated with POF rates of

20%–50%. Hematopoietic stem cell transplants (HSCTs) are associated with the highest rates of POF, due to the requirement for conditioning with high-dose chemotherapy with or without total-body irradiation in these patients.<sup>23</sup> Ultimately, 70%–100% of patients undergoing HSCTs will have POF.<sup>24</sup>

### Common regimens in non-Hodgkin's lymphoma

The CHOP (cyclophosphamide, hydroxydaunorubicin, oncovin, prednisone) regimen is associated with POF rates of approximately 5%, with pregnancy rates exceeding 50% after treatment.<sup>25</sup> Hyper-CVAD (cyclophosphamide, vincristine, adriamycin, dexamethasone, cytarabine, methotrexate) confers POF rates of approximately 14%, and consequent pregnancy rates after treatment are approximately 43%.<sup>26</sup> Similarly to Hodgkin's lymphoma, POF rates after HSCT are approximately 70%–100%.<sup>24</sup>

The cumulative dose of the cytotoxic drug being administered is a key factor impacting permanent ovarian failure. Goldhirsch et al demonstrated a steadily increasing rate of POF, ranging from 10% to 61% as the cumulative dose of cyclophosphamide increased.<sup>27</sup> Older women in particular are susceptible to gonadotoxic agents, with one study reporting POF rates approaching 34% in Hodgkin's lymphoma survivors over the age of 30.<sup>28</sup> Lower cumulative doses of cytotoxic drugs are required for older women to experience amenorrhea compared to younger women. In one study, all women above the age of 40 years had permanent amenorrhea after receiving more than 5.2 g of cyclophosphamide, whereas the dose required to

**Table 1** Effect of cell-cycle-specific and -nonspecific agents on ovarian function

Drug Type	G <sub>1</sub>	S	G <sub>2</sub>	M
<b>Cell-cycle-specific agents</b>				
Drug	L-Asparaginase Prednisone	Cytarabine 5-Fluorouracil Hydroxyurea Methotrexate Thioguanine	Bleomycin Etoposide	Vinblastine Vincristine Paclitaxel
Ovarian damage	No/low risk	No/low risk	No/low risk	No/low risk
<b>Cell-cycle-nonspecific agents</b>				
Drug type	<b>Alkylators</b>	<b>Antitumor antibiotics</b>	<b>Nitrosoureas</b>	<b>Miscellaneous</b>
Drug	Cyclophosphamide Ifosfamide Carboplatin Cisplatin Busulfan Chlorambucil Mechlorethamine Melphalan	Dactinomycin Daunorubicin Doxorubicin Mitomycin Mitoxantrone	Carmustine Lomustine Streptozotocin	Dacarbazine Procarbazine
Ovarian damage	High	Intermediate	Intermediate	High

**Note:** Data from Devita et al.<sup>19</sup>

cause amenorrhea in younger women was 9.5 g.<sup>29</sup> Similarly, prepubertal girls seem to be less susceptible than others to cytotoxic drugs.<sup>30</sup> Prepubertal girls may be able to tolerate up to 50 g of cyclophosphamide without adverse effects on long-term ovarian function. Previous studies have demonstrated posttreatment menarche and subsequent ovulatory cycles in prepubertal women undergoing even cytotoxic chemotherapy.<sup>31</sup> As such, it can be postulated that the higher the number of primordial follicles in a patient, the greater the prognosis in terms of preserved ovarian function. The prepubescent stage confers protection against gonadotoxicity, due to the presence of a larger pool of primordial follicles which are in a state of metabolic quiescence, thus reducing the deleterious effects of cytotoxic chemotherapy on the ovaries.

### Radiotherapy-induced ovarian failure

Radiation therapy utilized in the treatment of lymphoma may also have deleterious effects on future fertility. Total-body irradiation, direct pelvic, abdominal, and spinal irradiation or scatter irradiation, in doses as low as 1–2 Gy in girls<sup>32</sup> and 4–6 Gy in adults,<sup>33</sup> can have a permanent negative effect on the ovaries by causing the depletion of follicles. Irradiation results in ovarian atrophy and reduced follicle stores.<sup>34</sup> On the cellular level, oocytes show rapid onset of pyknosis, chromosome condensation, disruption of the nuclear envelope, and cytoplasmic vacuolization. Serum levels of FSH and LH rise progressively within 4–8 weeks following radiation exposure, along with a decline in serum E<sub>2</sub> levels, implying POF. Indeed, in the Childhood Cancer Survivor Study, pelvic radiation was found to be a significant risk factor for both acute ovarian failure and premature menopause.<sup>21</sup>

The degree and persistence of ovarian damage and suppression of ovarian function is related to the patient's age and the cumulative dose of ionic radiation to the ovaries (Table 2).<sup>34,35</sup> Similarly to chemotherapy exposure, older age

confers an increased risk. An effective sterilizing radiation dose at birth is estimated to be 20.3 Gy, while that number decreases to 14.3 Gy by 30 years of age.<sup>36</sup> Increasing cumulative doses of radiation and the schedule of delivery also determine the ultimate degree of ovarian damage.<sup>37</sup> Specifically, irradiation is more toxic when given in a single dose compared to fractionated doses.<sup>38</sup> Radiation doses as low as 2 Gy may cause ovarian damage.<sup>32</sup> Another study found ovarian failure rates of 11%–13% in women exposed to less than 3 Gy of radiation. Conversely, ovarian failure rates approached 60%–63% in women exposed to greater than 3 Gy.<sup>39</sup> Irradiation doses approaching 14–30 Gy may also cause permanent damage to uterine musculature and vessels, thereby potentially impeding the ability of a survivor to carry a full-term pregnancy.<sup>40,41</sup>

Concerns regarding pregnancy outcomes after pelvic irradiation have been addressed in the literature. Fenig et al reported an increased incidence in low birth weight and spontaneous abortions if pregnancy occurred within 1 year of irradiation.<sup>42</sup> Therefore, they recommended that pregnancy be delayed for at least 1 year after completion of radiation. Similarly, others have reported a small but absolute increase in adverse pregnancy outcomes in childhood cancer survivors, including those inflicted with Hodgkin's and non-Hodgkin's lymphoma.<sup>43,44</sup> The literature is not consistent with respect to this issue, however, as other studies have not found a similar increase in adverse pregnancy outcomes. De Sanctis et al reported no significant association between radiotherapy treatment and adverse birth outcomes, such as spontaneous abortions, premature birth and low birth weight, in Hodgkin's lymphoma survivors.<sup>45</sup> Additionally, Swerdlow et al reported no increase in stillbirths, low birth weight, congenital malformations, abnormal karyotypes, or cancer in the offspring of patients who underwent pelvic irradiation for Hodgkin's disease.<sup>46</sup>

Ultimately, as previously mentioned, chemotherapy- and radiotherapy-induced gonadal dysfunction depends on the age at first treatment and the treatment protocols. Therefore, it should be emphasized that all counseling on reproductive risks and options to preserve fertility after treatment must be individualized.

### Strategies to preserve fertility in females

A wide array of options are available for preservation of fertility in patients affected by lymphoma. Unfortunately, many of the available techniques are experimental and based only on case reports, retrospective studies, and expert opinions.

**Table 2** Effect of radiotherapy on ovarian function

Ovarian dose (cGy)	Risk of ovarian failure	
	Women aged 15–40 years	Women >40 years
<60	No adverse effects	No adverse effects
150	Minimal adverse effects in young women	Some risk of sterilization in women over 40 years
250–500	60% sterilized; remainder may experience transient amenorrhea	100% sterilized
500–800	60%–70% sterilized; remainder may experience transient amenorrhea	100% sterilized
>800	100% sterilized	100% sterilized

**Note:** Data from Damewood and Grochow.<sup>34</sup>

### Pharmacologic protection

As prepubertal females are the least susceptible to gonadotoxic therapies, administration of GnRH-as to mimic this state may prevent POF.<sup>47</sup> Indeed, there is some evidence in murine and rat models that adjuvant treatment with GnRH-as may protect gonads from the deleterious effects of chemotherapy.<sup>48,49</sup> Some retrospective studies have demonstrated a protective effect of GnRH-as against ovarian failure when initiated prior to chemotherapy.<sup>50</sup> However, there is controversy with regard to their efficacy in humans. To date, 20 studies (five prospective randomized controlled trials [RCTs] and 15 non-RCTs) comprising 1,837 patients have reported benefits of GnRH-a therapy during chemotherapy in various types of cancers with different chemotherapeutics with respect to prevention of ovarian failure.<sup>51,52</sup> Overall, patients treated with GnRH-as concurrently with chemotherapy preserved their cyclic ovarian function in 91% of cases compared to 41% of controls, with a pregnancy rate of 19%–71% in the treated patients in non-RCTs. Furthermore, seven meta-analyses consisting of RCTs and non-RCTs reported decreased rates of ovarian failure in GnRH-a-treated women compared to controls.<sup>6,47,53–57</sup> It should be noted, however, that most of these meta-analyses have included some RCTs and non-RCTs with methodological flaws. For instance, some included studies had short term follow-up of patients, some as short as 3 months after cessation of chemotherapy. Due to the acute effects of chemotherapy on cyclic ovarian function, few conclusions can be drawn from studies with such a short-term follow-up. Additionally, length of follow-up differed between treatment and control groups in some studies included, and definitions of ovarian failure were not uniform across studies. Most studies did not examine the effects on future fertility in these patients either, thus limiting our ability to draw significant conclusions in this regard.

Indeed, some authors have questioned the use of GnRH-as for gonadal suppression. Since primordial follicles that are primarily affected during chemotherapy are quiescent and their recruitment is gonadotropin-independent, the need for gonadal suppression is called into question.<sup>58</sup> As significant controversy regarding the utility of GnRH-as for prevention of ovarian failure exists in the literature, efforts should be made to counsel patients on methods with greater efficacy in preserving fertility.<sup>59,60</sup> It is important to note, however, that while GnRH-as may not definitively provide a protective effect on future ovarian function, they may facilitate menstrual cessation in patients with anemia as a result of lymphoma. Anemia is a relatively common occurrence in

lymphoma patients as a consequence of both lymphoproliferation and administration of cytotoxic therapy. As such, GnRH-a utilization may be beneficial to ameliorate symptoms of severe anemia through complete cessation of cyclic blood loss during menstruation.<sup>61</sup>

### Ovarian transposition

Ovarian transposition or oophoropexy may enable preservation of ovarian function after pelvic radiotherapy. Surgically transposing the ovaries outside the field of radiation may reduce the ovarian exposure by 5%–10% compared to in situ ovaries.<sup>62</sup> Initially, lateral ovarian transposition required laparotomy, division of bilateral utero-ovarian ligaments, and subsequent reposition and suture fixation of bilateral tubes and ovaries to ipsilateral paracolic gutters. Transposing the ovaries in this manner to the lateral paracolic gutters enables them to rest approximately 3 cm above the upper border of the radiation field, a distance that has been shown to minimize harm to ovaries.<sup>63</sup> Indeed, ovarian failure may result if the ovaries are not moved far enough out of the radiation field, or if they migrate back to their original position. Oophoropexy is now commonly performed through a laparoscopic approach, thereby minimizing patient burden.<sup>64</sup> In addition to expediting patient recovery, laparoscopic oophoropexy also offers the ability to initiate radiotherapy soon after surgery. Expediting radiotherapy may prevent ovarian damage as a result of ovaries migrating back to the pelvis, thus obviating the need for additional procedures to retranspose ovaries out of the radiation field. Laparoscopic ovarian transposition may preserve ovarian function in approximately 85% of patients aged under 40 years with normal ovarian reserve (relative supply of remaining oocytes) prior to treatment. Similarly, Terenziani et al reported 14 pregnancies in eleven girls afflicted with Hodgkin's lymphoma who underwent oophoropexy at the age of 13.<sup>65</sup>

### Assisted reproductive technology

Patients also have the option of preserving gamete function *ex vivo* prior to initiation of gonadotoxic chemoradiation with ART. ART affords patients the opportunity to cryopreserve either embryos or mature oocytes for future utilization. Other options include ovarian tissue and immature oocyte cryopreservation, which are still considered to be experimental.

### Embryo cryopreservation

Embryo cryopreservation is an established technology that offers fertility, regardless of posttreatment gonadal function. Studies on exact pregnancy rates of lymphoma

patients undergoing embryo cryopreservation are limited. Approximate pregnancy and live-birth rates for these patients may be inferred from infertile and donor populations (Table 3).<sup>66</sup> The Society for Assisted Reproductive Technology (SART) compiles national and clinic-specific data with regard to these outcomes.<sup>66</sup> As oocyte donors are generally young and presumably fertile, young lymphoma patients without a known history of infertility at the time of embryo cryopreservation would presumably have comparable pregnancy and live-birth rates as oocyte donors. In contrast, pregnancy and live-birth rates for older patients may be inferred from data from age-matched infertile couples, with the understanding that these data may have limitations, since these patients may not have infertility prior to gonadotoxic treatment. In general, postthaw survival rates of cryopreserved embryos range between 35% and 90%.<sup>67</sup> If multiple embryos are available for cryopreservation, cumulative pregnancy rates are in the order of 60%. The prognosis of future fertility with embryo cryopreservation is dependent on the age of the patient. Live-birth rates from cryopreserved embryos are highest among patients under the age of 35 years, at approximately 42% per cycle (Table 3). Cryopreserved embryos from donor oocytes result in a live-birth rate of approximately 37%.

However, direct comparisons of live-birth rates between the infertile population/donors and patients with lymphoma must be made judiciously, as recent data show that patients afflicted with both Hodgkin's and non-Hodgkin's lymphoma have lower ovarian reserves retrieved at baseline than healthy age-matched controls.<sup>68</sup> Furthermore, both types of lymphoma patients had significantly fewer oocytes retrieved than patients undergoing oocyte retrieval for breast cancer. Therefore, the diagnosis of lymphoma may confer disadvantages with respect to oocyte stimulation and thus embryo cryopreservation. However, due to a paucity of data on specific outcomes on lymphoma patients and despite these limitations of inference, national SART data may be the best-available option for counseling patients for fertility preservation prior to initiation of gonadotoxic therapy.

Embryo cryopreservation consists of undergoing controlled ovarian stimulation with gonadotropins lasting approximately 7–11 days. Serial transvaginal ultrasounds are utilized to measure oocyte response to stimulation. Once oocytes reach the appropriate size and are deemed mature, patients undergo retrieval of mature oocytes. Fertilization with sperm takes place using conventional IVF or intracytoplasmic sperm injection, utilizing either a slow-freeze method or ultrarapid vitrification.<sup>69</sup> Embryos can then be thawed and transferred to the uterus after completion of lymphoma treatment. The need for viable sperm is integral for embryo formation. As such, women without partners may opt to utilize sperm donors in order to accomplish fertilization. However, while this option offers excellent clinical outcomes, it may not be feasible to cryopreserve embryos from all patients, such as prepubescent girls, adolescent girls, or women without a partner who do not desire to utilize donor sperm. As such, other modalities for fertility preservation must be utilized in these patients.

#### Mature oocyte cryopreservation

For adolescent girls and women, mature oocyte cryopreservation may be more suitable than embryo cryopreservation. Early studies in mature oocyte cryopreservation yielded disappointing results with respect to oocyte survival, fertilization, and subsequent pregnancy rates. A variety of cellular and subcellular structures of the human oocyte, such as the zona pellucida and mitotic spindle, appear to be very sensitive to damage from cryopreservation.<sup>70</sup> However, the introduction of ultrarapid vitrification has improved success rates of mature oocyte cryopreservation tremendously.<sup>70–72</sup> With improvements in vitrification, postthaw survival rates of cryopreserved oocytes approach those seen with embryo cryopreservation.<sup>73–75</sup> Porcu et al reported outcomes on 18 patients who underwent mature oocyte cryopreservation secondary to the requirement of gonadotoxic treatment.<sup>76</sup> A 4-year patient follow-up revealed successful pregnancies after rapid cryopreservation of oocytes and subsequent thawing and fertilization. Pregnancy even occurred with oocytes that had been cryopreserved for years. Similarly, a recent

**Table 3** 2012 National in vitro fertilization live-birth rates per embryo transfer

	Oocyte donor	Age (years)				
		<35	35–37	38–40	41–42	>42
Fresh-cycle live birth per ET (%)	56.6	47.1	37.9	28.5	16.3	6.1
Cryopreserved/thawed-cycle live birth per ET (%)	37.2	42.4	39.8	33.9	26.4	17.8
Average number of embryos transferred	1.8	1.8	1.8	1.8	1.9	2.0

**Note:** Data from Society for Assisted Reproductive Technology.<sup>66</sup>

double-blinded RCT comprising 600 patients who underwent either IVF using fresh oocytes or mature cryopreserved oocytes noted no differences in pregnancy rates between the two modalities.<sup>74</sup> As such, mature oocyte cryopreservation is no longer considered experimental.<sup>77</sup>

Similarly to embryo cryopreservation, pregnancy rates following oocyte-cryopreservation decline with advancing age of the woman.<sup>78</sup> The process of controlled ovarian stimulation and oocyte retrieval required for mature oocyte cryopreservation is similar to the process of obtaining mature oocytes for embryo cryopreservation.

#### Cryopreservation and transplantation of ovarian tissue

Ovarian tissue cryopreservation and transplantation is an experimental procedure introduced to preserve fertility in women with threatened reproductive potential. Cryopreservation of cortical tissue may enable preservation of fertility in prepubescent females and females who are unable to delay treatment, and dispense the time necessary for undergoing embryo or oocyte cryopreservation.<sup>79</sup> Either laparoscopy or laparotomy can be utilized to obtain ovarian cortical tissue. Ovarian tissue is then cryopreserved using slow-freeze or ultrarapid vitrification. Upon completion of gonadotoxic therapy and at the time of desired childbearing, cryopreserved ovarian tissue can be thawed and implanted near the fallopian tube in an orthotopic fashion (physiologic location), allowing spontaneous ovulation and natural conception to take place.<sup>80,81</sup> In 2004, Donnez et al reported a case of a woman with stage IV Hodgkin's lymphoma who underwent ovarian cortex biopsy and cryopreservation prior to the initiation of chemotherapy.<sup>80</sup> The patient underwent orthotopic autotransplantation of freeze-thawed tissue 6 years after completion of therapy. Within 5 months of transplantation, the patient resumed monthly ovulation, as evidenced by a dominant follicle and monthly menstrual cycles. The patient spontaneously conceived an intrauterine pregnancy 11 months after ovarian tissue reimplantation. Similarly, numerous investigators have reported 24 spontaneous ovarian functions and pregnancies after 60 cases of ovarian cortex cryopreservation and subsequent orthotopic tissue reimplantation.<sup>81-90</sup> Ovarian tissue can also be reimplanted in a heterotopic (on the abdominal wall) location, necessitating ART for potential conception.<sup>81</sup>

Freezing cortical ovarian tissue does present some challenges. Unlike a suspended single cell, tissue cryopreservation presents serious physical constraints related to heat and mass transfer and the potential formation of ice crystals, rendering the tissue susceptible to freeze-thaw injury.<sup>91</sup>

While cortical ovarian tissue cryopreservation, thawing, and heterotopic transplantation followed by subsequent IVF have resulted in live births in animals, no live births have been reported in humans.<sup>92</sup> However, cortical ovarian tissue cryopreservation and subsequent autologous orthotopic transplantation has been successfully performed in humans, with resultant spontaneous conception and live births.<sup>82</sup> To date, six spontaneous pregnancies, resulting in seven live births, have been described in both Hodgkin's and non-Hodgkin's lymphoma patients who underwent this modality for fertility preservation.<sup>60</sup> Ovarian tissue function and spontaneous ovulation generally resumes between 60 and 240 days after orthotopic tissue reimplantation and lasts up to approximately 7 years.<sup>7,93</sup> Therefore, this modality may provide little hope toward ensuring long-term preservation of fertility after gonadotoxic treatment. As such, no live births have been reported in prepubescent girls who have undergone ovarian tissue cryopreservation at young ages.<sup>60</sup> However, as these patients have not undergone subsequent postthaw ovarian autotransplantation due to their young ages, few definitive conclusions can be made about the efficacy of this modality in fertility preservation in prepubescent patients.<sup>94</sup>

There are some limitations to ovarian tissue cryopreservation. Similarly to embryo and mature oocyte cryopreservation, the prognosis of ovarian tissue cryopreservation declines with age, and it may not be feasible in patients older than 40 years.<sup>95</sup> Additionally, concerns of reseeding cancerous tissue exist in autologous ovarian tissue reimplantation. While this risk appears to be higher in certain systemic conditions, such as leukemia, there have been no reports of reseeding and subsequent recurrence in lymphoma patients.<sup>96,97</sup> Other concerns include the loss of a large fraction of follicles during ovarian tissue cryopreservation secondary to initial ischemia after transplantation.<sup>98</sup> For this reason, there may be some utility of ovarian transplantation using a vascular graft. Due to a paucity of robust data with respect to long-term outcomes and safety of ovarian tissue cryopreservation, this modality is currently considered to be investigational, and should only be offered to patients after extensive counseling and referral to physicians with expertise in this procedure.

#### In vitro maturation of immature oocytes

In vitro maturation (IVM) refers to the maturation of immature oocytes in culture after their recovery from small antral follicles at early stages prior to selection and dominance. IVM of human oocytes was first reported in 1965, followed by successful pregnancy and delivery reported in 1989.<sup>99</sup> Folliculogenesis to early antral stages occurs independently of

gonadotropins. IVM can be performed urgently irrespective of the phase of the menstrual cycle without affecting the quantity and maturation rate of the oocytes. Therefore, this modality may be beneficial for patients who are unable to undergo controlled ovarian stimulation due to time constraints prior to initiation of gonadotoxic therapy. Exposure to gonadotropins and supraphysiologic levels of estradiol as a result of controlled ovarian stimulation may be detrimental to patients with estrogen-responsive carcinomas and patients predisposed to ovarian hyperstimulation (such as young patients with polycystic ovarian syndrome).<sup>100,101</sup> This approach also has fewer associated costs than controlled ovarian stimulation, as it does not involve expensive gonadotropin injections. Unfortunately, despite these advantages, maturation rates and the developmental potential of embryos derived from IVM oocytes are significantly lower than those of oocytes matured *in vivo*. Moreover, pregnancy rates with IVM are low compared with controlled ovarian stimulation with embryo/oocyte cryopreservation.<sup>60,102</sup> As a result of these challenges, IVM is considered to be an investigational modality for fertility preservation.

## Special considerations

### Prepubertal females

Fertility preservation in children and prepubertal adolescents remains a challenge. Unfortunately, well-established methods of fertility preservation in postpubertal females, such as embryo and mature oocyte cryopreservation, are not feasible in this patient population.<sup>103,104</sup> Prepubertal females are a unique subgroup of the fertility-preservation population in that they are commonly too young to understand the full scope of their disease and its implications on future fertility. As these patients have not yet commenced the hormonal changes associated with puberty and hypothalamic–pituitary–ovarian axis maturation, treatment options are limited. Cortical ovarian tissue cryopreservation and subsequent transplantation is the only modality that is efficacious for this patient population.<sup>94</sup> Unfortunately, to date there have been no reports of live births from ovarian tissue harvested prepubertally, due primarily to the young age of the patients at the time of treatment. In animals, however, IVM from immature oocytes harvested from ovarian tissue has yielded both successful embryo development and live births.<sup>105–112</sup> Fortunately, to ape these results in humans, improvements in IVM techniques in humans are at the fore of fertility-preservation research, and may be another option for fertility preservation in this population in the future.

## Men

### Male reproductive physiology

Spermatogenesis in the seminiferous tubules begins at puberty, and results in the transformation of immature germ cells into mature spermatozoa. Prior to pubertal development, the testis of a 10-year-old male is estimated to contain 83,000,000 diploid germ cells.<sup>113</sup> The process of transformation from spermatogonia to spermatozoa includes mitosis and two rounds of meiotic divisions over a period of approximately 74 days. Progenitor spermatogonia that remain mitotically active throughout the lifelong process of spermatogenesis are the most susceptible to gonadotoxic insult.<sup>114</sup> Successful completion of differentiation to mature spermatozoa is highly dependent on the specialized intratesticular microenvironment and hormonal milieu. At puberty, GnRH is released by the hypothalamus in a pulsatile fashion, which subsequently induces release of FSH and LH by the anterior pituitary gland.<sup>115</sup> FSH predominantly acts on Sertoli cells to promote spermatogenesis, whereas LH targets intratesticular Leydig cells to produce testosterone. Sertoli cells produce multiple regulating substances, such as anti-Müllerian hormone (during fetal life) and inhibin (negative feedback to FSH secretion) to regulate the hypothalamic–pituitary axis. Similarly, Leydig cell-produced testosterone provides negative feedback to pituitary secretion of LH.

### Effects of gonadotoxic therapy on testicular function

Male infertility associated with lymphoma and other malignancies may occur due to a myriad of factors, including the malignancy itself, radiation, gonadotoxic chemotherapy, or a combination of these factors. Although the underlying mechanism has not been fully elucidated, certain malignancies, including lymphomas, may have an adverse effect on testicular function in and of themselves.<sup>116,117</sup> Rueffer et al reported reduced semen quality in 70% of males with Hodgkin's lymphoma.<sup>118</sup> Some patients with lymphoma present with baseline oligospermia (low sperm count), azoospermia (absent sperm), or sperm chromosomal aneuploidy.<sup>118,119</sup> However, following treatment of their malignancy, some of these patients may experience improvement in semen parameters. These findings implicate the primary malignancy or the immune response to the cancer as a contributing factor to impaired fertility in these patients.<sup>120,121</sup> Additionally, both radiation and chemotherapy may result in damage to the seminiferous tubules, including spermatogonial cells and Sertoli cells, with resultant



detrimental effects on fertility. Active and inactive germ cells are also susceptible to gonadotoxic insult, possibly inducing permanent infertility.<sup>122,123</sup> This susceptibility to gonadotoxic insult may occur at all ages, including before puberty.<sup>124</sup> However, recovery may be possible. The degree of recovery attained depends on several factors, including the type of therapy, dose, and fractionation/delivery schedule.<sup>125</sup> Fortunately, in contrast to the seminiferous epithelium where spermatogenesis occurs, Leydig cells are relatively resistant to gonadotoxicity from chemoradiation, and hence cancer treatments rarely result in clinical hypogonadism.<sup>126</sup>

While little is known about the impact of biologic therapies, such as anti-20 monoclonal antibodies and proteasome inhibitors, on testicular function in humans, recent evidence in mice suggests that proteasome inhibitors may have long-term adverse effects on sperm concentrations, testicular weight, and Sertoli cell dysfunction, evidenced by persistently increased FSH levels.<sup>127</sup> Long-term studies in humans are needed, however, in order to delineate the effects of these therapies on future fertility.

## Effects of chemotherapy on testicular function

Chemotherapy can have a wide range of impact on testicular function and male fertility. Similarly to females, the effect of chemotherapy administration is age-dependent. Chromosomal abnormalities in spermatocytes may be detectable up to 24 months after cessation of treatment in Hodgkin's lymphoma patients.<sup>120</sup> Alkylating agents, such as cyclophosphamide, chlorambucil, procarbazine, and busulfan, are associated with the greatest risk of gonadotoxicity. Similarly, platinum-based agents also confer a high risk of impaired gonadal function in males.<sup>128</sup> Alkylating agents impede spermatogenesis in a dose-dependent manner.<sup>129</sup> Cyclophosphamide administered at smaller doses of 7.5–9 g/m<sup>2</sup> has been shown to impair sperm function, and dosages over 10 g/m<sup>2</sup> are associated with a high risk of gonadal damage. Dosages greater than 19–20 g/m<sup>2</sup> typically result in permanent sterility (Table 4).<sup>19,129,130</sup> Multiple smaller doses confer a greater risk to subsequent gonadal function than fewer larger doses of chemotherapy.<sup>131</sup>

## Effect of radiation on testicular function

The extent of testicular injury sustained by radiation therapy is directly related both to the dose of radiation delivered and the underlying cell type. For instance, seminiferous tubules are sensitive to radiation doses as low as 0.1 Gy, thereby resulting in temporary arrest of spermatogenesis.<sup>132</sup>

**Table 4** Effects of chemotherapeutic agents on male fertility

Chemotherapy (dose-to-cause effect)	Adverse effect on sperm count	
Chlorambucil (1.4 g/m <sup>2</sup> )	Prolonged or permanent azoospermia	
Cyclophosphamide (19 g/m <sup>2</sup> )		
Procarbazine (4 g/m <sup>2</sup> )		
Melphalan (140 mg/m <sup>2</sup> )		
Cisplatin (500 mg/m <sup>2</sup> )		
BCNU (1 g/m <sup>2</sup> )	Azoospermia in adulthood if treated before puberty	
CCNU (500 mg/m <sup>2</sup> )		
Busulfan (600 mg/m <sup>2</sup> )		
Ifosfamide (42 g/m <sup>2</sup> )		
BCNU (300 mg/m <sup>2</sup> )		
Nitrogen mustard		
Actinomycin D		
Doxorubicin (770 mg/m <sup>2</sup> )		When used alone, cause only temporary reduction in sperm count; in conjunction with above agents, may be additive in causing azoospermia
Thiotepa (400 mg/m <sup>2</sup> )		
Cytarabine (1 g/m <sup>2</sup> )		
Vinblastine (50 g/m <sup>2</sup> )		
Vincristine (8 g/m <sup>2</sup> )		
Amsacrine	When used in conventional regimens, cause only temporary reductions in sperm count; in conjunction with above agents, may be additive in causing azoospermia	
Bleomycin		
Dacarbazine		
Daunorubicin		
Epirubicin		
Etoposide		
Fludarabine		
Fluorouracil		
6-Mercaptopurine		
Methotrexate		
Mitoxantrone		
Thioguanine		

**Note:** Data from Devita et al.<sup>19</sup>

**Abbreviations:** BCNU, bis-chloroethylnitrosourea (carmustine); CCNU, 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (lomustine).

Azoospermia has been reported at radiation doses of 0.65 Gy, with doses of 1 Gy, 2–3 Gy, and 4–6 Gy causing azoospermia lasting 9–18 months, 30 months, and 5 years to permanently, respectively.<sup>132–134</sup>

Radiation may also directly damage other testicular cell types, including sperms themselves. Exposure to radiotherapy has been known to cause DNA fragmentation in sperm, which may have a negative impact on future fertility.<sup>135</sup> As previously mentioned, Leydig cells appear to be less susceptible to radiotherapy-induced damage. Radiation doses as high as 20 Gy in prepubertal males and 30 Gy in postpubertal males are required to induce damage to Leydig cells, and thereby clinical hypogonadism.<sup>136</sup>

In addition to the dose, testicular function is also directly affected by increasing the fractionation of ionizing radiotherapy. Increasing fractionation may lead to repeated sustained injury to testicular tissue and spermatogonia, causing an inability to repair and regenerate the reserve stem cell population.<sup>137,138</sup>

## Fertility-preservation options in males

Similarly to females, the ability to preserve fertility in males is dependent upon the age at presentation. Approximately 20% of males at Tanner stage III or above with testicular volumes greater than 10 mL have the ability to provide sperm for cryopreservation.<sup>139</sup> Prepubertal status in males, similarly to females, represents unique challenges, as the hypothalamic–pituitary–gonadal axis has not been activated. Currently, there are no widely accepted options for fertility preservation in prepubertal males.

### Ejaculated sperm cryopreservation

Sperm cryopreservation is a well-established procedure utilized for fertility preservation in postpubertal male patients undergoing gonadotoxic therapy. Sperm cryopreservation is achieved through semen collection by masturbation prior to the initiation of chemoradiation. Two to three samples are typically collected, due to frequently reduced semen quality in cancer patients, and samples are obtained prior to cancer treatment to ensure optimal DNA integrity and sperm quality.<sup>140</sup> Furthermore, in addition to preserving future fertility, many patients have reported that sperm banking is a method of coping with their malignancy, even if the samples remain unused.<sup>141</sup> Sperm banking may provide a sense of security and reassurance of the future to these patients. While all patients undergoing gonadotoxic therapy should be offered sperm cryopreservation, some patients will be able to recover from gonadotoxic insult and recover sufficient levels of spermatogenesis posttreatment to sustain normal fertility.<sup>142</sup>

Some postpubertal males will not be able to provide semen samples through masturbatory ejaculation due to anxiety or medical problems, such as hypogonadism, neurologic impairment, diabetes, pain, etc. Fortunately, there are other methods available to obtain adequate sperm samples in such cases. For instance, there may be utility in administration of phosphodiesterase type 5 inhibitors, due to their known efficacy in erectile dysfunction.<sup>143</sup> Other modalities used in this group of patients include penile vibratory stimulation (PVS) and electroejaculation.<sup>144</sup> PVS may be beneficial for obtaining sperm in patients with an abnormal ejaculatory reflex, such as those with neurologic impairment, provided that the sacral reflex arc is intact. In PVS, a vibrator is placed against the penis and mechanical stimulation is used to induce ejaculation in patients who are unable to achieve ejaculation physiologically. Conversely, electroejaculation is a more invasive procedure involving placement of a probe containing electrodes in the patient's rectum and is utilized in patients with a compromised sacral reflex arc. Electric stimulation is

subsequently delivered to the prostate and seminal vesicles to cause the emission of semen in patients with neurologic injury. This technique has also successfully been used in adolescents for fertility preservation.<sup>145</sup> Due to the invasive nature of this procedure, it must be conducted under anesthesia to minimize patient discomfort.<sup>146</sup>

Patients suffering from retrograde ejaculation, due to a myriad of causes including surgical or radiation injury to the bladder neck and autonomic nerves or due to the use of certain medications, such as antidepressants, may be candidates for medical therapy with sympathomimetics, such as pseudoephedrine and  $\alpha$ -agonists. Alternatively, urine collection and processing in these men will often result in adequate sperm counts for cryopreservation.<sup>147</sup>

Patients with baseline severe oligospermia present unique challenges. Often, aspiration and surgical extraction of sperm may be undertaken in these patients. Options for sperm aspiration include percutaneous epididymal sperm aspiration, microsurgical epididymal sperm aspiration (MESA), and testicular sperm aspiration (TESA). Options for surgical extraction involve testicular biopsy and subsequent testicular sperm extraction (TESE). Patients with both obstructive and nonobstructive azoospermia may undergo testicular tissue extraction and subsequent cryopreservation. At the time of desired childbearing, sperm may be isolated from thawed tissue and utilized for ART.<sup>148</sup> Rates of paternity utilizing ART with cryopreserved sperm from former cancer patients are reported to be between 33% and 56%.<sup>149</sup> Furthermore, successful paternity has been documented in patients with sperm preserved for up to 28 years.<sup>150</sup> Surgical procedures for sperm extraction require anesthesia, due to their invasive nature.

### Investigational modalities

#### GnRH-agonist therapy

Previous studies have examined the effects of GnRH-agonist therapy in patients undergoing gonadotoxic therapy. GnRH-agonist administration results in gonadotropin-dependent arrest of spermatogenesis, and as a result this has been utilized to mitigate gonadotoxic insult to testicular germ cells.<sup>151</sup> Unfortunately, despite success in rodent-models, human studies have failed to show any protective effects of GnRH-agonists on germ cells, likely due to the proliferation of germ cells through a gonadotropin-independent pathway.<sup>152</sup>

#### Testicular tissue cryopreservation in prepubertal boys

For prepubertal patients who have not initiated spermatogenesis, cryopreservation of testicular tissue through either cell

suspension or whole tissue may be a possible option for fertility preservation.<sup>153</sup> Tissue can be obtained through the techniques previously mentioned, including TESA, MESA, and TESE. Although prepubertal testicular tissue does not contain mature spermatozoa, it does demonstrate the presence of diploid spermatogonial stem cells, which have the capacity to differentiate into mature cells in an ideal microenvironment.<sup>154</sup> Ideally, this tissue could be autotransplanted at a later time in order to resume spermatogenesis after the completion of gonadotoxic therapy. Indeed, some studies have demonstrated successful transplantation of testicular tissue in immunodeficient mice.<sup>155</sup> Unfortunately, no studies in humans have demonstrated the ability to transform immature cryopreserved tissue into functional gametes for later use in ART.<sup>139</sup> Therefore, this procedure is offered at some centers to prepubertal boys only on an investigational basis.<sup>60</sup> Spermatogonial stem cell transplantation may prove to be another modality in the therapeutic arsenal in this patient population, due to its ability to initiate spermatogenesis. Animal studies in mice and rhesus monkeys with obliterated testicular function have demonstrated successful autologous transplantation of spermatogonial stem cells, with subsequent restoration of spermatogenesis.<sup>156,157</sup> While no such success has been documented in humans to date, ongoing research in this area may render this option feasible in this patient population.<sup>158</sup>

## Interdisciplinary care team

The American Society for Reproductive Medicine emphasizes the need for an interdisciplinary care team for these patients. This collaborative team may include oncologists, reproductive endocrinologists, urologists, and surgeons.<sup>60</sup> Additionally, the immense burden placed on these patients not only to cope with their illness but also to make rapid decisions with regard to fertility preservation underscores the need for thorough patient counseling. Mental health professionals should be included in this “care team” in addition to financial counselors to assist with coping with financial burden. While oncologists may initially counsel patients on their options for fertility preservation, reproductive endocrinologists and urologists should be consulted as soon as possible after initial diagnosis. Effective communication is crucial between all care providers to ensure optimal and expeditious patient care.

## Conclusion

The prevalence of lymphoma in young and reproductive-age patients underscores the need for effective fertility-preservation strategies. As the effects of gonadotoxic chemoradiation are

variable depending on the type, dose, and age of the patient, individualized counseling is essential for optimal care of these patients. Well-established fertility-preservation strategies, such as embryo cryopreservation for females and sperm cryopreservation for males, may not be feasible in all patients. Therefore, there is a significant need for further research into investigational methods, such as IVM and testicular tissue cryopreservation, in order to provide the ability to procreate to all patients afflicted with such cancers as lymphomas.

## Disclosure

The authors report no conflicts of interest in this work.

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