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ORIGINAL ARTICLE *Reproductive endocrinology*

Ovarian reserve and response to IVF and *in vitro* maturation treatment following chemotherapy

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BACKGROUND: Chemotherapy and radiotherapy can result in ovarian failure and premature menopause. However, there is still a paucity of information on the ovarian reserve and efficacy of assisted reproduction treatment (ART) procedures in patients with cancer previously exposed to chemotherapy or radiotherapy. The aim of our study was to evaluate the ovarian reserve and ovarian response to IVF or *in vitro* maturation (IVM) treatment in women who had previously been treated with chemotherapy.

METHODS: In this retrospective cohort study, we compared 23 women with cancer who had undergone chemotherapy and subsequently underwent fertility treatment with IVF ($n = 14$) or IVM ($n = 9$). In the IVF group, patients mostly had hematologic, gynecologic, gastro-intestinal, bone and soft tissue cancers, whereas in the IVM group patients had estrogen-receptor positive breast cancer, hematologic and brain cancers. The control (unexposed) group consisted of 70 age-matched women with male factor infertility undergoing the same treatment protocol (IVF $n = 42$ and IVM $n = 28$). All women were aged <42 years and undergoing their first cycle of ART.

RESULTS: There were no differences in age and FSH levels between the cancer and the control groups. However, the antral follicle count (AFC) was lower in the cancer-IVF group (median: 5, range: 3–12) than in the control group (median: 15, range: 12–18; $P = 0.0009$). Women with cancer treated with IVF had lower peak estradiol levels on the day of hCG administration than controls ($P = 0.006$) and lower number of oocytes retrieved [median: 4.5, range: 2–7; versus 12 (8–16) in controls; $P < 0.0001$]. In patients with cancer treated with IVM, the AFC was lower than in the control group (median: 14, range: 9.5–17; versus median: 20.5 range: 16–23, respectively; $P = 0.0007$). Likewise, the number of oocytes retrieved was lower in the cancer-IVM group (median: 6, range: 4–10) than that in the control group (median 10.5, range: 7.5–17; $P = 0.01$). The percentage of mature metaphase II oocytes was comparable in the cancer and control groups.

CONCLUSIONS: The ovarian reserve, response to gonadotrophins and number of oocytes retrieved are adversely affected by previous chemotherapy. This study reports the first series of IVM outcomes in cancer patients with a prior history of chemotherapy. In women with estrogen-receptor positive breast cancer, IVM of oocytes with cryopreservation of oocytes or embryos is a viable option. Since the efficacy of ART is significantly reduced after chemotherapy, early referral for fertility preservation before gonadotoxic treatment will give these young women the best chance to conceive.

Key words: fertility preservation / malignancy / chemotherapy / IVF / *in vitro* maturation

Introduction

With improved diagnosis and treatment of cancer, survival rates of patients have increased markedly. Chemotherapy and radiotherapy can indeed produce high rates of remission; however, they can have a devastating effect on future fertility, resulting in ovarian failure and premature menopause. The ovary is especially susceptible to chemotherapy and radiotherapy because of the normal decline in the number of primordial follicles after birth and the inability of follicles to replicate.

As increasing numbers of survivors seek fertility treatment after cancer therapy, it is important to evaluate the effect of cancer regimens on the ovarian reserve and the response to fertility treatment. Today, there is still a paucity of information on the ovarian reserve

and efficacy of assisted reproduction treatment (ART) in patients with cancer previously exposed to chemotherapy or radiotherapy.

The purpose of our study was to evaluate the ovarian reserve and the ovarian response to IVF or *in vitro* maturation (IVM) treatment, and oocyte maturity in women who had been previously treated with chemotherapy.

Materials and Methods

We evaluated the medical records of all women with malignancy who underwent IVF or IVM between 2003 and 2010 at the McGill University Health Centre, Montreal, Canada. Women who received chemotherapy or radiotherapy prior to undergoing fertility treatment were included in this study. Out of 23 women, 14 women underwent IVF and 9 others were treated with IVM. Patients who presented for fertility preservation prior to chemotherapy or radiotherapy were excluded from the analysis. None of the patients in the cancer group had a past history of infertility prior to the diagnosis of cancer. Patients were mostly referred to our center by their oncologist so that they could undergo fertility preservation treatment prior to undergoing another course of chemotherapy or additional treatment, including a bone marrow transplant, because their disease had relapsed. All decisions on ART and ovarian stimulation were made in consultation with the patient and her oncologist.

Age-matched control (unexposed) groups were selected among women undergoing IVF or IVM for male-factor infertility. For every patient with cancer, we included approximately three times the number of controls in the same age group undergoing ART for male infertility with the same treatment protocol during the same time period. The control group consisted of 42 women treated with IVF and 28 women treated with IVM for male-factor infertility. Only patients undergoing their first IVF or IVM cycle were included in the analysis. Patients more than 42 years of age were excluded from the analysis in both groups. The Research and Ethics Board of the McGill University Health Centre approved the study and each participant gave written informed consent.

All patients underwent serum measurements of basal FSH as well as transvaginal ultrasound examination for the antral follicle count (AFC) to estimate the ovarian reserve.

IVF cycles

In the IVF group, all patients underwent controlled ovarian stimulation with the GnRH antagonist protocol. GnRH antagonist (0.25 mg/day) was commenced when the diameter of the leading follicle was 14 mm and continued until the day of hCG administration. An hCG trigger was administered when two follicles reached 17 mm in diameter. Ultrasound-guided oocyte retrieval was performed 36 h later.

The cancer-IVF group underwent ICSI to avoid the risk of fertilization failure. Patients in the control-IVF group underwent ICSI for male-factor infertility. Mature metaphase II (MII) oocytes were either vitrified or fertilized using ICSI and preserved as vitrified embryos. Normal fertilization was documented by the presence of two pronuclei.

IVM cycles

Patients received one injection of 10 000 IU hCG s.c. when the endometrial thickness had reached at least 6.0 mm (Chian *et al.*, 2009; Son and Tan, 2010) and the largest follicle measured 10–12 mm. None of the patients received FSH in preparation for IVM. On an average, hCG was given on Days 8–11 of the cycle. Oocyte retrieval was performed 35–38 h later under transvaginal ultrasound guidance. None of the cycles were cancelled because of poor response. All patients in the IVM groups underwent ICSI to avoid the risk of zona hardening. The IVM procedure was performed using the protocol as previously described (Son and Tan, 2010).

Statistical analysis

We used the Shapiro–Wilks test to evaluate the distribution of the data. Comparisons were analyzed using Student's *t*-test or Mann–Whitney *U*-test when appropriate. Proportions were compared with the χ^2 test or Fisher exact test. All analyses were performed using StatsDirect statistical software (Cheshire, UK). *P*-value < 0.05 was considered significant. Primary outcome measures included the AFC, total dose of gonadotrophins required for stimulation, peak serum estradiol (E_2) level on the day of hCG administration (the IVF group) and number of oocytes retrieved. Secondary outcome measures included baseline FSH levels, number of days of stimulation in IVF cycles and number of MII oocytes.

Results

There were 14 women with cancer who underwent IVF and 9 others who underwent IVM. Most patients with hematologic, gynecologic and bone malignancies underwent IVF, whereas those with breast cancer which was estrogen-receptor positive underwent IVM. Tables I and II show the types of malignancy and chemotherapy received.

There were no significant differences in age and FSH level between the cancer and the control groups. However, the AFC was significantly lower in the cancer-IVF group than that in the control group ($P = 0.0009$, 95% confidence interval (CI): 4–12; Table III). The total dose of gonadotrophins and the duration of treatment were comparable in both groups of patients. Compared with the control women, women with cancer who were treated with IVF had significantly lower peak E_2 levels on the day of hCG administration ($P = 0.006$, 95% CI: 764–5288) and a lower number of oocytes retrieved ($P < 0.0001$, 95% CI: 4–10). However, the percentages of mature MII oocytes as well as the fertilization rates were comparable.

In patients with cancer treated with IVM, there were no significant differences in age and serum FSH between the cancer and control groups (Table IV). The AFC in the cancer group was significantly lower than that in the control group ($P = 0.0007$, 95% CI: 3–12). The number of oocytes retrieved was also significantly lower in patients with cancer when compared with the control group ($P = 0.014$, 95% CI: 1–11).

In cancer patients undergoing IVF, three patients underwent embryo transfer, of which one patient with breast cancer conceived and delivered a healthy baby girl. Another patient with Stage 2 Hodgkin's lymphoma underwent an IVF cycle between the first and second courses of chemotherapy. She elected to pursue surrogacy using her frozen embryos. The surrogate conceived and delivered dichorionic-diamniotic healthy male and female babies. The patient subsequently underwent a bone marrow transplant, following which she experienced premature ovarian failure. Of these three patients who underwent embryo transfer, only one patient underwent embryo transfer in a fresh cycle, 5 years after the completion of chemotherapy, but she did not conceive. No oocytes were retrieved in a patient with Grade 2 immature teratoma who was previously treated with a combination of bleomycin, etoposide and cisplatin.

In the cancer-IVM group, only one patient with Hodgkin's lymphoma underwent embryo transfer. However, an ultrasound scan 6 weeks following embryo transfer showed a gestational sac and fetal

Table I Type of malignancy in women undergoing post-chemotherapy IVF.

Type of cancer	Number of patients (*n*)	Type of chemotherapy	Total dose of gonadotrophins (IU)	Number of oocytes retrieved
Haematologic malignancy	Hodgkin lymphoma (6)	ABVD (doxorubicin, bleomycin, vinblastine, dacarbazine)	1837.5 (1200–3300)[a]	4.5 (2–6)[a]
		CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone)		
	Myelodysplasia (1)	Busulfan, methotrexate, cyclophosphamide	3150	10
Gynecologic malignancy	Ovarian cancer (3):	Bleomycin, etoposide, cisplatin		
	Immature teratoma-Grades 2–3 (1)		3000	0
	Ovarian endodermal sinus tumor (1)		4125	7
	Ovarian dysgerminoma (1)		2025	7
Gastrointestinal malignancy	Colon cancer (1)	5-flourouracil	5400	6
Bone and soft tissue malignancy	Ewing's sarcoma (1)	Cyclophosphamide, vincristine, ifosfamide, doxorubicin, etoposide	4800	1
	Undifferentiated pleomorphic sarcoma of thorax (1)	Doxorubicin, ifosfamide,	5250	2
Breast cancer	Estrogen-receptor negative breast cancer (1)	AC-Taxol (doxorubicin, cyclophosphamide, paclitaxel)	2400	3

[a]Values are median and inter-quartile range.

Table II Type of malignancy in women undergoing post-chemotherapy IVM.

Type of cancer	Number of patients (*n*)	Type of chemotherapy	Number of oocytes retrieved
Hematologic malignancy	Hodgkin lymphoma (2)	ABVD (doxorubicin, bleomycin, vinblastine, dacarbazine)	11
			6
	Non-Hodgkin lymphoma (1)	CHOP-rituximab (cyclophosphamide, doxorubicin, vincristine, prednisone, rituximab)	4
Breast malignancy	Estrogen-receptor positive breast cancer (5)	AC (doxorubicin and cyclophosphamide)	9 (5–10)[a]
		FEC (5-fluorouracil, epirubicin, cyclophosphamide)	
		Docetaxel, trastuzumab, carboplatin, bevacizumab	
Brain malignancy	Oligodendroglioma (1)	Temozolomide	3

[a]Values are median and inter-quartile range.

pole with no fetal heart activity. Only one oocyte could be retrieved from a patient with breast cancer, yet it did not mature after IVM. Two patients elected to have their embryos frozen, while the others opted for oocyte cryopreservation.

Discussion

The ability to conceive after cancer treatment remains a major concern for women with cancer. Despite the need for accurate information for appropriate counselling, there is a dearth of data on the efficacy of ART, such as IVF and IVM in these patients.

In agreement with previous studies (Meirow *et al.*, 1999; Larsen *et al.*, 2003; Lutchman Singh *et al.*, 2007) that anti-cancer regimens have a detrimental effect on the ovarian reserve, we found that the AFC was significantly decreased among patients post-chemotherapy. However, the follicular phase FSH levels were comparable between the cancer patients and controls. Meirow *et al.* (1999) observed that in young mice treated with different doses of cyclophosphamide,

Table III Clinical profile and treatment outcome in women with malignancy who underwent post-chemotherapy IVF.

Parameter	Cancer group	Control group	*P*-value
Number of patients	14	42	
Age (years)[a]	28.4 ± 1.2	30.2 ± 0.4	0.1
BMI (kg/m^2)[a]	23.4 ± 1.1	23.7 ± 0.8	0.8
Age at chemotherapy (years)[a]	25.7 ± 1.2	NA	NA
Interval between chemotherapy and IVF (months)[b]	24 (7–36)	NA	NA
Antral follicle count[b]	5 (3–12)	15 (12–18)	0.0009
FSH (IU/L)[b]	6.1 (4–9.8)	6.9 (5.8–8.4)	0.38
Total dose of gonadotrophins (IU)[a]	3075 (2025–4200)	1950 (1570–2400)	0.05
No. of days of stimulation[a]	8.5 (7–11)	8 (7–10)	0.85
Peak estradiol on day of hCG (pg/mL)[b]	2645 (1700.5–4072)	6085 (3204–8483.5)	0.006
No. of oocytes retrieved[b]	4.5 (2–7)	12 (8–16)	$P < 0.0001$
Percentage of total MII oocytes (%)[b]	100 (83.3–100)	83.3 (75–93)	0.96
Fertilization rate (%)[b]	71.4 (50–100)	75 (66.7–90)	0.84

NA, not applicable.
[a]Mean ± SEM.
[b]Median and inter-quartile range; fertilization rate (%): number of zygotes/number of MII oocytes.

Table IV Clinical profile and treatment outcome in women with malignancy who underwent post-chemotherapy IVM.

Parameter	Cancer group	Control group	*P*-value
Number of patients	9	28	
Age (years)[a]	30.7 ± 2.1	31.1 ± 0.6	0.93
BMI (kg/m^2)[a]	23.4 ± 0.8	22.4 ± 0.5	0.43
Age at chemotherapy (years)[a]	28.8 ± 2.2	NA	NA
Interval between chemotherapy and IVM (months)[b]	12 (12–36)	NA	NA
Antral follicle count[b]	14 (9.5–17)	20.5 (16–23)	0.0007
FSH (IU/l)[b]	6 (4.9–6.8)	6.7 (5.5–7.8)	0.35
No. of oocytes retrieved[b]	6 (4–10)	10.5 (7.5–17)	0.01
Percentage of total MII oocytes (%)[b]	66.7 (50–81.8)	69.6 (60.7–77.8)	0.91
Fertilization rate (%)[b]	75 (25–100)	72.7 (66.7–100)	0.86

[a]Mean ± SEM.
[b]Median and inter-quartile range; fertilization rate (%): number of zygotes/number of MII oocytes.

follicular depletion occurred in exponential proportion to increasing doses. In addition, Lutchman Singh *et al.* (2007) found that the AFC was significantly reduced in patients with breast cancer who received adjuvant cyclophosphamide, when compared with controls.

In a study comparing 10 cancer survivors and 11 controls with regular menstrual cycles, Bath *et al.* (2003) reported that cancer survivors had significantly higher early follicular phase FSH levels compared with controls, though the AFC was similar. In contrast, several studies have suggested that AFC and anti-Mullerian hormone are more sensitive markers of the ovarian reserve than FSH levels (Lutchman Singh *et al.*, 2007; Lie Fong *et al.*, 2008). This could be related to the fact that FSH can show considerable inter-cycle variability (Wallach, 1995; Sharara *et al.*, 1998). Moreover, AFC has been reported to be a better predictor of IVF outcome than age or FSH (Nahum *et al.*, 2001) and it is also the most reliable determinant of ovarian response to gonadotrophins and number of retrieved oocytes (Hsu *et al.*, 2011).

Our study concurs with and advances the findings of Ginsburg *et al.* (2001) in a study involving 15 women undergoing IVF after systemic cancer treatment. They reported that this group of women had a poorer response to gonadotrophins than did women who had undergone localized cancer treatment only. In contrast to our findings, the number of oocytes retrieved in their study did not differ significantly between the two groups. However, they did not compare the patients with cancer to a control group without cancer. Furthermore, the type of chemotherapy received by the patients was not analysed. We also observed that patients with cancer had a significantly diminished response to gonadotrophins and a significantly lower number of retrieved oocytes, when compared with the control women. Moreover, we found that the number of mature oocytes was significantly lower in the cancer group in comparison to controls. In agreement with their study, we noted that the fertilization rates were comparable in the two groups.

Dolmans *et al.* (2005) compared 4 patients who underwent IVF in the interval between two regimens of chemotherapy with 7 patients who underwent IVF before chemotherapy. In agreement with our findings, they found a notable reduction in the efficacy of IVF after just one round of chemotherapy. They noted that the total dose of gonadotrophins required was significantly higher, while the peak E_2 levels prior to hCG administration and the number of oocytes retrieved were significantly lower in patients undergoing IVF post-chemotherapy versus pre-chemotherapy. However, the ovarian reserve was not assessed in their patients and, moreover, patients with cancer were not compared with controls. We in fact observed that the maximum E_2 level in patients with cancer after chemotherapy, as well as the number of oocytes retrieved, were significantly lower than in the control group.

In our study, most patients who underwent IVF post-chemotherapy had hematological malignancies, mainly Hodgkin's lymphoma. The treatment regime commonly used was ABVD (doxorubicin, bleomycin, vinblastine and dacarbazine). It seems that treatment with ABVD is associated with less ovarian injury when compared with

other regimens (Hodgson *et al*., 2007). Nonetheless, even the ABVD treatment protocol can have a detrimental effect on the AFC and response to IVF treatment, as we noted in our study.

We report the first series of IVM outcomes in patients with cancer and a prior history of chemotherapy. All patients with estrogen-receptor positive breast cancer underwent IVM. The use of gonadotrophins for controlled ovarian stimulation in IVF is associated with a distinct increase in serum E_2 levels (Mitwally *et al*., 2006). Evidence indicates that exposure to estrogen is an important determinant of the risk of breast cancer (Yager and Davidson, 2006). IVM avoids the exposure to increased E_2 levels associated with IVF, thereby avoiding the risk of stimulating estrogen-sensitive neoplasias, such as breast cancer. Indeed, in IVM cycles the mean E_2 level following hCG trigger is within the physiological range (Elizur *et al*., 2008).

There are some reassuring data that ovarian stimulation performed with concurrent administration of the aromatase inhibitor, letrozole, may lessen, but not eliminate, the elevated estrogen levels. In this context, it has been shown that ovarian stimulation with FSH and letrozole was unlikely to result in a significant increase in recurrence of breast cancer, after a median follow-up of ~2 years (Azim *et al*., 2008). However, whether ovarian stimulation with gonadotrophins and letrozole has an impact on patient survival and long-term recurrence of cancer remains to be determined.

Animal studies suggest that there is an increased risk of birth defects in mice conceived soon after exposure to chemotherapeutic agents (Meirow *et al*., 2001). However, studies in humans involving offspring of cancer survivors have not reported an increased risk of genetic defects (Green *et al*., 2009). This could be related to the ability of the oocyte to recover over time. The median time interval between cancer therapy and fertility treatment in our study was 24 months in the IVF group and 12 months in the IVM group. More studies are necessary to further explore the possible associations between chemotherapy, radiotherapy and adverse pregnancy outcomes, especially in women who undergo ART for oocyte or embryo cryopreservation soon after chemotherapy.

Our study validates the fact that anti-cancer regimens have an adverse effect on fertility and response to ART. Anti-cancer treatment has been shown to decrease the finite primordial follicle pool by inducing apoptosis, ovarian atresia and impairment of the ovarian blood supply (Meirow, 2000) which can lead to early ovarian failure. We previously reported that in young women with cancer undergoing fertility preservation before chemotherapy, ovarian reserve, response to gonadotrophins, oocytes retrieved and oocyte maturity remain unaltered by the neoplastic process (Das *et al*., 2011). This implies that chemotherapy is the main factor that leads to a diminished ovarian reserve and early referral for fertility preservation will give these young women the best chance to have a family of their own.

We conclude that despite a depleted ovarian reserve and decreased response to ovarian stimulation after chemotherapy, young women with cancer can still attempt conception or banking of eggs or embryos with ART, such as IVF or IVM. In women with estrogen-receptor positive breast cancer, IVM of oocytes with cryopreservation of oocytes or embryos is a viable option and avoids the risk of inducing a high estrogen state by ovarian stimulation with FSH. Young women who are diagnosed with cancer should be referred for fertility preservation prior to undergoing gonadotoxic treatment as the ovarian reserve and the efficacy of ART is significantly reduced following chemotherapy. These findings will assist clinicians to more appropriately counsel young patients with cancer who wish to conceive.

Authors' roles

M.D. was involved in conception, study design, data acquisition, analysis and interpretation of data, writing the manuscript and finalising the manuscript. F.S. was involved in data acquisition, analysis of data, approval of final version. W.-Y.-S. was involved in data acquisition, analysis of data, critical discussion and approval of final version. T.T. was involved in conception, study design, analysis and interpretation of data, revising, editing and finalising the manuscript. H.H. was involved in critical discussion and approval of final version.

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Conflict of interest

None declared.

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