

Laparoscopy/Minimally Invasive Surgery

Retrieval of immature oocytes from unstimulated ovaries followed by in vitro maturation and vitrification: A novel strategy of fertility preservation for breast cancer patients

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Abstract

BACKGROUND: We report a novel fertility preservation strategy that may be useful for young breast cancer patients who present with time constraints or concerns about the effect of ovarian stimulation.

METHODS: The protocol involves retrieval of immature oocyte from unstimulated ovaries followed by in vitro maturation (IVM), and vitrification of oocytes or embryos.

RESULTS: Thirty-eight patients (age 24–45 years) underwent vitrification of oocytes (n = 18) or embryos (n = 20). The mean ages were 33.1 ± 5.0 years and 34.7 ± 4.8 years, respectively. The mean days required to complete the egg collection was 13 days. The median numbers of vitrified oocytes and embryos per retrieval were 7 (range 1–22) and 4 (range 1–13), respectively.

CONCLUSIONS: The strategy of immature oocyte retrieval without ovarian stimulation followed by IVM and oocyte or embryo vitrification, which does not increase the serum estradiol level and delay cancer treatment, represents an attractive option of fertility preservation for many breast cancer patients. © 2010 Elsevier Inc. All rights reserved.

Breast cancer is the most common form of malignancy in women of reproductive age. In the United States, close to 200,000 women are diagnosed with breast cancer each year,¹ a quarter of whom are premenopausal and up to 20% of childbearing age.² The treatment of breast cancer usually involves surgical resection, such as mastectomy or lumpec-

tomy, followed within 12 weeks by adjuvant chemotherapy and/or radiation treatment.³

Within the last few decades, there has been a decline in the mortality rate and an increase in the disease-free survival rate among breast cancer patients, particularly in women of reproductive age.⁴ One contributing factor is the use of adjuvant chemotherapy in most premenopausal breast cancer patients, which has been shown to reduce recurrence by up to 40% and death by 25%.⁵

Chemotherapy has an adverse effect on ovarian reserve, which may lead to premature ovarian failure and infertility.⁶

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At present, there are limited fertility preservation options for young breast cancer patients without a male partner. The only strategy of female fertility preservation recognized by the American Society of Clinical Oncology (ASCO) and the American Society of Reproductive Medicine (ASRM) is ovarian stimulation with follicle-stimulating hormone (FSH) followed by retrieval of mature oocytes, in vitro fertilization (IVF) using sperm from a male partner or a donor, and cryopreservation of the resultant embryos.^{7,8} However, this strategy may not be applicable to many breast cancer patients.

The primary concern is the safety of ovarian stimulation using FSH in breast cancer patients because of the induction of a high-estrogen state. The peak estradiol (E_2) levels can be 10 times higher than that seen in a natural menstrual cycle.⁹ Although the combination of letrozole and low-dose FSH has been used for ovarian stimulation in breast cancer patients,^{10,11} many patients and their oncologists have concerns about the long-term effects of ovarian stimulation on the risk of breast cancer recurrence, especially if the cancer is estrogen receptor-positive, as long-term safety data remain limited.

Another consideration is that ovarian stimulation with FSH may not be feasible for patients who do not wish to delay their cancer treatment. sFSH stimulation for 10 days to 14 days must be started in the early follicular phase of the menstrual cycle and there is, therefore, a delay of 2 weeks to 5 weeks between the initial consultation to oocyte retrieval depending on the timing of the consultation in relation to the onset of the next menstrual cycle.

Embryo cryopreservation is not a feasible option for women without a male partner, those who object to the use of donor sperm, and those who may not agree to embryo cryopreservation for various personal, religious, or moral reasons.

Ovarian tissue cryopreservation with orthotopic transplantation is another option that may temporarily restore the reproductive endocrine functions.^{12–15} However, this strategy requires 2 operations, is relatively inefficient,¹⁴ has produced only 3 published live births^{16–18} despite numerous attempts, and in cancer patients carries a possibility of reintroducing malignant cells.¹⁹

Oocyte cryopreservation represents a minimally invasive and possibly the only efficient option for patients wishing to delay their choice of a male partner. This approach does not require laparoscopy or removal of a piece of ovary. Conventional slow-freezing of oocytes is considered an experimental procedure because of the low oocyte survival and pregnancy rates and the limited number of live births achieved.^{7,20}

Recent advances in cryopreservation technique called vitrification, involving glasslike solidification without ice crystal formation, has markedly improved the efficacy of oocyte cryopreservation.^{21–23}

The McGill Reproductive Center (MRC) recently completed 2 prospective clinical trials on oocyte vitrification. The first trial involved 38 infertile women without cancer

who underwent FSH stimulation followed by oocyte vitrification using the McGill Cryoleaf (Medicult, Jyllinge, Denmark), resulting in a mean oocyte survival rate of 81% post-thawing, 76% fertilization rate, and clinical pregnancy rate per cycle started of 45%.²⁴ The above results suggest that pregnancy and live birth rates from vitrified-thawed oocytes are comparable to the rates achieved by conventional IVF treatment in many American, European, and Canadian Centers.

A novel fertility preservation strategy involves retrieval of immature oocytes without FSH stimulation, followed by in vitro maturation (IVM) and subsequent vitrification of the in vitro matured oocytes in the absence of a male partner^{25–28} or of the embryo in the presence of a male partner. In a second trial involving 20 infertile women without cancer, vitrification of in vitro matured oocytes resulted in a live birth rate of 20% per cycle started and 4 healthy newborns.^{24,29}

To date, our 2 oocyte vitrification trials have resulted in a total of 19 live births and 26 healthy newborns.²⁴ In a review of the obstetric and perinatal outcomes in 165 pregnancies and 200 infants conceived following oocyte vitrification cycles in 3 fertility centers, the birth weight and the incidence of congenital anomalies (2.5%) were comparable to those of spontaneous conceptions in fertile women or infertile women undergoing IVF treatment.³⁰

Based on the findings of the 2 clinical trials,²⁴ we offered this novel fertility preservation strategy to young women who were newly diagnosed with breast cancer, who were advised by their oncologists not to undergo FSH ovarian stimulation or had time constraints. The objective of this study is to report the feasibility of IVM followed by oocyte or embryo vitrification in these breast cancer patients.

Methods

Breast cancer patients from across Canada and the United States were referred by their oncologists to the fertility preservation program at the MRC. Candidates for this fertility preservation strategy of immature oocyte retrieval followed by IVM and vitrification of oocytes and embryos had the following characteristics: (1) they were women between the age of 18 and 45 years, (2) had histological confirmation of invasive breast cancer, (3) had no prior chemotherapy, (4) had both ovaries, (4) had regular menstrual cycles, and (5) were advised by their oncologists not to undergo FSH stimulation due to time constraints or concerns about effect on cancer progression and recurrence.

The protocols for IVM and vitrification of oocytes were approved by the Institutional Research Ethics Board of the McGill University Health Center (MUHC) and all patients provided written informed consent.

Immature oocyte retrieval

At the first consultation visit, the total numbers and sizes of the antral follicles in both ovaries were measured by ultrasound. Immature oocyte retrieval was performed throughout the menstrual cycle. Patients were given 10,000 of human chorionic gonadotropin (hCG) subcutaneously 36 hours before immature oocyte retrieval.^{31,32} Retrieval was performed under intravenous sedation (2 mg midazolam and 50 µg to 150 µg fentanyl) and a paracervical block using 20 mL of 1% lidocaine. Oocytes were retrieved with a specially designed 19-gauge single-lumen aspiration needle (K-OPS-7035-RWH-ET, Cook, Australia) under transvaginal ultrasound guidance. The aspiration pressure was 85 mm Hg and follicle aspirates were collected in 10-mL culture tubes (Falcon, Franklin Lakes, NJ) containing 2.0 mL warm .9% saline solution with 2 IU/mL heparin (Baxter, Mississauga, Canada).

Oocyte maturity was determined by the extrusion of the first polar body into the perivitelline space, indicating maturation to metaphase II stage (MII). All mature MII stage oocytes were subsequently cryopreserved by vitrification. The immature GV stage oocytes were matured by IVM culture.

In vitro maturation of immature oocytes

The GV stage oocytes were incubated in IVM Medium (Cooper Surgical, CT) supplemented with a final concentration of 75 mIU/mL of FSH and luteinizing hormone (LH) at 37°C in an atmosphere of 5% CO₂ in air with high humidity. Oocyte maturation was assessed after 24 hours IVM culture and the resultant matured oocytes were vitrified. The remaining immature oocytes were further cultured for another 24 hours for a total of 48 hours and any additional mature oocytes were vitrified.

Vitrification of oocytes

Mature MII stage oocytes were cryopreserved by vitrification using a specially designed vitrification device, the McGill Cryoleaf, the efficacy of which has been validated in both animal and human clinical trials.^{21–24,29} At room temperature, the oocytes were suspended in equilibration medium (EM) containing 7.5% vol/vol ethylene glycol (EG) + 7.5% vol/vol 1,2-propanediol (PROH) for 5 minutes and then transferred to vitrification medium (VM) containing 15% vol/vol EG + 15% vol/vol PROH + .5 mol/L sucrose) for 45 seconds to 60 seconds. They were then loaded on the McGill Cryoleaf and then plunged immediately into liquid nitrogen (LN₂) for storage.

Vitrification of embryos

Fertilized zygotes were either cryopreserved after fertilization or transferred to embryo maintenance medium for

further culture for 24 hours to 48 hours, and then cryopreserved by vitrification using the McGill Cryoleaf.

Results

From January 2003 to February 2008, a total of 38 women with invasive breast cancer fulfilled the characteristics mentioned and were offered the novel fertility preservation strategy as described above.

The mean age of patients in the oocyte vitrification group ($n = 18$) was 33.1 ± 5.0 years. Ten patients had stage I (56%), 7 had stage II (39%), and 1 had stage III (5%) breast cancer. The mean days required to complete the egg collection treatment cycle, which was defined as the period between the initial consultation at the MRC and the day of the first oocyte retrieval, was 9 days (range 0–29 days). In this cohort of patients, a total of 237 GV stage and 30 MII stage oocytes were retrieved from 21 oocyte retrieval cycles (Table 1). Three patients underwent 2 cycles of oocyte retrieval. To maximize the number of oocyte yield, 3 patients underwent oocyte retrieval during the luteal phase of the cycle because of time constraints. A mean oocyte maturation rate of $72.5\% \pm 19.4\%$ was achieved following IVM. A total of 191 MII stage oocytes were cryopreserved by vitrification. The median number of vitrified oocytes per retrieval was 7 (range 1–22).

In the embryo vitrification group ($n = 20$), the mean age was 34.7 ± 4.8 years. Thirteen patients had stage I (65%), 6 had stage II (30%), and 1 had stage IV (5%) breast cancer. The mean days required to complete the egg collection treatment was 16 days (range 1–55 days) (Table 1). The mean days required for both groups combined was 13 days. Two patients were seen at the MRC before undergoing their breast cancer surgery, leading to a delay of 43 days and 55 days, respectively, between the first consultation and oocyte retrieval. In this cohort of patients, a total of 192 GV stage and 16 MII stage oocytes were retrieved from 24 oocyte retrieval cycles (Table 2). Three patients had adequate time to undergo more than one cycle of oocyte retrieval during the 8-week period between the initial surgery and subsequent chemotherapy. One patient underwent sequential follicular and luteal phase oocyte collection. The oocyte maturation rate was $65.3\% \pm 25.2\%$ following IVM. A total of 144 oocytes were inseminated. The mean fertilization rate was $78.8\% \pm 21.1\%$. The median number of vitrified embryos per retrieval was 4 (range 1–13).

Comments

Fertility after cancer treatment represents a major concern for young women with breast cancer and may have an impact on their treatment decisions. Nearly one third of breast cancer patients reported that infertility concerns influenced treatment decisions.³³ Many of these patients may

Table 1 Oocyte yield and maturation rates in breast cancer patients who underwent IVM oocyte vitrification

Case no.	Menstrual cycle day	No. of oocytes retrieved		No. of oocytes underwent IVM	No. of matured oocytes at		Maturation rate (mean \pm SD)	No. of oocytes vitrified per retrieval (median; range)
		MII	GV		24 h	48 h		
1	10	0	19	19	6	11	89.5	17
2	10	0	1	1	0	1	100.0	1
3	12	2	19	19	16	1	89.5	19
4	10	0	2	2	1	1	100.0	2
5	10	0	10	10	4	1	50.0	5
6	9	0	18	18	4	5	50.0	9
7	8	3	7	7	4	1	71.4	8
8	1	0	9	9	1	3	44.4	4
9	10	0	3	3	3	0	100.0	3
9	10	2	3	3	3	0	100.0	5
10	19*	2	23	23	8	6	60.9	16
11	10	0	11	11	3	4	63.6	7
12†	23*	0	5	5	3	0	60.0	3
12†	10	3	18	18	7	6	72.2	16
13†	15*	0	7	7	2	3	71.4	5
13†	12	1	7	7	5	1	85.7	7
14	10	3	17	17	10	4	82.4	17
15	11	2	19	19	5	3	42.1	10
16	9	8	19	19	8	6	73.7	22
17	11	1	6	6	4	0	66.7	5
18	3	3	14	14	7	0	50.0	10
Total		30	237	237	104	57	72.5 \pm 19.4	191 (7; 1–22)

*Luteal phase immature oocyte retrieval.

†Patients underwent more than 1 egg collection. MII indicates mature metaphase–II stage; GV = immature germinal vesicle stage; IVM = in vitro maturation.

choose a less gonadotoxic chemotherapy regimen even if it is less effective. In fact, cancer patients were noted to be able to cope with chemotherapy with greater equanimity if the option of having a biological child in future was available to them.³³

Many young breast cancer patients feel their fertility concerns are not addressed adequately.³⁴ One of the problems is the limited options of fertility preservation applicable to young breast cancer patients, many of whom present with time constraints and concerns regarding the long-term effects of FSH stimulation.

In the present study, we reported a rapid and minimally invasive strategy of fertility preservation, involving immature oocyte retrieval without FSH stimulation, followed by IVM and vitrification of the matured oocytes or embryos.

In most breast cancer patients, there is usually a window of 8 weeks between the initial surgery and subsequent adjuvant chemotherapy³⁵ to allow these women to undergo ovarian stimulation, retrieval of mature oocytes, and subsequent cryopreservation of oocytes or embryos.

An established ovarian stimulation protocol for breast cancer patients that involves the combination of letrozole and low-dose FSH has been shown to generate similar oocyte and embryo yield as standard IVF cycles.^{9,10,36,37} Although this protocol resulted in lower mean serum E_2 level than standard protocols, the mean serum E_2 level at the time of hCG injection remains higher than physiological E_2

level, averaging 428 pg/mL.³⁶ In fact, using the FSH–letrozole protocol, some patients were reported to have high peak E_2 level exceeding 600 pg/mL. This tends to be an occurrence in young breast cancer patients with polycystic ovaries.⁹

An important benefit of IVM treatment in the case of breast cancer patients is avoiding the risk of stimulating estrogen-sensitive tumors, since in IVM cycles the mean E_2 level post-hCG injection has been shown to be within physiological range and averaged 73.3 \pm 24.5 pg/mL (270.6 \pm 90.5 pmol/L).³⁸

The safety of short-term exposure to even mildly elevated estrogen in breast cancers is unknown. In vitro studies demonstrated that short-term exposure to low estrogen concentration leads to accelerated malignant cell proliferation.³⁹ Although no increase in breast cancer recurrence has been reported following letrozole–FSH exposure,^{37,40} further long-term follow-up studies are needed to determine whether this exposure to elevated estrogen carries any absolute risk in cancer progression and recurrence since existing studies have only followed patients for 2 years and breast cancer recurrence can occur up to 10 years later.³⁷ One strategy of avoiding FSH stimulation in women with breast cancer has been natural cycle IVF, which involves collection of mature oocytes without FSH stimulation. However, this approach has been associated with an extremely low yield of mature oocytes for insemination. In

Table 2 Oocyte yield and maturation rates in breast cancer patients who underwent IVM embryo vitrification

Case no.	Menstrual cycle day	No. of oocytes retrieved		No. of oocytes underwent IVM	No. of matured oocytes at		Maturation rate (mean \pm SD)	No. of inseminated oocytes	No of fertilized oocytes	Fertilization rate (mean \pm SD)	No. of frozen embryos per retrieval (median; range)
		MII	GV		24 h	48 h					
1	10	0	8	8	7	0	87.5	7	6	85.7	6
2	7	0	7	7	2	2	28.6	4	3	75.0	3
3	11	0	22	22	10	2	20.0	12	9	75.0	9
4	14	0	4	4	2	2	50.0	4	2	50.0	2
5	12	1	1	1	0	0	0.0	1	1	100.0	1
6	12	0	4	4	4	0	100.0	4	3	75.0	3
7	10	0	10	10	7	1	80.0	8	6	75.0	6
8	12	2	12	12	2	4	60.0	8	6	75.0	5
9	8	2	4	4	1	2	75.0	5	5	100.0	5
10	11	0	5	5	4	1	100.0	5	5	100.0	5
11	6	0	7	7	1	1	28.6	2	1	50.0	1
12†	12	5	1	1	0	1	100.0	6	4	66.7	3
12†	10	1	6	6	2	3	83.3	6	6	100.0	4
12†	10	2	6	6	0	6	100.0	8	8	100.0	8
13	12	0	20	20	9	1	50.0	10	10	100.0	8
14	11	2	3	3	2	0	66.7	4	2	50.0	2
15	13	1	2	2	1	0	50.0	2	1	50.0	1
16	19	0	7	7	6	0	85.7	6	3	50.0	3
17†	13	0	7	7	6	0	85.7	6	6	100.0	5
17†	15	0	23	23	19	0	82.6	19	13	68.4	13
18	12	0	16	16	9	0	56.3	9	4	44.4	4
19†	9	0	2	2	2	0	100.0	2	2	100.0	2
19†	27*	0	9	9	3	1	44.4	4	4	100.0	3
20	7	0	6	6	2	0	33.3	2	2	100.0	2
Total		16	192	186	101	37	65.3 \pm 25.2	144	112	78.8 \pm 21.1	104 (4; 1–13)

*Luteal phase immature oocyte retrieval.

†Patients underwent more than 1 egg collection. MII indicates mature metaphase-II stage; GV = immature germinal vesicle stage; IVM = in vitro maturation.

one series, up to 40% of natural cycle IVF failed to generate any embryo.⁴¹

In addition to concerns regarding the effect of FSH exposure, the window of opportunity for fertility preservation is often shorter than 8 weeks. Cancer patients from across Canada and the United States were frequently referred by their surgeons and oncologists to our institution 1 to 2 weeks and occasionally a few days before their scheduled chemotherapy. There is often limited time to allow completion of 1 IVF cycle before starting their scheduled chemotherapy.²⁵ Using the FSH–letrozole protocol, Oktay et al reported the mean delay from surgery to the completion of the first IVF cycle was 39 days and close to 20% of patients required more than 8 weeks to complete their IVF cycles.¹⁰ Madrigano et al reported the mean interval from first evaluation to oocyte retrieval was 33.3 days (range 10–65 days) in 32 breast cancer patients.¹¹ Using the McGill IVM–oocyte and embryo vitrification protocol, the mean interval between initial consultation and completion of egg collection was only 13 days.

At the MRC, in breast cancer patients with adequate time and approval from their oncologists to undergo FSH stimulation, the letrozole–FSH stimulation protocol is offered as one of the fertility preservation options. However, many patients would have foregone fertility preservation if it meant delaying their cancer treatment. To these patients, retrieval of immature oocytes followed by IVM and oocyte vitrification represents an attractive option as it involves no stimulation and minimal delay. To date, only 4 patients have undergone the letrozole–FSH stimulation protocol at MRC. In fact, in a recent prospective trial involving 215 women of reproductive age with breast cancer, only 37% of them were willing to undergo fertility preservation using the letrozole–FSH stimulation protocol.³⁷

Another advantage of this fertility preservation strategy is the possibility to retrieve oocytes regardless of the phase of the menstrual cycle. Unlike conventional ovarian stimulation protocols that are dependent on the phase of the menstrual cycle and can only be initiated at monthly intervals, it is possible to perform immature oocyte retrieval sequentially in both luteal and follicular phase of the menstrual cycle without affecting the quantity and maturation rate of the oocytes as shown in this cohort of patients.

Retrieval of immature oocytes from unstimulated ovaries followed by IVM has become an effective treatment option for many infertile women, including those with polycystic ovaries or polycystic ovary syndrome, high antral follicle count, and those at risk of developing ovarian hyperstimulation syndrome, and more than 500 healthy infants have been born.^{32,42}

Caution should be exercised in extrapolating these results to breast cancer patients but the above studies provide important proof-of-principle that these strategies can work.²⁴ Since cancer patients may not have a second chance and cannot wait for IVM–oocyte vitrification to become an

established procedure, it is reasonable to offer this fertility strategy under institutional review board supervision.

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