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ARTICLE

Fertility preservation for breast-cancer patients using IVM followed by oocyte or embryo vitrification

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Abstract Unstimulated in-vitro maturation (IVM) cycles are considered for fertility preservation in breast cancer due to avoidance of ovarian stimulation and shortened time to oocyte retrieval. This study evaluated the efficacy of this approach in a retrospective cohort analysis of 66 patients with breast cancer. Immature oocytes were collected and matured *in vitro* and then either vitrified or fertilized and preserved as vitrified embryos. In group 1 (vitrified oocytes, $n = 35$), the average number of oocytes retrieved was 11.4 ± 8.8, the maturation rate was 64.2% and an average of 7.9 ± 6.6 oocytes were vitrified per patient treated. The median duration from the first evaluation to oocyte retrieval was 8 days. In group 2 (vitrified embryos, $n = 31$) the average number of oocytes retrieved was 9.7 ± 6.4, the maturation rate was 53.2% and an average of 5.8 ± 2.7 mature oocytes were available for fertilization/patient. The fertilization rate was 77.8%, resulting in 4.5 ± 2.7 vitrified embryos/patient. The median duration from the first evaluation to oocyte retrieval was 13 days. Calculated pregnancy rates per vitrified oocyte and embryo were 3.8% and 8.1%, respectively. IVM can be considered a useful option for fertility preservation in breast-cancer patients.

KEYWORDS: breast cancer, fertility preservation, in-vitro maturation, IVF, oocytes

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Introduction

Breast cancer represents about 30% of malignant tumours occurring in women of childbearing age (Jemal et al., 2004). Approximately 10—15% of breast cancers are diagnosed in women of reproductive age (Bines et al., 1996; Hankey et al., 1994). Postponing the age of motherhood increases the chance of women developing breast cancer before completing their family.

As a result of widespread mammographic screening, breast cancer is now diagnosed earlier in the evolution of the disease. Over the past two decades, earlier diagnosis and highly effective systemic therapies have led to reductions in mortality. Loco-regional treatment, chemotherapy, hormonal modulation (tamoxifen, gonadotrophin-releasing hormone (GnRH) analogues) (Goldhirsch et al., 2005, 2007) and anti-human epidermal growth factor receptor 2 agents (Hortobagyi, 2005) have improved survival rates. Unfortunately, while chemotherapy is crucial for fighting the disease, it may impair follicular maturation and also diminish the ovarian primordial follicle reserve (Falcone and Bedaiwy, 2005). As a result, an increasing number of women who are diagnosed with breast cancer in their reproductive years are seeking options to preserve their fertility. Various treatments are available, including embryo or oocyte freezing after in-vitro maturation (IVM) or IVF protocols, down-regulation of the ovaries with GnRH agonist and ovarian tissue cryopreservation. However, the only established procedure is embryo cryopreservation after ovarian stimulation.

IVM of oocytes has demonstrated good results in polycystic ovary syndrome (PCOS) patients (Reinblatt and Buckett, 2008). This procedure does not require ovarian stimulation, as oocytes are recovered from small antral follicles, generally before the dominant follicle emerges, and are matured *in vitro*. Oocyte collection may take place during the mid-follicular phase of the menstrual cycle (Chian et al., 2000) and even during the luteal phase in certain patients (Demirtas et al., 2008). After maturation, the oocytes are either vitrified or fertilized and then vitrified as embryos. The advantages of IVM-oocyte vitrification for breast-cancer patients are that women are not exposed to elevated oestradiol concentrations, which may preferentially aggravate their disease and that a shorter period of time is required to obtain mature oocytes or embryos for vitrification, which decreases the delay in chemotherapy. The aim of this study was to evaluate the efficacy of the IVM technique as a method of fertility preservation for breast-cancer patients.

Materials and methods

Between 2003 and 2009, 66 women with breast cancer were referred by their oncologists to the McGill Reproductive Centre, a university-based tertiary medical centre in Montreal, Canada. All patients recruited were evaluated by and consulted with a reproductive endocrinology and infertility specialist and a qualified coordinating nurse regarding the various fertility preservation options.

At the first consultation visit, the total numbers and sizes of the antral follicles in both ovaries were measured by ultrasound.

All women included in the study met the inclusion criteria for fertility preservation: (i) between the ages of 18 and 45; (ii) histological confirmation of invasive breast cancer with no prior chemotherapy; (iii) had regular menstrual cycles and had both ovaries; and (iv) were advised by their oncologists to avoid FSH stimulation due to time constraints or concerns about the effect of elevated oestradiol on cancer progression and recurrence.

The study only included IVM treatments, which were divided into two groups: oocyte vitrification group (group 1, $n = 35$) and embryo vitrification group (group 2, $n = 31$). The protocols for IVM and oocyte vitrification were approved by the institutional research ethics board of the McGill University Health Centre and all patients provided written informed consent.

Immature oocyte retrieval, maturation, fertilization and vitrification

Immature oocyte retrieval was performed throughout the menstrual cycle, either in the mid-follicular or luteal stage. Patients were given 10,000 IU human chorionic gonadotrophin (HCG) subcutaneously when the endometrial lining had reached 6—8 mm in thickness and the largest follicle measured 10—12 mm. Women who arrived during the luteal phase and could not postpone their treatment were also scanned and the size and number of follicles were recorded, after which 10,000 IU of HCG were administered. Retrieval was performed 38—40 h later under IV sedation (2 mg midazolam and 50—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 mmHg and follicle aspirates were collected in 10 ml culture tubes (Falcon, Franklin Lakes, NJ, USA) containing 2.0 ml warm 0.9% saline solution with 2 IU/ml heparin (Baxter, Mississauga, Canada).

Oocyte maturity was determined microscopically by the extrusion of the first polar body into the perivitelline space, indicating maturation to metaphase II (MII) stage. All mature MII stage oocytes were subsequently cryopreserved by vitrification or first fertilized (if there was an available partner). Immature germinal vesicle-stage oocytes were matured in an organ tissue culture dish (60—15 mm; Falcon) containing 1.0 ml of IVM medium (Cooper Surgical) supplemented with a final concentration of 75 mIU/ml of FSH and LH at 37°C in an atmosphere of 5% CO_2 in air with high humidity without mineral oil overlay. Oocyte maturation was assessed after 24 h of IVM culture based on the presence of the first polar body in the perivitelline space. The oocytes were denuded of the cumulus cells using a fine-drawn glass pipette after 1 min of exposure to 0.1% hyaluronidase (Cook). The resultant matured MII oocytes were vitrified. Any remaining immature germinal vesicle-stage oocytes were further cultured for another 24 h (for a total of 48 h) and any additional mature oocytes were similarly vitrified or fertilized by intracytoplasmic sperm injection (and then vitrified).

Mature oocytes were suspended in equilibration medium (Medicult, Jyllinge, Denmark) containing 7.5% (v/v) ethylene

glycol (EG) 7.5% (v/v) 1,2-propanediol (PROH) for 5 min at room temperature and then transferred to vitrification medium (Medicult) containing 15% (v/v) EG 15% (v/v) PROH 0.5 mol/l sucrose at room temperature for 45–60 s. They were then loaded on a specially designed vitrification device, the McGill Cryoleaf (Medicult) and plunged immediately into liquid nitrogen for storage.

Fertilized zygotes were either cryopreserved after fertilization or transferred to embryo maintenance medium for further culture for 24–48 h and then cryopreserved by vitrification using the McGill Cryoleaf.

Results

The study group was divided into two subgroups: group 1, cryopreservation of oocytes ($n = 35$); group 2, cryopreservation of embryos ($n = 31$). The decision to cryopreserve embryos was made according to the availability of a stable partner. The results are presented in **Table 1**.

In group 1, a total of 400 oocytes were retrieved, of which 59 were matured *in vivo* (matured on collection day). Out of the 341 immature oocytes, 64.2% were matured *in vitro* to MII, resulting in 278 vitrified oocytes. The average number of retrieved oocytes per patient was 11.4 ± 8.8 (range 1–38) and vitrified oocytes per patient 7.9 ± 6.6 (range 1–28). The median time needed from first consultation to oocyte retrieval was 8 days.

In group 2, a total of 301 oocytes were retrieved, of which 42 were matured *in vivo* (matured on collection day). Out of the 259 immatured oocytes, 53.2% were matured *in vitro* to MII, resulting in 180 oocytes. The fertilization rate was 77.8% resulting in 140 embryos for vitrification. The average number of retrieved oocytes per patient was 9.7 ± 6.4 (range 2–28) and the average vitrified embryos per patient 4.5 ± 2.7 (range 1–10). The median time needed from first consultation to oocyte retrieval was 13 days.

Theoretical expected calculated results

The data on pregnancy rates from IVM vitrified oocytes is limited (Reinblatt and Buckett, 2008) In order to quantify the fertility potential preserved by vitrifying IVM oocytes or embryos, recently published data were used (Chian et al., 2009), in which immature oocytes were collected in an IVM cycle, matured in the laboratory and vitrified. After thawing, the oocytes were fertilized and transferred. The oocyte survival rate after thawing was 67.5%, the fertilization rate was 64.2% and the cleavage rate was 95%. The implantation rate per embryo was 9.6%. Based on the data from Chian et al. (2009), the expected pregnancy rate per oocyte or embryo vitrified was calculated for this study's groups. The results are presented in **Table 2**.

Group 1 (vitrified oocytes)

Out of the 278 vitrified oocytes, 67.5% were expected to survive thawing; hence, a total of 187.6 oocytes would be available for fertilization and 64% (120.0) will be fertilized. With a cleavage rate of 95% (114.0) and implantation rate of 9.6%, 10.9 embryos were expected to implant. The expected pregnancy rate per oocyte collected or vitrified is 2.7% and 3.8%, respectively. For the average patient with 7.9 vitrified oocytes, the chance of pregnancy is expected to be 30.0%.

Group 2 (vitrified embryo)

Out of the 140 embryos and assuming a survival rate of 85% (119) (Shalom-Paz et al., unpublished data) and an implantation rate of 9.6%, 11.4 were expected to implant. Accordingly, the pregnancy rate per embryo vitrified is 8.1%. For the average patient with 4.5 vitrified embryos, the chance of pregnancy is expected to be approximately 36.0%.

Discussion

Most breast-cancer patients younger than 35 years will receive chemotherapy (Goldhirsch et al., 2005, 2007) with varying degrees of detrimental effect on ovarian function. Therefore, there is an increasing need for fertility preservation. The fertility preservation technique should be tailored specifically to each patient in order to achieve optimal results. Young and fertile cancer patients cope better

Table 1 IVM fertility preservation results for breast-cancer patients.

Characteristic	*Group 1 (n = 35)*	*Group 2 (n = 31)*
Age (years)	31.0 ± 5.2	34.2 ± 4.7
No. of oocytes retrieved	400 (11.4 ± 8.8)	301 (9.7 ± 6.4)
No. of in-vivo mature oocytes	59 (1.6 ± 2.7)	42 (1.3 ± 1.6)
No. of day-1 mature oocytes	136 (3.8 ± 3.2)	100 (3.2 ± 3.2)
No. of day-2 mature oocytes	83 (2.3 ± 2.6)	38 (1.2 ± 1.2)
No. of mature oocytes for vitrification or fertilization	278 (7.9 ± 6.6)	180 (5.8 ± 2.7)
Maturation rate (%)[a]	64.2	53.2
Median duration needed for fertility preservation(days)[b]	8	13

Values are number (mean ± SD) unless otherwise stated.

[a]Calculated as follows: matured oocyte on day 1 plus matured oocytes on day 2 divided by the total number of immature oocytes collected.

[b]From first day of evaluation to oocyte retrieval.

Table 2 Theoretical calculation of expected pregnancy rate per oocyte or embryo vitrified based on the centre results.

Calculation	*Expected rates*	*Vitrified oocytes (n = 278)*	*Vitrified embryos (n = 140)*
No. of oocytes surviving after thawing	0.67[a]	187.6	—
No. of embryos surviving after thawing	0.85[b]	—	119
No. of 2PN	0.64[a]	120	—
No. of cleaved embryos	0.95[a]	114	—
Expected no. of embryos implanted	0.096[a]	10.9	11.4
Pregnancy rate per oocyte collected (%)[c]	—	2.7	3.8
Pregnancy rate per oocyte or embryo vitrified (%)[d]	—	3.9	8.1
Expected live births	—	7.0[e]	7.4[e]

[a]Expected rates of survival after thawing, fertilization rate, cleavage rate and implantation rate for IVM vitrified embryos or oocytes according to Chian et al. (2009).
[b]McGill reproductive centre, unpublished data.
[c]Calculated as expected number of embryos implanted divided by number of oocytes collected.
[d]Calculated as expected number of embryos implanted divided by number of oocytes or embryos vitrified.
[e]Assuming 35% miscarriage rate (Shalom-Paz et al., unpublished data). — = not relevant.

emotionally with their disease and treatment if they feel that the option of having a biological child in the future is available for them (Partridge et al., 2004). At the diagnosis stage of breast cancer, plans for fertility preservation should be considered. Different fertility preservation options are available and include all the following: ovarian tissue freezing, IVF with oocyte or embryo freezing, IVM with oocyte or embryo freezing and ovarian suppression by GnRH agonist.

The different options for fertility preservation for breast-cancer patients and the pros and cons of each technique compared with IVM are discussed below. Each one of these options should be considered and should be raised as an alternative for the breast-cancer patients. Taking into consideration the priority of cancer, the best treatment will be prescribed to the patient by a multidisciplinary team including an oncologist, reproductive endocrinologist, psychologist and social worker in dialogue concerning the patient's wishes.

In-vitro fertilization

Ovarian stimulation for IVF requires exposure to hormones and elevation of oestradiol to supraphysiological concentrations. The influence of this elevation of hormonal concentration is certainly unclear. It is based only on the lowest evidence level (experts' opinion), which suggests avoiding hormone elevation (if possible) in breast-cancer patients regardless of their oestrogen receptor status (Gupta et al., 2007). The combination of ovarian stimulation with the protection of tamoxifen and or letrozole may be a good option for breast-cancer patients and has been suggested by others (Oktay, 2006; Oktay et al., 2007; Quintero et al., 2010). The idea behind this combination is to try to prevent the exposure of the cancer tissue to high oestradiol concentrations.

In contrast to IVF, the IVM technique does not cause hormonal elevation and the delay in cancer treatment according to this study's results is no longer than 8—13 days (probably shorter than the time needed for an IVF cycle which may delay cancer treatment for up to 5 weeks). The live-birth rate per vitrified oocyte in IVM cycles is about 20%, which is certainly lower than embryo freezing by IVF. Data concerning the results of IVM vitrification of oocytes and embryos is limited (Chian et al., 2009). Moreover, IVF is a well-established technique. Results show successful pregnancies with frozen IVF embryos with up to 57% delivery rate (Albani et al., 2008; Chian et al., 2009; Grifo and Noyes, 2010; Michaan et al., 2010; Porcu et al., 2008).

Ovarian tissue cryopreservation

Ovarian tissue can be obtained without additional hormonal stimulation and strips of ovarian cortical tissue can be cryopreserved directly for future use by either IVM or tissue transplantation. The ovarian cortex harbours primordial follicles that are more resistant to freezing damage, because the oocytes they contain have a relatively inactive metabolism and lack a metaphase spindle, zona pellucida and cortical granules (Dittrich et al., 2009). In early childhood or adolescence, when cancer treatment requires chemotherapy or pelvic radiotherapy, ovarian tissue cryopreservation may be the only reasonable option for fertility preservation (Sonmezer et al., 2005).

The risks of ovarian tissue cryopreservation include reimplantation of the primary tumour or its malignant transformation, as well as risks related to the invasiveness of the primary and recurrent procedures (Mueller et al., 2005). Various laboratory-based techniques are being used to screen ovarian tissue for the presence of metastatic disease before transplantation. These practices include preoperative imaging, histological analysis with immunohistochemical staining, polymerase chain reaction (PCR) amplification and real-time PCR (Elizur et al., 2004; Meirow et al., 2008). Additionally, immunoperoxidase broad-spectrum cytokeratin staining is performed on specimens from breast-cancer patients (Azem

et al., 2010). An alternative possibility, which may become available in the future is IVM of primordial ovarian follicles taken from the cryopreserved ovary. By that, the risk of cancer reimplantation will be eliminated (Abir et al., 2006; Smitz et al., 2010). Thus far, a few live births have been reported in cancer patients who underwent autologous transplantation of cryopreserved ovarian tissue (Andersen et al., 2008; Demeestere et al., 2007; Donnez et al., 2004; Meirow et al., 2005; Silber et al., 2008).

IVM may have several advantages over ovarian tissue cryopreservation: the surgical risks are minimal and the risk of reimplantating a metastatic tumour is eliminated. Importantly, IVM is becoming an accepted clinical practice with documented success rates.

Ovarian suppression

Although GnRH analogues are commonly used as ovarian protectant (Blumenfeld, 2007; Meistrich and Shetty, 2008); their ovarian-protective effect has never been proven in studies in humans. The treatment with GnRH analogues is lengthy, starting at least 10 days before the beginning of chemotherapy and at least 2 weeks after the end of treatment. GnRH agonist may cause severe menopausal symptoms and bone loss (Del Mastro et al., 2006) due to hypo-oestrogenic effects (Lee et al., 2006). Additionally, since a variety of human cancers, including those of the breast, ovary and endometrium, express GnRH receptors, (Oktay et al., 2007) the direct effects of GnRH agonists on human cancer cells are not known.

In-vitro maturation

IVM is becoming an accepted technique for treating PCOS patients as it avoids the use of hormones for stimulation of the ovaries and prevents ovarian hyperstimulation syndrome (Chian, 2004; Tan and Child, 2002). During IVM cycles, oestradiol concentrations are within the natural follicular phase range of up to 150 pmol/l (Child et al., 2002), far from the supra-high concentrations of oestradiol during ovarian stimulation. Breast-cancer cell proliferation can be induced by oestrogen (Allred et al., 2001; Platet et al., 2004; Prest et al., 2002); therefore, it is recommended to avoid high concentrations of oestradiol in these patients (Key and Allen, 2002; Spicer and Pike, 1993). In this aspect, the advantage of IVM treatment for breast-cancer patients is the reduced risk of stimulating oestrogen-sensitive tumours, which can enhance malignant cell proliferation (Azim et al., 2008; Elizur et al., 2008; Oktay et al., 2007; Platet et al., 2004).

As soon as breast cancer is diagnosed, the time for starting chemotherapy is limited. The time period needed for fertility preservation should minimally interfere with fighting the disease. Interestingly, this study included six (9.1%) patients who, due to shortage of time available before starting chemotherapy, underwent luteal-phase collection. In these patients, 57 oocytes were collected, eight of which were already mature on the day of collection (matured *in vivo*) and another 26 were matured *in vitro* and vitrified. The availability of oocytes during the luteal phase may demonstrate the enrolment of new viable follicles for the next cycle. Luteal phase oocyte retrieval might be considered whenever there is a significant shortage of time and waiting for conventional follicular-phase oocyte retrieval is not possible (Demirtas et al., 2008).

Two options are available for fertility preservation in women treated by IVM: embryo freezing and oocyte freezing. In recent years, and especially since the vitrification technique has been developed, oocyte freezing has became an option for fertility preservation. To date, embryo cryopreservation is still considered to be the most effective and reliable method. However, this approach requires a participating male partner or sperm donor. Embryo cryopreservation is offered only to couples who are in a stable relationship. Otherwise, oocyte vitrification is suggested in order to avoid custody issues.

When consulting with a cancer patient on fertility preservation, all the available options should be discussed and details concerning the success rates of each approach should be at hand. The expected pregnancy rates per vitrified oocyte or embryo were calculated in order to be able to provide this additional relevant data to patients. Detailed information is a mandatory tool for informed consent when different approaches (with pros and cons to each one of them) are considered and a decision must be made. The results in this study are in agreement with those existing in the literature concerning non-cancer patients. In a multicentre study, Parmegiani et al. (2009) reported the results of slow freezing and thawing oocytes and embryos. The pregnancy rates of frozen and thawed oocytes and embryos were 2.7% per oocyte and 5.7% per embryo. Paruco reported a 5–6% delivery rate per oocyte after oocyte freezing and thawing (Noyes et al., 2009; Parmegiani et al., 2009).

Data regarding pregnancy outcomes for cancer patients who have undergone IVM and oocyte/embryo vitrification is still limited. In order to maximize the chance of future fertility, it is reasonable to tailor the treatment to the patient by combining all the relevant options such as IVF and IVM oocyte/embryo freezing combined with ovarian tissue cryopreservation.

In conclusion, IVM and oocyte/embryo freezing may have a valuable role in fertility preservation, especially in breast-cancer patients. The option of luteal phase collection can be considered. This procedure offers the hope of future motherhood with comparable calculated pregnancy rate. It may be a combined with the different alternatives available for fertility preservation.

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