Immature oocyte retrieval and in vitro oocyte maturation at different phases of the menstrual cycle in women with cancer who require urgent gonadotoxic treatment

Helene Creux, M.D.,[a,b] Patricia Monnier, M.D., Ph.D.,[a,c] Weon-Young Son, Ph.D.,[a] Togas Tulandi, M.D., M.H.C.M.,[a] and William Buckett, M.D.[a]

[a] McGill University Health Centre, Department of Obstetrics and Gynecology, McGill University, Montreal, Quebec, Canada; [b] Reproductive Center, Department of Obstetrics and Gynecology, Hôpital Pellegrin, Bordeaux, France; and [c] Research Institute of McGill University Health Centre, McGill University, Montreal, Quebec, Canada

Objective: To evaluate the feasibility and the efficacy of in vitro maturation (IVM) when immature oocyte collection was performed in the early follicular, late follicular, or luteal phases in women with cancer who require urgent chemotherapy.
Design: Retrospective cohort study.
Setting: University teaching hospital.
Patient(s): One-hundred and sixty-four women with cancer undergoing IVM treatment for fertility preservation.
Intervention(s): Oocyte retrieval, IVM, cryopreservation.
Main Outcome Measure(s): Medians (interquartile range) of oocytes collected, maturation rates after 48 hours of culture, and metaphase II oocytes cryopreserved.
Result(s): The analysis included a total of 192 cycles grouped into early follicular phase (n = 46), late follicular phase (n = 107), or luteal phase (n = 39). Embryo cryopreservation was performed in 82 cycles, and oocyte cryopreservation in 105 cycles. Between the early follicular, late follicular, and luteal phases, no statistically significant differences were found in the number of oocytes collected (8.5 [4–15.8], 8 [5–14], and 7 [4–9], respectively), the maturation rates after 48 hours of culture (53.5% [39.8–77], 58% [44–82], and 50% [33–67], respectively), or the number of oocytes cryopreserved (3 [0–7.3], 3 [0–7], and 3 [1–5.5], respectively). Similarly, the fertilization rates (77 [62.8–92.5], 75 [60–100], and 63.5 [50–75], respectively) and number of embryos cryopreserved (3 [2–5.8], 3 [0.5–5], and 2 [1–3], respectively) were not statistically significantly different among the groups.
Conclusion(s): Our study confirms the feasibility of IVM collection at any time during the menstrual cycle. Treatment with IVM is an alternative method when chemotherapy cannot be delayed or ovarian stimulation is contraindicated. The long-term outcomes remain to be studied. (Fertil Steril® 2017;107:198–204. ©2016 by American Society for Reproductive Medicine.)
Key Words: Cancer, fertility preservation, in vitro maturation, luteal phase

Discuss: You can discuss this article with its authors and with other ASRM members at https://www.fertstertdialog.com/users/16110-fertility-and-sterility/posts/12274-22804

Chemotherapy and radiation treatment for malignancies have resulted in improved survival rates but may lead to sterility. To preserve the reproductive function, cryopreservation of oocytes, embryos, or ovarian tissue have been advocated. Currently, cryopreservation of oocytes or embryos after controlled ovarian hyperstimulation represents the established method for preserving female fertility (1). However, immature oocyte collection and subsequent in vitro maturation (IVM) of oocytes without any ovarian stimulation is an attractive alternative when chemotherapy cannot be delayed or ovarian stimulation is contraindicated because no ovarian stimulation is required. Treatment with IVM usually takes no more than 48 hours from the decision to perform

Received July 21, 2016; revised September 7, 2016; accepted September 22, 2016; published online October 31, 2016.
H.C. has nothing to disclose. P.M. has nothing to disclose. W.-Y.S. has nothing to disclose. T.T. has nothing to disclose. W.B. has nothing to disclose.
Reprint requests: Helene Creux, M.D., Reproductive Center, Department of Obstetrics and Gynecology, Hôpital Pellegrin, Place Amélie Raba-Léon, 33076 Bordeaux, France (E-mail: helenecreux@gmail.com).

Fertility and Sterility® Vol. 107, No. 1, January 2017 0015-0282/$36.00

http://dx.doi.org/10.1016/j.fertnstert.2016.09.041

oocyte retrieval (2). The metaphase II (MII) oocytes then can be cryopreserved, or they can be fertilized and the resulting embryos cryopreserved.

Conventional IVM oocyte retrieval is usually performed in the follicular phase. However, luteal phase IVM retrieval has been performed to avoid delaying cancer treatment (3–6). The concept of multiple major follicle recruitment waves during a normal menstrual cycle supports this practice (7–9). We evaluated the feasibility and the efficacy of IVM treatment when oocyte retrieval was performed in early follicular, late follicular, or luteal phase in women with cancer who required urgent gonadotoxic treatment.

MATERIALS AND METHODS

Subjects

We retrieved the data from our fertility preservation database. The study group consisted of women with cancer who underwent IVM treatment for fertility preservation during the period of January 2003 to December 2015. All cancer types were included. The exclusion criteria were age >40 years or <16 years, previous chemotherapy or oophorectomy, any ovarian stimulation, inadequate visualization of ovaries at transvaginal ultrasound, or incomplete data. We grouped the patients into three categories based on the phase of the cycle when human chorionic gonadotropin (hCG) priming was performed: early follicular phase, defined as before cycle day 7, and/or the absence of a dominant follicle (>10 mm), and/or endometrium (<6 mm); late follicular phase, defined as after cycle day 7, with one follicle >10 mm and combined with endometrium ≥6 mm; and luteal phase, defined as after spontaneous ovulation and/or the presence of a corpus luteum. The patients were offered oocyte or embryo cryopreservation according to their couple status and their wishes. The study was approved by our institutional review board (16-090 MUHC).

Oocyte Collection

Retrieval of the IVM oocytes was performed by an experienced and specifically trained physician 38 hours after a subcutaneous administration of 10,000 IU of hCG in accordance with the center's standard IVM practice (10). Transvaginal ultrasound-guided retrieval of oocytes was performed using a 19-gauge single lumen needle (K-OPS-7035-RWH-ET; Cook Australia) with a reduced aspiration pressure of 7.5 kPa, under conscious sedation. Retrieval of IVM oocytes requires training to acquire proficiency with a different aspiration system and method (particularly the smaller gauge needle and reduced suction pressure) because the follicles are smaller than those encountered during oocyte retrieval for conventional in vitro fertilization (IVF) cycles and the ovaries have greater mobility.

After the first evaluation under a stereomicroscope by an experienced embryologist, to avoid the possibility of missing oocytes with a small amount of cumulus cells (CC), the remaining follicular aspirate was filtered using 70-μm hole-size mesh (Falcon; Becton-Dickinson). Then the mesh was washed three times with oocyte wash medium (Cooper Surgical) that contained HEPES buffer supplemented with recombinant human serum albumin, and any further oocytes were identified under a stereomicroscope.

In Vitro Maturation

The oocytes collected were assessed for nuclear maturity under the dissecting microscope with high magnification (×80) using the spreading method (11, 12). Oocyte immaturity was assessed by the presence of the germinal vesicle (GV) and the first polar body, and mature oocytes were identified when the first polar body was extruded. If no GV was observed in the oocyte cytoplasm, the CCs were removed with 0.1% hyaluronidase solution and mechanical pipetting, and reassessment of maturity was performed. Oocytes that were mature on the collection day (day 0: 0–6 hours) were cryopreserved by vitrification on the same day; or they were inseminated, and the embryos were frozen on day 2 or day 3. The immature oocytes (GV or GV breakdown [GVBD] stage) were cultured in IVM medium (Cooper Surgical) supplemented with 75 mIU/mL follicle-stimulating hormone (FSH) and luteinizing hormone (LH). After culture on day 1 (24–30 hours), the oocytes were denuded from CCs with 0.1% hyaluronidase solution (Cook Australia) and mechanical pipetting.

After examination, the immature oocytes remaining at the GV or GVBD stage were further cultured in the same medium, and the meiotic status was reexamined on day 2 (48–52 hours' culture). Any immature eggs were discarded, and MII eggs were cryopreserved. The identification and maturation of immature oocytes in vitro requires appropriate training and experience.

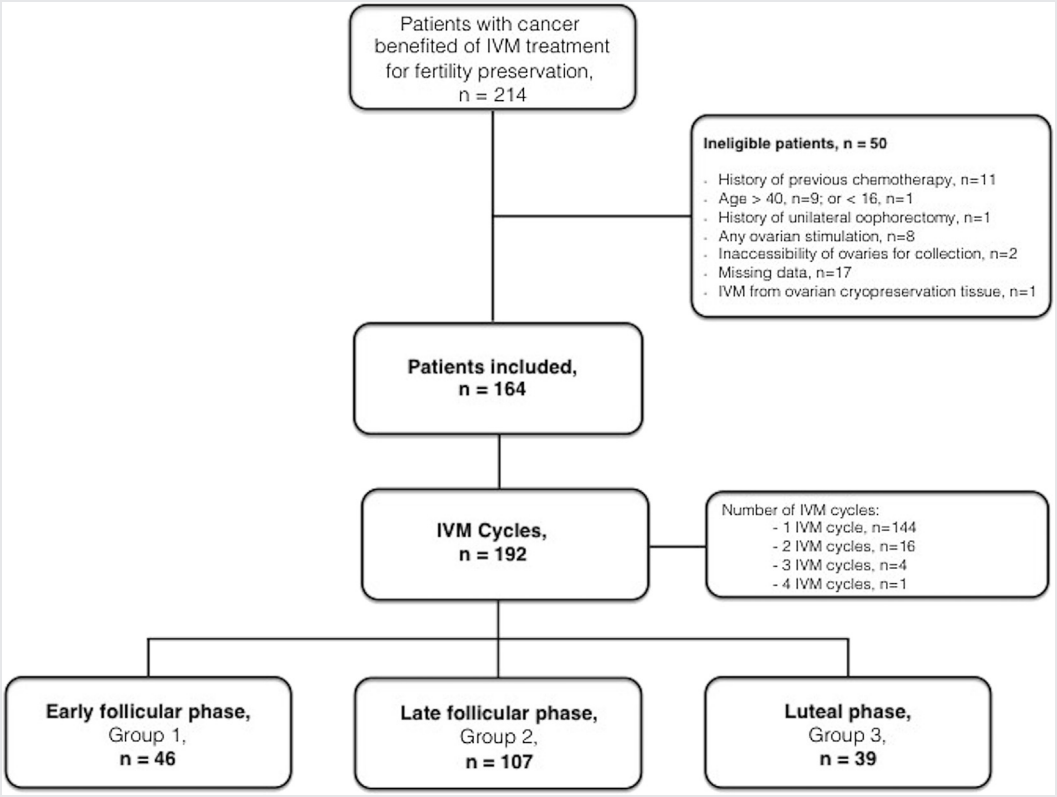
Fertilization and Embryo Development

Mature oocytes were inseminated by intracytoplasmic sperm injection (ICSI) using the partner's spermatozoa or donor sperm when appropriate. We performed ICSI at least 1 hour after observing the first polar body extrusion, as suggested by Hyun et al. (13). Fertilization was assessed 17 to 19 hours after insemination for the appearance of two distinct pronuclei and two polar bodies. The zygotes were cultured in Embryo Maintenance Medium (Cooper Surgical). Embryonic development was assessed on day 2 (41–43 hours) and on day 3 (65–67 hours) after insemination, according to the regularity of blastomeres, the percentage and pattern of anucleate fragments, and all dysmorphic characteristics of the embryos. The embryos were vitrified 2 or 3 days after ICSI.

Oocyte/Embryo Cryopreservation

For vitrification, the oocytes or embryos were suspended in equilibration medium containing 7.5% (v/v) ethylene glycol and 7.5% (v/v) dimethyl sulfoxide for 5 to 15 minutes at room temperature, then transferred to vitrification medium containing 15% (v/v) ethylene glycol, 15% (v/v) dimethyl sulfoxide, and 0.5 M sucrose at room temperature for 45 to 60 seconds. The oocytes or embryos were then loaded onto

FIGURE 1



Study flowchart of immature oocyte retrieval and in vitro oocyte maturation (IVM) at different phases of the menstrual cycle in women requiring urgent gonadotoxic treatment.

Creux. IVM throughout the menstrual cycle. Fertil Steril 2016.

a CryoTop (Kitazato Biopharma) and were immediately plunged into liquid nitrogen for storage.

Data Analysis

Patients characteristics collected for the study included age at oocyte retrieval, marital status, type of cancer, body mass index, gravidity, parity, early follicular phase serum FSH level, antral follicle count, cycle day, length of the menstrual cycle, last ultrasound parameters before hCG administration, type of cryopreservation performed (oocyte or embryo cryopreservation), and number of cycles per patient. The three groups were compared according to number of oocytes collected, number of MII oocytes on the day of collection, and maturation rates after 48 hours of culture. The fertilization rates and number of embryos cryopreserved were also evaluated where appropriate.

Statistical Analysis

Quantitative data were expressed as median and interquartile range (IRQ). To compare variables among the different phases of the menstrual cycle, we used nonparametric Kruskal-Wallis rank-sum tests with multiple comparison Z test. $P<.05$ was considered statistically significant. All statistical analyses were performed using NCSS 10 (NCSS Software).

RESULTS

Patient Characteristics

The study population consisted of 164 women with cancer who underwent IVM treatment for fertility preservation with a total of 192 cycles. Figure 1 shows the study's flowchart. Some patients underwent several IVM cycles during different phases of the menstrual cycle: 16 patients underwent two oocyte retrievals, four patients underwent three oocyte retrievals, and one patient underwent four oocyte retrievals.

The types of malignancy are demonstrated in Table 1. Breast cancer was the most frequent cancer in this population (68.8%), followed by hematologic cancers (15.9%). The median age of the population was 30 years (IRQ 27–35 years). Patients with breast cancer were the oldest with a median age of 32 years (IRQ 28–35 years), whereas patients with hematologic cancer or sarcoma were the youngest, with a median age of 26 years (IRQ 24–30 years) and 24 years (IRQ 23–40 years), respectively (see Table 1).

Among 192 IVM cycles, 105 IVM cycles (54.7%) resulted in cryopreservation of oocytes, and 82 IVM cycles (42.7%) resulted in cryopreservation of embryos. Of five cycles where cryopreservation (2.6%) was not performed, four IVM oocyte retrievals yielded no oocytes, and one cycle yielded immature

TABLE 1

Types of cancer in women with malignancies who underwent in vitro oocyte maturation treatment for fertility preservation.

Malignancy	No.	Proportion (%)	Age (y)
All kinds of cancer	164	100	30 (27–35)
Breast cancer	113	68.8	32 (28–35)
Hematologic cancer	26	15.9	26 (23.5–30)
Brain cancer	7	4.3	28.5 (25–29.8)
Gynecologic cancer	7	4.3	30 (27–37)
Sarcoma	7	4.3	24 (23–30)
Gastrointestinal cancer	4	2.4	29.5 (27.5–31)

Note: Age is median (interquartile range).

Creux. IVM throughout the menstrual cycle. Fertil Steril 2016.

oocytes but absence of maturation. Among 111 patients with a steady partner, 39 patients (35.1%) decided to freeze oocytes rather than embryos.

Comparison of IVM Results according to the Phase of the Menstrual Cycle

Oocyte retrieval was performed during the early follicular, late follicular, or luteal phases in 46, 107, and 39 cycles, respectively (Table 2). The three groups were comparable in terms of median age, body mass index, and ovarian reserve parameters including FSH and antral follicle count. In the early follicular, late follicular, and luteal phases, no statistically significant differences were found in the median of oocytes collected (8.5 [IRQ 4–15.8], 8 [IRQ 5–14], and 7 [IRQ 4–9], respectively), maturation rates after 48 hours of culture (53.5% [IRQ 39.8–77], 58% [IRQ 44–82], and 50% [IRQ 33–67], respectively), or number of oocytes cryopreserved (3 [IRQ 0–7.3], 3 [IRQ 0–7], and 3 [IRQ 1–5.5], respectively). Similarly, the fertilization rates (77 [IRQ 62.8–92.5], 75 [IRQ 60–100], and 63.5 [IRQ 50–75], respectively) and number of embryos cryopreserved (3 [IRQ 2–5.8], 3 [IRQ 0.5–5], and 2 [IRQ 1–3], respectively) were not statistically significantly different among the groups (see Table 2). We did not encounter any complications.

DISCUSSION

Our study shows that IVM oocyte retrieval is feasible and associated with good results among women with cancer who require urgent gonadotoxic treatment. The eggs can be obtained and potentially can be matured and developed, as reflected by oocyte fertilization and subsequent embryos through days 2 to 3. We also can confirm the feasibility of IVM treatment in any phase of the menstrual cycle. Initially, IVM was advocated for women with polycystic ovary syndrome to avoid the risks of ovarian hyperstimulation. Retrievals for IVM are usually performed during the follicular phase, but the best timing to proceed with IVM collection during the follicular phase remains controversial. Some investigators believe that a follicle size above 10 mm is detrimental (14, 15). For others, better outcomes have been reported when oocyte collection occurs when the leading follicle measures between 10–14 mm both in healthy women or women with polycystic ovary syndrome (10, 16, 17).

In our study, all cycles were triggered by hCG administration (18). Son et al. (18) found that hCG induced a dispersed cumulus pattern, faster maturation rates in vitro, and better embryonic developmental potential. Moreover, compared with oocytes matured in vitro, MII oocytes led to better quality embryos, better pregnancy rates, and better implantation rates (19). These results were corroborated by other groups as well (16, 20). The low number of MII oocytes retrieved in our study (median of 1) is reassuring in terms of our IVM procedure and definition. A recent study raised the issue of the standardization of IVM clinical definitions to allow transparency when comparing IVM results (21).

The feasibility of IVM treatment in the luteal phase is consistent with animal models, including bovines (22) and baboons (23), and also with the few previous publications in women with cancer (3–6). Oktay et al. (3) and Demirtas et al. (4) reported one and three cases, respectively, to stress the feasibility of the IVM procedure during the luteal phase. Maman et al. (5), in a retrospective study including 18 cancer patients (13 patients in the follicular phase and five patients in the luteal phase), found no statistically

TABLE 2

Comparable in vitro maturation results according to the phase of the cycle during which human chorionic gonadotropin priming was performed.

Parameter	Group 1 Early follicular phase (n = 46)	Group 2 Late follicular phase (n = 107)	Group 3 Luteal phase (n = 39)
Age, y	29.5 (27–34.3)	31 (27–35)	30 (24.8–36.3)
BMI, kg/m^2	21.4 (18.8–22.3)	21.4 (20–23.6)	22.9 (18.8–28.5)
FSH, IU/L	6.2 (4.7–7.7)	6.3 (4.5–7.7)	5.7 (3.8–7)
Antral follicle count	17 (10–30)	17.5 (11.3–22)	17.5 (12.8–22.3)
Collection day			
Total oocytes	8.5 (4–15.8)	8 (5–14)	7 (4–9)
MII oocytes	1 (0–2.3)	1 (0–2)	1 (0–1)
Maturation rate, %	53.5 (39.8–77)	58 (44–82)	50 (33–67)
Cryopreserved			
Oocytes	3 (0–7.3)	3 (0–7)	3 (1–5.5)
Embryos	3 (2–5.8)	3 (0.5–5)	2 (1–3)
Fertilization rate, %	77 (62.8–92.5)	75 (60–100)	63.5 (50–75)

Note: Data are median (interquartile range). BMI = body mass index; FSH = follicle-stimulating hormone; MII = metaphase II.

Creux. IVM throughout the menstrual cycle. Fertil Steril 2016.

significant differences in the mean ± standard deviation (SD) for the number of retrieved oocytes (17.3 ± 13.5 vs. 12.8 ± 8.4), maturation rates (57.8 ± 29.2 vs. 48.6 ± 18.3), fertilization rates (63.2 ± 27.3 vs. 69.2 ± 47.4), or mean total number of oocytes and/or embryos cryopreserved (7.8 ± 7.5 vs. 6.4 ± 6.6). Grynberg et al. (6), in a large prospective study including 248 breast cancer patients (127 patients in the follicular phase and 121 patients in the luteal phase), reported no statistically significant differences in the mean ± SD for the number of oocytes retrieved (9.3 ± 0.7 vs. 11.1 ± 0.8), in the median (range) for maturation rates after 48 hours of culture (66.7 [20–100] vs. 64.5 [0–100]), or the mean ± SD for the total number of oocytes cryopreserved (6.2 ± 0.4 vs. 6.8 ± 0.5). Yet Grynberg et al. (6) found a tendency toward a higher number of mature oocytes cryopreserved during the late follicular phase compared with the luteal phase. For these investigators, this trend can be related to the recovery of a mature oocyte from the dominant follicle. Also, Maman et al. (5) reported a trend to a slightly lower number of oocytes retrieved, lower maturation rates, and lower total number of oocytes and/or embryos cryopreserved in the luteal phase compared with the follicular phase. In our study, there was a tendency toward a higher number of oocytes retrieved, higher maturation rates, and higher number of matured oocytes cryopreserved in the follicular phase than in the luteal phase, but no statistically significant difference was established.

Several investigators have reported that in women with newly diagnosed cancer who require urgent oncologic treatment emergency fertility preservation by ovarian stimulation and IVF was possible at any time in the menstrual cycle (24–26). In the context of regular stimulation for IVF in noncancer patients, a recent report from Qin et al. (27) including 150 infertile patients demonstrated the efficacy of starting ovarian stimulation at no fixed time during the menstrual cycle. They reported a mean number of oocytes retrieved in the early follicular phase, late follicular phase, and luteal phase of 6.6 ± 3.8, 5.9 ± 4.3, and 5.9 ± 4.2, respectively, and a mean number of mature oocytes retrieved of 5.7 ± 3.6, 5.2 ± 3.7, and 5.2 ± 3.9, respectively.

Von Wolff et al. (28) have published another study of 684 patients before urgent chemotherapy. They also concluded that the outcome of ovarian stimulation was similar after stimulation initiation during any phase of the menstrual cycle. The mean number of oocytes retrieved in the early follicular, late follicular, and luteal phases was 11.6 ± 7.7, 13.9 ± 9.1, 13.6 ± 7.9, respectively, with a statistically significant increase in the late follicular phase and the luteal phase.

In our study, the analysis was performed using median values ± IQR because the variables were non-Gaussian. However, the mean number of oocytes retrieved in the early follicular, late follicular, and luteal phases was 11.3 ± 10.2, 9.9 ± 6.9, and 7.8 ± 5.3, respectively, and the mean number of mature oocytes cryopreserved was 5.1 ± 6.5, 4.6 ± 5.1, and 3.4 ± 2.9, respectively. These results remain close to those reported by Qin et al. (27) and Von Wolff et al. (28) in cases of random-start conventional IVF procedures. Whether there are differences in clinically important outcomes such as the number of embryos obtained after oocyte thawing and live births after treatment or between starting stimulation (or, in fact, IVM) at different stages of the menstrual cycle remains to be seen.

No study so far has compared IVF and IVM cycles results in patients with cancer. Several studies performed in infertile women have reported better pregnancy and live-birth rates after ovarian controlled hyperstimulation when compared with IVM procedures in polycystic ovary syndrome patients (29, 30) and normo-ovulatory patients (31). This would suggest that oocytes cryopreserved after ovarian stimulation may have a better developmental potential as compared with eggs matured in vitro. However, the average number of days from the start of stimulation to conventional oocyte retrieval in those studies was 10 to 12 days. The ovarian stimulation duration seems significantly longer with higher doses of gonadotropins in the late follicular and the luteal phases rather than in the early follicular phase as shown by Von Wolff et al. (28) with a stimulation duration in days in the early follicular, late follicular, and luteal phases of 10.8 ± 2.4, 10.6 ± 2.7, 11.5 ± 2.2, respectively. This delay may be too long when urgent chemotherapy is required.

In our study population, many women already had chemotherapy planned in the 3 to 5 days after the fertility preservation clinic counseling, especially in cases of aggressive hematologic cancers. For these patients, IVM treatment was a therapeutic option, but earlier referrals to fertility preservation counseling may perhaps change this practice. Twenty-one patients underwent more than one IVM cycle, and all were patients with a diagnosis of breast cancer before 2010. In these cases, IVM was not urgently indicated because of chemotherapy but rather was recommended because ovarian stimulation was contraindicated. Until 2010, our team managed all estrogen-dependent cancers with IVM procedures and contraindicated any ovarian stimulation. Our practice changed after the publication of the studies by Oktay et al. (32–35). After that point, retrievals in the setting of estrogen-dependent cancers, and especially breast cancers, were stimulated in our center via antiestrogen therapy.

Successful late follicular and luteal phase oocyte retrievals may be explained by the concept of multiple follicular waves. There has been increasing evidence to indicate that two or more cohorts of antral follicles are recruited during the human menstrual cycle, as was previously documented in several animal species (8).

Only a few live births have been reported in the literature after IVM and oocyte cryopreservation (36, 37), and in these cases oocyte retrieval was performed in the follicular phase. There have been a few reports of live births from cryopreserved embryos resulting from immature oocytes aspirated from surgically resected ovaries (38, 39). The fact that many of these young women are still recovering from cancer and have not yet tried to conceive may explain the lack of obstetric outcome data.

The limitations of our study include its retrospective nature and the limited ultimate use of the cryopreserved oocytes and embryos. However, we have included a relatively large number of patients, and we compared oocyte retrievals from three phases of the menstrual cycle. Moreover, the oocytes

were treated in one center in the same manner by an experienced team, which avoids a bias created by a center effect. Yet despite recent advances in ovarian stimulation protocols using antiestrogen therapy (40), there are still some concerns about the adverse effects of ovarian stimulation on the risk of cancer relapse in cases of hormone-dependent cancers. Some oncologists still take a cautious approach due to the limited availability of data from follow-up observations. Even if recent data have been reassuring and show no increased recurrence risk in breast cancer patients who underwent ovarian stimulation with letrozole during the 5 years after diagnosis (41), long-term follow-up evaluations are not yet available (42).

In conclusion, we have shown that IVM treatment combined with embryo or oocyte cryopreservation offers a viable option for fertility preservation at any time during the menstrual cycle. In patients who require urgent gonadotoxic therapy, IVM avoids the time needed for ovarian stimulation, even when ovarian stimulation is started at any phase of the menstrual cycle. However, the long-term outcomes remain to be studied.

Acknowledgments: The authors thank all the physicians, embryologists, nurses, and administrative personal working for our fertility preservation program at the Reproductive Centre of McGill University Health Centre. We also thank Dr. Christophe Cassinotto for his precious help in statistical analysis and manuscript editing.

REFERENCES

1. Loren AW, Mangu PB, Beck LN, Brennan L, Magdalinski AJ, Partridge AH, et al. Fertility preservation for patients with cancer: American Society of Clinical Oncology clinical practice guideline update. J Clin Oncol 2013;31:2500–10.
2. Berwanger AL, Finet A, El Hachem H, Le Parco S, Hesters L, Grynberg M. New trends in female fertility preservation: in vitro maturation of oocytes. Future Oncol 2012;8:1567–73.
3. Oktay K, Demirtas E, Son W-Y, Lostritto K, Chian R-C, Tan SL. In vitro maturation of germinal vesicle oocytes recovered after premature luteinizing hormone surge: description of a novel approach to fertility preservation. Fertil Steril 2008;89:228.e19–22.
4. Demirtas E, Elizur SE, Holzer H, Gidoni Y, Son W-Y, Chian R-C, et al. Immature oocyte retrieval in the luteal phase to preserve fertility in cancer patients. Reprod Biomed Online 2008;17:520–3.
5. Maman E, Meirow D, Brengauz M, Raanani H, Dor J, Hourvitz A. Luteal phase oocyte retrieval and in vitro maturation is an optional procedure for urgent fertility preservation. Fertil Steril 2011;95:64–7.
6. Grynberg M, Poulain M, le Parco S, Sifer C, Fanchin R, Frydman N. Similar in vitro maturation rates of oocytes retrieved during the follicular or luteal phase offer flexible options for urgent fertility preservation in breast cancer patients. Hum Reprod 2016;31:623–9.
7. Baerwald AR, Adams GP, Pierson RA. Characterization of ovarian follicular wave dynamics in women. Biol Reprod 2003;69:1023–31.
8. Baerwald AR, Adams GP, Pierson RA. Ovarian antral folliculogenesis during the human menstrual cycle: a review. Hum Reprod Update 2012;18:73–91.
9. Yang DZ, Yang W, Li Y, He Z. Progress in understanding human ovarian folliculogenesis and its implications in assisted reproduction. J Assist Reprod Genet 2013;30:213–9.
10. Son W-Y, Chung J-T, Chian R-C, Herrero B, Demirtas E, Elizur S, et al. A 38 h interval between hCG priming and oocyte retrieval increases in vivo and in vitro oocyte maturation rate in programmed IVM cycles. Hum Reprod 2008;23:2010–6.
11. Chian RC, Buckett WM, Tulandi T, Tan SL. Prospective randomized study of human chorionic gonadotrophin priming before immature oocyte retrieval from unstimulated women with polycystic ovarian syndrome. Hum Reprod 2000;15:165–70.
12. Son W-Y, Yoon S-H, Lim J-H. Effect of gonadotrophin priming on in-vitro maturation of oocytes collected from women at risk of OHSS. Reprod Biomed Online 2006;13:340–8.
13. Hyun C-S, Cha J-H, Son W-Y, Yoon S-H, Kim K-A, Lim J-H. Optimal ICSI timing after the first polar body extrusion in in vitro matured human oocytes. Hum Reprod 2007;22:1991–5.
14. Le Du A, Kadoch IJ, Bourcigaux N, Doumerc S, Bourrier M-C, Chevalier N, et al. In vitro oocyte maturation for the treatment of infertility associated with polycystic ovarian syndrome: the French experience. Hum Reprod 2005;20:420–4.
15. Cobo AC, Requena A, Neuspiller F, Aragon s M, Mercader A, Navarro J, et al. Maturation in vitro of human oocytes from unstimulated cycles: selection of the optimal day for ovum retrieval based on follicular size. Hum Reprod 1999;14:1864–8.
16. Mikkelsen AL, Smith S, Lindenberg S. Possible factors affecting the development of oocytes in in-vitro maturation. Hum Reprod 2000; 15(Suppl 5):11–7.
17. Fadini R, Dal Canto MB, Mignini Renzini M, Brambillasca F, Comi R, Fumagalli D, et al. Effect of different gonadotrophin priming on IVM of oocytes from women with normal ovaries: a prospective randomized study. Reprod Biomed Online 2009;19:343–51.
18. Son W-Y, Tan SL. Laboratory and embryological aspects of hCG-primed in vitro maturation cycles for patients with polycystic ovaries. Hum Reprod Update 2010;16:675–89.
19. Son W-Y, Chung J-T, Demirtas E, Holzer H, Sylvestre C, Buckett W, et al. Comparison of in-vitro maturation cycles with and without in-vivo matured oocytes retrieved. Reprod Biomed Online 2008;17:59–67.
20. Lim J-H, Yang S-H, Xu Y, Yoon S-H, Chian R-C. Selection of patients for natural cycle in vitro fertilization combined with in vitro maturation of immature oocytes. Fertil Steril 2009;91:1050–5.
21. Dahan MH, Tan SL, Chung J, Son W-Y. Clinical definition paper on in vitro maturation of human oocytes. Hum Reprod 2016;31:1383–6.
22. Chian R-C, Chung J-T, Downey BR, Tan SL. Maturational and developmental competence of immature oocytes retrieved from bovine ovaries at different phases of folliculogenesis. Reprod Biomed Online 2002;4:127–32.
23. Xu M, Fazleabas AT, Shikanov A, Jackson E, Barrett SL, Hirshfeld-Cytron J, et al. In vitro oocyte maturation and preantral follicle culture from the luteal-phase baboon ovary produce mature oocytes. Biol Reprod 2011;84: 689–97.
24. Cakmak H, Katz A, Cedars MI, Rosen MP. Effective method for emergency fertility preservation: random-start controlled ovarian stimulation. Fertil Steril 2013;100:1673–80.
25. Rashidi BH, Tehrani ES, Ghaffari F. Ovarian stimulation for emergency fertility preservation in cancer patients: a case series study. Gynecol Oncol Rep 2014;10:19–21.
26. Checa MA, Brassesco M, Sastre M, Gómez M, Herrero J, Marque L, et al. Random-start GnRH antagonist for emergency fertility preservation: a self-controlled trial. Int J Womens Health 2015;7:219–25.
27. Qin N, Chen Q, Hong Q, Cai R, Gao H, Wang Y, et al. Flexibility in starting ovarian stimulation at different phases of the menstrual cycle for treatment of infertile women with the use of in vitro fertilization or intracytoplasmic sperm injection. Fertil Steril 2016;106:334–41.
28. Von Wolff M, Capp E, Jauckus J, Strowitzki T, Germeyer A, FertiPROTEKT study group. Timing of ovarian stimulation in patients prior to gonadotoxic therapy: an analysis of 684 stimulations. Eur J Obstet Gynecol Reprod Biol 2016;199:146–9.
29. Child TJ, Phillips SJ, Abdul-Jalil AK, Gulekli B, Tan SL. A comparison of in vitro maturation and in vitro fertilization for women with polycystic ovaries. Obstet Gynecol 2002;100:665–70.
30. Gremeau AS, Andreadis N, Fatum M, Craig J, Turner K, McVeigh E, et al. In vitro maturation or in vitro fertilization for women with polycystic ovaries? A case-control study of 194 treatment cycles. Fertil Steril 2012;98:355–60.

31. Fadini R, Mignini Renzini M, Dal Canto M, Epis A, Crippa M, Caliari I, et al. Oocyte in vitro maturation in normo-ovulatory women. Fertil Steril 2013;99:1162–9.
32. Oktay K. Further evidence on the safety and success of ovarian stimulation with letrozole and tamoxifen in breast cancer patients undergoing in vitro fertilization to cryopreserve their embryos for fertility preservation. J Clin Oncol 2005;23:3858–9.
33. Oktay K, Buyuk E, Libertella N, Akar M, Rosenwaks Z. Fertility preservation in breast cancer patients: a prospective controlled comparison of ovarian stimulation with tamoxifen and letrozole for embryo cryopreservation. J Clin Oncol 2005;23:4347–53.
34. Oktay K, Türkçüoglu I, Rodriguez-Wallberg KA. GnRH agonist trigger for women with breast cancer undergoing fertility preservation by aromatase inhibitor/FSH stimulation. Reprod Biomed Online 2010;20:783–8.
35. Sönmezer M, Türkçüoglu I, Coskun U, Oktay K. Random-start controlled ovarian hyperstimulation for emergency fertility preservation in letrozole cycles. Fertil Steril 2011;95:2125.e9–11.
36. Chian R-C, Huang JYJ, Gilbert L, Son W-Y, Holzer H, Cui SJ, et al. Obstetric outcomes following vitrification of in vitro and in vivo matured oocytes. Fertil Steril 2009;91:2391–8.
37. Chian R-C, Gilbert L, Huang JYJ, Demirtas E, Holzer H, Benjamin A, et al. Live birth after vitrification of in vitro matured human oocytes. Fertil Steril 2009;91:372–6.
38. Prasath EB, Chan ML, Wong WH, Lim CJ, Tharmalingam MD, Hendricks M, et al. First pregnancy and live birth resulting from cryopreserved embryos obtained from in vitro matured oocytes after oophorectomy in an ovarian cancer patient. Hum Reprod 2014;29:276–8.
39. Uzelac PS, Delaney AA, Christensen GL, Bohler HC, Nakajima ST. Live birth following in vitro maturation of oocytes retrieved from extracorporeal ovarian tissue aspiration and embryo cryopreservation for 5 years. Fertil Steril 2015;104:1258–60.
40. Shapira M, Raanani H, Meirow D. IVF for fertility preservation in breast cancer patients–efficacy and safety issues. J Assist Reprod Genet 2015;32:1171–8.
41. Kim J, Turan V, Oktay K. Long-term safety of letrozole and gonadotropin stimulation for fertility preservation in women with breast cancer. J Clin Endocrinol Metab 2016;101:1364–71.
42. Reddy J, Oktay K. Ovarian stimulation and fertility preservation with the use of aromatase inhibitors in women with breast cancer. Fertil Steril 2012;98:1363–9.