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

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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

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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

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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