Outcome of immature oocytes collection of 119 cancer patients during ovarian tissue harvesting for fertility preservation

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Abstract

Purpose Few clinical options for fertility preservation are available to females with cancer, and data about clinical outcomes is limited. Potential supplementary approaches to fertility preservation include retrieval of immature oocytes followed by in vitro maturation (IVM) and storage. The aim of this study was to evaluate post-thawing outcomes of immature oocytes collected both by transvaginal aspiration and from excised ovarian tissue.

Methods We conducted a retrospective cohort study of patients treated in a single tertiary center. We reviewed the records of 119 cancer patients who underwent ovarian tissue cryopreservation and immature oocyte harvesting for fertility preservation. All embryos and oocytes that were frozen and thawed were included in the study. Post-thawing outcomes were evaluated.

Results Thirty-five stored embryos from eight patients were thawed. Twenty-nine embryos survived (82% survival rate) and were transferred. Six oocytes were thawed, two oocytes survived, and no oocytes were fertilized. Only one PCOS patient became pregnant, resulting in the normal delivery of a healthy baby.

Conclusions Although a relatively high number of mature oocytes and embryos can be stored with the combined procedure, the limited rate of pregnancies represents a poor reproductive outcome. Therefore, this approach should be reserved for special groups with limited options.

Keywords Fertility preservation · Oocyte retrieval · In vitro oocyte maturation

Introduction

Over the past four decades, advancements in cancer therapies, particularly chemotherapeutics, have led to dramatic improvements in patient survival. However, population-based studies have demonstrated a consistent 30–50% reduction in the probability of having a live birth in cancer survivors compared with control subjects, mainly due to treatment strategies such as chemotherapy and radiation [1–4].

There are relatively few available options for fertility preservation, and data on clinical outcomes is limited. For postpubertal females who have a committed male partner or who are prepared to use donor sperm, embryo cryopreservation is a well-established technology that provides a

predictable likelihood of success based on the number and quality of embryos stored [5]. Mature oocyte cryopreservation is an appropriate strategy for fertility preservation in postpubertal females who do not wish to use donor sperm and do not have a committed male partner [6]. However, embryo or mature oocyte cryopreservation should only be recommended if the patient has no contraindication to carrying out controlled ovarian stimulation (COS), and the patient has a reasonable chance of responding to COS and has adequate time to undergo COS and oocyte retrieval before initiation of gonadotoxic treatment. Theoretically, cryopreservation of ovarian cortical tissue represents an efficient way of preserving thousands of ovarian follicles in one procedure. This technique has been proposed principally for prepubertal females, for those who cannot delay cancer treatment or whenever hormonal stimulation treatment is contraindicated [7, 8]. Ovarian tissue banking may be the only acceptable method to preserve fertility for prepubertal girls since ovarian stimulation and in vitro fertilization (IVF) are not viable options [9, 10]. Orthotopic ovarian re-implantation has led to the birth of over 80 healthy babies [11–13]. However, this approach

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requires an additional operation and it does not always work. There is also a risk of ovarian involvement from the underlying cancer [14]. A combined approach of aspirating immature oocytes directly from the excised tissue has been suggested [15–17], and three live births were reported [18–20]. Another approach involves the retrieval of immature oocytes in vivo with or without minimal stimulation. This may provide an additional opportunity for female germ cell preservation in cancer patients. Both techniques of immature oocyte harvesting for IVM have the advantages of lessening the risk of transmission of malignant cells and of not requiring COS, thereby shortening time to initiation of cancer treatment and maintaining lower estrogen levels. IVM could also be implemented as a supplementary measure during ovarian cortex cryopreservation. Recently, our group demonstrated that retrieval of immature oocytes for IVM by transvaginal aspiration just prior to ovarian tissue cryopreservation is safe and yielded significantly more mature oocytes than ovarian tissue oocyte cryopreservation alone [16]. The aim of this study was to evaluate post-thawed fertility outcomes of immature oocytes collected both in vivo and from excised ovarian tissue.

Methods

Patients and fertility preservation methods

The files of all cancer patients in the Chaim Sheba Medical Center Infertility and IVF Unit who underwent fertility preservation procedures by excision and cryopreservation of ovarian tissue between the years 2007 and 2015 were reviewed. According to hospital policy, patients had been offered individualized fertility preservation methods, which were tailored according to parameters such as patient's age, marital status, disease type, treatment protocol, contraindications for hormonal treatment, and time constraints.

Patients were offered a combination of two fertility preservation methods: ovarian tissue cryopreservation and collection of immature oocytes, to be matured in vitro and fertilized or cryopreserved. However, patients who had been exposed to chemotherapy within 6 months preceding fertility preservation procedure were not eligible for cryopreservation of oocytes or embryos. This was due to extensive data indicating the damaging effect of chemotherapy on growing follicles and its effects on future pregnancy outcomes [21, 22] more]. Therefore, the only method of fertility preservation offered to these patients was ovarian tissue cryopreservation.

Immature oocyte collection

Two techniques of immature oocyte collection were used: (1) oocyte pick up (OPU) by transvaginal ultrasound-guided aspiration prior to laparoscopic ovarian tissue harvesting and (2)

collection of ovarian tissue oocytes (OTO), which were released to the medium during preparation of ovarian cortical tissue for freezing. In patients who were not eligible for OPU (children, virgin patients, or cervical cancer patients), oocytes were collected only during ovarian tissue processing.

OPU IVM protocol

Patients who were selected for OPU IVM first underwent an IVM cycle according to accepted IVM protocols [23]. On day 3 of a spontaneous menstrual cycle, patients had a baseline transvaginal ultrasound to assess ovarian morphology, antral follicle count (AFC), and endometrial thickness. Ultrasound evaluation and blood samples were taken to determine basal blood concentrations of estradiol and progesterone. Following that, 150 international units IU/day of recombinant follicle stimulating hormone (rFSH) were administered to the patients for 3 days. A second ultrasound evaluation and blood test was performed on day 6. An injection of 10,000 IU human chorionic gonadotropin (hCG) (Pregnyl; Organon, Oss, Holland) was administered subcutaneously when the leading follicle was 12 mm. Oocyte retrieval was performed 36 h later by transvaginal ultrasound-guided needle aspiration. Follicular fluid and oocytes were collected in culture tubes containing flushing medium with heparin (MediCult prod. no. 10760125, Denmark). In several urgent cases, OPU was performed without the routine preparation. In these cases, the patients did not receive hCG. In all cases of OPU, laparoscopy for ovarian tissue harvesting was performed immediately following the OPU process.

Ovarian tissue, oocytes, and embryos cryopreservation

Approximately one half to two-thirds of the ovarian cortex from one ovary was harvested from each patient by laparoscopic surgery. The tissue was immediately placed in an appropriate medium and transferred to an adjacent IVF laboratory where it was prepared for cryopreservation [11, 24]. Finally, ovarian tissue pieces were then frozen by slow freeze method [7].

Oocytes and embryos were vitrified and thawed using the SAGE vitrification cooling and warming kits (ORGIO, Denmark) with the Kitazato Cryotop (Kitazato, Japan) according to the manufacture instructions.

Oocyte isolation from ovarian tissue (OTO)

During the handling of the ovarian tissue samples, oocytes were collected from the ovarian tissue (OTO). Any small follicles observed were aspirated, and any oocytes observed in the medium were separated and saved for further maturation and handling in the laboratory. The medium in which the

ovarian tissue samples were prepared was also searched for oocytes, which were collected and preserved for further treatment [25, 26]. These procedures took place in an operating theatre adjacent to an IVF laboratory. This facilitated immediate preparation of the immature oocytes and enabled transportation of the tissue without ice, minimizing harm to the immature oocytes.

IVM procedure

Any metaphase II oocytes underwent intracytoplasmic sperm injection (ICSI) or were frozen immediately. Immature oocytes were incubated in IVM medium (Sage; Trumbull, CT) supplemented with 75 IU follicle-stimulating hormone (FSH) and 75 IU luteinizing hormone (LH) (Ferring, Keil, Germany). Oocyte maturation was assessed after 24 and 48 h in culture and mature oocytes were either frozen or fertilized by intracytoplasmic sperm injection (ICSI). Eighteen hours after the ICSI procedure, fertilization was assessed using the established pronuclei (PN) scoring system and embryos with a PN score of 2 were preserved.

Patients who underwent fertility preservation became sterile because of the cancer therapy and wished to conceive were referred for thawing and transplantation of stored oocytes or embryos. The main outcomes measured in this study were oocyte yield, maturation and fertilization rates, numbers of frozen oocytes and embryos and post-thawing and post-transplantation outcomes.

Frozen embryos transfer

All patients underwent an artificial cycle FET protocol. From day 1 of the cycle, patients commence oral estradiol (progynova, Bayer, Germany) 2 mg three times daily. After 7 days, an ultrasound is performed. If no leading follicle is present and the endometrial thickness is ≥ 8 mm, micronized progesterone (utrogestan Besins International, Belgium) is added to the regime, and thawing and transferring are commenced 4 or 5 days later according to the stage of cryopreservation.

Results

Patients' characteristics

A total of 119 patients underwent fertility preservation using ovarian tissue cryopreservation in combination with immature oocyte harvesting in our center between 2007 and 2015 and were included in the study. Table 1 summarizes the characteristics of the patients who participated in this study in each group. Fifty-three percent of the patients (64/119) suffered from hematological malignancies, mainly Hodgkin's disease

Table 1 Characteristics of all patients who underwent fertility preservation in the Chaim Sheba Medical Center Infertility and IVF Unit by ovarian tissue cryopreservation and immature oocyte harvesting for IVM between the years 2007 and 2015

Patient characteristics	All patients $n = 119$
Age, years	
Range	(9–41)
Mean $\pm$ SE	24.1 $\pm$ 0.6
Diagnosis, n (%)	
Hodgkin's	48 (40%)
Hematological	16 (13%)
Sarcoma	14 (12%)
Breast	15 (13%)
Cervix	7 (6%)
Other	19 (16%)
Chemotherapy before FP, %	22%

IVF in vitro fertilization, IVM in vitro maturation, FP fertility preservation

(48/119). There were no reports of unusual complications during the fertility preservation procedures. No signs of excessive bleeding from the aspirated ovaries were observed. There were no difficulties in excision of the ovarian cortices after 3 days of FSH administration and following oocyte aspiration, the ovaries were not enlarged and there was no need for excessive coagulation. No technical difficulties were encountered in preparation of the ovarian cortical slices for cryopreservation.

The yield of all immature oocyte collection and the results of the IVM procedures are presented in Table 2.

Post-thawing outcomes

At the time of writing, eight patients had become sterile as a result of the cancer therapy, wished to conceive, and were referred for thawing and transplantation of cryopreserved oocytes and embryos. Patient characteristics, thawing, and transfer results from these eight patients are described in Table 3. Two of the patients had chosen to preserve unfertilized mature oocytes; three oocytes had been vitrified for each of the two patients. Two oocytes survived thawing, but fertilization was unsuccessful. The other six patients had preserved embryos. Thirty-five embryos were thawed (all embryos were frozen using vitrification technique, 30 embryos from the OPU IVM and 5 embryos from OTO IVM); see Table 3. Twenty-four embryos were transferred from the OPU IVM (80% survival rate) and five embryos were transferred from the OTO IVM (100% survival rate). Out of 29 embryos, one pregnancy was reported and was followed by a normal pregnancy and delivery of a normal baby (Table 3). This patient had a profile of polycystic ovarian syndrome (PCOS). The other seven patients underwent ovarian tissue transplantation. At the time of

Table 2 Characteristics of oocytes and embryos from in vitro maturation (IVM) of Immature oocytes collected by OPU and OTO

Oocyte harvesting, IVM, IVF, and cryopreservation of oocytes and embryos	
Patients	
No. of patients who underwent oocyte harvesting for IVM	119
No. of patients from whom oocytes were collected (%)	90 (75%)
Oocyte harvesting yield	
Total no. of oocytes collected from patients	715
Mean $\pm$ SE ^a	7.9 $\pm$ 1.60
Range	(1–31)
Total no. of metaphase II oocytes on day 0	45
Mean $\pm$ SE ^a	0.5 $\pm$ 0.24
Range	(0–7)
IVM Results	
Total no. of metaphase II oocytes obtained after maturation	447
Mean $\pm$ SE ^a	4.9 $\pm$ 0.69
Range	(0–20)
Total maturation rate	62%
Total no. of injected oocytes	214
Mean $\pm$ SE ^a	1.79 $\pm$ 1.4
Total no. of pronuclei score 2	159
Mean $\pm$ SE ^a	2.3 $\pm$ 1.3
Fertilization rate	74%
Cryopreservation	
Total no. of cryopreserved oocytes	209
Mean $\pm$ SE ^a	2.3 $\pm$ 3.6
Total no. of cryopreserved embryos	148
Mean $\pm$ SE ^a	1.64 $\pm$ 3

OPU oocyte pick up, transvaginal aspiration of oocytes; OTO collection of ovarian tissue oocytes

^a Mean $\pm$ SE were calculated for patients from whom oocytes were obtained

writing, three pregnancies resulting in four babies have been recorded and one spontaneous pregnancy after ovarian tissue transplantation. An additional pregnancy was following ovarian tissue transplantation and IVF, and one pregnancy was from the native ovary.

Discussion

This study focuses on the outcomes and yield of collecting immature oocytes both in vivo, by transvaginal oocyte pick up, and ex vivo, from excised ovarian tissue, as potential techniques of fertility preservation. To our knowledge, at the time

Table 3 Patients characteristics, thawing, and transfer results from eight patients

Number of patients	8	
Age at OPU (years)	29 ± 3	
Age at FET (years)	33.3 ± 2.5	
FSH at FET (i.u.)	44 ± 10	
Menopause	1/8	
	OPU	OTO
Total oocytes (average ± SE)	82	25
	10 ± 1.2	15 ± 0.9
Total MII oocytes day 0	21	12
	2.6 ± 0.2	3.1 ± 0.3
Total MII oocytes	50	12
	6.2 ± 0.5	3.1 ± 0.3
Frozen oocytes	6	—
	3 ± 0	
Thawed oocytes	2	—
Transferred embryos (from frozen oocytes)	0	—
Cryopreserved embryos	30	5
	3.7 ± 0.4	1.2 ± 0.2
Transferred embryos (from frozen embryos)	24	5
	4	1.2 ± 0.2
Pregnancies	1	

of writing, this is the most extensive study on IVM using both techniques of immature oocyte collection for fertility preservation. Our results show that retrieval of immature oocytes by OPU just prior to ovarian tissue cryopreservation, followed by OTO, achieved a high yield of mature oocytes and embryos. However, reproductive outcomes were poor, with 1 pregnancy and live birth out of 35 thawed embryos and 3 thawed oocytes.

The yield of mature oocytes and embryos was high, achieving 447 mature oocytes per 90 patients and 148 frozen embryo (1.64 per patient). This is comparable or higher than previously described for IVM in fertility preservation patients [15, 19, 27, 28]. Table 2 shows that the yield from OPU was considerably higher than the yield from OTO, and in 50% of OTO, no oocytes were obtained, whereas oocytes were found in all of the OPU cycles. This is in keeping with data from a recent study of ours indicating that performing only OTO IVM yielded fewer oocytes with lower maturation rates and fewer cryopreserved oocytes and embryos compared to the combined OPU and OTO IVM approach [16].

It is possible that the higher yield from OPU is due to the fact that some oocytes were aspirated prior to the ovarian resection; however, the total number of oocytes was higher using the combined procedure. Consequently, using this approach has allowed us to cryopreserve significantly more oocytes. An additional reason for the higher yield in OPU is because the ultrasound guidance enables better visualization of available oocytes in the ovary compared to the limited visualization of the antral follicles in OTO. Another

explanation is that during OPU, we were able to aspirate follicles from both ovaries, whereas OTO is restricted only to the excised ovarian tissue, thereby reducing the final yield. Further limitations of OTO in comparison with OPU are that OTO is more time consuming than OPU and that the IVF laboratory must be next to the operating theater to enable warm tissue transfer. However, OTO-IVM has several advantages. It is the only available technique for fertility preservation in children [17]. It does not require patient synchronization or any hormonal treatments. In addition, when combined with ovarian tissue cryopreservation, the tissue is already excised, with no need for further operating facilities to carry out the oocyte retrieval.

This is the first study to show outcomes of thawed embryos after IVM of immature oocytes collected by both OPU and OTO techniques. Our data demonstrates good survival rates of thawed embryos (80%). However, out of 29 transferred embryos, only one pregnancy and delivery was recorded. This data summarized the fertility outcomes of only eight patients. As more patients return for fetal embryo transfer, the pregnancy rates may change; however, at present, clinical pregnancy rates per transfer are very low compared to pregnancy rates in IVM and IVF in non-oncological patients [29]. The sole pregnancy recorded in this study was that of a patient with PCOS. Unfortunately, we could not ascertain whether the origin of this embryo was OTO or OPU. This patient's age was unremarkable as her age at OPU and at fetal embryo transfer (29 and 33 years, respectively) matched the mean ages of all eight patients. However, in this patient, 29 oocytes were harvested compared with a mean of 12.25 oocytes for all 8 patients, and 8 thawed embryos were transferred, compared with a mean of 4.82 thawed embryos for the 6 patients who had cryopreserved embryos. It seems likely that the patient's underlying condition of PCOS, with a probably greater than average AFC, facilitated the high yield of oocytes, leading in turn to more transferred embryos and the desired pregnancy and delivery of a normal baby. These findings are also in line with a recent paper of Sonigo et al. [30]. The other seven patients were subsequently referred to ovarian tissue transplantation. This implies that IVM does not preclude the need for ovarian tissue transplantation in most patients. Given the time-consuming nature of immature oocyte harvesting, particularly OTO, IVM should perhaps be reserved for patients with a high AFC such as PCOS, or when time constraints, patient characteristics, or disease-type preclude the possibility of carrying out emergency IVF prior to ovarian tissue collection [31].

Number of patients is too small for a definite decision about the place of IVM in fertility preservation. However, this data presents the yield and outcomes of IVM in eight patients during the last 8 years in a large center of fertility preservation. In our opinion, publishing this data is essential for all physicians at the field of fertility preservation in order to reevaluate this technique and to stimulate more studies.

In summary, this study demonstrates that retrieval of immature oocytes by OPU and OTO just prior to ovarian tissue cryopreservation has the advantage of yielding numerous oocytes and embryos. However, this approach is time consuming and, because of the low pregnancy rates, does not replace ovarian tissue transplantation. IVM might be beneficial for young patients with a high AFC such as is generally found in PCOS patients. In addition, this approach may be the best method of fertility preservation in patients who are at high risk for reintroduction of hematological or other malignancies and cannot perform emergency IVF. Further research is needed with larger numbers of patients to confirm post-thawing outcomes of OTO and OPU.

Availability of data and materials The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

Ethics approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. For this type of study, formal consent is not required.

Conflict of interest The authors declare that they have no competing interests.

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