Oocyte cryopreservation: a feasible fertility preservation option for reproductive age cancer survivors

Nicole Noyes · Patty Ann Labella · James Grifo ·
Jaime M. Knopman

Received: 5 March 2010 / Accepted: 5 May 2010 / Published online: 18 May 2010
© Springer Science+Business Media, LLC 2010

Abstract

Purpose To compare oocyte cryopreservation cycles performed in cancer patients to those of infertile women.

Methods Cancer patients referred for fertility preservation underwent counseling in compliance with the ASRM; those electing oocyte cryopreservation were included. Ovarian stimulation was achieved with injectable gonadotropins and freezing was performed using slow-cooling and vitrification methods.

Results Fifty cancer patients (mean age 31 y) underwent oocyte cryopreservation; adequate ovarian stimulation was achieved in 10 ± 0.3 days. The outcome from these cycles included a mean peak estradiol of 2,376 pg/ml and an average of 19 oocytes retrieved (15 mature oocytes were cryopreserved/cycle). All patients tolerated ovarian hyperstimulation. There were no significant differences noted between cryopreservation cycles performed in cancer patients and in women without malignancy.

Conclusions Oocyte cryopreservation appears to be a feasible fertility preservation method for reproductive-age women diagnosed with cancer. This modality is not only effective but also, providing a multidiscipline effort, can be completed in timely fashion.

Keywords Cancer survivor · Fertility preservation · Oocyte cryopreservation · Quality-of-life

Introduction

In 2009, an estimated 713,000 women were diagnosed with a malignancy; approximately 9.6% occurred in women under the age of 45 [1, 2]. Early detection programs combined with improved treatment protocols have allowed these patients to live substantially longer lives. Therefore, quality-of-life issues, such as fertility preservation, have become increasingly important to survivors of reproductive age. Furthermore, pregnancies that occur after a cancer diagnoses and/or cancer treatment do not appear to have an impact on cancer recurrence, obstetrical outcome or neonatal development [3]. Previously, time constraints and poor success rates limited the effectiveness of fertility preservation procedures. However, recent advancements in the arena of fertility preservation, specifically oocyte cryopreservation (OC) technology, have revolutionized the options available to those who elect to maintain their reproductive potential. OC offers reproductive-age cancer patients several advantages. Two such widely recognized benefits include eliminating the need for donor gametes as well as many of the ethical, personal and religious constraints associated with embryo freezing. Therefore, although OC technology is still in its relative infancy, the American Society of Reproductive Medicine (ASRM) recently reported that they support its use as a “fertility preservation strategy for women with cancer and other illnesses requiring treatments that pose a serious threat to their future fertility” [4]. Despite this endorsement and the inherent potential in OC for cancer patients, the novelty of this procedure has limited its universality; only recently have oncologists begun to regularly refer patients for such treatment with even fewer patients yet returning to use these oocytes. To ensure feasibility, we compared outcomes of cancer patients undergoing OC to all cycles performed in

Capsule Oocyte cryopreservation has become increasingly successful, offering female cancer patients a viable means to preserve their fertility.

N. Noyes (✉) · P. A. Labella · J. Grifo · J. M. Knopman
NYU Fertility Center, NYU School of Medicine,
660 First Avenue, Fifth Floor,
New York, NY 10016, USA
e-mail: nnoyes01@gmail.com

non-cancer patients where an oocyte thaw was completed ($n=32$).

Materials and methods

Cancer patients referred to the New York University Fertility Center (NYUFC) from April 2005 to December 2009 for fertility preservation underwent extensive counseling in compliance with the ASRM [5]; those electing OC and considered appropriate candidates for treatment were included.

Outcome of cycles completed in cancer patients were compared to all OC thaw cycles ($n=32$) performed to date at the NYUFC in infertile women (mean age 32 ± 1). These latter cycles were completed from September 2004 to September 2009 and have been reported elsewhere [6]; they include 21 cycles where embryos were derived from autologous (mean age 33.3 y) and 11 from donated oocytes of younger women (mean age 28.4 y). All patients were consented and Institutional Review Board approval was obtained to report these results.

Baseline serum estradiol and FSH levels were assessed and ovarian stimulation included injectable gonadotropins (foliotropin beta, Schering Plough, NJ; Serono Pharmaceuticals, Rockland, MA; $\pm$ menotropins, Ferring, Parsippany, NJ) with LH suppression being achieved using GnRH agonist ($n=6$; leuprolide acetate, TAP Pharmaceuticals, Lake Forest, IL) or antagonist ($n=44$; ganirelix acetate, Schering Plough). Ovulation trigger was achieved with human chorionic gonadotropin ($n=48$; hCG: 10,000 units) or GnRH agonist ($n=2$; leuprolide acetate, 0.4 cc=2 mg; TAP Pharmaceuticals). The latter was chosen if the peak serum estradiol level was $>2,500$ pg/ml or the patient was scheduled for chemotherapy or surgery within 2 weeks of oocyte harvest. Women with a diagnosis of breast cancer were offered letrozole (5 mg/day; Novartis Pharmaceuticals, East Hanover, NJ) either during or immediately following ovarian stimulation to lower estradiol response.

In the OC/thaw cycles, clinical pregnancy and implantation were defined by a gestational sac visualized on first-trimester transvaginal ultrasound evaluation. Statistical analysis was performed using the Mann-Whitney Rank Sum test with significance set at $P<0.05$.

Oocyte cryopreservation and thawing

Both slow-cooling and vitrification methods were used to cryopreserve oocytes. Oocytes noted to be metaphase II (MII) when evaluated 1.5 h post-harvest were considered suitable for cryopreservation. OC and thawing methods were recently reported in detail by Grifo and Noyes [7] and are summarized below.

Slow cooling method

Oocytes were equilibrated using 1.5 mol/L propanediol (PROH), then placed in a loading solution containing 1.5 mol/L PROH plus 0.3 mol/L sucrose, then into cryopreservation straws (Conception Technologies, San Diego, CA). Temperature was decreased using a Planer Kryo controlled-rate freezer (Planer Products Limited, Sunbury, UK). Ice nucleation was induced manually at -7°C . At -150°C , straws were transferred to liquid nitrogen tanks for storage.

Slow-cooled oocytes were air-warmed followed by placement into a 30°C water bath. Cryoprotectants were removed using stepwise dilution and surviving oocytes were transferred to fresh media for culture.

Vitrification method

Vitrified oocytes were first equilibrated and then placed in sequential equilibration solutions containing ethylene glycol (EG) and dimethyl sulfoxide (DMSO) followed by placement in a vitrification solution containing EG, DMSO, and sucrose. The oocytes were then loaded into CryoTip (Irvine Scientific, Santa Ana, CA) or Cryolock (Bio Diseño, Bogota, Colombia) straws, which were immediately sealed/capped and plunged into liquid nitrogen.

Vitrified oocytes were warmed by first agitating straws in a 37°C water bath followed by releasing the straw contents into thaw media and then passing the oocytes through sequential equilibration solutions. Oocytes were finally washed in sucrose-free media and then cultured.

Thaw cycle

Patients who were scheduled for a thaw cycle underwent uterine preparation using sequentially increasing doses of oral estradiol (Barr Laboratories, Pomona, NY) adding progesterone in oil (50 mg/day; Watson Pharmaceuticals, Corona, CA) when the endometrium reached >7.5 mm in greatest diameter. On the day of thaw, a fresh semen sample was obtained from the male partners. Specimens were processed via isolate (Irvine Scientific) or swim-up techniques as described elsewhere [8] and intracytoplasmic sperm injection (ICSI) was performed.

Results

Cycle demographics and outcomes for all OC cycles are shown in Tables 1 and 2. Fifty cancer patients with a mean age of 31 ± 1 y (range: 19–43 y) completed treatment. Six patients had one child prior to presenting for OC. Malignant diagnoses included 22 gynecologic, 12 breast, 8 hematologic, three gastrointestinal, two central nervous system and

Table 1 Comparison of oocyte cryopreservation cycles completed in cancer patients vs. in women without cancer who have completed a thaw cycle

	OC—Cancer (<i>n</i> =50)	OC with thaw—Non-Cancer (<i>n</i> =32)	<i>p</i> ^b
Age (y)	31±1 (19–43)	32±1 (21–38)	0.9
Day 2 estradiol (pg/ml) and FSH (IU/L)	49±3 (20–129)	33±2 (2–59)	0.001
	6.1±0.5 (4.1–13.5)	6.4±0.4 (2.8–10.2)	0.6
No. days ovarian stimulation	10±0.3 (6–16)	9±0.2	0.03
Total gonadotropins administered (IU)	3,553±159	2,089±121	0.001
Estradiol—day ovulation trigger (pg/ml)	2,376±268	2,769±264	0.05
No. oocytes retrieved	19±2 (0 ^a –55)	22±2 (6–61)	0.2
No. mature oocytes cryopreserved	657 (15±2) (0 ^a –44)	438 (14±2) (5–55)	0.9
Delivered rate	1 thaw: negative	18/32 (56%)	

Values are mean±SEM (range)

^a One cancer patient had 0 oocytes retrieved. She was age 40 y old and 16-weeks pregnant at time of diagnosis

^b Mann-Whitney Rank Sum

three other. Seven of nine women age 38 or older had breast cancer. Fertility preservation treatment was completed in 12 ±0.3 days including an average of 10±0.3 days of ovarian stimulation. A mean of 19±2 oocytes were harvested with 15±2 mature gametes cryopreserved per treatment cycle. A total of 657 MII of 924 retrieved oocytes were frozen. No serious complications (intra-abdominal hemorrhage, severe ovarian hyperstimulation syndrome or infection) occurred in any of the above described patients. Of note, two patients were diagnosed with moderate ovarian hyperstimulation syndrome post-retrieval (peak serum estradiol levels >5,000 pg/ml, moderate intra-abdominal fluid, no hemo-concentration or electrolyte disturbances); their symptoms resolved with conservative management. One additional

patient with a large mediastinal mass was not included in the dataset. She initiated an OC cycle; however, ovarian stimulation was discontinued on treatment day 3 secondary to compromised respiratory status deeming her medically inappropriate for stimulation and retrieval.

Complete data on the 32 OC cycles of healthy women undergoing thaw have recently been reported elsewhere [6] (see Table 1). The mean age at oocyte retrieval was 32±1 y. A total of 438 (mean 14±2 per cycle) MII oocytes were cryopreserved. Seventy-one (mean 2.2±0.1 per patient) embryos were transferred resulting in 20 (63%) women achieving pregnancy; 18 women have delivered 24 liveborn infants (6 sets of twins). Two other women miscarried in the first trimester.

Table 2 Demographics and outcome data by type of malignancy

	Subdiagnosis	Age (y)	Estradiol day ovulation trigger (pg/ml)	No. mature oocytes cryopreserved
Gynecologic (<i>n</i> =22)	Ovarian (9) Cervical (7) Endometrial (5) Vaginal (1)	30 (19–40)	2,586	13 (0–33)
Breast (<i>n</i> =12)	DCIS (2) Invasive (10)	38 (33–43)	1,686	9 (3–26)
Hematologic (<i>n</i> =8)	AML (3) Hodgkin's Lymphoma (HL) (3) Non-H L (2)	25 (30–34)	3,181	24 (8–40)
Gastrointestinal (<i>n</i> =3)	Colon (2) Carcinoid (1)	32 (23–37)	990	3 (2–4)
Central nervous system (<i>n</i> =2)	Brain glioma (1) Spinal cord (1)	28 (27–29)	2,230	11 (6–15)
Other (<i>n</i> =3)	Rhabdomyosarcoma (1) Thymic (1) Peritoneal mesothelioma (1)	28 (27–29)	2,919	23 (11–35)

Values are expressed as means (range)

Discussion

Although OC is a relatively novel procedure, it can offer cancer patients, particularly those that are young and single women, a viable means to preserve their fertility. Despite its experimental label, several studies have recently reported pregnancy outcomes comparable to those of fresh IVF following OC in non-cancer patients [6, 9, 10]. However, these studies almost exclusively limited their subjects to healthy, yet infertile women, rather than cancer patients.

The male literature has demonstrated that the cancer process itself, irrespective of treatment, can have a deleterious effect on fertility. For example, studies have demonstrated that cancer, primarily testicular cancer and Hodgkin's Lymphoma, prior to initiation of treatment, can have a direct immunological or cytotoxic effect on germinal epithelium within the testes leading to fertility impairment [11, 12]. Although the same phenomenon has not been demonstrated in female cancer patients, there is a paucity of data. One small case series reported deterioration in the quality of oocytes retrieved from female cancer patients prior to the initiation of cancer treatment [13] while another evaluating the effect of a cancer diagnosis on IVF stimulation outcome found that cancer patients produce a similar number of oocytes after ovarian hyperstimulation compared with age-matched infertile controls [14]. Therefore, to assess the utility of OC in cancer patients, we compared cycles performed in cancer and non-cancer patients.

In our study, OC stimulation outcome data for cancer patients was comparable to that of women without cancer. Although we did note a slight delay in response to injectable gonadotropins in patients who had received chemotherapy in close proximity to the initiation of their fertility preservation treatment, ultimately all patients had an adequate response. We attributed this delay in stimulation to hypothalamic dysfunction and suppression, as a result of stress and medication. Therefore it is prudent to not only anticipate this delay but also to consider it when creating a stimulation protocol. In addition, in an effort to allow the cancer patient to initiate cancer treatment immediately after the retrieval, we have recently begun to utilize GnRH agonist, rather than hCG to induce final oocyte maturation. By avoiding hCG, the prolonged luteotropic effect often associated with this medication is also avoided. Thus, the GnRH agonist trigger translates into a rapid (~1 week) return to menses and lowering of the potential risk of ovarian hyperstimulation in young patients who are at higher risk for this condition [15]. As we continue to cryopreserve oocytes for cancer patients, we hope to tailor the stimulation cycle that best suits their needs and hasten their return to cancer therapy.

OC was originally touted for breast cancer patients as this is the most common tumor afflicting women of

reproductive age; however, our data suggests that patients with other malignancies that tend to strike at a younger age have better outcomes (for example, the mean age of the patients with hematologic malignancies was 25 y and gynecologic tumors 30 y vs. 38 y for breast cancer patients) thus highlighting the importance of considering patient age when assessing one's appropriateness for fertility preservation measures. In addition, although OC is optimal for single cancer patients, in our cohort, 7 partnered patients elected to cryopreserve half of their retrieved oocytes unfertilized, with the remainder being inseminated using the partner's sperm; resultant two-pronuclear zygotes were then frozen 18 h later. As the decision to create embryos can be quite difficult, particularly when considering disposition in the event of a death as well as the effect malignancy can have on one's personal relationship or future childbearing, we advocate an extensive counseling discussion, with a treatment plan tailored to the individual or couple before fertility preservation is initiated. At our center, the treating physicians have substantial experience with the effects of cancer treatment on fertility, and therefore are well suited to appropriately counsel such patients. In addition, the facility employs two psychologists whose services are readily available to these women/couples. In general, as recommended [3], patient age, partner status, type/stage of malignancy, overall health status, history of previous cancer treatment, future cancer treatment and ovarian reserve are assessed prior to the initiation of fertility preservation treatment. Regarding thaw, when deciding on the number of embryos to be transferred, we advocate applying the same program guidelines followed for fresh IVF attempts. Lastly, in an effort to optimize oncofertility interdisciplinary care, direct communication between all treating parties; reproductive endocrinologists, medical/surgical oncologists and psychosocial services exists. We feel direct communication is paramount in avoiding unnecessary delay in patient evaluation and treatment.

Although there was no difference noted between the two groups with regard to OC stimulation outcome, only one of the cancer patients has returned to use her frozen oocytes (three more are currently scheduled). Therefore, our conclusions regarding pregnancy outcome after OC, are based on the results from the OC performed in infertile patients. In the infertile patients who have undergone thaw to date, more than 50% have achieved delivery of a baby [6]. Recently, Porcu reported a healthy twin live birth in an ovarian cancer survivor who used her previously frozen oocytes [16]. Although we infer, based on the similarity between the two groups' stimulation cycles and our center's success with OC that the cancer patients will also have promising pregnancy outcomes following OC, we must currently offer this procedure with some reservation as this is an evolving field.

In summary, the cancer process itself, barring removal of reproductive organs, does not appear to impact ovarian reserve or stimulation response. In addition, OC can be performed in a timely and safe manner with limited delay of cancer treatment (average treatment time was 12 days). This, coupled with the diminution in the ethical, personal and religious constraints often associated with the use of donor gametes and embryos freezing make OC an ideal fertility preservation modality for cancer patients.

Conclusions

Fertility preservation is a burgeoning field undergoing constant growth and expansion. OC is at the forefront of these developments. To date, it has proven to be a viable fertility preservation method offering excellent outcomes in infertile women treated using this technology. In comparison to embryo freezing, OC has the added benefit of reproductive choice in women not currently in a committed relationship. Therefore, with a dedicated multidisciplinary team, OC can be performed expeditiously and successfully, minimizing interference with cancer therapy. As future parenthood greatly impacts quality-of-life in cancer patients, OC should be an integral component of fertility preservation counseling in reproductive-age women diagnosed with a treatable malignancy.

Acknowledgments The authors would like to thank the entire IVF team at the NYUFC for their efforts and participation in the oocyte cryopreservation program and all the oncologists who referred patients for fertility preservation treatment. Dr. Noyes would also like to thank Dr. Eleonora Porcu of Bologna, Italy for introducing her to oocyte cryopreservation technology and its application as a fertility preservation measure in cancer patients.

Financial Support Schering Plough provided fertility medication for some of the non-cancer patients.

Conflicts of Interest None.

References

1. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Thun M. Cancer statistics. *CA Cancer J Clin*. 2009;59:225–49.
2. Surveillance, Epidemiology and End Results data. <http://seer.cancer.gov/statfacts/html/all.html>. Accessed March 4, 2009.
3. Knopman JM, Papadopoulos E, Grifo J, Fino ME, Noyes N. Surviving childhood and reproductive-age malignancy: effects on fertility and future parenthood. *Lancet Oncol*. 15 Feb 2010. doi:10.1016/S1470-2045(09)70317-1, [Epub ahead of print].
4. American Society of Reproductive Medicine. Practice Committee response to Rybak and Lieman: elective self-donation of oocytes. *Fertil Steril*. 2009;92:1513–4.
5. Practice Committee of the Society for Assisted Reproductive Technology, Practice Committee of the American Society for Reproductive Medicine. Essential elements of informed consent for elective oocyte cryopreservation: a practice committee opinion. *Fertil Steril*. 2008;90:S134–5.
6. Noyes N, Knopman J, Labella P, McCaffrey C, Clark-Williams M, Grifo J. Oocyte cryopreservation outcomes including pre-cryo and post-thaw meiotic spindle evaluation following slow cooling and vitrification of human oocytes. *Fertil Steril*. 26 Feb 2010. doi:10.1016/j.fertnstert.2010.01.019, [Epub ahead of print].
7. Grifo JA, Noyes N. Delivery rate using cryopreserved oocytes is comparable to conventional in vitro fertilization using fresh oocytes: potential fertility preservation for female cancer patients. *Fertil Steril*. 2010;93:609–15.
8. Carrell D, Kuneck P, Peterson C, Hatasaka H, Jones K, Campbell B. A randomized prospective analysis of five sperm preparation techniques before intrauterine insemination of husband sperm. *Fertil Steril*. 1998;69:122–6.
9. Cobo A, Kuwayama M, Perez S, Ruiz A, Pellicer A, Remohi J. Comparison of concomitant outcome achieved with fresh and cryopreserved donor oocytes vitrified by the CryoTop method. *Fertil Steril*. 2008;89:1657–64.
10. Nagy ZP, Chang CC, Shapiro DB, Bernal DP, Elsner CW, Mitchell-Leef D, et al. Clinical evaluation of the efficiency of an oocyte donation program using egg cryo-banking. *Fertil Steril*. 2009;92:520–6.
11. Skakkebaek NE, Rajpert-De Meyts E, Main KM. Testicular dysgenesis syndrome: an increasingly common development disorder with environmental aspects. *Hum Reprod*. 2001;16:972–8.
12. Magelseen H, Brydoy M, Fossa SD. The effects of cancer and cancer treatments on male reproductive function. *Nat Clin Pract*. 2006;3:312–22.
13. Pal L, Leykin L, Schifren J, Isaacson K, Chang YC, Nikruil N, et al. Malignancy may adversely influence the quality and behaviour of oocytes. *Hum Reprod*. 1998;13:1837–40.
14. Knopman J, Noyes N, Talebian S, Krey L, Grifo J, Licciardi F. Women with cancer undergoing ART for fertility preservation: a cohort study of their response to exogenous gonadotropins. *Fertil Steril*. 2009;9:1476–8.
15. Engmann L, DiLuigi A, Schmidt D, Nulsen J, Maier D, Benadiva C. The use of gonadotropin-releasing hormone (GnRH) agonist to induce oocyte maturation after cotreatment with GnRH antagonist in high-risk patients undergoing in vitro fertilization prevents the risk of ovarian hyperstimulation syndrome: a prospective randomized controlled study. *Fertil Steril*. 2008;89:84–91.
16. Porcu E, Venturoli S, Damiano G, Ciotti PM, Notarangelo L, Paradisi R, et al. Healthy twins delivered after oocyte cryopreservation and bilateral ovariectomy for ovarian cancer. *Reprod Biomed Online*. 2008;17:267–9.