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FERTILITY PRESERVATION

Pronuclear embryo cryopreservation experience: outcomes for reducing the risk of ovarian hyperstimulation syndrome and for fertility preservation in cancer patients

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Abstract
Purpose To evaluate pregnancy rate (PR) and live birth rate (LBR) after freezing pronuclear (PN) embryos for two purposes: to reduce the risk of ovarian hyperstimulation syndrome (OHSS) and to bank embryos for cancer patients anticipating gametotoxic chemotherapy/radiotherapy.
Methods Data from 3,621 consecutive IVF cycles were retrospectively analyzed. PN freezing was offered to patients at risk for OHSS and for those wishing to preserve fertility prior to cancer therapy. Primary outcomes evaluated were PR and LBR. Outcomes were compared to patients who underwent fresh embryo transfer (ET) in 2006.
Results Sixty-six patients froze PN embryos. Thirty-eight were at risk for OHSS. The LBR was 34.3% after one transfer, and 51.4% after a mean of 1.4 transfers. Twenty-eight cancer patients froze embryos. The LBR was 16.7% after one transfer and 25.0% after a mean of 1.5 transfers. The LBR was 35.5% for patients who underwent fresh ET.
Conclusion PN freezing with delayed ET is an effective tool for achieving pregnancy for patients at risk of OHSS and for cancer patients wishing to preserve fertility.

Keywords Ovarian hyperstimulation syndrome · OHSS · IVF · Cancer · Fertility preservation

Capsule Pronuclear embryo freezing with delayed embryo transfer for patients at risk of ovarian hyperstimulation and cancer patients is effective for achieving pregnancy.

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Introduction

The first successful pregnancies and live births after thawing frozen human embryos were reported in the 1980s [1–4]. Now freezing and thawing embryos are considered common practice, and cryopreservation at PN, cleavage, and blastocyst stages has been described [5].

OHSS is an iatrogenic condition that is manifested by ovarian enlargement and electrolyte and fluid imbalances that can bring about tissue edema, pleural effusions, ascites, hypovolemia, oliguria, and hemoconcentration. In severe OHSS, morbidity and mortality can result from hypovolemic shock, respiratory distress, renal failure, and thromboembolic events.

There are two classifications of OHSS, early and late, depending on when symptoms develop. Regardless of classification, both seem to be fueled by hCG. Early OHSS develops in the first several days after oocyte retrieval after exogenous hCG is given to induce oocyte maturation. Late OHSS, which tends to be more severe, occurs after pregnancy when endogenous hCG rises.

Several strategies have been attempted to prevent OHSS including cycle cancellation [6], coasting [7, 8], altering the

dosage or type of medications [9, 10], administration of intravenous albumin [11, 12], dopamine agonists [13, 14] and avoidance of ET, and thus pregnancy, immediately after stimulation with freezing of embryos for subsequent use [12, 15–23].

There are two randomized, prospective studies that report on the PR and LBR resulting from thawed embryos frozen for the prevention of OHSS. Shaker et al. [12] compared the incidence of OHSS and PR between two groups of 13 patients who underwent either treatment with intravenous albumin and fresh embryo transfer or PN cryopreservation with subsequent transfer. Their results showed a similar incidence of OHSS but significantly higher PR in the cryopreservation group. Ferraretti et al. [15] demonstrated that the PR and LBR rate among patients who underwent subsequent thaw of cryopreserved embryos was similar to those who underwent fresh transfer. There were no cases of OHSS among the 58 patients who underwent cryopreservation with subsequent transfer of thawed embryos, and there were 4 patients among 67 who developed OHSS in the control group.

Embryos also are banked for cancer patients prior to chemotherapy/radiotherapy. There are few data reporting long-term outcomes of embryo freezing for fertility preservation for cancer patients [24–26].

The purpose of this study was to assess the outcomes for patients who cryopreserved PN embryos for avoidance of OHSS or for fertility preservation before cancer therapy and to evaluate these relative to outcomes of patients undergoing a fresh IVF transfer. In addition, secondary outcomes evaluated included embryo survival, implantation and multiple births.

Materials and methods

Patient selection

The study was approved by the Partners Healthcare Human Research Committee. Three-thousand six-hundred and twenty-one consecutive IVF cycles from January 1, 1997 to December 31, 2007 were reviewed.

Patients were identified at risk for OHSS based on an E_2 of greater than 4000 pg/ml and/or the presence of 20 or more dominant follicles on the day of hCG administration. All cancer patients who had undergone IVF for embryo banking prior to receiving chemotherapy/radiotherapy were identified. The comparison group consisted of 355 patients who had a fresh ET in 2006; the last year data was complete when the study was initiated.

Parameters evaluated included age, diagnoses, history of prior IVF, day 3 FSH levels, method of insemination—conventional insemination (CI) or intracytoplasmic sperm injection (ICSI), amount of medication used for controlled ovarian hyperstimulation (COH), peak E_2, number of oocytes retrieved, fertilization rate, embryo survival after thaw, number of embryos transferred, implantation rate, PR, LBR, and multiple gestations.

Stimulation, cryopreservation, thaw, and embryo transfer

Three COH protocols were used for all three groups as described by Styer, et al. [27]. The low-dose, luteal phase down-regulation protocol is preceded by a cycle of oral contraceptive pills (OCP). It consists of pituitary desensitization with the gonadotropin releasing hormone (GnRH) agonist, leuprolide acetate, 0.5 mg/d SC (TAP Pharmaceuticals Inc., Lake Forest, IL) from OCP cycle day 17 through 2 days after menses, followed by reduction of the leuprolide acetate dosage to 0.25 mg/d on day 3 after menses. Subcutaneous gonadotropins are administered beginning on cycle day 3. Flare protocol also is preceded by a cycle of OCP. On day 2 of menses, leuprolide acetate (1.0 mg/d SC) is administered and is continued through day 4 and reduced (to 0.25 mg) on day five. Exogenous gonadotropins are administered beginning on cycle day three. The antagonist protocol also is preceded by an OCP cycle, and gonadotropins are begun on the 3rd day after menses. The GnRH antagonist ganirelix acetate or cetrorelix, 250 μg SC per day (Organon Inc., West Orange, NJ; Merck, Inc, Whitehouse Station, NJ) was administered when the lead follicle reached 13 mm in size. Both the GnRH agonist and GnRH antagonist were continued through the day of trigger with hCG. Gonadotropins included Gonal-F (follitropin-alpha, Serono, Rockland, MA), Follistim (follitropin-beta; Organon Inc.), and Repronex or Menopur (hMG, Ferring Pharmaceuticals, Tarrytown, NY).

Pronuclear embryos were frozen as described [28] using controlled-rate slow-freeze methods that included a three step loading of the cryoprotectants propanediol (1.5 M) and sucrose (0.1 M). Thawing of the embryos utilized a three step cryoprotectant removal by stepwise reduction in sucrose (0.5, 0.2, 0 M). Patients who had transfer of thawed embryos underwent programmed hormone replacement [29]. The number of embryos transferred was based on American Society of Reproductive Medicine guidelines that existed at the time that the study was initiated [30].

Statistical analyses

Continuous baseline variables were compared among the three patient groups by one-way ANOVA with allowance for group-specific variance heterogeneity. Pair-wise comparisons were by t-test with unpooled variance and Satterthwaite degrees of freedom. Categorical baseline variables were compared by chi-square tests. Embryo survival rates and mean number of embryos transferred

were compared between OHSS and cancer patients across all transfers by mixed model analysis with a fixed term for group membership, random patient-specific intercepts, and group-specific variance heterogeneity. First transfers were compared without the random intercepts. Median time to first transfer was estimated by Kaplan-Meier product-limit estimates with time to transfer censored at the end of the study period for women who had not yet attempted a transfer. PR, implantation rate, and LBR were compared among groups by logistic regression with adjustment for age, insemination type, infertility diagnosis, and history of prior IVF cycles. When comparing all transfers, generalized estimating equations were use to adjust for multiple cycles per woman. Estimated odds and their 95% Wald confidence bounds were back-transformed to estimates of the probabilities of pregnancy, implantation, and live birth. All inference is based on two-tailed tests with significance declared for $p<0.05$. All analyses were performed in SAS (v. 9.2, Cary, NC).

Results

Baseline characteristics and IVF stimulation

During the study period, 56 patients were identified as at risk for OHSS. Eighteen cycles were cancelled, and 38 patients underwent retrieval and cryopreservation of all embryos.

Infertility diagnoses were similar between the group at risk for OHSS and the comparison group ($p=0.65$). Infertility diagnoses included tubal factor, ovulatory dysfunction, diminished ovarian reserve, endometriosis, uterine factor, male, other, unknown, multiple female diagnoses and multiple male and female diagnoses. The OHSS group had a lower mean age and day 3 FSH, was less likely to have previously attempted IVF, received less medication, had a higher peak E_2 and had more oocytes retrieved than the comparison group (Table 1). The fertilization rate was higher for the OHSS group relative to the comparison group.

Those undergoing IVF for embryo banking before cancer therapies included 28 patients. Cancer diagnoses included: 17 breast, 5 cervical, 1 endometrial, and 5 non-gynecologic cancers. None of the cancer patients presented with infertility; however, 5 (18%) were found to have evidence of sub-fertility: 3 male, 1 diminished ovarian reserve (DOR), and tubal with DOR.

The mean age, day 3 FSH, and frequency of prior IVF were lower in the cancer than the comparison group, but groups were similar with regard to amount of medication received, peak E_2, number of oocytes retrieved and fertilization rate (Table 1).

When comparing the OHSS to the cancer group, they were no statistical differences in age, day 3 FSH levels, history of prior IVF or fertilization rates (Table 1).

Table 1 Baseline characteristics of patients undergoing fresh ET or cryopreservation for OHSS or cancer

Characteristic	Group-specific estimates		
	Mean±SD		
	OHSS (n=38)	Cancer (n=28)	Fresh ET (n=355)
Age (years)	34.3±3.4[a]	33.9±3.4[d]	36.3±3.9
Prior IVF (%)	23.7[a]	10.7[d]	49.6
Gonadotropin (ampules)	24.2±11.7[b]	36.1±15.0[e]	37.9±15.1
Day 3 FSH (U/L)	6.3±2.0[a]	6.2±2.4[d]	7.5±2.5
Peak E2 (pg/mL)	3886±1006[b]	1918±1105[e]	1893±682
Oocytes (#)	18.9±7.4[b]	11.7±7.6[e]	10.0±5.1
Pronuclear embryos (#)	12.2±5.3[b]	6.6±5.7[e]	5.9±3.6
IVF by ICSI	42.1%[c]	28.6%[f]	47.6%
Fertilization rate (%)	77.2±15.6[a]	68.9±24.0[f]	69.2±21.7

[a] significantly different from comparison, not significantly different from cancer

[b] significantly different from comparison, significantly different from cancer

[c] not significantly different from comparison, not significantly different from cancer

[d] significantly different from comparison, not significantly different from OHSS

[e] not significantly different from comparison, significantly different from OHSS

[f] not significantly different from comparison, not significantly different from OHSS

Transfer outcomes

Of the 38 patients who cryopreserved embryos to avoid OHSS, 92% completed one or more transfers at the end of the study period. Fifty percent had one, 37% had two, and 5% had 3 or more transfers. Of the 3 patients who did not undergo transfer, 2 conceived naturally and elected to discard embryos. The third discarded embryos due to changes in social circumstances.

Forty-three percent of cancer patients have undergone a transfer. Of those who have undergone transfer, 67% had one, and 33% had 2 transfers. Two-thirds of the cancer patients who underwent transfer utilized a gestation carrier. Two patients elected to discard embryos without transfer, and one patient expired.

First transfer

Some patients from the OHSS and cancer groups had more than one opportunity for transfer from a single IVF retrieval, thus the analysis was broken down into results of the first transfer and cumulative results. By definition the comparison group had only one transfer. Many of these cycles resulted in supernumerary embryos being frozen which were available for subsequent transfer; however the results of these transfers are not included in this analysis.

The comparison group had an average of 5.9 PN embryos after retrieval. OHSS group had a mean of 5.9 PN embryos thawed for the first transfer (Table 2). The implantation rate, PR and LBR were similar between the OHSS and comparison groups (Table 2). The unadjusted LBR was 34.3% for the OHSS group and 35.5% for the comparison group. After adjusting for age, infertility diagnosis, prior IVF, and use of ICSI, estimated LBR for the OHSS group was slightly lower (30.3%) but still did not differ significantly from the comparison group.

The cancer group had a mean of 3.9 embryos thawed for the first transfer (Table 2). The implantation rate was not statistically different for cancer patients than for the comparison group (and also the OHSS group, Table 2). The PR and LBR were lower for the cancer group than the other groups; however, given the small sample size, the only statistically significant difference was a lower PR in the cancer group relative to women in the OHSS group (Table 2). Multiple birth rates were highest among the cancer patients; however, given small sample size the findings are not significant.

Cumulative transfer results

OHSS and cancer patients attempted roughly equal numbers of transfers (1.4 vs 1.5 respectively, Table 3). Across all transfers, OHSS group had a mean of 5.9 PN embryos thawed and cancer group had a mean of 3.7 embryos (Table 3). Percentage of embryo survival after thaw was similar for the cancer and OHSS groups. The unadjusted data are provided in Table 2. The adjusted cumulative LBR across all transfers was 46.9 for the OHSS group, 16.3% for the cancer group and 36.4% for comparison group (1 transfer). All groups have embryos remaining (Table 3).

Table 2 IVF outcomes on first transfer

Outcome	Group-specific estimates		
	Mean		
	Fresh ET (n=355)	OHSS (n=35)	Cancer (n=12)
Embryos thawed (#)		5.94*	3.92*
Survive rate (%)		73.4	63.2
Embryos transferred (#)	2.17	2.20	1.83
Implantation rate (%)	26.3	27.6	14.5
Pregnancy rate (%)	50.1	65.7	33.3
Live birth rate (%)	35.5	34.3	16.7
Multiples per live birth (%)	22.2	25.0	50.0

Unadjusted estimates

*The only significant difference between groups was the number of embryos thawed, p=0.025

Table 3 Cumulative IVF outcomes over all transfers

Outcome	Group-specific estimates	
	Mean	
	OHSS (n=35)	Cancer (n=12)
Transfers per patient (#)	1.42±0.67	1.51±0.61
Embryos thawed (#)	5.89*	3.72*
Survive rate (%)	72.0	62.8
Embryos transferred (#)	2.22	1.79
Implantation rate (%)	29.9	12.9
Pregnancy rate (%)	77.1	41.7
Live birth rate (%)	51.4	25.0
Multiples per live birth (%)	28.6	33.3
Percentage of patients with embryos remaining[a]	34.2%	66.7%
	Avg 7.2 embryos	Avg 3.9 embryos

Unadjusted estimates

[a] 21.7% of patients undergoing fresh transfer have embryos remaining, with a mean of 1.9 embryos per patient

*The only significant difference between groups was the number of embryos thawed, p= *0.003*

Discussion

The goal in this study was to evaluate IVF outcomes for patients who underwent transfer of cryropreserved PN embryos for two purposes: to reduce the risk of OHSS and to bank embryos for cancer patients anticipating gametotoxic chemotherapy/radiotherapy.

At the Massachusetts General Hospital (MGH) Fertility Center, viable supernumerary embryos from fresh cycles are frozen at the blastocyst stage. Pronuclear cryopreservation is carried out when there is no intention for immediate transfer. This primarily is done in an effort to avoid OHSS, however, it is also provided for patients who wish to preserve fertility after a diagnosis of cancer. For these groups PN freezing is performed because of reported clinical success rates [31] and because it ensures a larger potential cohort of embryos to be available to the patient should techniques for selecting embryos and thawing improve with time.

According to the MGH Fertility Center protocol, patients at risk for OHSS are offered cycle cancellation or a reduced dose of hCG (5000 IU instead of 10,000 IU) along with cryopreservation of PN embryos.

Retrospective studies on cryopreservation with subsequent thaw as a preventative measure for OHSS prevention in general provide evidence to suggest that the PR and LBR are acceptable (PR 40% to 58%; LBR 26.6% to 50%). These studies, however, are limited by a lack of a comparison group [15] or details of the comparison group [16–20].

The choice of comparison group for this study is not necessarily to serve as a control, but is provided only to provide the background for the center's general practice and IVF outcomes. The results of transfer of thawed PN embryos in this study are placed in the context of the experience of fresh transfer IVF patients. The PR and LBR for those at risk of OHSS who cryopreserved their embryos were favorable relative to those who underwent fresh transfers in the same center. All groups have embryos remaining, providing more opportunities for pregnancy. While one must be cautious to over interpreting these data based on inherent differences in the groups, it does allow one to counsel patients at risk of OHSS about their chances of live birth being approximately 50% and place this information with in the context of the center's outcomes in general.

In this study, none of the patients who cryopreserved embryos in an effort to prevent OHSS developed the illness. There was not a group at risk for OHSS as identified by the criteria above who underwent a fresh transfer, thus, this study could not specifically assess the intervention's ability to reduce the risk of OHSS; however, the incidence of moderate to severe OHSS requiring hospitalization after IVF was less than one patient in 700 during the study period. For patients at risk of OHSS, freezing embryos could be challenging because of postponement of anticipated pregnancy. Nevertheless, postponement of transfer provides a safe and effective means of achieving pregnancy.

Extremely limited data is available on long-term follow up for cancer patients undergoing embryo banking. Ginsburg et al. [26] found that a larger number of oocytes and embryos were obtained from women who underwent IVF prior to systemic chemotherapy compared to women who had IVF after local cancer treatment. They report that the number of deliveries was slightly less for the systemically treated patients than for the patients with locally treated cancers, although this finding was not significant and the findings were not compared to any other patient groups. Michaan et al. [25] compared IVF outcomes of 21 patients with cancer to 22 patients with isolated tubal factor infertility. Similar to the present study, they found that cancer patients and their comparison group had similar stimulation profiles, number of oocytes retrieved, peak E2 and fertilization rates. Their study is also somewhat limited in that only 4 patients (25%) had returned for transfer resulting 2 live births.

In the current study 43% of cancer patients had one or more transfers and while the LBR of 25% is less than the LBR for who underwent fresh transfer, it is important to emphasize that the number [12] of cancer patients who underwent transfer is still small. This study may help to contribute to our overall understanding of outcomes for cancer patients as more data build from this and many other centers. Fifty-seven percent of cancer patients have not yet returned for transfer, and 6 patients have had embryos in storage for 5 or more years. Given the large percentage of patients who potentially require long-term chemoprophylaxis, some delay is understandable. With time, a more accurate percentage of patients who return may be established.

Fewer PN embryos were thawed for cancer patients than for patients at risk of OHSS and fresh embryos available for the comparison group. This may be explained as an effort to preserve as many embryos as possible for subsequent attempts at pregnancy and live birth, since a fresh cycle of IVF is not an option for most of these patients after cancer therapy.

The distribution of cancer diagnoses is also noteworthy. Sixty one percent of all cancer patients had breast cancer and 21% had a gynecological cancer. The incidence of these cancers in our cohort is higher than the incidence of new cancer diagnoses reported by Jamal et al. [32] where 26% of cancers are breast and approximately 9% are gynecological. This distribution may be attributable to our referral base or to the age distribution differences in the two groups. Interestingly Michaan et al. [25] found that almost 60% of their cancer group consisted of those with breast cancer, but there were no gynecological cancers represented.

Due to the potential limitations of representation of cancer diagnoses, one must be cautious in over generalizing this study's findings, as different types of cancers and different therapies may result in different long-term outcomes. Further follow-up and a larger patient population representing a broader spectrum of cancers is needed to address this possibility.

Embryo banking for cancer patients may lead to successful outcomes. Patients should be carefully counseled to consider their reproductive goals and the potential effects of delaying cancer therapy. For women without a male partner or unable to use donor sperm, oocyte cryopreservation may offer an alternative experimental option under strict institutional review board (IRB) protocols with early data for outcomes promising [33]. Cryopreservation of ovarian tissue is a technique in development, although limited information is available regarding outcomes [34], and potential risk of cancer recurrence with reimplantation of tissue not exposed to chemotherapy may limit its use in cancer patients, at least until in vitro maturation techniques for immature oocytes becomes more standard [35, 36].

In summary, PN freezing with delayed ET results in favorable outcomes and can be an effective tool for achieving pregnancy and live birth for patients at risk of developing OHSS and for patients who wish to preserve fertility prior to cancer treatments.

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