Oocyte and Embryo Banking in Women Undergoing Fertility Preservation

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Scholarly Report Title: Oocyte and embryo banking in women undergoing fertility preservation

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Abstract

TITLE: Oocyte and embryo banking in women undergoing fertility preservation

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Purpose: Cancer treatments have significant negative impacts on female fertility. The impact of cancer itself on female fertility is unclear. The purpose of this study was to evaluate the effect of malignancy on ovarian stimulation during egg and embryo banking.

Methods: Data was prospectively collected from 13,221 consecutive ovarian stimulation cycles at a single, academic medical center between June 2007 and October 2014. One-hundred-forty-seven women underwent assisted reproduction to cryopreserve oocytes and/or embryos for the purposes of fertility preservation after a cancer diagnosis. Of these, 105 women were diagnosed with local cancer (Stage I-III solid malignancies) and 42 carried diagnoses of systemic cancer (Stage IV solid malignancies or hematologic malignancies). These women were compared to 664 healthy women undergoing their first ovarian stimulation cycle for in vitro fertilization due to male factor infertility, with no evidence of female causes of infertility. Multivariable linear, Poisson, and logistic regressions were applied to calculate β-coefficients, relative rates, and odds ratios, respectively, and 2-sided Wald p-values (p).

Results: Adjusting for age and BMI, women with systemic cancer had lower baseline antral follicle counts than women with no cancer or local cancer [relative risk (95% CI): 0.58 (0.41, 0.83) and 0.64 (0.42, 0.97)]. Women with systemic cancer required higher doses of gonadotropins than women with no cancer [2483.0 (2050.8, 2915.2)] or local cancer [1124.82 (380.5, 1869.2)]. Women with systemic cancer had greater odds of cycle cancellation as compared to women with no cancer or local cancer [odds ratio (95% CI): 14.41 (4.83, 42.98) and 17.03 (2.94, 98.71)]. No significant differences were observed regarding duration of stimulation, number of oocytes and mature oocytes retrieved, or number of embryos created. Fifteen women returned to use frozen embryos; 18 transfers resulted in 8 live births (44% live birth rate per cycle). One patient returned to use frozen oocytes, but did not achieve pregnancy.

Conclusions: Women with cancer achieve similar oocyte and embryo yields as women with no cancer, although they require higher FSH doses and are at greater risk of cycle cancellation.

Contribution to the Work

I had the unique experience of being involved in all aspects of this study, from conception to submission for publication.

With advising and mentoring from Dr. Ginsburg, I performed the initial literature review for the study, exploring what information already exists in the literature with regards to this topic and analyzing what the proposed study would add. Based on the background literature, and with help and guidance from Dr. Ginsburg, Dr. Missmer (epidemiology, research, and statistical expert for the division), and Dr. Racowsky (Director of the Assisted Reproductive Technology Laboratory at Brigham and Women's Hospital), I then wrote a project proposal, including the variables of interest and suggested analysis plan. I presented the proposal at a quarterly division meeting for commentary and approval. With approval from the division to pursue this study, I wrote, submitted, and secured IRB approval.

My mentors then granted me access to the existing database of all assisted reproductive cycles that have been performed at Brigham and Women's Hospital since July 2007. I received an overview and training on how to use the database. I used the database to isolate the patients and variables of interest, manually reviewed the electronic medical record to confirm multiple data points and pull out certain variables that were not available from the database, and compiled the data set for the purpose of this study.

Once all the data were compiled, I submitted it to Dr. Missmer and Leslie Farland for statistical analysis. This analysis went through multiple iterations based on discussions between Dr. Ginsburg, our statistical team, and me.

Once the data were analyzed, I wrote the abstract, background, methods, results, and discussion of the manuscript in preparation for submission. The manuscript was reviewed and edited by all co-authors. At the time of this writing, I have submitted the manuscript for publication and am awaiting news on its acceptance to a peer-reviewed medical journal.

Running Title: Effect of cancer on ovulation induction

Title: Responses to fertility treatment among patients with cancer

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Abstract

Study question: What is the effect of malignancy on ovarian stimulation during egg and embryo banking?

Summary answer: Women with cancer achieve similar oocyte and embryo yields as women with no cancer, although they require higher FSH doses and are at greater risk of cycle cancellation.

What is known already: Cancer treatments have significant negative impacts on female fertility. The impact of cancer itself on female fertility is unclear. While some studies have shown that women with cancer require higher doses of gonadotropins and decreased oocyte yields, others have shown comparable oocyte yields between women with cancer and healthy women.

Study design, size, duration: We prospectively collected data from 13,221 consecutive ovarian stimulation cycles at a single, academic medical center between June 2007 and October 2014. 881 cycles from women with a cancer diagnoses and from women with a partner with male factor infertility were included in this cohort study.

Participants/materials, setting, methods: One-hundred-forty-seven women in our study underwent assisted reproduction to cryopreserve oocytes and/or embryos for the purposes of fertility preservation after a cancer diagnosis. Of these, 105 women were diagnosed with local cancer (Stage I-III solid malignancies) and 42 carried diagnoses of systemic cancer (Stage IV solid malignancies or hematologic malignancies). These women were compared to 664 healthy women undergoing their first ovarian stimulation cycle for in vitro fertilization due to male factor infertility, with no evidence of female causes of infertility.

Main results and the role of chance: Adjusting for age and BMI, women with systemic cancer had lower baseline antral follicle counts than women with no cancer or local cancer [relative risk (95% CI): 0.58 (0.41, 0.83) and 0.64 (0.42, 0.97)]. Women with systemic cancer required higher doses of FSH than women with no cancer [2483.0 (2050.8, 2915.2)] or local cancer [1124.82 (380.5, 1869.2)]. Women with systemic cancer had greater odds of cycle cancellation as compared to women with no cancer or local cancer [odds ratio (95% CI): 14.41 (4.83, 42.98) and 17.03 (2.94, 98.71)]. No significant differences were observed regarding duration of stimulation, number of oocytes and mature oocytes retrieved, or number of embryos created. Fifteen women returned to use frozen embryos; 18 transfers resulted in 8 live births (44% live birth rate per cycle). One patient returned to use frozen oocytes, but did not achieve pregnancy.

Limitations, reasons for caution: Although the data were collected prospectively, this analysis cannot fully account for all possible biases, such as confounding by indication.

Wider implications of the findings: These data support fertility preservation in women with cancer and suggest that more aggressive ovarian stimulation may be needed in those with hematologic or advanced stage cancers.

Study funding/competing interests: Farland has been supported by T32HD060454 and 3R25CA057711

Keywords

Cancer, oncofertility; fertility preservation, assisted reproductive technology

Background

Approximately 47,500 women between the ages of 15 and 39 were diagnosed with cancer in 2012 in the United States (1). As new treatment options have developed, 5-year survival rates for female cancer patients have improved and an increasing number of women are looking forward to resuming life after treatment (2). However, the available therapies are often gonadotoxic and threaten women with fertility loss. While it is well established that cancer treatments have a significant negative impact on female fertility, whether the presence of cancer has a detrimental impact on ovarian function and/or the results of controlled ovarian hyperstimulation is still under debate.

Over the past few decades, several fertility preservation (FP) options have been developed, including the cryopreservation of oocytes and embryos. While the latter is a well-established option, mature oocyte cryopreservation has only recently been upgraded from an experimental strategy to an accepted therapeutic option for FP purposes (3, 4). We have previously shown that women with cancer undergoing FP in our program required a higher total dose of gonadotropins and produced a lower number of mature oocytes than non-oncologic *in vitro* fertilization (IVF) control patients, although the total number of oocytes retrieved and the number of embryos produced was not significantly different (5). Although both embryo and mature oocyte cryopreservation are now accepted and recommended techniques for FP, data regarding outcomes with thawed embryo transfer or thawed oocyte fertilization and transfer in cancer patients is limited.

The aims of this study are to expand our knowledge of possible associations between type of malignancy on ovarian function, ovarian stimulation (OS), and outcome following thawed oocyte/embryo transfer. These findings may provide valuable information to providers for more appropriate counseling of patients of child-bearing age diagnosed with malignancies about the efficacy of FP therapies.

Methods

IRB Approval

Institutional review board approval was obtained before the start of this study.

Selection Criteria

Between July 9, 2007 and October 31, 2014, 13,221 consecutive OS cycles, with or without intracytoplasmic sperm injection (ICSI), were performed at our institution. All cycle data are

prospectively reported and were retrospectively reviewed to identify women undergoing assisted reproduction to cryopreserve eggs and/or embryos for the purposes of FP in the setting of a cancer diagnosis (n=153 cycles). Of these cycles, four were excluded because they were performed in women who had already undergone prior OS cycles. One woman with two cycles was excluded because she had been diagnosed with colorectal cancer seven years prior to stimulation start, but had no active cancer at the time of stimulation. The remaining 147 cycles performed in 147 women were included in the final cancer patient dataset. A comparison group was identified, comprised of women undergoing their first OS cycle for IVF due to male factor infertility, with no evidence of female causes of infertility (n=664). These women were selected because they represent presumably fertile women, thus enabling identification of any differences in ovarian stimulation outcomes exclusively attributable to cancer in the cancer patients.

Exposure Definitions

We classified women with cancer in several ways. In primary analyses, they were grouped according to the distribution of their cancer as either local (stage I-III solid malignancy) or systemic (hematologic or stage IV solid malignancy). All women with cancer were also classified by their cancer treatment prior to FP as follows: 1) having had no chemotherapy or radiation; 2) exposure to any chemotherapy prior to OS; or 3) exposure to tamoxifen or letrozole during stimulation. Separately, women with systemic cancer were classified by their cancer treatment prior to FP as either 1) having had no exposure to chemotherapy or radiation or 2) having any prior chemotherapy and/or radiation. Because there is evidence that BRCA carrier status may affect outcomes (6), we also classified women with cancer as: 1) those with breast cancer who were carriers of BRCA gene mutations; 2) those with breast cancer who were not carriers of either BRCA-1 or BRCA-2 mutations; or 3) those diagnosed with other cancers. In analyzing the data based on BRCA carrier status, women with breast cancer without known BRCA-carrier status were excluded, as was one woman who was diagnosed with both breast and thyroid cancer.

Outcomes of Interest

The following parameters were assessed for all women included in this study: age, baseline antral follicle count (AFC), and anti-Müllerian hormone (AMH) levels. As there is often not enough time for assessment of early follicular serum FSH and estradiol levels, these were not universally performed. Due to the time-sensitive nature of FP treatments, menstrual cycle timing for start of stimulation was prompt and recorded. In addition, starting and total follicle stimulating hormone (FSH) doses were assessed, as

were serum estradiol levels at time of ovulatory trigger, the duration of stimulation, total follicle number at ovulation trigger, total number of oocytes and number of mature oocytes retrieved, number of two pronucleate (2PN) embryos obtained, and whether or not the cycle was cancelled. Exact cancer diagnoses were recorded, as were the types of cancer treatments each patient underwent prior to stimulation. For women with breast cancer, BRCA mutation carrier status was noted. For women who returned to use their frozen eggs and/or embryos in subsequent cycles, the duration of egg/embryo freezing and use of a gestational carrier were noted, as were the outcomes of the transfers, including pregnancy results, gestational age at delivery, and birth weights.

Stimulation Protocol and Retrievals

Women with no cancer were stimulated during the early follicular phase of their menstrual cycles, as is conventional. Due to the time-sensitive nature of cancer treatments, women with cancer were stimulated either during the early follicular phase or at another random point in their menstrual cycles.

Several different types of protocols were used for OS in infertile patients. Gonadotropin releasing hormone (GnRH) antagonist protocols involved therapy with cetrorelix or ganirelix acetate (0.25mg/d starting on stimulation day 6, and in some cases preceded by 5-21 days of oral contraceptive [OC] pills). Down-regulation protocols included: [1] “low dose” luteal phase down-regulation with leuprolide acetate (LA; 0.5mg/d from cycle day 21 to 2 days after menses followed by a reduction of LA to 0.25mg/d after gonadotropin administration began), [2] “very low dose” luteal phase down-regulation with LA (0.2mg/d from cycle day 21 to 2 days after menses followed by a reduction of LA to 0.1mg/d after gonadotropin administration). Poor-responder protocols included: [1] “micro dose” stimulation with LA (0.05mg administered twice daily from cycle day 1 preceded by 7-21 days of OC pills); [2] “mini dose” luteal phase down-regulation with LA (0.5mg/d from cycle day 21 to the day of gonadotropin initiation, then discontinued); [3] “ultra-low dose” luteal phase downregulation with 0.05 mg LA dropping down to 0.025 mg; and [4] a “estrogen priming” protocol with GnRH antagonist and transdermal estradiol starting 10 days after the prior cycle LH surge until the following cycle day 2 when stimulation began.

Women with estrogen-receptor positive breast cancers were offered letrozole or tamoxifen as adjunct medications during the stimulation cycle to reduce theoretical risk stemming from increased estrogen exposure. This approach was previously described (7, 8).

Cancer patients nearly always underwent GnRH antagonist protocols. For patients undergoing conventional stimulation in the early follicular phase, exogenous gonadotropins in the form of recombinant human FSH with or without human menopausal gonadotropin were administered beginning on cycle day 2 when possible. Follicular development was monitored (assessing for follicles >12mm) as is standard. hCG or leuprolide was administered for final follicular maturation 36 hours prior to oocyte retrieval when at least 2 follicles reached a mean diameter of 18mm. Oocyte retrievals were performed transvaginally under ultrasound guidance. For patients undergoing a random stimulation start, exogenous gonadotropins were administered at any time during the menstrual cycle.

Oocytes and embryos were cryopreserved either via slow cooling (prior to June 2012, described previously, (9)) or vitrification (June 2012 and after, as described in the protocol "Simplified Oocyte Vitrification Protocols for HSV Device", Irvine Scientific, Irvine, CA). For cancer patients freezing embryos, oocytes were fertilized using standard IVF or ICSI as indicated and all embryos were cryopreserved at the 2PN stage.

Statistical Analysis

Multivariable linear regression was used to calculate β-coefficients with 95% confidence intervals for starting and total FSH doses, and serum estradiol levels at the time of ovulation trigger. Poisson regression was used to calculate relative risks for baseline AFC, duration of stimulation, total follicle number at hCG trigger, the total number of oocytes and the number of mature oocytes retrieved, proportion of mature oocytes retrieved, the oocyte/AFC ratio, mature oocyte/AFC ratio, and the number of embryos created. Logistic regression was used to calculate an odds ratio of a woman experiencing cycle cancellation. These analyses were adjusted for the woman's age and body mass index (BMI) at the start of stimulation. As appropriate, the calculation for the number of embryos created was also adjusted for the use of ICSI. A Wald p-value of less than 0.05 was considered to be significant throughout. The SAS statistical software version 9.3 was used for all analyses (SAS Institute Inc., Cary, NC).

Results

The baseline characteristics of women undergoing OS for male factor infertility (no cancer) and FP (local cancer or systemic cancer) are presented in Table I. Women with systemic cancer were younger

(27.1±6.4 years, Mean±SD) than those with local cancer (33.6±4.8) and those with no cancer (34.6±4.2), but they had lower AMH levels (2.0±2.2ng/mL, Mean±SD) than women with local cancer (2.8±2.7) or no cancer (3.4±3.3). Women with cancer were stimulated using different protocols than women with no cancer. While the majority of women with no cancer were stimulated via down-regulation protocols, most women with systemic or local cancer were stimulated using GnRH antagonist protocols. Furthermore, not all women with cancer started stimulation in the early follicular phase. Specifically, 21.7% of the cancer patients, with 40.5% of women with systemic cancer and 14.3% of women with local cancer, were stimulated at a random time during their menstrual cycles. All stimulation protocol types were included in the analyses to minimize confounding by indication.

Table II shows the distribution of cancer patients regarding their cancer diagnoses. Approximately half (53.7%) had been diagnosed with breast cancer, with 79.4% reporting BRCA negative tumors (Table II). One woman was diagnosed with both breast and thyroid cancer at the time of OS.

Effect of Cancer

Comparisons of outcomes following OS for the three groups of patients are shown in Table III. After adjustment for age and BMI at cycle start, women with systemic cancer had significantly lower AFC at baseline, compared to those with no cancer ($p=0.003$) or local cancer ($p=0.04$). In general, women with either local or systemic cancer were started on significantly higher doses of FSH compared to women with no cancer (both $p<0.001$); and they both required higher total doses of FSH than women with no cancer (both $p<0.001$). Moreover, women with systemic cancer received significantly higher starting ($p<0.001$) and total doses of FSH ($p=0.0031$) than women with local cancer. Furthermore, women with systemic cancer had a significantly greater odds of undergoing cycle cancellation as compared to women with no cancer ($p<0.001$) or local cancer ($p=0.0016$). Those with systemic cancer who did not undergo cycle cancellation had a significantly lower total follicle number on the day of ovulatory trigger than women with no cancer; this was primarily driven by adjustment for age ($p=0.02$). Notably, there were no significant differences among the three groups regarding duration of stimulation, the number of oocytes and mature oocytes retrieved, or the number of 2PN embryos obtained.

Effect of Prior Chemotherapy

Further analyses showed that after adjusting for age and BMI, women with cancer who underwent any chemotherapy or radiation prior to FP (n=38) had significantly lower baseline AFC than women with no

cancer (4.7±4.5 vs. 9.4±7.2, p<0.0001). These women were started on significantly higher FSH doses (467.7±160 vs. 289.0±121.3 IU, p<0.0001), and received higher total doses of FSH (4168.7±160.0 vs. 1839.2±1294.7 IU, p<0.0001) over a longer stimulation (12.9±2.5 vs. 11.7±2.0 days, p=0.002). They had a significantly lower total follicle number at hCG trigger (11.5±6.3 vs. 12.9±6.6, p<0.0001), had fewer oocytes (14.6±9.3 vs. 15.7±8.6, p=0.001) and fewer mature oocytes retrieved (11.8±7.7 vs. 12.0±7.1, p=0.004), with fewer embryos obtained (6.2±6.7 vs. 8.9±6.3, p=0.0002). These women also had 22.2 times greater odds of having a cycle cancellation than women with no cancer.

Women with cancer but without any chemotherapy or radiation before OS (n=49) were also started on higher doses of FSH (348.2±163.9 IU, p<0.0001) and required significantly higher total FSH doses (2508.7±1697.5 IU, p<0.0001) than women with no cancer. The two groups did not differ for baseline AFC, total follicle number at hCG trigger, or the total number of oocytes or mature oocytes retrieved. Similar results were observed for the subset of women with cancer who had not been exposed to chemotherapy or radiation prior to OS, but who received either tamoxifen or letrozole as adjunct medications during their cycles (n=60). Notably, the serum estradiol levels (pg/mL) for women who had not been exposed to chemotherapy (1824.8±1091.6, p=0.001) and who had received prior chemotherapy or radiation (1344.8±685.1, p<0.0001) were significantly lower than women with no cancer (2229.0±905.7). In sensitivity analyses, after restriction to letrozole-treated cases only, similar significant trends were observed (812.9±600.0, p<0.0001).

Further analysis of only women with systemic cancer showed that after adjusting for age and BMI, women with systemic cancer who underwent any chemotherapy or radiation prior to FP had significantly lower baseline AFC than women with no cancer (p<0.0001). These women were started on significantly higher doses of FSH (p<0.001) and received higher total doses of FSH (p<0.0001) over a longer stimulation (p=0.003) than women with no cancer. They had significantly lower total follicle numbers at hCG trigger (p=0.0007), had fewer oocyte (p=0.01) and mature oocytes (p=0.02) retrieved, with fewer embryos (p=0.0008) obtained than women with no cancer. This group of women also had significantly greater odds of cycle cancellation than women with no cancer (p<0.0001). Women with systemic cancer who had not been exposed to any chemotherapy or radiation prior to FP, were started on significantly higher doses of FSH (p=0.001), but they did not receive significantly higher total doses of FSH than women with no cancer. They did have significantly higher number of oocytes and mature

oocytes retrieved (both p=0.03), and more embryos were obtained (p<0.0001) than in women with no cancer.

Notably, when we compared women with systemic cancer who had undergone chemotherapy or radiation prior to FP to women with systemic cancer who had not undergone chemotherapy or radiation prior to FP, we found that those with prior chemotherapy/radiation exposure were started on significantly higher doses of FSH (p=0.002) and received significantly higher total doses of FSH (p=0.0004). Furthermore, they had significantly fewer total follicles at hCG trigger (p=0.01), had fewer total oocytes (p=0.0004) and mature oocytes (p=0.0010 retrieved, and fewer embryos were obtained (p<0.0001).

Effect of Menstrual Cycle Phase

After adjusting for age and BMI, women with cancer, regardless of whether they were stimulated in the early follicular phase (n=109) or at a random time of their menstrual cycle (n=33), were started on significantly higher doses of FSH (383.6±175.4 and 319.3±162.5 IU, respectively) and used significantly higher total doses of FSH (2965.6±1961.7 and 2727.27±1617.2 IU, respectively) than women with no cancer [288.26±120.72 (starting) and 1835.0±1293.3 (total) IU]. Women with cancer who underwent random stimulation start tended towards lower starting and total FSH doses than women with cancer who underwent early follicular stimulation. Furthermore, more oocytes (23.0±18.9) and mature oocytes (16.1±9.5) were retrieved from women with cancer who underwent random stimulation start than women with no cancer (15.7±8.6 and 12.0±7.1, respectively) or women with cancer who underwent early follicular stimulation (16.6±14.7 and 12.5±10.6, respectively), although this did not reach statistical significance. However, women who underwent a random start had significantly greater odds of having a cycle cancellation compared to women with no cancer [OR: 6.95 (2.16-22.38), p<0.05].

Effect of Breast Cancer and BRCA Mutation

Because breast cancer is the most common malignancy in women of reproductive age (10), we specifically investigated the influence of BRCA mutation on the outcomes of OS (Table IV). After adjusting for age and BMI, we found similar overall patterns between breast cancer patients and women with no cancer as presented in Table III. Women with breast cancer were started on significantly higher doses of FSH and received significantly higher total units of FSH than their counterparts with no cancer. Furthermore, women with breast cancer who were BRCA-negative had significantly fewer mature

oocytes retrieved than women with no cancer, and subsequently had a fewer total number of 2PN embryos created. These differences were not seen in women with breast cancer who were carriers of BRCA 1 or 2 mutations, however we had limited power (n=13) to detect associations.

Cryopreservation and Pregnancy Outcomes

All women with cancer who were able to obtain eggs and/or embryos cryopreserved them. To date, 19 of the 147 cancer patients have died. Fifteen of the 147 patients with cancer returned to use their frozen embryos in 19 attempts after a median of 2.1 years following cryopreservation (range 1-7.6 years); seven of the 15 women used gestational carriers in nine cycles. Eight women had been diagnosed with breast cancer, two had been treated for cervical cancer, and one each had chronic myelogenous leukemia, myelodysplastic syndrome, endometrial cancer with endometrioid ovarian cancer, recurrent liposarcoma, and lung cancer. Eleven women underwent one embryo transfer attempt, two women had two embryo transfer attempts, and one woman underwent three embryo transfers. One woman had a cycle cancelled prior to embryo thawing. Of all nineteen cycles, eight resulted in live births (42.1% live birth rate per cycle start; 44.4% live birth per embryo transfer). Seven resulted in singleton births (gestational age at delivery ranging from 38+6/7 to 41+2/7 weeks), and one resulted in a set of twins (gestational age, 35+6/7 weeks). Four of the live births were from gestational carriers. One of the patients died soon after the birth of her child via a gestational carrier; this had been anticipated and extensive medical, social, and ethics consults were obtained prior to the cryopreserved embryo transfer cycle. Birth weights ranged from 2381-4706g with an average of 3478g. One pregnancy was ongoing at the time of writing and one pregnancy resulted in a spontaneous abortion.

To date, only one of the women returned to use cryopreserved oocytes (1.8 years after freezing them). Ten oocytes were thawed, six of which survived. ICSI was performed on 5 of the eggs, 4 of which fertilized. A single embryo was transferred into a gestational carrier but this transfer did not result in a pregnancy.

Discussion

In this study, we compared the ovarian response and outcomes of women diagnosed with cancer who used assisted reproductive technology for FP to those of presumably fertile women with no cancer whose partners had male factor infertility. Our data suggest that women with cancer whose cycles are not cancelled achieve similar oocyte and embryo yields compared to women with no cancer, although

they have lower antral follicle counts and require higher FSH doses to achieve those outcomes. Similar results were found in sensitivity analyses exploring only women with breast cancer with and without BRCA mutations. Nevertheless, women with systemic cancer who were exposed to chemotherapy or radiation prior to FP have lower oocyte and embryo yields and they are at greater risk of cycle cancellation than women with no cancer or local cancer.

The observation that women with cancer are started on significantly higher doses of FSH may suggest either an underlying trend in practice among providers treating patients with cancer or, in cases of women with systemic cancer who had been exposed to chemotherapy or radiation, a clinician response to their significantly lower baseline AFC. However, we also saw that the total dose of FSH administered is significantly higher in women with both local and systemic cancer, suggesting that the ovaries of women with cancer are less responsive to stimulation. Taken together, these observations suggest that a similar disruption of gonadal function may be found in women as reported in men diagnosed with advanced stage or systemic cancers. In men with Hodgkin's lymphoma, several studies have reported the presence of testicular dysfunction and semen abnormalities (11, 12). Notably, one study showed that the decreased fertility was most significant in the setting of elevated erythrocyte sedimentation rate and advanced-stage disease, suggesting that systemic inflammation may interfere with gonadal function (13).

Other studies have also investigated oocyte yields in patients with cancer compared with healthy women. While two studies showed significantly lower oocyte yields in cancer patients (14, 15), several reported comparable oocyte yields between groups (5, 16-20). Two of the latter studies suggested that women with malignancies have decreased gonadal function; one showed an increased incidence of poor response to OS (18), while the other noted reduced fertilization rates (19). This is in contrast to previously published results that demonstrated no significant difference in the total dose of gonadotropins needed to stimulate follicular development (5, 14, 16, 17, 19, 21, 22). A possible explanation for this difference is that in the current study we did not exclude patients who had been exposed to chemotherapy in the past. To our knowledge, this is the first study to evaluate the effect of prior chemotherapy on ovarian stimulation outcomes in the setting of fertility preservation in women with cancer. Most published studies exclude women who had been exposed to chemotherapy from their analyses. Only one other study specifically isolated women with cancer who had undergone

chemotherapy prior to FP; however, this study only had 7 patients in this group and no comparisons were drawn between women who had been exposed to chemotherapy and women who had not (15).

We believe that it is important to include all women presenting for fertility preservation as they accurately reflect patients presenting to fertility clinics. Our data show that both patients who were and were not exposed to chemotherapy required significantly higher doses of gonadotropins for OS, and that only the former had significantly lower oocyte and embryo yields. This phenomenon was especially prominent in women with systemic cancer who had been exposed to prior chemotherapy or radiation both when compared to their counterparts with no cancer and to women with cancer who had not been exposed to chemotherapy or radiation prior to FP. These points are consistent with the fact that chemotherapy is often gonadotoxic. Surprisingly, we found that women with systemic cancer who had not been exposed to chemotherapy or radiation prior to FP actually had significantly higher total number of oocytes and mature oocytes retrieved and significantly more embryos cryopreserved than women with no cancer. However, these data should be interpreted with caution due to the low sample size (n=11) of this subgroup. Altogether, these points reinforce the notion that it is imperative for women with cancer to be evaluated for and undergo FP treatments prior to chemotherapy if at all possible.

Few studies have reported on OS outcomes in the BRCA mutation carrier population. One study demonstrated that women with BRCA-positive breast cancers undergoing FP had significantly lower oocyte yields than women with BRCA-negative malignancies (6). In contrast, another study showed no significant differences in the number of oocytes collected or zygotes produced between women with BRCA-positive versus BRCA-negative breast cancers (23). Similar to the second study, our results suggest BRCA-positivity does not have a negative impact on oocyte yield, although the higher FSH dose requirements suggest a lower ovarian response among these patients. Given our small number of women with BRCA-positivity, further studies are needed to explore the biological effect of deleterious BRCA mutations on ovarian response and whether or not more aggressive stimulation protocols are needed for these patients.

Finally, very few publications have reported on women returning after oncologic therapy to use their cryopreserved oocytes and/or embryos. All published studies have small sample sizes ranging from 4 to 33 patients. Among these, the live-birth rate has ranged from 12-75% (5, 17, 19, 24-29). The results from our FP population are consistent with what has been previously shown, with a 44% live birth rate per

embryo transfer. It is unclear at this time why so few patients come back to use their cryopreserved oocytes and/or embryos. More long-term follow-up studies are needed as a greater number of women take advantage of FP options.

This is the second largest study to examine OS outcomes during FP in women with malignancy. In addition to providing greater statistical power than previous studies, our large population allowed us to make comparisons to healthy women undergoing OS, between women with different types and stages of cancers, and to perform subgroup analyses that are not feasible with smaller patient populations. While several studies attempted to stratify women with cancer by cancer type (e.g. hematologic, breast, gastrointestinal tract, etc.), their group sizes were small, limiting the interpretation of the data (18, 21, 22). In an attempt to overcome this issue, this is the first study to examine ovarian stimulation differences between women with localized cancers and women with cancers that are more systemic in nature. Furthermore, the high number of unexposed patients (i.e. women with no cancer) increases the robustness of our analysis. However, similar to all other published literature, it is difficult to tease out the effects the malignancies themselves have on OS versus the different protocols used on cancer patients at non-conventional times in their menstrual cycles. We have presented subgroup analyses among women with systemic cancer who had and who had not undergone chemotherapy and radiation which resulted in several smaller samples that may limit interpretation. While a randomized controlled trial could overcome this confounding by indication, it is unlikely that such work is ethically plausible.

In summary, women with cancer undergoing OS for the purposes of FP are able to obtain equivalent numbers of oocytes and embryos to women with no cancer, especially if the ovarian stimulation is performed prior to chemotherapy. However, women with cancer require higher doses of gonadotropins to achieve those yields and they are at higher risk of cycle cancellation, suggesting an underlying adverse effect of malignancy on ovarian responsiveness. Furthermore, cancer patients who undergo FP are able to achieve live births of biological children using their cryopreserved embryos. Although these results are reassuring, more studies are required to evaluate the true effect of malignancy on ovarian function.

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Table I: Baseline characteristics of women without cancer and women with local or systemic cancer

Characteristics at cycle start	No Cancer (n=664)	Local Cancer (n=105)	Systemic Cancer (n=42)
Woman's Age at Stimulation, years (mean ± SD)	34.6±4.2	33.6±4.8	27.1±6.4
Baseline Antral Follicle Count (mean ± SD) (missing, n=48)	9.4±7.2	10.1±7.4	7.3±7.5
AMH, ng/mL (mean ± SD)	3.4±3.3	2.8±2.7	2.0±2.2
Cycle Day 2-4 FSH, mIU/mL (mean ± SD) (missing, n=91)	7.2±2.3	9.6±19.8	9.1±6.3
Woman's BMI at Stimulation, kg/m^2 (mean ± SD)	25.7±4.2	26.0±6.3	24.9±5.9
Woman's Race (n [%])			
Caucasian	466 (70.2%)	88 (86.2%)	36 (85.7%)
Other	198 (29.8%)	14 (13.7%)	6 (14.3%)
Current Smoker (n [%])	17 (2.6%)	6 (5.7%)	0 (0%)
Gravida (n [%])	186 (28.3%)	35(33.3%)	7 (16.7%)
Cycle Type (n [%])			
GnRH antagonist	88 (13.3%)	103 (98.1%)	36 (85.7%)
Down-regulation	533 (80.3%)	1 (1.0%)	4 (9.5%)
Gonadotropin only	3 (0.5%)	1 (1.0%)	2 (4.8%)
Poor-responder protocols	40 (6.0%)	0 (0%)	0 (0%)
Start of Stimulation (n [%])			
Conventional/Early Follicular	664 (100.0%)	84 (80.0%)	25 (59.5%)
Random Start	0 (0%)	15 (14.3%)	17 (40.5%)
Late Follicular	0 (0%)	3 (2.9%)	2 (4.8%)
Luteal	0 (0%)	7 (6.7%)	5 (11.9%)
Total Motile Post-wash Sperm Count, 10^6 (mean±SD)	5.66±16.88	28.9±33.4	34.8±41.5
ICSI (n [%])	547 (85.6%)	21 (21.7%)	4 (11.4%)

Table II: Cancer diagnoses of women undergoing fertility preservation

Pre-therapy diagnosis	n (%)
Breast	79 (53.7)
Breast cancer type[a]	
Estrogen Receptor Positive	64 (81.0)
Estrogen Receptor Negative	15 (19.0)
Inflammatory	2 (2.5)
HER-2 Positive	18 (22.8)
BRCA status[b]	
Negative	51 (64.6)
BRCA-1 Positive	7 (8.9)
BRCA-2 Positive	6 (7.6)
BRCA-1 and -2 Positive	0 (0.0)
Gynecologic	8 (5.4)
Ovarian	1 (0.7)
Endometrial	4 (2.7)
Cervical	5 (3.4)
Hematologic	38 (25.9)
Leukemia	11 (7.5)
Hodgkin's lymphoma	18 (12.2)
Non-Hodgkin's lymphoma	1 (0.7)
Myelodysplasia	2 (1.4)
Gastrointestinal	6 (4.1)
Brain	6 (4.1)
Other	11 (7.5)
Total	147[c] (100)

[a]Breast cancer type percentages reported as percent of all breast cancers.

[b]BRCA status percentages reported as percent of all breast cancers. 15 patients had an unknown BRCA mutation carrier status.

[c]One patient was diagnosed with both primary breast and thyroid (other) malignancy at the same time, and is thus only represented once in the Total row.

Table III: Ovarian stimulation cycle outcomes among women without cancer and women with local or systemic cancer

Cycle characteristic	No Cancer (n=664)	Local Cancer (n=105)	Systemic Cancer (n=42)	Systemic Cancer No prior chemotherapy (n=11)	Systemic Cancer Prior chemotherapy (n=31)
Baseline AFC (n)[1]	9.4±7.2 1.00 (Ref)	10.1±7.4 1.03 (0.89,1.21) 1.00 (Ref)	7.3±7.5 0.58 (0.41,0.83)* 0.64 (0.42,0.97)*	14.5±8.5 1.26 (0.88,1.83) 1.00 (Ref)	4.5±4.8 0.33(0.23-0.48)* 0.27(0.17-0.44)*
Starting FSH dose (IU)[2]	289.0±121.3 0.00 (Ref)	354.2±170.6 80.9 (58.2,103.6)* 0.00 (Ref)	417.8±180.7 252.4 (215.8,289.1)* 158.80 (95.7,221.9)*	320.5 ±156.8 102.6 (41.4,163.8)* 0.00 (Ref)	452.39±178.0 306.3 (266.7,345.9)* 178.2(66.9,289.4)*
Total FSH dose (IU)[2]	1839.2±1294.7 0.00 (Ref)	2813.6±1785.6 1094.0 (825.9,1362.1)* 0.00 (Ref)	3358.9±2147.3 2483.0 (2050.8,2915.2)* 1124.82 (380.5,1869.2)*	1847.7±1117.1 594.5 (-125.8, 1314.7) 0.00 (Ref)	3895.2±2179.8 3245.7 (2779.36,3712.0)* 2389.7 (1060.2,3719.1)*
Duration of stimulation (days)[1]	11.7±2.0 1.00 (Ref)	11.7±2.3 1.00 (0.96,1.04) 1.00 (Ref)	12.2±2.2 1.06 (0.99,1.14) 1.03 (0.94,1.12)	11.2 ± 2.0 0.96 (0.87,1.08) 1.00 (Ref)	12.6±2.2 1.12(1.04,1.21)* 1.12 (0.98,1.28)
Total follicle number at hCG trigger (n) [1]	12.9±6.6 1.00 (Ref)	12.2±8.4 0.94 (0.85,1.04) 1.00 (Ref)	13.2±5.90 0.81 (0.68,0.96)* 0.91 (0.75-1.12)	15.5±5.0 1.07(0.85,1.32) 1.00 (Ref)	12.3 ± 6.1 0.71 (0.57,0.86)* 0.68 (0.51,0.93)*
Number of oocytes retrieved (n) [1]	15.7±8.6 1.00 (Ref)	16.8±13.6 1.04 (0.89,1.21) 1.00 (Ref)	20.6±21.0 1.03 (0.68,1.55) 1.05 (0.60,1.86)	33.7±32.6 1.92 (1.07,3.43)* 1.00 (Ref)	15.0±9.8 0.70 (0.53,0.92)* 0.37(0.22,0.64)*
Number of mature oocytes retrieved (n) [1]	12.0±7.1 1.00 (Ref)	12.2±8.4 0.99 (0.86,1.13) 1.00 (Ref)	16.0±14.8 1.02 (0.70,1.50) 1.12 (0.68,1.84)	26.2±22.4 1.89 (1.07,3.33)* 1.00 (Ref)	12.0±8.09 0.73 (0.55,0.96)* 0.41 (0.24,0.70)*
Proportion of mature oocytes (n/n) [1]	0.76±0.19 1.00 (Ref)	0.76±0.20 0.96 (0.81,1.14)	0.78±0.15 0.93 (0.60,1.42)	0.71±0.10 0.44(0.25,0.80)*	0.80±0.16 1.54(1.14,2.07)*

		1.00 (Ref)	0.94 (0.57,1.52)	1.00 (Ref)	3.27 (1.68,6.34)*
Oocytes/AFC ratio [1]	2.28±2.87 1.00 (Ref)	2.06±2.11 0.93 (0.67,1.28) 1.00 (Ref)	3.92±7.35 2.29 (1.01,5.23)* 2.15 (0.86,5.40)	2.06±1.47 0.64 (0.40,1.04) 1.00 (Ref)	4.76±8.3 6.89(2.21,21.48)* 27.26(6.15,120.75)*
Mature oocytes/AFC ratio [1]	1.76±2.33 1.00 (Ref)	1.56±1.59 0.90 (0.65,1.25) 1.00 (Ref)	2.82±4.97 2.09 (0.94,4.65) 2.01 (0.81,4.99)	1.45±1.04 0.58(0.36,0.94)* 1.00 (Ref)	3.4±5.9 6.10(2.06,18.03)* 26.8(6.39,112.13)*
Number of embryos (n)[3]	8.9±6.3 1.00 (Ref)	8.8±6.4 0.93 (0.85,1.03) 1.00 (Ref)	12.3±11.7 1.03 (0.88,1.19) 1.16 (0.98,1.39)	20.6±16.6 2.0(1.62,2.45)* 1.00 (Ref)	9.08±7.8 0.72(0.59,0.87)* 0.39 (0.29,0.53)*
Cycle Cancelled [n (%)][4]	14(2.1) 1.00 (Ref)	2(1.9) 0.92 (0.21,4.11) 1.00 (Ref)	9(21.4) 14.41 (4.83,42.98)* 17.03 (2.94,98.71)*	1(9.1) 5.3(0.6,45.6) 1.00 (Ref)	8(25.8) 20.8(6.0,72.2)* 4.0(0.33,46.3)

All results reported as mean ± standard deviation, unless otherwise noted.

[a] *indicates significance (p-value <0.05)*

b. Poisson regression estimate, RR (95% CI), Adjusted for age and BMI at cycle start
c. Linear regression estimate, β (95% CI), Adjusted for age and BMI at cycle start
d. Poisson regression estimate, β (95% CI), Adjusted for age and BMI at cycle start, and ICSI use
e. Logistic regression estimate, OR (95% CI), Adjusted for age and BMI at cycle start

Table IV: Ovarian stimulation cycle outcomes among women with no cancer, women with breast cancer with and without BRCA mutations, or other cancer

Cycle characteristic	No Cancer (n=664)	BRCA- Breast Cancer (n=49)	BRCA+ Breast Cancer (n=13)	Other Cancer (n=68)
Woman's Age at Stimulation (years)	34.6±4.2	34.7±3.7	32.3 ± 4.0	28.5 ± 6.4
Baseline AFC (n)[b]	9.4±7.2 1.00 (Ref)	11.5±7.7 1.22 (0.99,1.50)	7.7±6.3 0.76 (0.50,1.15)	8.3±7.8 0.70 (0.54,0.90)[a]
Starting FSH dose (IU)[c]	289.0±121.3 0.0 (Ref)	342.0±159.6 53.5 (21.1,85.9)[a]	395.2±236.5 142.8 (81.5,204.1)[a]	375.5±174.5 183.2 (153.3,213.1)[a]
Total FSH dose (IU)[c]	1839.2±1294.7 0.00 (Ref)	2498.0±1570.9 637.7 (302.4,1044.9)[a]	4326.9±3046.6 2775.0 (2072.0,3478.0)[a]	2994.5±1715.8 1916.5 (1573.7,2259.4)[a]
Duration of stimulation (days)[b]	11.7±2.0 1.00 (Ref)	11.3±2.4 0.96 (0.91,1.02)	13.0±3.0 1.12 (0.98,1.28)	12.1±2.0 1.05 (1.00,1.10)[a]
Total follicle number at hCG trigger (n)[b]	12.9±6.6 1.00 (Ref)	12.1±5.8 0.95 (0.83,1.09)	12.1±5.5 0.87 (0.68,1.12)	13.3±5.9 0.85 (0.75,0.97)[a]
Number of oocytes retrieved (n)[b]	15.7±8.6 1.00 (Ref)	14.9±9.6 0.94 (0.78,1.12)	15.5±7.1 0.86 (0.67,1.12)	20.8±19.7 1.11 (0.84,1.45)
Number of mature oocytes retrieved (n)[b]	12.0±7.1 1.00 (Ref)	10.2±5.9 0.85 (0.72,0.99)[a]	11.5±5.6 0.90 (0.69,1.16)	15.7±12.4 1.08 (0.85,1.38)
Proportion of mature oocytes (n/n)[b]	0.76±0.19 1.00 (Ref)	0.78±0.21 0.97 (0.78,1.19)	0.79±0.07 1.23 (0.94,1.60)	0.80±0.16 0.94 (0.69,1.26)
Oocytes/AFC ratio (n/n)[b]	2.28±2.87 1.00 (Ref)	1.85±2.15 0.73 (0.46,1.18)	2.29±1.25 1.37 (0.66,2.82)	3.19±5.71 1.69 (0.95,2.98)
Mature oocytes/AFC ratio (n/n)[b]	1.76±2.33 1.00 (Ref)	1.29±1.43 0.66 (0.41,1.06)	1.77±0.94 1.37 (0.66,2.80)	2.39±3.93 1.62 (0.94,2.80)
Number of embryos (n)[d]	8.9±6.3 1.00 (Ref)	8.0±10.0 0.84 (0.74,0.97)[a]	6.4±5.7 0.90 (0.67,1.21)	6.2±6.7 1.09 (0.98,1.23)
Cycle cancelled [n (%)][e]	14 (2.1) 1.00 (Ref)	0 (0) --	2 (15.4) 8.55 (1.70,42.88)[a]	8 (11.8) 6.06 (2.12,17.35)[a]

All results reported as mean ± standard deviation, unless otherwise noted.

[a] *indicates statistical significance (p-value <0.05)*

b. Poisson regression estimate, RR (95% CI), Adjusted for age and BMI at cycle start
c. Linear regression estimate, β (95% CI), Adjusted for age and BMI at cycle start
d. Poisson regression estimate, β (95% CI), Adjusted for age and BMI at cycle start, and ICSI use
e. Logistic regression estimate, OR (95% CI), Adjusted for age and BMI at cycle start