Published in final edited form as:
Reprod Biomed Online. 2013 April ; 26(4): 337–344. doi:10.1016/j.rbmo.2013.01.003.

Response to ovarian stimulation in patients facing gonadotoxic therapy

Lauren N. C. Johnson, MD[1], **Katherine E. Dillon, BA**[1], **Mary D. Sammel, ScD**[2], **Brenda L. Efymow, RN, BSN**[1], **Monica A. Mainigi, MD**[1], **Anuja Dokras, MD, PhD**[1], and **Clarisa R. Gracia, MD, MSCE**[1]

[1]Reproductive Endocrinology and Infertility, University of Pennsylvania, 3701 Market St Ste 800 Philadelphia, PA, United States, 19104

[2]Biostatistics and Epidemiology, University of Pennsylvania, 605 Blockley Hall 423 Guardian Drive Philadelphia, PA, United States, 19104.

Abstract

Chemotherapy naïve patients undergoing embryo/oocyte banking for fertility preservation (FP) were assessed for response to ovarian stimulation. Fifty FP patients facing gonadotoxic therapy were matched by age, race, cycle number, date of stimulation and fertilization method to patients undergoing IVF for infertility or oocyte donation. There were no differences in baseline FSH, anti-Müllerian hormone, antral follicle count and total gonadotrophin dose. FP patients had more immature oocytes (2.2 versus 1.1; $P = 0.03$) and lower fertilization rates per oocyte retrieved (52% versus 70%; $P = 0.002$.) There were no differences in numbers of oocytes retrieved, mature oocytes or fertilized embryos. Subgroup analysis revealed that FP patients taking letrozole required higher gonadotrophin doses (3077 IU versus 2259 IU; $P = 0.0477$) and had more immature oocytes (3.4 versus 1.2; $P = 0.03$) than matched controls. There were no differences in gonadotrophin dose or oocyte immaturity among FP patients not taking letrozole. Chemotherapy naïve FP patients had ovarian reserve, response to stimulation and oocyte and embryo yield similar to controls. Patients who received letrozole required higher gonadotrophin doses and produced more immature oocytes, suggesting that response to ovarian stimulation may be impaired in patients with hormone-sensitive cancers receiving letrozole.

Keywords

chemotherapy; embryo banking; fertility preservation; letrozole; oocyte banking; oncofertility

Introduction

Advances in early detection and treatment of cancer over the past two decades have led to improved survival rates for patients. In 2004, the average 5-year survival for all women

Corresponding author: Lauren N. C. Johnson, M. D. 3701 Market St, Ste 800 Philadelphia, PA 19104 Lauren.johnson2@uphs.upenn.edu 215–662–2971 (Office) 215–349–5512 (Fax) Reproductive Endocrinology and Infertility 619 Pine Street, Apt 2 Philadelphia, PA 19106 Home: (919) 696-8037 laurenncollins@gmail.com.

Declaration: The authors report no financial or commercial conflicts of interest.

diagnosed with cancer before age 49 was 83% (Horner *et al.*, 2011). With improved survival, there has been a shift in attention toward management of long-term consequences of cancer therapy, including infertility. Many young women with cancer, particularly those facing gonadotoxic therapies, pursue fertility preservation (FP) strategies – mainly ovarian stimulation – for the purpose of banking oocytes or embryos for future use.

There has been growing interest in FP stimulation cycles and development of optimum IVF protocols. Unlike other patients undergoing IVF, most women who choose to undergo ovarian stimulation for embryo or oocyte banking have no history of infertility; therefore, response to stimulation may be difficult to predict. Furthermore, as many of these patients are preparing for difficult and life-saving therapies, optimizing patient safety is critical. Minimizing delays in cancer therapies often involves the use of short gonadotrophin-releasing hormone (GnRH) antagonist protocols, and GnRH agonists may be used to trigger final oocyte maturation in order to reduce the risk of ovarian hyperstimulation syndrome (Acevedo *et al.*, 2006; von Wolff *et al.*, 2009). Additionally, aromatase inhibitors may be added to gonadotrophin stimulation protocols to limit exogenous oestrogen exposure in individuals with oestrogen-sensitive cancers, such as breast and endometrial cancers (Oktay *et al.*, 2010a, Azim *et al.*, 2008).

Several recent studies suggest that response to ovarian stimulation is poorer than expected in the population of patients pursuing FP with embryo or mature oocyte banking (Friedler *et al.*, 2012; Domingo *et al.*, 2012). Thus, consensus regarding optimal protocols and expected success of oocyte/embryo banking in this population is currently lacking.

The purpose of this study was to examine how patients without prior exposure to chemotherapy who are facing gonadotoxic therapy respond to ovarian stimulation compared with healthy controls. Additionally, this study sought to determine the impact of letrozole, an aromatase inhibitor, on ovarian stimulation. The primary outcome was the total number of oocytes retrieved. Secondary outcomes included embryo yield, number of mature oocytes, fertilization rate and total gonadotrophin dose.

Materials and methods

The study was approved by the Institutional Review Board at the University of Pennsylvania (reference number UPCC 04812, 20 December 2012). Patients with cancer or medical conditions requiring gonadotoxic therapy who underwent embryo and/or oocyte banking at Penn Fertility Care between January 2005 and August 2012 were identified. These patients were matched 1:1 by age, race, IVF cycle number, date of stimulation (within 2 years) and fertilization method (conventional or intracytoplasmic sperm injection, ICSI) to patients undergoing IVF for tubal factor infertility and male factor infertility or as oocyte donors. Women previously exposed to chemotherapy were excluded.

Ovarian stimulation was performed using either luteal-phase GnRH agonist or GnRH antagonist protocols. Recombinant FSH (Gonal-F; Merck-Serono; or Follistim; Organon) was initiated with or without LH support (Menopur; Ferring; or Luveris; Merck-Serono) during the early follicular phase of the cycle. For patients using antagonist protocols initiated in the early follicular phase, GnRH antagonist (Cetrotide; Merck-Serono; or Ganirelix; Organon) was started when the lead follicle was 14 mm. Two patients received the GnRH antagonist at the time of FSH initiation and throughout the cycle (random start protocol) because they were not in the early follicular phase of the cycle and ovarian stimulation had to be expedited. Once the dominant follicle reached a mean diameter of ≥18 mm, patients were instructed to take recombinant human chorionic gonadotrophin (HCG; Novarel; Ferring; or Ovidrel; Merck-Serono) and/or GnRH agonist (luprolide acetate; Sun

Pharma Global) to induce final oocyte maturation. Most patients with breast and endometrial cancer also received an aromatase inhibitor. Under this protocol, letrozole 5 mg (Mylan Pharmaceuticals or Teva Pharmaceuticals) was administered daily starting the second or third day of a spontaneous menstrual cycle and continuing until the day of HCG or GnRH agonist administration. Recombinant FSH was started 0–2 days after the initiation of letrozole, and the GnRH antagonist was added when the lead follicle was ≥14 mm. Final oocyte maturation was triggered with recombinant HCG and/or GnRH agonist when the lead follicle reached at least 20 mm in mean diameter. Oocyte retrieval was performed 35–36 h after trigger. Oocytes were fertilized via conventional insemination or ICSI in the case of severe oligospermia. In women facing gonadotoxic therapy, embryos were cryopreserved at the 2 pronuclear (2PN) stage. For oocyte cryopreservation, oocytes were stripped on day of retrieval to assess maturity prior to cryopreservation. Some patients elected to cryopreserve both oocytes and embryos. Embryos were cryopreserved using slow freezing and oocytes were cryopreserved via vitrification.

Demographics, baseline ovarian reserve measurements, IVF stimulation parameters and embryology data were collected from medical records. The number of mature and immature oocytes was determined on the day of retrieval for those undergoing ICSI or oocyte cryopreservation. For those undergoing conventional insemination, immature oocytes were recorded the day after retrieval. Maturation rate was defined by number of mature oocytes divided by total number of oocytes retrieved. Fertilization rate was calculated by the ratio of 2PN embryos on the day after retrieval divided by number of oocytes retrieved.

Statistical analysis

A priori power calculations based on an alpha of 0.05 and a power of 0.8 determined that 44 patients were needed in each arm of the study in order to detect a 20% difference in oocyte yield. Statistical significance was defined as $P < 0.05$.

Demographic characteristics for the cases and controls were summarized and compared using paired t-tests, Fisher's Exact test, Wilcoxon signed-rank tests and McNemar's tests as appropriate. Log-transformed hormone concentrations and outcome variables were also compared between cases and controls using paired t-tests. Linear regression models examined the difference in log-transformed hormones and IVF outcomes between the case and control pairs while allowing for control of non-matching factors such as protocol and trigger. Subgroup analyses were performed using Student's t-tests, Mann–Whitney test and Pearson chi-squared tests as appropriate. Statistical analysis was performed using STATA version 12.0 (StataCorp, College Station, TX, USA).

Results

From January 2005 to August 2012, 50 chemotherapy-naïve patients with cancer or medical illnesses requiring gonadotoxic therapy pursued ovarian stimulation for embryo and/or mature oocyte banking. These 50 patients completed 56 IVF cycles: 37 cryopreserved embryos, nine cryopreserved oocytes and eight cryopreserved both oocytes and embryos. In two cycles, there were no embryos or oocytes available for cryopreservation. There were no cancelled cycles.

Baseline characteristics are presented in **Table 1**. Of the FP patients, the mean age was 31.2 years, the mean body mass index was 24 kg/m^2, 88% were Caucasian and 68% were nulligravid compared with 52% of controls. There were no differences in age, BMI, race or pregnancy history. The majority of patients had breast cancer (58%.) Gynaecological and haematological malignancies occurred in 16% and 12% of patients, respectively. Diagnoses

for patients without malignancy included myasthenia gravis, sickle cell disease, *BRCA1* mutation, mixed connective tissue disease and sarcoidosis. Six patients (12%) had a history of infertility. Partner semen analysis was abnormal for 11 FP patients, and four required ICSI for severe male factor infertility. None of the patients had undergone ovarian tissue cryopreservation prior to stimulation. Of the control patients, 34% were oocyte donors, 54% had tubal factor infertility and 12% had male factor infertility.

The first-cycle stimulation protocols were significantly different between groups as only patients with breast or endometrial cancer received letrozole as part of the protocol and more controls received luteal-phase lupron protocols ($P < 0.001$, **Table 1**). The number of patients who received HCG versus GnRH agonist to trigger final oocyte maturation was similar in both groups.

Data from the first stimulation cycle for each patient (100 cycles total) were compared between cases and controls (**Table 2**). Compared with controls, chemotherapy naïve cancer patients had no differences in baseline FSH, anti-Müllerian hormone, antral follicle count, total gonadotrophin dose and number of oocytes retrieved. Baseline oestradiol concentrations were significantly higher among cases compared with controls, but the difference was small and clinically insignificant: 48.1 pg/ml (95% CI 40.9–56.8) versus 39.2 pg/ml (95% CI 35.5–43.4; $P = 0.04$.) FSH was greater than 10 mIU/ml in 9.5% of FP patients (4/42) compared with 2.2% (1/46) of controls. Prior to administration of HCG, there were no differences in the total number of follicles (21.6 versus 22.8) or number of follicles greater than 14 mm (11.1 versus 11.8). FP patients had more immature oocytes (2.2 versus 1.1; $P = 0.03$) and lower fertilization rates per oocyte retrieved (52% versus 70%; $P < 0.01$). There were no differences in number of oocytes retrieved, number of mature oocytes, maturation rate or number of fertilized embryos obtained. For FP patients who underwent ICSI for male factor infertility ($n = 4$), there was no statistical difference in fertilization rate compared with controls.

A subgroup analysis was performed comparing women on letrozole to their matched controls (**Table 3**). FP patients taking letrozole had a higher starting gonadotrophin dose (317 IU versus 203 IU; $P < 0.01$), higher total gonadotrophin dose (3077 IU versus 2259 IU; $P = 0.0477$ and more immature oocytes obtained (3.4 versus 1.2; $P = 0.03$). There was a trend toward a lower fertilization rate in the letrozole group compared with controls (47.1% versus 66.6%), but this result did not reach statistical significance. There were no differences in number of follicles, number of oocytes retrieved or number of embryos obtained. FP patients on non-letrozole protocols had a lower peak oestradiol on the day of trigger (1664 versus 2705 pg/ml; $P = 0.01$) and lower fertilization rates (55% versus 72%; $P = 0.02$) compared with their matched controls. Despite these significant differences, there were no differences in number follicles, oocytes retrieved, number of immature oocytes or number of embryos in non-letrozole cycles compared with matched controls.

The impact of discordance in stimulation protocols was examined using linear regression on the difference in each outcome between cases and controls. When stimulation protocols were discordant, there were significant differences in follicle number, number of mature oocytes, maturation rate and number of embryos. Discordant protocols did not influence number of immature, fractured or atretic oocytes, fertilization rate or total gonadotrophin dose. When cases and controls were discordant for type of final maturation trigger used, there was a significant difference in number of immature oocytes obtained.

To date, six cancer patients have returned to thaw embryos (**Table 4**). Four patients successfully conceived after transfer of thawed embryos. Three patients had singletons and one had twins. Two of the four used a gestational carrier. The remaining two patients (ages

40 and 43 years) did not undergo embryo transfer because their embryos did not survive after thawing.

Discussion

Although women with cancer are increasingly choosing to preserve their fertility through oocyte and embryo banking, there are many unanswered questions regarding optimal protocols, expected response to ovarian stimulation and potential for live birth in this population. Recent data suggest that women with cancer do not respond as well to ovarian stimulation as would be expected given their age and unproven fertility (Friedler *et al.*, 2012; Domingo *et al.*, 2012). The current study sought to evaluate ovarian reserve and response to ovarian stimulation in a FP population without previous exposure to chemotherapy and to determine the extent to which any difference in oocyte and embryo yield can be attributed to underlying patient characteristics and differences in IVF protocols.

With respect to ovarian reserve and response to ovarian stimulation, there were no overall differences in baseline measures of ovarian reserve, total gonadotrophin use or mean number of retrieved oocytes (12 oocytes) in the cohort of chemotherapy naïve FP patients and matched controls. Several other studies have found that the number of retrieved oocytes is similar (with a mean ranging from 9 to 14 oocytes) in newly diagnosed cancer patients undergoing ovarian stimulation compared with infertile controls (Oktay *et al.*, 2006; Knopman *et al.*, 2009; Michaan*et al.*, 2010; Quintero *et al.*, 2010; Robertson *et al.*, 2011; Pal *et al.*, 1998). However, a recent meta-analysis, which included these six studies and an additional study (Klock *et al.*, 2010), with 218 cancer patients total, concluded that FP patients had fewer oocytes retrieved overall compared with controls (Friedler *et al.*, 2012). It is important to note that this meta-analysis also found that cancer patients received significantly lower total doses of gonadotrophins (3031 1726 IU versus 3387 1763 IU; $P = 0.008$), which could have contributed to the lower number of oocytes obtained compared with controls. A major limitation of this study was the inability to compare differences in response to stimulation according to stimulation protocols. Indeed, the main challenge in trying to understand differences in response to ovarian stimulation in cancer and non-cancer populations is the fact that stimulation protocols tend to be different for cancer patients.

Patients with oestrogen-sensitive cancers such as breast cancer are routinely prescribed letrozole as part of stimulation. However, patients with infertility and non-estrogen-sensitive cancers undergo more traditional protocols.

In order to better elucidate the role of different simulation protocols on response, this study performed subgroup analyses comparing outcomes from cycles in FP patients using letrozole and non-letrozole protocols compared with matched controls. In the study clinic, women with breast and endometrial cancer routinely receive letrozole during ovarian stimulation to minimize any potential adverse impact of super-physiological oestradiol concentrations. Within each stimulation group (letrozole and non-letrozole), no difference was observed in the number of retrieved oocytes compared with controls. However, patients who received the letrozole protocol received a higher starting dose and total dose of gonadotrophins compared with controls, suggesting that response to stimulation may be impaired in patients with hormone-sensitive cancers receiving letrozole protocols. A reduced response to ovarian stimulation in patients with hormone-dependent cancers undergoing letrozole stimulation was also suggested in a recent study assessing response to ovarian stimulation in 223 cancer patients undergoing oocyte vitrification for FP. The authors found that patients with hormone-dependent tumours (Domingo *et al.*, 2012) had lower numbers of retrieved oocytes compared with controls even when similar doses of gonadotrophins were administered. These findings suggest that women with hormone-dependent malignancies,

primarily breast cancer, may have a different response to stimulation. This finding may be due to the stimulation protocol or physiological differences in this population. It has been previously observed that patients with *BRCA1* mutations have decreased response to ovarian stimulation, suggesting that difference in underlying genetic make up may influence stimulation (Oktay *et al.*, 2010b).

Similarly to the current findings, patients with non-hormone-dependent cancers who did not receive letrozole had no difference in the number of oocytes retrieved compared with controls (12.2 versus 12.4 oocytes). Unlike their findings, however, the current study found that peak oestradiol concentrations were significantly lower in the non-letrozole group. It is not clear what might be driving this association. Perhaps differences in the hypothalamic–gonadal axis from underlying disease or stress or differences in ovarian reserve may be responsible for lower oestradiol concentrations (Lawrenz *et al.*, 2012).

In addition, this study observed that FP patients had more immature oocytes compared with controls. One explanation for this finding would be the tendency of physicians to access all follicles during retrieval, including small follicles, in FP patients when they would not normally do so. If that had been the case, then one would expect to see higher rates of oocyte immaturity regardless of protocol. While a significant difference in oocyte immaturity in the entire FP cohort was observed, this difference was driven by the high rate of immaturity among those receiving letrozole. Indeed, there was no difference in oocyte immaturity among those who received nonletrozole protocols. Thus, this observation suggests that there is a difference in response to stimulation in women with breast cancer or a difference in response based on stimulation protocol. Earlier studies of letrozole use in breast cancer patients have reported lower rates of oocyte maturity (73.2 22.9% for letrozole versus 86.3 12.7% for controls; $P = 0.003$) However, this phenomenon was overcome by delaying administration of HCG until the lead follicle reached 20 mm in mean diameter (Oktay *et al.*, 2006). In the current study, it is interesting to note that, even though the trigger was delayed to meet those criteria, higher rates of oocyte immaturity were still observed in this group. It should also be noted that the assessment of oocyte maturity was limited since conventional insemination was used in most cases and therefore the oocytes were not stripped on the day of oocyte retrieval. For oocytes undergoing conventional insemination, oocyte maturity was recorded on the day after retrieval. Using this method may have caused an underestimation of the number of immature oocytes as oocytes undergoing conventional insemination have the opportunity to mature and fertilize in the 24 h following retrieval. Despite a potential underestimation of oocyte immaturity, there was still a statistically significant difference in number of immature oocytes obtained, especially among those using letrozole. Thus, the rate of oocyte immaturity may be higher than reported here.

Interestingly, this study also observed that FP patients had lower fertilization rates compared with controls though a similar number of embryos were obtained overall. However, this phenomenon was only observed in cycles utilizing conventional insemination. The reduced fertilization rate did not appear to be related to the type of stimulation protocol as it was observed in FP patients who received letrozole and non-letrozole protocols. Similar findings have been observed in some but not all studies. For example, a small study by Pal *et al.* (1998) reported fewer mature oocytes and significantly lower fertilization rates (51% versus 81%; $P < 0.05$) in cancer patients compared with infertile controls. Similarly, Knopman *et al.* (2009) reported decreased fertilization rates in 28 patients undergoing embryo banking for cancer. These findings are in contrast to the previously mentioned meta-analysis, which did not demonstrate differences in fertilization rates or numbers of embryos obtained when compared with infertile controls (Friedler *et al.*, 2012). It is important to note that the method of insemination varies between studies and may explain the discrepant findings. Since fertilization rates by insemination method were not reported in previous studies, it is

unclear whether insemination method impacts fertilization rates in FP patients. The study clinic does not routinely recommend ICSI in FP patients since cost is often a concern. Only four patients had ICSI in this study for severe oligospermia. Several other FP studies have reported using ICSI rather than conventional insemination in the FP population (Robertson *et al.*, 2011; Knopman *et al.*, 2009). While it is not clear if utilization of ICSI in this population improves fertilization and live birth rates, given the observation that fertilization rates are lower with conventional insemination, the use of ICSI should be considered.

Strengths of this study include the control cohort design, which was matched for multiple factors that influence IVF outcomes, including age, race and fertilization method. Furthermore, this study used a matched statistical analysis, which is a more rigorous and appropriate methodology compared with the cross-sectional method utilized in many other studies.

This study has several limitations. One of the principal limitations is the inclusion of a heterogenous group of cancer patients with a variety of diagnoses. If ovarian response to stimulation is influenced by cancer diagnosis, then inclusion of various types of cancer patients in this study could have biased the results by obscuring a real association. Indeed, there is some evidence that patients with lymphoma, in particular, may have impaired baseline measures of ovarian reserve (Lawrenz *et al.*, 2012).

Additionally, because ovarian stimulation in breast cancer patients is systematically different than for infertility patients, it was not possible to control for stimulation protocols through matching. Breast cancer made up more than 50% of the case population, and almost all of these patients were treated with a letrozole protocol. Since letrozole is not routinely used in IVF protocols, any comparison with controls is limited. Thus, differences in study protocol are an inherent limitation in this type of research. This limitation was addressed by matching for study protocol when possible and by assessing the impact of protocol discordance on the data. Protocol discordance did not impact number of immature oocytes or fertilization rate, indicating that the differences observed were not due to differences in protocol.

In conclusion, chemotherapy naïve cancer patients have similar ovarian reserve, response to stimulation and oocyte and embryo yield compared with infertile and donor controls, despite more immature oocytes and lower fertilization rates. These differences were more prominent in letrozole protocols. While these data are helpful in counselling patients, systematic differences in treatment protocols among cancer patients and the heterogeneous population make it difficult to draw definitive conclusions about expected success rates. Furthermore, only a handful of patients that have undergone IVF for fertility preservation have returned to use their embryos. Thus, data on pregnancy and live birth rates using cryopreserved embryos and oocytes in this population are lacking. Further studies are needed to determine both the clinical implications as well as potential underlying mechanisms of the observed associations in this population.

Acknowledgments

This was supported by the National Institutes of Health (K01 L:1-CA-133839-03 to CRG) and a Doris Duke research fellowship (KED).

Biography

Body Mass Index and Severe Postpartum Hemorrhage

Michael J. Paglia, Chad A. Grotegut, Lauren N. C. Johnson, Betty Thames, Andra H. James. Gynecologic and Obstetric Investigation, January 2012.

Bleeding per Vaginam is associated with Funisitis in Women with Preterm, Prelabour Rupture of the Fetal Membranes

Chad A. Grotegut, Lauren N. C. Johnson, C. Brennan Fitzpatrick, R. Phillips Heine, Geeta K. Swamy, Amy P. Murtha. British Journal of Obstetrics and Gynecology, May 2011.

Oxytocin Exposure During Labor Among Women with Postpartum Hemorrhage Secondary to Uterine Atony

Chad A. Grotegut, Michael J. Paglia, Lauren N. C. Johnson, Betty Thames, Andra H. James. American Journal of Obstetrics and Gynecology, January 2011.

Eligibility and Accessibility of Magnetic Resonance-Guided Focused Ultrasound (MRgFUS) for the Treatment of Uterine Leiomyomas

Millie A. Behera, Madeline Leong, Lauren N. C. Johnson, Haywood L. Brown. Fertility and Sterility, October 2010.

With improvement in cancer survival rates, there has been a shift in attention toward management of long-term consequences of cancer therapy, including infertility. Many young women with cancer, particularly those will be treated with chemotherapy, pursue fertility preservation (FP) strategies for the purpose of banking oocytes or embryos for future use. We examined patients with no prior exposure to chemotherapy who underwent IVF to freeze embryos or oocytes for FP. Fifty FP patients were identified and matched to healthy controls by age, race, cycle number, date of stimulation and fertilization method. There were no differences in baseline measures of ovarian reserve or amount of medication needed to stimulate the ovaries. FP patients had more immature oocytes and lower fertilization rates than controls. There were no differences in number of oocytes retrieved, number of mature oocytes, rate of maturity or number of fertilized embryos. Subgroup analysis revealed that FP patients taking letrozole required higher gonadotrophin doses and had more immature oocytes compared with matched controls. There were no differences in gonadotrophin dose or oocyte immaturity among FP patients not taking letrozole. We demonstrated that FP patients not previously exposed to chemotherapy have similar ovarian reserve, response to stimulation and oocyte and embryo yield compared with infertile and donor controls. Patients who received letrozole required higher gonadotrophin doses and produced more immature oocytes, suggesting that response to ovarian stimulation may be impaired in patients with hormone-sensitive cancers receiving letrozole.

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Table 1

Demographic information and first-cycle IVF protocols for fertility preservation patients and matched controls.

	Cases (n = 50)	*Controls (n = 50)*
Age (years)	31.2 (19–13)	31.2 (21–41)
Body mass index (kg/m^2)	24.2 (19–47)	24.4 (18–44)
Race (Caucasian)	88 (44/50)	88 (44/50)
Previous pregnancy	32 (16/50)	48 (24/50)
Previous pregnancy[a]	1 (1–5)	2 (1–8)
Cancer type		
Breast	58 (29/50)	–
Hodgkin's lymphoma	4 (2/50)	–
Myelodysplastic syndrome	6 (3/50)	–
Ovarian cancer	6 (3/50)	–
Cervical cancer	6 (3/50)	–
Endometrial cancer	4 (2/50)	–
Other cancer	6 (3/50)	–
Non-malignancy	10 (5/50)	–
First-cycle protocol[b]		
Letrozole	44 (22/50)	0 (0/50)
Antagonist without letrozole	44 (22/50)	40 (20/50)
Luteal-phase lupron	12 (6/50)	60 (30/50)
Trigger medication		
HCG	78 (39/50)	86 (43/50)
GnRH	16 (8/50)	14 (7/50)
Both	6 (3/50)	0 (0/50)

Values are median (range) or % (*n*).

GnRH = gonadotrophin-releasing hormone; HCG = human chorionic gonadotrophin.

[a] For those with previous pregnancy.

[b] $P < 0.001$.

Table 2

Unadjusted analysis of first IVF cycle characteristics and outcomes for fertility preservation patients and matched controls.

	Cases (n = 50)	*Controls (n = 50)*	*P-value*
Baseline oestradiol (pg/ml)[a]	48.1 (40.9–56.8)	39.2 (35.5–13.4)	0.040
Baseline FSH (mIU/ml)[a,b]	5.3 (4.3–6.7)	5.7 (5.1–6.4)	NS
Baseline AMH (ng/ml)[a,c]	2.0 (1.6–2.8)	2.5 (1.5–4.1)	NS
Baseline antral follicle count	21.1 (17.6–24.7)	19.4 (16.8–22.0)	NS
Days of stimulation	9.9 (9.2–10.5)	10.1 (9.6–10.6)	NS
Starting gonadotrophin dose (IU)	281.2 (247.5–315.0)	238.8 (204.8–272.7)	NS
Total gonadotrophin dose (IU)	3009 (2573–3444)	2556 (2079–3033)	NS
Total follicle number	21.6 (18.2–25.1)	22.8 (19.4–26.1)	NS
Total follicles >14 mm	11.1 (9.5–12.7)	11.8 (10.6–12.9)	NS
Oestradiol at trigger (pg/ml)[a]	933 (709–1228)	2730 (2453–3044)	<0.001
Endometrial thickness at trigger (mm)	9.6 (8.7–10.4)	10.6 (9.8–11.4)	NS
No. of oocytes retrieved	12.4 (10.1–14.7)	11.7 (10.1–13.3)	NS
No. of oocytes mature (M2)	9.0 (7.4–10.5)	8.9 (7.6–10.2)	NS
No. of immature (GV + M1)	2.2 (1.2–3.3)	1.1 (0.6–1.5)	0.030
Maturation rate	74.1 (67.6–80.6)	76.7 (69.2–84.3)	NS
No. of 2PN embryos	5.4 (4.1–6.6)	6.0 (4.9–7.2)	NS
Fertilization rate	51.6 (43.4–59.5)	69.5 (60.8–78.3)	0.002

Values are mean (95% CI).

AMH = anti-Mullerian hormone; NS = not statistically significant.

[a] Geometric mean presented.

[b] Data available for 42 cases and 46 controls.

[c] Data available for 27 cases and 14 controls.

Table 3

Unadjusted subanalysis of IVF cycle characteristics and outcomes for patients taking letrozole.

	Letrozole cases (n = *22*)	*Controls* (n = *22*)	P-*value*	*Non-letrozole cases* (n = *28*)	*Controls* (n = *28*)	P-*value*
Baseline oestradiol (pg/ml)[a]	46.6 (36.7–59.1)	38.5 (32.4–45.6)	NS	49.2 (38.9–62.3)	39.6 (34.8–45.3)	NS
Baseline FSH (mIU/ml)[a,b]	4.7 (3.4–6.5)	5.8 (5.0–6.8)	NS	6.1 (4.4–8.5)	5.6 (4.6–6.7)	NS
Baseline AFC	21.9 (17.1–26.6)	21.1 (17.2–25.1)	NS	20.6 (15.2–25.9)	18.0 (14.4–21.6)	NS
Days of stimulation	9.0 (8.0–10.1)	10.2 (9.4–11.0)	NS	10.5 (9.6–11.4)	10.0 (9.3–10.7)	NS
Starting gonadotrophin dose (IU)	317 (276–358)	203 (172–235)	<0.01	267 (212–321)	253 (202–304)	NS
Total gonadotrophin dose (IU)	3077 (2416–3738)	2259 (1580–2938)	<0.05	2789 (2099–3479)	2955 (2339–3571)	NS
Total follicles	21.8 (16.2–27.4)	26.5 (20.4–32.7)	NS	21.5 (16.9–26.1)	19.8 (16.2–23.4)	NS
Total follicles >14 mm	11.4 (8.8–14.1)	12.9 (11.1–14.7)	NS	10.8 (8.6–12.9)	10.9 (9.3–12.4)	NS
Oestradiol at trigger (pg/ml)[a]	459 (317–665)	2766 (2361–3239)	<0.01	1664 (1310–2113)	2705 (2310–3165)	0.01
Endometrial thickness at trigger (mm)	9.3 (7.7–10.9)	10.5 (9.0–11.9)	NS	9.8 (8.9–10.7)	10.8 (9.9–11.6)	NS
No. of oocytes harvested	14.6 (10.3–19.0)	13.1 (10.7–15.5)	NS	10.7 (8.4–13.0)	10.6 (8.3–12.9)	NS
No. of mature	10.0 (7.3–12.6)	10.0 (7.6–12.3)	NS	8.2 (6.3–10.2)	8.1 (6.7–9.6)	NS
No. of immature	3.4 (1.5–5.4)	1.2 (0.5–1.8)	0.03	1.1 (0.5–1.8)	1.0 (0.3–1.6)	NS
Maturation rate	73.6 (65.9–81.3)	72.3 (63.8–81.0)	NS	77 (71–83)	81 (74–88)	NS
No. of 2PN	5.3 (3.4–7.2)	6.5 (4.4–8.6)	NS	5.4 (3.7–7.2)	5.7 (4.2–7.2)	NS
Fertilization rate	47.1 (33.0–61.1)	66.6 (53.6–79.6)	NS	55 (45–65)	72 (59–85)	0.02

Values are mean (95% CI). Fertility preservation patients are compared with their matched controls.

AMH = anti-Mullerian hormone; NS = not statistically significant.

[a]Geometric mean presented.

[b]Data available for 42 cases and 46 controls.

Table 4

Cycle characteristics and pregnancy outcomes for fertility preservation patients who thawed embryos.

Patient	Age (years)	Cancer type	IVF protocol	Oocytes retrieved	2PNs obtained	Oocytes cryopreserved	Embryos cryopreserved	Embryos thawed	Quality of embryos transferred[a]	Gestational carrier	Pregnancy complications	Mode of delivery	Outcome (weighting)
1	26.0	Breast	A and /L	10	4	0	4	4	4cell grade 1.0; 5cell grade 1.5)	Yes	Twin gestation, preeclampsia	Caesarean	Preterm delivery at 36 weeks (2381 and 2580)
2	34.9	Breast	LPL	28	19	0	19	4	2 cell; 5 cell[b]	No	Preterm labour	Vaginal	Term delivery (2920)
3	26.8	Breast	1st cycle LPL	10	4	0	4	4	5 cell grade 2.0; 3 cell grade 2.0	No	Large for gestational age	Caesarean	Term delivery (4309)
			2nd cycle A	18	10	0	10						
4	34.2	Cervical	A	8	7	0	7	4	4 cell grade 2.0; 4 cell grade 2.0	Yes	None	Vaginal	Term delivery (3401)
5	43.1	Breast	A	5	2	0	2	2	0	–	–	–	Embryos did not survive thaw
6	40.8	Breast	A	10	2	4	2	2	0	–	–	–	Embryos did not cleave, oocytes still vitrified

All patients had day-3 embryos transferred.

A = antagonist; LET = letrozole; LPL = luteal-phase lupron.

[a]Quality assessed using the Veeck's grading system (1989).

[b]Embryo grades not available.