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ORIGINAL ARTICLE ***Reproductive endocrinology***

Effects of cancer stage and grade on fertility preservation outcome and ovarian stimulation response

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STUDY QUESTION: Do the stage and grade of malignancy affect the fertility preservation outcome in females?

SUMMARY ANSWER: Patients with high-grade cancer have a decreased number of retrieved mature oocytes and cryopreserved embryos.

WHAT IS KNOWN ALREADY: Cancer has local and systemic effects on the host. The effects of cancer spread and aggressiveness on the ovarian function and stimulation response remain unclear.

STUDY DESIGN, SIZE, DURATION: Retrospective cohort study evaluating data of all fertility preservation treatment cycles among women with cancer at the reproductive unit of the McGill University Health Centre in the period from 2008 to 2017.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Study inclusion criteria were age 18–38 years, first stimulation cycle, GnRH-antagonist protocol and early follicular phase stimulation start. Only one stimulation cycle per patient was included. Patients with ovarian pathology, previous ovarian surgery and previous chemo- or radiotherapy were excluded. The outcomes of women with low-stage cancer (local tumor Stage I–II, no lymph node involvement, no metastases) were compared with those with high-stage disease (local tumor Stage III–IV, lymph node involvement or metastases). Similarly we compared those with low-grade ($G_{1–2}$) and high-grade ($G_{3–4}$) malignancies. The primary outcome measure was the number of mature oocytes retrieved. The secondary outcomes included the total number of retrieved oocytes, the number of vitrified oocytes, and the number of frozen embryos. We used Student's t-test for normally distributed data and Wilcoxon test for skewed data. To determine factors associated with good fertility preservation outcome defined as over 10 retrieved mature oocytes, we used multivariate logistic regression.

MAIN RESULTS AND THE ROLE OF CHANCE: A total of 147 patients were included in the final analysis. Age, body mass index, ovarian reserve parameters of the study groups in stage- and grade-based analyses were similar. Compared to women with low-stage cancer (n = 83), those with high-stage cancer (n = 64) required a higher dose of gonadotropin (P = 0.02). The number of retrieved mature oocytes (9 (7–13) versus 8 (5–12); P = 0.37) and vitrified oocytes (10 (7–15) versus 10 (7–13); P = 0.53) were similar between the two groups. However, in cycles where fertilization of all retrieved oocytes was performed, the fertilization rate (82.7% versus 71.5%; P = 0.03) and the number of vitrified embryos (6.2 ± 3.2 versus 4.3 ± 2.1; P = 0.01) were higher in the low-stage group. Compared to patients with low-grade cancer (n = 62), those with high-grade disease (n = 85) had significantly lower number of retrieved mature oocytes (11 (7–15) versus 8 (5–11); P = 0.002) and vitrified oocytes (12 (8–15) versus 10 (7–11); P = 0.005). The number of vitrified embryos was lower in high-grade group (6.5 ± 3.5 versus 4.6 ± 2.3; P = 0.03) in cycles where the fertilization was performed. In multivariate logistical analysis, the low-grade cancer was significantly associated with retrieval of over 10 mature oocytes (OR = 4.26; 95% CI 1.82–9.98; P = 0.0009).

LIMITATIONS, REASONS FOR CAUTION: The main limitations of the study include its retrospective design and the relatively small sample size in the embryological outcome analysis. The results of our study should be viewed with caution as different malignancy types were included in the study groups, although their distribution between the study groups was similar.

WIDER IMPLICATIONS OF THE FINDINGS: Cancer grade seems to have a negative impact on the fertility preservation outcome and the ovarian stimulation response.

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Key words: fertility preservation / stage of cancer / grade of cancer / number of oocytes / vitrification

Introduction

Strategies to preserve fertility in women undergoing gonadotoxic treatment include ovarian transposition, ovarian suppression by gonadotropin-releasing hormone (GnRH) agonist, embryo cryopreservation, oocyte cryopreservation and ovarian tissue cryopreservation (Donnez and Dolmans, 2015). The most established and successful method is embryo cryopreservation followed by oocyte cryopreservation. The latter is considered safe, effective and reproducible (Noyes *et al.*, 2013) and has been suggested as a better option for all women to maintain their reproductive autonomy and potential (Mahajan, 2015).

The counseling and treatment decisions for women with cancer are individualized and based on multiple conventional factors, such as the patient's age, body mass index and ovarian reserve status. However, in cancer patients the presence of malignancy and the type of cancer have been proposed as factors that can also affect ovarian function. We previously reported that the ovarian reserve and number of oocytes obtained in women with cancer were similar to those with male factor infertility (Das *et al.*, 2011). Alvarez and Ramanathan demonstrated higher number of mature oocytes in patients with hematologic malignancies and lower numbers of mature oocytes in women with gynecological cancer compared to those with gastrointestinal and breast cancer (Alvarez and Ramanathan, 2018). In the study of Almog *et al.*, it was found that ovarian response parameters in women with breast cancer, soft tissue sarcoma, hematologic malignancies, and gastrointestinal tract cancers were similar. Although aromatase inhibitors (AI) were not administered, significantly lower maximal estradiol levels were found in cancer patients comparing with control group (Almog *et al.*, 2012). The authors of this study assumed that this finding may represent an early sign of the possible negative effect of cancer state on granulosa-cell performance. In meta-analysis of Friedler *et al.* a reduced number of oocytes in patients with malignancies undergoing controlled ovarian hyperstimulation for fertility preservation was found. The increased catabolic state, malnutrition, and increased stress hormone levels associated with malignant disease were proposed as factors may affect the hypothalamic-gonadal axis, decrease ovarian reserve and affect oocyte quality (Friedler *et al.*, 2012).

The effects of stage and grade of cancer on the outcome of fertility preservation remain unclear. Advanced cases of cancer can cause a number of systemic effects including elevated level of matrix metalloproteinases (MMPs), disruption of immune response by lymphocyte function abnormalities (Rubin, 2005), discoordination of cell–matrix and cell–cell signaling (Friedl and Alexander, 2011; Sansing *et al.*, 2011), and increased release of tumor growth factor-β (TGF-β) (Jiang *et al.*, 2015). Some of these effects may have negative effects on ovarian function. Increased expression of MMPs may facilitate the follicular atresia process and granulosa cell apoptosis (Smith *et al.*, 2002). Abnormal expression of TGF-β causes a dysregulation of human ovarian functions, including follicle development and maturation (Peng, 2003). Studies in animals showed that overexpression of TGF-β can cause a block of folliculogenesis (Lin *et al.*, 2003).

The purpose of our study was to evaluate the effects of stage and grade of any malignancy on ovarian response to stimulation in women undergoing fertility preservation treatment before their chemo- and/or radiotherapy treatment.

Materials and Methods

Population

We conducted a retrospective cohort study evaluating the data of all fertility preservation treatment cycles among women with cancer at the reproductive unit of the McGill University Health Center in the period from 2008 to 2017. *In-vitro* maturation (IVM) and ovarian tissue cryopreservation cycles were excluded from the current analysis, as the goal of the study was to evaluate the ovarian response to gonadotropin stimulation.

The inclusion criteria were age 18–38 years, early follicular phase stimulation start (Day 2–3), and use of the GnRH-antagonist protocol. A single stimulation cycle of each patient was included in the analysis. If the patient underwent more than one stimulation cycle, the first treatment cycle was chosen.

We excluded the cycles started in the luteal phase, as the stimulation start phase can be a significant confounder. Studies show a longer FSH treatment period and higher FSH doses have been required using luteal phase protocols (von Wolff *et al.*, 2016). Additionally, we did not have a full data about their baseline ovarian reserve, as the treatment was usually started in the luteal phase of the same menstruation cycle they were referred. Patients who had ovarian pathology, previous ovarian surgery or those who received chemo- or radiotherapy were excluded from the study.

Study groups

All malignancies were staged and graded according to the American Joint Committee of Cancer (AJCC) (Edge *et al.*, 2010) and the cancer protocols of the College of American Pathologists (Amin, 2010). Since the tumor, node and metastasis (TNM) classification has not been used for staging of lymphoid neoplasms, we used revised Ann Arbor staging system (Edge *et al.*, 2010) for patients with Hodgkin and non-Hodgkin lymphomas.

The gynecological tumors were additionally staged according to the International Federation of Gynecologists and Obstetricians (FIGO) staging systems (Belhadj *et al.*, 2014; Prat *et al.*, 2015) and no discrepancy between the AJCC and FIGO staging was found.

There is no TNM staging system for primary central nervous system neoplasms. Accordingly, we used the World Health Organization (WHO) grading system (Louis *et al.*, 2007).

Patients with Stage I–II tumors, no lymph node involvement and no metastases ($T_{1–2}N_0M_0$) including those with Stage I–II lymphoid neoplasms (localized on the same side of the diaphragm) were allocated to the low-stage group. They were compared with those allocated to the high-stage disease group having Stage III–IV tumors ($T_{3–4}N_0M_0$) including Stage III–IV lymphoid neoplasms (involvement of lymph node regions on both sides of the diaphragm and/or extralymphatic organs), lymph node involvement ($T_{any}N_1M_{any}$) or metastatic disease ($T_{any}N_{any}M_1$).

Eight of 10 patients with brain tumors were allocated to low-stage group, as the risk of hematologic and lymphatic extracranial brain tumor spread is extremely rare (Ray et al., 2015), and the disease was found to be localized on examination and imaging of these patients. Two others with glioblastomas and neck metastases were allocated to the high-stage group.

In view of cancer grades, patients with Grade I and II ($G_{1–2}$) were allocated to low-grade group and others with Grade III and IV ($G_{3–4}$) to high-grade group.

Patients who underwent fertilization of all retrieved oocytes were allocated to study groups according to their malignancy stage and grade and their embryological outcome was compared. The sperm concentration and motility parameters of male partners (Björndahl et al., 2016) were compared in this analysis.

The data of all patients were collected from the medical records including the year of referral and treatment, age, body mass index (BMI), current smoking behavior, antral follicle count (AFC), baseline FSH level, the type, stage and grade of cancer, stimulation protocol, dose of gonadotropin used, number of retrieved and cryopreserved oocytes, fertilization rate and number of frozen embryos.

Stimulation protocol

All patients underwent ovarian stimulation using early follicular phase start GnRH-antagonist fixed protocol as previously described (Lambalk et al., 2017). Briefly, recombinant FSH (Gonal-F, Merck-Serono, Geneva, Switzerland) or urinary human menopausal gonadotropin (Repronex, Ferring Pharmaceuticals, NY, USA) was administered starting in the early follicular phase of the cycle (Day 2–3). GnRH-antagonist 0.25 mg ganirelix (Orgalutran, MSD Organon, Oss, Netherlands) or 0.25 mg cetrorelix (Cetrotide, Merck-Serono, Geneva, Switzerland) was added on Day 6 of stimulation. We also prescribed aromatase inhibitor 5–7.5 mg daily (Femara, Novartis, NJ, USA) to patients with hormone-dependent cancers during the period of the stimulation (Oktay et al., 2005). Final oocyte maturation was triggered with subcutaneous injection of 0.25 mg recombinant hCG (Ovidrel, EMD Serono, MA, USA) or 1 mg of GnRH-agonist buserelin (Suprefact, Sanofi-Aventis, QB, Canada) when 2–3 follicles reached 17 mm in diameter. Ultrasound-guided retrieval was performed 36 h later under conscious sedation using a 17-gauge single lumen needle (K-OSN-1735-A-90-US, Cook Brisbane, Australia).

After collection the oocytes were either cryopreserved or fertilized. All oocytes and embryos were cryopreserved using vitrification technique (Chian et al., 2009; Creux et al., 2017).

Outcomes and definitions

The primary outcome of the study was the number of retrieved mature oocytes. The secondary outcomes included the total number of retrieved oocytes, the number of vitrified oocytes, fertilization rate and the number of frozen embryos.

Statistical analysis

Data were analyzed using JMP Pro 13.2.0 software (SAS Institute Inc., USA). We used the Shapiro–Wilk test to evaluate the distribution of the data. Data are presented as mean ± standard deviation (SD) or median (with inter quartile range) for normally distributed or skewed data, respectively. We used Student's *t*-test for normally distributed data and Wilcoxon test for skewed data. Categorical data were compared using chi-squared test.

We performed univariate analyses to evaluate factors associated with good fertility preservation outcome defined as over 10 retrieved mature oocytes. This was based on the previous studies that demonstrated high live birth rate (33.4%) in the fresh IVF cycles and cumulative live birth (50.5%) in 18–38 years age group as the number of retrieved oocytes was more than 10 (Sunkara et al., 2011; Drakopoulos et al., 2016). To determine factors independently associated with retrieval of over 10 mature oocytes the multivariate logistic regression model was conducted. The determinants from the univariate analysis with *P*-value <0.1 were subsequently included in the multivariate analyses. According to the data distribution Pearson or Spearman correlation test was calculated. If determinants were highly correlated, only one of them was included in the multivariate model. Backward stepwise regression was conducted and the odds ratios (OR) with 95% confidence intervals (95%CI) were calculated.

Ethical approval

The study was approved by McGill University Health Centre Research Ethics Board (MUHC 2018-4279).

Results

A total of 402 fertility preservation treatment cycles among women with cancer were conducted at the reproductive unit of the McGill University Health Center in the period of 2008–2017. They included 212 ovarian stimulation cycles, 157 *in vitro* maturation (IVM) cycles and 33 ovarian tissue cryopreservations. Patients who had ovarian pathology ($n = 3$), previous ovarian surgery ($n = 3$) or those who received chemo- or radiotherapy ($n = 5$) were excluded from the study.

A total of 147 patients were included in the final analysis according to the inclusion and exclusion criteria. Fifty three patients underwent conventional IVF or ICSI of all retrieved oocytes (Fig. 1).

Age, body mass index, current smoking behavior, ovarian reserve parameters of the study groups in stage- and grade-based analyses were similar (Tables I and II). The distribution of patients with different malignancy types as well as proportion of patients who received AI, different gonadotropin types and different type of final maturation trigger was also similar.

Compared to women with low-stage cancer, those with high-stage cancer required a higher dose of gonadotropin ($P = 0.02$). In 19 patients (30%) with high-stage disease, the daily dose of gonadotropins was subsequently increased during the stimulation compared with 18 patients (22%) with low-stage cancer ($P > 0.05$). Additionally, there was no difference in FSH start dose between the groups (Table I).

The number of retrieved mature and vitrified oocytes, the number of immature oocytes (GV and MI) and the maturation rate were similar (Table I). The analysis of patients with different cancer stage did not show a significant correlation between the number of retrieved mature oocytes and the stage of disease.

In grade-based analysis the dose of gonadotropin was similar between low-grade and high-grade groups. The number of follicles > 14 mm on the trigger day was significantly higher in low-grade group ($P = 0.01$). The number of retrieved mature oocytes in the low-grade group was higher than in patients with high-grade disease (11 (7–15)

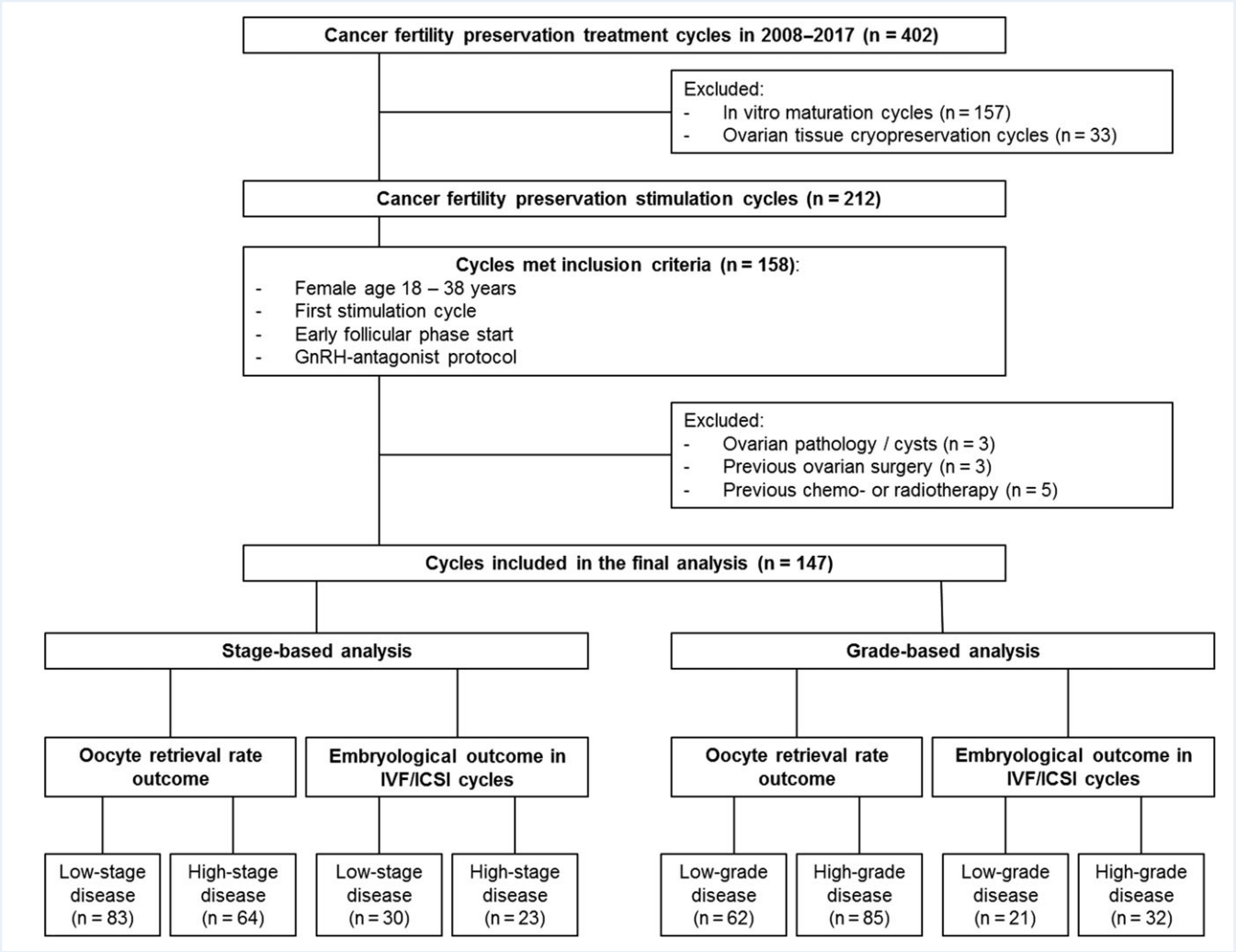

Figure 1 Flowchart of patient selection process. GnRH-antagonist, gonadotropin-releasing hormone antagonist; IVF, *in-vitro* fertilization; ICSI, intracytoplasmic sperm injection.

versus 8 (5–11); $P = 0.002$). The total number of retrieved and vitrified oocytes in the low-grade group were also significantly higher than in the high-grade group ($P = 0.008$, $P = 0.005$, respectively) (Table II). The analysis of patients with different grades (G_1–G_4) revealed a trend of a decreasing number of retrieved mature oocytes with increasing grade. The most significant was difference between Grade 2 and 3 (11 (8–13) versus 7 (5–11); $P = 0.01$).

A total of 12 patients (8%) had three or less oocytes retrieved. The distribution of these patients was similar in stage- and grade-based analyses (Tables I and II).

Factors potentially associated with retrieval of over 10 mature oocytes were tested in the univariate analysis (Supplementary Table SI). AFC, AI addition, breast cancer, trigger type and grade of cancer were included in the multivariate model. Multivariate logistic regression demonstrated that AFC (OR 1.11 per unit change; 95% CI 1.05–1.17), GnRH-agonist trigger (OR 3.49; 95% CI 1.48–8.28) and low malignancy grade (OR = 4.26; 95% CI 1.82–9.98) were significantly associated with good fertility preservation outcome (Table III).

When the embryological outcome of patients with low-stage ($n = 30$) and high-stage ($n = 23$) disease was compared, the demographic, ovarian reserve, stimulation cycle, sperm concentration and motility parameters were found to be similar. The number of retrieved mature and immature oocytes was similar between the groups. The number of cycles with Day-3 and Day-5 embryo cryopreservation as well as the separate number of Day-3 and Day-5 embryos obtained was not significantly different. Compared to those in the high-stage group, the fertilization rate (82.7% versus 71.5%; $P = 0.03$) and the total number of vitrified embryos (6.2 ± 3.2 versus 4.3 ± 2.1; $P = 0.01$) were significantly higher in the low-stage group (Supplementary Table SII).

The demography, ovarian reserve, stimulation cycle, sperm quality parameters as well as the number of retrieved mature and immature oocytes were similar between the low-grade ($n = 21$) and the high-grade ($n = 32$) disease groups. The number of cycles with Day-3 or Day-5 embryo cryopreservation and the number of Day-3 and Day-5 embryos achieved were not significantly different. However, the total number of vitrified embryos was significantly higher in low-grade group (6.5 ± 3.5 versus 4.6 ± 2.3; $P = 0.03$) (Supplementary Table SIII).

Table I Patients' characteristics and oocyte retrieval outcome according to the cancer stage.

	Low-stage disease	High-stage disease	*P*-value
Number of patients	83	64	
Age (years)	29 (24–34)	29 (25–33)	0.94
BMI (kg/m^2)	23.6 (22.5–25.1)	23.5 (20.9–25.6)	0.22
Smoking [*n* (%)]	9 (11)	4 (6)	0.33
Malignancy type [*n* (%)]:			0.11
Breast	28 (34)	24 (38)	
Lymphoma	31 (37)	19 (30)	
Brain	8 (10)	2 (3)	
Colon	4 (5)	9 (14)	
Endometrium	3 (4)	1 (2)	
Cervix	6 (7)	1 (2)	
Ovary	1 (1)	1 (2)	
Lung	0 (0)	1 (2)	
Soft tissue sarcoma	2 (2)	5 (8%	
Thyroid	0 (0)	1 (2)	
AFC (*n*)	16 (11–23)	14 (8–23)	0.11
Baseline FSH (IU/L)	4.9 (4.0–5.9)	6.4 (4.3–8.2)	0.09
Stimulation [*n* (%)]:			0.73
rFSH	58 (70)	43 (67)	
HMG	25 (30)	21 (33)	
rLH addition [*n* (%)]	14 (17)	10 (16)	0.84
Total FSH dose (IU)	1600 (1200–2400)	2038 (1575–2681)	0.02
FSH start dose (IU)	200 (150–218.8)	225 (150–237.5)	0.32
Days of stimulation	8 (7–9)	8 (7–10)	0.38
Cycles with addition of AI [*n* (%)]:	30 (36)	24 (38)	0.87
Trigger [*n* (%)]:			0.60
HCG	55 (66)	45 (70)	
GnRH agonist	28 (34)	19 (30)	
Estradiol on trigger day (pmol/L)	3224 (1238.0–5426.5)	3288 (1020.8–5857.0)	0.69
Follicles >14 mm on trigger day (*n*)	6 (5–9)	5 (3.25–9)	0.21
Oocytes (*n*):			
Retrieved	13 (9–17)	11 (7–17)	0.55
MII at retrieval	9 (7–13)	8 (5–12)	0.37
GV at retrieval	1 (0–3)	1 (0–3)	0.64
MI at retrieval	1 (0–2)	1 (0–1)	0.38
Vitrified	10 (7–15)	10 (7–13)	0.53
Patients with three or less retrieved oocytes [*n* (%)]	6 (7)	6 (9)	0.64
Maturation rate of GV and MI oocytes (mean %)	34	34	0.83

Data are median (quartiles) unless stated otherwise.
AFC, antral follicles count; AI, aromatase inhibitor; MI/II, metaphase I/II; GV, germinal vesicle.

Discussion

The aim of our retrospective cohort study was to evaluate the possible effects of cancer stage and grade on ovarian response and fertility preservation outcome. The results of our study demonstrate that the grade of malignancy has a significant impact on the number of retrieved mature oocytes and vitrified oocytes. The results of multivariate logistic regression showed that low cancer grade is significantly associated with good fertility preservation outcome. Among patients who underwent fertilization of all retrieved oocytes, high-stage and high-grade cancer patients had significantly lower number of cryopreserved embryos comparing to those with low-stage and low-grade disease, respectively. These results show that a more aggressive malignancy

Table II Patients' characteristics and oocytes retrieval outcome according to the cancer grade.

	Low-grade disease	High-grade disease	*P*-value
Number of patients	62	85	
Age (years)	27.5 (24–34)	29 (25–33)	0.89
BMI (kg/m^2)	23.5 (22.1–25.1)	23.5 (22.2–25.6)	0.85
Smoking [*n* (%)]	7 (11)	6 (7)	0.37
Malignancy type [*n* (%)]:			0.80
Breast	22 (36)	30 (35)	
Lymphoma	22 (36)	28 (33)	
Brain	3 (5)	7 (8)	
Colon	5 (8)	8 (9)	
Endometrium	2 (3)	2 (2)	
Cervix	5 (8)	2 (2)	
Ovary	1 (2)	1 (1)	
Lung	0 (0)	1 (1)	
Soft tissue sarcoma	2 (3)	5 (6)	
Thyroid	0 (0)	1 (1.2)	
AFC (*n*)	15 (11–23)	17 (10–23)	0.90
Baseline FSH (IU/L)	4.6 (4.0–5.7)	5.8 (4.2–7.8)	0.10
Stimulation [*n* (%)]:			0.89
rFSH	43 (69)	58 (62)	
HMG	19 (31)	27 (32)	
rLH addition [*n* (%)]	10 (16)	14 (17)	0.96
Total FSH dose (IU)	1575 (1350–2306)	2000 (1575–2562)	0.10
FSH start dose (IU)	225 (162.5–225)	187 (150–212.5)	0.19
Days of stimulation	8 (7–9)	8 (7–10)	0.34
Cycles with addition of aromatase inhibitor [*n* (%)]:	21 (34)	33 (39)	0.54
Trigger [*n* (%)]:			0.67
HCG	41 (66)	59 (69)	
GnRH agonist	21 (34)	26 (31)	
Estradiol on trigger day (pmol/L)	3430.5 (1490.8–5453.5)	2514.5 (1020.8—5637.8)	0.35
Follicles >14 mm on trigger day	7 (5–10)	5 (3.5–8.5)	0.01
Oocytes (*n*):			
Retrieved	14 (9–19)	11 (7–15)	0.008
MII at retrieval	11 (7–15)	8 (5–11)	0.002
GV at retrieval	1 (0–3)	1 (0–3)	0.48
MI at retrieval	1 (0–2)	1 (0–2)	0.92
Vitrified	12 (8–15)	10 (7–11)	0.005
Patients with three or less retrieved oocytes [*n* (%)]	5 (8)	7 (8)	0.97
Maturation rate of GV and MI oocytes (mean %)	34	34	0.89

Data are median (quartiles) unless stated otherwise.
AFC, antral follicles count; AI, aromatase inhibitor; MI/II, metaphase I/II; GV, germinal vesicle.

can have a more significant negative effect on fertility preservation outcome.

Although the stimulation duration was similar, the total dose of gonadotropin stimulation used was higher in patients with high-stage malignancy. Similar trend was shown in the study of Almog *et al.* comparing cancer patients with controls (Almog *et al.*, 2012). Although not statistically different, the upper quartile of FSH dose in the study group (300 IU) was higher than the upper quartile dose of the controls (225 IU). Authors proposed that possible reason for this observation may be the tendency to achieve a maximal number of oocytes in patients destined to undergo chemotherapy and less concern of ovarian hyperstimulation syndrome as all embryos in the study group were frozen.

In our study the FSH start dose was similar between the patients in the low- and high-stage groups. However, the daily dose of

Table III Multivariate logistic regression analysis of factors associated with retrieval of over 10 mature oocytes.

Parameter	OR	95% CI
AFC	1.11*	1.05–1.17*
Low-grade cancer (versus high-grade)	4.26	1.82–9.98
GnRH-agonist trigger (versus HCG trigger)	3.49	1.48–8.28
Breast cancer (versus other cancer types)	2.62	0.53–12.96
AI addition (versus no AI)	0.88	0.19–4.07

*OR and 95% CI per unit change in parameter.
AFC, antral follicles count; AI, aromatase inhibitor.

gonadotropins was increased during the stimulation in 29.7% of patients with high-stage disease compared with 21.7% of low-stage patients. This trend can partially explain the difference in gonadotropin doses in stage-based analysis suggesting lower response of patients with high-stage cancer. Yet, the number of retrieved mature and vitrified oocytes was similar to those with low-stage tumors.

Univariate and multivariate analyses demonstrated the factors significantly associated with retrieval of over 10 mature oocytes, which included AFC, GnRH-agonist trigger and low-grade cancer. AFC is a well-established predictor of ovarian response and pregnancy rates (Jayaprakasan *et al.*, 2012). GnRH-agonist triggering was mostly used in patients at risk of ovarian hyperstimulation syndrome, who achieve higher number of retrieved oocytes. Additionally low-grade of cancer was significantly associated with good fertility preservation outcome. This association was not found for low-stage cancer group.

Ovarian tissue has an exceptional metabolic activity and is highly susceptible to different factors such as Bisphenol A (Chianese *et al.*, 2018), alcohol, cigarette smoking (Bressler *et al.*, 2016), chemotherapy or radiation (De Vos *et al.* 2014; Donnez and Dolmans, 2017). Some autoimmune diseases (e.g. thyroiditis, Addison's disease, Crohn's disease, rheumatoid disease) are associated with systemic effects including deleterious effect on ovarian reserve and stimulation response by formation of perivascular and perineural inflammation infiltrates, as well as, lymphocytic and plasma cell infiltrates in proximity to the follicles (Reato *et al.*, 2011; Ayesha *et al.*, 2016).

Cancer is another condition that along with local effect on the impaired organ, cause multiple systemic endocrine, immune and metabolic changes affecting the function of other systems and organs (Friedl and Alexander, 2011; Sansing *et al.*, 2011; Bruzzese *et al.*, 2014; Jiang *et al.*, 2015) including ovarian function (Smith *et al.*, 2002; Peng, 2003). Yet, clinical studies evaluating fertility preservation outcome in women with different malignancies as well as comparing oncological and healthy patients have shown conflicting results (Das *et al.*, 2011; Almog *et al.*, 2012; Alvarez and Ramanathan, 2018).

Our study was not designed to identify the physiological mechanisms underlying our results. However, the systemic effect of advanced stage malignancies has been previously described. Cancer growth is accompanied by progressive infiltration, invasion, and destruction of the surrounding tissue causing activation of the systemic inflammatory responses involving many organ systems (Roxburgh and McMillan, 2014), that can be similar to the previously described systemic effect of autoimmune and chronic diseases. In animal models, altered endothelial integrity and recruited immune cells can affect malignant progression directly by altering the milieu in organs that represent sites for metastasis – even before the tumor cells arrive. Furthermore, recent data show that vascular function is impaired in distant organs not directly affected by either the primary tumor or metastases (Cedervall *et al.*, 2015). In human, different families of proteases, such as matrix metalloproteinases (MMPs), cathepsin D and urokinase plasminogen activator have been implicated in tumor cell invasion. Also, catabolic effects of advanced cancer cause increased level of circulating MMPs (Rubin, 2005).

Controlled turnover of extracellular matrix by MMPs and tissue inhibitors of metalloproteinases (TIMPs) may be essential for creating and/or preserving microenvironments conducive to follicular and luteal ovarian function and is likely dependent upon the ratio of enzyme to inhibitor. Increasing the MMP:TIMP-ratio may have functional implications in processes associated with ovarian function such as folliculogenesis inhibiting (Smith *et al.*, 2002; Kim *et al.*, 2014). Catabolizing the extracellular matrix, MMPs alternate the expansion of the cumulus oocyte complex matrix, affect follicular wall breakdown, or stigma formation, which can results in retained or entrapped oocytes (Curry and Smith, 2006). Activation of MMPs including MMP-1, -2 and -9 contribute to follicular atresia modulation and luteolysis (Yang *et al.*, 2011). The development and maintenance of a systemic inflammatory response has been consistently observed in both early and advanced stage disease (Roxburgh and McMillan, 2014). This finding can partially explain the similar fertility preservation outcome in the low- and high-stage groups in our study.

The association between grade of cancer and ovarian function is unclear. Progression to a higher grade reflects underlying changes in heterotypic signaling interactions including proteases, tumor growth factor (TGF) and vascular endothelial growth factor (VEGF) (Hanahan and Weinberg, 2011). Studies in animals have shown that TGF-β superfamily is involved in regulating ovarian functions, such as follicular cell proliferation and differentiation, follicle development, and atresia, as well as oocyte maturation. Inhibition of ovulation by TGF-β may play a role in the kinetics of ovulation, preventing premature follicle rupture by a mechanism involving inhibition of macrophage activation, thereby allowing completion of oocyte cytoplasmic maturation. Results from clinical studies suggest that the TGF-β superfamily also plays important regulatory roles in folliculogenesis, and production of mature oocytes (Ingman and Robertson, 2002). In many tumors, TGF-β signaling is redirected away from suppressing cell proliferation and is found instead to activate a cellular program, termed the epithelial-to-mesenchymal transition (EMT) (Hanahan and Weinberg, 2011). EMT is a physiological process contributing to folliculogenesis, ovarian surface epithelium plasticity, healing the wound after ovulation. However, the overexpression of TGF-β and disregulation of EMT cause abnormal granulosa cell growth and differentiation affecting folliculogenesis (Bilyk *et al.*, 2017). These physiological mechanisms could explain the correlation between the tumor grade and the fertility preservation outcome observed in the current study.

To the best of our knowledge, this was the first clinical study evaluating the effects of stage and grade of malignancy on the fertility preservation outcome in women with cancer.

In our study different malignancy types were included in the study groups, which can be a limitation. Since the largest cancer types

included only 52 cases for breast cancer and 50 cases for lymphomas, the separate analysis of specific cancer type was not conducted. Focusing on the specific cancer type was also problematic, as previous studies (Das *et al.*, 2011; Almog *et al.*, 2012; Alvarez and Ramanathan, 2018) demonstrated conflicting results of the association between cancer type and impact on the ovarian function. Since malignant diseases of different location may cause multiple universal effects on host's homeostasis (Friedl and Alexander, 2011; Sansing *et al.*, 2011; Jiang *et al.*, 2015), we decided to evaluate the cohort of all oncological patients and effect of cancer as a systemic disease. Even in cases of brain tumors the systemic effect cannot be excluded. Cytokines cross the blood-brain barrier (BBB) using specific transport systems as demonstrated in previous radio-imaging studies (Konsman *et al.*, 2004). Prostaglandins released in response to cytokine activation can alter BBB permeability leading to increased cytokine movement and leukocyte migration across the BBB (Saligan and Kim, 2012).

Regardless of cancer type, the allocation of patients to study groups was based on clear difference between local (Stage I and II) and extended (lymph node involvement, Stage III and IV) disease in stage-based analysis and well to moderate versus poorly differentiated malignancies in the grade-based analysis. Moreover the distribution of different cancer types was similar between the study groups.

Additional limitation of our study was its retrospective design. Nevertheless, we used strict inclusion and exclusion criteria reducing the possible impact of various treatment protocols and techniques. Since only 18 embryo transfers were performed to 10 out of 147 study patients and five clinical pregnancies were achieved, we could not evaluate the pregnancy outcome.

The absolute difference between the medians of MII oocytes at retrieval in low- and high-grade groups was three (11 (7–15) versus 8 (5–11); $P = 0.002$). However, the importance of this difference is that the high-grade group patients had 27% less mature oocytes, which can have a significant clinical impact. For total number of retrieved and vitrified oocytes this difference is 21% and 17%, respectively.

We conclude that the grade of malignancy has a significant impact on the number of retrieved mature oocytes and vitrified oocytes as well as on the total number of cryopreserved embryos during the fertility preservation treatment. We propose that the grade and stage of malignancy should be taken into account in the clinical evaluation and consultation of females with malignancy before fertility preservation treatment. According to our results the decision to start stimulation with relatively higher doses of gonadotropins in patients with more aggressive cancer can be considered. Further studies are needed to determine the physiological mechanisms underlying the systemic effect of cancer on ovarian function as well as on the pregnancy outcome.

Supplementary data

Supplementary data are available at *Human Reproduction* online.

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Authors' roles

A.V-P.'s role included study design, data collection, statistical analysis and manuscript writing. Y.C. was involved in data collection and statistical analysis. S.A.'s role was data collection. W-Y.S. performed the primary data collection. T.T., W.B., M.D. were directly involved in the clinical management of the patients. E.S.'s role included statistical analysis and manuscript review. M.H.D. was involved in study design and manuscript writing. T.T. performed data analysis and manuscript writing and review. W.B.'s role included study design, manuscript writing and review.

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Conflict of interest

None declared.

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