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ORIGINAL ARTICLE *Infertility*

Efficacy of ovarian tissue cryopreservation for fertility preservation: lessons learned from 545 cases

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STUDY QUESTION: How effective is ovarian tissue cryopreservation (OTC)?

SUMMARY ANSWER: In our cohort of patients who underwent OTC, premature ovarian failure (POF) rates, return rates and pregnancy rates after autotransplantation were 31.5, 4.4 and 33%, respectively.

WHAT IS KNOWN ALREADY: OTC for fertility purposes has been performed for >20 years now. With over 86 live births reported worldwide and success rates of ~30% after autotransplantation of frozen-thawed ovarian cortex, the procedure should no longer be considered experimental. However, very few publications report the efficacy of this procedure.

STUDY DESIGN, SIZE, DURATION: Cases of ovarian tissue cryobanking for fertility preservation performed between 1997 and 2013 in a single institution were reviewed by analysis of the cryobank database and a prospective questionnaire sent out in March 2015.

PARTICIPANTS/MATERIALS, SETTING, METHODS: There were 545 patients who underwent OTC during this period. The analysis included indications for OTC, survival rates, ovarian function and spontaneous pregnancies after OTC, come-back rates for ovarian tissue transplantation, pregnancy rates after transplantation, and complication and satisfaction rates.

MAIN RESULTS AND THE ROLE OF CHANCE: OTC was performed in this cohort at a mean age of 22.3 ± 8.8 years for oncological indications (79%), benign gynecological pathologies (17.5%) and genetic risks of POF (3.5%). Of the 545 patients, 29% were under 18 years of age at the time of OTC and 15% were prepubertal. While 10% of patients died from their disease, 21 patients (3.9%) underwent autotransplantation, 7 of whom delivered a healthy baby, yielding a post-transplantation live birth rate of 33%. Of 451 patients who were sent the questionnaire, 143 agreed to respond (32%). Nevertheless, ovarian function could not be evaluated in 36% of those who answered. Of 92 evaluable patients, 31.5% were menopausal and 68.5% showed persistent ovarian function. Of 52 women who attempted to conceive naturally, 37 were successful (71%). Among 140 patients who answered the questionnaire, 96% were satisfied with the procedure and only 1 major complication (intra-abdominal hemorrhage) was encountered. Among all the patients, 12% have donated their ovarian cortex for research purposes or have had it destroyed.

LIMITATIONS, REASONS FOR CAUTION: The questionnaire participation rate (32%), limited follow-up (mean 7.6 ± 3.5 years) and use of only clinical criteria for evaluation of ovarian function made it difficult to accurately assess the risk of POF and efficiency of OTC.

WIDER IMPLICATIONS OF THE FINDINGS: Our findings confirm a 30% pregnancy rate after ovarian cortex autotransplantation but also stress the difficulties of evaluating the real efficacy of OTC.

STUDY FUNDING/COMPETING INTEREST(S): No funding was sought for this study and none of the authors have any conflict of interest.

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Key words: fertility preservation / ovarian cortex cryopreservation / ovarian cortex autotransplantation / ovarian function / satisfaction rates / efficacy

Introduction

Ovarian tissue cryopreservation (OTC) for fertility purposes has been performed for >20 years. Since the first report of a live birth after thawing and autotransplantation of ovarian cortex in 2004 (Donnez *et al.*, 2004), followed by a second a year later (Meirow *et al.*, 2005), the procedure has gained ground and is now accepted and performed all over the world (Donnez and Dolmans, 2015; Donnez *et al.*, 2015; Jensen *et al.*, 2015; Meirow *et al.*, 2016; Van der Ven *et al.*, 2016). With >86 live births reported to date (Donnez and Dolmans, 2015; Jensen *et al.*, 2015, 2016; Van der Ven *et al.*, 2016) and success rates ~30% after autotransplantation of frozen-thawed ovarian cortex (Donnez *et al.*, 2013, 2015; Dittrich *et al.*, 2015; Jensen *et al.*, 2015; Meirow *et al.*, 2016; Van der Ven *et al.*, 2016), we believe that this procedure should no longer be considered experimental. However, very few publications report its effectiveness (Schmidt *et al.*, 2013; Imbert *et al.*, 2014; Wallace *et al.*, 2014; Jensen *et al.*, 2015; Lotz *et al.*, 2016; Van der Ven *et al.*, 2016). We therefore set out to review our cohort of 545 patients who underwent OTC between April 1997 and December 2013. The main objective was to try to determine for which patients the procedure is truly beneficial by analyzing patient outcome in terms of survival rates, ovarian function after the procedure, demand for ovarian transplantation, patient satisfaction and intended use of their cryopreserved tissue, all in relation to specific indications for fertility preservation. Analysis of these outcomes is essential to identify patients who will most likely benefit from the procedure, and improve the efficacy of the technique.

Materials and Methods

Study population, OTC and grafting

The study population included all patients who underwent OTC for fertility preservation between April 1997 and December 2013 in our academic institution (Cliniques Universitaires Saint-Luc, Brussels) (n = 545). Our selection criteria for OTC, as well as freezing and transplantation protocols, have been previously described (Donnez *et al.*, 2008; Donnez and Dolmans, 2013). In brief, all patients at risk of premature ovarian failure (POF) due to chemo- and/or radiotherapy or other reasons were able to undergo the procedure unless the surgical risk was judged to be too high. We did not limit our indications only to patients at high risk of POF. Indeed, in our early analysis (Jadoul *et al.*, 2010), we observed that patients initially at low risk of POF could change risk category and become high risk due to treatment switches. Our upper age limit was 35 years, but we had no lower age limit. Ovarian cortex was harvested in most cases by laparoscopy. Laparotomy was only performed when harvesting of ovarian tissue was concomitant with removal of an abdominal mass. The slow-freezing procedure was used in all cases.

Data collection and survey

Our cryobank database was used to select patients and review cryopreservation data (Dolmans *et al.*, 2013a). In addition, a questionnaire was developed to assess a patient's ovarian function before and after OTC, complications related to the procedure and the patient satisfaction. Questions were related to the development of puberty in prepubertal patients, persistence of natural menstrual cycles in women of reproductive age, use of contraception or hormone replacement therapy, attempts to conceive, and occurrence and outcome of pregnancies. All questions had multiple-choice answers and a free-text option to add explanatory notes if necessary. Questionnaires were sent out in March 2015 to surviving patients in whom the procedure had been performed prior to January 2014 (n = 491), in order to exclude patients still undergoing treatment for their disease and to have at least 1 year of follow-up since the procedure. Patients had until 31 January 2016 to return the questionnaire. Informed consent was obtained from all patients participating in the questionnaire, or from a parent or legal guardian if the subject was under the age of 18.

Ethics approval

Approval was obtained from the ethics committee of the Cliniques Universitaires Saint-Luc (reference 2015/22JAN/023 -B40320153403) on 9 March 2015.

Statistical analysis

Wilcoxon–Mann–Whitney tests were used to compare mean age at OTC and at the time of questionnaire completion between responding and non-responding groups. Indications for cryopreservation in the different groups were compared using the chi-square test.

Results

Indications and age at OTC

Mean age at the time of OTC was 22.3 ± 8.8 years (range: 6 months–39 years). Of the 545 women, 29% (n = 157) were under 18 years of age at the time of the procedure (n = 157) and 15% were prepubertal (n = 80). Mean follow-up time in the surviving patients was 7.6 ± 3.5 years at the time questionnaires were sent. In 79% of cases, cryopreservation was performed in patients needing to undergo chemotherapy. The main indications were hematological pathologies (35%), with lymphomas in 23%, leukemia in 9% and benign hematologic pathologies requiring bone marrow transplantation in 3% of patients. Further indications were breast cancer (17%), sarcoma (9%), gynecological malignancies (6%), neurological malignancies (5%), gastrointestinal malignancies (3%) and systemic diseases requiring chemotherapy (2%). Benign and borderline ovarian pathologies accounted for 17.5% of cases, while genetic risks of POF, such as Turner syndrome, family history of early menopause or galactosemia, made up 3.5% (Table I). Non-malignant ovarian pathologies included 48 cases of borderline ovarian tumors, 12 recurrent bilateral endometriomas and 35 recurrent ovarian dermoid, serous or mucinous cysts, including 5 cases of ovarian torsion.

The number of annual procedures grew steadily from 1997 (3 procedures) to 2010 (68 procedures), with a large increase noted after 2004 (Fig. 1), but after 2010 the numbers fell again. Indeed, from 2010

Table I **Follow-up of patients undergoing OTC.**

	Whole cohort			Questionnaire study					
	n (%) patients	n (%) deaths	n autografts	n (%) patients who responded	n (%) patients with unknown ovarian function	n (%) evaluable patients	n (%) patients with POF	n (%) patients with clinical ovarian function	Spontaneous pregnancy rates
Hematological pathology	191 (35)	15 (8)	11	50 (35)	21 (42)	29 (58)	7 (24)	22 (76)	12/17 (71)
Lymphoma	127 (23)	6 (5)		36 (25)	15 (42)	21 (58)	3 (14)	18 (86)	11/15 (73)
Leukemia	50 (9)	9 (18)		13 (9)	5 (38)	8 (62)	4 (50)	4 (50)	1/2 (50)
Benign	14 (3)	0		1 (0.7)	1 (100)	0 (0)	0	0	
Breast cancer	94 (17)	5 (5)	1	22 (15)	5 (23)	17 (77)	6 (35)	11 (65)	8/13 (62)
Sarcoma	51 (9)	17 (33)		18 (12.6)	8 (44)	10 (56)	4 (40)	6 (60)	3/4 (75)
Gynecological malignancy	33 (6)	4 (12)	2	8 (5.6)	2 (25)	6 (75)	5 (83)	1 (17)	
Neurological malignancy	26 (5)	7 (27)	1	7 (5)	3 (43)	4 (57)	0	4 (100)	1/1 (100)
Gastrointestinal malignancy	16 (3)	5 (31)	1	2 (1.4)	1 (50)	1 (50)	1 (100)	0	
Systemic disease	11 (2)	0	2	4 (2.8)	1 (25)	3 (75)	0	3 (100)	0/1 (0)
Benign and borderline ovarian pathology	95 (17)	0	3	20 (14)	7 (35)	13 (65)	2 (15)	11 (85)	10/13 (77)
Genetic disease	19 (3)	0		9 (6.3)	3 (33)	6 (67)	3 (50)	3 (50)	3/3 (100)
Other	9 (2)	1 (11)		3 (2)	0	3 (100)	1 (33)	2 (67)	
Total	545 (100)	54 (10)	21 (3.9)	143 (100)	51 (35.7)	92 (64.3)	29 (31.5)	63 (68.5)	37/52 (71)

OTC, ovarian tissue cryopreservation; POF, premature ovarian failure.

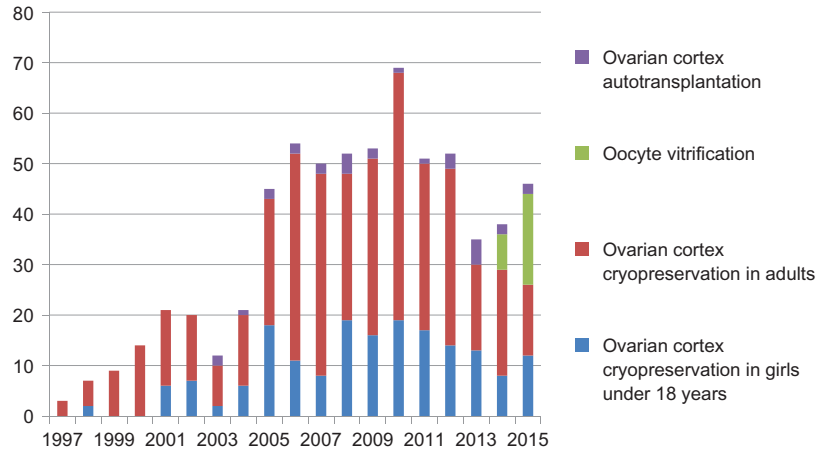

Figure 1 Numbers of patients undergoing ovarian tissue cryopreservation (OTC), oocyte vitrification and ovarian tissue transplantation between 1997 and 2015.

on, oocyte vitrification became available in other institutions in our country and some of our patients were referred to these centers when oocyte vitrification was considered a better option. In 2014, we started to perform oocyte vitrification in our institution. Figure 1 shows all cases of OTC, oocyte vitrification and ovarian cortex transplantation carried out since 1997.

Response to questionnaire

After excluding deceased patients ($n = 54$) and those with no further postal address ($n = 40$), 451 patients were sent the questionnaire. Of that number, 143 patients returned the questionnaire (31.7%), yielding a 6.8% margin of error for a 95% confidence level. There was no statistically significant difference in indications for OTC between the responding and non-responding groups ($P = 0.47$). Indications for cryopreservation in the responding group are presented in Table I. There were significant differences in age at the time of cryopreservation and at the time of questionnaire completion between responders and non-responders ($P = 0.03$ and 0.02, respectively). Indeed, both mean age at OTC (21.4 ± 8.5 years) and mean age upon questionnaire completion (29.5 ± 9.5 years) in the responding group were slightly lower than in the non-responding group (22.9 ± 9.0 years and 31.3 ± 10.1 years). Age and follow-up data on responders and non-responders are shown in Table II. Patients under 18 years of age at the time of OTC were significantly more inclined to respond than older patients ($P < 0.005$).

Deceased patients

Fifty-four patients had died from their disease since the procedure (9.9%) (Table I). The highest death rates were observed in women with sarcoma (33% of those with the disease), gastrointestinal malignancies (31%) and neurological malignancies (27%), and cervical malignancies (20%). Death rates were low in case of hematological pathologies (8%), breast cancer (5%), and borderline and malignant ovarian pathologies (3%). Among patients under 18 years of age at the time of OTC, 24 girls had died (13.5%), 23.5% of those with sarcoma, 22% of those with neurological malignancies and 13% of girls with hematological malignancies.

Ovarian cortex autotransplantation

Return rate

Between January 2003 and December 2015, 24 of the 545 patients (4.4%) came back to undergo autotransplantation of their ovarian cortex. Indications for OTC in these patients were hematological diseases ($n = 14$), benign ovarian pathologies ($n = 3$), gynecological malignancies ($n = 2$), two systemic conditions ($n = 2$), breast cancer ($n = 1$), rectal cancer ($n = 1$) and neurological cancer ($n = 1$). In three of these patients who had suffered from leukemia, we refused to reimplant tissue because of the possible risk of grafting leukemic cells.

Pregnancy rate

Seven of the 21 patients who underwent autografting of their ovarian cortex conceived after the transplantation (33%), one of whom delivered twice and another one delivered three times.

Ovarian function after OTC

Of the 143 responders, only 92 patients (64%) were evaluable by questionnaire in terms of clinical ovarian function. Of these 92 patients, 31.5% ($n = 29$) were menopausal and 68.5% ($n = 63$) had functional ovaries. Among the 51 patients who were not evaluable by questionnaire (prepubertal girls ($n = 4$), patients using hormone treatment ($n = 46$) and a woman presenting with pituitary insufficiency ($n = 1$)), 25 (49%) had proven ovarian function before starting hormone treatment, 11 having achieved pregnancy and 14 with regular menstruation before starting contraception. The remaining 26 had no proof of ovarian function, being either prepubertal or having used hormone treatment since the cryopreservation procedure.

Of the 29 menopausal patients, 5 underwent bilateral oophorectomy for ovarian pathologies and 3 underwent prophylactic oophorectomy for BRCA mutations, while 8 patients underwent bone marrow transplantation. Rates of menopause in evaluable patients were 50% in

Table II Characteristics of responders and non-responders to the questionnaire.

	Whole cohort ($n = 545$)	Questionnaire study ($n = 451$)		
		Responders ($n = 143$)	Non-responders ($n = 308$)	*P*-value
Age at OTC (years)	22.3 ± 8.8	21.4 ± 8.5	22.9 ± 9	0.03
Age at time of questionnaire (years)		29.5 ± 9.5	31.3 ± 10.1	0.02
Follow-up at time of questionnaire (years)	7.6 ± 3.5	7.7 ± 3.5	7.8 ± 3.6	
Patients aged under 18 years at time of OTC (%)	29	37	24	0.004

women with leukemia, 14% in case of other hematological diseases, 35% in women with breast cancer, with half of these cases attributed to bilateral oophorectomy and the other half to repeat chemotherapy for recurrence, 40% in sarcoma patients, 83% in case of gynecological malignancies and 50% in women with genetic pathologies (Table I). Menopause also occurred in one patient with nephroblastoma and one with gastrointestinal malignancy.

Of the 143 patients who responded to the questionnaire, 52 (36.4%) attempted to conceive naturally, 37 of whom succeeded (71%). Pregnancies were also obtained by oocyte donation ($n = 1$), transfer of embryos frozen before gonadotoxic therapy ($n = 2$) and after ovarian cortex autotransplantation ($n = 1$). The 37 natural pregnancies occurred in women in remission after leukemia ($n = 1$), breast cancer ($n = 9$), sarcoma ($n = 3$), lymphoma ($n = 11$), ovarian pathologies ($n = 9$), genetic diseases ($n = 3$) and neurological malignancy ($n = 1$) (Table I). It is worth mentioning that four spontaneous pregnancies were achieved in women who were menopausal and 11 pregnancies were in women who were non-evaluable at the time of the questionnaire (see above).

Complication rates

Five minor complications and one major adverse event were encountered among 140 patients. Reported complications classified as minor included raised temperature, labial hematoma, urinary infection, bowel irritation and psychological distress. One of the 140 patients had to undergo a second laparoscopy for intra-abdominal hemorrhage due to ovarian biopsy, which was considered a major complication. Hence, at least 96% of patients did not report any complications, and only one major complication was noted.

Satisfaction rates

Among the 140 patients who responded to the satisfaction questionnaire, 96% were satisfied with the procedure, while three (2%) were not. One woman stated she had not completely understood the purpose of the intervention, one found the intervention unnecessary, and the third was dissatisfied because of our refusal to perform ovarian tissue autotransplantation due to the risk of grafting leukemic cells. The patient who considered the procedure unnecessary had conceived spontaneously, but all of the other women who achieved pregnancy without needing to use their ovarian cortex did not regret undergoing the procedure. Three patients were not sure of what to think about the procedure.

Disposal of stored ovarian tissue

Of the 545 patients evaluated in the study, 66 (12%) donated their ovarian cortex for research purposes (83%), or had it destroyed (17%). Of these 66 women, 29 answered the questionnaire and are currently aged 39 ± 5.8 years. Of these 29 women, 21 had pregnancies and 19 have given birth.

Of 37 patients participating in the questionnaire who have already delivered or have ongoing pregnancies, 53% have withdrawn their ovarian cortex from storage, while 47% wish to keep their tissue cryopreserved.

Discussion

We report one of the largest series of OTC in a single institution, numbering 545 cases between 1997 and 2013. Our objective was to analyze the efficiency of the technique in different patient categories. On the one hand, we identified patients in whom the procedure is known to be justified, namely women who returned for autotransplantation and those with POF. On the other hand, we determined patients in whom for the moment, or possibly definitively, the procedure has proved of little value, namely patients who died, those who conceived without use of their frozen cortex, and those exhibiting persistent ovarian function.

Effectiveness and ineffectiveness of procedures

In our series, we can first conclude that the procedure was effective in the seven patients who delivered after autografting, representing a 33% delivery rate after ovarian autotransplantation. This is similar to figures obtained in other series (Dittrich *et al.*, 2015; Donnez and Dolmans, 2015; Jensen *et al.*, 2015; Meirow *et al.*, 2016; Van der Ven *et al.*, 2016). Out of 545 patients, 24 came back for autotransplantation and 21 underwent the procedure. This return rate of 4.4% and utilization rate of 3.9% are comparable to Lotz' series, where 5 out of 306 patients came back for reimplantation (Lotz *et al.*, 2016), and the 3 and 5% (74/2500 and 41/800) transplantation rates reported by Van der Ven *et al.* (2016) and Jensen *et al.* (2015). It is also consistent with available figures from oocyte vitrification programs. In Martinez's oocyte vitrification study, 11 out of 357 patients (3%) returned to use their oocytes after cancer treatment (Martinez *et al.*, 2014). When embryos are cryopreserved, patient return rates appear to be much higher (17–37%) (Courbiere *et al.*, 2013; Cardozo *et al.*, 2015;

Dolmans *et al.*, 2015), which is logical, as these patients already have a partner at the time of embryo cryopreservation, so might be more inclined to start a family as soon as possible. In case of male fertility restoration, utilization rates by cancer survivors who banked sperm prior to cancer treatment are <10% (Magelssen *et al.*, 2005; Pacey *et al.*, 2012). Because the mean age of our patients is still low (29.5 ± 9.5 years) demand for transplantation of ovarian cortex may well increase in the coming years; thus, a longer duration of follow-up would yield a more accurate picture.

The rather low return rate could indeed increase with time, as 31% of our evaluable patients in the questionnaire study presented with POF. Other follow-up studies of patients after OTC showed similar results. In Schmidt's questionnaire study, 22% of women were menopausal (Schmidt *et al.*, 2013). In Lotz' series involving 147 patients, 33% presented with amenorrhea, but this included 3 patients who had undergone hysterectomy and 17 taking anti-hormonal treatment (Lotz *et al.*, 2016). In Imbert's study investigating 225 patients, 30% were menopausal (Imbert *et al.*, 2014), while in Wallace's report, 43% of girls were menopausal (Wallace *et al.*, 2014). Smaller follow-up studies have shown rates of menopause to be between 0 and 57% (Anderson *et al.*, 2008; Desvignes *et al.*, 2014). The main causes of POF in our study were bilateral oophorectomy and bone marrow transplantation (each accounting for 28% of women with POF). Bilateral oophorectomy was also responsible for ~30% of POF in Imbert's study (Imbert *et al.*, 2014). In Desvignes' study (Desvignes *et al.*, 2014), all women affected by POF had undergone bone marrow transplantation, as had 55% of women with POF in Schmidt's study (Schmidt *et al.*, 2013). In Biasin's report, 93% of 47 women who underwent OTC before hematopoietic stem cell transplantation subsequently became menopausal after the stem cell transplantation (Biasin *et al.*, 2015). In case of breast cancer, our study confirms a low risk of POF in the absence of prophylactic bilateral oophorectomy (13%, comparable to 8 and 11% in Schmidt's and Imbert's studies, respectively) (Schmidt *et al.*, 2013; Imbert *et al.*, 2014). We can therefore conclude that besides bilateral oophorectomy, the risk of POF is highest in women undergoing bone marrow transplantation. Importantly, we also demonstrated that menopause rates are over 30% in women with sarcoma, gynecological malignancies, gastrointestinal malignancies and genetic diseases. According to both our and other studies, POF appears to occur in ~30% of patients.

For some patients, the procedure will prove to have been unnecessary to restore their fertility. First, a non-negligible number of our patients will achieve spontaneous pregnancy without use of their frozen ovarian cortex. In our questionnaire study, 71% of women who tried to conceive succeeded without the need for ovarian transplantation, oocyte donation or cryopreserved embryos. For these women, cryopreservation was clearly not really required for fertility preservation purposes, although POF still cannot be excluded. For this reason, 47% of these women have chosen to keep their ovarian cortex cryobanked for possible future use.

For the 10% of our patients who unfortunately died from their disease, the procedure may also be considered needless. Death rates were high in case of certain pathologies. Our study shows that at least one in four women with neurological malignancies, gastrointestinal cancer or sarcoma will not survive their disease. However, for the three out of four patients who will survive, it would be unethical to deny them OTC, as POF rates are especially high in individuals with sarcoma and gastrointestinal malignancies.

Other patients who will not use their banked ovarian tissue are those who donated their tissue for research or had it destroyed.

Other arguments in favor of OTC

Our review yields two further arguments in favor of OTC, namely low complication rates and high satisfaction rates. Indeed, 96% of patients were found to be satisfied with the procedure, even those who did not require reimplantation and donated their tissue for research or opted to have it destroyed. We believe that discussing and addressing the question of future fertility has a positive psychological impact on these women facing cancer and possible death. It allows them to picture themselves in the future bringing a sense of hope, which might be an explanation for the high satisfaction rates, even if the procedure later turns out to be fruitless. We only encountered one major complication when a second laparoscopy was required for intra-abdominal bleeding. In the literature, one death has been associated with the procedure (Imbert *et al.*, 2014), and Rosendahl reported a 3% rate of additional surgery needed to manage complications after OTC (Rosendahl *et al.*, 2008). It is therefore essential to conduct a thorough preoperative evaluation to exclude women at high risk from surgery and to remain mindful of the fact that our patients are already weakened by their oncological condition. Even if 96% of patients were satisfied with the procedure, our attention is inevitably drawn to the one individual who regrets the procedure, because ultimately she could not undergo autotransplantation due to the risk of reimplanting the initial pathology. It is clear that this risk of reintroducing the initial malignancy remains the major drawback of OTC (Meirow *et al.*, 2008; Abir *et al.*, 2010; Bastings *et al.*, 2013; Dolmans *et al.*, 2013b). Whenever this risk is present, and if time allows, oocyte or embryo cryopreservation should preferentially be performed. However, OTC remains the only possibility in children and when chemotherapy cannot be postponed. We believe that these patients should not be denied the opportunity of having their ovarian cortex cryopreserved, with the perspective of future in vitro maturation and advances in the development of an artificial ovary (Telfer *et al.*, 1990; Donnez and Dolmans, 2013; Telfer and Zelinski, 2013; Luyckx *et al.*, 2014; Shea *et al.*, 2014; Kniazeva *et al.*, 2015; Paulini *et al.*, 2016; Yin *et al.*, 2016), as long as they have been clearly informed.

Limitations of our and other studies

In our study, as in others, estimating the true incidence of POF is difficult. First, follow-up can be problematic. For 40 of our 491 surviving patients, we no longer had a valid address and only 32% agreed to participate in the questionnaire study. This response rate is much lower than in Schmidt's and Lotz' studies respectively, where 78 and 48% of patients responded (Schmidt *et al.*, 2013; Lotz *et al.*, 2016). We were surprised that only 1 of the 21 patients who underwent ovarian autotransplantation answered our questionnaire, while other non-transplanted patients followed in our gynecology unit did not respond. Patients seen on a regular basis might have assumed that their medical history is already known to us and may not have respond for that reason. Unfortunately, we could not use this information, as they had not given their informed consent for the study. Questionnaires were only six pages long and were sent with a personalized cover letter and postage-paid return envelope, which should have boosted response rates (Sahlqvist *et al.*, 2011). Sending the questionnaire a second time

or calling patients might have made a difference, but not necessarily. Indeed, even when we contacted patients by letter twice to invite them to renew their consent for cryopreservation, 25% did not answer. While the low response rate could potentially invalidate our results, our figures remain comparable to those of Schmidt and Lotz, where the response rates were considerably higher (Schmidt *et al.*, 2013; Lotz *et al.*, 2016).

Accurate evaluation of ovarian function is another issue. Like Schmidt *et al.* (2013) and Lotz et al. (2016), we also used clinical parameters to evaluate ovarian failure. Imbert used anti-Müllerian hormone (AMH) when available to assess ovarian function (Imbert *et al.*, 2014), but this was not the case in other studies. In our opinion, interpretation of AMH levels after gonadotoxic treatment remains challenging, as a number of pregnancies have occurred with undetectable AMH levels (Janse *et al.*, 2011; Hamy *et al.*, 2016). On the other hand, it is widely recognized that ovarian activity and spontaneous pregnancy may ensue even several years after ovarian failure has been established (Bath *et al.*, 2004). Third, in 36% of patients, prepubertal stage or use of hormone therapy made evaluation of ovarian function difficult or impossible. A fourth concern is the limited duration of follow-up. In our study, mean age at the time of evaluation was 29.5 years and the mean follow-up was 7.6 ± 3.5 years. The absence of clinical signs of POF at that age cannot rule out its occurrence in subsequent years. Indeed, when bilateral oophorectomy or bone marrow transplantation is performed, POF is usually immediate and hence easily diagnosed. In other pathologies, POF may be delayed, so an accurate diagnosis is more dependent on the duration of follow-up. Long-term follow-up is therefore essential to evaluate the true efficacy of OTC. For example, Wallace recommends OTC only in case of high risk of POF, and reports a 43% rate of POF in evaluable patients who underwent the procedure, and persistent ovarian function in >99% of girls who were not offered OTC (Wallace *et al.*, 2014). In that study, however, the median age at follow-up was only 16.9 years. Longer follow-up might considerably change the picture, and we may well see increased rates of POF in patients to whom OTC was not proposed.

A further limitation of our study is use of a questionnaire. It is difficult to know whether we can extrapolate our results from the questionnaire study to the complete cohort of women who underwent OTC. Indeed, we cannot exclude the possibility that women able to fulfill their wish to conceive are more inclined to participate in questionnaire studies. Moreover, we could only evaluate ovarian function based on self-reported clinical signs of ovarian activity. Although we did not observe any discrepancy between the patients' different answers, we cannot be sure of the accuracy of their responses. All these factors make it difficult to evaluate the real risk of POF in our cancer patients.

Conclusions

Based on our results and literature findings, return rates after OTC are currently low, estimated to be between 3 and 5% (Jensen *et al.*, 2015; Lotz *et al.*, 2016; Van der Ven *et al.*, 2016; present study). Nevertheless, at least 30% of women undergoing ovarian tissue autotransplantation will go on to give birth (Donnez *et al.*, 2013, 2015; Dittrich *et al.*, 2015; Jensen *et al.*, 2015; Meirow *et al.*, 2016; Van der Ven *et al.*, 2016). Despite, or maybe because of, the limitations encountered in our study, we feel now is not the time to modify the indications for cryopreservation. Although come-back rates are somewhat low, spontaneous pregnancy rates are high, and while a large proportion of women will not need their banked ovarian tissue, it is impossible to tell in advance. Moreover, the figures are comparable to those observed in women undergoing oocyte vitrification for oncological indications (Martinez *et al.*, 2014).

We have four key arguments why OTC should be offered to any woman at risk of POF. First, as reported by our team, even in women considered to be at low risk of POF at initial diagnosis, treatment can change and a significant number of patients will require more gonadotoxic treatment, which will alter their risk category (Jadoul *et al.*, 2010). Second, in our present investigation and other existing studies, follow-up was not long enough to evaluate the real risks of POF or return rates. These risks and rates may well be higher than currently estimated. Third, we observed a very high patient satisfaction rate. Indeed, 96% of patients were satisfied with the procedure, even those who did not require reimplantation and donated their tissue for research or had it destroyed. Last but not least, complication rates are very low. In order to extend our knowledge on the efficiency and value of OTC, we encourage authors to publish their long-term results on patients undergoing the procedure.

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Authors' roles

P.J. performed a significant number of cryopreservation and grafting procedures, designed the study, analyzed questionnaires and wrote the manuscript. A.G. sent, collected and analyzed questionnaires. J.S., M.L. and R.V. participated in cryopreservation procedures and reviewed the manuscript. C.W. is the director of the cryobank. M.M.D. is responsible for the ovarian tissue cryobank, played a significant part in cryopreservation and grafting procedures and reviewed the manuscript.

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Conflict of interest

None declared.

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