Oocyte vitrification versus ovarian cortex transplantation in fertility preservation for adult women undergoing gonadotoxic treatments: a prospective cohort study

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Objective: To compare the efficacy of oocyte vitrification (OV) with that of ovarian cortex cryopreservation and transplantation (OCT) in women undergoing gonadotoxic treatments.
Design: Prospective observational cohort study.
Setting: Not applicable.
Patient(s): Candidates for chemo-/radiotherapy who joined our fertility preservation (FP) program were included in this study between 2005 and 2015. One cohort included 1,024 patients undergoing OV; the other cohort included 800 patients undergoing OCT.
Intervention(s): OV using the cryotop device and OCT using a slow freezing protocol.
Main Outcome Measure(s): Live-birth rate (LBR) and clinical pregnancy rate (CPR).
Result(s): Basal antimüllerian hormone levels of the patients revealed no differences in ovarian reserve before FP (OV, 11.6 pM [5.4–24.7]; OCT, 11.8 pM [6.4–21.9]). In the OV cohort, 49 patients used the vitrified oocytes after a mean storage time of 3.9 years. In the OCT cohort, 44 sought pregnancy after a mean storage time of 5.5 years. A trend toward higher CPR and LBR (per patient) was observed in the OV group (risk ratio [RR_{CPR}], 1.31 [95% confidence interval, 0.90–1.92]; RR_{LBR} 1.39 [95% confidence interval, 0.95–2.03]), although differences were not statistically significant. In the OCT group, 46.7% of pregnancies occurred spontaneously and no pregnancy was achieved when the tissue was harvested beyond the age of 36 years. All patients except three undergoing OCT resumed or improved endocrine ovarian function.
Conclusion(s): Although we observed a trend toward higher LBR after OV, OCT is a very effective method to preserve fertility, allows for natural pregnancy, and restores ovarian function. In clinical scenarios where OV is not feasible, OCT remains the FP technique of choice and should no longer be considered experimental. (Fertil Steril® 2018;109:478–85. ©2017 by American Society for Reproductive Medicine.)
Key Words: Oocyte vitrification, ovarian cortex cryopreservation and transplantation, fertility preservation

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Received July 13, 2017; revised November 14, 2017; accepted November 15, 2017; published online February 7, 2018.
C.D.-G. has nothing to disclose. J.D. has nothing to disclose. J.A.G.-V. has nothing to disclose. S.H. has nothing to disclose. V.M. has nothing to disclose. I.I. has nothing to disclose. A.C. has nothing to disclose. J.R. has nothing to disclose. A.P. has nothing to disclose.
C.D.-G. and J.D. should be considered similar in author order.
This study was partially funded by a PROMETEOII/2014/045 grant of the Regional Valencian Ministry of Education. C.D.-G. is partially supported by a FIS-P16/01664 grant from the Spanish Ministry of Economy and Competitiveness.
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Fertility and Sterility® Vol. 109, No. 3, March 2018 0015-0282/$36.00

https://doi.org/10.1016/j.fertnstert.2017.11.018

Fertility preservation (FP) procedures are increasingly requested because of higher cancer survival rates associated with oncological treatments, making FP an integral component of the holistic management of oncologic patients (1–4). However, close to 30% of women undergoing gonadotoxic treatments are not properly informed of FP options (5). Counseling should be individualized based on the risk of gonadal failure, depending upon multiple factors including the prognosis of the patient, her age and ovarian reserve, the level of gonadotoxicity linked to specific drugs and regimens, and the amount of time available before the start of chemotherapy, radiotherapy, or surgery (2).

Currently, embryo and oocyte vitrification (OV) are the established FP methods (1–4). Embryo vitrification requires a male counterpart and permanently links further fertility to that same partner. OV, however, postpones the contribution of a male counterpart and is a reproducible, safe, and effective technique (6, 7). Live-birth rates (LBRs) as a result of OV depend on the number of available mature oocytes vitrified; this number will, in turn, depend on the number of ovarian stimulation cycles done before the gonadotoxic treatment is administered, as well as the age of the patient at the time of vitrification, which may affect survival rates after warming, embryo development, and LBRs (7).

Ovarian cortex cryopreservation and transplantation (OCT) is considered an experimental technique (1, 3), mainly due to a lack of evidence regarding its efficiency and the risk of reintroduction of malignant cells. Despite an increasing number of successful reports of OCT procedures (8–13), with more than 75 live births reported so far, there is a paucity of data concerning retransplantations. OCT is often considered as the first option for FP when there is not enough time to complete ovarian stimulation for OV or in prepubertal patients (2). Orthotopic OCT can restore ovarian function and allow natural fertility, with unlimited oocyte retrievals, as long as the graft is active.

We sought to compare the results of OCT with that of OV in two large cohorts of patients to establish the efficacy of the latter as compared with that of the former. In 2005, we implemented an FP program for oncology patients. Due to national regulations, OCT could only be offered in tertiary hospitals, while OV could be performed in both hospitals and fertility clinics. For this reason, all patients undergoing OCT were centralized in one institution (La Fe University Hospital), while the patients undergoing OV were referred to any of the institutions participating in our network (La Fe University Hospital-IVF Unit or IVI clinics). To date, the number of FP procedures performed in our centers accounts for more than 57% of the OV procedures and more than 72% of the OCT done for medical reasons in Spain, according to the registry of the Spanish Fertility Society (www.registrosef.com).

MATERIAL AND METHODS

Study Design and Study Population

Female patients with medical conditions requiring gonadotoxic treatments were sent to one of the program's institutions (La Fe University Hospital, n = 1,150; or IVI clinics, n = 895) between January 1, 2005 and December 31, 2015. Our FP program grants access to FP techniques without any economic cost to the patients and covers the same population nationwide. All referred patients were counseled according to the same management algorithm (Supplemental Fig. 1). Patients undergoing pelvic radiotherapy, whole-body irradiation, or chemotherapy including alkylating agents were in general proposed to undergo FP, although the risk of infertility was adjusted on individual parameters described as predictors of ovarian failure in the literature: age, ovarian reserve markers (antral follicle count and antimüllerian hormone [AMH]), and treatment protocol. Patients postponing maternity for medical reasons beyond the age of 38 were also included (for example, patients needing long-term hormonotherapy after breast cancer, even if they did not receive any type of chemotherapy). Patients were included in the study if they had 5–10 year survival rates over 50% and if they underwent OCT or OV. Patients older than 40 years, undergoing both OCT and OV, undergoing embryo vitrification, or not undergoing any of the techniques were not included in the study. Patients requiring OCT were redirected to La Fe University Hospital, and patients requiring OV were treated either in La Fe University Hospital or in IVI clinics. Two observational cohorts were defined: the OV cohort included patients undergoing ovarian stimulation for OV, and the OCT cohort included patients undergoing ovarian cortex retrieval and cryopreservation. Patients wishing to conceive and considered to be disease free with no formal contraindication for pregnancy were offered the use of their cryopreserved oocytes or tissue if they presented with amenorrhea, had a premature ovarian insufficiency (POI) defined according to the European Society of Human Reproduction and Embryology criteria (14), were over 40 years of age, or had undergone assisted reproductive technology treatments without success.

Variables regarding the demographic characteristics of the patients, the diseases motivating the FP, and the parameters of ovarian stimulation and ovarian cortex retrieval were prospectively collected using the SIVIS and DONAGEST databases. The ovarian reserve of the patients before FP was quantified using AMH determinations (Beckman Coulter AMH Gen II ELISA and automated chemiluminescence AMH assay on Roche Modular E170 analyzer from 2014).

Ethical Approval

This study was approved by the Institutional Review Board of La Fe University Hospital (2011/0018) and IVI group (1505-VLC-033-AC), and informed consent was obtained from the patients.

Oocyte Vitrification

Our protocols for controlled ovarian stimulation (COS), OV and warming, endometrial preparation, and luteal phase support have been described in detail elsewhere (15) and are available as Supplemental Material.

Ovarian Cortex Transplantation

Ovarian tissue was retrieved laparoscopically unless contraindications existed for general anesthesia. In such cases, the tissue was obtained by minilaparotomy under spinal anesthesia.

The collected tissue was fragmented, and one of the fragments was sent for histological/reverse transcriptase–polymerase chain reaction (RT-PCR) examination to rule out the presence of malignant cells (16). The other tissue fragments were cryopreserved and stored in ethyl-vinyl-acetate bags (17). Detailed information about the ovarian cortex cryopreservation, thawing, and reimplantation procedures is described as Supplemental Material.

Study Outcomes

The primary outcome of our study was LBR defined as the number of births of live infants beyond viability (>24 weeks). Secondary outcomes included pregnancy rates diagnosed by serum β-hCG determination 15 days after ET and clinical pregnancy rates (CPRs) defined as a visible fetal pole with normal fetal heartbeat observed after 2 weeks of β-hCG determination. Implantation rates were calculated as the number of visible sacs 4 weeks after ET/number of embryos transferred. Other outcome variables related to the OV procedures included cancelation rates, duration of stimulation, total doses of FSH employed, numbers of oocytes vitrified, oocyte survival rates, and fertilization rates. Other variables related to OCT procedures were the resumption of endocrine function defined as resumption of E_2 production and FSH normalization or restoration of menses; the duration of graft viability was defined as the time between OCT and cessation of E_2 production or amenorrhea > 1 year. The utilization rate (number of patients using their oocytes or ovarian cortex) was calculated in both groups as was the number of relapses/recurrences of disease after OV/OCT.

Statistical Methods

Data are expressed as mean (standard deviation) or median [interquartile range] if not normally distributed. Categorical variables are given as raw numbers (percentages). Patients undergoing OV were contrasted to those undergoing OCT. Percentages were compared using χ^2 and Fisher's tests; continuous variables were compared using the Student's t or Mann-Whitney tests, and $P<.05$ was considered statistically significant. Risk estimates were calculated as risk ratios (RR) with 95% confidence intervals (CI). Suggestive associations were identified if the CI did not include 1. Sensitivity analyses for pregnancy and LBRs were carried out by stratification based on age. All calculations were computed using SPSS software (IBM SPSS Statistics 20.0.0 SPSS).

RESULTS

Fertility Preservation Procedures

Starting in 2005, 2,045 patients were evaluated for FP. Among them, 1,024 patients underwent OV, and 800 underwent OCT. The number of cortex retrievals performed by laparoscopy were 738; 32 cases were done by minilaparotomy due to mediastinal bulky contraindicating general anesthesia, and in 30 cases the underlying disease required laparotomy, so the retrieval was done during the same procedure. The flow of included and excluded patients is shown in Figure 1. The median follow-up was 4.89 [0.39–10.97] years; baseline characteristics of the patients participating in the study are shown in Table 1. Patients undergoing OCT were younger (OV, 31.7 ± 6.4 years vs. OCT, 28.2 ± 7.3 years; $P<.001$), although the ovarian reserve evaluated by AMH did not differ between groups (OV, 15 [7.5–25] pM vs. OCT, 11.4 [6–21] pM). The most prevalent pathologies were breast cancer (OV, 60.3%; OCT, 53.9%) and Hodgkin lymphoma (OV, 14.2%; OCT, 19.9%).

The number of overall FP procedures significantly increased between 2005 and 2014 (P trend < .001) and then reached a plateau (Supplemental Fig. 2). The number of OV procedures increased between 16% and 38% per year during this period, and the number of OCT procedures increased until 2012 and then experienced a 15%–25% reduction per year until 2015.

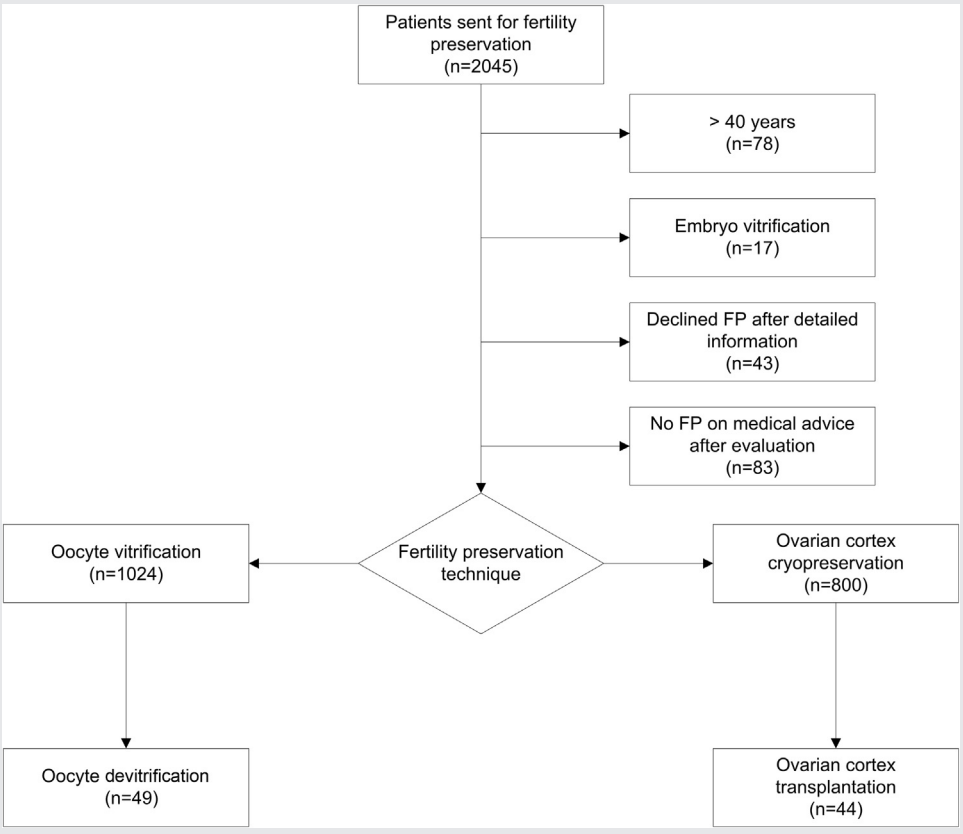
In the OV cohort, 1,024 ovarian stimulation cycles for FP were performed, and in the OCT cohort, 800 ovarian cortex retrieval procedures were completed. The results of the ovarian stimulation and the number of oocytes vitrified are shown in Supplemental Table 1. The time interval between the first visit and the date of the end of the FP procedure was shorter in the OCT group (OV, 24.0 ± 6.2 days vs. OCT, 4.5 ± 4.1 days; $P<.001$). Seven patients underwent OCT the same day of the first visit: two patients with Burkitt lymphoma, who started chemotherapy the same day at the ICU; two patients with Hodgkin lymphoma and mediastinal bulky compressing the airways; and three patients with locally advanced breast cancer.

Use of the Cryopreserved Oocytes/Ovarian Tissue

Forty-nine patients came back to use vitrified oocytes (return rate of 4.8%), and 50 patients came back to have their tissue reimplanted (return rate of 6.2%): six for endocrine purposes and 44 seeking pregnancy. Tables 2 and 3 show the characteristics of the patients at the time of use of the cryopreserved material and the fertility outcomes. There were no patients lost to follow-up.

In the OV group, thawing of the oocytes occurred after a mean storage time of 3.9 years. Twenty-eight positive pregnancy tests were obtained after 51 fresh ET cycles and 17 frozen ETs, eight of which were biochemical pregnancies and four were clinical miscarriages (8.2%; Table 3). In the OCT cohort, patients came back to use the tissue after a mean storage time of 5.5 years; 41 out of 44 patients (93.2%) resumed menstruation or improved their menopausal symptoms. Among the three patients who did not show ovarian function, one was 35 years old and had a Hodgkin lymphoma and previous infertility; another was 35 years old and had breast cancer; and the last one was only 26 years old and had Hodgkin lymphoma, although she underwent four cycles of adriamycin, bleomycin vinblastine, and dacarbazine and two cycles of etoposide, solumedrol, high-dose cytarabine, and platinum (ESHAP) before ovarian cortex retrieval. This patient underwent another OCT, but ovarian function did not resume. A lack of ovarian follicles was evidenced during the histological examination of the ovarian cortex of the three patients. The mean time to ovarian function resumption was 94.3 (61.1) days. Seven spontaneous

FIGURE 1



Patients flowchart.

Diaz-Garcia. Fertility after oocyte vitrification and ovarian transplantation. Fertil Steril 2017.

pregnancies and eight pregnancies after IVF were observed (46.7% natural conception), yielding an overall CPR and LBR per patient of 27.3% and 18.2%, respectively (Tables 2 and 3). One patient delivered twice, another patient delivered once and had two miscarriages, and a third patient delivered once and had one miscarriage. When CPR and LBR (per patient) were compared between groups, the CRP and LBR appeared higher in the OV group (RR_{CPR}, 1.31 [95% CI 0.90–1.92]; RR_{LBR}, 1.39 [95% CI 0.95–2.03]), although the differences were not statistically significant (Table 3). A sensitivity analysis revealed that no pregnancy occurred in the OCT group when the ovarian tissue was harvested beyond 36 years old, while six of 20 (30%) of the patients who achieved pregnancy in the OV group had vitrified their oocytes beyond that age (Supplemental Table 2). No live births occurred in the OCT group beyond the age of 36, while six of 16 (37.5%) of the patients who delivered in the OV group were older than 36.

One patient diagnosed with Hodgkin lymphoma relapsed after using her cyropreserved oocytes and delivering twice. One patient treated for triple-negative, BRCA-2-positive, bilateral breast cancer who underwent OCT 2 years after diagnosis experienced bone metastasis 1 year after OCT. One year after diagnosis of the metastasis there is no progression of the disease and no evidence of malignancy has been found in the ovarian site. Both patients are alive.

DISCUSSION

To the best of our knowledge, these are the largest prospective cohorts that have been used to compare the results of OCT and OV in a controlled study, evaluating the current state of the art in FP in adult women desiring pregnancy and analyzing the efficacy of an established technique (OV) compared with that of an experimental one (OCT).

The overall LBR in the OV cohort was 32.6%. When we compared our results in OV patients under 36 years old (to exclude the effect of age on egg quality) with those from an external cohort of patients of similar characteristics undergoing FP for social reasons in our clinics (7), we found lower LBR in our cohort of patients preserving for medical reasons (OV-medical LBR, 28.6% vs. OV-social LBR, 50.0%; $P<.001$). These data support the hypothesis that cancer in young women could affect oocyte quality even in the absence of any gonadotoxic treatment (18).

LBRs after OCT in our series (18.2%) were similar to those described by other large programs (16/74, 21.6% [12]; 6/20, 30% [19]; 6/25, 24% [20]; 5/19, 26.3% [21]). It is of note

TABLE 1

Baseline characteristics of the patients.

Characteristic	OV (n = 1,024)	OCT (n = 800)	*P* value
Age, y	31.7 (6.4)	28.2 (7.3)	< .001
BMI, kg/m^2	22.5 (3.6)	21.8 (3.5)	NS
AMH, pM	11.6 (5.4–24.7)	11.8 (6.4–21.9)	NS
Nulliparous	952 (89.8)	722 (90.2)	NS
Duration of the FP procedure, d[a]	24.0 (6.2)	4.5 (4.1)	< .001
Conditions motivating FP			
Breast	618 (60.3)	431 (53.9)	< .001
Hodgkin lymphoma	145 (14.2)	159 (19.9)	
Non-Hodgkin lymphoma	61 (6.0)	24 (3.0)	
Gynecological	44 (4.3)	25 (3.1)	
Sarcoma	16 (1.6)	52 (6.5)	
Leukemia	12 (1.2)	54 (6.7)	
Autoimmune disease	8 (0.8)	20 (2.5)	
Other solid organ tumors	120 (11.7)[b]	35 (4.4)[c]	

Note: Values of quantitative variables are shown as mean (standard deviation), median (interquartile range). AMH = antimüllerian hormone; BMI = body mass index; FP = fertility preservation; NS = not significant; OCT = ovarian cortex cryopreservation and transplantation; OV = oocyte vitrification.
[a] From day of first visit until discharge of the patient.
[b] Includes the following cancers: 49 digestive tract, 43 thyroid, 21 central nervous system, three nose-ear-throat, four desmoids (various locations).
[c] Includes the following locations: 13 digestive tract, 12 central nervous system, eight thyroid, two lung, one suprarenal.

Diaz-Garcia. Fertility after oocyte vitrification and ovarian transplantation. Fertil Steril 2017.

that miscarriages occurred in two out of seven patients (28.6%) after spontaneous conception and in three out of eight patients after IVF (37.5%). Previous studies suggest that both cryopreservation and retransplantation of the ovarian tissue can cause dysfunctional folliculogenesis and asynchrony between the oocyte and the granulosa cells and alter oocyte morphology (22). All these events would subsequently result in decreased rates of oocyte maturity, lower fertilization rate, and poorer embryo quality.

Despite the fact that OV showed a trend toward higher CPR and LBR, it should be highlighted that OCT allowed for natural conception in almost half of the patients as well as the restoration of ovarian function in 93.2% of patients after 3 months. In patients who got spontaneously pregnant, it is virtually impossible to be certain that the oocyte did not come from the remaining native ovary. Nevertheless, taking into account the above-mentioned ovarian function restoration rate and also the much higher pregnancy rates compared with a native POI population, it is very likely that the OCT played a role. The three patients who did not resume the endocrine function showed characteristics that could be pointed to as bad prognosis markers in terms of ovarian function: advanced age, previous chemotherapy, and previous infertility. All three had a complete absence of follicles at pathological examination of the cortex, although it has to be pointed out that the validity of an ovarian biopsy to predict the ovarian reserve remains limited (23).

We hypothesize that the differences in CPR and LBR can be explained by the following: [1] the broader selection criteria for OCT at the beginning of the FP program, without restrictions based on age or ovarian reserve (this is consistent with the absence of viable pregnancy when tissue was harvested after the age of 36). In 2013 our patient management algorithm changed, and patients over 35 years were only offered OV if there was proof of a good ovarian reserve. While some groups advocate the broadening of the criteria necessary to perform OCT (19), we advocate keeping them strict so as to improve efficacy, especially in cases of advanced maternal age. The low success rate of OCT in older patients has been reported by other groups and is also supported by our study (12, 19–21). [2] Restrictions in the follow-up: in contrast to OV, OCT patients have potentially unlimited spontaneous cycles while the orthotopic graft is active; the median life span of the grafts was 98 months in patients who ceased ovarian function, and 37 of them are still active. This allowed for spontaneous pregnancies in seven out of 15 patients. It has to be kept in mind that, given the broad CI of our estimations and the fact that our cohort continues growing every day, our data are not definitive and they should be confirmed in further studies including more patients.

We observed a trend toward fewer OCT procedures and more OV procedures. There are different reasons that could explain such a tendency: [1] the implementation of the Cryotop OV method in our programs in 2008 and 2012 matches with an increase in the number of OV procedures at those time points; [2] as the FP program progressed, patients were referred more quickly, and, therefore, longer times allowing for ovarian stimulation were available; [3] there is an increasing body of evidence suggesting that COS for OV in patients with hormone-dependent malignancies is a safe option (24, 25); [4] on an equal basis, both patients and physicians preferred the easier technique and to avoid a surgical procedure.

The safety of OCT is under evaluation since it is still considered an experimental technique. The presence of ovarian metastasis in the initial stages of breast cancer should be regarded as an exceptional event (26). Leukemia, neuroblastoma, and Burkitt lymphoma are cancers with high risk of ovarian metastasis, but moderate risk has also been described in breast cancer stage IV, colon, and cervix cancer, Ewing sarcoma, and some non-Hodgkin lymphoma (27). Despite this risk of malignant cell contamination, histology

TABLE 2

Fertility results after use of vitrified oocytes or cryopreserved ovarian tissue.

	Breast			Hodgkin lymphoma			Non-Hodgkin lymphoma			Other conditions			All		
Variable	OV (n = 38)	OCT (n = 31)	*P*	OV (n = 2)	OCT (n = 9)	*P*	OV (n = 3)	OCT (n = 0)	*P*	OV (n = 6)[a]	OCT (n = 4)[b]	*P*	OV (n = 49)	OCT (n = 44)	*P*
Status of patient at reimplantation			NS			NS		–	–			NS			.04
Amenorrhea >1 y	5 (13.2)	11 (35.5)		1 (50.0)	7 (77.8)		1 (33.3)			2 (33.3)	2 (50.0)		9 (18.4)	20 (45.4)	
POI without amenorrhea	30 (78.9)	19 (61.3)		0 (0)	2 (22.2)		2 (66.7)			2 (33.3)	0 (0)		34 (69.4)	21 (47.8)	
Regular menstruations	3 (7.9)	1 (3.2)		1 (50.0)	0 (0)		0 (0)			2 (33.3)	2 (50.0)		6 (12.3)	3 (6.8)	
Age at retrieval, y	35.5 (3.1)	35.8 (3.3)	NS	32.5 (4.9)	27.1 (3.7)	NS	34.5 (2.1)	–	–	34.2 (3.7)	29.5 (0.2)	NS	35.2 (3.1)	34.3 (7.2)	NS
Age at reimplantation, y	40.0 (3.3)	41.0 (2.4)	NS	33.5 (3.5)	33.8 (3.1)	NS	39.0 (1.0)	–	–	37.0 (4.2)	32.9 (1.7)	NS	39.0 (3.8)	38.9 (4.1)	NS
AMH before reimplantation, pM	0 [0–1.33]	0 [0–0]	NS	2.1 [0–4.2]	0 [0–1.00]	NS	0 [0-0]	–	–	0 [0–1.26]	0.37 [0–1.47]	NS	0 [0–1.29]	0 [0–0.30]	NS
No. of pregnant patients	13 (34.2)	5 (16.1)	NS	1 (50.0)	5 (55.5)	NS	2 (66.7)	0	NS	4 (66.7)	2 (50.0)	NS	20 (40.8)	12 (27.3)	NS
No. of patients with live births	11 (28.9)	2 (6.4)	NS	1 (50.0)	4 (44.4)	NS	2 (66.7)	0	NS	2 (33.3)	2 (50.0)	NS	16 (32.6)	8 (18.2)	NS

Note: Values of quantitative variables are shown as mean (standard deviation) or median [interquartile range]; values of categorical variables are shown as n (%). AMH = antimüllerian hormone; BMI = body mass index; FP = fertility preservation; NS = not significant; OCT = ovarian cortex cryopreservation and transplantation; OV = oocyte vitrification; POI = premature ovarian insufficiency.

[a] Includes the following cancers: two endometrium, one rectum, one myeloma, one sarcoma, one acute myeloid leukemia.

[b] Includes the following cancers: two rectum, one medulloblastoma, one persistent trophoblastic disease.

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TABLE 3

Efficiency of oocyte vitrification and ovarian cortex cryopreservation in fertility preservation.

OV	Patients (n = 49)
Warmed oocyte/patient	5.1 (3.5)
Oocyte survival rate, %	77.3
No. of ET (fresh-frozen)	68
Surplus embryos/patient	2.7 (2.2)
Warmed embryo/patient	2.0 (1.7)
Embryo survival rate, %	91.7
No. of embryos transferred	1.42
CPR/fresh cycle (%)	14/51 (27.4)
LBR/fresh cycle (%)	11/51 (21.6)
CPR/transfer (%)	20/55 (36.4)
LBR/transfer (%)	16/55 (29.1)
No. of pregnancies	21 (42.9)
No. of live births	17 (34.7)
No. of pregnant patients	20 (40.8)
No. of patients with live births	16 (32.6)
OCT	**Patients, n = 44 (%)**
Surgical approach	
Laparoscopy	1 (2.3)
Laparotomy	41 (93.2)
Surgical technique/sites	
Subcortical pouches	24 (54.5)
Cortical microsurgical sutures	26 (59.1)
Subperitoneal pouches	27 (61.4)
Ovarian function after graft	43 (97.7)
CPR after spontaneous pregnancy	7 (15.9)
LBR after spontaneous pregnancy	5 (11.4)
No. of patients undergoing IVF	28
CPR after IVF	8 (18.2)
LBR after IVF	5 (11.4)

Note: Values of quantitative variables are shown as mean (standard deviation) and values of categorical variables are shown as n (%). CPR = clinical pregnancy rate; ET = embryo transfer; LBR = live-birth rate; OCT = ovarian cortex cryopreservation and transplantation; OV = oocyte vitrification.

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(26) and RT-PCR panels (16) are employed to rule out the presence of malignant cells in such scenarios. We found that the disease-free survival rate did not differ from that expected in patients of similar characteristics (28, 29).

Despite this being the first study to compare OV and OCT in two large cohorts, some limitations exist: [1] The utilization rate was close to 7% in both groups. Although this may seem to be a low utilization rate, it is consistent with that observed in male FP programs (30) and in other female FP programs for social (7) and medical (12, 19–21) reasons. In our series, breast cancer was the most prevalent disease and modern chemotherapy for breast cancer will rarely result in ovarian failure, except for the use of cyclophosphamide, methotrexate and fluorouracil regimes typically used for advanced disease. Most of the patients who came back to use their cryopreserved gametes/tissue did so in their late 30s, approximately 5 years after diagnosis. This is not surprising if we take into account that half of our population cryopreserved under the age of 30 and the mean age of a woman at the birth of a first child in Spain is 30.4 years, with 64.5% of first children born in women age 30 or more, according to Eurostat (http://ec.europa.eu/eurostat/statistics-explained/index.php/Fertility_statistics). [2] The limited number of patients who come back to use the cryopreserved material could limit the sensitivity of the statistical tests to detect differences. A power analysis was performed assuming an alpha error of 5%, and it revealed that our study reached a power of 64.3% to detect differences in LBR of at least 14% (the differences found in our study), but it reached up to 87.2% to detect differences in LBR of at least 20%. [3] The prepubertal girls are the main group of patients who could benefit from OCT since ovarian stimulation is not feasible in this population. Nevertheless, efficiency parameters could not be calculated for these patients because younger patients are not ready to become mothers and no reimplantation of ovarian tissue harvested during the prepubertal age has been done in our program. On the other hand, there are several cases in the literature reporting the use of this tissue and proving its effectiveness to induce puberty and to achieve motherhood (9, 31).

In summary, this study presents useful information to counsel patients and oncologists on the indications and real possibility of achieving motherhood after either FP approach in reproductive-age cancer patients. Despite the fact that comparisons between groups are inherently difficult given the number of patients who attempted pregnancy, our data suggest that both methods are effective in preserving fertility, offering real possibilities for future maternity. OCT allows for natural pregnancy and restores ovarian function. In clinical scenarios where OV is not feasible, OCT remains the FP technique of choice. Taking into account that data from other series support our findings and that our findings probably under estimates the potential LBRs in prepubertal populations given their higher follicular densities, we believe that OCT should no longer be considered as experimental when OV is not a feasible alternative.

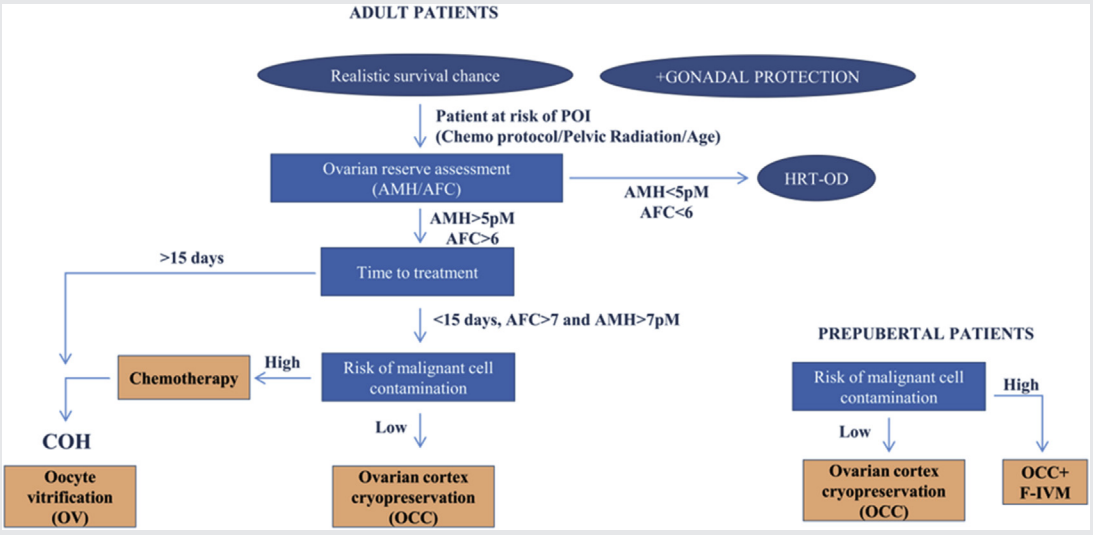
Acknowledgments: The authors thank all the medical staff, nurses and biologists who make the fertility preservation program possible, especially Dr. Mara Andrés, pediatric oncologist and coordinator of the fertility preservation prepubertal patients subprogram, for providing her data.

REFERENCES

1. Loren AW, Mangu PB, Beck LN, Brennan L, Magdalinski AJ, Partridge AH, et al. Fertility preservation for patients with cancer: American Society of Clinical Oncology clinical practice guideline update. J Clin Oncol 2013;31:2500–10.
2. Committee IP, Kim SS, Donnez J, Barri P, Pellicer A, Patrizio P, et al. Recommendations for fertility preservation in patients with lymphoma, leukemia, and breast cancer. J Assist Reprod Genet 2012;29:465–8.
3. Practice Committee of American Society for Reproductive M. Fertility preservation in patients undergoing gonadotoxic therapy or gonadectomy: a committee opinion. Fertil Steril 2013;100:1214–23.
4. Peccatori FA, Azim HA Jr, Orecchia R, Hoekstra HJ, Pavlidis N, Kesic V, et al. Cancer, pregnancy and fertility: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2013;24(Suppl 6):vi160–i170.
5. Letourneau JM, Ebbel EE, Katz PP, Katz A, Ai WZ, Chien AJ, et al. Pretreatment fertility counseling and fertility preservation improve quality of life in reproductive age women with cancer. Cancer 2012;118:1710–7.
6. Cobo A, Diaz C. Clinical application of oocyte vitrification: a systematic review and meta-analysis of randomized controlled trials. Fertil Steril 2011;96:277–85.

7. Cobo A, Garcia-Velasco JA, Coello A, Domingo J, Pellicer A, Remohi J. Oocyte vitrification as an efficient option for elective fertility preservation. Fertil Steril 2016;105:755–64.e8.
8. Donnez J, Dolmans MM, Pellicer A, Diaz-Garcia C, Ernst E, Macklon KT, et al. Fertility preservation for age-related fertility decline. Lancet 2015; 385:506–7.
9. Demeestere I, Simon P, Dedeken L, Moffa F, Tsepelidis S, Brachet C, et al. Live birth after autograft of ovarian tissue cryopreserved during childhood. Hum Reprod 2015;30:2107–9.
10. Donnez J, Dolmans MM. Ovarian cortex transplantation: 60 reported live births brings the success and worldwide expansion of the technique towards routine clinical practice. J Assist Reprod Genet 2015;32:1167–70.
11. Rodriguez-Wallberg KA, Tanbo T, Tinkanen H, Thurin-Kjellberg A, Nedstrand E, Kitlinski ML, et al. Ovarian tissue cryopreservation and transplantation among alternatives for fertility preservation in the Nordic countries—compilation of 20 years of multicenter experience. Acta Obstet Gynecol Scand 2016;95:1015–26.
12. Van der Ven H, Liebenthron J, Beckmann M, Toth B, Korell M, Krussel J, et al. Ninety-five orthotopic transplantations in 74 women of ovarian tissue after cytotoxic treatment in a fertility preservation network: tissue activity, pregnancy and delivery rates. Hum Reprod 2016;31:2031–41.
13. Stoop D, Silber S, Cobo A. Fertility preservation for age-related fertility decline—authors' reply. Lancet 2015;385:507–8.
14. European Society of Human Reproduction and Embryology Guideline Group on Premature Ovarian Insufficiency, Webber L, Davies M, Anderson R, Bartlett J, Braat D, et al. ESHRE Guideline: management of women with premature ovarian insufficiency. Hum Reprod 2016;31:926–37.
15. Garcia-Velasco JA, Domingo J, Cobo A, Martinez M, Carmona L, Pellicer A. Five years' experience using oocyte vitrification to preserve fertility for medical and nonmedical indications. Fertil Steril 2013;99:1994–9.
16. Rodriguez-Iglesias B, Novella-Maestre E, Herraiz S, Diaz-Garcia C, Pellicer N, Pellicer A. New methods to improve the safety assessment of cryopreserved ovarian tissue for fertility preservation in breast cancer patients. Fertil Steril 2015;104, 1493–502.e1–2.
17. Herraiz S, Novella-Maestre E, Rodriguez B, Diaz C, Sanchez-Serrano M, Mirabet V, et al. Improving ovarian tissue cryopreservation for oncologic patients: slow freezing versus vitrification, effect of different procedures and devices. Fertil Steril 2014;101:775–84.
18. van Dorp W, van den Heuvel-Eibrink MM, de Vries AC, Pluijm SM, Visser JA, Pieters R, et al. Decreased serum anti-Mullerian hormone levels in girls with newly diagnosed cancer. Hum Reprod 2014;29:337–42.
19. Meirow D, Ra'anani H, Shapira M, Brenghausen M, Derech Chaim S, Aviel-Ronen S, et al. Transplantations of frozen-thawed ovarian tissue demonstrate high reproductive performance and the need to revise restrictive criteria. Fertil Steril 2016;106:467–74.
20. Andersen CY. Success and challenges in fertility preservation after ovarian tissue grafting. Lancet 2015;385:1947–8.
21. Donnez J, Dolmans MM, Diaz C, Pellicer A. Ovarian cortex transplantation: time to move on from experimental studies to open clinical application. Fertil Steril 2015;104:1097–8.
22. Dolmans MM, Donnez J, Camboni A, Demylle D, Amorim C, Van Langendonckt A, et al. IVF outcome in patients with orthotopically transplanted ovarian tissue. Hum Reprod 2009;24:2778–87.
23. Kwok R, Johnson NP. Ovarian biopsy has no role as a routine diagnostic test of ovarian reserve: a systematic review. Reprod Biomed Online 2012;24:492–5.
24. Azim AA, Costantini-Ferrando M, Oktay K. Safety of fertility preservation by ovarian stimulation with letrozole and gonadotropins in patients with breast cancer: a prospective controlled study. J Clin Oncol 2008;26:2630–5.
25. Kim J, Turan V, Oktay K. Long-term safety of letrozole and gonadotropin stimulation for fertility preservation in women with breast cancer. J Clin Endocrinol Metab 2016;101:1364–71.
26. Sanchez-Serrano M, Novella-Maestre E, Rosello-Sastre E, Camarasa N, Teruel J, Pellicer A. Malignant cells are not found in ovarian cortex from breast cancer patients undergoing ovarian cortex cryopreservation. Hum Reprod 2009;24:2238–43.
27. Dolmans MM, Luyckx V, Donnez J, Andersen CY, Greve T. Risk of transferring malignant cells with transplanted frozen-thawed ovarian tissue. Fertil Steril 2013;99:1514–22.
28. Swisher SK, Vila J, Tucker SL, Bedrosian I, Shaitelman SF, Litton JK, et al. Locoregional control according to breast cancer subtype and response to neoadjuvant chemotherapy in breast cancer patients undergoing breast-conserving therapy. Ann Surg Oncol 2016;23:749–56.
29. Sieniawski M, Franklin J, Nogova L, Glossmann JP, Schober T, Nisters-Backes H, et al. Outcome of patients experiencing progression or relapse after primary treatment with two cycles of chemotherapy and radiotherapy for early-stage favorable Hodgkin's lymphoma. J Clin Oncol 2007;25:2000–5.
30. Meseguer M, Molina N, Garcia-Velasco JA, Remohi J, Pellicer A, Garrido N. Sperm cryopreservation in oncological patients: a 14-year follow-up study. Fertil Steril 2006;85:640–5.
31. Poirot C, Abirached F, Prades M, Coussieu C, Bernaudin F, Piver P. Induction of puberty by autograft of cryopreserved ovarian tissue. Lancet 2012; 379:588.

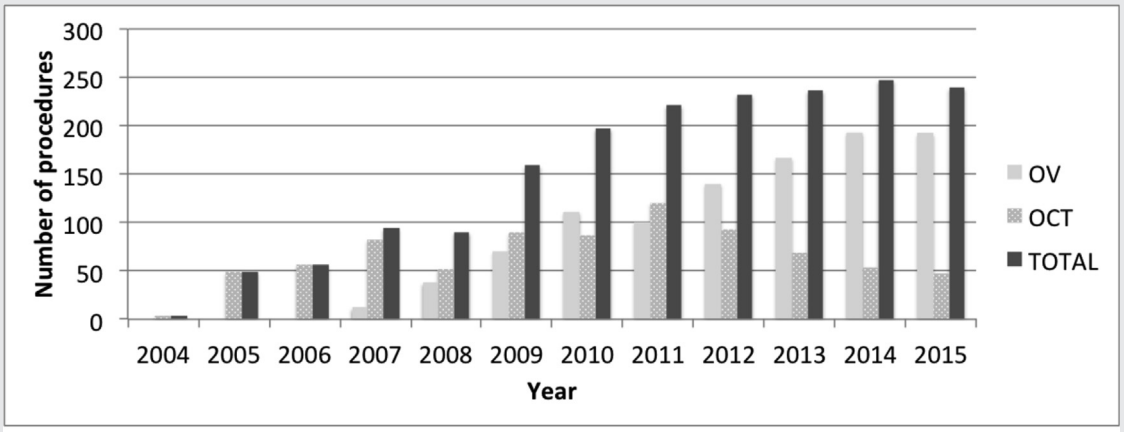
SUPPLEMENTAL FIGURE 1



Fertility preservation decision tree. Since ovarian stimulation requires 10 days on average (8), patients with more than 15 days before the beginning of gonadotoxic treatment were counseled to undergo either oocyte vitrification or ovarian cortex cryopreservation and transplantation. Patients with less than 15 days were offered or ovarian cortex cryopreservation and transplantation. Alternatively, if the risk of malignant cell contamination was high, defined by the presence of clinical signs suggesting ovarian involvement or in face of any type of leukemia/Burkitt lymphoma, patients were offered to undergo chemotherapy and COH after a reasonable wash-out period, if any follicular reserve remained. From 2013 onward, patients over 35 year old with an antral follicle count fewer than seven or antimüllerian hormone levels less than 7 pM were only offered oocyte vitrification. The time limit before the beginning of the gonadotoxic treatment was always set by the referring doctor.

Diaz-Garcia. Fertility after oocyte vitrification and ovarian transplantation. Fertil Steril 2017.

SUPPLEMENTAL FIGURE 2



Number of procedures stratified by fertility preservation technique and year.

Diaz-Garcia. Fertility after oocyte vitrification and ovarian transplantation. Fertil Steril 2017.