Evaluation of ovarian tissue transplantation: results from three clinical centers

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Objective: To report ovarian tissue autotransplantation (AT) results and describe the relationship between technical and clinical factors and outcomes.
Design: Multicenter retrospective cohort study.
Setting: Tertiary medical centers.
Patient(s): Infertile patients who had stored ovarian tissue before sterilizing treatment and returned for AT with the aim of conceiving.
Interventions(s): Ovarian tissue cryopreservation (OTC) and AT, endocrine monitoring, in vitro fertilization.
Main Outcome Measure(s): Endocrine performance, pregnancy and live-birth rates.
Result(s): From 2004 to 2018, 70 patients underwent 87 ATs. Sixty patients undergoing 70 ATs met the inclusion criteria. After AT, menses returned in 94% of patients and median FSH dropped from 68 to 19 IU/mL. Fifty pregnancies and 44 deliveries were attained, with 50% of women achieving at least one pregnancy and 41.6% at least one delivery. Twelve patients underwent AT more than once and had their endocrine activity restored in case menses recurred after the first transplantation. Repeated transplantations yielded five live births in three patients, two of whom had already given birth after the first transplantation. Preharvesting chemotherapy was not associated with inferior outcomes. Of seven patients whose pelvis was exposed to radiation before AT, four conceived and delivered. Neither tissue dimensions nor surgical approach affected fertility outcomes.
Conclusion(s): OTC is highly effective at restoring fertility in sterilized patients, and prior exposure to chemotherapy should not be considered a contraindication. Repeated AT should be contemplated in case of graft malfunction, especially if previous transplantation was successful. In selected cases, conception and delivery may be feasible after pelvic exposure to radiation (Fertil Steril® 2020;114: 388–97. ©2020 by American Society for Reproductive Medicine.)
El resumen está disponible en Español al final del artículo.

Key Words: Ovarian autotransplantation, ovarian cryopreservation, fertility preservation, cancer

In recent years, we have become increasingly aware of the long-term adverse effects of cytotoxic treatment, such as ovarian failure and infertility, among young cancer survivors (1). This has prompted frequent inclusion of fertility preservation measures in the management of patients likely to overcome their disease but at major risk of significant ovarian injury (2, 3). Although storage of embryos and oocytes has become an accepted approach in the field, it is only recently that ovarian cortex cryopreservation has been adopted by a number of centers as a standard procedure for fertility preservation (4–6).

Ovarian tissue cryopreservation (OTC), which enables preservation of many thousands of primordial follicles at once (7), offers some major advantages over embryo and oocyte cryopreservation. It does not require ovarian stimulation and is the only option available for prepubertal girls and patients who cannot delay their cancer treatment to undergo stimulation (8). In terms of efficacy and success rates, according to some publications

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reporting cumulative data from several centers, ~30% of autotransplanted patients conceive and deliver with the aid of this procedure (9–11). However, the current literature indicates that despite being increasingly applied, OTC and autotransplantation (AT) are practiced quite differently by various teams worldwide. There is inconsistency regarding selection criteria for the procedure (age and chemotherapy exposure), laboratory handling of harvested tissue (fragment dimensions, freezing protocols), and the transplantation technique itself (transplantation site, surgical strategy). Thus, there is a growing need to better define selection criteria and determine the optimal technical approach.

The present report presents the combined results of OTC and AT from three different centers each with more than 15 years of experience in tissue transplantation. Each participating center independently coordinates the process of OTC and AT from beginning to end, meaning that patient selection, tissue harvesting, preparation, cryopreservation, storage, AT, and patient follow-up are performed exclusively on site. This study considers the different strategies of each of the centers to provide generalizable OTC-AT success rates and investigate the relationship between key technical and clinical factors and procedure outcomes.

METHODS

Patients

This multicenter cohort study included three participating centers: the Sheba Medical Center (Israel), Cliniques Universitaires Saint-Luc (Belgium), and the Infertility Center of St. Louis (U.S.). Included in this study were patients who underwent OTC for the purpose of fertility preservation before potentially sterilizing treatment and were autotransplanted years later with the aim to conceive, while suffering from ovarian failure or after failing to conceive on IVF. Patients who were lost to follow-up, who had undergone OTC owing to other indications, or whose tissue was cryopreserved with the use of vitrification were excluded.

As of June 2018, a total of 1,314 patients have undergone OTC in these three centers (545 in the Sheba Medical Center, 706 in Cliniques Universitaires Saint-Luc, and 63 in the Infertility Center of St. Louis), From January 2004 to June 2018, 70 women who had had their ovarian tissue stored came back to have it thawed and autotransplanted (34 from the Sheba Medical Center, 25 from the Cliniques Universitaires Saint-Luc, and 11 from the Infertility Center of St. Louis), leading to a combined return rate of 5.32%. Of the 70 returning patients, 10 were excluded for the following reasons: Four had their tissue cryopreserved with the use of vitrification (Infertility Center of St. Louis), two were lost to follow-up, two were harvested because of premature ovarian insufficiency, one had borderline tumor, and one was found to be 6 weeks' pregnant a month after transplantation. Thus, a total of 60 transplanted patients who met the inclusion criteria were finally included in the study.

Patient age at the time of OTC was from 14 to 39 years (mean 26.3 ± 6.26 y) and the majority of subjects were nulliparous (83.05%). Most of them were diagnosed with hematologic malignancies (23 Hodgkin lymphoma, 11 non-Hodgkin lymphoma, five leukemia). Five patients had breast cancer, two cervical cancer, two central nervous system tumors, two sarcomas, and one colorectal cancer. The remaining nine patients underwent OTC for a variety of nonmalignant conditions that required potentially sterilizing medical treatment, including endometriosis resection surgery involving bilateral salpingo-oophorectomy, bone marrow transplantation for sickle cell disease, and chemotherapy including alkylating agents for systemic lupus erythematosus, scleroderma, and Wegener granulomatosis. Twenty-four patients were treated with nonsterilizing chemotherapy before tissue harvesting: Ten patients had tissue harvested on initial diagnosis after nonsterilizing chemotherapy but before more intensive chemotherapy, and 13 patients at the time of a later relapse that required second-line therapy. In one sarcoma patient, tissue was harvested during surgery to remove a large abdominal tumor after several chemotherapy cycles. Oncologic treatment included pelvic exposure to radiation in seven patients: Two cervical cancer patients and one colorectal cancer patient were given pelvic radiotherapy, two leukemia patients and one Hodgkin lymphoma patient were treated with total body irradiation, and one non-Hodgkin lymphoma patient underwent lumboaortic radiation.

At the time of AT, a median of 7 years after OTC (interquartile range [IQR] 3.8–10.2), mean patient age was 32.7 ± 5.6 years (range 21–45 years). Most patients showed clear evidence of ovarian failure, with persistent amenorrhea and hormone profiles indicative of menopause; median FSH level was 63.8 IU/mL (IQR 38.5–90.3). However, eight patients did show some signs of residual ovarian activity, with seven experiencing regular menses and one oligomenorrhea. Transplantation was performed in these cases to treat their infertility and enhance their chances of future pregnancy. Five of these patients manifested low (<0.1 ng/mL) antimüllerian hormone or high (>15 IU/mL) FSH levels. Each underwent several in vitro fertilization (IVF) cycles, all of which resulted in up to one embryo transfer per patient, but no pregnancies were achieved. Two 45-year-old patients underwent transplantation because of age-related poor reproductive performance, one having suffering repeated implantation failure, and the other multiple early miscarriages. Finally, the colorectal cancer patient had one ovary harvested and the other transposed to the upper abdomen. Laparoscopy was subsequently performed to replace the right ovary inside the pelvis, and this opportunity was taken to transplant ovarian tissue fragments to boost the chances of pregnancy by means of IVF. Institutional review board approval was obtained for the study.

Ovarian Tissue Cryopreservation and Transplantation

For a detailed description of laboratory and surgical methods, readers are referred to previous publications (4, 12–14). In brief, after tissue extraction, the tissue was cut into fragments measuring 1–2 mm in thickness. The median surface area of these fragments was 50 mm^2, ranging from 2 mm^2 to 60 mm^2 (Table 1). Slow freezing was applied in all cases, using the protocol described by Gosden et al. with

TABLE 1

Surgical methods practiced by each participating center.

Variable	Sheba Medical Center	Cliniques Universitaires Saint-Luc	Infertility Center of St. Louis
No. of transplanted patients (no. of ATs)	34 (39)	25 (34)	11 (14)
No. of eligible patients[a] (no. of ATs)	32 (37)	23 (32)	5 (7)
Tissue fragments dimensions. median	50 mm^2 (IQR 50–50, range 2–60)	19.5 mm^2 (IQR 7–45, range 4–64)	50 mm^2 (IQR 50–50, range 36–50)
No. of fragments transplanted, median	4 (IQR 3.8–6, range 2–20)	13 (IQR 5.1–22, range 3–70)	5 (IQR 2.8–7.5, range 2–9)
AT surgical approach	Minilaparotomy: 32/37; Laparoscopy: 5/37	Minilaparotomy: 3/32; Laparoscopy: 29/32	Minilaparotomy: 7/7
Graft site	Ovary-subcortical pockets: 22/37; Peritoneal window: 1/37; Both: 14/37	Ovary-denuded medulla: 18/32; Peritoneal window: 5/32; Both: 9/32	Ovary-denuded medulla: 7/7

Note: AT = autotransplantation; IQR = interquartile range.
[a] Patients whose tissue was vitrified or whose tissue was harvested because of premature ovarian insufficiency were not included. See additional exclusion criteria in the text.

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minor modifications (15). Tissue harvesting, preparation, OTC, and storage were done on site in all three centers, with no transportation needed.

Most patients included in the study underwent AT only once. However, in several women, repeated transplantation was considered in case of graft malfunction, as indicated by persistently high FSH levels and cessation of menses, or no resumption of menses in the first place. Thus, 12 patients were grafted more than once, the number of transplantations totaling 76 for all 60 patients (Table 1).

Orthotopic reimplantation was performed in all cases, using the minilaparotomy approach in 42 transplantations and the laparoscopic approach in 34 transplantations. The median number of strips grafted on each occasion was 5.5 (IQR 4–13). Small ($\leq$9 mm^2) tissue strips were transplanted in ten patients, medium (10–49 mm^2) tissue strips in nine patients, and large ($\geq$50 mm^2) tissue strips in 37 patients. One patient was grafted with both medium and large strips. In three patients, tissue dimensions were not recorded. The fragments were grafted to the ovary inside subcortical ovarian pockets (16) or onto a denuded ovarian medulla (12, 14). Another site used was a specially created peritoneal window in the lower pelvis (Table 1).

After AT, ovarian function was closely monitored on site, including keeping track of the first menstrual bleed and repeated day 3 FSH measurements on return of menses. The three participating centers differed from one another regarding clinical management after confirmation of graft activity. In Israel, assisted reproductive technology (ART) is relatively accessible and IVF treatments are generally funded by national authorities. Therefore, in the Sheba Medical Center, after resumption of menses, increase in E_2 levels, and demonstration of follicle growth by ultrasound, proceeding to IVF was promptly considered. IVF was performed using either modified natural cycles (17) or the standard GnRH antagonist protocol, as dictated by individual endocrine profiles and antral follicle counts. In contrast, at the Infertility Center of St. Louis (USA), patients were encouraged to conceive spontaneously without any intervention such as IVF or hormonal stimulation. At Cliniques Universitaires Saint-Luc (Belgium), IVF was not routinely performed after transplantation. It was proposed in the presence of male-factor or mechanical infertility or when spontaneous pregnancy had not occurred within 1 year after AT. The protocol involved mild stimulation, followed by GnRH antagonist administration once a leading follicle measuring >10 mm was detected (18).

Statistical Analysis

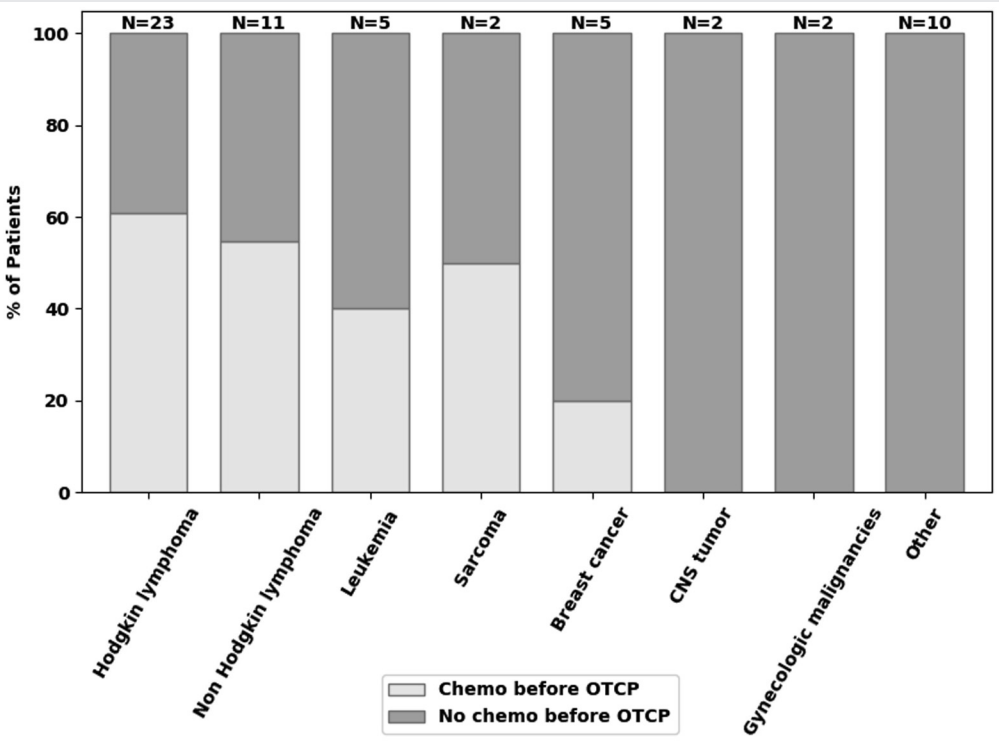
Statistical analysis was conducted with the use of SciPy, version 1.0.0. Normality of distribution was evaluated with the use of histograms and Q-Q plots. Continuous variables are presented as mean ± SD or median (IQR) as appropriate. Categoric variables are presented as n (%). Normally distributed continuous variables were compared by means of Student *t* test, and nonnormally distributed numbers were compared by means of Mann-Whitney test. Categoric variables were compared by means of chi-square or Fisher exact test as appropriate. A *P* value of $<.05$ was considered to be statistically significant.

RESULTS

During the study period, 60 patients that met the inclusion criteria underwent 76 ATs. After the first transplantation, there was evidence of ovarian activity in 55 women. Of the 52 patients who showed no ovarian function before AT, 47 (90.4%) resumed menses, at an average of 3.64 ± 1.79 months after transplantation. Among the five women who did not recover menses, one had undergone hysterectomy and another was treated with chemotherapy after being diagnosed with secondary malignancy shortly after transplantation. Therefore, excluding these two patients, the restoration rate of menses was 94% (47/50). Median FSH levels were 63.8 IU/mL (IQR 38.5–90.3) before AT and 19 IU/mL (IQR 10.7–36.5) after AT.

After transplantation, the patients were followed for a median duration of 64 months (IQR 36–82). During this time period, 33 of them underwent ovarian stimulation and

FIGURE 1



Chemotherapy exposure according to diagnosis. Hematologic malignancies were more commonly associated with chemotherapy before ovarian tissue cryopreservation (OTCP).

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IVF, and 14 (42.4%) conceived at least once. A total of 117 cycles were completed, with a median three cycles per patient (IQR 2–5). In 25 of these cycles (21.4%), attempts to retrieve oocytes revealed empty follicles. One hundred sixty-nine oocytes were collected and 88 were fertilized, yielding a fertilization rate of 52.1%. Altogether, 71 embryos were transferred. Mean oocyte number per cycle was 1.44, with clinical pregnancy and live birth rates per cycle of 14.5% and 12.8% respectively. Ultimately. A total of 17 pregnancies and 15 live births were obtained following IVF.

A total of 50 pregnancies were achieved (33 spontaneous, 17 IVF), 44 of which resulted in live births. Three of the pregnancies were still ongoing at the time of writing. Six patients conceived and delivered more than once: Four patients gave birth twice and two patients had three deliveries (one of them conceived four times and had spontaneous miscarriage). The overall miscarriage rate was 6%: 11.8% for IVF pregnancies and 3.0% for spontaneous pregnancies ($P=.29$). Thirty patients conceived at least once (50.0%) and 25 gave birth at least once (41.6%). Two additional pregnancies were obtained in two of four patients transplanted with vitrified tissue at the Infertility Center of St. Louis (12), although, as previously stated, these women were excluded from the analysis. Patients who conceived were compared with those who did not. Mean age at OTC was 25 ± 5.5 years versus 27.6 ± 6.8 years, respectively, and this difference did not reach statistical significance ($P=.1$). However, there was a trend toward a higher proportion of women aged ≥35 years on OTC among those who did not conceive (53.3% vs. 26.6%; $P=.06$). In addition, a significantly younger age at AT was observed among those who became pregnant (31.6 ± 5.2 vs. 34.8 ± 6.2; $P=.03$). Hematologic malignancy was the most common diagnosis in both groups (70% vs. 60%).

Among patients grafted with small tissue fragments, 58% conceived at least once, compared with 46% of those transplanted with medium or large tissue fragments, but the difference was not statistically significant ($P=.41$). The surgical approach did not affect outcomes: Of those transplanted with the use of a laparoscopic approach, 44.73% conceived at least once, compared with 55.26% of those undergoing AT by means of minilaparotomy ($P=.7$). Of those whose tissue had been transplanted into the ovarian site, 51.5% conceived at least once, compared with 50% of those whose tissue had been transplanted into peritoneal pockets ($P=.71$).

Twenty-four women had their tissue stored after chemotherapy. Patients affected by hematologic malignancies were most likely to be exposed to chemotherapy before OTC (Fig. 1). Table 2 presents transplantation outcomes according to chemotherapy treatment before OTC. Those who received and those who did not receive chemotherapy were of similar age. There was no significant difference between the two groups in terms of posttransplantation FSH values,

TABLE 2

Ovarian transplantation outcomes according to preharvesting chemotherapy exposure.

Variable	Total	Chemotherapy	No chemotherapy	*P* value
Patients, n	60	24	36	
Age at OTC, y	26.3 ± 6.26	25.83 ± 5.65	26.55 ± 6.71	.66
Age at AT, y	32.7 ± 5.6	32.96 ± 6.1	33.36 ± 5.64	.80
Tissue transplanted, area, mm^2	250 (150–300)	200 (150–214)	200 (165–310)	.11
FSH after AT, IU/mL	19 (10.7–36.5)	14 (8.8–42.5)	23.65 (12–34.2)	.26
Menses recurrence[a]	94.0% (47/50)	95.0% (19/20)	93.3% (28/30)	.81
At least 1 pregnancy	50.0% (30/60)	58.3% (14/24)	44.4% (16/36)	.32
At least 1 live birth	41.7%(25/60)	45.8% (11/24)	38.9% (14/36)	.79
No. of pregnancies (no. via IVF)	50 (17)	25 (8)	25 (9)	
No. of live births (no. via IVF)	45 (15)	21 (5)	24 (10)	
No. of ongoing pregnancies (no. via IVF)	3 (1)	1 (1)	2 (0)	
Miscarriages	6% (3/50)	12% (3/25)	0% (0/25)	.24

Note: Values are presented as mean ± SD or median (interquartile range) unless otherwise indicated. AT = autotransplantation; FSH = follicle-stimulating hormone; IVF = in vitro fertilization; OTC = ovarian tissue cryopreservation.

[a] Ten patients were excluded from the analysis: eight who had residual ovarian activity before transplantation, one who underwent hysterectomy, and one patient who was treated with chemotherapy after being diagnosed with secondary malignancy shortly after transplantation.

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restoration of menses, pregnancy, live birth, or miscarriage rates. Ten patients were treated with alkylating agents before OTC and exhibited no ovarian activity before AT. After AT, seven had their menses restored and four conceived at least once. The four patients who conceived were aged 23–32 years at OTC. One patient with non-Hodgkin lymphoma had been treated with six cycles of VACOP-B (etoposide, doxorubicin, cyclophosphamide, vincristine, prednisolone, bleomycin) before OTC and conceived via IVF after transplantation. The second, a patient with Hodgkin lymphoma, had been given five cycles of ABVD (doxorubicin, bleomycin, vinblastine, dacarbazine) and one cycle of MOPP (mechlorethamine, vincristine, procarbazine, prednisone) and conceived spontaneously after AT. The third, another patient with Hodgkin lymphoma, had undergone six cycles of BEACOPP (bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, prednisone), conceived after two IVF attempts, and at the time of writing was 8 weeks' pregnant. Finally, a patient with breast cancer who received four cycles of AC (adriamycin and cyclophosphamide) and a further two cycles of cyclophosphamide conceived after transplantation following just one IVF attempt.

Seven patients had their pelvis exposed to radiation before AT. All four leukemia or lymphoma survivors who underwent total body or lumboaortic radiation, receiving up to 20 Gy pelvic radiation, regained their menses after transplantation. Three of them conceived and delivered at term, with no obstetrical complications, demonstrating preserved uterine function despite exposure to radiation. One colorectal cancer patient, who was given pelvic radiation at a total dose of 45 Gy, also conceived after AT and replacement of a previously transposed ovary back to the pelvis. She delivered prematurely at 32 weeks' gestational age. In contrast, the two cervical cancer patients did not recover endocrine function and did not achieve pregnancy.

Twelve patients underwent more than one transplantation (Table 3): Four of them underwent three ATs, and the remaining eight underwent two ATs. Mean age at OTC was 24.17 ± 4.62 years, whereas at first and last ATs it was 29.92 ± 3.84 years and 32.83 ± 3.66 years, respectively. The median interval between transplantations was 2 years (range 1–5). All those who had their menses restored after the first transplantation also recovered menses after subsequent transplantations. In three patients, repeated ATs resulted in a total of five pregnancies and live births. Two of these women had also conceived after their first transplantations.

DISCUSSION

The years 2004–2005 saw some groundbreaking advances in the world of reproduction. In 2004, the first live birth to be achieved spontaneously after OTC-AT was reported in Belgium (14). In 2005, the first live birth resulting from ovarian stimulation and IVF after OTC-AT in a woman with documented longstanding ovarian failure was announced in Israel (16). During that same year, a live birth obtained after transplantation of fresh ovarian cortex from a monozygotic twin was also recorded for the first time in the USA (19). In the 15 years that have passed since those transformative times, OTC-AT has gained ground as an accepted means of fertility preservation, practiced in multiple centers around the world on both experimental and nonexperimental bases. As of June 2017, it was estimated that OTC-AT had yielded more than 130 live births worldwide (20). This figure is expected to swell over the coming years, as the appeal of OTC-AT grows by virtue of its low surgical complication rates (21) and favorable outcomes. In a meta-analysis, OTC-AT was found to restore endocrine function in 64% of cases, with an ongoing pregnancy or live birth rate of 28.4% (10). Similar live birth rates are often observed when scrutinizing data from other teams or networks, for example, 32.6% in the Spanish group in 2018 (22), 31% in the Danish group in 2015 (9), and 25% in the German FertiPROTEKT network in 2016 (11).

TABLE 3

Repeated ovarian tissue transplantations: patient characteristics and results.

			OTC			Before AT			After AT			
Patient #	AT#	Diagnosis	Age, y	Chemotherapy	Radiation	Age, y	Menses	FSH, IU/mL	Menses	Time to menses, mo	FSH, IU/mL	Pregnancies/live births
1	1	CML	21	–	TBI	27	no	116	yes	3	7.2	
	2					28	no	44	yes	3	100	
	3					33	no	50	yes	3	14	
2	1	Ewing sarcoma	24	–	–	28	no	107	yes	3	24.3	1/1 (IVF)
	2					31	no	80	yes	2	9	1/1 (IVF)
3	1	Hodgkin lymphoma	21	ABVD	–	28	no	71	yes	8	21.5	
	2					29	no	35	yes	0.5	31	
4	1	Non-Hodgkin lymphoma	36	RCHOP	–	40	no	87	no	–	90	
	2					41	no	90	no	–	78	
5	1	Hodgkin lymphoma	25	–	–	30	no	91	yes	4	34	
	2					31	yes	34	yes	4	NA	1/1 (spontaneous)
6	1	Sickle cell disease	20	–	–	26	no	44	yes	5	22	
	2					27	yes	25	yes	4	NA	
	3					31	yes	NA	yes	4	25	
7	1	Non-Hodgkin lymphoma	28	ABVD	–	34	no	65	yes	6	80	
	2					36	yes	80	yes	2	78	
8	1	Wegener's granulomatosis	22	–	–	27	no	114	yes	5	60	
	2					28	yes	60	yes	4	IVF	
9	1	Hodgkin lymphoma	23	ABVD	–	30	no	72	yes	6	103	
	2					33	yes	103	yes	5	64	
	3					35	yes	64	yes	2	NA	
10	1	Hodgkin lymphoma	22	MOPP/ABVD	–	28	yes	62	yes	6M	118	
	2					30	yes	41	yes	NA	NA	
11	1	Hodgkin lymphoma	28	–	–	30	no	50	yes	9	48	
	2					33	no	48	yes	6	35	
12	1	Hodgkin lymphoma	20	ABVD	TBI	31	no	64.2	yes	4	8.2	1/1 (spontaneous)
	2					33	no	62.4	yes	4	3.9	
	3					36	no	61.2	yes	4	5	3/3 (spontaneous)

Note: ABVD = doxorubicin, bleomycin, vinblastine, dacarbazine; AT = autotransplantation; CML = chronic myeloid leukemia; MOPP = mechlorethamine, vincristine, procarbazine, prednisone; NA = not available; OTC = ovarian tissue cryopreservation; RCHOP = rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone; TBI = total body irradiation.

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Our first three reports of successful ovarian tissue transplantation from 2004–2005 triggered a learning curve that continues to this day, and the present paper presents the actual clinical findings of this ongoing process. Restoration of ovarian activity was observed in 94% of patients who had shown no ovarian activity before AT. At least one pregnancy was obtained in 50% of patients, and 41.6% of subjects delivered at least once. Such encouraging figures support use of OTC when fertility preservation is desired, emphasize its role in restoration of endocrine function, and, importantly, advocate implementation of OTC as a nonexperimental procedure. The positive results obtained in the current multicenter study may be attributed to extensive long-term experience in OTC-AT, as well as complete centralization of the entire process by each of the centers involved. Compared with other series in the literature, a younger study population may account for the higher pregnancy rate observed in our study; the present study included a large number of hematologic patients who were in their 20s at presentation. In contrast, other series in the literature included a higher proportion of breast cancer patients, who underwent OTC at an older age (9, 11, 22). Substantiating this explanation was a trend toward a higher proportion of patients aged $\geq$35 years at OTC among those who did not conceive in our cohort. Age differences could also explain the low miscarriage rate observed in this study (6%) compared with that described by other groups (~33%) (9, 22).

It is still unclear whether OTC is preferable to oocyte cryopreservation in postpubertal patients who have sufficient time for ovarian stimulation before chemotherapy. A study comparing the two techniques reported a trend toward higher pregnancy (40.8% vs. 32.6%) and live birth (27.3% vs. 18.2%) rates in patients undergoing oocyte vitrification (22). However, it is noteworthy that the favorable outcomes of oocyte vitrification presented in the study above are from fertility centers with vast experience in oocyte freezing. On the other hand, as previously stated, a number of groups around the world have achieved live birth rates of >18% following OTC-AT, and the results of the present analysis considerably outperform this figure. Moreover, OTC has several advantages over oocyte vitrification. It restores ovarian function and enables natural conception, as reflected by the 33 spontaneous pregnancies achieved in this series (leading to a spontaneous clinical pregnancy rate of 55%). Another advantage demonstrated by the present cohort is that OCT-AT may result in several pregnancies. These are less likely to occur after a single cycle of IVF for oocyte cryopreservation. When possible, a combination of OTC and oocyte cryopreservation can be offered to further increase the chances of future pregnancy (23).

Importantly, ovarian stimulation and oocyte retrieval become irrelevant immediately after chemotherapy, and for at least another 6 months (8), resulting in a diminished response to ovarian stimulation (24, 25) and genetically abnormal oocytes, as demonstrated in nonhuman animal models (26–28). Therefore, OTC is the only fertility preservation option available to patients who cannot postpone chemotherapy on primary diagnosis or disease relapse. There is still ongoing debate around the efficacy and feasibility of OTC in the context of previous chemotherapy, with some centers restricting the procedure to chemotherapy-naive patients only (29). However, in the present cohort, both chemotherapy-naive and chemotherapy-exposed patients showed similar ovarian activity restoration rates of ~94%. The proportions of women who conceived and delivered at least once were actually slightly higher among chemotherapy-exposed patients (58% vs. 42% and 46% vs. 39%, respectively) despite being of similar age, although the difference was not statistically significant. These results corroborate those observed in a smaller recent study reporting outcomes of 31 autotransplanted patients (30). By 1 year after AT, ovarian function was restored in 93% of chemotherapy-exposed subjects, compared with 67% of nonexposed subjects (P=.14). None of the chemotherapy-naive patients conceived, whereas 32% of the chemotherapy-exposed group delivered at least once, although lower pregnancy rates were encountered among patients treated with alkylating agents. In our study, however, seven of ten subjects previously exposed to alkylating agents regained ovarian function following AT. Four of them conceived and gave birth, despite exposure to cyclophosphamide. Owing to a lack of statistical power, the findings of the present study cannot refute a possible decline in AT outcomes in case of preharvesting chemotherapy exposure, especially when alkylating agents are involved. Nevertheless, chemotherapy-exposed patients did not show inferior results to chemotherapy-naive patients. Withholding OTC-AT from chemotherapy-exposed patients therefore seems to be unjustified.

The value of AT in patients with previous exposure to pelvic radiation is unclear. Assuming orthotopic transplantation is performed, poor pelvic vascularization may compromise graft function, making the procedure somewhat ineffective. Importantly, even if ovarian function is preserved or restored, radiation-induced uterine injury may impede uterine capability to carry a pregnancy, so surrogacy is often required. Our data suggest that AT may be appropriate in patients whose pelvis is exposed to radiotherapy, as four of five patients receiving pelvic radiation at doses up to 45 Gy conceived and delivered. Whereas patients who received radiation at doses $\leq$20 Gy delivered at term without any complications, the patient who was treated with a dose of 45 Gy delivered prematurely at 32 weeks. A higher dose of pelvic radiation (external and brachytherapy) is given in cases of cervical cancer (31), and none of the patients with cervical cancer in our cohort managed to conceive. To the best of our knowledge, there have been no reports of successful transplantation in such cases. When contemplating fertility preservation in a patient destined to undergo pelvic radiotherapy, informed discussion is essential. Limited experience with the procedure in such circumstances should be communicated to the patient, as should the possibility of alternate options and potential need for surrogacy in the future. A higher risk for numerous obstetrical complications should be conveyed to the patient as well, including miscarriage, preterm labor, and intrauterine growth restriction (32). If AT is pursued, tissue should be grafted to a vascularized site, such as an ovary replaced back inside the pelvis after transposition.

Graft dimensions have been shown to play a significant role in determining the extent of follicle loss during cryopreservation, thawing and transplantation. On freezing, thinner cortical strips allow good penetration of cryoprotective agents and decrease the likelihood of ice crystal formation and injury (33). On transplantation, smaller dimensions reduce penetration depth for new blood vessels, potentially shortening the ischemia period (34, 35). On the other hand, grafting smaller fragments may boost follicle activation, resulting in increased loss of dormant follicles (36). The present study has enabled us to describe AT clinical results according to fragment dimensions for the first time. Patients transplanted with small tissue strips (≤ 9 mm^2) achieved pregnancy and live birth rates similar to those achieved by patients transplanted with larger pieces. Tissue dimensions may determine a graft's life span, but such a hypothesis could not be evaluated in the present study. To determine optimal tissue strip dimensions, if they exist at all, larger multicenter studies, also evaluating grafts' life span, should be conducted. As experience with OTC and transplantation continues to grow globally, such studies will undoubtedly be available in the near future.

In the event of graft malfunction, repeated AT can be considered if a sufficient amount of ovarian tissue was stored. To prevent complications of repeated surgical procedures, it is important to identify patients most likely to benefit from additional transplantation. In the present study, 12 patients underwent more than one AT. All those whose menses resumed after their first transplantation also exhibited ovarian activity after their subsequent transplantations. In contrast, the only woman not to recover her menses after her first transplantation did not show any ovarian activity after her second transplantation either. Repeated ATs yielded a total of five live births in three patients, two of whom had been transplanted for the second or third time after already giving birth after their first AT. These patterns may suggest that first and repeated transplantations share similar course and outcomes. Therefore, patients who manage to initially conceive after AT but later experience diminished or absent graft activity may be encouraged to pursue a second transplantation if an additional pregnancy is desired.

Several strength and limitations of the present study should be acknowledged. Among the strengths is the centralization practiced by the three participating centers, exclusively managing every case through all aspects of patient care, from OTC to AT and subsequent follow-up. In addition, to the best of our knowledge, this study presents the largest series to evaluate the effect of chemotherapy exposure on AT outcomes and is the first to describe pregnancy rates according to different surgical strategies. Yet because of the limited years of OTC-AT practice, combined with a low return rate of 5.3%, and despite the integration of data coming from three centers, the modest number of patients who underwent AT could limit the sensitivity of statistical tests. The effect size observed when comparing technical strategies was small, limiting the statistical power to detect differences in pregnancy rates. The return rate in the present cohort is rather low, but nevertheless similar to utilization rates observed by other groups in oocyte vitrification (22), OTC-AT (11, 22), and male fertility preservation programs (37, 38). Improved identification of those likely to utilize their tissue is of great importance and should be addressed in future studies.

CONCLUSION

This multicenter study reinforces the role of OTC as an effective tool for fertility preservation, resulting in conception and live birth in up to 50.0% and 41.6% of patients, respectively. This study reveals that once a previously functioning graft is depleted, repeated AT is likely to trigger endocrine activity and conception once again. OTC is highly appropriate in case of recent chemotherapy and should not be excluded in such scenarios. AT after pelvic exposure to radiation can yield satisfactory results in selected cases, not always requiring surrogacy. However, the efficacy of AT remains questionable in cases of cervical cancer. Regarding techniques used when handling harvested tissue, neither tissue dimensions nor the surgical approach to transplantation appears to affect reproductive outcomes, but these should be further evaluated in larger studies in the future. It is expected that in the coming years, implementation of OTC-AT will continue to expand globally, with increasing numbers of procedures performed for nononcologic indications as well. We can therefore expect a growing body of data to accumulate in the near future, allowing for large-scale studies to better understand and address the impact of technical aspects and selection criteria on outcomes, further improving efficiency and performance. In the meantime, it is fair to say that in the hands of experienced practitioners, OTC-AT is a highly effective approach to fertility preservation.

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Revisión del trasplante de tejido ovárico: resultados de tres centros clínicos.

Objetivo: Comunicar los resultados del autotrasplante de tejido ovárico (AT) y describir la relación entre los factores técnicos y clínicos y los resultados.

Diseño: Estudio retrospectivo de cohortes multicéntrico.

Entorno: Centros médicos terciarios.

Paciente(s): Pacientes infértiles que habían guardado tejido ovárico antes del tratamiento causante de infertilidad y retornaron para AT con el objetivo de lograr gestación.

Intervención(es): Criopreservación de tejido ovárico (OTC) y AT, monitorización hormonal, fertilización in vitro.

Medida(s) del resultado principal: perfil hormonal, tasas de embarazo y de nacidos vivos.

Resultado(s): Entre 2004 y 2018, 70 pacientes se sometieron a 87 AT. Sesenta pacientes sometidas a 70 AT cumplieron los criterios de inclusión. Después del AT, recuperaron ciclos menstruales el 94% de las pacientes y el valor medio de FSH descendió desde 68 a 19 UI/mL. Se obtuvieron cincuenta embarazos y 44 partos, logrando el 50% de las pacientes al menos un embarazo y el 41,6% al menos un parto. Doce pacientes se sometieron a AT más de una vez y lograron recuperar su actividad endocrina si se había recuperado la menstruación después de su primer trasplante. Los trasplantes recurrentes dieron lugar a cinco nacidos en tres pacientes, dos de las cuales ya habían dado a luz después del primer trasplante. La quimioterapia pre-preservación no estuvo asociada a resultados inferiores. De siete pacientes que fueron sometidas a radioterapia pélvica antes del AT, cuatro lograron embarazo y dieron a luz. Ni el tamaño del tejido ni la técnica quirúrgica influyeron en los resultados de fertilidad.

Conclusión(es): OTC es altamente efectiva para restaurar la fertilidad en pacientes con esterilidad debida a tratamiento y la exposición previa a quimioterapia no debería considerarse una contraindicación. Deberían considerarse AT repetidos en casos de no funcionamiento del injerto, especialmente si un trasplante previo había tenido éxito. En casos seleccionados, puede ser factible el embarazo y parto después de la exposición pélvica a radioterapia.