

## Review

# Current Success and Efficiency of Autologous Ovarian Transplantation: A Meta-Analysis

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## Abstract

**Context:** Ovarian cryopreservation followed by autotransplantation is still considered an experimental strategy for fertility preservation (FP) mainly because the success rates are unknown. **Objective:** To determine cohort epidemiologic characteristics and success rates of autologous ovarian tissue transplantation (OTT) with previously cryopreserved tissue. **Materials and Methods:** Literature review from 1999 to October 1, 2016. Additional cases were retrieved from meeting abstracts and own database. We selected studies that reported autologous OTT with previously banked tissue in humans. We did not include any cases involving fresh ovarian tissue transplantation or those performed to treat idiopathic premature ovarian failure/insufficiency. Both authors reviewed and selected studies for eligibility, which resulted in 59 full-text studies assessed for eligibility. Cases were extracted from original reports and reviews by the junior author, and the senior author reviewed and verified the extracted data. **Results:** Nineteen reports were included for qualitative synthesis. In 10 studies, detailed data were available to determine clinical and live birth + ongoing (LB + OG) pregnancy as well as endocrine restoration rates. Three hundred nine OTTs were performed with cryopreserved tissue, resulting in the birth of 84 children and 8 OG pregnancies. The cumulative clinical and LB + OG rates were 57.5% and 37.7%, respectively, and the endocrine restoration rate was 63.9%. **Conclusion:** Success rates with cryopreserved OTT have reached promising levels. Given these recent data, ovarian tissue cryopreservation should be considered as a viable option for FP.

## Keywords

ovary, transplantation, cryopreservation, fertility preservation

## Introduction

Ovarian cryopreservation is a success story for translational research. The concept of ovarian cryopreservation for fertility preservation was first introduced in animal studies performed beginning in 1950s.<sup>1,2</sup> This concept was further solidified by studies with human ovarian tissue in vitro<sup>3</sup> and xenograft models.<sup>4</sup> Following the successful in vivo studies in the sheep,<sup>5</sup> the first report of restoration of ovarian function in human with the transplantation of autologous frozen-thawed tissue was published in 2000.<sup>6</sup> Subsequently, pregnancies were reported and the research has gained speed in the area of ovarian cryopreservation and transplantation.

Ovarian tissue cryopreservation (OTC) enables preservation of a potentially large number of primordial follicles, bringing the possibility of not only restoring spontaneous fertility but also the endocrine function upon transplantation. It does not require ovarian stimulation, avoiding significant delays in the initiation of cancer treatments, and can be utilized in prepubertal children.<sup>7</sup> A simple outpatient laparoscopic procedure typically suffices to obtain the tissue for cryopreservation. Though tissue preparation for cryopreservation requires some expertise

and in most cases a programmable freezer, given its relative simplicity and unique advantages over oocyte and embryo freezing, ovarian freezing presents itself as a strong option for fertility preservation for medical indications.

However, despite the clinical progress within the past 2 decades, the procedure still remains in the experimental realm. Among others, one of the major reasons why ovarian tissue is considered experimental is the unavailability of clear data on success rates. This is largely due to the methodology, as most studies are case reports or case series where the total number of attempts (denominator) is typically not specified. Therefore, we conducted this meta-analysis with the primary

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aim of determining the per-patient success, specifically the cumulative clinical pregnancy (CP) and live + ongoing (LB + OG) pregnancy rates and fertility preservation rate with ovarian tissue freezing and autologous transplantation. Moreover, since ovarian transplantation (OTT) is the only FP method that can also reverse ovarian insufficiency, we also calculated the success rates for endocrine function restoration. Secondarily, we sought to provide a bird's eye view of the current state and safety of the procedure and assess whether it still belonged to experimental category. To achieve this, we analyzed the published and unpublished data including our own (Table 1).

## Materials and Methods

The institutional board at New York Medical College approved the ovarian cryopreservation and transplantation protocol. We searched PubMed, EMBASE, and Cochrane Library in English language from 1999, the year when the first OTT with cryopreserved tissue was performed by us, until October 1, 2016. We used the following keywords controlled by medical subject headings and their synonyms: "transplantation," "ovary," "cryopreservation," and "fertility preservation." Other references were obtained from review articles, original reports embedded in reviews, abstract presentations, and from our own case series.

## Selection of Studies

Because the aim of this study was to investigate the success of ovarian cryopreservation as a fertility preservation method, we did not include any cases involving fresh ovarian tissue transplantation or those performed for follicle activation and autotransplantation to treat premature ovarian failure/insufficiency.

While we considered all studies for calculating the total number of OTTs, demographic characteristics, live births (LBs) and OG pregnancies, and graft function longevity, we only included original data reporting the total number of transplanted women (denominator) when calculating the success rates. Hence, the denominators for calculating the clinical pregnancy rate (CPR), live birth rate (LBR) + OG, and endocrine function rates differed and were numerically smaller than the total number of ovarian transplant attempts to date.

We used the most up-to-date data from each group when it reported on incremental progress. For specific information such as the OTT technique, amount of tissue transplanted, transplantation site and longevity of the graft transplanted, we only utilized original data.

## Success Rate Definitions

We defined CP as the presence of a heartbeat (pregnancies with multiple gestational sacs and heartbeats are counted as single CP) during the first trimester. Live birth was defined as

**Table 1.** Own Ovarian Transplantation Cases that Were Included in the Meta-analysis.

Indication for OTC	Age at OTC	Amount of Tissue Transplanted	Age at OTT	Ovarian Transplant Type	Outcome	Longevity
Endometriosis (oophorectomy) <sup>6,10</sup>	28	Cortex equivalent of ~1 ovary	29	Orthotopic—subperitoneally to pelvic side wall	Endocrine function restoration, ovulation	9 months
Breast cancer (chemo) <sup>8,10</sup>	30	Cortex equivalent of ~1 ovary	36	Heterotopic—subcutaneously to the abdominal wall	Oocyte retrievals, embryo development	Lost to follow-up
Hodgkin lymphoma (chemo for HSCT) <sup>9</sup>	29	Cortex equivalent of ~1 ovary	31	Heterotopic—subcutaneously to the abdominal wall	One MII oocyte retrieved from the graft + 4 spontaneous pregnancies and 3 live births within 5 years after OTT	Irregular menses, follicle development 144 months post OTT
Breast cancer (chemo) <sup>a</sup>	34	Cortex equivalent of about 1/10th of an ovary	38	Orthotopic—ovarian medulla (suture and injection under cortex)	Short-lived follicle growth	No function (tissue frozen elsewhere, occult POI prior to ovarian freezing, very low initial follicle density)
HLH syndrome—HSCT <sup>11</sup>	23 <sup>b</sup>	Cortex equivalent of ~1/2 of an ovary	30	Orthotopic—ovarian medulla	1 Live birth (IVF)	Regular cycles, normal baseline FSH, and ovulation 36 months post OTT
Non-Hodgkin lymphoma—HSCT <sup>11</sup>	23 <sup>b</sup>	Cortex equivalent of ~1/2 of an ovary	35	Orthotopic—ovarian medulla	1 Live birth (IVF)	E2 production but irregular menses 32 months post-OTT

Abbreviations: E2, estradiol; FSH, follicle stimulating hormone; HLH, Hemophagocytic lymphohistiocytosis; HSCT, hematopoietic stem cell transplantation; IVF, in vitro fertilization; MII, metaphase II; OTC, ovarian tissue cryopreservation; OTT, ovarian transplantation; POI, premature ovarian insufficiency.

<sup>a</sup>Unpublished.

<sup>b</sup>Updated from previous publication.

the live delivery of a baby beyond the 25th week of amenorrhea. The delivery of twins or other multiples was considered as single event. Ongoing pregnancy was defined as a conception with at least 1 fetal sac demonstrating heartbeat beyond the 12 weeks of amenorrhea. Cumulative CP or LB + OG pregnancy rates were calculated by dividing the total number of pregnancies in each category to number of women undergoing OTT.

Women who had at least an OG pregnancy were considered to have preserved fertility. Based on this, we determined a fertility preservation rate by calculating the percentage of women who at least had 1 LB or OG pregnancy after OTT.

For success rate calculation, we excluded the cases where (1) there was no desire to conceive, (2) there was prior hysterectomy, (3) there was a contraindication for pregnancy because of a second malignancy or recurrence, (4) there was no follow-up, (5) the transplant was to a heterotopic site where conception was only possible via in vitro fertilization (IVF).

We considered ovarian endocrine function to have been restored when there was a minimum of 4-month follow-up and at least one of the following occurred: (1) resumption of cyclic menstrual function for at least 6 months, (2) ovarian follicular growth monitored by ultrasound monitoring, or (3) pregnancy post OTT.

Given the small number of the heterotopic OTT procedures and because the process of follicle development is drastically different than with orthotopic transplants,<sup>8</sup> they were considered together with orthotopic OTT cases when evaluating endocrine function restoration but not included in the pregnancy rate calculation.

### Statistical Methods

Where applicable, we used the Student *t* test to compare patient demographics and age differences between women who did and did not conceive after OTT using SPSS 15 for Windows software (IBM Corp, Armonk, New York). Same was also used for comparing mean ages between those ovarian endocrine function was restored versus was not. Summary data were expressed as mean (standard deviation [SD]). A *P* value of <.05 was considered significant.

## Results

### Eligible Studies

Overall, we identified 708 reports through PubMed, EMBASE and Cochrane Library. Fifteen additional records were identified from review articles, original reports embedded in reviews, and abstract presentations. Fifty-nine full-text articles were assessed for eligibility according to their title and abstract. Of those, one study was excluded because the indication for OTT was to treat primary ovarian insufficiency through follicle activation, whereas 39 were excluded because of data duplication. After these exclusions, 19 original studies or reviews embedded with original data were included for qualitative synthesis (Table 2). Of those, 10 were eligible for quantitative synthesis (Table 3), where a denominator for calculating CP, LB + OG, or endocrine function restoration rates was available (Figure 1).

**Table 2.** Total Data on Pregnancies After Orthotopic and Heterotopic Transplantation of Cryopreserved Ovarian Tissue.

Sources	Patients/ OTT/PW and FUP (n)	LB (OG) (n)	Twins (n)	Babies Born (n)
Azem et al <sup>12,a</sup>	1/1/1	1	0	1
Donnez et al <sup>13,b</sup> ; Donnez and Dolmans <sup>14,b</sup>	62/66/62	26 (5)	1	26
Dunlop et al <sup>15,c</sup>	1/1/1	1	0	1
Fabbri <sup>16,c</sup>	3/3/2	0	0	0
Imbert et al <sup>17,c</sup>	8/9/6	3 (1)	0	3
Demeestere et al <sup>18,c</sup>				
Jensen et al <sup>19,c</sup>	41/53/32	12 (1)	1	13
Kim et al <sup>20,c</sup>	4/7/3	NE	0	0
Kiseleva et al <sup>21,c</sup>	1/1/1	0	0	0
Lorenzo et al <sup>22,a</sup>	1/2/1	2	0	2
Meirow et al <sup>23,c</sup>	20/21/19	9 (1)	1	10
Oktay and Oktay <sup>10,c</sup> ; Oktay et al <sup>11,b</sup>	6/6/5	2	0	2
Póvoa et al <sup>24,c</sup>	1/1/1	0	0	0
Silber et al <sup>25,c</sup>	8/8/8 <sup>d</sup>	4 <sup>d</sup>	0	4 <sup>d</sup>
Stern et al <sup>26,a</sup>	22/33/21	2	1	3
Tanbo et al <sup>27,c</sup>	2/2/2	2	0	2
Van der Ven et al <sup>28,c</sup>	74/95/49 <sup>e</sup>	17	0	17
Total	255/309/214	81 (8)	4	84

Abbreviations: CP, clinical pregnancy; CPR, clinical pregnancy; FUP, number of patients with appropriate follow-up; LB, live birth; LBR, live birth rate; NE, not eligible; OTT, number of ovarian transplant procedures; POI, premature ovarian insufficiency; PW, number of patients with pregnancy wish.

<sup>a</sup>Meeting/abstracts.

<sup>b</sup>Review paper, including author's own data.

<sup>c</sup>Original report.

<sup>d</sup>Three patients and related pregnancies (1 CP and 2 LB) were excluded as they underwent OTT for the treatment of POI.

<sup>e</sup>In the study by Van der Ven H et al, 49 patients with at least 1-year follow-up were included to estimate CPR, LBR, and endocrine function rate.

For cumulative CPR calculation, 7 studies (Fabbri et al, 2009; Imbert et al, 2014; Jensen et al, 2015; Tanbo et al, 2015; Silber et al, 2015; Meirow et al, 2016; Van der Ven et al, 2016) plus 6 cases of our group (Oktay et al 2010; 2016) qualified. For cumulative LB + OG pregnancy rate calculation, 8 studies (Fabbri et al, 2009; Imbert et al, 2014; Jensen et al, 2015; Donnez et al, 2015; Tanbo et al, 2015; Silber et al, 2015; Meirow et al, 2016; Van der Ven et al, 2016) plus 6 cases of our group met the criteria. Finally, 9 studies (Kim, 2009; Donnez, 2013; Fabbri, 2014; Imbert et al, 2014; Jensen et al, 2015; Tanbo et al, 2015; Silber et al, 2015; Meirow et al, 2016; Van der Ven et al, 2016) plus 6 of our own cases were eligible for endocrine function rate calculation.

### Characteristics of the Overall Study Population and the Procedures

We identified 309 OTTs, which were performed following cryopreservation of ovarian tissue in 255 patients. These included 6 of our own cases (Table 1). Of those, 246 patients

**Table 3. Studies Eligible for Ovarian Transplantation Success Rate Calculation.**

Sources	Country of origin	Study Period	Diagnosis Before OTC	Mean Age at OTC in Years (SD), Range	Number of Cases	Mean Age at OTT in Years (SD), Range	Mean Follow-Up in Months (SD), Range	Cumulative CP Rate	Cumulative LB + OG Rate	% LB + OG via ART	FP Rate	Endocrine Function Rate
Donnez et al <sup>13</sup>	Multicenter (Belgium, Spain, Denmark)	NA	Nonhematologic malignancies (27) Hematologic malignancies (21) Benign diseases (12)	NA	60	NA	NA	NE <sup>a</sup>	NE <sup>a</sup>	33.3% (2/6)	NE <sup>a</sup>	92.8% (52/56) <sup>b</sup>
Donnez and Dolmans <sup>14</sup>	Multicenter (Belgium and Spain)	NA	NA	NA	52	NA	NA	28.8% (15/52)	34.6% (18/52)	NA	17.3% (9/52)	NA
Fabbri et al <sup>16</sup>	Italy	December 2002 to August 2013	Nonhematologic malignancies (2) Hematologic malignancies (1) Benign diseases (0)	29 (5.0), 24-34	3	35.6 ± 4.5 (31-40)	19 (3.75), 15-22.7	0% (0/2) <sup>c</sup>	0% (0/2) <sup>c</sup>	NE	0% (0/2) <sup>c</sup>	100% (3/3)
Imbert et al <sup>17,d</sup>	Belgium and Spain	March 1999 to ?	Nonhematologic malignancies (2) Hematologic malignancies (3) Benign diseases (1)	25.3 (7.5), 13-35	8	30.1 (6.3), 22-39	NA (5-84)	83.3% (5/6) <sup>e</sup>	66.6% (4/6) <sup>e</sup>	0% (0/4)	33% (2/6) <sup>e</sup>	83.3% (5/6) <sup>e</sup>
Jensen et al <sup>19</sup>	Denmark	2003 to June 2014	Nonhematologic malignancies (23) Hematologic benign diseases (15) Benign diseases (3)	28.8 (5.9), 9.5-38.7	41	32.9 (5.6), 13.8-43.2	40.5 (32.2), 6-60	77.4% (24/31) <sup>c,f</sup>	41.9% (13/31) <sup>c,f</sup>	46.1% (6/13)	32.2% (10/31) <sup>c,f</sup>	97.5% (40/41)
Kim et al <sup>20</sup>	Multicenter (United States and South Korea)	2002 to 2005	Nonhematologic malignancies (4) Hematologic malignancies (0) Benign diseases (0)	NA (29-38)	4	NA	NA (2-96)	NE <sup>c</sup>	NE <sup>c</sup>	NE <sup>c</sup>	NE <sup>c</sup>	100% (4/4)
Meirow et al <sup>23</sup>	Israel	2004 to 2015	Nonhematologic malignancies (5) Hematologic malignancies (15) Benign diseases (0)	28.8 ± 7.4 (14-39)	20	34 ± 6.9 (21-45)	38.2 (NA), (7-141)	84.2% (16/19) <sup>g</sup>	52.6% (10/19) <sup>g</sup>	50% (5/10)	36.8% (7/19) <sup>g</sup>	89.5% (17/19) <sup>g</sup>
Oktay et al <sup>10,11</sup>	United States	1999 to October 2016	Nonhematologic malignancies (2) Hematologic malignancies (2) Benign diseases (2)	27.8 ± 4.2, 23-34	6	33.16 (3.65), 29-38	44.4 (19.1), 3-144	66.6% (2/3) <sup>h</sup>	66.6% (2/3) <sup>h</sup>	100% (2/2)	66.6% (2/3) <sup>h</sup>	83.3% (5/6)
Silber et al <sup>25</sup>	United States	2004 to 2013	Nonhematologic malignancies (3) Hematologic malignancies (3) Benign diseases (2)	NA	11	NA	18.9 (10.2), 9.8-38.5	50% (4/8) <sup>i</sup>	50% (4/8) <sup>i</sup>	0% (0/4)	50% (4/8) <sup>i</sup>	100% (8/8)
Tanbo et al <sup>27</sup>	Norway	2004 to 2014	Nonhematologic malignancies (2) Benign diseases (0)	23 ± 1.4 (22-24)	2	29.5 ± 2.1 (28-31)	38 (2.1) 36-40	100% (2/2)	100% (2/2)	50% (1/2)	100% (2/2)	100% (2/2)
Van der Ven et al <sup>28</sup>	Multicenter (16 centers in Germany, Switzerland, Austria)	2008 to July 2015	Nonhematologic malignancies (31) Hematologic malignancies (16) Benign diseases (2)	31 ± 5.9 (17-44)	74	34 (4.9), NA	NA (12-60)	32.6% (16/49) <sup>j</sup>	24.4% (12/49) <sup>j</sup>	NA	24.4% (12/49) <sup>j</sup>	67.3% (33/49) <sup>j</sup>

Abbreviations: ART, assisted reproductive technology; CP, clinical pregnancy; FP, fertility preservation; LB + OG, live birth + ongoing; NA, not available; NE, not eligible (numerator not available for this particular success rate calculation); OTC, ovarian tissue cryopreservation; OTT, ovarian transplantation; POI, premature ovarian insufficiency; SD, standard deviation.

<sup>a</sup>Update data available in Donnez et al, 2015.<sup>14</sup>

<sup>b</sup>Four patients excluded for short follow-up (<3 months).

<sup>c</sup>Patients with grafts to heterotopic sites only, from Fabbri et al, 2014 (1 patient); Jensen et al, 2015 (1 patient); Kim et al, 2009 (4 patients) and Oktay et al<sup>10</sup> (2 patients) were excluded from the calculation of pregnancy rates but not from the calculation of endocrine function rate.

<sup>d</sup>Data were complemented by Demeestere et al.<sup>18</sup>

<sup>e</sup>Two patients excluded from the original study for short follow-up (<5 months).

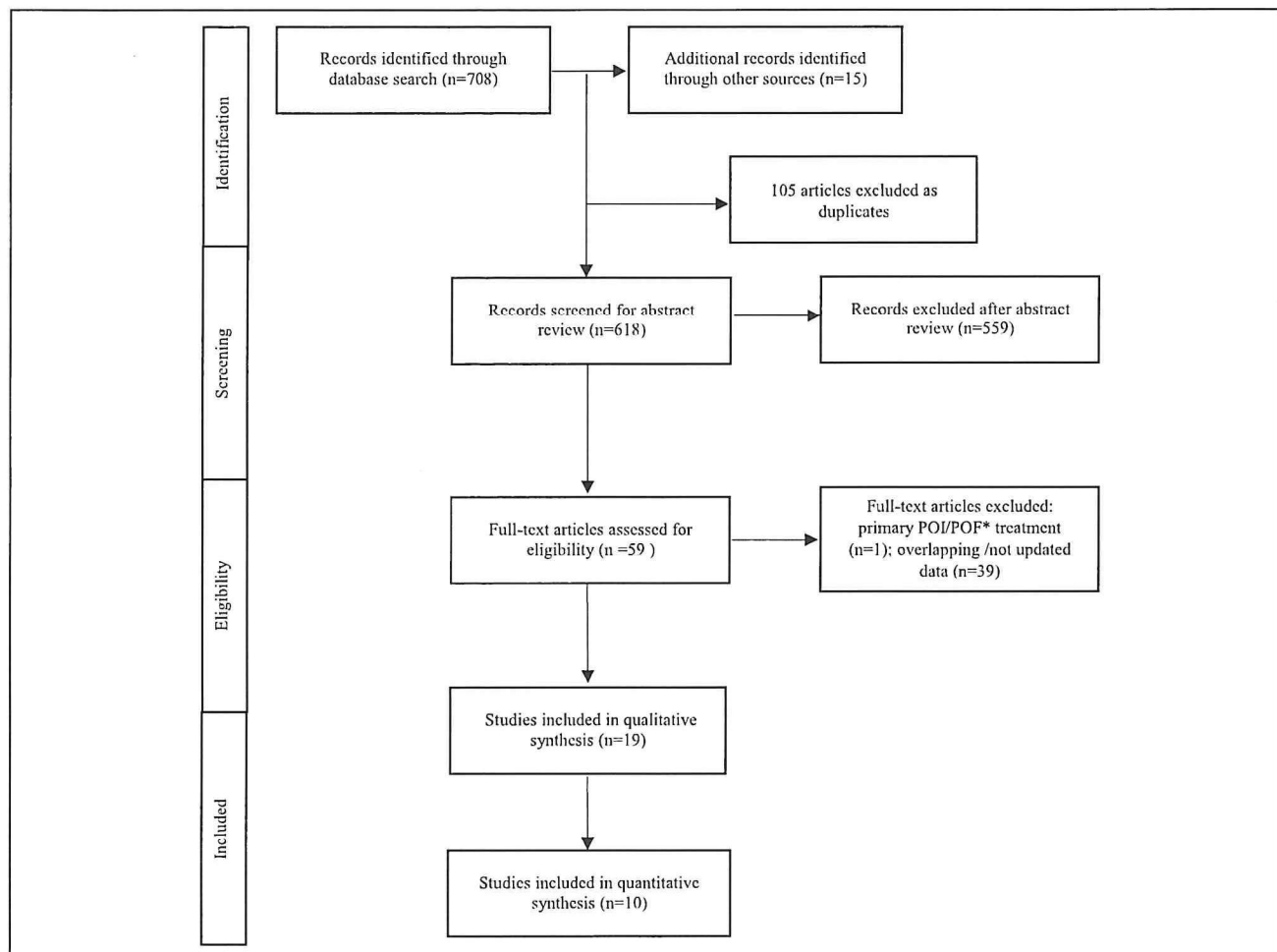
<sup>f</sup>Besides 1 patient with grafts to heterotopic sites only, 9 more women were excluded from the calculation of pregnancy rates for 2 different reasons: (1) they did not desire to conceive, they underwent OTT for endocrine reasons only (6) and (2) they had originally a hysterectomy and required surrogacy (2).

<sup>g</sup>One patient excluded from analysis because she lost to follow-up (she was diagnosed with a secondary malignancy of breast cancer 4 months after OTT).

<sup>h</sup>One patient excluded because had no pregnancy wish.

<sup>i</sup>Three patients and related pregnancies (1 CP and 2 LB) were excluded as they underwent OTT for the treatment of POI.

<sup>j</sup>49 patients with at least 1-year follow-up were included to estimate CPR, LBR, and endocrine function rate.



**Figure 1.** The study flow diagram. POF indicates premature ovarian failure; POI, premature ovarian insufficiency.

received OTT to restore fertility and 9 to restore endocrine function only. The mean age at OTC was 29.3 (6.5) years (range: 9–44 years) for patients who subsequently underwent OTT (Table 4). The mean age was 33.0 (5.7) years (range: 13.8–45 years) at the time of the first OTT.

Twenty-two patients received chemotherapy prior to OTC, including 5 who received an alkylating agent as part of the chemotherapy protocol. Of the cases where the reason for fertility preservation was specified, the indications for OTC included malignancy in 78% (160 of 205) and a nonmalignant condition in the remainder, where chemotherapy, pelvic radiation, hematopoietic stem cell transplantation, or sterilizing surgery was required.

In 4 retrospective reports<sup>17,23,27,28</sup> plus our own data that included 5 published<sup>8,9</sup> and 1 unpublished case, encompassing 113 OTTs in total, we were able to compare the mean age at OTC for those who had at least 1 LB or OG pregnancy versus those who did not. Those who conceived were significantly younger at the time of OTC, 27.1 (5.8) years, range: 13–38 years vs 31.0 (6.2) years, range: 14–44 years;  $P = .001$ .

The amount of tissue cryopreserved and transplanted varied. A bilateral oophorectomy was performed in 4 patients, while an entire ovary was removed and cryopreserved in 69 women. In 106 women, only ovarian biopsies were frozen. We could evaluate the percentage of tissue transplanted in 7 studies,<sup>11,18,19,30–33</sup> where 100% corresponds to the surface area of 1 ovary. On average, the transplanted tissue percentage was 34% (16%) in the first and 30% (7.7%) in the second procedure. Forty-five patients required 2 or more transplantations before achieving pregnancy. In all OTTs resulting in LBs, the tissues had been frozen with the slow freezing technique.

There were 84 babies born and 8 pregnancies were OG (Table 2); 8 women had 2 or more children after OTTs (Table 4). Of the 77 LB + OG pregnancies where the method of conception was specified, 37.6% was with assisted reproduction, while the remainder (62.3%) was spontaneous conceptions. While the details were not available in most cases, no anomalies were reported in 84 babies born after OTT, with the exception of a child with arthrogryposis.<sup>23</sup>

**Table 4.** Outcomes of Ovarian Tissue Cryopreservation and Autotransplantation.

Age at Cryopreservation (range)	29.3 (6.5) years, 9-44 years
Age at first transplantation <sup>a</sup> (range)	33.0 (5.7) years, 13.8-45 years
Maternal age at delivery	30.4 (4.2) years, 23-40 years
Gestational age at delivery <sup>b</sup>	38.2 (1.8) weeks, 33-41.2 weeks
Cumulative clinical pregnancy rate	57.5% (69/120) <sup>c</sup>
Cumulative live + ongoing pregnancy rate	37.7% (65/172) <sup>d</sup>
Fertility preservation rate (%)	28.4% (49/172) <sup>d</sup>
Endocrine function rate	63.9% (55/86) <sup>e</sup>

<sup>a</sup>Forty-five patients had repetitive transplants from the same cryopreserved tissue.

<sup>b</sup>Eight patients had consecutive deliveries after transplant.

<sup>c</sup>Based on Fabbri et al, 2014, Imbert et al, 2014, Jensen et al, 2015, Tanbo et al, 2015, Silber et al, 2015, Meirow et al, 2016, Van der Ven et al, 2016, and 6 cases of our group.

<sup>d</sup>Based on same cases as above plus Donnez, 2015.<sup>14</sup>

<sup>e</sup>Based only on cases where pretransplant postmenopausal status was verified: Kim, 2009, Fabbri, 2014, Imbert et al, 2014, Jensen et al, 2015, Tanbo et al, 2015, Meirow et al, 2016, Van der Ven et al, 2016, and 6 of our own cases (Oktay et al, 2010,<sup>10</sup> Oktay et al, 2016,<sup>11</sup> and 1 unpublished case).

### Transplantation Techniques

Of the 267 cases where the surgical technique for OTT was described, 195 were performed laparoscopically (3 with robotic assistance) and 72 via laparotomy. The definition of heterotopic versus orthotopic transplantation varied among the reports, but we considered those in the pelvis and with potential possibility of restoring spontaneous conception as orthotopic and others as heterotopic. Of the 228 OTTs where the grafting site was described, 195 were performed exclusively to an orthotopic site and 3 to a heterotopic site. Orthotopic and heterotopic sites were combined in 30 cases.

The variations in the orthotopic technique included grafting of ovarian cortical pieces to the pelvic sidewall,<sup>34</sup> underneath the cortex of a menopausal ovary,<sup>35</sup> and onto a denuded menopausal ovary.<sup>36</sup> Of note, some surgeons performed the transplantation in 2 steps with the intention to enhance revascularization at the site of grafting.<sup>31,37-40</sup> In addition, a method of the utility of a human extracellular matrix scaffold with robot-assisted transplant into bivalved contralateral menopausal ovary was reported in 2 patients, with both resulting in an LB.<sup>11</sup> The heterotopic sites included subcutaneous areas in the forearm<sup>41</sup> or abdomen<sup>8</sup> and retroperitoneal space under the abdominal wall.<sup>42</sup>

### Cumulative Clinical and LB + OG Pregnancy Rates After Ovarian Transplant

In only 7<sup>16,17,19,23,25,27,28</sup> and 8<sup>16,17,19,23,25,27-29</sup> reports, the numerators and denominators could be determined for CP and LB + OG pregnancy rates, respectively. We also included the data from our 3 cases (2 patients with heterotopic grafts plus 1 patient with no pregnancy wish were excluded) in the success rate analysis. The CP and LB + OG pregnancy rates were 57.5% (69 of 120) and 37.7% (65 of 172) per woman

undergoing OTT and desiring pregnancy. This translated into a 28.4% fertility preservation rate, with 49 of 172 women achieving at least 1 OG pregnancy or LB after an OTT. The majority of the LBs occurred after 2010, compared to the prior period (59 vs 10, respectively), indicating accelerated progress with the procedure.

### Restoration of Ovarian Endocrine Function

In most studies, at least the presence of a grown follicle was shown as evidence of follicular function. There was not uniform monitoring of the entire cycle to confirm continued follicle growth but most coupled this information with restoration of menstrual flow and in some cases occurrence of pregnancies as well.

For the restoration of ovarian endocrine function analysis, we included 9<sup>13,16,17,19,20,23,25,27,28</sup> studies where the denominator was known plus 6 of our own cases. While in 1 study with 41 patients<sup>19</sup> as much as 17% underwent OTT for the sole purpose of ovarian endocrine function restoration, this number was 3.5% when the entire cohort was considered. Overall, OTT restored endocrine function in 85.2% (144 of 169) of the patients as reported by the authors. However, when we considered only the 7 studies<sup>16,17,19,20,23,27,28</sup> which had defined ovarian insufficiency,<sup>43</sup> menopause (a minimum of 12-month amenorrhea), or bilateral oophorectomy before OTT, the ovarian endocrine function restoration rate was 63.9% (55 of 86).

In cases where ovarian function was not restored, the lack of endocrine restoration was attributed to the preexisting occult primary ovarian insufficiency and small quantity of ovarian tissue cryopreserved in 1 case (own data), absence of follicles in the frozen-thawed tissue in 3 cases,<sup>13</sup> harvesting and cryopreservation at a remote center without sufficient expertise in 3,<sup>13</sup> and advance age (range: 34-39 years) at OTC in 3 cases.<sup>19,23,28</sup> The mean age between those who had endocrine function restored versus not was similar, 28.5 (6.0) versus 31.0 (10.0),  $P = .89$ .

### Ovarian Tissue Transplant Longevity

Follow-up information on continued ovarian function was available in 75 women. In 24 cases, we had to base the ovarian transplant longevity on the time between the OTT and the last reported pregnancy as there was no further follow-up. We excluded cases with recurrent OTTs, as the indications for repeat procedures were in general unclear. We found that the mean graft longevity from the time of OTT was 26.9 (25.6) months (range: 4-144 months). However, this information may not be precise because of lack of specific information as to what constituted continued ovarian function and the absence of systematic follow-up in many cases.

### Discussion

In this meta-analysis, we sought to determine the overall success and efficiency of OTC as a method for fertility preservation and restoring ovarian endocrine function. We found that

among those who desired to restore fertility, cumulative live and OG pregnancy rate was nearly 38%, with approximately 1 of 3 to 4 women attempting OTT being able to have at least 1 child. Moreover, over 60% of those who received orthotopic ovarian transplants were able to conceive naturally, and based on strict criteria, in nearly two thirds, the ovarian endocrine function was restored for over 4 months. These data portray an encouraging and acceptable efficacy profile for this fertility preservation strategy.

As is the case for all meta-analyses, these encouraging data may have some limitations. While we based our success rate calculation on a respectable 67% of all known OTT cases where the denominator (total number of transplanted women) was known, we cannot rule out underreporting of failed cases by others. On the contrary side of the argument, since the acceptance of small reports of OTT for publication became more challenging in scientific journals, many successful attempts may also be underreported. Moreover, as follow-up is in general limited in OTT cases and as the cumulative pregnancy rates will likely increase over time, the true potential of OTT may also be underestimated. Nevertheless, despite the lack of uniformity in the cryopreservation expertise, surgical techniques, and patient populations among the centers, the success rates calculated in this study portray a relatively successful profile for OTC as a method of fertility preservation.

Another argument for keeping OTC in the experimental category is the lack of sufficient safety data. Data based on autopsy studies indicate that for the major indications of FP including lymphomas, the risk of occult ovarian metastasis is extremely low.<sup>44</sup> Breast cancer has a low to intermediate risk of ovarian involvement in early stages.<sup>45</sup> In the absence of clinical and radiological evidence of distant metastasis, ovarian involvement is extremely rare, and most cases could be detected by a thorough clinical and radiological evaluation.<sup>46</sup> Moreover, with the advent of aromatase inhibitor protocols and given that women with breast cancer often have sufficient time to undergo ovarian stimulation for oocyte or embryo cryopreservation before chemotherapy, these patients less commonly require OTC.<sup>47</sup>

In a recent report,<sup>19</sup> 2 of 11 patients with breast cancer relapsed locally after OTT, one during pregnancy prompting pregnancy termination. Another patient relapsed with Ewing sarcoma in the hemithorax. The relapse rate of the transplanted patients was 7% (3 of 41), which was similar to the relapse rate (7%, 48 of 691) among women who underwent OTC but did not yet undergo transplantation. Though this is a very small study, the authors concluded that the recurrences were not related to OTT.

In a 2015 report of 20 cancer survivors who underwent OTT,<sup>32</sup> 3 carried higher recurrence risk. These included non-Hodgkin lymphoma (highly malignant B-cell lymphoma, stage IVa; the patient was lost to follow-up 4 months after OTT), dysgerminoma, and serous adenocarcinoma of the ovary (International Federation of Gynecology and Obstetrics [FIGO] Stage IIIC). Some frozen-thawed tissue fragments were histologically screened prior to OTT in all 3 cases. The patient with a dysgerminoma showed no hormonal function or follicular

activity 11 months post OTT, and the graft was removed. The patient with ovarian cancer conceived and the removal of the ovarian tissue was planned 6 weeks after delivery. Authors surmised that OTT can safely be performed even in cases of higher risk of ovarian involvement, especially if the tissue removal is planned immediately after the pregnancy is successfully completed.

A 2016 case series<sup>23</sup> described 20 cancer survivors who underwent OTT. Of those, 3 were considered high risk for ovarian involvement. One woman with a history of Ewing sarcoma developed a secondary cancer in the breast, which was diagnosed during IVF treatment following OTT. The other 2 high-risk patients had chronic myelogenous leukemia and acute myelogenous leukemia (AML) at the time of the OTC. For safety, both patients had received chemotherapy before the tissue harvesting and before OTT; their tissues were screened via histological analysis, immunohistochemistry, PCR markers, as well as xenografting in the case of AML. There were no relapses in either of the women after the mean follow-up of 5 years and 8 months, respectively. The patient with the history of AML conceived and was in the third trimester of pregnancy at the time of the report. The tissues of the 2 other patients with leukemia who did not receive chemotherapy before OTC revealed cancer cells and they were excluded from undergoing OTT. The authors concluded that leukemia should not be a contraindication for OTT, especially when chemotherapy was performed prior to OTC because it eliminates malignant cells from the peripheral blood and the ovarian tissue. However, there is no definitive test to conclusively rule out the risk of reseeding of leukemia cells and this risk should be fully disclosed to all prospective patients.

At a recent abstract presentation,<sup>26</sup> 15 of 22 transplanted patients had a malignant disease before OTC. The diagnoses included Hodgkin lymphoma ( $n = 4$ ), non-Hodgkin lymphoma ( $n = 3$ ), sarcoma ( $n = 1$ ), cervical cancer ( $n = 4$ ), breast cancer ( $n = 1$ ), endometrial cancer ( $n = 1$ ), and ovarian cancer ( $n = 1$ ). The latter was a case with prior granulosa cell tumor, where metastasis was discovered on peritoneal surfaces and the diaphragm at the time of cesarean section for twin delivery. All macroscopic tumors and the ovarian tissue graft were resected at the operation. Histology confirmed metastasis in the peritoneum and diaphragm but not in the ovarian graft.<sup>48</sup> Patient remained disease free at 1-year follow-up.

Overall, these data suggest a good safety profile for OTT, though greater caution is required in cases where there is high risk of occult ovarian metastasis. Preoperative evaluation of representative ovarian samples by histological and molecular means may reduce the likelihood of introducing occult cancer cells to the patient, but the efficacy of such screening has not been tested in prospective studies. In such cases, full informed consent and participation in an institutional review board–approved research protocol may be preferred.

Because the number of babies born is currently at 84 after OTT with cryopreserved tissue, it is not possible to comment with a high degree of certainty on the well-being of children born. The baby with arthrogryposis was the only case description

of a malformation among children following OTTs. Among all the babies born and reported after OTT worldwide, this yields an anomaly rate of 1.2%, which is not different than the 1% to 2% major malformation rate seen in the general population. While there needs to be a larger number of births to have a reliable assessment, given that OTC primarily preserves primordial follicles that have much smaller volume and that are less differentiated than the M-II oocytes, it is unlikely that there will be a higher risk of genomic damage with OTC compared to M-II oocyte cryopreservation, which is now considered an established method of fertility preservation.

Finally, one of the main limitations of OTT is the variability of the longevity of the grafts. Our meta-analysis suggests that though the mean length of longevity is 24.9 months, there is a large variation. The variation in the longevity could be due to various factors including the age at OTC, history of chemotherapy exposure, the method of cryopreservation, the transplantation technique, and the amount of tissue transplanted. While the earlier data suggested that cellular insults from freezing and thawing are responsible for a small fraction of loss of primordial follicles, the most significant loss is during the ischemic phase following tissue transplantation.<sup>49</sup> While whole ovary cryopreservation followed by vascular reanastomosis met some degree of success in animal models,<sup>50</sup> this is not yet practically possible in humans.<sup>51</sup> Among the other potential improvements is the utility of vascularization-enhancing agents such as sphingosine-1-phosphate,<sup>52</sup> as well as extracellular matrix scaffolds and robotic surgery.<sup>9</sup> The clinical translation and the investigation of these and other potential approaches will be needed in larger trials to see whether OTT longevity can be uniformly extended.

## Conclusion

In conclusion, the efficacy of orthotopic transplantation with cryopreserved ovarian tissue has reached acceptable levels. While the data are still limited on the effectiveness of heterotopic ovarian transplant techniques, the current success data on orthotopic transplantation with cryopreserved tissue warrant some discussion on the current experimental status of this fertility preservation technique. Given that ovarian cryopreservation followed by orthotopic OTT is the only FP approach that can restore ovarian endocrine function and natural fertility, and given that current LB and ongoing pregnancy rates exceed 37% per women, we suggest that ovarian cryopreservation should be strongly considered among the other established fertility preservation options.

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