Ovarian transplantation with robotic surgery and a neovascularizing human extracellular matrix scaffold: a case series in comparison to meta-analytic data

Kutluk Oktay, M.D., Ph.D.,[a,b] Loris Marin, M.D.,[a,c] Giuliano Bedoschi, M.D.,[a,d] Fernanda Pacheco, M.D.,[b,e] Yodo Sugishita, M.D., Ph.D.,[a,f] Tai Kawahara, M.D.,[a,f] Enes Taylan, M.D.,[a] Carlo Acosta, B.S.,[b] and Heejung Bang, Ph.D.[g]

[a] Department of Obstetrics, Gynecology and Reproductive Sciences, Laboratory of Molecular Reproduction and Fertility Preservation, Yale University School of Medicine, New Haven, Connecticut; [b] Innovation Institute for Fertility Preservation, New York, New York; [c] Department of Women's and Children's Health, University of Padua, Padua, Italy; [d] Division of Reproductive Medicine, Department of Gynecology and Obstetrics, Faculty of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, Brazil; [e] Classiclínica, Porto Alegre, Rio Grande do Sul, Brazil; [f] Department of Obstetrics and Gynecology, St. Marianna University, Yokohama, Japan; and [g] Division of Biostatistics, Department of Public Health Sciences, University of California, Davis, California

Objective: To report our experience with robot-assisted (RA) autologous cryopreserved ovarian tissue transplantation (ACOTT) with the use of a neovascularizing extracellular matrix scaffold.
Design: Case series with meta-analytic update.
Setting: Academic.
Patient(s): Seven recipients of RA-ACOTT.
Intervention(s): Before or shortly after initiating chemotherapy, ovarian tissue was cryopreserved from 7 women, who then underwent RA-ACOTT 9.9 ± 1.8 years (range, 7–12 years) later. Perioperatively, they received transdermal estrogen and low-dose aspirin to enhance graft vascularization. Ovarian cortical pieces were thawed and sutured on an extracellular matrix scaffold, which was then robotically anastomosed to the bivalved remaining ovary in 6 cases and retroperitoneally (heterotopic) to the lower abdomen in 1 case.
Main Outcome Measure(s): Ovarian function return, the number of oocytes/embryos, aneuploidy %, live births, and neonatal outcomes were recorded. Graft longevity was compared with the mean from the meta-analytic data.
Result(s): Ovarian function returned 13.9 ± 2.7 weeks (11–16.2 weeks) after ACOTT, and oocytes were retrieved in all cases with 12.3 ± 6.9 embryos generated. In contrast to orthotopic, the heterotopic ACOTT demonstrated low embryo quality and an 80% aneuploidy rate. A recipient did not attempt to conceive and 2 needed a surrogate, whereas 4 of 4 delivered 6 healthy children, compared with 115 of 460 (25% pregnancy rate) from the meta-analytic data (n = 79). The mean graft longevity (43.2 ± 23.6/47.4 ± 22.8 months with/without sensitivity analysis) trended longer than the meta-analytic mean (29.4 ± 22.7), even after matching age at cryopreservation.
Conclusion(s): In this series, RA-ACOTT resulted in extended graft longevity, with ovarian functions restored in all cases, even when the tissues were cryopreserved after chemotherapy exposure. (Fertil Steril® 2022;117:181–92. ©2021 by American Society for Reproductive Medicine.)
El resumen está disponible en Español al final del artículo.

Key Words: Fertility preservation, cryopreservation, ovarian tissue transplantation, robotic surgery, extracellular matrix

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Reprint requests: Kutluk H. Oktay, M.D., Ph.D., Department of Obstetrics, Gynecology and Reproductive Sciences, Laboratory of Molecular Reproduction and Fertility Preservation, Yale University School of Medicine, 333 Cedar St, New Haven, Connecticut 06510 (E-mail: correspondence@fertilitypreservation.org).

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Autologous cryopreserved ovarian tissue transplantation (ACOTT) is a key fertility preservation technique. Autologous cryopreserved ovarian tissue transplantation was considered an experimental approach until recently, but it is now recognized as a valid clinical technique by the American Society for Reproductive Medicine and several other specialty societies worldwide (1, 2). The first ACOTT with previously cryopreserved tissue was performed in 1999 and reported in 2000 (3). In that orthotopic laparoscopic approach, ovarian cortical pieces were sutured onto a polycellulose scaffold (Surgicel; Johnson and Johnson, NJ) and then grafted subperitoneally on the pelvic side wall (3, 4). The procedure resulted in the restoration of ovarian endocrine and ovulatory functions, although the patient did not desire to conceive. Subsequent 2 decades of progress resulted in an estimated >130 live births (LBs) (5–8).

In a recent meta-analysis performed by our group, we found that the worldwide LB rate was 37.7% per woman after ACOTT, with at least 63.9% of the recipients having ovarian endocrine function of >6 months' duration. However, the average duration of ovarian function was only 26.9 months, a major limitation of the ACOTT procedure. On the basis of the cumulative experience from 2000 to 2017, we found that the mean graft longevity was 26.9 ± 25.6 months when approximately half of the cortex of an ovary was transplanted (8). Because ovarian cortical grafts have to acquire their blood supply through neovasculogenesis, which can take up to 10 days, a significant proportion of primordial follicles are lost by the time the graft is fully vascularized (7, 9). A xenografting study estimated that nearly two-thirds of the ovarian reserve is lost during the initial ischemic period after transplantation (10, 11). As our meta-analysis showed, these ovarian reserve losses are reflected in the limited longevity of ovarian autotransplants.

With the aim of improving ovarian transplant survival and longevity and addressing this *Achilles' heel* in fertility preservation by ovarian tissue cryopreservation, we previously developed and reported a surgical approach where the thawed cortical pieces were first sutured onto a neovascularizing extracellular matrix (ECM) scaffold (AlloDerm) and then grafted to the patient's remaining ovary with robot-assisted (RA) laparoscopy (12). That previous report included the short-term follow-up of the technique with 2 cases and only reported on 1 LB and 1 ongoing (OG) pregnancy. Here, we report our extended experience with RA ovarian autotransplantation with AlloDerm on 7 cases with additional LBs in 4 patients, with the hypothesis that our ovarian transplantation approach improves transplantation longevity compared with the historical data. To that end, we updated the longevity data from our 2017 meta-analysis (8).

MATERIALS AND METHODS

Patient Selection and Preoperative Preparation

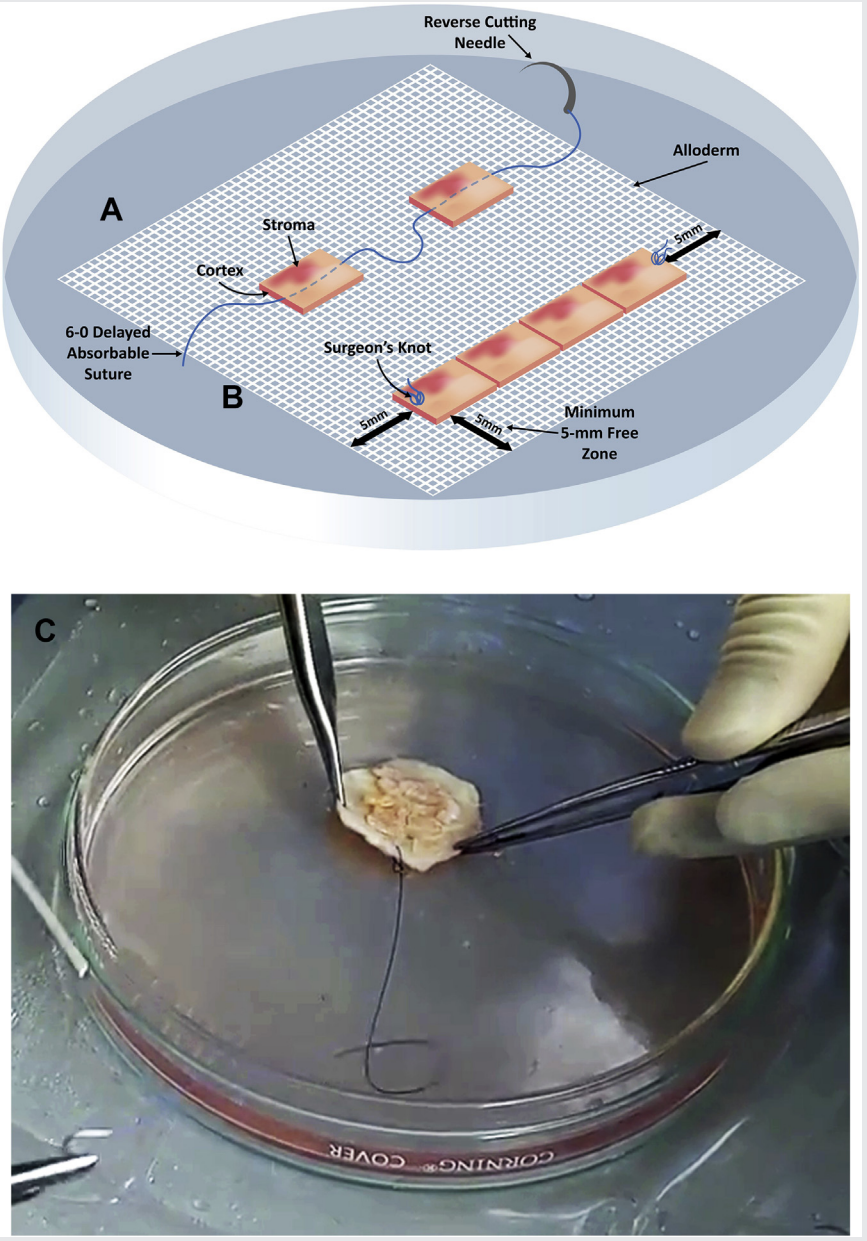
Seven consecutive women who provided informed consent for an institutional review board–approved ovarian tissue cryopreservation and transplantation study and who subsequently underwent ACOTT were included. The cases shared the following characteristics: slow freezing technique; ovarian failure caused by gonadotoxic treatments (chemotherapy and/or radiation therapy); perioperative pharmacologic support (transdermal estrogen 4 weeks prior and postoperative and baby aspirin 10 days prior); RA-ACOTT by a single surgeon (K.O.); use of an ECM scaffold (AlloDerm LifeCell Corp., Branchburg, NJ); and a minimum of 1-year follow-up. Of the 7 patients, cases 1–5 and 7 had already received some form of chemotherapy (Supplemental Table 1, available online), but ovarian cryopreservation was performed before they received the brunt of the gonadotoxic regimens, before hematopoietic stem cell transplantation (Supplemental Table 1).

The clinical characteristics of the participants are shown in Supplemental Table 1. Before the ovarian transplant, an extensive medical evaluation was performed to determine the suitability for the procedure. Medical clearances were obtained from the patients' medical and/or surgical oncologists or hematologists, where applicable. For every patient, a sample of the cryopreserved ovarian tissue was thawed and histologically analyzed for primordial follicle density and to rule out the presence of cancer cells (12). There are currently no established guidelines in determining the amount of tissue to thaw for transplantation. Because pre-ovarian tissue cryopreservation ovarian reserve assessment was not reliable due to 6 patients having already received chemotherapy and 1 having had a prior unilateral oophorectomy, the follicle density and patients' preferences empirically guided us in deciding how much tissue to thaw and transplant. Before the transplant, each patient was treated with transdermal estradiol (E2) for 4 weeks, and low-dose aspirin for 10 days to aid the graft revascularization, as we reported previously (12). Transdermal E2 was continued postoperatively, until the documentation of ovarian follicle growth.

Ovarian Tissue Cryopreservation, Thawing, and the Surgical Technique

All ovarian tissues were cryopreserved with the slow freezing method and thawed with the rapid thaw approach, as we previously reported (3, 12). All transplants were performed with robotic assistance, after suturing the cortical pieces onto an ECM scaffold (AlloDerm) under a surgical microscope (Fig. 1). We have previously described our surgical approach in detail, including in a video format (12, 13). When an ovary was present, it was bivalved, and the graft was anastomosed to that bivalved in situ ovary with the stromal side of the cortical pieces and the recipient site juxtaposed. If the ovary was too atrophic, the mesosalpinx was denuded to provide additional vascular surface, and then transplant was extended and sutured onto this surface (13). The use of perioperative baby aspirin ensured that early microclotting does not occur and the graft receives extended perfusion before revascularization. In one case, where total abdominal hysterectomy, pelvic lymph node dissection, and bilateral salpingo-oophorectomy were performed for pelvic cancer, the graft was transplanted to the abdominal wall retroperitoneally (13).

FIGURE 1



The technique of graft preparation with the extracellular matrix (AlloDerm) scaffold. (**A**) Ovarian cortical pieces are sutured to the "shiny" side of hydrated medium-thickness AlloDerm, with stromal surface facing up (shiny-to-shiny principle). A 6-0 delayed absorbable suture is first tied 5 mm from the matrix edge and then threaded through the stroma of the first piece, through the matrix, and then back through the stroma of the next piece, avoiding any piercing through the cortex. Depending on the dimensions of the recipient site, numerous pieces can be strung together, and several rows are created on AlloDerm in this fashion. A surgeon's knot is tied again when the row is completed. (**B**) The rows are created with at least 5-mm clearance from the edges so that this margin can be used to anastomose the graft to the recipient surface. (**C**) A photograph of a completed ECM graft, ready for transplantation. Medical illustration drawn by K.O.; artwork by G.B.

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Postovarian Transplant Follow-Up

In each case, we performed a color Doppler ultrasound within 7–10 days of the surgery to document blood flow and retention of the graft. Afterward, the patients were kept on hormone replacement with continuous transdermal E2 and cyclical micronized progesterone. Starting 4–6 weeks after the transplantation, the graft was evaluated by ultrasound examination every 2–4 weeks. Once the follicle growth was documented, hormone replacement was discontinued. We then periodically measured serum E2, follicle-stimulating hormone (FSH), luteinizing hormone (LH), and progesterone levels and performed pelvic ultrasound examinations to

monitor ovarian follicle growth. Because all patients preferred to cryopreserve embryos or oocytes in case their grafts prematurely ceased function and/or they needed to use a gestational carrier due to a past hysterectomy or radiation-induced uterine damage, they underwent ovarian stimulation and oocyte retrievals, as was described previously (12). In general, an early-follicular-start antagonist protocol was used with a recombinant FSH preparation, and the trigger was performed singularly with 250 μg of recombinant human chorionic gonadotropin (Ovidrel, Merck, NJ) or dually by adding 1 mg of leuprolide acetate. In general, we performed oocyte retrieval sooner (34–35 hours) after the trigger with ovarian transplant patients compared with similar-age nontransplant patients because of the higher risk of premature LH surge with the former. If a premature LH surge was encountered ahead of the trigger, we switched to a long protocol with low-dose luteal phase leuprolide acetate suppression. In the case of the heterotopic transplant, no antagonist was needed because our previous experience showed that the heterotopic transplants do not trigger premature LH surge. In such transplants, we administered gonadotropins and human chorionic gonadotropin near the graft area with the aim of direct delivery to the graft. A more detailed account of the ovarian stimulation approaches in ovarian transplantation can be found in a forthcoming book chapter (14). Three patients consented to preimplantation genetic testing for aneuploidy (PGT-A), and their embryos were tested via next-generation sequencing at the blastocyst stage. In 1 of those 3, a noninvasive PGT-A approach, through the evaluation of DNA in spent embryo culture media, was also used.

Update of the 2017 Meta-Analysis

To serve as a historical reference point, we updated our 2017 meta-analysis as of November 2020 from peer-reviewed manuscripts and meeting abstracts or through personal correspondence with the investigators (Supplemental Fig. 1, available online) (8). From this updated dataset, we identified the studies that reported graft longevity. From those, we only included studies ("inclusion criteria") with recipients who had clearly defined ovarian insufficiency or menopause (a minimum of 12-month amenorrhea) or underwent bilateral oophorectomy before ACOTT, and had at least 1 year of follow-up to match our case series dataset. Those who had repetitive ovarian transplant procedures were excluded. To summarize the current state of success from all ACOTT worldwide, we also updated the total number of LBs + OG pregnancies from the same meta-analytic dataset. To calculate the LB + OG pregnancy rate, only studies that specify the total number of transplant recipients (denominator) were included (8, 15–31). Although we included the 6 births from this report in the total LB + OG count worldwide, those were not included in the meta-analytic LB + OG pregnancy rate.

Statistical Methods

Data were analyzed using SAS version 9.4. Continuous variables are reported as mean/median with standard deviation (SD) and range, whereas categorical variables are presented as frequency and percentage. Because of the skewed distribution, the differences between groups were evaluated using the nonparametric Wilcoxon test. A sensitivity analysis was performed for longevity comparisons to account for a recipient with underlying premature ovarian insufficiency (POI). Additionally, longevity data were further compared with age matching.

Institutional Review Board Approval

All patients underwent ovarian tissue cryopreservation with informed consent under an institutional review board–approved ovarian tissue cryopreservation and transplantation protocol. The results of the transplant procedures were reviewed retrospectively (Yale IRB Protocol ID 2000030279). No institutional review board approval was required for the meta-analytic update because the raw data were obtained from publicly available publications.

RESULTS

Patient Population

On average, patients had an RA-ACOTT approximately 10 years (range, 7–12 years) after the cryopreservation. The mean ages at cryopreservation and transplantation were 22.0 ± 5.1 (range 16–32) and 31.9 ± 5.6 (range, 26–42) years, respectively. The indications for ovarian tissue cryopreservation are shown in Supplemental Table 1. In each case, after the initial primordial follicle density assessment and on the basis of the patient or the couple's goals, an amount of cryopreserved tissue equivalent of 41%–58% of the entire cortex of an ovary was thawed and transplanted. One woman received ovarian tissue transplantation after acute lymphoblastic leukemia. The test sample did not show any leukemic or malignant cells by expert histopathological analysis under light microscopy, and because no specific markers were available, no further tissue testing was recommended. All but 1 woman underwent orthotopic ovarian transplantation onto the remaining ovary with or without extension onto the mesosalpinx. In 1 case, ovarian autotransplantation was performed retroperitoneally to the lower abdominal wall. In that case, the patient had initially undergone unilateral salpingo-oophorectomy due to an ovarian mass, which was later diagnosed as an endometrioid carcinoma on permanent sections. Then, the patient was scheduled for total abdominal hysterectomy with salpingo-oophorectomy of the remaining adnexa. Although the peritoneal washings were positive for adenocarcinoma at the time of this second surgery, her remaining ovary appeared normal and approximately half of the cortex was harvested and cryopreserved. Ovarian biopsies from that ovary were negative for malignancy. Before the ovarian transplant, histological evaluation of the test tissue also did not reveal any malignant cells. However, because of the extensive pelvic scarring due to previous radical surgery and out of abundance of caution for a recurrence in the transplanted tissue, a heterotopic approach was chosen.

Ovarian Transplantation Outcomes

All transplants resulted in the resumption of E2 production, follicle growth, and ovulation. The mean length of time from

TABLE 1

Outcomes with robot-assisted ovarian transplantation.

No.	Age at transplantation (y)	Type of transplant	Time to function (wk)	Longevity (mo)	Follicle density (/mm^2) (mean ± SD)	% of ovary cortex grafted	No. of M2 oocytes retrieved	Fertilization rate	No. of nonarrested embryos/PGT-A results if any	Pregnancy outcome
1	35	Orthotopic	11	49	0.39 ± 0.17	50%	11	100%	11 (no PGT-A)	Live birth × 2 (frozen ET × 2)
2	29	Orthotopic	11.5	89 (ongoing)	1.28 ± 0.75	41%	12	92%	9 (no PGT-A)	Live birth × 2 (fresh ET, spontaneous)
3	33	Orthotopic	16	18	0.43 ± 0.64	50%	1	N/A	N/A (oocyte cryo only)	For endocrine function
4	26	Orthotopic	11	50 (ongoing)	1.17 ± 0.6	48%	24	100%	4 euploid 4 aneuploid 2 no PGT-A	Live birth (fresh ET)
5	26	Orthotopic	16	38.3 (ongoing)	0.40 ± 0.34	58%	30	93%	5 euploid 5 aneuploid	Live birth (frozen ET)
6	42	Heterotopic	15	36.3 (ongoing)	0.45 ± 0.58	46%	16	87.5%	3 aneuploid[a] (noninvasive PGT-A) 1 untested	Hysterectomy; GC being arranged
7	32	Orthotopic	23	22 (ongoing)	2.87 ± 0.88	58%	3	100%	0	RT damage: GC being arranged

Note: ET = embryo transfer; N/A = not applicable; GC = gestational carrier; PGT-A = preimplantation genetic testing for aneuploidy; RT = radiation therapy.

[a] The only euploid result came from an early blastocyst that was submitted in its entirety for PGT-A after it failed to expand and began degenerating. Hence, it was not included in the table.

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the day of transplant to the restoration of ovarian function, as defined by follicle growth and an accompanying increase in serum E2 levels, was 14.8 ± 4.3 weeks (range, 11–23 weeks). Of the 7 recipients, 4 attempted pregnancy as of the time of this report, and 2 are in the process of undergoing in vitro fertilization (IVF) to accumulate embryos to be used with a gestational carrier. The motive of the remaining recipient was to restore ovarian endocrine function (case 3, Table 1), but her ovarian follicle density was low, likely due to a pre-existing autoimmune polyglandular disease combined with prior chemotherapy exposure. She had inconsistent follow-up, and her ovarian function ceased 16 months after the transplantation. However, 50% of her ovarian cortical tissue remains in cryostorage, and consequently, she has the option to return in the future for a second ovarian transplant to restore her fertility.

Ovarian Stimulation Cycles and IVF Cycle Outcomes

All patients underwent ovarian stimulation and oocyte retrieval. However, case 3 dropped out after 1 retrieval for oocyte cryopreservation, and case 7 paused treatment after 3 retrievals for embryo cryopreservation. These retrievals resulted in 13.9 ± 10.5 (range, 1–30) metaphase II oocytes, 16 ± 9.7 (range, 3–30) when excluding case 3 with a single cycle of egg freezing. Of those who underwent IVF, the fertilization rate with intracytoplasmic sperm injection was 95.4 ± 5.4% (range, 87.5%–100%). This yielded 12.3 ± 6.9 (range, 2–22) day 3–5 embryos; 14.4 ± 5.2 (range, 9–22) day 3–5 embryos excluding case 7 who has not completed her attempts. Of those, 8.8 ± 2.8 (range, 4–11) were nonarrested embryos (Table 2). Of all embryos, 52% were high grade (grade 1 for cleavage stage or grade A for blastocyst stage), 39% were medium grade (grades 1.5 and 2 for cleavage and B for blastocyst), and only 9% were low grade (≤grade 3 for cleavage stage and C for blastocyst). Of the 7 cases, 3 had a total of 23 embryos analyzed with PGT-A at the blastocyst stage. This analysis revealed a mean aneuploidy rate of 56.5% of all embryos tested for all transplant types (50%–80%). When broken down on the basis of the ovarian transplantation type, 9 of 18 (50%) embryos from the orthotopic ACOTT and 4 of 5 embryos (80%) from the heterotopic ACOTT recipient were aneuploid. Three of the latter were tested using a noninvasive PGT-A approach, and the only euploid result came from an early blastocyst, which was submitted in its entirety for analysis after it failed to expand and began degenerating.

Pregnancy and Neonatal Outcomes

Of the 4 women who attempted pregnancy (cases 1, 2, 4, and 5), all conceived at least 1 child at the time of this report. Two patients, whose first pregnancies were reported previously, had 1 additional LB each during this study period (cases 1 and 2) (12). In case 1, both pregnancies were from a single frozen embryo transfer. In case 2, the first pregnancy was from a fresh embryo transfer, and the second one was spontaneously conceived. The remaining 2 patients (cases 4 and 5) had 1 child each at the time of this report. Case 4 conceived

TABLE 2

Summary outcomes for robotic ovarian transplantation and in vitro fertilization.

Variable	Mean ± SD (range)
Age at cryopreservation	22 ± 5.1 (16–32)
Age at transplantation	31.9 ± 5.6 (26–42)
Primordial follicle density (mm^2)	1 ± 0.9 (0.39–1.28)
% ovary transplanted	50.1 ± 6.2 (41–58)
Time to function (wk)	14.8 ± 4.3 (11–23)
Longevity (mo)	43.2 ± 23.6 (18–89)
Number of oocytes retrieved[a]	16 ± 9.7 (3–30)
Fertilization %[a]	95.4 ± 5.4 (87.5–100)
Number of embryos/patient with and without case 7 included[b]	12.3 ± 6.9 (2–22)/14.4 ± 5.2 (9–22)
Number of nonarrested embryos/patient with and without case 7 included[b]	7.3 ± 4.4 (0–11)/8.8 ± 2.8 (4–11)
% aneuploid embryos of tested	56.5% (50%–80%)

Note: Data are from 7 recipients, unless otherwise stated.
[a] Case 3 not included as transplanted for endocrine function only.
[b] Case 7 paused treatment after having 3 retrieval attempts.

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with single fresh embryo transfer, and case 5 conceived with single frozen embryo transfer (Table 3). Case 4 is currently being prepared for a frozen embryo transfer to attempt second childbirth.

All deliveries except 1 were at term with a mean gestational age of 38.7 ± 2.2 weeks, and there were no significant antepartum, postpartum, or neonatal complications. Case 2 underwent labor induction at 35 gestational weeks for mild preeclampsia. The infant was admitted to the neonatal intensive care unit because of prematurity but was discharged without any complications. Case 5 was induced at 37 weeks due to pregnancy-induced hypertension. Cases 2, 4, and 5 underwent C-section due to failure to progress, and case 2 delivered the second infant via repeat C-section due to the previous uterine surgery. At the time of this report, all children were developing without any health problems.

As a reference point, we updated the LB + OG pregnancy rates from our previous meta-analytic dataset. After the update from peer-reviewed manuscripts, meeting abstracts or through personal correspondence with investigators, and including the 6 LBs (6 infants) from our cohort, we identified 518 recipients who received 631 ACOTTs. Those resulted in 141 LBs (147 infants) + 11 OG pregnancies worldwide. To calculate the LB + OG pregnancy rate, we only included studies that specify the total number of transplant recipients (denominator) (8, 14–31). After the exclusion of 23 recipients who did not intend pregnancy, the LB + OG pregnancy rate per woman receiving a transplant was 25% (115 LB + OG in 460 recipients). Although all 4 women who attempted pregnancy had 1–2 LBs each in our series, a statistical comparison was not justified because of the small numbers.

TABLE 3

Pregnancy outcomes.

Child no.	ACOTT case no.	Mode of conception	Gestational age	Complications	Mode of delivery	Gender and birth weight	Neonatal complications
1	1 (First birth)	Frozen embryo transfer	39 wk 5 d	None	Vaginal delivery	Female 3,600 g	None
2	1 (Second birth)	Frozen embryo transfer	35 wk 4 d	Pre-eclampsia	Vaginal delivery after labor induction	Male 2,863 g	NICU admission for preterm birth, discharged without complications
3	2 (First birth)	Fresh embryo transfer	41 wk 3 d	None	C-section due to failure to progress	Female 3,827 g	None
4	2 (Second birth)	Spontaneous	38 wk 4 d	Pregnancy-induced hypertension at 38 weeks	Repeat C -section due to previous C-section	Male 3,175 g	None
5	4[a]	Fresh embryo transfer	40 wk 4 d	None	C-section due to failure to progress	Male 3,827 g	None
6	5	Frozen embryo transfer	37 wk 3 d	Pregnancy-induced hypertension at 33 weeks	C-section due to failure to progress	Female 3,629 g	None

Note: NICU = neonatal intensive care unit.
[a] Currently attempting for a second child via frozen embryo transfer.

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Graft Longevity

Five of the 7 transplants were still functioning at the time of this report. The mean longevity of the transplants was 43.2 ± 23.6 months (18–89 months). Of the 2 recipients whose grafts ceased function (cases 1 and 3), 1 (case 1) did so after 49 months (Table 1). This patient had received several courses of gonadotoxic chemotherapy before her ovarian tissue was cryopreserved. After her graft stopped functioning, she conceived her second child via a frozen embryo transfer. In case 3, the graft function ceased after 18 months. However, this patient was suffering from a polyglandular autoimmune disorder and had low pretransplant follicular density, presumably caused by autoimmune oophoritis and the 1 course of gonadotoxic cyclophosphamide, hydroxydaunorubicin, vincristine sulfate, prednisone regimen (Supplemental Table 1) she received before ovarian tissue cryopreservation. When case 3, whose premature graft failure was likely due to preexisting autoimmune POI was excluded, the graft longevity was 47.4 ± 22.8 months (22–89 months).

To assess whether our RA ovarian transplantation approach with AlloDerm improves ovarian graft longevity, we compared the mean ovarian function length from this case series to the data from the updated meta-analytic database (8, 15, 16, 18, 21, 22, 24–28, 30–56). After the exclusion of 17 cases with repetitive transplants, these updated data showed a mean/median (SD) longevity of 29.2/22.9 (22.6) months from 79 transplants worldwide. In comparison, the graft mean/median longevity from the 7 recipients was 43.2/38.3 (23.6) months in our cohort. The 2-group comparison of longevity showed a trend for extended longevity with the RA ovarian transplant technique compared with those previously reported (2-sided $P=.057$ from the Wilcoxon test). In a sensitivity analysis after excluding case 3 in our cohort who had pre-existing autoimmune POI (sample sizes of 79 and 6, respectively), the graft longevity was 47.4/43.7 (22.8) months, and the Wilcoxon test yielded $P=.015$ for the same comparison.

Age at cryopreservation can affect ovarian transplant longevity. Although we lacked sufficient sample size for more definitive confounder adjustment, we performed an exploratory comparison with age-matched samples. In the original samples, the mean age at cryopreservation was 22.0 (n = 7) in our case series vs. 28.6 in the meta-analytic data (n = 79), with similar SDs of 5–6 (Supplemental Table 2, available online). After matching age, both groups had a mean age of 22 years, and the mean (SD) longevity was 33.7 (22.1) months in the meta-analytic group (n = 28) vs. 43.2 (23.6) months in our case series (n = 7), with $P=.21$. When we excluded case 3 in our group (who had preexisting autoimmune POI), the mean (SD) longevity was 47.4 (22.8) in our case series (n = 6), where the 2-group comparison yielded $P=.07$ (Supplemental Table 2). Thus, a robust trend for a longevity of over 10 months longer was demonstrated in our case series despite reduced statistical power because of a lower sample size after age matching.

Of note, this improvement was observed even though a statistically significantly higher proportion of our patients received chemotherapy before ovarian tissue cryopreservation compared with those in the age-matched samples (86% [6/7] in our series vs. 27% [7/26] in the meta-analytic data; $P=.008$).

DISCUSSION

Here, we report the first extended series of RA-ACOTT with an ECM scaffold (AlloDerm). In this cohort, the procedure resulted in the restoration of ovarian function in all recipients. Despite the relatively small numbers and the fact that 6 of 7 recipients had already received some chemotherapy before the tissue cryopreservation, the mean longevity exceeded that of previously reported procedures by at least 10 months (8). The ovarian transplants resulted in embryo generation and/or 6 LBs in all recipients who intended pregnancy. Although we suspect that the high success with our approach, relative to the meta-analytic data, is likely because of the improved surgical precision with robotic surgery and potential vascularization re-enhancing features of AlloDerm, numerous other factors could have also contributed (12). These may include our unique pharmacologic preoperative priming and the fact that all procedures were performed by the same team with extensive experience.

There are 2 sides to the estrogen priming before and during ovarian transplantation. One may be concerned that E2 may lower serum FSH levels and, hence, negatively affect revascularization as a rodent study suggested that gonadotropins aid in that process (57). However, our preoperative transdermal estrogen treatment (Climara 0.1 mg/wk) is administered for a brief period of time (approximately 4 weeks) and does not significantly lower FSH levels in these patients with established ovarian failure, and hence, our patients may benefit from both. Estrogenization may also enhance reproductive tissue quality including those of the recipient areas, in addition to providing neoangiogenic effects. Furthermore, recent data in rodents show that estrogen action reduces primordial follicle activation, and hence, estrogen replacement therapy may prevent ischemia-triggered primordial follicle activation and depletion, until full vascularization is achieved (58).

Although encouraging, our report also identified areas that need further investigation. First, in the only case of a heterotopic ovarian transplant in this series, we consistently encountered poor embryo development. After 11 months of attempting IVF, we could not obtain a blastocyst. In the hopes of improving oocyte quality and following the encouraging preliminary results of a primate study, we performed a laparoscopic omental flap procedure on this patient to improve graft vascularization (Supplemental Video 1, available online) (59). After the omental flap procedure, we were able to cryopreserve a 7-cell day 3 embryo and 2 blastocysts from this patient, although the latter were aneuploid. Further studies will be needed to determine if a routine vascular omental flap is beneficial to improve heterotopic ovarian transplant outcomes. In our prior experience with heterotopic ovarian transplants, we also encountered poor embryo development, although those grafts were implanted subcutaneously, in either the forearm or lower abdomen (60, 61). Because of the medical risks with invasive surgery, we recently

performed a subcutaneous heterotopic ovarian autotransplantation under local anesthesia (the third procedure that is shown in the video, which is available at https://youtu.be/CqMl_WXtCmM) (13). In that case as well, repeated retrievals only resulted in poor-quality oocytes and embryos. In the current robotic technique, the tissues are placed retroperitoneally below the rectus abdominis muscle, a deeper location than that of subcutaneous transplants. Live births have been reported with a similar heterotopic ovarian transplantation technique, and it is plausible that the currently cryopreserved embryos will result in a conception when transferred to a gestational carrier in the patient reported here (54, 55).

For the RA heterotopic transplant case reported here, there could be other reasons for the poor response, including the relatively advanced age of the patient at the time of the cryopreservation and the potential negative impact of pelvic cancer on oocyte quality. Although the ovary where the cryopreserved tissues originated from was found to be free of cancer by gross and microscopic examination, peritoneal washings were positive for adenocarcinoma. Consequently, it is possible that the tumor cells induced inflammatory changes that had a detrimental effect on the germ cell pool (62). It is also possible that the blood flow, vascularization patterns, and paracrine/endocrine factors may not be optimal for oocyte development in heterotopic sites. Future studies will determine whether heterotopic ovarian transplants should have room in fertility restoration or whether they should be reserved to restore ovarian endocrine function only.

One advantage of heterotopic ovarian transplantation is easy access to the transplanted tissue for monitoring and removal, should there be a risk of disease recurrence. In case 6, we are regularly monitoring the graft volume and anatomy for any signs of cancer recurrence, and the graft may be easily removed once the pregnancy goal is achieved. However, in instances where ovarian tissue was cryopreserved in the presence of a malignant tumor in the same ovary, removal of the graft soon after the achievement of fertility is recommended (54). In fact, in the first report of LB after abdominal wall ovarian transplantation, the graft was removed during the C-section delivery, and it was found to contain local recurrence of a granulosa cell tumor (54).

In this manuscript, we reported the live delivery of a child from a woman who received ovarian tissue transplantation after acute lymphoblastic leukemia (Supplemental Table 1). Concerns have been expressed in autotransplanting cryopreserved ovarian tissue from women with history of acute leukemias because these tissues may harbor leukemic cells (63). However, ovarian cryopreservation is typically performed (as was the case with our patient) after the initial consolidation treatment, when the patient is in remission and about to receive gonadotoxic preconditioning chemotherapy for hematopoietic stem cell transplantation. During the remission, there are no circulating leukemic cells (31, 64). In fact, a quantitative polymerase chain reaction analysis showed that there is no or negligible degree of malignant cell contamination in ovarian tissues of patients with acute leukemia who were in remission at the time of the harvesting; the xenografting of these tissues did not transmit the disease to immunodeficient mice. (64). In a case reported by Shapira et al. (31) in a leukemia survivor, there was no recurrence during the 28-month follow-up, and the patient had 2 children from the transplant. Sonmezer et al. (56) reported an acute lymphoblastic leukemia survivor who underwent multiple IVF cycles after ovarian transplantation. The recipient conceived, and the graft was removed during the C-section 25 months after the transplantation. Histopathological examination revealed no leukemic cells in the excised graft, and because there were no specific cancer markers available, no further testing was recommended by the pathologist. However, when there are specific markers available for a given leukemia (e.g., Philadelphia chromosome in chronic myeloid leukemia), a polymerase chain reaction or fluorescence in situ hybridization analysis can be performed with such markers (31). Some proposed xenografting experiments, but such experiments are not only costly, but their clinical usefulness has not been established (64). In our case, the patient delivered an infant after the orthotopic ovarian tissue transplantation, and the patient is currently disease-free for nearly 4 years after the transplantation. Although xenografting studies and a limited number of clinical observations suggest that performing ovarian tissue cryopreservation in patients with acute leukemia during remission is safe, larger clinical studies will be needed to confirm this conclusion.

Although on the basis of a small number of cases, we observed seemingly high aneuploidy rates from embryos in women whose ovarian tissues were cryopreserved at a relatively young age. We performed PGT-A on the embryos of the recipients who were 16, 18, and 32 years old, respectively, at the time of tissue cryopreservation. Of the 21 embryos tested, a mean of 56.5% were aneuploid (50%–80%). The aneuploidy rate was 80% for the heterotopic transplant case and 50% for the orthotopic transplant patients. A recent large study indicated that the aneuploidy rates may show a biphasic age pattern, highest in extremes of reproductive life span (65). In that study, the rate of aneuploidy by PGT-A was 44.4% in embryos from women with a mean age of 22 years. Consequently, the aneuploidy rate for the 2 recipients in our series whose tissues were cryopreserved at ages 16 and 18 years does not seem to be increased.

However, an 80% aneuploidy rate appears to be higher than expected for the recipient (case 6) who underwent ovarian tissue cryopreservation at the age of 32 years. The study by Franasiak et al. (65) reported an aneuploidy rate of 31% for a woman aged 32 years. Previous studies suggest that the ovarian milieu can affect meiotic integrity and oocyte quality (66, 67). Thus, it is possible that the heterotopic environment impairs oocyte development and quality. We prefer performing PGT-A in ovarian transplant patients, especially for those planning on using a gestational carrier, to increase the likelihood of implantation and reduce the number of attempts. Although the suitability of PGT-A for improving IVF outcomes has been questioned in young women, because of the high incidence of aneuploidy in ovarian autotransplant recipients, it may be a strategy that is worth further exploration (68).

Another clinically relevant observation from this report is that despite the chemotherapy exposure before ovarian

cryopreservation, 4 transplant recipients cryopreserved numerous good-quality embryos and had 1–2 LBs. Of those, 3 had received alkylating agent regimens. While some questioned the feasibility of tissue cryopreservation after gonadotoxic chemotherapy exposure, our data show that sufficient primordial follicle reserve survives in young women to enable successful outcomes after ovarian transplants (69, 70). This could be because of the presence of large ovarian reserve in young women as well as the higher ability of the primordial follicle oocytes to repair chemotherapy-induced DNA damage (71–75). Hence, ovarian cryopreservation should not be routinely withheld from young women who have already begun receiving gonadotoxic chemotherapy. However, in such patients, primordial follicle density should be assessed to determine the feasibility before ovarian autotransplantation.

Although this case series reports on novel advances in transplantation techniques, it is based on a relatively small number of recipients at a single center. In addition, age at cryopreservation and whether the ovary was exposed to previous gonadotoxic chemotherapy can affect the graft longevity. Although we attempted an exploratory analysis with age-matched data, this reduced the sample size and statistical power. Of note, after age matching, we still found a trend for an extended longevity of 10–14 months with our approach, despite the fact that a statistically significantly larger proportion of our patients had already received gonadotoxic chemotherapy before tissue cryopreservation compared with the age-matched meta-analytic controls. Thus, the trend for extended longevity with our approach on the basis of historical controls justifies larger prospective studies to further test the ACOTT technique reported herewith. However, ovarian transplantation is still a relatively rare procedure because of its currently low utilization, and thus, such prospective studies may not yet be feasible. Despite more than 2 decades of progress, the number of reported LB + OG pregnancies is 158 worldwide, on the basis of our update as of November 2020, including the livebirths we reported here. In the meantime, our series represents a meaningful patient population relative to the entirety of published reports on ovarian transplantation success. However, given that ACOTT is no longer considered experimental by the American Society for Reproductive Medicine, we expect its more widespread use (1). In that context, we believe that this report will provide guidance to those who are planning to establish an ovarian transplantation program in their centers and will stimulate further research to improve ACOTT success.

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Trasplante ovárico con cirugía robótica y una matriz extracelular humana de neovascularización: una serie de casos en comparación con datos meta-analíticos.

Objetivo: Reportar nuestra experiencia con trasplante de tejido ovárico autólogo criopreservado (ACOTT) asistido por robot (RA) con el uso de una matriz extracelular estructurada de neovascularización.

Diseño: Serie de casos con actualización meta analítica.

Escenario: Académico.

Paciente(s): Siete receptoras de RA-ACOTT.

Intervención(es): Antes e inmediatamente después de iniciar quimioterapia, el tejido ovárico fue criopreservado en 7 mujeres que fueron sometidas a RA-ACOTT 9.9 ± 1.8 años (rango 7-12 años) después. En el periodo perioperatorio, recibieron estrógenos transdérmicos y aspirina a bajas dosis para favorecer la vascularización del injerto. Segmentos de corteza ovárica fueron descongelados y suturados en una matriz extracelular estructurada, la cual posteriormente fue anastomosada robóticamente al ovario remanente en 6 casos y retroperitonealmente (heterotópico) en el abdomen inferior en un caso.

Medida(s) de resultado(s) principal(es): Recuperación de la función ovárica, número de ovocitos/embriones, % de aneuploidía, recién nacidos vivos, y resultados neonatales fueron registrados. La longevidad del injerto se comparó con la media de los datos meta analíticos.

Resultado(s): La función ovárica se recuperó 13.9 ± 2.7 semanas (11-16.2 semanas) después del ACOTT, y se recuperaron ovocitos en todos lo casos con 12.3 ± 6.9 embriones generados. En contraste con los trasplantes ortotópicos, los ACOTT heterotópicos presentaron baja calidad embrionaria y 80% de tasa de aneuploidía. Una receptora no intentó gestación y 2 necesitaron subrogación uterina, mientras que 4 de 4 tuvieron 6 recién nacidos saludables, comparados con 115 de 460 (25% de gestación) de los datos meta analíticos (n=79). La media de longevidad del injerto (43.2 ± 23.6/47.4 ± 22.8 meses con/sin análisis de sensibilidad) tendió a ser mayor que la media de los datos meta-analíticos (29.4 ± 22.7), incluso después de emparejar por edad al momento de la criopreservación.

Conclusión(es): En esta serie, RA-ACOTT presentó una longevidad del injerto extendida, con recuperación de la función ovárica en todos los casos, aun cuando los tejidos se criopreservaron después de exposición a quimioterapia.