Articles

Embryo development after heterotopic transplantation of cryopreserved ovarian tissue

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Summary

Background Cancer treatments, including chemotherapy, radiotherapy, and radical surgery, can induce premature menopause and infertility in hundreds of thousands of women of reproductive age every year. One of the ways to possibly preserve fertility before these treatments is to cryopreserve ovarian tissue for later transplantation. We aimed to restore fertility by cryopreservation and transplantation of ovarian tissue.

Methods Ovarian tissue was cryopreserved from a 30-year-old woman with breast cancer before chemotherapy-induced menopause, and this tissue was transplanted beneath the skin of her abdomen 6 years later.

Findings Ovarian function returned in the patient 3 months after transplantation, as shown by follicle development and oestrogen production. The patient underwent eight oocyte retrievals percutaneously and 20 oocytes were retrieved. Of the eight oocytes suitable for in-vitro fertilisation, one fertilised normally and developed into a four-cell embryo.

Interpretation Fertility and ovarian endocrine function can be preserved in women by long-term ovarian tissue banking.

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Introduction

Chemotherapy, radiotherapy, and radical surgery can result in ovarian failure and infertility in hundreds of thousands of women of reproductive age with cancer in the USA alone. Thousands more patients receive chemotherapy and radiation for the treatment of disorders such as collagen vascular, haematological, and idiopathic diseases.[1] Women of reproductive age and their partner can undergo a cycle of in-vitro fertilisation (IVF) to cryopreserve their embryos before chemotherapy.[2] However, most cancer patients do not have sufficient time to complete the necessary ovarian stimulation for IVF. This option is also not acceptable for single women who do not want to use donor sperm and for children. Oocyte cryopreservation can be considered for adult single women who understand that pregnancy rates are low with this experimental strategy, but this approach, similar to embryo freezing, also needs several weeks of ovarian stimulation. Against this background, an experimental ovarian cryopreservation and autotransplantation technique has been developed. After success by other researchers in animals,[3,4] we reported ovarian function after orthotopic transplantation of frozen-thawed and heterotopic grafting of fresh human ovarian cortical pieces.[5,6] In all women, ovarian function was restored, at least temporarily. In one patient with fresh ovarian tissue that was transplanted subcutaneously in the forearm, an intact oocyte was retrieved percutaneously but it did not become fertilised. In this study we aimed to restore fertility by subcutaneous transplantation of frozen banked ovarian tissue.

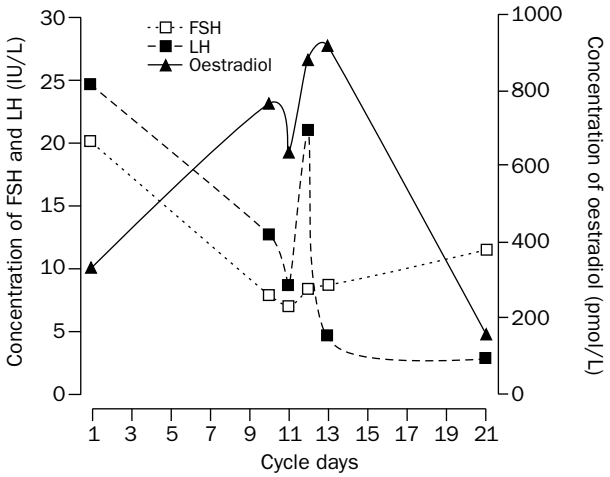

Figure 1: **Resumption of ovarian function after ovarian transplantation**
Peak oestradiol concentration accords with that of a typical cycle and is accompanied by a luteinising hormone (LH) surge, but no FSH increase is seen.

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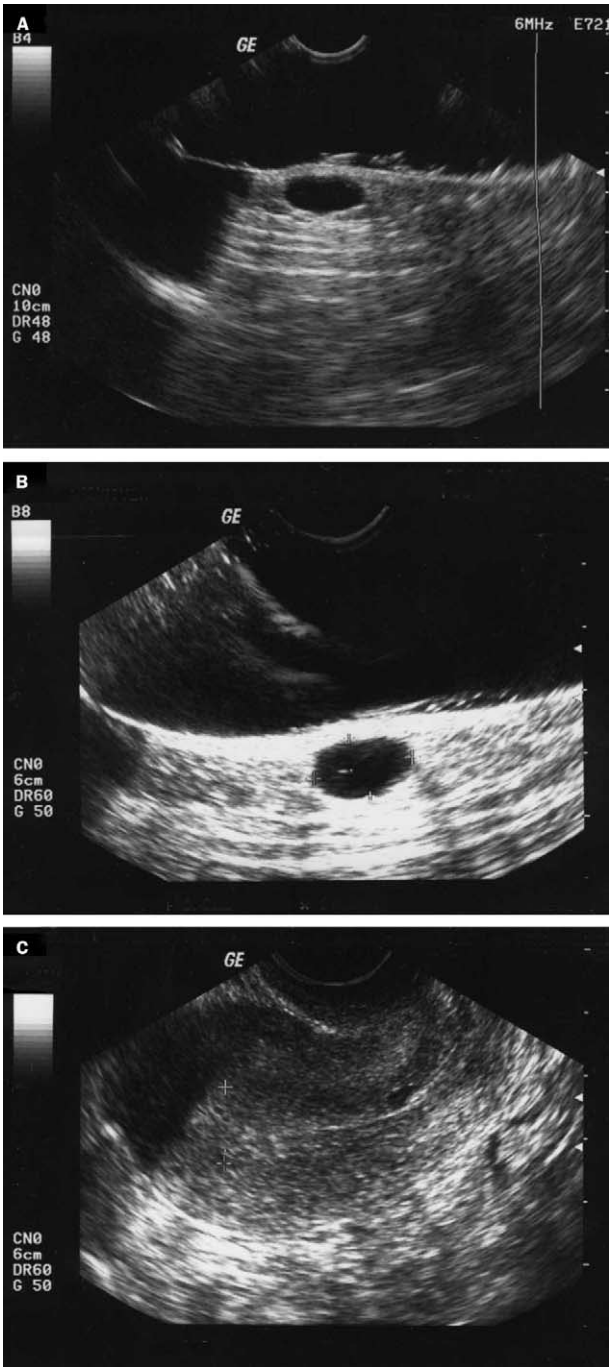

Figure 2: **Subcutaneous ovarian follicle growth**
(A) Day 12 follicle. (B) Day 14 follicle. (C) Trilaminar endometrial development in response to oestrogen production.

Methods

Patient's information

The patient was a woman who was diagnosed with stage IIB breast cancer at age 30 years. Before she received high-dose gonadotoxic chemotherapy, including cyclophosphamide, we removed one ovary laparoscopically. Cortical pieces, which invariably contain some stroma, were cryopreserved with a slow freezing protocol, with dimethylsulfoxide as a cryoprotectant.[7] After completing her chemotherapy and bone-marrow transplantation, the patient ceased to menstruate, and a raised follicle-stimulating hormone (FSH) value of 117 IU/L confirmed menopause. The study was approved by the institutional review board of Weill Medical College of Cornell University and the patient gave written informed consent for experimental procedures.

	Stimulation dose (FSH/HMG) and duration*	Peak oestradiol (pmol/L)†	Follicle size (mm)†	Oocyte
Retrieval				
1	None	888	10·9	GV
3	3675/2175, 13 days	980	14·2	FZ
			10·7	Degenerated
			8·9	GV
			7·3	FZ
4	None	1093	9·1	FZ
5	3225/1725, 11 days	2257	14·5	FZ
			13·1	M-I
			9·5	FZ
			6·4	GV
6	2025/2475, 10 days	1868	12·8	Mature
			10·8	FZ
			8·4	FZ
7	2100/2400, 10 days	921	11·8	Mature
			10	GV
			6·7	Degenerated
8	2025/1500, 10 days	987	11	Degenerated
			9·9	Mature
			9·8	GV
			6·4	GV

HMG=human menopausal gonadotropins. FZ=fractured zona pellucida. GV=germinal vesicle. M-I=metaphase-I. No oocytes were recovered during the second retrieval. Embryos were generated after in-vitro maturation during the fifth (M-I oocyte) and eighth (GV oocyte) IVF cycles. Immature oocytes are further categorised as GV or M-I stage. *Numbers represent total dose of FSH and HMG in IU. †On the day human chorionic gonadotropin was injected.

Stimulation type, peak oestradiol concentration, and follicle sizes on day of human chorionic gonadotropin administration, and oocyte retrieval outcome from ovarian transplant

Procedures

6 years after ovarian cryopreservation, the patient's tissues were transported to our laboratory. We thawed one vial of tissue and tested the piece histologically to rule out ovarian involvement with cancer and to establish the density of primordial follicles. On the basis of follicle density and the number of pieces available, we estimated that the patient had about 11 000 follicles, which could potentially last for a year.[8] Under local anaesthesia, we implanted all 15 ovarian cortical pieces—with sizes ranging from 5×5×1 mm to 15×5×2 mm—beneath the skin of the patient's lower abdominal wall with a suture pull-through technique, as described.[9,10] Before every cycle of ovarian stimulation, we achieved ovarian suppression by administration of gonadotropin-releasing hormone antagonist or agonist. We did ovarian stimulation with a combination of FSH and human menopausal gonadotropins. Oocyte maturity was triggered by 250 μg of recombinant human chorionic gonadotropin (Ovidrel, Serono, Norwell, MA, USA), and we undertook oocyte retrieval 36–40 h later. We gave progesterone supplements in every cycle; thus, we could not fully assess spontaneous luteinisation. For IVF, the intracytoplasmic sperm injection technique was used for all but one oocyte. We used a complex, non-commercial sequential culture medium, which we prepared every week onsite at the Weill Cornell facility. We matured and fertilised the oocyte and cultured the embryo in phase 1 of this medium until about 48 h after injection. We did preimplantation genetic diagnosis by fluorescence in-situ hybridisation, as described.[11]

Role of the funding source

The sponsor had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

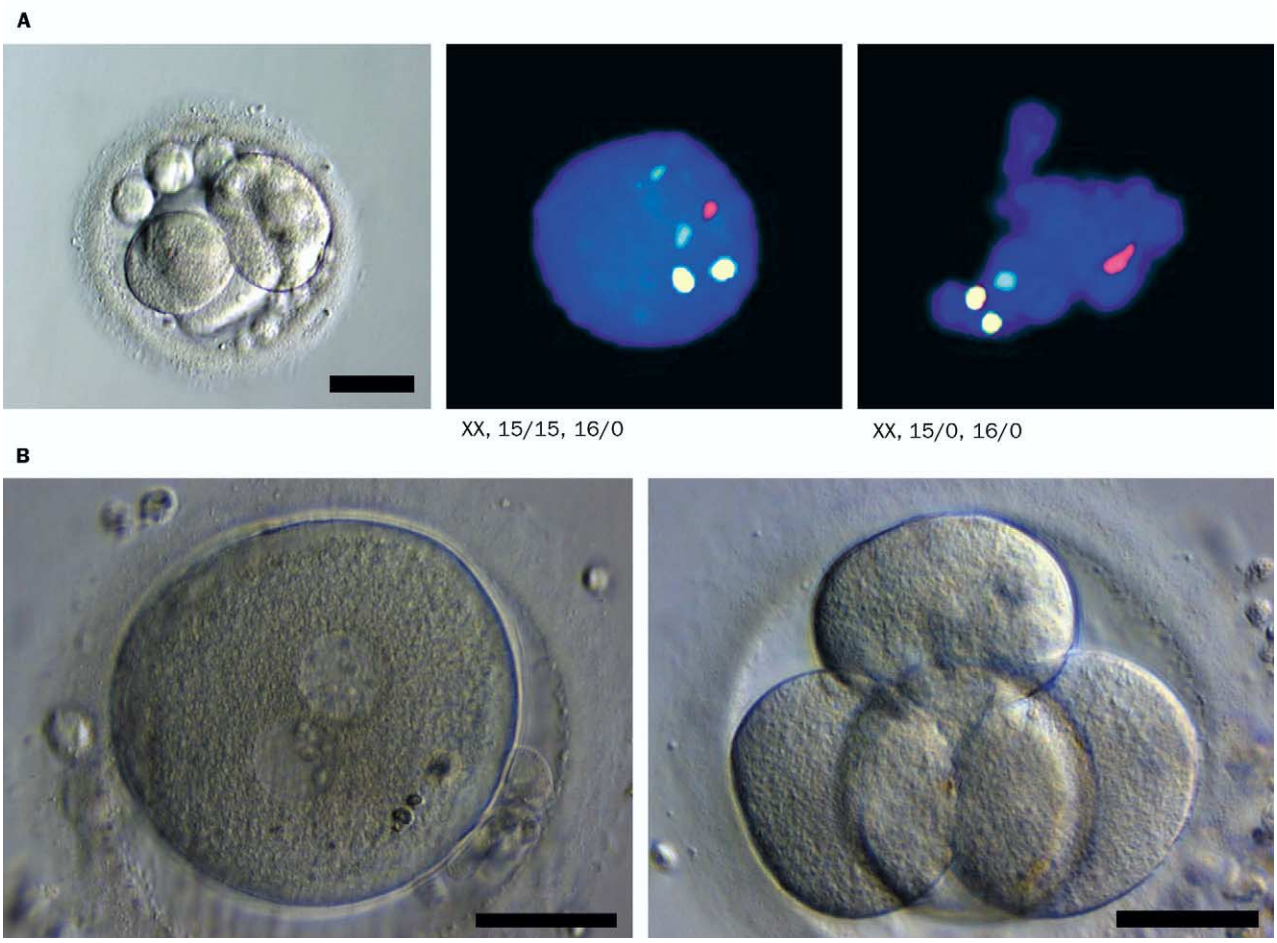

Figure 3: **Embryo development with in-vitro fertilised oocytes retrieved from transplanted ovarian tissue**
(A) Abnormal three-cell embryo with unequal blastomeres and extensive fragmentation (left). Multicolour fluorescence in-situ hybridisation shows that one cell was aneuploidic for chromosome 16 (middle) and the other for chromosomes 15, 16 (right), and 13, 18, 21 (not shown). X=yellow; 15=green; 16=orange. Scale bar=50 μm. (B) Fertilisation and normal embryo formation after intracytoplasmic sperm injection. 18 h later, a two-pronuclear stage embryo was seen (left), which progressed to a high-grade four-cell stage embryo with minimal fragmentation (right). Scale bar=50 μm.

Results

85 days after ovarian transplantation, the patient noticed a pea-size lump at the transplant site. Resumption of ovarian endocrine function was confirmed by raised oestradiol concentrations (477 pmolL), by lowered FSH concentrations after 11 days (7·9 IU/L; figure 1), and by demonstration of ovarian follicle development by ultrasound (figure 2). Follicles could also be palpated underneath the skin. Simultaneous ultrasound monitoring of the menopausal ovary did not show ovarian follicle development in any of the cycles.

We did eight consecutive percutaneous oocyte retrievals over the next 8 months, six after ovarian stimulation (table). Of the 20 oocytes obtained from the patient's transplanted tissue, eight were suitable for IVF with her husband's sperm. Three of these were mature at retrieval and five had to be matured in vitro. Although mature oocytes did not fertilise, two of the oocytes that were matured in vitro were fertilised via intracytoplasmic sperm injection. One embryo showed abnormal morphology and its growth halted at the three-cell stage (figure 3, A). Fluorescence in-situ hybridisation analysis was done on two cells,[11] which showed an XX embryo with aneuploidy for several chromosomes. A second oocyte that matured in vitro in 24 h showed signs of normal fertilisation (two pronuclei) 18 h after intracytoplasmic sperm injection, which then progressed to a high-grade[12,13] four-cell stage embryo 24 h later (figure 3, B). The embryo was then transferred to the patient's uterus.

Discussion

Development of a morphologically normal four-cell embryo indicates that ovarian follicles with viable and functional oocytes can develop in ovarian tissue thawed and transplanted to a heterotopic location. In addition to normal morphological findings, the rate of in-vitro maturation[14] and growth to four-cell stage accords with embryo viability.[12,15–17] Our results also suggest that, even though oestrogen production is seemingly typical, oocyte quality could be compromised at this heterotopic location. Alteration in quality could potentially be attributable to freezing, thawing, and initial ischaemia after transplantation.[18] Oocyte quality could also be altered because of differences in temperature and blood flow in the subcutaneous environment compared with pelvic (orthotopic) location. If this change is the case, the longer the follicles are allowed to develop before retrieval the higher the likelihood of compromise. This hypothesis is lent support by the fact that the only viable embryo we generated was from a germinal vesicle stage oocyte that was retrieved from a small antral follicle. By contrast, an abnormal embryo was obtained from an oocyte of a larger follicle matured from metaphase-I stage, and mature oocytes failed to fertilise. Because of this difference, aspiration of immature oocytes from smaller follicles

followed by in-vitro maturation could be the preferred approach to preserve competence to undergo fertilisation. Our findings also showed that ovarian follicles do not grow to the same sizes in heterotopic locations compared with pelvic placement,[5] which accords with findings of previous xenografting experiments.[19] Oocyte maturity seems to be attained at 10–11 mm diameter, contrasting with 16–17 mm in orthotopic ovaries.[20] Our findings show that fertility and ovarian endocrine function can be preserved in women by long-term ovarian tissue banking. Because the probability of pregnancy with one embryo originating from an in-vitro matured oocyte is 6–12%,[13,21] many attempts or simultaneous transfer of multiple embryos might be needed to achieve a pregnancy. Even though the final proof of success of ovarian cryopreservation and transplantation procedure will be a viable pregnancy in human beings, with the development of a human embryo, prospects for a pregnancy and liveborn are now more promising.

Contributors

K Oktay was responsible for study design, invention and performance of the transplantation technique, ovarian stimulation, cycle monitoring, oocyte retrievals, embryo transfer, data analysis, and preparation of the report. E Buyuk planned and did tissue thawing and preparation, undertook viability tests, collected and analysed data, and prepared the report. Z Rosenwaks assisted with tissue preparation and gave technical advice in IVF-embryo transfer. K Xu undertook and interpreted the analysis of fluorescence in-situ hybridisation. L Veeck and N Zaninovic provided IVF laboratory support, did in-vitro maturation, and undertook embryo culture and assessment. T Takeuchi did intracytoplasmic sperm injection. M Opsahl removed and cryopreserved ovarian tissue.

Conflict of interest statement

None declared.

Acknowledgments

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