



Two live births following orthotopic ovarian tissue autotransplantation: A case report of cycle monitoring and (modified) natural-cycle IVF in one patient

Nicole Francisca Klijn*, Leoni Albertine Louwé, Gonneke Saskia Kirsten Pilgram, Lucia Alida Johanna van der Westerlaken

Department of Obstetrics & Gynaecology, Leiden University Medical Center, Albinusdreef 2, 2333, ZA, Leiden, The Netherlands

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ABSTRACT

Introduction: Ovarian tissue cryopreservation (OTC) is an option to preserve fertility. This article describes the effect of cycle monitoring on the chances of pregnancy following orthotopic ovarian tissue autotransplantation.

Case presentation: A 32-year-old woman diagnosed with breast cancer underwent right-sided oophorectomy and OTC prior to chemotherapy. Three years later she was diagnosed with premature ovarian insufficiency and ovarian tissue chips were transplanted in the remaining ovary and a peritoneal window. Eight months after transplantation, activity was seen in the tissue placed in the peritoneal window. With a low natural chance of conception, modified natural-cycle (MNC) IVF was started by administration of a GnRH antagonist. Ovulation was triggered with hCG. One oocyte was retrieved by transvaginal ovum pick-up. After fertilization, a six-cell-stage embryo of good quality was transferred. This resulted in pregnancy and a healthy girl was born at 41 weeks. In the second monitored cycle after her delivery, a follicle was seen in the remaining ovary. Two weeks later a pregnancy test was positive and after an uncomplicated pregnancy a healthy boy was delivered spontaneously at 41 weeks.

Conclusions: This case reports shows that MNC IVF conception after OTC is successful in patients with tissue partially placed in a peritoneal window. If the tissue placed in the peritoneal window becomes active, MNC IVF can be performed. If the tissue placed in the remaining ovary becomes active, natural conception should be pursued.

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1. Introduction

Premature ovarian insufficiency is one of the most common long-term adverse effects of the treatment of malignant diseases with alkylating agents or radiotherapy [1]. This results in loss of both endocrine and reproductive function of the ovary. Several options are available to preserve fertility. These include cryopreservation of oocytes, embryos and ovarian tissue. Only ovarian tissue cryopreservation (OTC) can start without delaying the gonadotoxic treatment, as no hormonal stimulation is needed.

When the patient is disease free and premature ovarian insufficiency has been diagnosed, the cryopreserved ovarian tissue can be thawed and transplanted into an orthotopic site (i.e. the remaining

ovary or a peritoneal window) or a heterotopic site (i.e. the forearm) [2].

In 2004, the first child born after autotransplantation was documented [3]. Since then, the number of live births has exceeded 130 [4]. Naturally conceived pregnancies and pregnancies after in vitro fertilization are described in case reports and case series [5,6].

This case report concerns a woman who gave birth to two children after ovarian tissue autotransplantation. This unique case shows that after ovarian tissue autotransplantation, cycle monitoring is crucial, especially when tissue is placed in a peritoneal window.

2. Case Presentation

2.1. Patient History

The patient was diagnosed with ductal carcinoma of the right breast, stage T2N1M0, triple negative, at age 32. Genetic testing for

* Corresponding author at: Leiden University Medical Center (H3-P), Postbus 9600, 2300 RC Leiden, The Netherlands.

E-mail address: n.f.klijn@lumc.nl (N.F. Klijn).

BRCA mutations was negative. Her oncologist advised her not to postpone the cancer treatment for an ovarian stimulation to obtain oocytes. Therefore, before neoadjuvant chemotherapy, and after the patient gave signed written informed consent, the right-side ovary was removed by laparoscopy for ovarian tissue cryopreservation.

After oophorectomy, she was treated with four cycles of dense adriamycin cyclophosphamide (AC) and two cycles of intermediate high-dose chemotherapy with cyclophosphamide, carboplatin and thiotepa. This treatment was followed by right-sided mastectomy and post-operative local radiation therapy (64 Gy).

Three years after this treatment she was considered disease-free. There was a desire to conceive and because of secondary amenorrhoea, with less than one hot flush a day, hormonal tests were performed. Post-menopausal status was confirmed (FSH 185.8 U/L, estradiol 22.5 pmol/L, AMH < 0.1 µg/L).

Autotransplantation was decided on at a multidisciplinary team meeting. The patient was counselled by a fertility specialist about the procedure. The oncologist had no objections to transplantation and potential pregnancies, as there was a low estimated risk of recurrence of the breast cancer, based on the tumour characteristics, cancer treatment and disease-free interval. Semen analysis of her partner showed normospermia.

2.2. Procedure of Ovarian Tissue Cryopreservation and Autotransplantation

Immediately after the oophorectomy, the cortex of the ovary was isolated, whereas the medulla was discarded. Freezing of ovarian tissue was undertaken according to the protocol described by Radford et al. [7]. The frozen ovarian tissue chips were stored in cryovials in liquid nitrogen.

Before autotransplantation, the first vial was thawed and the tissue tested negative for malignant cells by negative keratin AE1/AE3 colouring. Primordial follicles were seen in this tissue.

For autotransplantation, five cryovials, each containing one ovarian cortical strip, were thawed according to the protocol of the Andersen group [8]. They were transferred to a container containing PBS and transported to the operating theatre, where it arrived within 30 min after starting the thawing process. Four of the five pieces were transplanted; one was discarded because of abnormal colouring of the tissue and sent for further examination to the pathology department. There, only ovarian stromal cells were seen, and no malignancy.

The transfer of the tissue was performed by laparoscopy. Two cortical chips were placed on the medullar incision of the remaining ovary with the cortical side positioned to the surface. Due to lack of space for the other two chips, a peritoneal window was made at the right side of the uterus.

Antibiotic prophylaxis was (due to allergy) clindamycin 600 mg and ciprofloxacin 400 mg intravenous administered 30 min before surgery.

2.3. Ethical Approval

The Medical Ethical Committee of the LUMC approved all the procedures. In the Netherlands, there is consensus for OTC and autotransplantation by the Dutch Society of Obstetrics and Gynaecology (NVOG) and the Society of Clinical Embryology (KLEM).

2.4. Results

In follow-up after transplantation, first follicular activity was seen on ultrasound in the remaining ovary after four months. This was confirmed by a drop in FSH level to 23 U/L and a rise in estradiol level (245 pmol/L). The patient mentioned disappearance of



Fig. 1. The 6-cell-stage embryo on day 2 just before transfer.

hot flushes and an increase of vaginal discharge. Cycle monitoring showed continuing follicle growth (17 mm) and increasing estradiol levels (up to 502 pmol/L). Unfortunately, luteinisation could not be confirmed. One month later, recovery of the menstrual cycle was observed. This was followed by another menstrual bleed after 22 days, but cycle monitoring thereafter did not show any follicular activity and higher FSH levels were measured (FSH 62 U/L).

At a follow-up appointment, eight months after the autotransplantation, activity was seen in the ovarian tissue placed in the peritoneal window. With a low chance of natural conception from this side and low ovarian reserve, the cycle was converted to a MNC IVF cycle with the start of a GnRH-antagonist (Cetrotide®) to prevent a spontaneous LH surge. One day later, the follicle diameter was 17 mm, estradiol 515 pmol/L and ovulation triggered with choriogonadotropin alpha (Ovitrelle®) 250 µg. The transvaginal ovum pick-up 36 h later retrieved one oocyte. After conventional IVF, the oocyte was fertilised and two days later a six-cell-stage embryo of good quality (see Fig. 1) was transferred under abdominal ultrasound guidance. Luteal phase was supported with vaginal progesterone capsules (Utrogestan®) three times a day 200 mg. Two weeks later the pregnancy test was positive.

Ultrasound at 6 weeks and 4 days of gestational age confirmed a viable pregnancy. The pregnancy was uncomplicated and at 41 weeks she delivered spontaneously a healthy girl of 3850 g.

Five months after delivery she returned to our clinic with recovery of her menstrual cycle. Transvaginal ultrasound showed a follicle in the tissue in the peritoneal window with a confirmatory estradiol level (1300 pmol/L). The patient and her partner were not ready to pursue another ART treatment, but would welcome a spontaneous conception. The next cycle, monitoring was performed and for the first time a follicle was seen in the remaining ovary with an estradiol level of 1000 pmol/L and a starting LH surge (15 U/L). In all other monitored cycles in this patient, activity in this ovary had never been observed. Two weeks later a pregnancy test was positive.

Ultrasound at 7 weeks and 1 day of gestational age confirmed a viable pregnancy. The pregnancy was uncomplicated and at 41 weeks she delivered spontaneously a healthy boy of 3800 g.

3. Discussion and Conclusions

This article describes two successful pregnancies after ovarian tissue autotransplantation in one woman. The first pregnancy is the result of MNC IVF, the second the result of natural conception. The conversion to MNC IVF was started when cycle monitoring showed follicular activity in the tissue placed in the peritoneal window. With absence of a fallopian tube on the right side and follicle

growth in this tissue, it was argued that the chance of natural conception from this side was low. It could be argued that there was a chance of natural conception and that conversion to MNC-IVF was not necessary. However, the antral follicle count in this patient after autotransplantation was low and together with the location of the growing follicle, the overall chances of natural conception were estimated as low. This information was discussed with the patient and her partner and in mutual agreement conversion to MNC IVF was undertaken.

Although other articles describe pregnancies after ovarian tissue autotransplantation with a complete IVF stimulation, this is not always the optimal treatment. The benefit of stimulation can be argued in patients with a low antral follicle count. Furthermore, in patients with a history of estrogen receptor-positive breast cancer, stimulation might be harmful. In these situations, cycle monitoring without added hormonal stimulation and with selected MNC IVF might give higher chances of pregnancy than would attempts at natural conception.

This case reports shows that follow-up by cycle monitoring increases the chances of pregnancy after OTC in patients with ovarian tissue partially placed in a peritoneal window. Follicular activity can be detected during cycle monitoring. When there is follicular growth in the tissue placed in the peritoneal window, MNC IVF can start. If the ovarian tissue placed in the remaining ovary is active, natural conception can be pursued.

Contributors

Nicole Francisca Klijn performed the embryo transfer and wrote the first manuscript draft.

Leoni Albertine Louwé performed the oophorectomy and autotransplantation of the ovarian tissue.

Gonneke Saskia Kirsten Pilgram performed the cryopreservation procedure of the ovarian tissue and the laboratory phase of the natural cycle IVF treatment.

Lucia Alida Johanna van der Westerlaken performed the cryopreservation procedure of the ovarian tissue and the laboratory phase of the natural cycle IVF treatment.

All authors contributed substantially to the interpretation of the procedures in this case. They all critically reviewed and approved the manuscript.

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Ethical Approval

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Patient Consent

Obtained.

Provenance and Peer Review

This case report was peer reviewed.

Declaration of Competing Interest

The authors declare that they have no conflict of interest regarding the publication of this case report.

References

- [1] D. Meirou, Reproduction post-chemotherapy in young cancer patients, *Mol. Cell. Endocrinol.* 169 (2000) 123–131, [http://dx.doi.org/10.1016/S0303-7207\(00\)00365-8](http://dx.doi.org/10.1016/S0303-7207(00)00365-8).
- [2] K. Oktay, K. Economos, M. Kan, J. Rucinski, L. Veeck, Z. Rosenwaks, Endocrine function and oocyte retrieval after autologous transplantation of ovarian cortical strips to the forearm, *JAMA*. 286 (2001) 1490–1493, <http://dx.doi.org/10.1001/jama.286.12.1490>.
- [3] J. Donnez, M.M. Dolmans, D. Demylle, P. Jadoul, C. Pirard, J. Squifflet, B. Martinez-Madrid, A. Van Langendonck, Livebirth after orthotopic transplantation of cryopreserved ovarian tissue, *Lancet* 364 (2004) 1405–1410, [http://dx.doi.org/10.1016/S0140-6736\(04\)17222-X](http://dx.doi.org/10.1016/S0140-6736(04)17222-X).
- [4] J. Donnez, M.M. Dolmans, Fertility preservation in women, *N. Engl. J. Med.* 377 (2017) 1657–1665, <http://dx.doi.org/10.1056/NEJMra1614676>.
- [5] F. Pacheco, K. Oktay, Current success and efficiency of autologous ovarian transplantation: a meta-analysis, *Reprod. Sci.* 24 (2017) 1111–1120.
- [6] A.K. Jensen, K.T. Macklon, J. Fedder, E. Ernst, P. Humaidan, C.Y. Andersen, 86 successful births and 9 ongoing pregnancies worldwide in women transplanted with frozen-thawed ovarian tissue: focus on birth and perinatal outcome in 40 of these children, *J. Assist. Reprod. Genet.* 34 (Mar 2017) 325–336, <http://dx.doi.org/10.1007/s10815-016-0843-9>.
- [7] J.A. Radford, B.A. Lieberman, D.R. Brison, A.R.B. Smith, J.D. Critchlow, S.A. Russell, A.J. Watson, J.A. Clayton, M. Harris, R.G. Gosden, et al., Orthotopic reimplantation of cryopreserved ovarian cortical strips after high-dose chemotherapy for Hodgkin's lymphoma, *Lancet*. 357 (2001) 1172–1175, [http://dx.doi.org/10.1016/S0140-6736\(00\)04335-X](http://dx.doi.org/10.1016/S0140-6736(00)04335-X).
- [8] M. Rosendahl, K.T. Schmidt, E. Ernst, P.E. Rasmussen, A. Loft, A.G. Byskov, A.N. Andersen, C.Y. Andersen, Cryopreservation of ovarian tissue for a decade in Denmark: a view of the technique, *Reprod. BioMed. Online* 22 (2011) 162–171, <http://dx.doi.org/10.1016/j.rbmo.2010.10.015>.