

# The first woman to give birth to two children following transplantation of frozen/thawed ovarian tissue

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Worldwide eight children have been born as a result of transplanting frozen/thawed ovarian tissue. Two of these children were born in Denmark following transport of the ovarian tissue for a period of 5 h prior to cryopreservation. One of these women, who was originally transplanted with six pieces of ovarian cortex, after having experienced a period of menopause has now conceived again following natural conception. She gave birth to a healthy girl on 23 September 2008 and is therefore the first woman in the world to have had two children, from separate pregnancies, born as a result of transplanting frozen/thawed ovarian tissue. This result encourages further development of cryopreservation of ovarian tissue for fertility preservation as a clinical procedure for girls and young women facing gonadotoxic treatment.

**Key words:** ovarian transplantation / ovarian cryopreservation / natural conception

## Introduction

Cryopreservation of ovarian tissue is a new method, which has been developed in an attempt to circumvent the long-term ablative effect on reproductive performance that certain types of gonadotoxic treatment may impose (Lamar and Decherney, 2009). Recent developments now provide a realistic hope to this group of patients of becoming biological parents after having recovered from the malignant disease (review von Wolff *et al.*, 2009). Although cryopreservation of ovarian tissue is still an experimental procedure, it is slowly becoming accepted as a valid alternative to other methods, especially because it can be performed at short notice and because current experience with transplanting the tissue has resulted in both the birth of healthy children and prolonged periods of return of ovarian function (review von Wolff *et al.*, 2009). Currently, eight healthy children have been born as a result of transplanting frozen/thawed ovarian tissue (review: von Wolff *et al.*, 2009; Sánchez-Serrano *et al.*, 2009). Two of these children were a result of the programme in our centre (Andersen *et al.*, 2008). The present paper reports that one of these women became pregnant again and has now given birth to a healthy baby.

## Materials and methods

The patient was aged 27 years when diagnosed with Ewing's sarcoma, which developed from the right VII and VIIIth costae. She initially received six series of VIDE (vincristin/ifosfamid/doxorubicin/ethoposin) chemotherapy, after which the remaining tumour was removed surgically together with the two costae. Her chemotherapy continued with three series of VAI (vincristin/actinomycinD/ifosfamid). Thereafter, her menstrual bleedings stopped and postmenopausal levels of gonadotrophins and hot flashes developed.

The patient had only the right ovary present at the time of freezing ovarian tissue since the left ovary previously had been removed because of a dermoid cyst involving the whole ovary. Prior to any gonadotoxic treatment part of the right ovary (approximately one-third) was retrieved on 22 March 2004. The tissue was transported on ice for 5 h and cryopreserved as described (Andersen *et al.*, 2008). Transplantation was performed on 14 December 2005. Thawing of six pieces of cortex was performed using the following steps: quick thawing by placing the ampoules in a 37°C water bath, and immediately after thawing the pieces of cortex were placed into a solution at room temperature of 0.75 mol/l ethyleneglycol and 0.2 mol/l sucrose in phosphate-buffered saline (PBS) for 10 min, then 0.1 mol/l sucrose in PBS for 10 min and finally 10 min in PBS. Sterile forceps were used to move the pieces of cortex from one solution to another. A biopsy obtained from the ovary

*in situ* revealed no remaining follicles upon histological examination. After tissue transplantation levels of gonadotrophins gradually returned to premenopausal levels and estradiol levels increased. As previously described, she conceived her first child following a mild ovarian stimulation and on 8 February 2007 delivered a normal healthy girl, following Caesarean section, weighing 3204 g in week 39 after an otherwise uneventful pregnancy (Andersen *et al.*, 2008).

## Results

The patient was breastfeeding her first baby until October 2007 and returned to our fertility clinic in January 2008 for an additional IVF treatment. However, a pregnancy test revealed that she had already conceived naturally, without any treatment. The gestational age was 7 + 4 estimated by transvaginal ultrasonography. After an uneventful pregnancy, the patient delivered a normal healthy girl on 23 September 2008, weighing 3828 g and 54 cm in length.

## Discussion

This report demonstrates for the first time a woman who has given birth to two healthy children, one in each of two pregnancies, as a result of one transplantation of frozen/thawed ovarian tissue. Therefore, this study shows that transplantation of just six pieces (i.e. around 15–20% of one entire ovary) of frozen/thawed ovarian cortex to a woman who experienced chemotherapy-induced menopause can result in the production of fully mature oocytes for a period exceeding 4 years and that the capacity to sustain embryogenesis and give birth to healthy children remains.

Taken together, the present results extend the number of children born as a result of transplanting frozen/thawed ovarian tissue to nine globally and encourage a continued effort to develop this technique as a valid method for fertility preservation.

The woman had originally 13 pieces of cortex frozen (around one-third of an ovary), and six were transplanted whereas seven remain in liquid nitrogen. The transplanted tissue has remained active for more than 4 years (at February 2010) and in combination with the pieces of cortex which are still frozen, this woman may experience several more years of ovarian function if she chooses to have the remaining tissue transplanted.

The patient is still breastfeeding almost one year after delivery and still has not regained regular periods. However, ovarian function appears to be normal, as judged by transvaginal ultrasound, since several antral follicles of different sizes were visible in the ovary. The patient now uses pregnancy prevention measures.

Previously, eight children have been reported to be born as a result of transplanting frozen/thawed ovarian cortex (Donnez *et al.*, 2004; Meirow *et al.*, 2005; Demeestere *et al.*, 2007; Andersen *et al.*, 2008; Silber *et al.*, 2008; Sánchez-Serrano *et al.*, 2009). The woman in the current study had her first child following an IVF procedure, whereas the second child was conceived naturally. Thus, in total, three of the nine women have conceived naturally, whereas six

women have conceived followed IVF. This suggests that there is a relatively good chance of restoring natural fertility following the transplantation of ovarian tissue and that the cryopreservation and transplantation procedure does not in itself harm normal ovarian function.

In conclusion, the present result supports cryopreservation of ovarian tissue as a valid method of fertility preservation and encourages development of this technique to be used in a clinical setting in women facing gonadotoxic treatment.

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