Fertility preservation in women: exploring clinical dilemmas
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CHAPTER 7

Reproductive choices and outcomes after freezing oocytes for medical reasons: a follow-up study

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CHAPTER 7

Abstract

Study question: What reproductive choices do women make after they have cryopreserved oocytes for medical reasons?

Summary answer: Women who had cryopreserved oocytes for medical reasons and tried to become pregnant, either attempted natural conception or resorted to assisted reproduction with fresh oocytes.

What is known already: Women confronted with a risk of premature ovarian insufficiency, due to gonadotoxic therapy, ovarian surgery or genetic predisposition, have an indication to cryopreserve oocytes. Many of these women will retain ovarian function, thus will retain the possibility of natural conception. The added value of cryopreserved oocytes to reproductive outcomes is unknown as there is a lack of follow-up of women who have cryopreserved oocytes for medical reasons.

Study design, size, duration: This follow-up study included a cohort of 85 women who cryopreserved their oocytes for medical reasons between 2009 and 2012.

Participants/materials, setting, methods: Medical data from women who cryopreserved their oocytes at the Centre for Reproductive Medicine in the Academic Medical Centre in Amsterdam were extracted and self-report questionnaires were disseminated. The collected data considered demographics, outcomes of ovarian stimulation, fertility-threatening treatments, menstrual cycle changes, pregnancy attempts and outcomes and intended plans for the cryopreserved oocytes.

Main results and the role of chance: A total of 68 women, followed up for an average 25.3 months, returned the questionnaire (response rate: 80%). None of the women had used her cryopreserved oocytes although 16 women had tried to conceive. Of these women, eight were trying to conceive naturally, five had conceived naturally within 2 months and three had conceived with assisted reproduction not requiring cryopreserved oocytes (two women with conventional IVF because of tubal pathology and endometriosis and one woman with IUI because of polycystic ovary syndrome). Three out of the eight pregnancies had resulted in live births, two resulted in miscarriages and three were ongoing. Most women (71%) intended to conceive with their cryopreserved oocytes as a last resource option.
Limitations, reasons for caution: Transferability of our findings is challenged by the small sample but positively affected by our high response rate. As the time span between cryopreservation of oocytes and follow-up was short, follow-up of the cohort should be repeated in 2 years.

Wider implications of the findings: After a mean follow-up of 2 years, none of the women with a medical reason to cryopreserve oocytes had used her oocytes. Women who were trying to conceive during follow-up were doing so without using their stored oocytes. It is unclear whether starting assisted reproduction while having cryopreserved oocytes is the most appropriate clinical decision. Our findings emphasize the relevance of taking the chances of natural conception into account in counselling women about cryopreservation of oocytes.

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Introduction

Women confronted with a risk of premature ovarian insufficiency (POI) due to gonadotoxic therapy, ovarian surgery or genetic predisposition have a medical reason to undergo fertility preservation (FP) (Bedoschi and Oktay, 2013). Cryopreservation of embryos has long been the only option to preserve fertility, but it is slowly being overtaken by cryopreservation of oocytes since this technique allows for reproductive autonomy and has similar pregnancy rates (Kuwayama et al., 2005; Grifo and Noyes, 2010; Rienzi et al., 2010). The efficacy of this technique and the reassuring data on the health of babies conceived with cryopreserved oocytes (Noyes et al., 2009) led to the removal of the experimental connotation of cryopreservation of oocytes by the American Society of Reproductive Medicine (Loren et al., 2013). Nevertheless, the timely need to evaluate the added value of newly introduced techniques by means of follow-up has been acknowledged (Harper et al., 2012).

The likeliness of chemotherapy to induce POI depends on the dose, agent, number of cycles of chemotherapy and age of the patient (Bines et al., 1996; Meirrow, 1999; Burstein and Winer, 2000; Meirow et al., 2010). A recent follow-up study on fertility in women who cryopreserved ovarian tissue because of planned gonadotoxic
chemotherapy, revealed that the majority retained ovarian function with an overall risk of POI of 22% after a mean follow-up of 58 months (Schmidt et al., 2013). Pregnancy rates of 65% are described in childhood cancer survivors (Nielsen et al., 2013), even when trying to conceive naturally for longer than 1 year (Barton et al., 2013). Data on the risks of POI in women with Mosaic Turner Syndrome are based on a limited number of studies indicating loss of ovarian reserve in women with karyotypes associated with poor fertility prognosis (such as Xq deletions and 45XO) but natural conceptions rates are rarely mentioned in literature (Tarani et al., 1998; Sybert, 2002; Purushothaman et al., 2010). Women with a mother with a history of POI are at risk for developing POI themselves, but natural conception rates are unknown (Bentzen et al., 2013).

Considering the increased awareness about FP, a result of the tremendous efforts of organizations like the American Society for Clinical Oncology, Fertile Hope, the International Society for Fertility Preservation, the Oncofertility Consortium and local initiatives (i.e. the DutchNetwork for FP), it is likely that we will enter an era in which many women have anticipated their risk of POI and cryopreserved their oocytes. Since many of these women will retain ovarian function, chances of natural conception will be present while cryopreserved oocytes are stored. The added value of cryopreserved oocytes to reproductive outcomes is unknown in these women, as there is a lack of a comprehensive follow-up of women who have cryopreserved oocytes for medical reasons and insight into women’s intentions for their cryopreserved oocytes is lacking. This study therefore aimed to examine the reproductive choices and outcomes of women who have cryopreserved their oocytes for medical reasons.

**Methods**

*Ethical approval*

This follow-up study was conducted in August 2013. The Institutional Review Board of the Academic Medical Centre (AMC) Amsterdam (project no W13_091), stated that the study was not subject to the Dutch ‘Medical Research Involving Human Subjects Act’, meaning that no further approval was required.
**Study population**

A cohort of 85 women, who had cryopreserved oocytes between January 2009 and December 2012, because of medical reasons, at the Centre for Reproductive Medicine of the AMC in Amsterdam was eligible for follow-up.

**Data collection**

Medical data on the indication for cryopreservation of oocytes, ovarian stimulation and the results of oocyte cryopreservation were extracted from medical files. A paper–pencil questionnaire was developed and consecutively pilot tested. The questionnaire finally covered five topics: established medical diagnosis and fertility-threatening treatment received, menstrual cycle changes and use of contraception, attempts to conceive and intended plan for (residual) cryopreserved oocytes. All 14 questions had a multiple choice response scale but for each, the possibility to add explanations was provided (see Supplementary data, File 1). Up to date contact information (i.e. postal addresses) of the entire cohort of women was retrieved from the central hospital register of the AMC. The questionnaire was coded and sent by mail in August 2013 accompanied by an invitation and information letter and possibility to declare no interest in participation. Three weeks later, non-responders were reminded by mail and again by telephone after another 3 weeks.

**Data-analysis**

Data were entered and analyzed using SPSS statistics 20. Descriptive statistics were computed to describe demographics, indications for oocyte cryopreservation, reproduction after oocyte cryopreservation, menstrual cycle changes and intended use of (residual) cryopreserved oocytes.
CHAPTER 7

Results

Respondents

The flowchart of the recruitment of the study cohort is shown in Fig. 1. A total of 85 questionnaires were sent, and 68 women completed the questionnaire and returned it by post (80%). Demographics of these women are shown in Table I. The mean age at time of oocyte retrieval for cryopreservation was 30.2 years (range 18–40 years) and only two women (3%) had children at the time of cryopreservation of oocytes. The majority (75%) of the women were highly educated. Women went through one (n = 30; 44%), two (n = 23; 34%), three (n = 13; 19%) or four consecutive cycles (n = 2; 3%). Three women (5%) cryopreserved oocytes in 2009, 13 women (19%) in 2010, 21 women (31%) in 2011 and 21 women (31%) in 2012. The mean time to follow-up was 25.3 months (range 9–50 months).

Figure I: Flowchart study cohort of women who cryopreserved oocytes for medical reasons from 2009 to 2012.

![Flowchart](https://example.com/flowchart.png)
Table I: Demographics of 68 women who cryopreserved oocytes for medical reasons from 2009 to 2012.

<table>
<thead>
<tr>
<th>Demographic characteristic</th>
<th>Mean (range)</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age at time of oocyte retrieval</td>
<td>30.2 years (18–40)</td>
<td></td>
</tr>
<tr>
<td>Previous children</td>
<td>2/65 (3)</td>
<td></td>
</tr>
<tr>
<td>Higher professional school or university</td>
<td>41/55 (75)</td>
<td></td>
</tr>
<tr>
<td>Dutch origin</td>
<td>52/55 (95)</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.3 (17.3–33.7)</td>
<td></td>
</tr>
<tr>
<td>Non-smokers</td>
<td>47/60 (78)</td>
<td></td>
</tr>
<tr>
<td>Regular cycle</td>
<td>29/38 (76)</td>
<td></td>
</tr>
<tr>
<td>Women undergoing one cycle</td>
<td>30 (44)</td>
<td></td>
</tr>
<tr>
<td>Women undergoing two cycles</td>
<td>23 (34)</td>
<td></td>
</tr>
<tr>
<td>Women undergoing three cycles</td>
<td>13 (19)</td>
<td></td>
</tr>
<tr>
<td>Women undergoing four cycles</td>
<td>2 (3)</td>
<td></td>
</tr>
<tr>
<td>Mean number of cryopreserved oocytes per woman</td>
<td>16.5 (1–36)</td>
<td></td>
</tr>
<tr>
<td>Mean number of cycles per woman</td>
<td>1.8 (1–4)</td>
<td></td>
</tr>
<tr>
<td>Mean time of follow-up</td>
<td>25.3 months (9–50)</td>
<td></td>
</tr>
</tbody>
</table>

BMI, body mass index.

Indications for cryopreservation of oocytes

At the time of follow-up, 35 women (51%) reported having undergone therapy which contained cyclophosphamide. Other fertility-threatening treatments were chemotherapy consisting of doxorubicin, bleomycin, vinblastine and dacarbazine (ABVD) (n = 4), chemotherapeutic preparation for bone-marrow transplantation (n = 1), endocrine therapy only (n = 1), methotrexate (n = 1) and bilateral salpingo-oophorectomy (BSO) because of a BRCA1 gene mutation (n = 1).

Out of the 34 women with breast cancer, 23 women (68%) were undergoing additional endocrine therapy because of hormone-sensitive breast cancer at time of follow-up, which made conception ill advised (11 women were using tamoxifen; 9 women were using tamoxifen and GnRH analogues, 2 women were using GnRH analogues and 1 woman did not remember the name of her endocrine breast cancer treatment). One of these women however was trying to conceive after a wash out period to clear the tamoxifen.
Reproduction after cryopreserving oocytes

Out of the 68 women, 16 women had tried to conceive after cryopreservation of oocytes; eight women were trying to conceive naturally at time of follow-up (of whom one woman was using donor insemination) and eight women had already became pregnant without using their cryopreserved oocytes. Of the eight women who had already became pregnant, five conceived naturally and all within the first 2 months of trying. These five pregnancies resulted in one live birth, two miscarriages and two ongoing pregnancies (at gestational ages of respectively 8.5 and 9 weeks). The three other women became pregnant with assisted reproductive techniques (two using conventional IVF and one using IUI). The reasons for starting conventional IVF after cryopreservation of oocytes were bilateral tubal pathology in one woman who had undergone unilateral ovariectomy and endometriosis in the other woman. Both women had live births after conventional IVF. IUI with ovarian stimulation was performed in a woman with a family history of POI and polycystic ovarian syndrome, which became apparent after cryopreservation of oocytes. This woman reported a pregnancy at 9 weeks gestational age. Of the remaining 52 women who were not trying to conceive, 22 were using endocrine therapy for breast cancer which made conception ill-advised, two women had a contraindication for pregnancy due to unstable auto-immune disease and 14 women did not have a male partner. In total, 14 women (21%) were not trying to conceive despite having a male partner and being medically allowed a pregnancy.

Menstrual cycle changes

Questions about changes in menstrual cycle could be answered by 28 women as they did not use any medication that could mask their menstrual cycle (i.e. oral contraception). All 28 women retained their menstrual cycle at time of follow-up. One woman with Mosaic Turner syndrome reported having noticed a change from a regular cycle (28–30 days) to an irregular cycle of 30–35 days after a follow-up of 15 months.

Intended use of cryopreserved oocytes

The majority of the responding women (n = 48; 71%) reported that they would use their cryopreserved oocytes if they had difficulties conceiving naturally. Additionally, 19 women (28%) had no specific plan with their cryopreserved oocytes and one
woman wanted to donate her cryopreserved oocytes to research. When asked their intentions in case of residual cryopreserved oocytes, some women reported not to have a plan in mind (35%), whereas others planned to donate their residual oocytes in the future to research (26%), either to research or other women (13%), or only to other women (13%), whereas others would wish to destroy their residual cryopreserved oocytes (10%) or did not answer this question (3%).

Discussion

Cryopreservation of oocytes is becoming mainstream for women opting for FP because this technique does not require a male partner and is no longer considered experimental (Loren et al., 2013). This follow-up study found that after a mean follow-up of 25.3 months, women who had cryopreserved oocytes for medical reasons and tried to become pregnant, had attempted natural conception or resorted to assisted reproduction techniques with fresh oocytes for various reasons. So far, none of the cryopreserved oocytes had been used. Half of the women attempting to conceive became pregnant, mostly by natural conception. This is the first follow-up study in women who have cryopreserved oocytes for medical reasons, to report all reproductive outcomes, including outcomes resulting from the choice not to use cryopreserved oocytes.

None of the women in our study reported clinical symptoms indicating an onset of menopause (defined as an absence of menstrual cycle for longer than one year without using hormonal contraception or endocrine therapy for breast cancer). It was impossible to detect symptoms of impaired ovarian function for the total study cohort, since most women in this study used hormonal therapy masking their menstrual cycle.

Our study found that 50% of the women cryopreserving oocytes for medical reasons did so because of breast cancer. The relatively large proportion of women with breast cancer opting for FP in our study was also found in a large retrospective cohort of 475 oncological patients, in which the majority of women cryopreserving oocytes were diagnosed with breast cancer (Garcia-Velasco et al., 2013). Breast cancer is the most common malignancy of women of reproductive age and its management usually includes gonadotoxic chemotherapy regimens with cyclophosphamide. In case of hormone-sensitive breast cancer, prolonged endocrine treatment with tamoxifen or aromatase-inhibitors for 5 years is recommended after chemotherapy (Davies et al., 2011). Women are frequently advised by their oncologists to postpone motherhood during therapy with tamoxifen because of possible teratogenicity (Barthelmes and Gateley, 2004;
Braems et al. (2011). In case women do opt for a pregnancy during tamoxifen use, such as one woman in our cohort, a wash out period of at least 2 months is advised (Braems et al., 2011). Moreover, as was the case in one woman of our cohort, women who carry a BRCA1/2 gene mutation may have to prophylactically undergo BSO before they have had a chance to conceive. Therefore, the fertility of women with breast cancer is at an even greater threat.

As reported in this study, women who have cryopreserved oocytes in the past may not rely on their cryopreserved oocytes in the future due to maintenance of natural chances of conception. This is in accordance with data from women who had cryopreserved oocytes for age-related decline of fertility, where <1% of women who achieved a pregnancy after cryopreservation of oocytes made use of her cryopreserved oocytes (Hodes-Wertz et al., 2013). Additionally, one study reported that in 143 women who cryopreserved ovarian tissue because of gonadotoxic treatment, 46 women became pregnant after treatment (32%), of whom 41 women (89%) conceived naturally (Schmidt et al., 2013). This is a higher proportion than that found in our study. The difference might be explained by a longer follow-up time in this study compared with our study (mean of 58 versus 25 months) and the relatively high number of women with breast cancer in our study, who were still undergoing additional endocrine therapy at time of follow-up, disabling them from conceiving. It is difficult to define the appropriate timespan after which to start follow-up. Our relatively short time to follow-up (mean of 25.3 months) results in the need to repeat this study in 2 years. On the other hand, the need for timely critical reflection on newly introduced techniques (Harper et al., 2012) and the finding in our study, that half of the women who were already trying to conceive did not need to rely on their cryopreserved oocytes, demonstrates the timeliness of our study.

Of the 52 women who were not trying to conceive, only 14 women had a male partner and had no medical contraindication to pregnancy. Although this study did not thoroughly question motivations for not conceiving, fears about pregnancy-related risks after surviving cancer may contribute to postponing motherhood (Schover, 2005; Gonçalves et al., 2013).

Transferability of our findings is challenged by the small sample size but positively influenced by our high response rate. Another limitation of our study was that assessment of ovarian function (i.e. blood sampling or transvaginal ultrasound) was not included in the study design. Therefore, the decisions of women (and their advising physicians) on when and how to start trying to conceive was not influenced by knowledge about ovarian reserve. It is likely that having undergone fertility-threatening
In our study population, 75% of women were highly educated (higher professional school or university), whereas only 28% of the general Dutch population is highly educated. The overrepresentation of highly educated women opting for cryopreservation of oocytes is also found in other cohorts of women cryopreserving oocytes (Stoop et al., 2011; Hodes-Wertz et al., 2013). A possible explanation is that highly educated women are prone to delay motherhood (Heck et al., 1997) and may be more assertive in asking about possible fertility related risks of medical treatments and subsequent options to preserve fertility. Data available to gain insight into the pregnancy chances for this study population are based on comparisons of pregnancy rates of fresh oocytes by conventional IVF with cryopreserved and thawed oocytes, showing no superiority of fresh oocytes over cryopreserved oocytes (Cobo et al., 2008; Rienzi et al., 2012). Our study showed that two women with a pregnancy wish after cryopreservation of oocytes relied on fresh conventional IVF. It is debatable whether conventional IVF/ICSI indications justify the use of conventional IVF in women who have already undergone IVF for cryopreservation of oocytes, which in this respect can be regarded as an ‘unfinished IVF’ treatment. From a pragmatic and cost-effectiveness point of view, one might opt for using cryopreserved oocytes first before initiating a new fresh IVF cycle. On the other hand, from the perspective of age-related fertility decline, one might choose to undergo conventional fresh IVF first before using the cryopreserved oocytes. This implies that the rationale for cryopreserving oocytes has then changed from a medical reason into a non-medical reason, which may be an ethical dilemma.

In conclusion, this study found that after a mean follow-up of 2 years, none of the women with a medical reason to cryopreserve oocytes had used any of these oocytes, and all women attempting to conceive after cryopreservation of oocytes were doing so without the use of the stored oocytes. There is a need to develop tailored prognostic and prediction models to personalize chances of obtaining a pregnancy with or without use cryopreserved oocytes. It may be too soon to predict these chances because cryopreservation of oocytes has been introduced only recently and rates at which these oocytes are used are inherently low. The results of this study emphasize the importance of taking the chances of obtaining a pregnancy without use of cryopreserved oocytes (e.g. by natural conception) into account when counselling women on live birth rates after cryopreserving oocytes and when designing new studies that evaluate pregnancy rates in the setting of oocyte cryopreservation.
References


