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Timing of fertility preservation procedures in a cohort of female patients with cancer

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ABSTRACT

Objective: Comparison of time intervals from diagnosis to chemotherapy between patients opting for embryo cryopreservation or ovarian tissue cryopreservation.
Study design: Retrospective analysis.
Setting: University hospital in the Netherlands.
Patients and methods: Thirty-five female patients undergoing fertility preservation procedures before treatment with chemotherapy for cancer. Embryo cryopreservation was performed in 12 patients and ovarian tissue cryopreservation in 23 patients. We investigated differences in time intervals (from diagnosis to start of chemotherapy) between patients opting for embryo cryopreservation and patients opting for ovarian tissue cryopreservation. We calculated time intervals between the moment of diagnosis, the moment of referral, the moment of consultation, the moment of finishing of the fertility preservation procedure and the start of chemotherapy.
Results: The median time between diagnosis and referral (median = 18 days) and between referral and consultation (median = 5 days) was comparable in both groups. A significant difference was found between ovarian tissue cryopreservation and embryo cryopreservation for the time interval between consultation and cryopreservation ($p = 0.001$). Ovarian tissue cryopreservation was completed for half of the patients within 6 days after consultation with the gynecologist, and the hormonal stimulation for embryo cryopreservation was completed for all patients within four weeks (median = 18 days), with a median of 11 days of hormonal stimulation. A significant difference was found between ovarian tissue cryopreservation and embryo cryopreservation in the time interval between fertility preservation and start of chemotherapy (median = 7 vs 19 days, $p = 0.019$). In sum, the total duration between diagnosis and chemotherapy was significantly shorter for ovarian tissue cryopreservation patients than for embryo cryopreservation patients (median = 47 vs 69 days, $p = 0.042$).
Conclusion: Embryo cryopreservation can be performed within the standard timeframe of cancer care in patients with breast cancer receiving adjuvant chemotherapy, but if delay of the start of chemotherapy is harmful, ovarian tissue cryopreservation can be done within one week.

1. Introduction

As a result of improvement in oncological treatments, most young cancer patients achieve prolonged survival in which quality of life issues are emphasized [1–3]. In particular, the consequences for family planning due to premature ovarian failure are of major concern in premenopausal women. Multidrug chemotherapy, especially with alkylating agents, radiation therapy or surgery can permanently or temporarily impair future fertility [4]. The risk of premature ovarian failure depends mainly on the age of the patient, the type and dose of chemotherapy, and the irradiation settings. Moreover, the resumption of cyclic menses after oncological treatment does not guarantee normal fertility [5]. However, studies suggest that cancer survivors do want to have children that are biologically theirs, and some even experience increased value on parenthood because of their experience with cancer [1,2,6–10]. Fertility preservation has therefore become a main issue over the past decades as an integral part of the care for cancer patients, recognizing the importance of fertility in future life.

After fertility preservation became a subject of interest, several procedures were investigated. In our hospital in vitro fertilization

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with embryo cryopreservation, ovarian tissue cryopreservation and transposition of the ovaries are available techniques for fertility preservation. Currently, only ovarian transposition and conventional in vitro fertilization (IVF) with embryo cryopreservation are considered standard treatment options for fertility preservation with reasonable success rates. Although embryo cryopreservation after oocyte retrieval and IVF is an effective and widely available procedure, the necessity of a male partner and time-consuming hormonal stimulation means it is not applicable to single women, or to patients who need to start cancer therapy immediately and it is less suitable in patients with hormone-sensitive malignancies. For those patients, options are limited to experimental approaches like ovarian tissue and oocyte cryopreservation. Although cryopreservation of ovarian tissue prior to gonadotoxic treatments is considered an experimental procedure, nevertheless 14 livebirths after transplanting frozen/thawed ovarian tissue have been reported [11–18]. Cryopreservation of oocytes utilizing vitrification, an ultra-rapid freezing protocol, which avoids ice crystal formation in the cytoplasm, offers future possibilities for restoring fertility, especially in single women [19]. The combination of in vitro maturation (IVM) with oocyte cryopreservation prevents any delay in cancer treatment and avoids risks associated with high estradiol levels in hormone-sensitive tumors [20]. Cryopreservation of oocytes for fertility preservation is not yet available for the indication of fertility preservation in cancer patients in the Netherlands. Surgically transposing the ovaries out of the radiation field before pelvic radiation therapy reduces radiation exposure to the ovaries to 5–10% [21], but transposition is not applicable for patients being treated with chemotherapy. There is controversy about the effects of pharmacologic methods for protecting ovarian function by using gonadotropin analogues [22,23].

There are many variables to take into consideration when deciding upon fertility preservation procedures. These include delaying cancer treatment, surgical complications, ovarian hyperstimulation with high hormone levels, reintroducing cancer cells, low success rates and the experimental nature of some of the fertility preservation procedures. However, it remains very important for the physician to inform the patient about all the different treatments so she may make an informed decision regarding the fertility preservation options. In addition, to preserve the full range of options, fertility preservation procedures should be considered as early as possible during treatment planning [24].

Fertility preservation procedures will always take time regarding the steps of referral, counselling and the procedure itself. Whether delay of cancer treatment for fertility preservation procedures is acceptable or not, is to be discussed by the medical oncologist and the patient. In this descriptive study we retrospectively analysed data on the time intervals between the moment of diagnosis of cancer, the moment of referral to a gynecologist, the moment of consultation with a gynecologist, the moment of finishing of fertility preservation procedures (embryo cryopreservation or ovarian tissue cryopreservation) and the start of chemotherapy.

2. Materials and methods

Thirty-five female cancer patients underwent a procedure to preserve their fertility before the start of chemotherapy as cancer treatment. The study period was between November 2003 and March 2008, in the Leiden University Medical Center (LUMC), the Netherlands. Fertility preservation therapy (FPT) consisted of embryo cryopreservation (EC) or ovarian tissue cryopreservation (OTC). The decision to perform FPT was made by consensus among the referring clinician and the institutional multidisciplinary team, including a medical oncologist, a gynecologist and a surgeon. Preferably, EC was performed. However, in patients without a partner and patients with insufficient time to perform an IVF cycle, ovarian tissue cryopreservation was proposed according to a protocol "Cryopreservation of ovarian tissue". Approval for this protocol and for use of the computerized database of the patients referred for fertility consultation, was obtained from the Institutional Review Board of the Leiden University Medical Center. Informed consent was signed by the patient or a patient's parent in under-age patients.

The ovarian stimulation procedure for hormone-sensitive breast cancer is based on a protocol by Oktay [25]. These patients started on day 2 or 3 of the menstrual cycle with a short protocol of tamoxifen alone or tamoxifen plus low dose follicle stimulating hormone (FSH). However, from August 2007 the protocol of tamoxifen alone was abandoned because of low embryo yield. The standard IVF protocol, FSH plus a gonadotropin-releasing agonist, was applied in patients without a hormone-sensitive tumor [26]. IVF during an unstimulated cycle was considered if hormonal stimulation was contraindicated or after patient non-approval. A single dose of recombinant human chorionic gonadotropin was given when the lead follicle had a mean diameter of 18 mm (measured in two directions). Ultrasound-guided oocyte retrieval was performed 36 h later. IVF was performed via intracytoplasmatic sperm injection and embryos were cryopreserved with a slow freezing protocol until further use.

The OTC procedure consisted of a laparoscopic unilateral oophorectomy under general anesthesia. The oophorectomy was performed by laparotomy if a surgical procedure was already planned. In the operating room the ovarian tissue was dissected into small slices of ovarian cortex (10 mm × 5 mm × 1 mm) according to the description of Radford [27]. The slices were transferred in vials to the IVF laboratory. After cryopreservation with a slow freezing protocol, they were stored in liquid nitrogen, until required. No complications related to the fertility preservation procedures were reported.

Clinical charts and a computerized database were reviewed retrospectively for date of diagnosis, date of referral for fertility consultation, date of first consultation for FPT, date of finishing FPT (in OTC day of operation, in EC day of ovum pick-up) and date of start of chemotherapy. The date of diagnosis was the date on which the histologic diagnosis was definitive.

The Statistical Package for the Social Sciences, SPSS version16 was used to perform descriptive statistics. A comparison between OTC and EC was made by using the Mann–Whitney *U*-test and chi-squared test in the case of respectively continuous or ordinal and nominal variables. p-Value <0.05 was considered statistically significant.

3. Results

3.1. Patients' characteristics

Twelve of the 35 patients (34.3%) opted for IVF in order to cryopreserve embryos (EC), ten patients underwent one, and two patients two IVF cycles. In 9 of the 12 patients who started an IVF cycle, embryos were cryopreserved (median = 4; range 1–16). Ovarian tissue was cryopreserved (OTC) in 23 of the 35 patients (65.7%).

The mean age of the patients undergoing OTC or EC was 29.3 ± 5.8 years (range 14–39). Among the total study group 27 patients were nulligravid, four patients had previous pregnancies with elective terminations ($n = 2$) or miscarriages ($n = 2$). Four patients had full-term pregnancies prior to FPT. All patients undergoing EC were diagnosed with invasive ductal carcinoma of the breast. In the OTC group 14 patients were diagnosed with invasive

ductal carcinoma, four patients had a bone or soft tissue sarcoma (Ewing's sarcoma $n = 1$; osteosarcoma $n = 2$; myxoid liposarcoma $n = 1$), two patients had non-Hodgkin's lymphoma and one patient Hodgkin's disease; one patient had rectal cancer and one patient cervical cancer. All received combination chemotherapy. Patients with a malignancy other than breast cancer ($n = 9$) received neo-adjuvant chemotherapy.

3.2. Embryo cryopreservation

Ten patients started one IVF cycle. The median number of embryos that could be cryopreserved was 3 (range 0–16). After two stimulation cycles one patient had 7 embryos cryopreserved and another patient had 12 embryos cryopreserved.

The outcome of zero embryos for cryopreservation ($n = 3$) was caused by a premature luteinizing hormone (LH) surge in one patient. Another patient preferred natural-cycle IVF and therefore only ultrasound monitoring of the non-stimulated ovaries was performed. Unfortunately on the day of oocyte pick up, the ovaries were in a postovulatory state. The third patient stopped the procedure during hormonal stimulation because of relational problems.

The median length of hormonal stimulation cycle, between start of hormonal stimulation and oocyte pick up, was 11 days (range 7–16).

3.3. Time intervals from diagnosis to start of chemotherapy

No significant differences were observed between the EC patients and the OTC patients for the time interval between diagnosis and referral (median = 18 days; range 2–77) and for the time interval between referral and consultation (median = 5 days; range 0–25) (Table 1).

The median time interval for OTC patients between consultation and oophorectomy was 6 days (range 1–36) and was significantly ($p = 0.001$) shorter than the median time interval for EC patients between consultation and oocyte pick up of 18 days (range 10–28). The time interval between ovarian tissue cryopreservation and start of chemotherapy was significantly shorter ($p = 0.019$) in OTC patients (median = 7 days, range 1–41) than the time interval between oocyte pick-up and start of chemotherapy in EC patients (median = 19 days, range 5–42).

The total time interval between diagnosis and start of chemotherapy, based on the separate time intervals, was significantly shorter ($p = 0.042$) for OTC patients (median = 47 days, range 9–111) than for EC patients (median 69 days, range 33–118). One breast cancer patient (EC) postponed chemotherapy for 60 days to perform a second IVF cycle to preserve fertility and an OTC patient postponed it for three days due to personal reasons. After implementation of the protocol for cryopreservation of ovarian tissue in the LUMC in January 2007, the mean time interval between referral and fertility preservation consultation reduced significantly from 9 to 3 days ($p = 0.001$).

4. Comment

In this descriptive study we retrospectively analysed data about the timing of fertility preservation procedures (embryo cryopreservation and ovarian tissue cryopreservation) with respect to the diagnostic and therapeutic interventions for the diagnosed cancer in 35 female cancer patients. FP consultations were performed within five days from referral in half of the patients (no difference for OTC or EC). Half of the patients opting for OTC underwent oophorectomy within six days after consultation with a gynecologist. A first IVF cycle for embryo cryopreservation was completed in four weeks after consultation. In 9 of the 12 EC patients one or more embryos could be cryopreserved. We concluded that embryo cryopreservation can be performed within the standard timeframe of cancer care in patients with breast cancer receiving adjuvant chemotherapy. If delay of the start of chemotherapy is harmful, ovarian tissue cryopreservation can be done within one week.

Since successful procedures on future fertility were established within weeks, performing the actual procedure is probably not a limiting factor in referral for fertility consultation. Although ovarian tissue cryobanking is still in an experimental stage, worldwide thousands of young females have decided to freeze ovarian tissue.

The significant difference between OTC and EC patients in the median time intervals between diagnosis and ovarian tissue cryopreservation or oocyte pick-up can be explained by the fact that hormonal stimulation for EC takes more time than an oophorectomy for OTC.

All EC patients were breast cancer patients receiving adjuvant chemotherapy. Standard treatment for breast cancer consists of primary surgery followed by radiotherapy when breast conserving therapy is performed and adjuvant chemotherapy in lymph-node positive or intermediate/high risk lymph-node negative disease. Radiotherapy usually starts within four to six weeks after surgery, and chemotherapy two to four weeks after radiotherapy [28]. In this schedule hormonal stimulation is easily implemented. There is little information about the outcome after delay of adjuvant chemotherapy. The International Breast Cancer Study Group concluded that early start of adjuvant chemotherapy improved outcome for premenopausal breast cancer patients with tumors not expressing estrogen receptors [29]. Madrigano et al. stated that egg retrieval does not delay breast cancer treatment [30]. In other malignancies, like osteosarcoma and hematological malignancies, patients frequently require immediate chemotherapy. In these cases OTC is recommended because of the little delay of start of chemotherapy. This study has shown that fertility preservation can be performed within the standard timeframe of cancer care.

Results from earlier studies suggest an 'under-referral' for fertility preservation in, e.g. the Netherlands [31,32]. Discussing fertility preservation procedures is possibly limited by the lack of knowledge about the risk of infertility with current cancer treatments. This risk is multifactorial and depends on the patient's age, the treatment modality and dose and the ovarian reserve prior

Table 1
Time intervals in days from diagnosis to start of chemotherapy.

	Total group ($N = 35$) Median (range)	OTC ($N = 23$) Median (range)	EC ($N = 12$) Median (range)	p-Value[a]
Diagnosis –referral	18 (2–77)	18 (2–77)	19 (2–63)	0.932
Referral-consultation	5 (0–25)	5 (1–25)	4 (0–15)	0.503
Consultation-cryopreservation	7 (1–36)	6 (1–36)	18 (10–28)	0.001
Cryopreservation-chemotherapy	8 (1–42)	7 (1–41)	19 (5–42)	0.019
Total diagnosis-chemotherapy	56 (9–118)	47 (9–111)	69 (33–118)	0.042

OTC = ovarian tissue cryopreservation. EC = embryo cryopreservation.
[a] Differences between OTC and EC.

to gonadotoxic treatment [33]. After treatment, clinical information, such as a monthly menstrual cycle or normal hormone levels [5,28], does not automatically imply that the ovaries are undamaged. After apparently normal ovarian function, premature ovarian failure (POF), can occur [25]. In general approximately 60% of women treated for cancer experience POF [34]. Among younger women treated for cancer POF occurs in 17% of women from 15 to 30 years and in 42% of women in the third decade (34). An exact individual risk concerning ovarian function loss is, however, difficult to determine, as recovery from treatment-induced menstrual changes occurs in 80% of women under the age of 35 years and in 25% of women under 40 years of age. However, the vast majority of women who remain amenorrheic one year following treatment will not regain ovarian function [35]. This study stresses the importance of providing the opportunity for an informed decision regarding fertility preservation options on time and without delay to all young patients who require potentially sterilizing treatments for cancer. Therefore access to professional fertility consultation and services provides an added measure of potential long-term well-being and a message of hope for premenopausal women who need to postpone childbearing because of cancer treatment. We advise using an up-to-date protocol for indication and referral for fertility preservation procedures to make access as easy as possible.

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