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FERTILITY PRESERVATION

Combined strategy for fertility preservation in an oncologic patient: vitrification of in vitro matured oocytes and ovarian tissue freezing

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Introduction

Breast cancer is the most common malignancy in women and nearly 7% of cases are diagnosed before age 40 [1]. Diagnosis at early ages is associated with a worse prognosis and therefore, these cases are usually managed with adjuvant chemotherapy and/or hormonal therapy. These treatments are effective in reducing recurrence rates and improving survival rates; however, they have a negative impact on ovarian function and fertility [2]. Fertility preservation in these cases may be of interest also due to the current trend towards delaying motherhood, which leads to a situation where many women may have not fulfilled their motherhood desire at the time of breast cancer diagnosis.

There are several strategies to preserve fertility. Choosing one or the other will depend on patient's age, tumour biology and, above all, time availability until the start of gonadotoxic treatment.

Capsule In vitro matured oocyte vitrification combined with ovarian tissue cryopreservation represents a strategy for fertility preservation when ovarian stimulation is contraindicated.

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Case report

We report a case of a 28-year-old patient without relevant past medical history. Menarche occurred at 13 years; she was null gravid and had used oral contraceptives during 6 years. Her routine gynaecological check-ups were all within normality. She presented family oncologic background in second and third grade relatives but none of the tumours were of gynaecological origin. Due to the finding of a painful lump on the right breast, mammographic, ultrasound and magnetic resonance screenings were performed. Mammography revealed a group of microcalcifications in the retroareolar region and the ultrasound scan and magnetic resonance showed a mass at the same location, which was biopsied. An infiltrating ductal carcinoma (histological grade II, estrogen receptors (ER) 100%, progesterone receptors (PR) 70%, Her 2 (1+) negative, Ki67 15%) was identified. Radiologic extension studies did not reveal distant disease. A tumorectomy with the sentinel node technique was performed, micrometastases were found in 2/2 nodes, and surgery was completed with lymphadenectomy (0/9 affected nodes). Final diagnosis was pT1cNmicM0 HR positive Her 2 negative and adjuvant treatment was recommended with chemotherapy, radiotherapy and hormonal therapy (doxorubicin and cyclophosphamide x 4 cycles and weekly taxol x 12 cycles, followed by radiotherapy and tamoxifen x 5 years). Given the patient's desire for fertility preservation, her oncologist suggested treatment with GnRH agonists before initiation of chemotherapy. The patient came for reproductive medicine consultation to discuss other options for fertility preservation.

Due to the tumour's biology, controlled ovarian hyperstimulation was contraindicated and ovarian tissue cryopreservation was recommended; additionally, the possibility of freezing

in vitro matured oocytes obtained from the visible antral follicles present in the excised ovarian tissue was considered.

Two fragments of right ovarian cortex measuring 15× 0.5 mm were obtained by laparoscopy and two antral follicles were macroscopically identified. The samples were sent to the IVF laboratory in L-15 Medium Leibovitz with L-glutamine (Sigma-Aldrich UK) in cold. The ovarian tissue fragments were placed in a sterile glass plate and the visible follicles were punctured. Two GV oocytes were retrieved. After washing them in culture medium (G-IVF plus, Vitrolife Serie V), they were incubated in IVM medium MediCult (IVM® System, Origio, Denmark) supplemented with rFSH and HCG at 37°C and 5% CO_2. After 24 h of culture the two GV oocytes reached metaphase I (MI). IVM culture was prolonged under the same conditions. After 48 h in IVM medium, cumulus cells were displaced using 175 μm diameter capillars (Stripper tips Origio, Midatlantic devices, NJ, USA). Oocytes were observed at the inverted microscope to check for complete maturation to MII stage. Both oocytes were vitrified following the Cryo Top method [3]. The rest of the ovarian cortex was placed on a sterile glass plate with L-15 at 4°C. Fragments of 5×10 mm were obtained after dissection of the tissue with scissors and scalpel and transferred to cryotubes containing 800 μl of L-15 with cryoprotectant, human serum albumin (HSA) and dimethyl sulfoxide (DMSO). The ovarian fragments were cryopreserved in a programmable cooler, (Minicool 40PC, Air Liquide), following the methodology described by Donnez in 2006 [4].

One piece of ovarian cortex was sent to the pathology laboratory to check for the presence of primordial follicles and absence of malignant cells.

Discussion

Breast cancer in young women is associated with adverse prognostic features and higher rates of recurrence when compared to older women and, as a consequence, most women of reproductive age diagnosed with breast cancer will receive adjuvant chemotherapy [5].

One of the chemotherapy regimens more frequently used nowadays in breast cancer includes doxorubicin, cyclophosphamide and taxanes. The combination of doxorubicin and cyclophosphamide leads to amenorrhea in 34% of cases, being frequently permanent in women >40 years and temporary at younger ages [6].

In premenopausal women, around 60% of breast cancers are hormone receptor — positive and will need hormonal therapy, usually with tamoxifen during 5 years; this treatment has a temporary and reversible effect on the ovary but delays pregnancy during those 5 years, so the age effect is added to the chemotherapy effect in the reduction of the ovarian reserve [2].

If there is sufficient time and ovarian stimulation is not contraindicated, embryo or oocyte cryopreservation are the preferred options. Ovarian stimulation protocols with aromatase inhibitors have been described in order to avoid excessive high estradiol levels and no increase of recurrence rate of breast cancer has been observed with this treatment [7].

Nonetheless, there are breast tumours with an extremely high expression of hormonal receptors in which ovarian stimulation should preferably be avoided and other fertility preservation options should be considered.

Retrieval of immature oocytes followed by in vitro oocyte maturation is one of the strategies in cases in which ovarian stimulation is not possible (time constraints or oestrogen sensitive tumours). Oocyte retrieval is usually performed prior to ovulation but immature oocytes can also be recovered during both the follicular and luteal phase [8]. Oocyte cryopreservation after IVM can be performed using vitrification or slow freezing techniques. Vitrification of IVM oocytes has resulted in a live-birth rate of 20%; nonetheless, survival and fertilization rates of IVM oocytes are lower than those of in vivo matured ones [9]. Smitz et al. report implantation rates 2–3 times lower in embryos coming from IVM oocytes when compared to embryos coming from in vivo matured oocytes, early miscarriage rates also being higher in the first group [10]. On the other hand, in a recently published study, Oktay suggests IVM as an additional strategy in all fertility preservation cycles, even if ovarian stimulation is carried out, as it results in a 45% increase in mature oocyte yield [11].

Another alternative when there is not sufficient time or ovarian stimulation is not recommended is ovarian tissue freezing. One of the concerns regarding transplantation of the thawed ovarian tissue is the risk of re-seeding of malignant cells, although in early stage breast cancer the risk of occult ovarian involvement is very low [12]; this risk might be higher in BRCA mutation carriers and in invasive lobular carcinomas but these are <15% of all breast cancers and occur most often in postmenopausal women [13, 14].

Up to now, 15 births have been described after fresh or frozen/thawed ovarian tissue transplantation [15]. There is a limited functionality of the ovarian graft and this may be partially due to the initial ischemic injury after transplantation. Several techniques have been proposed to minimize ischemic damage to follicles. Cryopreservation of ovaries with vascular pedicle has been proposed by some authors as an alternative to cryopreservation of ovarian cortex fragments, but nowadays it is not a realistic option due to the complexity of the technique [16]. Also it has to be considered that the risk of reintroducing malignant cells is increased when transplanting full ovary [17]. As an alternative, In vitro follicular culture has been proposed

by some groups. The aim of this technique is to develop an in vitro system which allows growth of primordial and primary follicles to antral stages in order to obtain mature oocytes [18]. Although in vitro follicular culture has reached antral stages, it is necessary to optimize In vitro culture protocols in order to achieve results that can be applied in clinical practice.

In the case that we report, fertility preservation is performed with the use of ovarian tissue cryopreservation as well as IVM oocyte vitrification. The combination of the two options, improves future expectations of fertility preservation. This approach has also been reported by other authors and can be performed at any time of the menstrual cycle [19, 20].

Immature oocyte collection and further IVM and vitrification must be considered as a complementary strategy of fertility preservation in cases of ovarian tissue freezing.

In the present case, should the patient desire pregnancy and not achieve it spontaneously once cured from the disease, warming of vitrified oocytes should be proposed first. If pregnancy after embryo transfer is not achieved, ovarian tissue thawing and transplantation should follow.

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