World Cancer Research Journal WCRJ 2014; 1 (4): e409

GONADOTOXIC EFFECTS OF BREAST CANCER TREATMENT AND FERTILITY PROTECTION STRATEGIES: EVIDENCE BASED ANSWERS TO THE MAIN QUESTIONS THE PATIENTS ASK

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Under the auspices of PROFERT (Società Italiana di Conservazione della Fertilità)

Abstract: *Nowadays cancer is diagnosed earlier and age at pregnancy is delaying, making fertility counseling an issue of greater importance. Not only it can help younger cancer patients to fulfill the desire to have a child, but it also increases the quality of life, giving hope for the future, even if the patient will not get pregnant or if she will not pursue active strategies to protect fertility before chemotherapy or radiotherapy.*

All professionals who deal with breast cancer patients have the duty to know it and to actively inform patients in fertile age about how to limit the gonodotoxic effects of cancer treatments. However, the oncofertility is a rapidly evolving, complicated, and still partly experimental issue. This article is intended to help giving evidence based answers to the main questions the patients ask.

KEY WORDS: *Breast cancer, Chemotherapy, Ovarian toxicity, Ovarian reserve, Fertility preservation, Counselling.*

All physicians involved in the treatment of breast cancer patients have the duty to explain the potential gonadotoxic effects of antiblastic therapies and the fertility protection strategies. Patients will preferably be rapidly sent to subspecialist in fertility preservation in oncology for a timely and complete information[1]. This article is composed in a questions and answers format in order to help breast cancer surgeons, medical oncologists, radiotherapists and nurses to better counsel patients since the first breast cancer diagnosis.

WHY IS FERTILITY COUNSELLING SO IMPORTANT?

Fertility counseling is not only mandatory according to ASCO guidelines[2], it can also improve the quality of life (QoL) of cancer patients even though they will not try to get pregnant. In a study[3] including 1,041 cancer women aged 18-40 years counseling about reproductive loss and pursuing fertility was associated with less regret and greater QoL for survivors, even though (96%) patients did not do active strategies to preserve fertility.

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Loss of reproductive potential as a consequence of anticancer treatment negatively impacted the QoL in young survivors[4,5]. As showed in recent studies, the potential iatrogenic loss of fertility, which also means loss of a potential child, had a profound impact on young women and in some ways may was more stressful than the cancer diagnosis itself[6,7]. An active approach to counseling made a huge psychological difference[8]. Authors assessed, in women under the age of 45 at the time of diagnosis of breast cancer, how many of them wanted and tried to become pregnant after breast cancer treatment, the effects of pre-treatment counseling and their prognosis. They showed a higher rate of pregnancy than expected, possibly due to newer treatments including fertility preservation and also possibly due to the active counseling program in the unit. Authors concluded that "*the positive attitude of the breast team towards pregnancy may also help reduce the fear of pregnancy after breast cancer and consequently also reduce the elective abortion rate*".

HOW TO SUCCESSFULLY PERFORM FERTILITY COUNSELING?

Patients should have active counseling about fertility when planning treatment, and fertility preservation can then be incorporated into a treatment plan[9]. An informed choice about whether to access any available fertility preservation strategy can only be made after a proper discussion of their risks, success rates and costs. On the other hand, being some fertility preservation strategies still experimental and difficult to access in some centers, it is mandatory for oncologists and gynecologists to work together[10]. Further research is needed to improve the efficacy and safety of the available strategies, and an effective collaboration between oncologists and gynecologists should be implemented to improve patients access to reproductive technologies.

Before giving information, patients must be properly listened. It seems obvious, but active hearing is frequently neglected. Physician must not only listen at the words but also at the non-verbal communication which informs about three very important elements[11]. The first is the kind of patient through her appearance and the way of speaking. This can, for example, help to modulate our information according to her level of education and to mirror her attitudes or to modulate her anxiety. The second non verbal communication to be careful for is how she relates with the physician. If she sets herself in a position as an adult to an adult, she needs more technical, scientific based information. If she relates like a frightened daughter asking help to a parent she will better not be submerged by too many information. Maybe she needs mostly to be listened and more actively helped to make a difficult fertility preservation choice. The third part of information that lies behind the words and/or it is vehiculated by the non-verbal communication (mimic, posture, tone of voice, etc.) is what she really needs. This is the most difficult thing to understand, but the most important. Even the patient herself could be unaware of that and if the physician can help her to understand that or if the physician answers that need, the patient-doctor alliance will be greatly improved. Giving the proper information about fertility protection in relatively easy, while a good communication is difficult but essential, it should not be delegated only to psychologists, it improves QoL and this also could reduce legal litigation.

WHAT ARE THE EFFECTS OF ANTIBLASTIC BREAST CANCER TREATMENTS ON MENSTRUATION AND FERTILITY?

With the increased screening and education, the incidence of breast cancer in young patients is improving. In fact, at the time of diagnosis, approximately 30% of patients are premenopausal and 10% are aged between 35-44 years[12]. In addiction breast cancer, even in early stages, presents more aggressive features and worst prognosis in younger compared with older people[13-15].

Adjuvant chemotherapy prolongs disease-free periods and overall survival in patients with breast cancer but, on the other hand, it is also responsible for a long-time side effects as ovarian damage and failure[16]. In fact, in a variable percentage of pre and peri-menopausal women, chemotherapy may cause amenorrhea or premature menopause with the consequent loss of child-bearing potential, menopause symptoms and prolonged exposure to menopausal risks such as osteoporosis[17]. The reduction of fertility potential in these patients may be considered in family planning decisions through the diagnosis and follow up, by an adequate counseling.

The exact mechanism of chemotherapy-induced amenorrhea is not clearly understood although *in vitro* models have demonstrated that chemotherapeutic agents may act directly on primordial follicles through the induction of apoptotic changes in pregranulosa cells. This mechanism may lead to the irreversible loss of follicles and oocytes[18]. Recently, a correlation between acute vascular damage and alteration in ovarian blood flow, size, and function, in association with an abnormal hormonal profile and clinical symptoms, has been demonstrated in a small cohort of 20 premenopausal patients with EBC who have been treated with neoadjuvant/adjuvant CT[19].

Acute amenorrhea occurring during systemic treatment, may be temporary or permanent. The incidence of chemotherapy-induced amenorrhea by regimen ranges from 9% to 75%[20].

The clinical degree of ovarian dysfunction (i.e., oligomenorrhea, transient/prolonged amenorrhea, and true menopause) is essentially related to the extent of injury caused by CT. Oligomenorrhea or temporary amenorrhea are the consequence of damage to both steroid-producing cells (granulosa cells and theca cells) and the oocytes of growing follicles. When exposure to CT induces near complete follicular depletion or few follicles remain viable (approximately <1000), periods may cease and menopause will occur[21,22]. However this condition may be only temporary. Cessation of menses is not synonymous with true ovarian failure because estrogen levels can remain in a premenopausal range despite 1 year or longer of chemotherapy-induced amenorrhea. The incidence of permanent amenorrhea after systemic treatment for breast cancer is estimated to be between 33% and 76% in women age 50 or younger[23].

Physiologically, the remaining follicles may be recruited from the primordial pool and accordingly to this event the levels of gonadotropins may raise the normal range and menstrual cycles may resume months to years after withdrawal/end of CT[24]. Resumption of menses can occur even after 2 to 3 years of chemotherapy-induced amenorrhea and the majority of patients younger than 40 years recover menses within 1 year from cessation of treatment[25]. Notwithstanding, it has been demonstrated that women who continue to menstruate after treatment with chemotherapy for breast cancer have a significant reduction of fertility and they enter menopause earlier[26].

Amenorrhea rates may underestimate reproductive impairment, so it is important to inform patients with a diagnosis of breast cancer eligible for chemotherapy regimens that their reproductive potential may be impaired even if menses are regular.

Ovaries are variably sensitive to most cytotoxic drugs and many factors may influence the onset of chemotherapy induced amenorrhea and or menopause such as: age at diagnosis, type, duration and total cumulative dose of a drug, and use of TAM (TAM)[27].

Previous studies showed an higher incidence of amenorrhea with alkylating agent-based regimens. These drugs are most commonly associated with permanent and irreversible gonadal damage. Cycle-specific agents, such as methotrexate, 5 fluorouracil, bleomycin and vinca alckaloids are less gonadotic. Cisplatin and adriamycin modestly affect ovarian function[24-28].

Secondarily, several studies showed that menses are more likely to return not only in women receiving less gonadotoxic regimens but also in younger ones and, in any case, in those with a higher basal number of follicles.

However, the estimation of the individual risk to develop chemotherapy induced amenorrhea and or menopause remains inappropriate and these symptoms may only represent late signals. Evaluation of ovarian reserve in breast cancer patients represents a challenge issue to predict more reliable assessments of ovarian function and at present, the levels of AMH, antral follicular count (AFC) and inhibin-B are considered predictive preclinical signs of ovarian function compromise[29].

HOW TO ESTIMATE THE GONADOTOXIC EFFECTS OF SPECIFIC ANTIBLASTIC TREATMENTS?

Early-stage invasive breast cancer with negative estrogen receptor are treated with adjuvant cytotoxic therapy, while hormone therapy is used for estrogen positive receptor tumors. The majority of young women diagnosed with breast cancer are estrogen receptor negative and have a worst prognosis so they will undergo adjuvant chemotherapy. Compared to untreated women, patients receiving chemotherapy showed a significantly lower follicle counts and produce significantly less estradiol compared with controls. The amount of the effect is very different. When considering the treatment related effects, these are the main factors to assess: dose, dose-intensity, method of administration, size and location of the radiation field, the radiation delivered dose and its fragmentation. Among chemotherapy agents, the greatest risk is associated with alkylating agents, particularly cyclophosphamide, but also carboplatin and cisplatin can have a negative effect[30,31].

Alkylating agents are associated with a high gonadal toxicity because of the vascular damage to the ovary[32] and the direct induction of follicle and oocyte apoptosis[33].

The National Surgical Adjuvant Breast and Bowel Project (NSABP) B-30 trial[34], a study carried on in 5,300 women with early-stage, node-positive breast cancer, has demonstrated that adjuvant therapy with sequential doxorubicin (A) and cyclophosphamide (C) followed by docetaxel (T; AC→T), compared with four cycles of AT or TAC, improved survival. The rates of prolonged amenorrhea (>6 moths) after one year was 69.8% for AC→T, 37.9% for AT, and 57.7% for TAC ($p < 0.001$). The amenorrhea rates were higher with the addition of TAM. The AT group without TAM showed the lowest rate of amenorrhea, hovering around 20-30% across the 24-month period of ob-

servation. Approximately 61% of women under the age of 40 experienced at least 24 months of amenorrhea contrasting with nearly 100% among patients older than 40 years.

A low risk of treatment-related ovarian failure is associated with methotrexate and fluorouracil. Few data are available for newer agents such as taxanes[35]. The addition of taxanes to anthracycline-containing chemotherapy for breast cancer has shown a similar rate of amenorrhea compared to historical controls in a case series of 230 women younger than 40 years[20].

A prospective observational study that assessed ovarian function, used the surrogate of monthly bleeding, after breast cancer treatment in 595 premenopausal patients[25]. Patients younger than 35 years had rapid menstrual cycling recovery with the proportion with bleeding rising to approximately 85% at 6 months following the end of chemotherapy, and remaining relatively constant. The recovery was less pronounced for patients between the ages of 35 and 40. The majority of women aged 40 years or older had no menstrual bleeding at the end of chemotherapy and no recovery of bleeding in the follow up years compared with younger women. Concerning the chemotherapy regimen administered, treatment with AC alone resulted in an important decrease in the proportion of patients with periods. Paclitaxel or taxol added to adrimycin-cyclophosfamide led to a small further decline in the number of patients with bleeding, while CMF (cyclophosfamide, metotrexate, 5-Fluorouracil) resulted in a greater proportion of patients with monthly bleeding in the initial months but with a progressive decrease in the follow-up years. Finally, the addition of TAM resulted in a decrease in the proportion of patients with monthly bleeding by 1 year following chemotherapy, but this effect became non significant by 3 years. Chemotherapy acted primarily on primordial follicles, through the induction of apoptotic changes in pregranulosa cells, leading to irreversible loss of follicles and oocytes. There was also an indirect effect on vascularization and fibrosis.

Combination chemotherapy is used more often than single agents, and it is therefore difficult to evaluate the contribution of each individual drug.

WHAT ARE THE EFFECTS OF GN RH, TAM ALONE OR FOLLOWING CHEMOTHERAPY?

For young women with receptor-positive breast cancer, endocrine therapy including ovarian suppression/ablation with gonatrophin releasing hormone (GnRH) analogs and TAM is an alternative or complement to conventional chemotherapy. Ovarian medical suppression combined with TAM is currently accepted as an adjuvant endocrine treatment for premenopausal receptor-positive breast cancer[36].

This only treatment represents a reasonable alternative for women with good risk early-stage breast cancer (receptor-positive, lymph node-negative disease), particularly those wishing to preserve fertility. The association of GnRH agonist and TAM offers excellent protection against the endometrial side-effects induced by TAM. Moreover, TAM appears to be able to reduce the significant bone loss induced by GnRH agonist in young women[37,38].

TAM alone is associated with a low risk of premature menopause, which is age related: over the age of 45, the risk of infertility seems 10% higher than in controls. The administration of TAM sequentially to chemotherapy causes a statistically significant increase in the risk of infertility compared to chemotherapy alone[39,40].

HOW TO MEASURE OVARIAN RESERVE BEFORE AND AFTER CHEMOTHERAPY?

Ovarian reserve measurements are essential to estimate expected damage of anticancer therapies on fertility[41]. The number of the remaining oocytes are reduced by age, chemotherapy, but also pelvic surgery, heavy smoking, environmental and genetic factors[42,43]. Ovarian reserve is assessed by hormonal assays and evaluation of antral follicular count (AFC) with transvaginal ultrasound[44]. Anti-mullerian hormone (AMH) has been proven the more accurate in predicting ovarian response to stimulation both in IVF than in fertility preservation cycles, among hormonal markers[45,46]. AMH concentration measurements are also useful in the evaluation of chemotherapy induced ovarian damage and may become a tool for the comparison of ovarian toxicity of different chemotherapy regimens[47-50]. Since AMH concentrations are stable throughout the menstrual cycle, differently from other hormonal markers such as basal follicle-stimulating hormone (FSH) and 17 beta estradiol which must be dosed early in the follicular phase (day 2-4), AMH evaluation should be done as soon as possible to make results available at the time of consultation. The oocyte quality is also important but more difficult to evaluate, and more strictly related with patients' age.

Assisted fertility after preservation techniques may be efficacious only in cases with a good ovarian reserve. It has been reported a low response to stimulation with letrozole and gonadotropins for oocytes recovery in breast cancer patients when the AMH level is ≤1.2 ng/mL[46].

DO GN RH AGONISTS REALLY PROTECT FERTILITY?

The gonadotropin-releasing hormone analogues (Gn RHa) causes an ovarian suppression which is only temporary in 90% of patients under the age of 40 and in 70% of women older than 40 years[51].

Randomized trials have studied the GnRH agonist to preserve ovarian function in breast cancer patients during chemotherapy reporting conflicting results[52-56].

A meta-analysis to evaluate the role of Gn RHa in the prevention of chemotherapy-induced premature ovarian failure (POF) has been presented: a total of seven randomized clinical trials involving 745 premenopausal patients randomly assigned to receive chemotherapy or chemotherapy plus Gn RHa were included in the analysis; 5 trials were carried out in breast cancer patients and two trials in lymphoma patients. The pooled odds ratio estimate for chemotherapy induced POF was 0.46 (95% CI: 0.3-0.72) showing an important benefit of this strategy in reducing the gonadal toxicity of cytotoxic therapy in premenopausal cancer patients[57]. So the temporary ovarian suppression induced by Gn RHa significantly reduced the risk of chemotherapy-induced POF in young cancer patients if given before and during chemotherapy[58].

Another recent meta-analysis assessed the efficacy of Gn RHa administration to prevent chemotherapy induced ovarian toxicity specifically in premenopausal breast cancer women has been published[59]. Five randomized clinical trials (total number of patients: 528) were included in the analysis: significantly fewer women treated with Gn RHa during chemotherapy experienced post-treatment POF (RR: 0.40; 95% CI: 0.21-0.75). However, both treatment groups had similar rates of resumed menses (RR: 1.31; 95% CI: 0.93-1.85) and spontaneous pregnancy (RR: 0.96; 95% CI: 0.20-4.56). That's why this subject remains controversial and this strategy is still considered experimental[60,61].

IS OVARIAN STIMULATION AFTER BREAST CANCER A TRULY SAFE PROCEDURE?

Cryopreservation of embryos and oocytes are considered standard strategies and are the advised fertility preservation options for breast cancer patients[62].

Controlled ovarian stimulation (COS) with gonadotropins is needed to obtain more than one oocyte and it is a key component in the success of *in vitro* fertilization (IVF), as well as in cycles aiming to preserve fertility by oocyte or embryo cryostorage[63]. In women diagnosed with breast cancer, there is usually sufficient time to undergo COS to preserve fertility by embryo or oocyte cryopreservation, especially when adjuvant chemotherapy is planned. Chemotherapy is typically initiated 3 to 6 weeks after breast surgery, thus providing enough time to undergo COS, especially if patients are referred early in the process[64].

There are a few data on pregnancies obtained with oocytes or and embryos cryopreserved in cancer patients: therefore, to estimate the potential of these fertility preservation techniques it is necessary to consider data derived from an age-matched infertile population[65].

There are still some concerns about the impact of the COS on hormone responsive tumors[62,66]. There are no studies demonstrating the absolute safety of IVF in breast cancer patients, especially in case of estrogen-responsive tumors, as there is a potential risk that supra-physiologic estradiol (E2) levels could promote the growth of estrogen receptor-positive breast cancers cells. At this stage, patients have not completed treatment for breast cancer, and it is plausible that cancer cells may still be present in the body and will respond to high E2 levels, as documented in *in-vitro* studies and in patients with metastatic disease[67-69].

The rise in E2 is directly proportional to the number of growing follicles; for this reason, alternative and potentially safer protocols have been introduced for these patients, including natural cycle IVF, stimulation protocols with TAM alone or combined with gonadotropins and stimulation protocols with aromatase inhibitors[70].

Natural cycle IVF does not allow to obtain more than one oocyte or embryo per cycle, thus resulting ineffective.

TAM is a selective estrogen-receptor modulator (SERM) with antiestrogenic actions on breast tissue leading to inhibition of the growth of breast tumors due to competitive antagonism of E2 at its receptor site[71]. TAM can be used in ovulation induction starting on day 2-5 of the menstrual cycle in doses of 20–60 mg/day; it may be used alone or in combination with gonadotropins[72]. Recently Meirow demonstrated that high E_2 serum levels in young breast cancer patients treated with TAM are very common[73]. Irrespectively of these high E_2 serum levels, TAM has a proven protective effect for young E2 receptor–positive patients, both those with ovarian failure after chemotherapy and those with functioning ovaries[71,74-76]. Meirow demonstrated both the safety and the effectiveness of TAM co-administration during COS: he added 20 mg TAM to various ovarian-stimulation protocols in patients with hormone receptor-positive tumors. Both protocols with GnRH antagonists or GnRH agonists were effective

and the numbers of oocytes collected and embryos stored were higher with the co-administration of TAM, especially for the older age group. The author suggested a protective action of TAM co-treatment, based on the long-standing protective effects of TAM in the high-E2 environment.

Aromatase inhibitors, such as Letrozole, suppress plasma E2 levels by competitively inhibiting the activity of the enzyme aromatase[77]. Oktay proposed the so-called "COST-LESS" protocol, in which letrozole is administered in association with gonadotropins and a GnRH-antagonist to induce multiple ovulation in breast cancer patients[78]. He administered letrozole orally from day 2 or 3 of the cycle at a dose of 5 mg day, whereas gonadotropins (150-300 UI) were started two days later. A GnRH-antagonist was added when E2 levels exceeded 250 pg/ml, or when the leading follicle reached 14 mm diameter, in order to prevent premature LH surge. All medications were discontinued the day of human chorionic gonadotropin (hCG) trigger, and letrozole was reinitiated after oocyte retrieval and continued until E2 levels fell to 550 pg/ml. hCG trigger was later replaced by GnRH-agonist trigger, leading to significantly faster drop in E2 levels, significantly lower rate of moderate/severe OHSS and comparable number of mature oocytes[79]. Compared to a conventional IVF protocol, the COST-LESS protocol resulted in a significantly lower peak estradiol level and in a 44% reduction in gonadotropin requirement, while the length of stimulation, the number of embryos obtained and the fertilization rate were similar[78].

The largest experience with the use of oocyte cryopreservation strategies in breast cancer patients has been reported by Azim et al[80]. These authors prospectively evaluated 215 breast cancer patients, 79 of which undergoing embryo or oocyte cryopreservation, and 136 controls who did not undergo any fertility-preserving procedure. After a median follow up of 23.4 months after chemotherapy, the risk of recurrence after IVF was not significantly higher (HR 0.56, 95% CI: 0.17-1.9) and the survival rate of patients that underwent cryopreservation strategies was not lower compared with controls ($p = 0.36$). Anyway, long-term follow-up and future research are needed to confirm these promising results.

IS OVARIAN TISSUE CRYOPRESERVATION A SUITABLE ALTERNATIVE FOR BREAST CANCER PATIENTS?

Ovarian tissue cryopreservation is an experimental technique for female fertility preservation offering very encouraging results.

This strategy is the only option for pre-pubertal girls and for women who cannot delay the beginning of chemotherapy, as well as for women who cannot do or who refuse COS. It is the main option for those women who require urgent cancer treatment, such as neo-adjuvant chemotherapy. Ovarian tissue retrieval does not require COS and it is independent from the menstrual cycle phase, as it can be planned and performed in a few days. Moreover, ovarian tissue cryopreservation allows to store a great number of primordial follicles that are relatively resistant to cryodamage (about 70%-80% survival)[81]. Finally this technique allows to restore endocrine function after re-transplantation of ovarian tissue, which is not desirable in patients with an estrogen sensitive breast cancer[82].

As an adequate ovarian reserve is mandatory for the success of the technique, the age at ovarian retrieval is one of the most important issues. Since the follicular reserve of the ovary is age-dependent, the technique should be offered to patients younger than 38[83].

The main disadvantage of ovarian tissue cryopreservation is the need of invasive procedures both for tissue harvesting and for transplantation, even if the use of micro-invasive surgery, when possible, can minimize the risk of complications and adverse events. Ovarian tissue retrieval can be performed using multiple ovarian biopsies, partial oophorectomy and total oophorectomy including the vascular pedicle. The ovarian tissue can be harvested by simple laparoscopic procedure, under general anaesthesia. The general advantages of laparoscopy compared to laparotomy are well established. Different studies concluded that laparoscopy is a safe and effective procedure for ovarian tissue harvesting, and should be considered as the gold standard. Obviously ovarian tissue can be obtained during contingent laparotomic surgery performed to treat pelvic or abdominal malignancies[84].

After ovarian tissue is recovered, rapid move to the laboratory is performed in transport medium on ice. Transport from the place of removal to the tissue bank is also possible over a longer period of time, up to 20 hours[85]. Then the ovarian tissue is prepared for the freezing procedure: ovarian cortex is enucleated from medullary compartment with sharp scalpel dissection and cut in small strips or cubes. Finally the tissue is stored in liquid nitrogen after the freezing procedure.

Even if ovarian tissue cryopreservation is now relatively well established, the use of the cryopreserved ovarian cortex in order to restore fertility remains a challenge. Up to now, orthotopic or heterotopic transplantation is the only available option to restore fertility using cryopreserved ovarian tissue, as other techniques still require additional research before be-

coming available for humans. The timing of transplantation has to be decided in agreement with the oncologists, and is usually performed when the patient is willing to get pregnant, as the duration of transplanted ovarian tissue is limited in time (the longer duration ever reported is 7 years)[86]. The fragments of ovarian cortex that are thawed and transplanted are not all those available, as some of them are kept, if available, to establish a reserve of tissue.

Transplantation can take place either into the pelvic cavity, in the orthotopic transplant, or in alternative sites in the heterotopic transplant. To date almost 30 live births have been reported worldwide after orthotopic autologous ovarian transplant[87-104], whereas heterotopic graft has led to one twin pregnancy[105], a biochemical pregnancy[106] and four spontaneous pregnancies with three live births as a result of a reactivation of the native ovary[107].

A very important concern about the application of this technique is the potential reintroduction of cancer cells during transplantation[108-110]. Indeed the risk of reintroducing malignant cells theoretically exists in breast cancer[111]. Breast cancer can metastasize to the ovaries, more commonly in advanced-stage cancer, even if the development of an ovarian tumor is more likely to be of primary ovarian origin than a breast cancer metastasis[112]. In a study by Azem, histological examination of cryopreserved ovarian tissue from 13 women with breast cancer using histology and immunohistochemistry revealed no evidence of malignant cell involvement[113]. Similarly, Sánchez-Serrano did not find histological evidence of malignant cells in the ovarian cortex of 100 biopsies from 63 women with breast cancer stage I-IIIa[114]. Rosendahl examined the ovarian cortical biopsy of 51 patients with breast cancer (grade I-III) and did not detect the presence of ovarian metastases by morphological or immunohistochemical methods[115].

On the other hand, however, in a very large review of 5,571 female autopsies, Kyono evidenced ovarian metastases in 24.2 % of breast cancer patients[116]. Obviously as these data are obtained as results of autopsies, they may not reflect the risk of ovarian involvement in patients who are normally offered ovarian tissue cryopreservation, who typically have a minimal risk of dissemination and ovarian involvement; anyway a great caution is necessary when transplanting the tissue. A pilot study by Donnez evaluated the risk of cancer cell contamination of cryopreserved ovarian cortical fragments with conventional screening methods (histology and immunoistochemistry) combined with a single-marker PCR assay, confirmed by gene sequencing and xenotransplantation. The study demonstrated that cryopreserved ovarian tissue from patients with advanced-stage breast cancer may contain cells expressing the MGB2 gene, even if the real malignant potential of these cells is not yet known[117]. Also Ernst reported the case of a 33 year-old patient affected by breast cancer who spontaneously conceived after ovarian tissue transplant and had a legal termination of pregnancy because of cancer recurrence, even if it was unclear whether the transplanted tissue had any effect on the recurrence[118].

Besides the possibility of tumour contamination of the cryopreserved tissue, the return of natural ovarian function may have an impact on the course of breast cancer.

For patients at risk of having malignant cells in their cryopreserved ovarian tissue, other options could be follicle culture with *in vitro* maturation[119-121], grafting of isolated follicles[122], ovarian tissue purging to eliminate malignant cells[123] or artificial ovaries of primordial follicles combined with disease-free stromal elements placed in an alginate matrigel matrix[124].

So far, ovarian tissue cryopreservation has to be considered still experimental and should be performed only in centers with the necessary expertise under approved clinical protocols. Furthermore, particular attention should be paid to the follow-up of these patients for recurrent or re-implanted cancer, particularly in breast cancer patients.

WHAT IS THE ROLE OF OOCYTE IN VITRO MATURATION FOR PATIENTS WITH BREAST CANCER?

Cryopreservation of immature or matured oocytes is an experimental strategy to preserve fertility, representing an emerging option for women who need to start chemotherapy urgently as well as for prepubertal girls who cannot undergo ovarian stimulation.

maturation (IVM) of immature oocytes obviates the need for ovarian stimulation as immature oocytes can be collected without hormonal stimulation or with a short stimulation lasting 3-5 days. Immature oocytes can be collected from the ovaries both in the follicular and luteal phases, maximizing the possibility of fertility preservation for cancer patients. Anyway, immature oocytes require further *in vitro* maturation, which is not perfectly efficient and it is only available in a small number of labs worldwide[125].

To date, only a few live births have been obtained from cryopreserved *in vitro* matured oocytes, and no pregnancies concerned fertility preservation of cancer patients[126,127].

A major issue is whether immature oocytes should be cryopreserved before or after IVM. It has been proposed that cryopreservation at the im-

mature germinal vesicle (GV) stage might reduce the damage of freezing procedure, but difficulties still exist with IVM of frozen GV-stage oocytes after thawing. With the development of vitrification techniques, it was found that there is no difference in the survival rate between oocytes vitrified at the immature GV stage and those vitrified at the mature metaphase II (MII) stage. As a consequence, oocytes should be preferably vitrified at the mature MII stage after having accomplished IVM[128-131]. *Ex vivo* retrieval of immature oocytes, IVM and vitrification of matured oocytes can be associated with ovarian tissue cryopreservation[132].

Moreover IVM can be a further strategy to improve the mature oocyte yield in breast cancer patients who are undergoing ovarian stimulation with a modified letrozole-FSH protocol[133].

CONCLUSIONS

Preservation of fertility in breast cancer survivors in reproductive age has become an important issue, even regarding the quality of life. Proper counseling needs not only knowledge of the subject but good communication skills too. Gonadotoxicity of antiblastic therapy for breast cancer depends mostly on age, ovarian reserve, type and dose of drugs and genetic predisposition. There are several potential options, including all available assisted technologies, such as *in vitro* fertilization and embryo transfer, *in vitro* maturation, oocyte and embryo cryopreservation, and cryopreservation of ovarian tissue. Because increased estrogen levels are thought to be potentially risky in breast cancer patients, recently developed ovarian stimulation protocols with the aromatase inhibitor letrozole and tamoxifen appear to provide safe stimulation with endogenous estrogen. Embryo cryopreservation is the most established fertility preservation strategy. Ooocyte freezing can be considered as an alternative in patients who are single and in those who do not wish a sperm donor. Although ovarian tissue harvesting appears to be safe, experience regarding ovarian transplantation is still limited due to low utilization and fear of re-transmitting cancer cells. Other options, that can be combined are immature oocyte retrieval, *in vitro* maturation of oocytes. The access to Reproductive Medicine Centers because of infertility after cancer treatments will increase due to the growing number of young breast cancer patients and to the reassurance on the safety of their pregnancy. Unfortunately because of gonadotoxic therapies, reduced success is obtained compared with non-cancer patients.

Conflict of Interests:
The Authors declare that they have no conflict of interests.

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