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Review article

Fertility preservation options in breast cancer patients

Running title: Fertility options in breast cancer patients

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The purpose of this review is to analyse current options for fertility preservation in young women with breast cancer (BC). Considering an increasing number of BC survivors, owing to improvements in cancer treatment and delaying of childbearing, fertility preservation appears to be an important issue. Current fertility preservation options in BC survivors range from well-established standard techniques to experimental or investigational interventions. Among the standard options random-start ovarian stimulation protocol represents a new technique, which significantly decreases the total time of the in vitro fertilisation cycle. However, in patients with oestrogen-sensitive tumours, stimulation protocols using aromatase inhibitors are currently preferred over tamoxifen regimens. Cryopreservation of embryos and oocytes are nowadays deemed the most successful techniques for fertility preservation in BC patients**.** GnRH agonists during chemotherapy represent an experimental method for fertility preservation due to conflicting long-term outcome results regarding its safety and efficacy. Cryopreservation of ovarian tissue, in vitro maturation of immature oocytes and other strategies are considered experimental and should only be offered within the context of a clinical trial. An early pretreatment referral to reproductive endocrinologists and oncologists should be suggested to young BC women at risk of infertility, concerning the risks and benefits of fertility preservation options.

Introduction

Fertility preservation in breast cancer (BC) patients makes sense, since women who got pregnant following BC had a 41% reduced risk of death compared to women who did not get pregnant (PRR: 0.59), suggesting that pregnancies do not have an adverse effect on the outcome of BC. Therefore, BC survivors should not be denied the opportunity of future conception [1]. Although most physicians recommend that pregnancy should be delayed by 2 to 3 years after BC**,** in early BC patients younger than 45 years of age, pregnancy that occurs at least 10 months after diagnosis does not jeopardize the prognosis and may actually confer significant survival benefit [2]. An increase in the incidence rate of BC in European women in their 20s and 30s has recently been reported, with the mean annual changes during the 1995-2006 decade being 1.032 and 1.014, in women aged 20-29 and 30-39 years, respectively [3]. In the USA, the incidence of BC increased in 25- to 39-year-old women from 1.53 per 100,000 in 1976 to 2.90 per 100,000 in 2009 [4]. However, the mortality rate in women under the age of 40 with BC has steadily been decreasing since the mid 80s, due to earlier diagnosis and recent advances in treatment [5].

In the past decades more women have delayed childbearing until their 30s or later for different reasons. Unfortunately, there appears to be an increased BC risk with advancing maternal age at first childbirth: a 3,7 relative risk in women with an estimated first median age of 41 years, compared with those with an estimated first birth age of 23 years [6]. Consequently, a greater number of BC survivors are faced with reproductive concerns prior to completing their family. In a pilot survey of survivors' attitudes, 76% of young childless women with cancer intended to have a child in the future and 35% of the survivors who already had at least one child wanted to have another [7].

Therefore, international recommendations have been suggested regarding fertility preservation in these patients with cancer desiring pregnancy. The Update Panel of the American Society of Clinical Oncology (ASCO) recommends to health care providers, including oncologists, urologists, haematologists, surgeons and reproductive specialists to inform patients regarding potential threats to fertility, as early as possible, to allow the widest array of options for fertility preservation [8]. The European Society Medical Oncology (ESMO) Clinical Practice Guidelines recommended that young women desiring pregnancy following cancer diagnosis should be counselled on available fertility preserving options soon after diagnosis, to allow prompt referral to fertility specialists [9]. The First international consensus guidelines suggest that young women with BC, faced with specific physical, psychosocial and sexual issues, should address themselves to a multidisciplinary team of providers, including oncologists, breast nurses, social workers, psycho-oncologists, gynaecologists and fertility experts [10]. Although Anti-Mullerian hormone may be a useful marker of ovarian reserve before gonadotrophin administration in young BC patients at risk for poor-response or no response to ovarian stimulation, its clinical role in early BC and in predicting treatment-induced infertility needs to be defined [11].

Currently available options to BC patients protecting their fertility range from well-established standard techniques, such as ovarian stimulation for in vitro fertilisation (IVF) and embryo/oocyte cryopreservation, to investigational or experimental interventions as are, ovarian tissue cryopreservation, in vitro maturation (IVM) of immature oocytes and ovarian suppression with gonadotrophin releasing hormone (GnRH) agonists during chemotherapy [12]. Although many young women (68%) with BC do discuss fertility issues with their physicians before starting therapy and more than a half (51%) are concerned about becoming infertile after treatment, only a minority of women (10%) chooses to pursue available fertility preservation strategies [13]. Therefore, more efforts are necessary in order to achieve better

patient understanding, to improve physicians' ability to convey of fertility preservation possibilities to all women of reproductive age with cancer and to encourage participation in informed decisions about their therapeutic strategy and future reproductive ability [14].

As the number of younger BC patients increases the aim of this review is to summarize the current knowledge on various fertility preservation options and consequently improve the communication between health care providers and patients about their benefits and disadvantages.

Ovarian stimulation

Ovarian stimulation for oocyte/embryo cryopreservation is currently the best established and the most preferred method for fertility preservation in BC patients. Although most young patients may conceive spontaneously, it is important to choose the appropriate ovulation induction protocol, because these patients have only one opportunity to undergo a single cycle of IVF before starting oncologic treatment. In most cases, ovarian stimulation protocols using gonadotrophins with GnRH antagonists and GnRH agonists for trigger should be preferred in fertility preservation cycles due to time restraints and the risk of ovarian hyperstimulation syndrome (OHSS) [15]. Instead of traditional ovarian stimulation with GnRH agonists, currently GnRH antagonists are routine practice in fertility preservation. Advances have been made from GnRH agonists to GnRH antagonists and to random start stimulation, in order to minimize the time from patient presentation to oocyte retrieval [16]. However, in cases of BC with oestrogen positive receptors several alternative and potentially safer protocols have been introduced using tamoxifen and aromatase inhibitors [17].

Conventional-start ovarian stimulation with GnRH antagonists can be used in a fixed manner or in a flexible manner during the gonadotrophin stimulation. Although this approach

still requires awaiting spontaneous menses, it decreases the interval to oocyte retrieval compared to traditional stimulation protocols. Alternatively, the administration of GnRH antagonists in the midluteal phase results in quicker initiation of gonadotrophins and GnRH antagonists, further reducing delays for BC treatment. Nevertheless, adhering to the conventional-start antagonist protocols could result in significant delay of cancer treatments [16,18].

Random-start ovarian stimulation protocol represents a new fertility preservation technique, when waiting for the next menstrual period to start the ovulation induction is not advisable, due to the urgency of the cancer treatment. It has been proposed to start in the late follicular phase or the luteal phase following spontaneous luteinizing hormone (LH) surge or after the ovulation induction with human chorionic gonadotrophin (hCG) or a GnRH agonist. Therefore, random-start ovarian **stimulation** provides a significant advantage by decreasing the total time of the IVF cycle, without compromising the oocyte yield and maturity before the cancer treatment. Since oocytes can be obtained before cancer treatment irrespective of the phase of the menstrual cycle, both random-start ovarian stimulation protocols are as effective as conventional-start regimens in the early follicular phase [17,19]. The average number of oocytes retrieved was similar between the groups (12.5 vs.15) [16].

Moreover, double ovarian stimulations during the follicular and luteal phases in the same menstrual cycle, provide more opportunities for retrieving more oocytes, in a short period of time in poor responders and newly diagnosed cancer patients needing fertility preservation. The primary outcome measured was the number of oocytes retrieved: stage one 1.7 and stage two 3.5. Out of 167 oocytes collected 26 succeeded in producing one to six viable embryos cryopreserved for later transfer [20]. In assesing the clinical pregnancy rate in recipients of embryos from the same oocyte donor, obtained after ovarian stimulation initiated on day 2 or day 15 of the menstrual cycle, no differences were noted in pregnancy rates

(62.5% vs. 58.3%) after both types of ovarian stimulation. Good pregnancy rates achieved on day 15 of the cycle may be useful information for patients with cancer undergoing fertility preservation [21].

In the evaluation of the safety and efficacy of tamoxifen co-administration during conventional ovarian stimulation for fertility-preservation in BC patients, it was found that the high serum estradiol levels should be considered safe and that tamoxifen does not interfere with IVF results [22]. Aromatase inhibitors significantly reduce plasma oestrogen levels by competitively suppressing the activity of the aromatase enzyme. The third generation aromatase inhibitor, letrozole, is a drug of choice for the treatment of BC in women with oestrogen positive receptors and has recently been introduced as a new option for ovulation induction [23]. Furthermore, the use of aromatase inhibitors with gonadotrophins represents a safe and effective protocol for fertility preservation for women with BC [24]. Moreover, in patients requiring emergency fertility preservation, ovarian stimulation with the use of letrozole 2.5 mg/d, gonadotrophins, GnRH antagonists and hCG can be started at a random cycle date without compromising fertilization rates [25]. The use of GnRH agonist trigger instead of hCG, reduces oestrogen exposure and improves cycle outcomes, by increasing the yield of mature oocytes and embryos as well as decreasing the incidence of OHSS during ovarian stimulation with letrozole (5 mg/d) and gonadotrophins [26]. The largest prospective data with the longest follow up on the safety of ovarian stimulation with letrozole and gonadotrophins for fertility preservation suggests that it is unlikely to cause a substantially increased recurrence risk in BC patients, even in women who have not yet undergone breast surgery [27]. However, anastrozole, another third-generation aromatase inhibitor has a minimal suppressive effect on rising estradiol levels and its use is not recommended in fertility preservation cycles of oestrogen-sensitive cancer patients [28].

Embryo and oocyte cryopreservation

Embryo cryopreservation is the most established technique for fertility preservation for women with available partner or for women using donor semen, which combines ovarian stimulation, oocyte retrieval and IVF. It usually takes 2–5 weeks and is therefore not applicable to patients who cannot delay BC treatment. According to *Society for Assisted Reproductive Technologies*, the current live-birth rate per transfer using frozen-thawed embryos in women under 35 years across the U.S. is 35.6%. [15,29,30]. The main methods of embryo cryopreservation are slow freezing and vitrification, with the latter gaining more popularity in recent years. Both vitrification methods (Irvine and Vitrolife) are more efficient (89.4% vs. 87.6%) than slow freezing (63.8%) for cryopreservation of human cleavage stage embryos in terms of post-warming survival rate [31]. Furthermore, vitrification of cleavage stage embryos yields a significantly better cycle outcome than slow freezing cryopreservation, according to the clinical pregnancy rate (20% vs.11.9%, respectively) [32]. The effectiveness of cryopreservation techniques is best evidenced by the success of cryopreserved embryos and the IVF outcome of frozen-thaw cycles, which have shown a satisfactory live birth rate (22%) in cancer patients for fertility preservation who have returned to use their embryos, over a 15-year period of follow-up (1996–2011) [33].

Freezing of mature oocytes for fertility preservation is an alternative to embryo cryopreservation and it is the preferred strategy regardless of the presence or absence of a partner [34]. The patient has to undergo ovarian stimulation and egg retrieval to obtain oocytes before chemotherapy, in order to avoid fertilizing a damaged egg and does not require IVF. Unlike embryo freezing, oocyte cryopreservation is an alternative option that can avoid ethical, religious and legal concerns [35]. Although human oocytes are extremely sensitive to temperature changes, cryoprotectants and ice formation, vitrification is more effective than slow cooling, as shown by higher survival rate and spindle assessment. Since embryos

resulting from vitrified oocytes have significantly enhanced clinical pregnancy rates (38%), compared with embryos resulting from slow-rate freezing oocytes (13%), vitrification/warming is currently the most efficient method of oocyte cryopreservation [36,37]. These results were confirmed recently showing that vitrification success rates are superior to slow freezing, while the success rates of both techniques match those of fresh embryo transfer. However, the success rates with either technique tend to meaningfully decline after the age of 36 [38]. It should be noted that vitrification, a relatively recent clinical programme, has yielded over 1000 infants born worldwide and these figures are constantly increasing [39]. Consequently, in 2012, the American Society of Reproductive Medicine removed oocyte freezing from the experimental category for the patients who are unable to cryopreserve embryos and facing infertility due to gonadotoxic therapies, but not for the sole purpose of circumventing reproductive aging in healthy women [40].

Cryopreservation of immature oocytes represents an attractive alternative to cryopreservation of mature oocytes, because it does not require ovarian stimulation for BC patients with oestrogen sensitive tumours or who cannot delay chemotherapy. Following retrieval, immature oocytes can be cryopreserved either before or after IVM, which is mostly used in combination with other strategies rather than alone. Immature oocytes survive cryopreservation better than mature oocytes and after thawing these oocytes can be successfully matured in vitro and fertilized. Since the efficacy of cryopreservation of immature oocytes is very low, it still remains an experimental option for fertility preservation [29,41-43]. However, IVM of immature oocytes prior to cryopreservation optimizes the reproductive potential because combined survival and maturation were significantly higher in fresh oocytes undergoing IVM (63.8%), compared with the postthaw IVM group (33.3%) [44]. Additionally, the combination of ovarian tissue cryopreservation with immature oocyte

collection from the tissue followed by oocyte vitrification via IVM, represents another promising approach to fertility preservation in young women with cancer [45].

Ovarian tissue cryopreservation

According to a group of experts ovarian tissue preservation is the only fertility preservation possibility for prepubertal girls with cancer [34]. For BC patients who require urgent cancer treatment such as neo-adjuvant chemotherapy, ovarian tissue cryopreservation for future autotransplantation should be considered as the only fertility preservation option [46]. Ovarian tissue can be extracted by laparoscopy in order to harvest the ovarian cortex containing a lot of primordial follicles that are relatively resistant to freeze-thaw injury. It appears that the slow freezing technique makes the oocyte and granulosa cells more vulnerable whereas vitrification is a safer and more efficient method. Autotransplantation of the cortical strips after BC treatment is typically performed orthotopically by laparoscopy into the ovary or the pelvic peritoneum allowing natural conception [47]. The age limit for cryopreservation is a crucial factor because the chance of restoring the ovarian function is closely correlated to the number of follicles in the ovarian graft. Women should be up to the age of 35 to 37 because follicular density is still sufficient, otherwise the chance of pregnancy is low [48]. Worldwide, at least twenty two singletons and two sets of twins have so far been born with a total of 26 healthy children born as a result of orthotopic retransplantation of cryopreserved ovarian tissue [49]. In appears that the success rate may be higher in experienced transplantation centres because following transplantation into the peritoneum delivery rates were in 23% of cases and following overnight transportation of ovarian tissue delivery rates were up to 29% [50]. Unfortunately, the future of heterotopic grafting of

cryopreserved ovarian tissue subcutaneously to the forearm or the suprapubic region and its clinical practicability for fertility preservation is still debatable [51].

However, in women with Breast cancer antigen (BRCA) mutation–positive BC there are safety concerns considering fertility preservation. An early oophorectomy may be recommended to cryopreserve ovarian tissue of these patients before their risk for ovarian cancer increases with age. It appears that ovarian tissue reimplantation (rather than cryopreservation) is not considered to be a safe procedure in BRCA mutation carriers nor in advanced stage BC nor in invasive lobular BC. The use of preimplantation genetic diagnosis during IVF may be acceptable despite psychosocial and emotional difficulties [52]. Therefore, ovarian tissue transplantation remains an experimental fertility preservation approach due to insufficient data on the efficacy, safety and the reproductive outcomes – a possibility for patients carefully selected by centres with the necessary laboratory and surgical expertise [53].

Ovarian suppression with GnRH analogues

Although adjuvant chemotherapy with tamoxifen have conferred significant improvements in the overall survival of young BC patients, long-term adverse effects of cytotoxic treatment, such as premature ovarian failure (POF) and infertility have become increasingly important. A potential fertility preservation strategy may be administration of GnRH agonists before and during adjuvant chemotherapy. However, the results of a recent meta-analysis, suggest that concurrent use of GnRH agonists and chemotherapy in BC patients who did not use tamoxifen after chemotherapy, may not preserve ovarian function [54]. Therefore, ovarian suppression with GnRH agonists during chemotherapy has been considered experimental,

although several recent studies offer both safety and efficacy for the use of GnRH agonists [55]. Adjuvant therapy with GnRH agonists alone or in combination with tamoxifen, produces results at least similar to those obtained with the different chemotherapy protocols in patients with hormone receptor-positive BC, with respect to overall survival. Therefore, it is advisable to choose GnRH agonist with tamoxifen for oestrogen receptor-positive BC patients, whereas for hormone receptor-negative women chemotherapy is possible to lead to reduction of the risk of recurrence. However, before asserting that adjuvant endocrine therapy produces similar results to adjuvant chemotherapy, it would be useful to assess the lymph node status, mitotic index and HER2 expression which may also influence the prognosis of BC [56]. The annual report of the ASCO about the Clinical cancer advances in 2015, suggests good news for women with early-stage BC, desiring to have a child after BC treatment. Although POF is a common adverse effect in young women undergoing chemotherapy, new studies represent a promising way to preserve fertility, improve the long-term outcome results and the safety issue in BC patients. It appears that GnRH agonists temporarily shut down ovarian function, by introducing the patient in a postmenopausal state, protecting follicles and developing oocytes from chemotherapy damage in young women with BC [57]. In the first study, GnRH analogue administration with chemotherapy was associated with **less** POF and more pregnancies in premenopausal women with BC and hormone receptor–negative disease. Adding the hormone drug goserelin to standard chemotherapy cut the rate of POF from 22 to 8%. After an extended follow-up period of 11,3 years the combination treatment approach resulted in successful pregnancies in 22 (88%) of the 25 women who attempted pregnancy, compared with 12 (67%) of 18 women who attempted pregnancy after receiving standard chemotherapy alone. The hormonal treatment did not increase the risk of miscarriage, pregnancy termination, or delivery complications and it even extended women's survival, compared with chemotherapy alone [58]. Another study reported similarly encouraging

assesing results in mostly hormone receptor–positive BC, assessing the effect of triptorelin, on preventing POF in women with early-stage BC undergoing chemotherapy. After an average follow-up period of 7.3 years, there were eight pregnancies among the 148 women who received chemotherapy plus triptorelin with four pregnancies among the 133 women who received chemotherapy alone and triptorelin did not affect survival [59]. (Table 1)

Fertility preservation options /Characteristics	**Studies**	**Advantages**	**Disadvantages**	**Live-birth rates**
Embryo cryopreservation	Bedoschi G et al. [15] Kim SS et al. [29] Lawrenz B et al. [30] Barcroft J et al. [33]	Most established and successful technique Pregnancy outcome similar or higher than with fresh embryo transfer	Ovarian stimulation, oocyte retrieval and IVF Required time 2–5 weeks Need a partner or donor Delay of treatment required Risk of OHSS	22%-35,6%
Mature oocytes cryopreservation	Konc J et al. [35] Smith GD et al. [37] Cil AP et al. [38] Rodriguez-Wallberg KA et al. [39]	Alternative to embryo freezing Partner not required No ethical, religious and legal concerns Over 1000 babies born wotldwide	Not for the sole purpose in healthy women Ovarian stimulation, oocyte retrieval Cellular damage of oocytes Risk of OHSS Lower success rates after the age of 36	13%-38%
Cryopreservation and transplantation of ovarian tissue	Mathias FJ et al. [47] von Wolff M et al. [48] Macklon KT et al. [49] Liebenthron JR et al. [50] Rodriguez-Wallberg KA et al. [52]	Urgent BC treatment Prepubertal girls with cancer Partner not required Overnight tissue transportation to experienced transplantation centres 26 healthy children born	Laparoscopic removal with orthotopic retransplantation and heterotopic grafting of ovarian tissue Women should be up to the age of 35 to 37 Risk of reintroduction cancerous cells Experimental option	10%-29%
Ovarian suppression with GnRH analogues during chemotherapy	Vitek WS et al. [54] Franco JG et al. [56] Masters GA et al. [57] Moore HCF et al. [58] Lambertini M et al. [59]	Reduction in the rate of POF from 22 to 8% Increase in pregnancy rates from 11 to 21% Non-invasive strategy No operation required	No preservation of ovarian function Controversial results regarding efficacy and safety Experimental method	18%

Table 1 Comparison of characteristics and outcomes of commonly used fertility preservation options in BC patients preservation options in BC patients

Other experimental strategies

Significant progress has been made in the development of other improved methods for fertility preservation such as in vitro follicle maturation, isolation of primordial follicles with transplantation in scaffolds, the potential role of AS 101, S1P and imatinib, which may revolutionise fertility preservation practice. However, further technological advances and research are required before these strategies can be utilised therapeutically in humans [60-64]. Ovarian tissue banking of thin cortical surface biopsies that contain predominantly primordial follicles is being increasingly offered to a variety of patients as a means of fertility preservation. The potential of this tissue could be realized by the development of in vitro systems, to support complete growth from the early primordial stages through to maturity. However, complete oocyte development in vitro from the primordial stage has been achieved only in mice [60]. Comparing macroporous alginate scaffolds with Matrigel for culturing frozen-thawed human primordial follicles in organ culture, it was shown that three dimensional alginate scaffolds are a promising putative in vitro technology for developing human primordial follicles [61]. Although cyclophosphamide activates the growth of the quiescent primordial follicles in mice and leads to loss of ovarian reserve, coadministration of an immunomodulator, AS101, reduces follicle activation, thereby increasing follicle reserve and rescuing fertility after cyclophosphamide and increasing the efficacy of cyclophosphamide against BC cell lines. Therefore, AS101 may be useful as an ovarian-protective agent, which may be able to preserve fertility in female cancer patients [62]. Furthermore, Sphingosine-1-phosphate (S1P), a ceramide-induced death pathway inhibitor, can prevent cyclophosphamide or doxorubicin induced apoptotic follicle death in human ovarian xenografts. Therefore, S1P and its future analogues may be desirable for preserving fertility for patients undergoing chemotherapy [63]. Although two commonly used chemotherapeutic agents (cisplatin and doxorubicin) induce follicle loss in a markedly

different pattern, imatinib mesylate provides selective protection only against cisplatin. Therefore, any treatment designed to protect the adverse effects on the ovary needs to be specific to the drug regimen to which the patient is exposed [64].

Conclusions

Fertility is a major concern for young BC patients and information about fertility preservation options is of crucial importance. Random-start ovarian stimulation protocol represents a commendable technique, advisable in cases of urgent cancer treatment. In patients with oestrogen-sensitive tumours, stimulation protocols using letrozole are currently preferred over tamoxifen regimens. The most successful techniques for fertility preservation in BC patients are cryopreservation of embryos and oocytes. Ovarian suppression with GnRH agonists during chemotherapy represents an experimental method for fertility preservation due to conflicting results regarding its efficacy. Cryopreservation of ovarian tissue, IVM of immature oocytes and other strategies are considered investigational or experimental and should only be offered within the context of a clinical trial. The patient′s future chance of pregnancy should be discussed within an interdisciplinary medical team, made up of oncologists and fertility specialists, early in the early pretreatment consultation.

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