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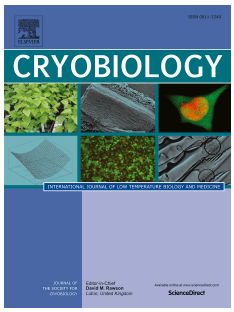

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PII: S0011-2240(19)30535-8

DOI: https://doi.org/10.1016/j.cryobiol.2019.12.001

Reference: YCRYO 4156

To appear in: *Cryobiology*

Received Date: 2 November 2019

Revised Date: 17 December 2019

Accepted Date: 17 December 2019

Please cite this article as: R. Sciorio, R.A. Anderson, Fertility preservation and preimplantation genetic assessment for women with breast cancer, *Cryobiology* (2020), doi: https://doi.org/10.1016/j.cryobiol.2019.12.001.

Fertility preservation and preimplantation genetic assessment for women with breast cancer

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Abstract

Breast cancer is the most common cancer diagnosed among reproductive aged women, and its treatment can compromise future fertility. Options for fertility preservation include oocyte or embryo cryopreservation after ovarian stimulation (OS), which are the most established choices and are applicable for adult women with cancer. Ovarian tissue freezing may also be appropriate, as it offers potentially the least delay. The recognistion of the role of BRCA1 and BRCA2 mutations in some women has led to the involvement of preimplantation genetic diagnosis (PGD), recently renamed preimplantation genetic testing for monogenic disorder (PGT-M), whereby embryos are created by IVF and cell(s) are removed and genetically analyzed for specific disease-related mutations. PGT-M offers a valid option for women wishing to avoid transmission of the predisposition for hereditary breast cancer to their offspring. The aim of this paper is to provide an overview of the factors that influence fertility preservation in newly diagnosed breast cancer patients, and to illustrate the option of PGT-M to enable conception of an unaffected child.

KEYWORDS: Breast cancer, Ovarian stimulation, PGT-M, Fertility preservation, Oocyte cryopreservation, Embryo cryopreservation, Ovarian tissue cryopreservation.

Background

Breast cancer (BC) is the most common cancer in women of reproductive age, with more than 10% of new cases diagnosed in women younger than the age of 40 years [41]. Currently, with the social trend to delaying motherhood until later in life, there are an increasing number of women who have

not completed childbearing at the time of cancer diagnosis, and therefore are likely to desire pregnancy following the chemotherapy [56]. In 2018, has been calculated that 2.1 million new cases of BC were diagnosed worldwide [12]. For many years, BC has been considered the most important cancer in reproductively-aged women, both in terms of incidence and mortality. However, for a range of reasons including improved screening methods and therapies, the number of deaths has been decreasing. Whereas in 2009, estimated deaths were 21.1% of estimated new cases, they were 15.4% in 2018, with a reduction of 27% over the last decade [22]. However, a potential side effect is the loss of fertility or impaired reproductive function [81]. Additionally, women with hormone receptor positive disease may also be advised to take hormonal therapy for up to 10 years after chemotherapy. This, also impact on the complexity of reproductive choices they have to make, facing declining fertility through increasing age as well as from effects of chemotheraphy. Fertility concerns among young cancer patients have an important role in determining quality of life [69]. At the time diagnosis, about half of young women are concerned about becoming infertile or having reduced reproductive function after BC treatment, and while a survey 5 years ago indicated that only a small minority of 10% take up fertility preservation (FP) options [80], this proportion is increasing. There have also been concerns about whether a subsequent pregnancy may increase the chance of recurrence of breast cancer, but it is now clear that this is not the case [44].

Fertility Preservation: Available Options

The many advances in assisted reproductive technologies (ART) over the forty years since its introduction include the development of methods and strategies for FP in women with BC and other conditions whose treatment risks their future fertility, before initiation of anti-cancer therapy. These include cryopreservation of oocytes or embryos after OS, or ovarian tissue cryopreservation (OTC) [3, 27]. The recognition of the role of mutations in the BRCA1 and BRCA2 genes in the aetiology of breast and other cancers in some women also introduces consideration of the use of PGT-M in women with these genetic mutations in order to avoid transmitting the mutation to their offspring. These considerations have raised some concerns, but the possible health and psychological consequences of this particular condition are considered to justify its use [86]. These complex issues will be occurring at a time of great stress and uncertainty to patients in the immediate

aftermath of a new diagnosis. This and the very limited time available for discussion, decisions and potential interventions requires excellent lines of communication between the oncology setting and reproductive medicine. This review discusses the available methods for FP in women with breast cancer, and the role of PGT-M in this context. Protection of the ovary from chemotherapy-induced damage has also been the subject of significant investigation. This has recently been reviewed by Spears and colleagues [87], but of particular importance to women with breast cancer is the demonstration, now confirmed in several large RCTs, that administration of GnRH agonists during chemotherapy for breast cancer reduces the risk of premature ovarian insufficiency (POI). This has been subject to recent meta-analysis [45], thus will not be discussed in detail here. However it is important to recognize that while there seems good evidence regarding risk of POI, whether there is an increased chance of a subsequent pregnancy is unclear, and this approach should not be regarded as an effective form of FP where other interventions are possible.

Risks to fertility in breast cancer patients

Advances in chemotherapy and anti-cancer treatment have resulted in higher survival rates among cancer patients. The most common malignancy in adult women is breast cancer, affecting one in nine women [88]: the five-year survival rate for women treated for breast cancer in the UK is more than 80% [63]. Unfortunately, a side effect of chemotherapeutic drugs is the risk of developing POI, which is dependent on various factors. Most important is the chemotherapy regimen used and the drug doses: the alkylating agents are particularly gonadotoxic, but taxanes also have a negative effect [51]. The age of the patient is also important, as older women have a much higher reported incidence of POI after treatment, compared to the younger women [70, 49]. It is also clear that pre-chemotherapy ovarian reserve, as reflecting in serum concentrations of anti-Mullerian hormone (AMH) are also predictive of long-term ovarian function. This has been demonstrated in several prospective studies in women with BC [6, 7] also showing the interaction with age [89]. Pretreatment antral follicle count (AFC) may also be predictive, but there are few data clarifying this [90].

Effect of chemotherapy

Chemotherapy can have two different effects on ovarian function. The first is immediate, during or following the treatment, with loss of the growing follicle population resulting in amenorrhea. However, if sufficient primordial follicles remain in the resting pool upon the cessation of treatment, the population of growing follicles will then be restored, and menses resume. In contrast, the second is a longer term effect, caused by the depletion of the primordial follicle pool, and results in a shortened reproductive lifespan and POI. If there is only partial loss of primordial follicles, this longer term effect may not manifest itself until years following treatment [58]. Where the reduction in the primordial follicle pool is near complete, the effect is acute, and the patient undergoes immediate POI [70]. This results from the primordial pool of follicles being formed before birth, such that at birth, the ovary has a fixed amount of oocytes. Primordial follicles are continuously recruited out of the resting pool and activated to grow, but from each cohort of this follicles, only very few will go to through to the pre-ovulatory stage and eventually only one will ovulate: the majority of follicles become atretic and will die at some point during development [36]. Chemotherapeutic agents can directly affect the resting pool of primordial follicles or the growing follicles. The loss of the growing population of follicles may lead to increased activation of primordial follicles and so the accelerated loss of that reserve. Chemotherapeutic agents target not only the germ cells, but also the somatic cells. Granulosa cells surround the oocyte proliferate during follicle maturation. Given the essential nature of the contact and communication between the oocyte and the granulosa cells, damage to granulosa cells will result in indirect damage to the oocyte, leading to follicle loss (Figure 1) [58].

It is difficult to predict the exact risk for future fertility. A population-based analysis of pregnancy after cancer showed that women with breast cancer diagnosis before the age of 40 had a markedly reduced chance of post-cancer pregnancy compared to age-matched controls, with a standardized incidence ratio of 0.39 (95% confidence interval 0.36-0.42), but also that there have been significant improvements in the chances of a post-cancer pregnancy over recent years (Figure 2) [4]. As stated earlier, the gonadotoxic effect of chemotherapy is directly associated to female age at the time of treatment and depends considerably on the agent used and the duration of treatment [51, 55]. With

reference to agents commonly used for breast cancer, alkylating agents have the strongest gonadotoxic potential. These agents, directly affect cell proliferation and primordial follicles [9], and promote cell apoptosis and follicle depletion [55]. Cyclophosphamide is one of the most effective drugs used for BC, is also the one of the most investigated compound in connection with gonadal toxicity: the risk of amenorrhea is high, and there is a four-fold higher risk of developing POI as compared with other agents [48, 51]. A high risk of amenorrhea, particularly in women in their later reproductive years, is also associated with other drugs such as fluorouracil, epirubicin and fluorouracixorubicin, which are often used in women with breast cancer. Taxanes cause an intermediate ovarian damage, whereas methotrexate and 5-fluorouracil are associated with a lower toxicity risk [25, 48]. Limited clinical data are currently available regarding newer agents such as trastuzumab, bevacizumab, and cetuximab [91]. Abusief and colleagues [29] suggested that trastuzumab might not induce amenorrhea in premenopausal women with breast cancer. However, further studies are needed to clarify the effect of these agents on ovarian function.

Oocyte Cryopreservation: from slow-freezing protocol

In the last decades, the cryopreservation of mature oocyte has become an established procedure in ART, and represents a safe and effective method for patients wishing to preserve their fertility [64, 97]. Oocyte quality is one of the most important factor influencing the vitrification-warming survival rate, and the subsequent fertilization and embryo development [31]. Cryopreservation involves freezing cells and subsequent storage in liquid nitrogen or its vapour at -196 °C. The first birth from a cryopreserved oocyte was reported in Australia in 1986, using a slow-freezing procedure [16]. Oocytes are extremely difficult cells to freeze successfully, mainly due to the large size cell, and the high content of water which during the freezing process might be converted to intracellular ice, which can induce damage and cell death [68]. Early studies highlighted difficulties in predicting the membrane permeability characteristics of human oocytes along with other biophysical components [29]. Several studies reported the negative effects of cryopreservation on the stability of microtubules and the spindle in mammalian oocytes [72]. In addition, zona pellucida (ZP) hardening after cryopreservation was reported as an extra complication from the freezing

process [97] although this can be overcome by the use of intracytoplasmic sperm injection (ICSI) [73]. Other possible injuries resulting from cooling and warming procedures include DNA fragmentation [33], damage to intracellular organelles [38] and epigenetic risks [99].

To Vitrification

A massive breakthrough in ART cryopreservation was reported with the introduction of "vitrification" in the late 1990s [43]. Vitrification was proposed as an alternative to the slow-freezing technique for human oocytes and embryos and was expected to give superior success rates in term of cryo-survival and pregnancy outcomes. The Human Fertilisation and Embryology Authority (HFEA) has allowed the use of frozen oocytes for infertility treatment in the UK since 2000 [98] and the American Society for Reproductive Medicine (ASRM) in 2013 removed the experimental label applied to oocyte freezing [74] following randomized controlled studies [18, 77] which reported that IVF using vitrified-warmed oocytes could produce similar pregnancy outcomes to IVF with fresh oocytes. A recent meta-analysis confirmed that results from vitrification are superior to those achieved with slow freeing protocols [78]. An important consideration to make is the choice of the carrier used for vitrification, especially in terms of whether liquid nitrogen comes in contact with the droplet containing the embryo (open vitrification) or not (closed vitrification). The issue with open vitrification is that liquid nitrogen itself can contain microbes or pathogens, therefore concerns have been raised over the sterility of open systems due to potential cross contamination between the vitrification sample and liquid nitrogen [10]. Published studies have shown that closed vitrification devices can be used for successful cryopreservation of human embryos [82, 83, 96]. While some IVF scientists remain concerned that closed systems may reduce the survival rates, in the UK 75% of clinics use closed rather than open devices for vitrification [13].

Oocyte cyopreservation in cancer patients

The developments in oocyte cryopreservation described above can be considered a major advance in FP. Prior to the development of vitrification, slow freezing of oocytes had a very low success rate, and the more effective option of embryo cryopreservation was only available to women with a

partner, other than with the use of donated sperm. Cryopreservation of immature oocytes with subsequent in vitro maturation is a potential option but still considered experimental [47], thus in this section cryopreservation of mature oocytes (ie at metaphase II, MII) only will be discussed. A key aspect of this approach is the need for OS, which takes at least 2 weeks, despite the development of 'random start' protocols to minimise delay. These involve the administration of FSH to stimulate multi-follicular development, which can be started at any stage in the menstrual cycle, with co-administration of GnRH antagonists to prevent premature ovulation [23]. In general, women with breast cancer respond to OS with the number of mature oocytes collected that would be expected based on their age and pretreatment ovarian assessment [75]. Exposure to supraphysiological levels of estrogen as a result of OS, albeit briefly, may be a particular risk for patients with a hormone receptive cancer, and the aromatase inhibitor letrozole is widely used to minimise this [94] without apparent detrimental effect on the ovarian response or the quality of the oocytes recovered. Oktay and co-workers [64] analyzed the efficacy of oocyte cryopreservation by vitrification in a meta-analysis, and reported live birth rates per oocyte warmed of 6.6%. A recent study investigated the pregnancy outcome in fertility preservation after oocyte freezing for age-related fertility decline and for patients before cancer treatment. This showed that overall oocyte survival was comparable between the two groups, but implantation, ongoing pregnancy and live birth rates were lower in cancer patients [20]. A live birth rate of 61.9% was reported from 12 cryopreserved oocytes in women ≤35 years and of 43.4% from 10 oocytes in those >35 years, illustrating the importance of both the number of oocytes that can be collected and cryopreserved (which of course declines with age), and the decline in oocyte quality with age. Another aspect to be mentioned is the ideal number of oocytes to freeze in order to obtain a pregnancy after warming. This is a critical point that could be very useful and help clinicians to inform correctely their patients and plan their treatments accordingly [34]. This aspect was investigated in a recent multicenter retrospective study, incluted a total on 6,362 women who underwent to oocyte vitrification for FP, due to age-related fertility decline (5,289 women) or for oncological reasons (1,172 women). The authors reported an increased cumulative live birth rate from 15,8% with 5 oocytes to 32.0% with 8 oocytes. For younger patients (≤ 35 years old) 10 or 15 oocytes provided success rates of 42.8% and 69.8%. The highest cumulative live birth rate of 94.4% was obtained in younger patients when

number of oocytes vitrified was 24 [20]. Another study, evaluated the minimum number of mature oocytes to achieve at least one euploid blastocyst for transfer. The study found that the age of the woman was the most critical predictor for the likelihood of achieving one euploid blastocyst. Based on this model a patient of 37 years-old undergoing ART treatment using ejaculated sperm needs between 9 to 13 mature oocytes to obtain at least one euploid blastocyst to transfer [28]. Regarding the safety of the procedure, studies have analyzed the long term obstetric and perinatal outcomes associated with oocyte vitrification. An analysis of 165 pregnancies and 200 infants found that the mean birth weight and incidence of congenital abnormalities were similar in infants born following oocyte vitrification to those born from spontaneous conception or through standard ART treatment [17]. Another review of 936 infants, born following either slow-freezing or vitrification of oocytes, also reported a comparable incidence of congenital abnormalities [61]. A large study published in 2014 reported births of 1027 babies derived from vitrified-warmed oocytes and suggested that oocyte vitrification does not increase adverse obstetric and perinatal outcomes [19]. Thus, clinical outcomes using vitrified-warmed oocytes followed by IVF or ICSI appear to be similar to outcomes using fresh oocytes. However, these data were mainly reported for oocyte donation cycles and for standard ART cycles. Comparable data for women after cancer treatment who became pregnant and delivered a child after oocyte cryopreservation are not yet available.

Embryo cryopreservation in cancer patients

Oocytes obtained from OS can be fertilized using the partner's sperm, and cryopreserved for future use. The first pregnancy from cryopreserved embryos was reported in Australia in 1983 [93] and the first baby born after transfer of a cropreserved-thawed blastocyst was announced in 1985 [21]. Initially, slow-freezing was the method used, but as with oocytes, this has now been replaced by vitrification. Embryo cryopreservation is the most established FP option for BC patients who have a male partner [39, 40] or for those women who are using donor sperm. Although this option is the most widely used globally, is not an option for couples who might have personal religious or moral objections. In addition, it is essential that the patient is informed and recognizes that any such embryos will require consent from both her and her partner for their subsequent use, and that may be problematical if the relationship is not continuing at the time of use [48]. Embryo

cryopreservation implies OS: as described above, recently studies have reported the use of OS protocols that can be started at anytime during the menstrual cycle [23]. Comparison of patients with and without cancer who underwent IVF and embryo cryopreservation have shown no difference in the number of collected oocytes, fertilization rates and number of live births, although patients with cancer had fewer good quality embryos [64]. Published studies have reported pregnancy outcomes comparable to those of non-oncological populations after IVF. Muñoz and collaborators performed a cohort study including 259 patients with early BC scheduled to receive chemotherapy (age 18 to 40 years old) divided into patients who wished to preserve their fertility (exposed group; n = 148), and underwent OS and chose to vitrify their oocytes, and patients with the same characteristics, but who did not want to preserve their fertility (non-exposed group; n = 111). The primary endpoint was disease free survival time and overall survival rate, with a follow-up of 5 years. Recurrences occurred in 9/148 women (6.1%) in the exposed group and 15/111 women (13.5%) in the non-exposed group, with no significant difference. The overall survival rates were comparable: 2/148 (1.4%) and 4/111 (3.6%) patients died, in exposed and non-exposed groups, respectively, therefore the authors concluted that ovarian stimulation in patients with early stage breast cancer appears safe in the long term [59]. A study published by Oktay and coauthors analysed OS with the concurrent use of letrozole in 131 women with BC with the purpose of FP via embryo freezing. Of the 131 women undergoing embryo cryopreservation, 33 come back to thaw their embryo and use in frozen embryo transfers. Post thaw survival rate of embryos was 98 (84.4%) and the mean number of embryos transferred was 1.97 ± 0.7. They reported an overall clinical pregnancy per transfer of 65.0% (26 of 40), live birth per transfer of 45.0% (18 of 40), which is comparable to those in a non-cancer population undergoing ART treatment [66]. Table 1 displays published trials performed to assess ovarian performance in cancer, in which breast cancer disease was a predominant diagnosis.

Ovarian Tissue Cryopreservation (OTC)

OTC is a potential option for young women with breast cancer, though relatively infrequently used where oocyte vitrification is available. Although there are historic reports of ovarian transplantation in humans [62], the technique came to the fore following its successful development in the sheep,

where ovarian function and fertility were demonstrated after cryopreservation and autotransplantation of ovarian cortical tissue [8, 32]. The first live birth was annouced in 2004 [27], and now more than 130 live births have been reported worldwide [30], demonstrating that this strategy is viable in adults, although the success rate is unclear because the total number of attempts performed is unknown. OTC involves the surgical removal (or dissection following oophorectomy in many cases) and cryopreservation of the ovarian cortex. Later, upon completion of oncologic treatment, the ovarian tissue can be thawed and transplanted back into the patient, either to orthotopic (into the pelvic cavity; on the atrophic ovary) or heterotopic sites (outside of the pelvis; subcutaneous regions such as the forearm) although only limited success has been reported from the latter. It can be performed at any time during the menstrual cycle, there is no need for OS, and therefore no delay in cancer treatment, and it results in storage of a large number of primordial follicles, depending on the patient's age [27]. After reimplantation, ovarian function is expected to be restored after 4-5 months, normally in more than 90% of patients. Regarding the freezing procedure, slow freezing is most widely used: most centres use Gosden's protocol with dimethylsulfoxide [60]. The efficiency of vitrification for freezing human ovarian tissue remains controversial [1] but there have been two reports of births from vitrified and replaced ovarian tissue [30]. Ovarian graft longevity is very variable but the woman's age is a crucial factor in determining success, and many centres use an upper age limit of 35 years, in addition to criteria regarding risk of infertility and chance of survival [3, 27]. Although, more than 130 live births have been reported worldwide [30], there are still unresolved concerns, as substantial loss of primordial follicles is known to occur after transplantation. This event seems to be related to the early hypoxia state that characterizes the post-grafting period [52]. However, this loss of dormant follicles is accompanied by an increase in the growing follicle population, suggesting a double mechanism of follicle death and activation [53]. The greatest concern about this method is safety of the procedure relating to that the replaced ovarian tissue might reimplant the cancer, therefore ovarian tissue should be properly inspected, both by histology and immunohistochemistry (with additional molecular analyses where possible) for malignant involvement of the ovarian tissue. This risk is however considered low in early breast cancer [5].

Preimplantation genetic testing for monogenic disorder (PGT-M) to avoid BRCA transmission

The mean age at diagnosis of breast cancer for BRCA1 and BRCA2 mutation carriers is 43 and 47 years, respectively [96], but with a significant number of cases diagnosed before age 35. In BRCA1 carriers, the cancer incidence per year is 10/1000 in women between 20 and 29 years, 17/1000 between 30 and 39, and 20/1000 between 40 and 49 years. For BRCA2 carriers, the incidence peaks at age 40 to 49 (41/1000 cases per year) [54]. These women are therefore encouraged to undergo risk reducing salpingo-oophorectomy at ages 35-40 for BRCA1-carriers and between 40 and 45 for BRCA2-carriers [50]. PGT-M offers a valid option for BRCA-carriers women wishing to avoid transmission of the mutation to their offspring and being able to conceive an unaffected child. Preimplantation genetic testing in the human was successfully introduced in the late 1980s for fertile couples at risk of transmitting X chromosome-linked diseases to their children [35]. The process involves the aspiration of one or more cells from an embryo generated through IVF, subsequent genetic analysis, and the transfer into the uterus of only unaffected embryos [11, 35]. As stated earlier, the evolution of pre-implantation genetic assessment started with the analysis of limited number of chromosomes using the fluorescence in situ hybridization (FISH) technology in the late 1980s [11, 35]. It was soon replaced by analysis of the whole chromosome set by using different genetic platforms, such as metaphase Comparative Genomic Hybridization (CGH), array based Comparative Genomic Hybridization (aCGH), single nucleotide polymorphism (SNP) microarray, and quantitative polymerase chain reaction (qPCR). At present, the most advanced technique is Next Generation Sequenzing (NGS), whiche refers to a DNA sequencing technology that enables sequencing of millions of small DNA fragments in unison. NGS has revolutionized genomic research studies, and is currently the gold standard for the analysis of monogenic diseases or single single gene mutations [84]. As an autosomal dominant, women with a BRCA mutation have a 50% chance of transferring it to their offspring. BRCA1 and BRCA2 are members of the ATM (ataxia teleangiectasia mutation) protein family, involved in DNA double strand damage detections and repairs. Loss of ATM function in human and mouse causes defects in DNA repair and cell cycle checkpoint control and thus predisposes to cancers. BRCA1 is also highly expressed in germ cells and blastocysts, suggesting a possible role in gametogenesis and embryogenesis. In

the oocytes of primordial follicles in BRCA mutation carriers, it has been suggested that DNA damage may accumulate over time: this may lead to loss of some follicles, with a reduction in the ovarian reserve. This correlation has been demonstrated in mice model, where BRCA1 mutation is associated with lower primordial follicle counts and AMH levels compared to normal controls [92] and there are data suggesting the same in women, for BRCA1 but not BRCA2 [65, 71, 92]. Women with BRCA mutations may show a reduced ovarian response to OS [46] although not all studies have confirmed this [85]. With the PGT-M technique, embryos cultured in vitro are genetically tested for the presence of the mutation, in order to transfer only BRCA negative embryos to the uterus. Couples undergoing PGT-M are usually fertile but they have to undergo IVF treatment, which can be costly and stressful. These couples also have to face the possibility that all embryos might be affected, and that the transfer of an unaffected embryo may not lead to a successful pregnancy. In 2003, despite uncertainties about prospective improvements and therapeutic opportunity, the European Society of Human Reproduction and Embryology (ESHRE) ethics taskforce considered genetic testing acceptable for hereditary conditions and multifactorial diseases such as BC or other cancer dispositions [86]. A major benefit compared to the alternative approach of prenatal testing is the avoidance of consideration of termination of an otherwise viable pregnancy. It is important to recognize that PGT-M is not a therapy, but only a selection tool. As an autosomal dominant condition, half of the embryos will be expected to test positive for the relevant BRCA mutation and thus will be discarded. As the number of available embryos will decline with the woman's age and the number of oocytes collected, it seems more appropriate only in young BC patients. As discussed above, being a carrier of a BRCA mutation may also reduce the number of embryos available for testing. Moreover, for PGT-M a physically demanding in vitro fertilization treatment is required regardless of couple's fertility, and OS is necessary, which can delay cancer treatment [39, 40]. Opinion studies among women affected by BC have shown that the majority, after being informed about PGT-M, are in favour of offering PGT-M for BRCA1 and BRCA2 mutations, although only a minority would consider this option for themselves [24, 67]. PGT-M for BRCA mutations is growing; a survey of 1081 BRCA mutation carriers highlighted that patients are keen to have reproductive counseling, with more than 50% stating that PGT-M should be offered.

The most frequently quoted reason in considering PGT-M was, in all categories of couples, to protect their future child from the physical and psychological impact of the BRCA mutation [15].

Conclusion

FP is a rapidly developing area of medicine, and the provision of information to patients facing the loss of fertility throught treatment for cancer and other conditions has become standard of care. Women should be informed not only about advantages of oocyte and embryo cryopreservation, as established technology that will contribute to achieve a pregnancy after cancer, but also about the general risks, cost and effectiveness of the procedures to reach a shared decision. Reproductive decision-making regarding PGT-M is complex for BRCA mutation carriers. For some couples, the emotional impact of the decision is substantial and long-lasting, therefore reproductive and dynamic counselling over time is crucial, considering that a women's aspirations may change with age. All women about to receive chemotherapy for a newly diagnosed BC should receiveprope r and complete oncofertility counselling regarding the possible gonadotoxic risk and potential approaches for FP, to allow them to take fully informed decisions about the proposed therapy and its long-term consequences. This requires as a minimum the development of optimized communication between specialities, with referral to reproductive medicine clinics for ART becoming an integrated part of cancer care. The development of national and international registries is required to monitor the techniques used, the success rates achieved and the long-term follow-up of children born from these procedures.

Conflict of interest. RS declares that he has no conflict of interest. RAA is past coordinator of the ESHRE Special Interest Group in Fertility Preservation.

Compliance with ethical standards

Human and animal rights. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and with the 1964 Helsinki declaration and its later amendments. For this type of study, formal consent is not required.

Funding and role of the funding body RAA is at the MRC Centre for Reproductive Health, which is funded by MRC Centre grant MR/N022556/1

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Fertility preservation and preimplantation genetic assessment for women with breast cancer

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Figure 1: Potential targets of chemotherapeutic damage within the ovary. **(A)** Chemotherapeutic agents could be directly affecting the resting pool of primordial follicles or the growing follicle population. As growing follicles inhibit the recruitment of primordial follicles, the loss of this growing population will lead to increased activation of primordial follicles and so loss of that reserve. **(B)** Chemotherapeutic agents could be directly targeting the oocyte or the somatic cells. Oocyte death would result from death of the follicular somatic cells, as the oocyte is dependant on these for its survival.

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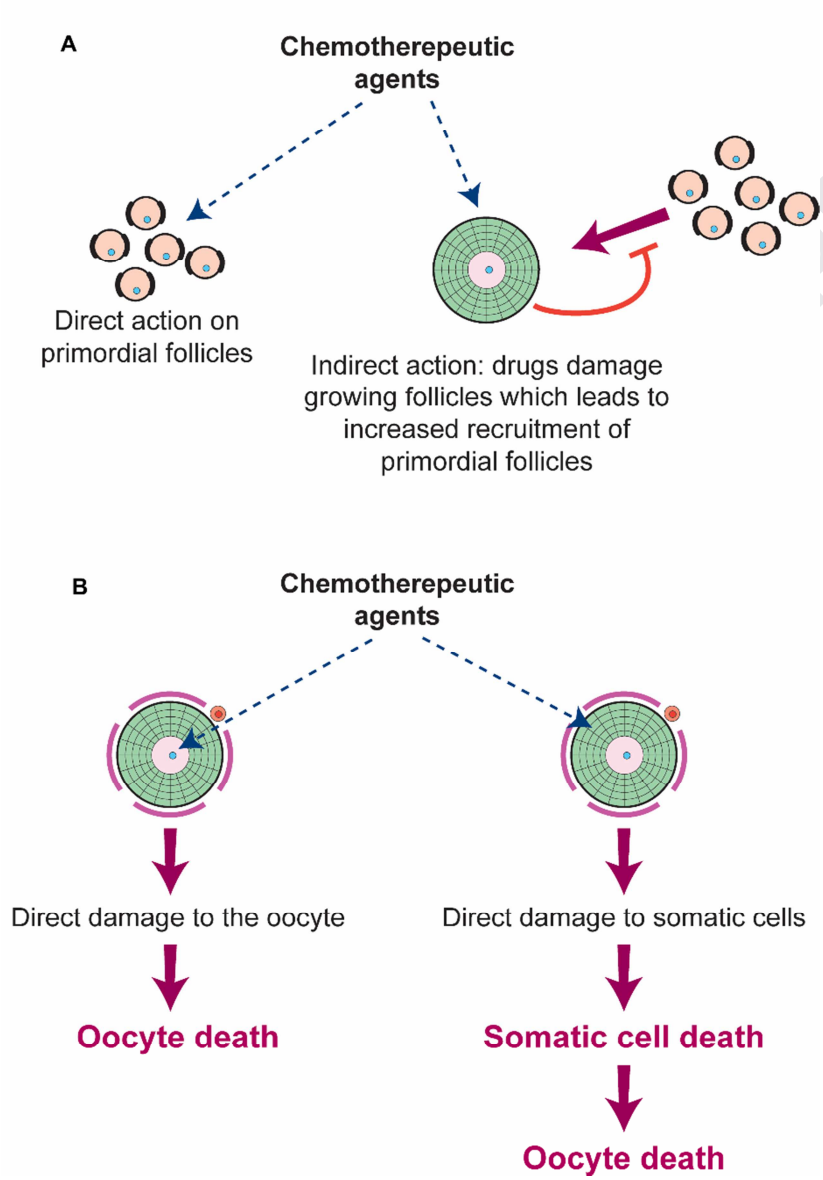

Figure 2. **(A)** Probability of pregnancy after cancer diagnosis in women with breast cancer (red) compared to matched population controls (blue). Table under the panel indicate the number of women at each 10 year interval. **(B)** Hazard ratio for first pregnancy after breast cancer diagnosis by period of diagnosis. Reprinted from Anderson et al, 2018 [4], with permission.

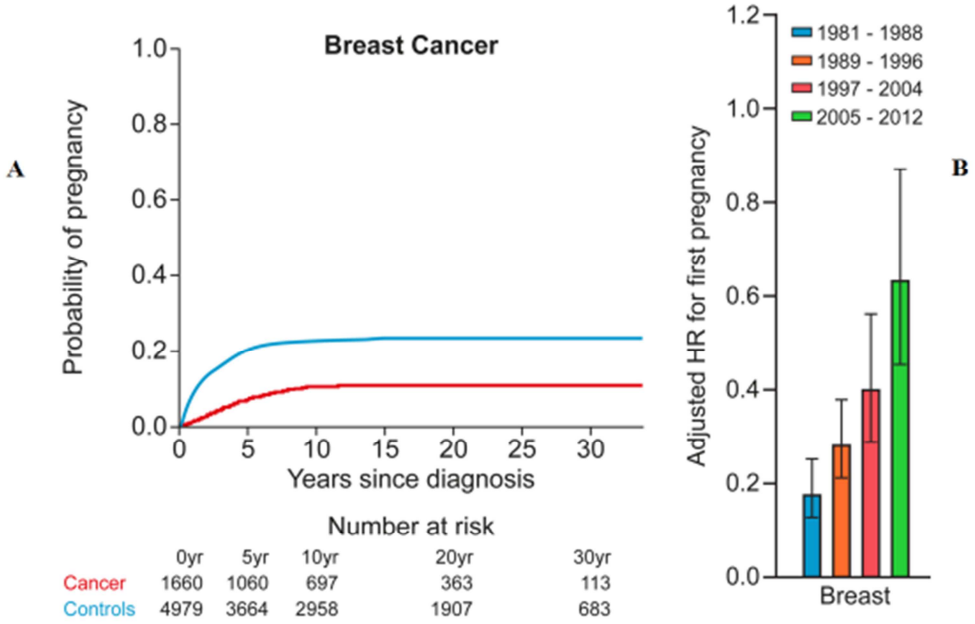

Table 1: Compares IVF outcomes in cancer patients versus non cancer patients

Studies	Number of Patients [1]	% Breast Cancer [2]	Mature Oocytes [3]		Fertilized 2 PN	
			Cancer	Control	Cancer	Control
Cardozo et al. [14]	63	41 (65%)	12.4	10.9	6.6	7.1
Domingo et al. [26]	208	142 (69%)	10.5	12.4	N/A	N/A
Knopman et al. [42]	26	10 (38%)	14	12	N/A	N/A
Michaan et al. [57]	22	12 (55%)	8.8	8.8	5.4	5
Robertson et al. [79]	38	16 (42%)	12	14	6	7
Quintero et al. [76]	50	28 (56%)	11.5	13	6.8	7.4
Johnson et al. [37]	50	29 (58%)	12.4	11.7	5.4	6

1 Number of cancer patients included in trial

2 Number and percentage of breast cancer patients included in study

3 Mean number of oocytes collected for cancer patients and control patients.