# A retrospective study evaluating the impact of scattering radiation from imaging procedures on oocyte quality during ovarian stimulation for fertility preservation in young breast cancer patients

Margherita Condorelli[1,2], Maëlle Sens[2], Ornit Goldrat[1], Anne Delbaere[1], Judith Racapé[3,4], Matteo Lambertini[5,6*], Isabelle Demeestere[1,2*]

*Co-last authors

[1]Fertility Clinic, Department of Obstetrics and Gynecology, Hôpital Erasme, Université Libre de Bruxelles, Brussels, Belgium.

[2]Research Laboratory on Human Reproduction, Université Libre de Bruxelles, Brussels, Belgium.

[3] Chair in Health and Precarity, Faculty of Medicine, Université Libre de Bruxelles, Brussels, Belgium

[4]Research Center in Epidemiology, biostatistics and clinical research, School of Public Health, Université Libre de Bruxelles, Brussels, Belgium.

[5]Medical Oncology Department, U.O.C. Clinica di Oncologia Medica, IRCCS Ospedale Policlinico San Martino, Genova, Italy

[6]Department of Internal Medicine and Medical Specialties (DiMI), School of Medicine, University of Genova, Genoa, Italy.

Corresponding author:

Isabelle Demeestere

isabelle.demeestere@ulb.be

Tel: +32 (0) 2 555 6358

Fax: +32 2 5554520

**ORCID of the authors:**

Margherita Condorelli: 0000-0002-1104-8208

Ornit Goldrat: 0000-0002-7794-3133

Anne Delbaere: 0000-0001-7236-0687

Judith Racapé: 0000-0002-1266-0191

Matteo Lambertini: 0000-0003-1797-5296

Isabelle Demeestere: 0000-0002-3192-6565

**Abstract**

**Purpose**: Ovarian stimulation for oocyte and embryo cryopreservation is the standard of care for fertility preservation in young breast cancer patients before gonadotoxic chemotherapy. The procedure should be started as soon as possible to avoid delay of treatment; thus, it is often performed concomitantly with tumor staging assessments. However, questions remain regarding the potential negative impact on oocyte quality that may occur due to exposure to scattered ionizing radiation from imaging techniques when staging assessment is conducted at the same time as ovarian stimulation.

**Methods**: We conducted a retrospective study on all breast cancer patients who received ovarian stimulation for fertility preservation at our center between November, 2012 and May, 2020.

**Results**: Gynecologic and oncologic characteristics were similar between patients exposed (n=14) or not (n=60) to ionizing radiation. Exposed patients started the ovarian stimulation sooner after diagnosis than non-exposed patients (11.5 vs 28 days, respectively, P<0.01). Cycle parameters, including the median number of oocytes collected (10.5 vs 7, P=0.16), maturation rates (92.5% vs 85.7%, P=0.54), and fertilization rates (62.2% vs 65.4%, P=0.70) were similar between groups.

**Conclusions**: This study shows that scattered ionizing radiation due to staging assessment appears to be safe without compromising follicular growth and maturation. Larger studies on fertility and obstetrical outcomes are needed to confirm these preliminary data.

Keywords: breast cancer, fertility preservation, ionizing radiation, staging and risk assessment, oocyte maturation

## Background

Breast cancer in young women is of great concern as it is the most common cancer diagnosed in women aged between 20 to 39 years old [1]. A recent study has shown an increasing incidence of breast cancer cases in premenopausal women in countries with a high human development index over the last 15 years [2]. In the last few decades, progress in oncological treatments has led to an improvement in overall survival for these patients which now exceeds 80% at 5 years [3]. At the same time, increasing efforts are being devoted to the care of survivors in order to improve their quality of life. Particular attention is being paid to fertility counseling and to the implementation of fertility preservation programs for younger cancer patients. Several international oncological and reproductive scientific societies have highlighted the importance of such programs in recent guidelines [4-8]. The first option recommended to preserve fertility in breast cancer patients is the cryopreservation of oocytes and/or embryos after letrozole-associated controlled ovarian stimulation [7, 8]. This approach has been proven to be safe, and as efficient as standard ovarian stimulation protocols [9].

Once the diagnosis of breast cancer is confirmed, an oncological staging assessment is performed to exclude metastasis using various radiological exams, such as thoraco-abdominal scan, bone scan, and positron emission tomography (PET)-CT [9, 10]. In addition, an echocardiographic or multi-gated acquisition (MUGA) scan is recommended to exclude any existing cardiac pathology when patients are candidates for anthracycline and/or trastuzumab treatment [10]. During these exams, a small quantity of scattered radiation can be absorbed by the pelvis and consequently reach the ovaries. In the early setting, oncologists generally start chemotherapy as soon as the staging assessment is completed. Thus, the lapse of time during the staging assessment is of great value for the fertility specialist, who needs an average of 2 weeks to complete the ovarian stimulation cycle before (neo)adjuvant therapy [5]. Previous study did not observe delay related to fertility preservation procedure before the start of (neo)adjuvant therapy [11], but it may occur and have potential detrimental oncological consequences if the ovarian stimulation cycle started after completion of the staging and risk assessment.

Although the option of starting ovarian stimulation as soon as possible is recommended, and usually offered, concerns have also been raised regarding the potential negative impact of imaging procedures

using ionizing radiation and/or nuclides on the performance of embryo/oocyte cryopreservation. Early preclinical studies on murine models showed a significant increase in the number of malformations in the litter when mice were exposed to radiation or cyclophosphamide 3 weeks before conception, corresponding to the follicular growth phase [12, 13]. In contrast to primordial follicles that have a high sensitivity to gonadotoxic treatment and rapidly go into apoptosis, oocytes progressing beyond prophase at the final stage seem to have a high tolerance for DNA damage with possible consequences on the offspring [14, 15]. Sublethal damage to oocytes in growing stage follicles and defects in DNA repair mechanisms may lead to hereditary disorders, fetal malformations, or in-utero death [13]. As the time for a follicle to grow from the primordial stage to the preovulatory stage in humans is estimated to be around 220 days [16], it is recommended that women avoid conceiving for at least one year following treatment to avoid oocyte exposure during the growing phase and allow DNA repair mechanisms to occur [13, 16]. In this context, the question of the effect of scattered radiation during the fertility preservation procedure appears to be particularly relevant but has never been investigated.

This study aimed to compare the impact of scattered radiation during staging and risk assessment on the performance of ovarian stimulation for fertility preservation in a cohort of young women with newly diagnosed breast cancer.

## Materials and Methods

### Study design and patients

This was a retrospective study including all breast cancer patients who underwent ovarian stimulation for fertility preservation prior to chemotherapy at CUB-Hôpital Erasme between the 29$^{th}$ of November, 2012 and May 1$^{st}$, 2020. Ovarian stimulation was conducted using a random start antagonist protocol, with simultaneous administration of letrozole 5 mg/day until the ovulation trigger, as previously described [17]. Patients with metastatic breast cancer, or a previously diagnosed neoplasia, or aged over 41 years were excluded. Patients who were exposed to any one of the imaging procedures releasing ionizing radiation during ovarian stimulation (PET scan, bone scan, CT scan, and/or MUGA scan) were included in the exposed group. Patients who completed their staging and risk assessment before starting

ovarian stimulation or after oocytes collection were included in the non-exposed group. In this group, patients did not undergo any of the above-mentioned imaging procedures involving ionizing radiation during ovarian stimulation. Ionizing radiation techniques differed according to the markers used: fluorodeoxyglucose labelled with fluorine 18 ($^{18}$F-FDG) and a low-dose total body scanner was used for PET-scan, red blood cells labelled with Technetium 99 ($^{99}$Tc-RBC) was used for MUGA scan, and methyl-diphosphonate labelled with $^{99}$Tc ($^{99}$Tc-MDP) was used for bone scan. We extrapolated the scattered pelvic irradiation doses based on the existing literature on conceptus dosage in pregnant women, taking into account the highest estimate as follows: 25 mGy for a thoraco-abdominal CT [18], 20 mGy for a PET scan [18], 5 mGy for a bone scan [19], and 0.5 mGy for a MUGA scan [18]. Data were collected from the electronic medical records from CUB-Hôpital Erasme and/or the referring centers. Data were registered and managed using the REDCap software.

Statistical analyses

Statistical analyses were performed using IBM SPSS Statistics 27.0 (Armonk, NY, USA) and Stata 16.0 software (Stata Corporation, Texas, USA). The primary endpoint to evaluate the performance of the ovarian stimulation cycle was the comparison of collected mature oocytes between exposed and non-exposed groups. Continuous variables are reported as means and standard deviations (SD) for symmetrical distributions, or medians and ranges (minimum–maximum values) for asymmetrical distributions and compared using Student's T-test or the Mann–Whitney–Wilcoxon test according to the distribution of variables. Fisher's exact test was used for categorical variables. The association between the number of mature oocytes and the possible explanatory variables was analyzed using a negative binomial regression model (overdispersion). Incidence rate ratios are presented with their 95% confidence intervals (CIs). Univariate and multivariate models were constructed including exposure to imaging procedures releasing ionizing radiation, age, anti-Müllerian hormone (AMH), and germline *BRCA* pathogenic variants. A P-value $<0.05$ was considered statistically significant.

## Results

Between the 29th of November 2012 and the 1st of May 2020, 82 breast cancer patients underwent ovarian stimulation for fertility preservation. A total of 8 patients were excluded for premature LH surge/premature triggering (n=4), non-compliance to the ovarian stimulation protocol (n=2), fertility preservation for breast cancer relapse (n=1), and expression of formal refusal communicated to the institution to use their clinical data for clinical trials (n=1). Among the 74 patients included in the study, 14 patients were exposed to at least one imaging procedure involving ionizing radiation during ovarian stimulation (exposed group) and 60 patients had already had their staging and risk assessment before starting ovarian stimulation (non-exposed group).

Exposed and non-exposed patients had similar baseline and oncological characteristics, except for BMI at diagnosis (median BMI of 24.4 and 22.1 in exposed and non-exposed groups, respectively; P=0.04) (Table 1 and Table S1). Mean age at diagnosis was 31.2 years in the exposed cohort and 32.6 years in the non-exposed cohort (P=0.21). A total of 23 patients (31.1%) had children at the time of diagnosis. Ovarian reserve was similar in both cohorts, with a median AMH level of 2.5 µg/L (range: 0.2-13) in the exposed group, compared to 1.9 µg/L (range: 0.3-7.1) in the non-exposed group (P=0.20) (Table 2).

The majority of the patients had a stage 2 tumor (57.1% and 50% in the exposed and non-exposed groups, respectively), without nodal invasion (71.4% and 60% in the exposed and non-exposed group, respectively), with positive hormone receptors (57.1% and 63.3% in the exposed and non-exposed cohort, respectively), and HER2 negative status (71.4% and 66.7% in the exposed and non-exposed group, respectively) (Table 1).

### Radiation exposure

Among the 14 exposed patients, 5 (35.7%) underwent a PET scan, 9 (64.3%) a bone scan, 2 (14.3%) a CT scan, and 1 patient underwent a MUGA scan (7.1%) during the ovarian stimulation cycle. Four patients underwent 2 imaging procedures during the ovarian stimulation cycle, and one patient underwent 3 imaging procedures (Table S2).

The mean time between the beginning of ovarian stimulation and the first ionizing radiation exposure was 3.9 days (range: 0-7). The mean time between first exposure and oocyte collection was 8.7 days (range: 4-12). Taking into account the highest estimated scattering dose according to the literature [18, 19], patients were exposed to a median pelvic radiation exposure of 0.7 mGy (range: 0.5-45.5).

Fertility preservation outcomes

All the patients exposed to ionizing radiation had one ovarian stimulation cycle while 9 out of 60 patients in the non-exposed group had two consecutive stimulation cycles. The median time between diagnosis and the beginning of the first ovarian stimulation cycle was shorter in the exposed cohort (11.5 days, range: 5-33) than in the non-exposed group (28 days range: 1-164) ($P<0.01$). The characteristics of the ovarian stimulation cycles were similar in both groups (Table 2). hCG triggering was used at the beginning of the protocol and then replaced by GnRH analogues [17].

Median number of collected oocytes was similar in both groups (10.5 versus 7 in the non-exposed and exposed group, respectively; $P=0.16$) as well as the maturation rate (92.5% versus 85.7% in the exposed and non-exposed groups, respectively; $P=0.54$). Incidence rate ratios (IRR) of ionizing radiation exposure on the number of mature oocytes was 1.37 (IC: 0.94-2.0; $P=0.10$) in the univariate model and 1.13 (IC: 0.77-1.65; $P=0.53$) in the multivariate model. The increasing exposure dose was not associated with a decrease in the number of oocytes collected (Suppl Fig 1).

Age (IRR=0.95; IC: 0.91-0.99; $P=0.02$) and AMH (IRR 1.18; IC: 1.09-1.28; $P<0.0001$) were both significantly associated with the number of mature oocytes in the univariate model. AMH was still significantly associated with the number of mature oocytes collected in the multivariate model (IRR 1.16; IC: 1.07-1.27; $P=0.001$), while age was not (IRR 0.98 IC: 0.94-1.02; $P= 0.40$). In contrast, the presence of a germline *BRCA* pathogenic variant was not significantly associated with the number of mature oocytes collected (IRR 0.92; IC: 0.62-1.36; $P=0.67$ and IRR 1.06; C: 0.72-1.56; $P= 0.75$) in the univariate and multivariate models, respectively (Table 3).

A total of 45 and 132 mature oocytes were fertilized in the exposed and non-exposed groups, respectively. Fertilization rates were similar in both groups (Table 2).

Oncological and fertility outcomes

Patients had a median follow-up of 3.7 years from breast cancer diagnosis (range: 0.8-7.5). Twelve and 58 patients had at least one year of follow-up after treatment in the exposed and non-exposed groups, respectively. Three patients out of 14 in the exposed cohort experienced a relapse (21.4%) compared to 6 out of 60 (10%) in the non-exposed cohort (P= 0.4) (Table S2). No patients died in the exposed cohort, while there was one death in the non-exposed cohort (1.7%).

Among exposed patients, none returned to the clinic to recover cryopreserved material or had a pregnancy after their breast cancer. In the non-exposed cohort, 11 patients out of 60 (18.3%) used their frozen oocytes (n=4), embryo (n=6), or both (n=1) to achieve pregnancy. The mean time between fertility preservation and oocyte/embryo thawing was 3.4 ±1.5 years. Mean survival rates after thawing were 55.6% for the oocytes (10/18) and 84.6% for the embryos (11/13). Fifteen embryos were transferred into 10 patients and 10 pregnancies were obtained (implantation rate 66.7%) leading to 5 live births, 3 miscarriages, and 2 ongoing pregnancies. All patients who received an embryo transfer from cryopreserved oocytes/embryos had at least one positive hCG test (10/10). In addition, 7 patients had at least one spontaneous pregnancy and 2 others became pregnant using fresh oocytes retrieved in IVF/ICSI cycle.

## Discussion

In daily practice, patients are referred to the oncofertility unit soon after diagnosis, when disease staging assessment has not been performed yet or is ongoing. The safety of starting the stimulation cycle before completion of staging assessment is a matter of debate as uncertainties remain regarding the impact of scattering ionizing radiation on oocyte quality. Based on the “precautionary principle” and the ALARA (“As Low As Reasonably Achievable”) principle, some physicians avoid the exposure of their patients to radiation and radionuclides during ovarian stimulation considering the lack of data, leading to fertility

preservation cycles cancellation or postponement of oncological treatment with potential safety consequences.

This pilot study did not show a detrimental impact on the number of mature oocytes collected in breast cancer patients who underwent staging and risk assessment imaging during ovarian stimulation compared to those who had already completed their assessment before starting the ovarian stimulation cycle.

It is well established that ionizing radiation causes DNA damage through double-strand breaks (DSBs) [20]. Although primordial follicles decrease due to apoptosis following DNA damage, this response is less prevalent in the population of growing follicles[14]. Sterilizing doses inducing acute premature ovarian insufficiency (POI) was observed after an irradiation doses on the ovaries >20Gy at birth and decreased with age [21]. Although low doses of irradiation applied during staging and risk assessment in oncology are not at risk of inducing POI, they could impact the acquisition of oocytes competence in growing follicles. Using a mouse follicular culture model, Jacquet et al showed that irradiation (2-4 Gy) does not alter follicular growth but has a dose-dependent effect on oocyte maturation progression and on chromosomal aberrations [22]. Others have confirmed that in vitro oocyte maturation completion can be disrupted by toxic agents that induce non-repairable DNA damage, especially DSBs [23]. The block of metaphase I progression that is associated with DNA damage is due to the activation of specific checkpoint signals that prevent damaged oocytes from becoming fertilized [24]. Thus, if DSBs occur during ovarian stimulation, it may lead to a decrease in the number of mature oocytes collected.

In experimental studies, extensive apoptosis and primordial follicle depletion was observed after ovarian exposure to 0.45 Gy [25]. Suh et al. also reported that 3 DSBs occur in oocytes exposed to 0.1 Gy, and 10 for exposure to 0.45 Gy [25]. During staging and risk assessment for breast cancer before chemotherapy, we have estimated that ovaries were exposed to a median of 0.7 mGy (range: 0.5-45.5), which is lower than doses required to induce significant oocyte DNA damage. Therefore, many centers assume that staging assessment can be performed during ovarian stimulation, although no study was available until now to confirm the safety of this practice.

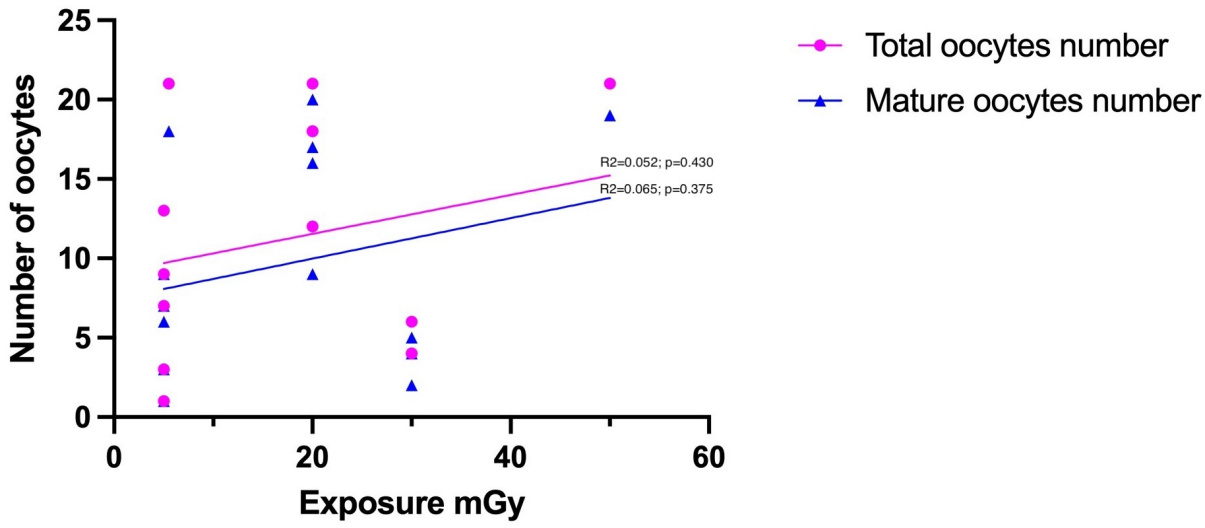
This study provides reassuring preliminary data on the safety of oocyte collection when staging and risk assessment has been conducted during ovarian stimulation. The major limitation of the study was the limited number of patients included. However, the most relevant parameters that could impact the number of mature oocytes such as the age or the ovarian reserve were similar in both groups. Patients in the exposure group had a higher median BMI but it is unlikely that it constitutes a major bias for the interpretation of the data. Fertilization rates were similar in both cohorts, but no patients in the exposed cohort have used the cryopreserved material to achieve pregnancy yet. Although we did not observe any detrimental effects of staging assessment during ovarian stimulation on oocyte maturation rate and fertilization capacity, additional studies on fertility and obstetrical outcomes are needed to further confirm these findings. Considering these limitations, it is recommended to limit overlap with particularly high-exposure imaging as much as possible during ovarian stimulation and to increase liquid intake to avoid prolonged exposure to nuclides in the bladder (i.e. close to the ovaries) when used.

## Conclusion

The choice of the best fertility preservation strategy for oncologic patients has to take into account several factors, one of them being the lapse of time available before starting chemotherapy or any gonadotoxic treatment. It is usually recommended to start the fertility treatment as soon as possible in order to avoid any delay of chemotherapy in the neoadjuvant setting. This study showed that starting ovarian stimulation while patients still have to complete their staging and risk assessment does not appear to be detrimental in terms of number of mature oocytes collected. This serves as a proof-of-concept study that supports the hypothesis that ovarian stimulation can be started soon after the initial diagnosis and treatment decision, irrespective of staging assessment. These data also highlight the importance of collecting information on the use of imaging procedures during ovarian stimulation in order to expand our knowledge of the potential impact of these procedures on human oocytes in large prospective trials.

**Legend of the figure:**

*Figure S1: Correlation between radiation doses and total number of oocytes collected (pink) and total number of mature oocytes (blue) in the exposure group*




**Declarations**

**Funding:** Margherita Condorelli and Isabelle Demeestere acknowledge Télévie-FNRS and Fonds Erasme for their financial support. This work was supported by Télévie-FNRS (Grant number: 7.6508.20) and Fonds Erasme (Grant number: not applicable). Matteo Lambertini acknowledges the support from the Italian Association for Cancer Research ("Associazione Italiana per la Ricerca sul Cancro", AIRC; MFAG 2020 ID 24698) and the Italian Ministry of Health (5 x 1000 funds 2017).

**Ethics declarations**

Conflicts of interest/Competing interests: Matteo Lambertini acted as a consultant for Roche, Lilly, AstraZeneca and Novartis and has received honoraria from Sandoz, Roche, Lilly, Pfizer, Novartis and Takeda, outside the submitted work. Anne Delbaere received grants from Ferring Pharmaceuticals and

consultancy or lecture fees from Merck, Gedeon-Richter Ferring Pharmaceuticals and OVVI Diagnostics, outside the submitted work. Isabelle Demeestere received research grants from Ferring and Roche, consultancy or lecture fees from Roche, Novartis and support for attending meetings from Ferring, Theramex, outside the submitted work. The remaining authors have no conflicts of interest to declare.

Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

This study was approved by the ethics committee of CUB-Hôpital Erasme (P2020.328).

Informed consent: The need for obtaining informed consent was waived by the Hôpital Erasme ethical committee, given that this study was retrospective and non-interventional. Patients have the right to refuse to participate in any clinical trial by informing the hospital which keeps a record of their choice. We hereby confirm that we took into account patient preference, and consequently excluded all patients that refused their participation in clinical trials.

**Availability of data and material**: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Code availability:** Not applicable

**Authors' contributions:** MC, ML and ID conceived and designed the study, OG, MS, AD and MC acquired the data. JR, ML, MS, and MC analyzed the data. MC and ID wrote the manuscript and all other authors revised it critically and finally approved it.

**Acknowledgements**

The authors would like to acknowledge the contribution of a medical writer, Sandy Field, PhD, for English language editing of this manuscript.

## References


1. Fidler MM, Gupta S, Soerjomataram I *et al* (2017) Cancer incidence and mortality among young adults aged 20-39 years worldwide in 2012: a population-based study. The lancet oncology 18:1579-1589. https://doi.org/10.1016/S1470-2045(17)30677-0
2. Heer E, Harper A, Escandor N *et al* (2020) Global burden and trends in premenopausal and postmenopausal breast cancer: a population-based study. Lancet Glob Health 8:e1027-e1037. https://doi.org/10.1016/S2214-109X(20)30215-1
3. Copson E, Maishman T, Gerty S *et al* (2014) Ethnicity and outcome of young breast cancer patients in the United Kingdom: the POSH study. British journal of cancer 110:230-241. https://doi.org/10.1038/bjc.2013.650
4. Anderson RA, Amant F, Braat D *et al* (2020) ESHRE guideline: female fertility preservation. Hum Reprod Open 2020:hoaa052. https://doi.org/10.1093/hropen/hoaa052
5. Lambertini M, Peccatori FA, Demeestere I *et al* (2020) Fertility preservation and post-treatment pregnancies in post-pubertal cancer patients: ESMO Clinical Practice Guidelines. Ann Oncol. https://doi.org/10.1016/j.annonc.2020.09.006
6. Paluch-Shimon S, Cardoso F, Partridge AH *et al* (2020) ESO-ESMO 4th International Consensus Guidelines for Breast Cancer in Young Women (BCY4). Ann Oncol 31:674-696. https://doi.org/10.1016/j.annonc.2020.03.284
7. Oktay K, Harvey BE, Partridge AH *et al* (2018) Fertility Preservation in Patients With Cancer: ASCO Clinical Practice Guideline Update. J Clin Oncol 36:1994-2001. https://doi.org/10.1200/JCO.2018.78.1914
8. Practice Committee of the American Society for Reproductive Medicine. Electronic address aao (2019) Fertility preservation in patients undergoing gonadotoxic therapy or gonadectomy: a committee opinion. Fertility and sterility 112:1022-1033. https://doi.org/10.1016/j.fertnstert.2019.09.013
9. Bonardi B, Massarotti C, Bruzzone M *et al* (2020) Efficacy and Safety of Controlled Ovarian Stimulation With or Without Letrozole Co-administration for Fertility Preservation: A Systematic Review and Meta-Analysis. Front Oncol 10:574669. https://doi.org/10.3389/fonc.2020.574669
10. Cardoso F, Kyriakides S, Ohno S *et al* (2019) Early breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 30:1674. https://doi.org/10.1093/annonc/mdz189
11. Chien AJ, Chambers J, McAuley F *et al* (2017) Fertility preservation with ovarian stimulation and time to treatment in women with stage II-III breast cancer receiving neoadjuvant therapy. Breast cancer research and treatment 165:151-159. https://doi.org/10.1007/s10549-017-4288-3
12. Kirk M, Lyon MF (1982) Induction of congenital anomalies in offspring of female mice exposed to varying doses of X-rays. Mutat Res 106:73-83. https://doi.org/10.1016/0027-5107(82)90191-9
13. Meirow D, Epstein M, Lewis H *et al* (2001) Administration of cyclophosphamide at different stages of follicular maturation in mice: effects on reproductive performance and fetal malformations. Human reproduction (Oxford, England) 16:632-637. https://doi.org/10.1093/humrep/16.4.632
14. Stringer JM, Winship A, Zerafa N *et al* (2020) Oocytes can efficiently repair DNA double-strand breaks to restore genetic integrity and protect offspring health. Proceedings of the National Academy of Sciences of the United States of America 117:11513-11522. https://doi.org/10.1073/pnas.2001124117
15. Marangos P, Carroll J (2012) Oocytes progress beyond prophase in the presence of DNA damage. Current biology : CB 22:989-994. https://doi.org/10.1016/j.cub.2012.03.063
16. Gougeon A (1996) Regulation of ovarian follicular development in primates: facts and hypotheses. Endocrine reviews 17:121-155.

17. Goldrat O, Gervy C, Englert Y *et al* (2015) Progesterone levels in letrozole associated controlled ovarian stimulation for fertility preservation in breast cancer patients. Human reproduction (Oxford, England) 30:2184-2189. https://doi.org/10.1093/humrep/dev155
18. Radiology ACo (2018) ACR–SPR PRACTICE PARAMETER FOR IMAGING PREGNANT OR POTENTIALLY PREGNANT ADOLESCENTS AND WOMEN WITH IONIZING RADIATION.
https://wwwacrorg/Clinical-Resources/Radiology-Safety/Radiation-Safety.
19. McCollough CH, Schueler BA, Atwell TD *et al* (2007) Radiation exposure and pregnancy: when should we be concerned? Radiographics 27:909-917; discussion 917-908. https://doi.org/10.1148/rg.274065149
20. Borrego-Soto G, Ortiz-Lopez R, Rojas-Martinez A (2015) Ionizing radiation-induced DNA injury and damage detection in patients with breast cancer. Genet Mol Biol 38:420-432. https://doi.org/10.1590/S1415-475738420150019
21. Wallace WH, Thomson AB, Saran F *et al* (2005) Predicting age of ovarian failure after radiation to a field that includes the ovaries. International journal of radiation oncology, biology, physics 62:738-744. https://doi.org/10.1016/j.ijrobp.2004.11.038
22. Jacquet P, Adriaens I, Buset J *et al* (2005) Cytogenetic studies in mouse oocytes irradiated in vitro at different stages of maturation, by use of an early preantral follicle culture system. Mutat Res 583:168-177. https://doi.org/10.1016/j.mrgentox.2005.03.008
23. Yuen WS, Merriman JA, O'Bryan MK *et al* (2012) DNA double strand breaks but not interstrand crosslinks prevent progress through meiosis in fully grown mouse oocytes. PloS one 7:e43875. https://doi.org/10.1371/journal.pone.0043875
24. Collins JK, Lane SIR, Merriman JA *et al* (2015) DNA damage induces a meiotic arrest in mouse oocytes mediated by the spindle assembly checkpoint. Nat Commun 6:8553. https://doi.org/10.1038/ncomms9553
25. Suh EK, Yang A, Kettenbach A *et al* (2006) p63 protects the female germ line during meiotic arrest. Nature 444:624-628. https://doi.org/10.1038/nature05337

**Table 1**. Breast cancer characteristics

| | Ionizing radiation exposed cohort (n=14) | Non-exposed cohort (n=60) | P-value |
|---|---|---|---|
| **Mean age at diagnosis (SD)** | 31.2 (3.4) | 32.6 (4.0) | 0.21 |
| ***BRCA* pathogenic variants – n (%)** | 5 (35.7) | 9 (15.0) | 0.12 |
| *Of which:* | | | |
| *BRCA1* | 3 (21.4) | 5 (8.3) | |
| *BRCA2* | 2 (14.3) | 4 (6.7) | |
| **Histology - n (%)** | | | 0.72 |
| Ductal carcinoma | 13 (92.9) | 54 (90.0) | |
| Lobular carcinoma | 0 (0) | 1 (1.7) | |
| Other | 0 (0) | 1 (1.7) | |
| Unknown | 1 (7.1) | 2 (3.3) | |
| **Tumor grade - n (%)** | | | 0.06 |
| 1-2 | 1 (7.1) | 22 (36.7) | |
| 3 | 13 (92.9) | 36 (60.0) | |
| Unknown | 0 (0) | 2 (3.3) | |
| **Tumor size - n (%)** | | | 0.85 |
| T1 | 4 (28.6) | 22 (36.7) | |
| T2 | 8 (57.1) | 30 (50) | |
| T3-T4 | 2 (14.3) | 8 (13.3) | |
| **Nodal status - n (%)** | | | 0.63 |
| N0 | 10 (71.4) | 36 (60.0) | |
| N1-N3 | 4 (28.6) | 22 (36.7) | |
| Unknown | 0 (0) | 2 (3.3) | |
| **Hormone receptor status - n (%)** | | | 0.76 |
| ER and/or PR positive | 8 (57.1) | 38 (63.3) | |
| ER and PR negative | 6 (42.9) | 22 (36.7) | |
| **HER2 status - n (%)** | | | 1.00 |
| HER2 negative | 10 (71.4) | 40 (66.7) | |
| HER2 positive | 4 (28.6) | 20 (33.3) | |

Abbreviations: HER2, human epidermal growth factor receptor 2; ER, estrogen receptor; PR, progesterone receptor.

393 **Table 2**. Ovarian stimulation and oocyte retrieval

| | Ionizing radiation exposed cohort (n=14) | Non-exposed cohort (n=60) | P-value |
|---|---|---|---|
| **Basal AMH (**µg/L)- median (range) | 2.5 (0.2-13) | 1.9 (0.3-7.1) | 0.20 |
| **Number of cycles** | 14 | 69 | |
| **Time between breast cancer diagnosis to day 1 of OS -** in days- median (range) | 11.5 (5-33) | 28 (1-164) | <0.01 |
| **Time between imaging and oocyte retrieval,** in days - median (range) | 8.7 (4-12) | Not applicable | |
| **Type of ovarian stimulation cycle** - n (%) | | | 0.92 |
| Standard | 9 (64.3) | 39 (56.5) | |
| Random follicular | 1 (7.1) | 5 (7.2) | |
| Random luteal | 4 (28.6) | 24 (34.8) | |
| Unknown | 0 (0) | 1 (1.4) | |
| **Gonadotropins** | | | 0.72 |
| Recombinant FSH- n (%) | 12 (85.7) | 53 (76.8) | |
| HMG- n (%) | 2 (14.3) | 16 (23.2) | |
| Total FSH dose (IU) - mean (SD) | 2794.6 (892.3) | 2524.1 (950.1) | 0.33 |
| **Stimulation**, in days - median (range) | 11 (8-14) | 10 (3-16) | 0.40 |
| **Triggering method - n (%)** | | | 0.54 |
| hCG | 3 (21.4) | 22 (31.9) | |
| GnRH agonists | 11 (78.6) | 47 (68.1) | |
| **Data at triggering** - median (range) | | | |
| E2 (ng/l) | 319.8 (95-1345) | 317 (20-1024) | 0.44 |
| Progesterone (µg/l) | 0.8 (0.4-2.4) | 1 (0.2-5.7) | 0.53 |
| Number of follicles > 18 mm | 2.5 (1-11) | 2 (0-7) | 0.76 |
| Number of follicles 15-18 mm | 3 (1-17) | 4 (0-20) | 0.82 |
| Number of follicles < 15 mm | 5 (1-15) | 6 (0-24) | 0.89 |
| **OS outcomes** | | | |
| Number of oocytes collected- median (range) | 10.5 (1-21) | 7 (0-23) | 0.16 |
| Number of oocytes collected- mean (SD) | 11.3 (7.4) | 8.1 (5.1) | |
| Number of mature oocytes median (range) | 8 (1-20) | 6 (0-19) | 0.17 |

| | | | |
|---|---|---|---|
| Number of mature oocytes collected- mean (SD) | 9.7 (6.9) | 6.7 (4.4) | |
| Maturation rate - % median (range) | 92.5 (46.2-100) | 85.7 (0-100) | 0.54 |
| **Fertilization outcomes** | | | |
| Total number of oocytes fertilized | 45 | 132 | |
| Fertilization rate (%) | 62.2 | 65.4 | 0.70 |
| Total number of frozen embryos | 22 | 89 | |

Abbreviations: OS, ovarian stimulation; AMH, anti-Müllerian hormone; FSH, follicle-stimulating hormone; HMG, human menopausal gonadotropin; IU, international units; SD, standard deviation; hCG, Human chorionic gonadotropin; GnRH, Gonadotropin-releasing hormone.

**Table 3.** Association between parameters and number of mature oocytes

| Negative Binomial | IRR (IC95%) | P-value | aIRR (IC95%) | P-value |
|---|---|---|---|---|
| **Treatment** exposed vs non-exposed to ionizing radiation | 1.37 (0.94-2.0) | 0.10 | 1.13 (0.77-1.65) | 0.53 |
| **Age** | 0.95 (0.91-0.99) | 0.02 | 0.98 (0.94-1.02) | 0.40 |
| **AMH** | 1.18 (1.09-1.28) | <0.0001 | 1.16 (1.07-1.27) | 0.001 |
| **BRCA** BRCA PV vs BRCA VUS, negative or unknown | 0.92 (0.62-1.36) | 0.67 | 1.06 (0.72-1.56) | 0.75 |

Abbreviations: IRR, incidence rate ratios; aIRR, adjusted incidence rate ratios; AMH, anti-Müllerian hormone; PV, pathogenic variant; VUS, variants of unknown significance.

404 **Table S1**. Medical and reproductive history before breast cancer diagnosis

| | Ionizing radiation exposed cohort (n=14) | Non-exposed cohort (n=60) | P-value |
|---|---|---|---|
| **Median BMI** (range) | 24.4 (20.1-37.5) | 22.1 (17.5-30.8) | 0.04 |
| **Toxics intake-** n (%) | | | |
| Tobacco | 3 (21.4) | 10 (16.7) | 0.70 |
| Alcohol | 1 (7.1) | 6 (10) | 1.00 |
| Drugs | 0 (0) | 4 (6.7) | 1.00 |
| **Gynecological history with a possible impact on fertility -** n (%) | | | |
| PID | 0 (0) | 1 (1.7) | 1.00 |
| Endometriosis | 0 (0) | 3 (5.0) | 1.00 |
| PCOS | 0 (0) | 2 (3.4) | 1.00 |
| **Obstetrical history-** n (%) | | | |
| Gravidity | | | 0.37 |
| 0 | 10 (71.4) | 33 (55.0) | |
| ≥1 | 4 (28.6) | 27 (45.0) | |
| Parity | | | 0.20 |
| 0 | 12 (85.7) | 39 (65.0) | |
| ≥1 | 2 (14.3) | 21 (35.0) | |
| Miscarriages | 1 (7.1) | 7 (11.7) | 1.00 |
| Termination | 2 (14.3) | 7 (11.7) | 0.68 |
| Extra-uterine | 0 (0) | 1 (1.7) | 1.00 |
| **Use of hormonal contraception -** n (%) | | | 0.17 |
| Yes | 6 (42.9) | 17 (28.3) | |
| No | 7 (50.0) | 42 (70.0) | |
| Unknown | 1 (7.1) | 1 (1.7) | |
| **History of infertility-** n (%) | 0 (0) | 7 (11.7) | 1.00 |

405 Abbreviation: BMI, body mass index; PID, pelvic inflammatory disease; PCOS, polycystic ovary
406 syndrome.

407

408

409

**Table S2**. Baseline characteristics, cycle outcome and radiation exposure for each exposed patient

| Patient ID | Age | AMH value | *BRCA* | Number of oocytes collected | Number of mature oocytes | Fertilization rate * | Type of exam | Max radiation exposure (mGy) |
|---|---|---|---|---|---|---|---|---|
| 4 | 37.1 | Unknown | BRCA2 VUS | 7 | 7 | NA | Bone scan | 5 |
| 7 | 35.2 | 0.16 | BRCA1 PV | 3 | 3 | NA | Bone scan | 5 |
| 9 | 26.3 | 13 | Neg | 13 | 6 | NA | Bone scan | 5 |
| 20 | 28.8 | 6.1 | Neg | 21 | 18 | 55.6 (9/5) | Bone scan and MUGA scan | 5.5 |
| 22 | 32.6 | 0.65 | BRCA2 VUS | 1 | 1 | 100 (1/1) | Bone scan | 5 |
| 24 | 29.8 | 6.6 | Neg | 9 | 9 | 100 5(2/2) | Bone scan | 5 |
| 25 | 33 | 2.43 | Neg | 18 | 16 | 70 (10/7) | PET scan | 20 |
| 41 | 30.2 | 4 | BRCA2 PV | 21 | 20 | 16.7 (16/2) | PET scan | 20 |
| 44 | 29.5 | 2.4 | BRCA2 PV | 4 | 2 | 50 (2/1) | Bone scan and CT scan | 30 |
| 52 | 29.5 | 4.7 | BRCA1 PV | 21 | 19 | NA | Bone scan, CT scan and PET scan | 50 |
| 59 | 26.1 | 1.59 | Neg | 4 | 4 | 25 (4/1) | Bone scan and CT scan | 30 |
| 66 | 35.5 | Unknown | BRCA1 PV | 6 | 5 | 80 (5/4) | Bone scan and | 30 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | CT scan | |
| 72 | 30.4 | 2.55 | Neg | 18 | 17 | NA | PET scan | 20 |
| 74 | 33.6 | 1.85 | Neg | 12 | 9 | NA | PET scan | 20 |

*(number of oocyte used for ICSI/number of embryos obtained)

Abbreviation: AMH, anti-Müllerian hormone; PV, pathogenic variant; VUS, variants of unknown significance; Neg, negative; PET, positron emission tomography; CT, computerized tomography.

**Table S3**. Oncological outcomes

| Oncological outcomes | Ionizing radiation exposed cohort (n=14) | Non-exposed cohort (n=60) | P-value |
|---|---|---|---|
| **Follow-up from breast cancer diagnosis,** in years- median (range) | 2.9 (0.8-6.1) | 3.9 (0.8-7.5) | 0.31 |
| **Number of patients with at least 1 year of follow-up**- n (%) | 12 (85.7) | 58 (96.7) | 0.24 |
| **Relapse -** n (%) | | | 0.36 |
| No | 11 (78.6) | 54 (90.0) | |
| Yes | 3 (21.4) | 6 (10.0) | |
| **Type of relapse** | | | 0.79 |
| Loco-regional | 2 (66.7) | 1 (16.7) | |
| Distant | 0 | 1 (16.7) | |
| Metastatic | 1 (33.3) | 3 (75.0) | |
| Missing | 0 | 1 (16.7) | |
| **Death -** n (%) | | | 1.00 |
| No | 14 (100.0) | 59 (98.3) | |
| Yes | 0 (0) | 1 (1.7) | |