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# Ovarian follicular density in women with BRCA1 and BRCA2 mutations: new insights into the negative impact on ovarian reserve

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## Abstract

**Background** Germline mutations of BRCA1 and BRCA2 may impair DNA repair in the ovarian cortex, leading to increased oocyte apoptosis, thus, affecting ovarian reserve. Aim of this study was to assess follicular density in ovarian biopsies from women with breast cancer carrying BRCA1 and BRCA2 mutations who underwent ovarian tissue cryopreservation (OTC) at our center.

**Methods** This was a single center, observational, cross-sectional study carried out in a tertiary level referral center for fertility preservation treatment. Exclusion criteria were: patients aged < 18 years or > 38 years, patients who had already undergone chemotherapy/pelvic radiotherapy at the time of OTC, patients without data on follicular density and those with unknown BRCA mutational status. Follicular density was defined as the number of primordial, intermediate primordial, small primary, large primary, secondary, preantral, and antral follicles per 1 mm<sup>2</sup> of cortical section area.

**Results** Out of 216 patients, 21 women reported germline mutation: 9 (4.2%) were carriers of the BRCA1 mutation and 13 (6%) of the BRCA2 mutation. The mean age at OTC was 31.5 ± 3.6 years, and the median age was 32.4 years (range, 21–38). No significant difference in follicular density was observed among women without BRCA mutations, those with BRCA1 mutations, and those with BRCA2 mutations. The median follicular density was 4.0/mm<sup>2</sup> (range 0–74.5) in BRCA-negative women, 3.5/mm<sup>2</sup> (range 0–20) in women with BRCA1 mutations, and 4.0/mm<sup>2</sup> (range 0–32) in women with BRCA2 mutations ( $p=0.272$  and  $p=0.703$ , respectively). After adjusting for age, no statistically significant differences in follicular density were observed according to BRCA1 and BRCA2 mutation status: the median follicular density was 4.6/mm<sup>2</sup> in BRCA-negative women, 3.1/mm<sup>2</sup> in women with BRCA1 mutations, and 3.6/mm<sup>2</sup> in women with BRCA2 mutations ( $p=0.428$  and  $p=0.385$ , respectively).

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**Conclusions** No significant difference in follicular density was observed between women with BRCA1/BRCA2 mutations and those without. Our findings suggest that the presence of a BRCA mutation does not have a significant negative clinical impact on the follicular population of the ovarian cortex. Larger studies are needed to further validate these findings.

**Keywords** Cancer, Tumor, Preservation, Ovar\*, Germline mutations, Fertility, Follicles

## Introduction

BRCA1 (Breast Cancer Gene 1) and BRCA2 (Breast Cancer Gene 2) are genes that play a critical role in maintaining genomic integrity by repairing DNA damage [1]. Germline mutations in BRCA1 or BRCA2, significantly increase risk of high-grade serous ovarian cancer, fallopian tube cancer, primary peritoneal cancer and breast tumor [2]. However, deficiencies in DNA repair caused by mutations in these genes may extend beyond carcinogenesis, potentially affecting other biological processes. Mutations in the BRCA1 and BRCA2 genes have been linked to oocyte aging, as they impair DNA damage repair in the ovarian cortex [3]. This impairment results in the progressive accumulation of unrepaired DNA damage over time, which in turn promotes increased oocyte apoptosis [4], and potentially affects ovarian reserve.

The impact of BRCA1/BRCA2 germline mutations on ovarian reserve has been mainly evaluated using Anti-Müllerian Hormone (AMH) [5]: BRCA1 mutation carriers have been reported to experience a 25% reduction in AMH levels [1], whereas evidence regarding the association between BRCA2 mutations and AMH levels remains inconsistent [1, 6]. However, AMH is recognized as a serum marker of ovarian reserve, which appears to have a stronger correlation with pool of developing follicles, oocyte yield and ovarian responsiveness in vitro fertilization than with actual follicular density [7]. Conversely, only one study has examined follicular density in ovarian biopsy tissue from women with breast cancer carrying BRCA1 or BRCA2 mutations [8]. In this study, Lambertini et al. reported no significant differences in follicular density between 19 BRCA-positive women and 53 BRCA-negative patients undergoing ovarian tissue cryopreservation (OTC) for fertility preservation [8].

The aim of this study was to assess follicular density in ovarian biopsies from women with breast cancer carrying BRCA1 and BRCA2 mutations who underwent OTC at our center, with the goal of externally validating the preliminary findings reported by Lambertini et al. [8].

## Material and methods

### Study protocol, selection criteria and study outcomes

This was a single center, observational, cross-sectional study following an a priori defined study protocol. The study was carried out in a tertiary level referral center for fertility preservation treatment (IRCCS Azienda

Ospedaliero-Universitaria di Bologna, Bologna, Italy). The whole study was reported according to the STrengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement and checklist [9]. From the biobank of our center, all consecutive patients who underwent OTC for breast cancer from January 1st, 2002 (date of establishment of the biobank) to September 30th, 2024, were included in the study.

Exclusion criteria were: patients aged <18 years or >38 years, patients who had already undergone chemotherapy/pelvic radiotherapy at the time of OTC, patients without data on follicular density from the ovarian biopsy and those with unknown BRCA mutational status.

Medical reports were searched for extraction of the following patient data: age at OTC, body mass index (BMI), breast cancer treatments before OTC, antral follicular count on preoperative transvaginal ultrasound, anti-müllerian hormone concentration (ng/ml) prior to surgery, follicular density of ovarian tissue sample. Specifically, stage of breast cancer according to 2019 Tumor Node Metastasis (TNM) staging system, breast cancer histotypes according to 2019 World Health Organization (WHO) classification, hormonal receptors status of the tumor were also collected. Since the WHO classification for breast cancer histotypes and the TNM staging system have changed over the last twenty years, all included cases were reported according to the latest editions [10, 11].

### Surgical procedure for ovarian tissue collection

In this study OTC included a large biopsy from one or both ovaries through laparoscopy [12], followed by cryopreservation of the excised tissue for future transplantation after cancer remission.

### Assessment of follicular density

The analysis was conducted by a biologist specialized in fertility preservation techniques. Histological analysis was performed at the time of laparoscopy, using fresh samples, to assess the follicular density. In detail, one fragment ( $\pm 2 \text{ mm} \times 2 \text{ mm} \times 1 \text{ mm}$ ) from one ovary (or both ovaries in cases of bilateral biopsies) was fixed in 4% formaldehyde, embedded in paraffin and serially sectioned at a thickness of 4 micron. The serial sections obtained per block were stained with hematoxylin and eosin (Merck, Darmstadt, Germany) to assess the

follicular density. Sections were observed at 10× magnification to detect artefacts, erroneous sampling and to determine follicular density. At 25× magnification different types of follicles could be identified (primordial, intermediary primordial and small primary follicles, large primary, secondary, preantral and antral follicles). Follicles containing empty oocytes or oocytes with large cytoplasmic vacuoles, dark and granular cytoplasm, hyperchromatic nuclear staining and that were detached from surrounding granulosa cells were considered degenerated and were not included in the follicular count. For the purpose of this study, follicular density was defined as the number of follicles per 1 mm<sup>2</sup> of cortical section area, including primordial follicles (primordial and intermediate primordial) and growing follicles (small and large primary, secondary, preantral, and antral follicles). For bilateral OTC, mean value of the two follicular density values was considered in the analysis.

Determination of BRCA1/BRCA2 mutational status

The BRCA1/BRCA2 mutational status was assessed for all patients included in the study. Specifically, patients were contacted to confirm their mutational status if it was not documented in the medical records. However, over the time span during which ovarian tissue biopsies were performed, the criteria for testing germline in breast cancer patients have being evolved [13]. BRCA1/BRCA2 mutational status was considered negative if the genetic test results were reported as negative for either mutation or if the woman had not been recommended by her oncologist to undergo genetic testing. Patients with BRCA mutations of uncertain significance were classified as negative [14], as these mutations are currently considered not-pathogenic variants [14].

Statistical analysis and power analysis

The 95% confidence intervals (CIs) for the median follicular density in the study groups were calculated using a binomial-based exact method, with linear interpolation applied to improve the accuracy of the estimated CIs. The extremes were fixed at the minimum and maximum sample value [15]. We used the Wilcoxon rank-sum test to evaluate whether follicular density was distributed

differently according to BRCA1 and BRCA2 mutation status. Due to the presence of extreme outliers and small sample sizes, a Monte Carlo permutation version of the test was used to estimate the *p*-values for the Wilcoxon rank-sum *z* statistic. The same approach was used to assess differences in baseline characteristics across the study groups. To control for the potential confounding effect of age, the association between follicular density and mutation status was reanalyzed with covariate adjustment using bootstrapped quantile regression [16]. Specifically, we applied simultaneous-quantile regression ('sqreg' command in Stata) with 1000 bootstrap replications to model the median follicular density (50th percentile) as a function of BRCA1/2 status and age, which was categorized via median split to enhance model stability. This approach allows robust postestimation of adjusted medians and confidence intervals, minimizing the influence of extreme values. Quantile regression was chosen because it yields a direct estimate of covariate-adjusted medians and is particularly suited for non-normally distributed outcomes such as follicular density. All analyses were performed using Stata 18 (StataCorp. 2023. *Stata 18 Base Reference Manual*. College Station, TX: Stata Press). The significance level was set at 0.05, and all tests were two-sided.

Ethical statement

The study received approval from the Institutional Review Board (CE-AVEC 320/2021/Oss/AOUBo) and was carried out in accordance with the Helsinki Declaration. All patients signed an informed consent for the use of their anonymized data for the study.

Results

Study population

During the study period, 231 consecutive women with breast cancer underwent OTC. Fifteen patients (6.5%) were excluded from the study analyses since they did not meet the selection criteria. As a result, a total of 216 (93.5%) patients were considered for the analyses (Table 1).

Out of 216 patients, 21 women reported germline mutation: 9 (4.2%) were carriers of the BRCA1 mutation

Table 1 Demographic and clinical characteristics of the whole study population and per study groups

|                                                 | BRCA1 Mutation   |                 |                |                 | BRCA2 Mutation  |                 |                 |
|-------------------------------------------------|------------------|-----------------|----------------|-----------------|-----------------|-----------------|-----------------|
|                                                 | All<br>(n = 216) | No<br>(n = 207) | Yes<br>(n = 9) | <i>p</i> -value | No<br>(n = 203) | Yes<br>(n = 13) | <i>p</i> -value |
| Age [years], mean ± SD                          | 31.5 ± 3.6       | 31.5 ± 3.6      | 30.4 ± 3.2     | 0.296           | 31.6 ± 3.5      | 29.9 ± 3.6      | 0.083           |
| BMI [kg/m <sup>2</sup> ], mean ± SD             | 21.7 ± 3.7       | 21.7 ± 3.6      | 23.9 ± 4.6     | 0.160           | 21.7 ± 3.6      | 22.2 ± 4.8      | 0.875           |
| Antral Follicle Count at ultrasound, mean ± SD  | 20.2 ± 11.8      | 20.2 ± 11.9     | 20.4 ± 7.2     | 0.471           | 20.1 ± 11.8     | 21.8 ± 11.2     | 0.468           |
| AMH concentration before OTC [ng/ml], mean ± SD | 3.6 ± 2.5        | 3.5 ± 2.5       | 3.4 ± 5.4      | 0.771           | 2.9 ± 2.3       | 2.7 ± 1.6       | 0.565           |
| Bilateral ovarian biopsy, n (%)                 | 114 (52.8)       | 110 (53.1)      | 4 (44.4)       | 0.738           | 109 (53.7)      | 5 (38.5)        | 0.392           |

SD Standard deviation, BMI Body Mass Index, OTC Ovarian tissue cryopreservation

and 13 (6%) of the BRCA2 mutation. The mean age at OTC was similar across groups, with no significant difference between patients with and without BRCA1 mutation ( $30.4 \pm 3.2$  vs  $31.5 \pm 3.6$  years,  $p=0.296$ ) or BRCA2 mutation ( $29.9 \pm 3.6$  vs  $31.6 \pm 3.5$  years,  $p=0.083$ ). BMI was also comparable, with no significant differences observed. Mean AFC was similar across groups, with an overall mean of  $20.2 \pm 11.8$ , and no significant differences observed between BRCA1-mutated ( $20.4 \pm 7.2$ ) and non-BRCA1-mutated women ( $20.2 \pm 11.9$ ;  $p=0.471$ ), or between BRCA2-mutated ( $21.8 \pm 11.2$ ) and non-BRCA2-mutated women ( $20.1 \pm 11.8$ ;  $p=0.468$ ). This data was not available for 18 (8.3%) women. Regarding AMH concentration before OTC, no significant differences were observed between BRCA1-mutated women ( $3.4 \pm 5.4$  ng/ml) and non-BRCA1-mutated women ( $3.5 \pm 2.5$  ng/ml;  $p=0.771$ ), or between BRCA2-mutated women ( $2.7 \pm 1.6$  ng/ml) and non-BRCA2-mutated women ( $3.4 \pm 2.6$  ng/ml;  $p=0.565$ ). However, AMH data were missing for 96 (44%) of patients. The distribution of cancer stages according to the 2019 TNM staging system showed a significant difference between the BRCA1 group and other groups ( $p=0.009$ ), with a higher percentage of BRCA1 mutation carriers being diagnosed at stage I (66.7%) compared to the other groups (1.1% in BRCA2 and 28.2% in the overall population). However, no significant differences were found for the stage distribution between BRCA2 mutation carriers and non-BRCA1 mutated women ( $p=0.209$ ). Regarding the 2019 WHO Breast Cancer Histotype, the most common histologic type across all groups was invasive ductal

carcinoma (85.2% in the overall cohort, 77.8% in BRCA1 carriers, and 84.6% in BRCA2 carriers). There was no significant difference in histotype distribution between BRCA1, BRCA2, and the overall population ( $p=0.355$  and  $p=0.454$ , respectively). For hormone receptor status, most patients were classified as luminal A (67.1% in the overall cohort, 66.7% in BRCA1, and 61.5% in BRCA2). The basal-like subtype was observed in 20.8% of overall population, with a higher proportion in BRCA1 mutation carriers (33.3%). There were no significant differences in hormone receptor status between the groups ( $p=0.613$  and  $p=0.754$  for BRCA1 and BRCA2 mutation status, respectively) (Table 2).

### Study outcomes

No significant difference in follicular density was observed among women without BRCA mutations, those with BRCA1 mutations, and those with BRCA2 mutations. The median follicular density was  $4.0/\text{mm}^2$  (range 0–74.5) in BRCA-negative women,  $3.5/\text{mm}^2$  (range 0–20) in women with BRCA1 mutations, and  $4.0/\text{mm}^2$  (range 0–32) in women with BRCA2 mutations ( $p=0.272$  and  $p=0.703$ , respectively) (Table 3; Fig. 1). Since age was an independent risk factor for reduced follicular density, we conducted an additional analysis to assess follicular density independently of age. After adjusting for age, no statistically significant differences in follicular density were observed according to BRCA1 and BRCA2 mutation status: the median follicular density was  $4.6/\text{mm}^2$  in BRCA-negative women,  $3.1/\text{mm}^2$  in women with BRCA1 mutations, and  $3.6/\text{mm}^2$  in women with BRCA2

**Table 2** Breast cancer characteristics of the study population and per study groups

|                                         | BRCA1 Mutation   |                 |                | p-value | BRCA2 Mutation  |                 | p-value |
|-----------------------------------------|------------------|-----------------|----------------|---------|-----------------|-----------------|---------|
|                                         | All<br>(n = 216) | No<br>(n = 207) | Yes<br>(n = 9) |         | No<br>(n = 203) | Yes<br>(n = 13) |         |
| 2019 TNM staging system, n (%)          |                  |                 |                | 0.009*  |                 |                 | 0.209   |
| Stage I                                 | 61 (28.2)        | 55 (26.6)       | 6 (66.7)       |         | 60 (29.6)       | 1 (7.7)         |         |
| Stage II                                | 120 (55.6)       | 119 (57.5)      | 1 (11.1)       |         | 112 (55.2)      | 8 (61.5)        |         |
| Stage III                               | 35 (16.2)        | 33 (15.9)       | 2 (22.2)       |         | 31 (15.3)       | 4 (30.7)        |         |
| 2019 WHO Breast Cancer Histotype, n (%) |                  |                 |                | 0.355   |                 |                 | 0.454   |
| Lobular in Situ                         | 1 (0.5)          | 1 (0.5)         | 0 (0.0)        |         | 1 (0.5)         | 0 (0.0)         |         |
| Ductal in Situ                          | 5 (2.3)          | 5 (2.4)         | 0 (0.0)        |         | 4 (2.0)         | 1 (7.7)         |         |
| Invasive Ductal                         | 184 (85.2)       | 177 (85.5)      | 7 (77.8)       |         | 173 (85.2)      | 11 (84.6)       |         |
| Invasive Lobular                        | 2 (0.9)          | 2 (1.0)         | 0 (0.0)        |         | 2 (1.0)         | 0 (0.0)         |         |
| Infiltrating of No Special Type         | 13 (6.0)         | 11 (5.3)        | 2 (22.2)       |         | 13 (6.4)        | 0 (0.0)         |         |
| Others                                  | 11 (5.1)         | 11 (5.3)        | 0 (0.0)        |         | 10 (4.9)        | 1 (7.7)         |         |
| Hormone Receptors Status, n (%)         |                  |                 |                | 0.613   |                 |                 | 0.754   |
| Basal-like                              | 45 (20.8)        | 42 (20.3)       | 3 (33.3)       |         | 42 (20.7)       | 3 (23.1)        |         |
| Luminal A                               | 145 (67.1)       | 139 (67.1)      | 6 (66.7)       |         | 137 (67.5)      | 8 (61.5)        |         |
| Luminal B                               | 20 (9.3)         | 20 (9.7)        | 0 (0.0)        |         | 20 (9.9)        | 0 (0.0)         |         |
| HER2 + + +                              | 4 (1.9)          | 4 (1.9)         | 0 (0.0)        |         | 4 (2.0)         | 0 (0.0)         |         |
| Not reported                            | 2 (0.9)          | 2 (1.0)         | 0 (0.0)        |         | 0 (0.0)         | 2 (15.4)        |         |

\*TNM Tumor Node Metastasis, WHO World Health Organization, HER2 + + + overexpression of Human Epidermal growth factor Receptor 2

**Table 3** Follicular density (n/mm2) by BRCA1 and BRCA2 mutation status

|       | All OTC<br>(n=216) | Follicular density<br>[n/mm <sup>2</sup> ], median<br>[range] | 95% CI  | p-value |
|-------|--------------------|---------------------------------------------------------------|---------|---------|
| BRCA1 |                    |                                                               |         |         |
| No    | 207                | 4.0 (0–74.5)                                                  | 3.0–6.0 | 0.272   |
| Yes   | 9                  | 3.5 (0–20)                                                    | 0.0–5.5 |         |
| BRCA2 |                    |                                                               |         |         |
| No    | 203                | 4.0 (0–74.5)                                                  | 3.0–5.5 | 0.703   |
| Yes   | 13                 | 4.0 (0–32)                                                    | 0.6–8.6 |         |

OTC ovarian tissue cryopreservation, n/mm<sup>2</sup> number of follicles per 1 mm<sup>2</sup> area of ovarian cortex, BRCA1 Breast Cancer Gene 1, BRCA2 Breast Cancer Gene 2

mutations ( $p=0.428$  and  $p=0.385$ , respectively) (Supplementary Table 1).

Post-hoc analysis

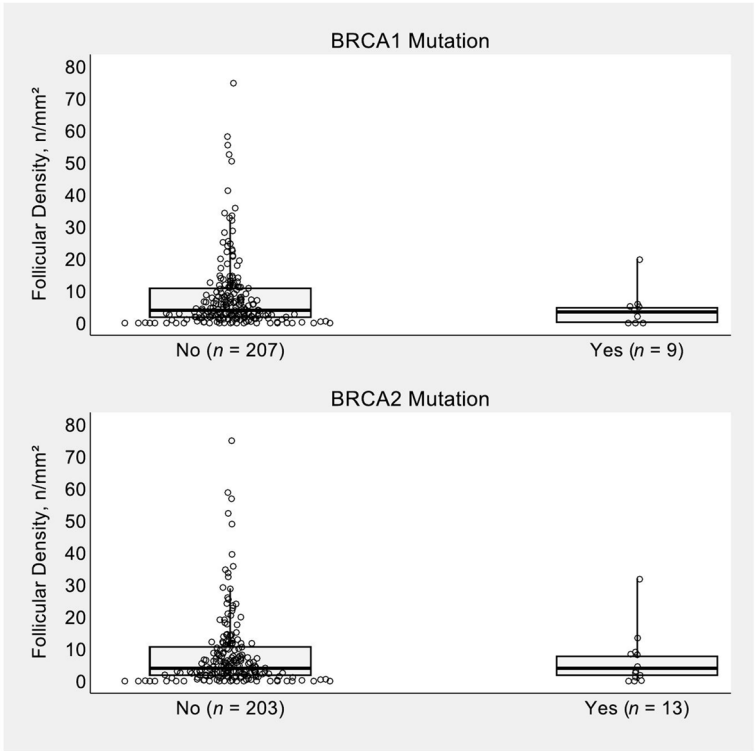
In a *post-hoc* analysis, we re-evaluated the data after excluding seven non-BRCA-mutated patients who had not been initially referred for genetic testing according to clinical criteria at that time. Following this exclusion, no significant difference in follicular density was observed among women without BRCA mutations, those with BRCA1 mutations, and those with BRCA2 mutations. The median follicular density was 4.0/mm<sup>2</sup> (range 0–74.5) in BRCA-negative women, 3.5/mm<sup>2</sup> (range 0–20)

in women with BRCA1 mutations, and 4.0/mm<sup>2</sup> (range 0–32) in women with BRCA2 mutations ( $p=0.288$  and  $p=0.707$ , respectively) (Supplementary Fig. 1). After adjusting for age, no statistically significant differences were observed according to BRCA1 and BRCA2 mutation status (Supplementary Table 3).

Discussion  
Main findings

In this study, we found no significant differences in follicular density between ovarian tissue biopsies from women carrying BRCA1 and BRCA2 mutations and those from non-carrier women, and these findings were further confirmed after adjusting for age.

BRCA1 and BRCA2 are tumor suppressor genes primarily recognized for their role in repairing DNA damage, as well as in chromatin remodeling [17], telomere maintenance [18], and embryonic development [19]. Regarding how mutations in these genes may compromise oocyte numbers, it has been hypothesized that loss-of-function of BRCA mutated genes compel cells to utilize a non-homologous and non-conservative repair pathway, which is more susceptible to errors during DNA repair damage [3]. If these errors become fixed, they can propagate during replication unless apoptosis occurs to prevent their spread. Thus, this mechanism negatively



**Fig. 1** Follicular density by BRCA1 and BRCA2 mutation status. The median is shown by the line that divides the box into two parts; the range of scores from the lower to upper quartile is marked by the box; the whiskers stretch from the 5th percentile to the 95th percentile. n/mm2= number of follicles per 1 mm2 area of ovarian cortex; BRCA1 =Breast Cancer Susceptibility Gene 1; BRCA2=Breast Cancer Susceptibility Gene 2

affects the cell division process in non-dividing cells, such as those in the ovaries, and raises oocyte loss through increased rate of apoptosis [3].

We confirmed the findings of Lambertini et al. [8], which reported no statistically significant differences in follicular density between ovarian biopsies from BRCA-mutated and non-mutated women. A trend toward lower follicular density in BRCA-mutated women, with a more pronounced reduction in the BRCA1 group, was also observed in our study, both in the entire population and in the age-adjusted analyses. Specifically, BRCA1 carriers showed a median follicular density 0.5 units lower than healthy controls, which decreased to 1.5 units lower when adjusted for age. Regarding the impact of the BRCA2 mutation, we confirmed the findings of Lambertini et al. in a larger cohort of BRCA2 carriers, reporting a median follicular density similar to that of non-mutated patients in the whole population, which became 1 unit lower than healthy controls after age adjustment.

These results could be explained by considering the characteristics of folliculogenesis that occur in the cortex of the human ovary [20]. During folliculogenesis, follicles progressed through various stages of growth, development, or atresia, ranging from the primordial to the ovulatory phase. Primordial follicles were composed of primary oocytes arrested in meiotic prophase, where they could remain for extended periods until they left the resting pool [21]. Primary, secondary, and antral follicles progressively increased in size and were distinguished by the accumulation of additional layers of granulosa cells. These stages were marked by a significant rise in the mitotic activity of somatic cells, accompanied by the formation of blood and lymphatic networks within the surrounding tissue [21]. AMH was produced mainly by follicles containing granulosa cells: as a result, serum AMH levels reflected the proportion of developing follicles in the ovaries [22]. The lack of a significant difference between follicular density of BRCA-mutated women and those of non-BRCA-mutated women could help clarify the mechanisms through which these mutations can affect ovarian reserve. The increased rate of apoptosis associated with loss-of-function mutations in BRCA genes may occur mainly during the more dynamic phases of folliculogenesis, characterized by higher mitotic activity of growing follicles (i.e., primary, secondary, and antral follicles) [21]. Since the oocyte population included in follicular density was primarily composed of primordial follicles, with a smaller proportion of growing follicles, follicular density was not markedly reduced in mutated women compared to non-mutated ones: primordial follicles were quiescent and, thus, were less susceptible to impaired DNA damage repair.

The results of our study supported the need to offer fertility preservation techniques to women with

BRCA-mutated breast cancer. Oocyte cryopreservation may be the preferred option for BRCA-mutated patients with breast cancer [23, 24]. However, the final choice of fertility preservation technique — or a combined approach involving both oocyte and ovarian tissue cryopreservation — should be individualized and discussed with each patient, carefully outlining the advantages and limitations of each procedure.

### Strengths and limitations

This study represented the first external validation of the exploratory analysis performed by Lambertini et al. [8]. To overcome the limits of Lambertini et al. [8], we included only a population of women with a recent diagnosis of breast cancer who had not undergone any type of oncological treatment. Moreover, our findings were further strengthened by adjustment for age, a critical confounding factor for evaluation of ovarian reserve, aiming to refine the analysis by controlling for the most significant confounding factor. In addition, the analysis of follicular density was performed by experienced personnel following the tissue processing protocol previously published [25], minimizing inter-operator variability in the assessment of follicular density. Finally, we included a slightly larger number of BRCA-mutated patients and carried out the investigation on a significantly higher number of BRCA2-mutated patients (compared to the five previously reported [8]).

However, our study has some limitations. The first limit was the relatively small sample size due to the low prevalence of women with BRCA1 and BRCA2 mutations, which may have reduced the power to detect subtle differences between these groups. However, the follicular density values observed were remarkably similar across groups, suggesting that the study was not markedly underpowered. Additionally, the use of advanced statistical techniques, such as Monte Carlo permutation tests, bootstrapped quantile regression, and exact confidence intervals, allowed for robust analyses despite the small sample size and the presence of extreme outliers. These methods minimized the impact of distributional assumptions and increased the reliability of the results, enhancing the validity of the findings. Another limitation of the study was the absence of preoperative AMH values in nearly half of the patients that prevented an evaluation of the correlation between follicular density and AMH.

### Conclusion

Although a different trend in follicular density was noted between women with and without BRCA1/BRCA2 mutations, this variation did not reach statistical significance. Our results indicate that the presence of a BRCA mutation does not appear to exert a significant negative clinical impact on the follicular population of the ovarian

cortex. However, larger studies are warranted to confirm and further investigate these findings.

## Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s13048-025-01901-1>.

Supplementary Material 1: Supplementary Table 1. Age-adjusted follicular density (n/mm<sup>2</sup>) by BRCA1 and BRCA2 mutation status.

Supplementary Material 2: Supplementary Table 2. Distribution of pathogenic variants in BRCA1- and BRCA2-mutated women

Supplementary Material 3: Supplementary Table 3. Age-adjusted follicular density (n/mm<sup>2</sup>) by BRCA1 and BRCA2 mutation status (*post-hoc* analysis).

Supplementary Material 4: Supplementary Fig. 1. Follicular density by BRCA1 and BRCA2 mutation status (*post-hoc* analysis).

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Not applicable.

## Authors' contributions

All the authors contributed to the present manuscript as follows: M. M. Conceptualization, Investigation, Methodology, Investigation, Writing—Review & Editing, Project administration. R. V. Formal analysis, Writing—Review & Editing. C. F. Conceptualization, Investigation. S. Di S. Conceptualization, Investigation. J. L. Formal analysis. A. R. Conceptualization. M. D. Conceptualization. L. C. Conceptualization. A. P. Conceptualization. E. S. Conceptualization, Methodology, Resources, Writing—Review & Editing, Supervision, Funding acquisition. R. S. Conceptualization, Methodology, Resources, Writing—Review & Editing, Supervision, Funding acquisition. D. R. Conceptualization, Methodology, Resources, Writing—Review & Editing, Supervision, Funding acquisition.

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## Data availability

The data of this study are available at the following link: <https://zenodo.org/records/14681137>.

## Declarations

### Ethics approval and consent to participate

The study received approval from the Institutional Review Board (Comitato Etico Area Vasta Emilia Centro 320/2021/Oss/AOUBo) and was carried out in accordance with the Helsinki Declaration. All patients signed an informed consent for the use of their anonymized data for the study.

### Consent for publication

All patients signed an informed consent for the use of their anonymized data for the publication.

### Competing interests

The authors declare no competing interests.

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