The Breast 59 (2021) 239–247

Contents lists available at ScienceDirect

The Breast

journal homepage: www.elsevier.com/brst

Anti-müllerian hormone levels and antral follicle count in women with a *BRCA1* or *BRCA2* germline pathogenic variant: A retrospective cohort study

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ARTICLE INFO

Article history:
Received 22 May 2021
Received in revised form
5 July 2021
Accepted 8 July 2021
Available online 12 July 2021

Keywords:
*BRCA*1
*BRCA*2
Anti-müllerian hormone
Antral follicle count
Ovarian reserve

ABSTRACT

Background: Some studies suggested a decreased ovarian reserve among *BRCA1/2* pathogenic variant carriers, with conflicting results.
Methods: We conducted a retrospective single-center observational study of ovarian reserve and spontaneous fertility comparing *BRCA1/2* pathogenic variant carriers to controls (women who attended consultations to discuss fertility preservation before gonadotoxic treatment). Measures of associations between plasma AMH concentration, AFC and *BRCA1/2* status were modelled by nonlinear generalized additive regression models and logistic regressions adjusted for age at plasma storage, oral contraceptive use, body mass index, cigarette smoking, and the AMH assay technique.
Results: The whole population comprised 119 *BRCA*1/2 pathogenic variant carriers and 92 controls. A total of 110 women (42 carriers, among whom 30 were cancer-free, and 68 controls) underwent an ovarian reserve evaluation. Spontaneous fertility analysis included all women who previously attempted to become pregnant (134 women).
We observed a tendency towards a premature decrease in ovarian reserve in *BRCA1/2* pathogenic variant carriers, but no difference in mean AMH or AFC levels was found between *BRCA1/2* pathogenic variant carriers and controls. An analysis of the extreme levels of AMH ($\leq$5 pmol/l) and AFC ($\leq$7 follicles) by logistic regression suggested a higher risk of low ovarian reserve among *BRCA1/2* pathogenic variant carriers (adjusted odds ratio (OR) = 3.57, 95% CI = 1.00–12.8, p = 0.05; and adjusted OR = 4.99, 95% CI = 1.10–22.62, p = 0.04, respectively).
Discussion: Attention should be paid to *BRCA1/2* pathogenic variant carriers' ovarian reserve, considering this potential risk of premature alteration.

1. Introduction

Patients with germline *BRCA1* or *BRCA2* (Breast Cancer Gene 1 and 2) pathogenic variants are at high risk of developing breast and/or ovarian carcinoma [1]. The prevalence of *BRCA1* or *BRCA2* pathogenic variants is estimated to be 0.102% (CI = 0.042%–0.250%)

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https://doi.org/10.1016/j.breast.2021.07.010

Abbreviations

AFC	antral follicle count
AMH	anti-Müllerian hormone
ART	assisted reproductive technology
BMI	Body mass index
BRCA1/2	Breast Cancer Gene 1/2
ECLIA	automated electrochemiluminescence immunoassays
FMR1	fragile X mental retardation 1
GAMs	Generalized additive regression models
pvBRCA	BRCA germline pathogenic variant
RCS	restricted cubic spline

or 1/980 in the general population, with autosomal dominant inheritance [2,3]. These genes are involved in double-stranded DNA damage repair by homologous recombination.

Fertility and pregnancy planning in *BRCA1* and *BCRA2* pathogenic variants carriers *(pvBRCA1/2)* can be impacted by the occurrence of cancer and its treatment. Their window of fertility is also shortened as a prophylactic bilateral oophorectomy is recommended after the age of 40. Additionally, it has been suggested that the mutation in itself could be linked to a premature diminution of ovarian reserve [4–7].

The Anti-Mullerian hormone (AMH) level decreases is a reliable marker of ovarian reserve and ageing [8], and is also considered by certain authors to be predictive of the age of menopause [8]. Currently, the AMH level is considered to be the best predictive marker of the ovarian response to stimulation in assisted reproductive technology (ART) [9].

The antral follicle count (AFC) is obtained by measuring and counting the antral follicles between 2 and 9 mm in size on pelvic ultrasound. AFC indirectly reflects the ovarian reserve. Similar to the AMH level, the AFC decreases with age and is considered to be a predictive marker of ovarian stimulation in ART [10].

The objectives of our study were to describe ovarian reserve (evaluated with the AMH level and the AFC) and the clinical data linked to fertility in a cohort of *pvBRCA1/2* females and to compare the findings to the same parameters in a control population of noncarriers.

2. Material et methods

We conducted a retrospective monocentric observational study at the Cancer Center Léon Bérard, Lyon, France.

2.1. Population

All women carrying a pathogenic *BRCA1/2* variant who attended a consultation at the gynaecology clinic in Leon Berard Cancer Center between January 2013 and November 2016 constituted the study population. Women of childbearing age, newly diagnosed with any type of cancer, and attending a consultation to discuss fertility preservation before gonadotoxic treatment were eligible for inclusion as controls. Breast cancer patients having a family history, and/or younger than 36, and/or triple negative breast cancers had been addressed for *BRCA1/2* mutations testing. As a result, breast cancer patients, without any pathogenic variant at genetic analysis, or breast cancer patients older than 36 at diagnosis, who had neither family history, nor triple negative breast cancer, were classified as controls. All patients were systematically interviewed and examined by the same gynaecologist practitioner.

The whole population comprised 119 *pvBRCA1/2* women and 92 controls (Fig. 1).

Given the limited number of women in this study, statistical analyses did not distinguish between *BRCA1* and *BRCA2* carriers. Similarly, non-carriers and low-risk control women were considered in a single category “Not mutated/analysis not done”. To analyse ovarian reserve markers (AMH and AFC), we excluded menopausal women and patients with a history of unilateral or bilateral oophorectomy, chemotherapy or pelvic radiation, or any condition associated with premature ovarian failure. All the women who previously attempted to become pregnant were included in the spontaneous fertility analysis.

2.2. Data collection

All clinical and paraclinical data were collected from the clinical records and recorded in a secure database. Spontaneous fertility was evaluated with clinical data that were systematically collected during the patient interview: pregnancy attempts, parity, time to conception for each pregnancy, risk factors for fertility disorders (endometriosis, previous ovarian surgery, unilateral oophorectomy, polycystic ovary syndrome, ovulation disorders), history of infertility (failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse and/or the use of ART to achieve pregnancy), cycle regularity, and age at menarche.

2.3. AMH analysis

The main outcome measure of the study was the AMH level. The serum AMH level was measured the day of the gynaecological consultation or in the following days. Women underwent a blood test in Léon Bérard Cancer Center. Blood samples were sent to the hormonology laboratory of the Biology and Est Pathology Center (Hospices Civils de Lyon). Serum AMH concentrations were measured beginning in June 2015 by automated electrochemiluminescence immunoassays (Roche Diagnostics®, Mannheim, Germany: limit of quantification 0.21 pmol/l, coefficient of variation 1.8% for repeatability and 4.4% for intermediate precision). Serum AMH tests performed before June 2015 in this laboratory and tests performed in external laboratories (for a minority of patients) were measured by AMH Gen II assay®, an enzymatically amplified two-site immunoassay (Beckman Coulter®, France) [11]. To make the values comparable, a correction factor routinely used by biologists was applied for the AMH values ssayed from the latter Y-type technique $= 0.797X + 0.847$ (Y being the corrected AMH value, and X being the measured value according to the Gen II assay® technique). The values are expressed in pmol/l. We defined AMH values $\leq$ 5 pmol/l (0.681 ng/ml) as very low serum AMH values (threshold considered low-normal for the ECLIA Roche technique) (Data sheet AMH dosage Roche), which is a threshold usually used in the literature [13].

2.4. AFC analysis

Secondary outcome measures were the antral follicle count (AFC) and clinical measure of spontaneous fertility. AFC was defined as the total number of follicular structures of 2–9 mm in diameter in both ovaries [14]. This count was performed during transvaginal sonography. This ultrasound (Philips HD11XE®) was

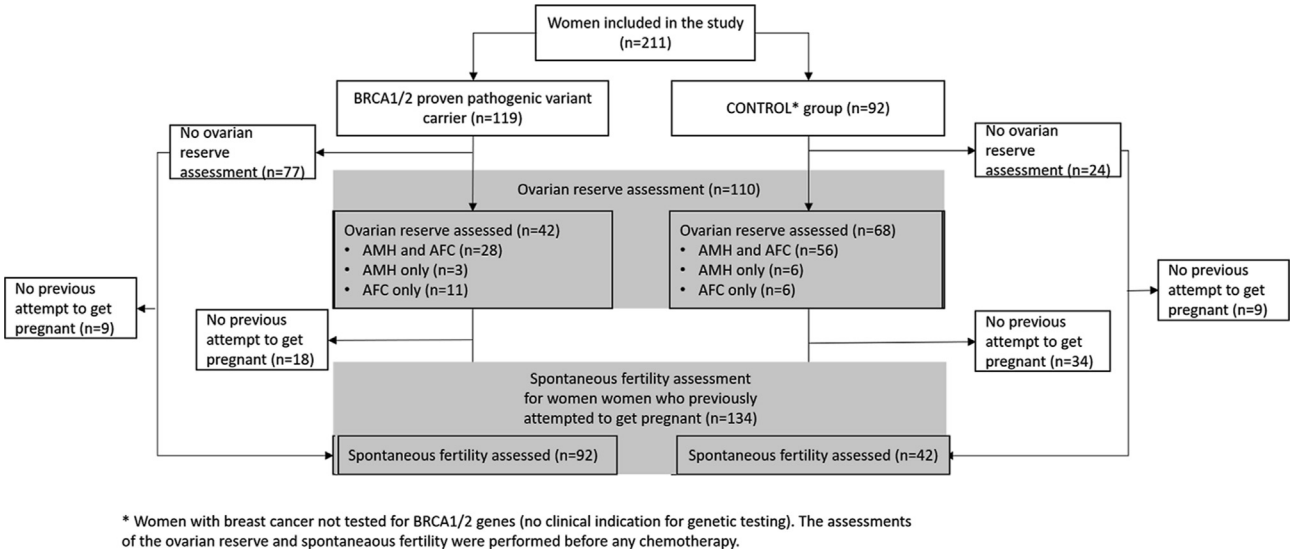

Fig. 1. Study flow diagram.

performed for most patients by the same gynaecologist. A few patients underwent AFC with an external radiologist. To take into account the interobserver variability, the location of ultrasound realization was specified during data acquisition. Low AFC was defined as an AFC $\leq$7, which is a threshold usually associated in the literature with lower pregnancy rates in patients undergoing ART [13].

2.5. Statistical analysis

Generalized additive regression models (GAMs) [15] were used to model both the AMH levels and the AFC after log-transformation. The nonlinear effects of age on AMH and AFC were fitted using restricted cubic spline (RCS) functions with 3 degrees of freedom. The RCS functions were chosen because they represent a good compromise between model robustness and flexibility [16].

Factors described in the literature as influencing AMH levels were retained for multivariable regression modelling. The following variables were retained:

- Age modelled by RCS to fit a nonlinear effect [17].
- Body mass index (BMI) [18].
- Smoking status (never/former/current) and past tobacco consumption (pack_year) [19].
- Current or recent (stopped within the last 6 weeks) oral contraceptive use at the time of ovarian reserve assessment [20].
- The AMH assay technique: automated electrochemiluminescence immunoassays

(ECLIA Roche®) or enzymatically amplified two-site immunoassay (Gen II Assay Beckman Coulter®) [21].

AMH values obtained in our study were compared with those found in a population of fertile women and published by Tehrani et al. [22], where the serum AMH concentrations were measured by an AMH Gen II assay. The same correction factor as described above was used to make the measures with the ECLIA automated assays in our study comparable.

Using logistic regression, we studied the link between mutation status, AMH value $\leq$ 5 pmol/l and AFC $\leq$7. AFC data from our study were compared with AFC data collected from the general population in the la Marca et al. study [23]. For the 134 women who attempted to become pregnant, clinical measures of fertility were compared according to the mutation status with Fisher's exact test for categorical variables and Student's test for continuous variables. All statistical analyses were performed with R [24] and the rms package.

2.6. Ethical approval

The study was approved by the local ethics committee (declaration number Commission Nationale Informatique et Libertés CNIL n°2056206).

3. Results

3.1. Participants

A total of 211 women were included in our study: 64 (30%) *pvBRCA1* carriers, 55 (26%) *pvBRCA*2 carriers, 29 (14%) proven noncarriers, and 63 (30%) individuals with unknown *BRCA* status (genetic testing not done). A total of 110 patients underwent an ovarian reserve assessment, including serum AMH levels and/or an AFC (Fig. 1). The remaining 101 participants were excluded from ovarian reserve assessment (menopausal or history of oophorectomy, chemotherapy or pelvic radiation, or any condition associated with premature ovarian failure).

Out of 110 females of childbearing age who underwent a gynaecology consultation and for whom an AMH test was prescribed, only 17 did not go through with it.

Among the 110 women with ovarian reserve assessment data, 42 were *pvBRCA1/2* carriers (30 cancer-free women, and 12 breast-cancer patients at the time of the ovarian reserve evaluation), and 68 women constituted the control group (Table 1). Among the control group, all the 68 women had cancer at the time of the ovarian reserve evaluation (39 with breast cancer, and 29 other cancers, mainly lymphoma and sarcoma). Among the 39 control women with breast cancer, 26 were proven non-carriers, and 13 women were not tested. The mean age of women in this population was 31.9 years (range 16–46 years). There were no statistically significant differences between these 2 groups in terms of mean age, BMI, smoking, age at menarche, menstrual cycles, contraception, pelvic ultrasound location for AFC, and serum AMH test

Table 1
Clinical characteristics and ovarian reserve assessments of the 110 women according to *BRCA* status.

	N	*pvBRCA1/2* carriers (N = 42)	CONTROL group (N = 68)	ALL (N = 110)	p-value
Age (years)	110				0.089 (a)
Mean ± SD		33.18 ± 6.06	31.16 ± 5.96	31.93 ± 6.05	
[Min.–Max.]		[18–46]	[16–41]	[16–46]	
AMH level (pmol/l)	93				0.43 (b)
Median (mean)		11.7 (15.52)	13.92 (17.82)	12.8 (17.05)	
[Min.–Max.]		[0.3–43.81]	[0.3–95.3]	[0.3–95.3]	
AMH assay technique	93				0.11 (c)
Gen II Assay Beckman Coulter		7 (23%)	25 (40%)	32 (34%)	
ECLIA Roche		24 (77%)	37 (60%)	61 (66%)	
AFC (number)	101				0.40 (b)
Median (mean)		15 (19.08)	16 (21.05)	16 (20.29)	
[Min.–Max.]		[2–57]	[4–62]	[2–62]	
Location of AFC assessment	101				0.17 (c)
Léon Bérard Center		32 (82%)	43 (69%)	75 (74%)	
Other location		7 (18%)	19 (31%)	26 (26%)	
Body mass index (kg/m^2)	109				0.19 (c)
16.5–18.5		5 (12%)	6 (9%)	11 (10%)	
18.6–25		25 (61%)	49 (72%)	74 (68%)	
25.1–30		6 (15%)	11 (16%)	17 (16%)	
30.1–35		2 (5%)	2 (3%)	4 (4%)	
35.1–40		3 (7%)	0 (0%)	3 (3%)	
Smoking status	107				0.76 (c)
Never smoker		24 (62%)	44 (65%)	68 (64%)	
Former smoker		6 (15%)	7 (10%)	13 (12%)	
Current smoker		9 (23%)	17 (25%)	26 (24%)	
Former tobacco consumption (pack-year)	12				0.74 (b)
Median (mean)		5 (5.2)	6 (6.14)	5 (5.75)	
[Min.–Max.]		[3–10]	[2–10]	[2–10]	
Current tobacco consumption (pack-year)	24				0.35 (b)
Median (mean)		3 (4.56)	5 (5.93)	4.5 (5.42)	
[Min.–Max.]		[1–10]	[2–12]	[1–12]	
Overall tobacco consumption (pack-year)	110				0.91 (b)
Median (mean)		0 (1.6)	0 (1.94)	0 (1.81)	
[Min.–Max.]		[0–10]	[0–12]	[0–12]	
Age at menarche	109				0.87 (c)
Physiological (10–15 years)		37 (88%)	61 (91%)	98 (90%)	
Early menarche (<10 years)		1 (2%)	1 (1%)	2 (2%)	
Late menarche (>15 years)		4 (10%)	5 (7%)	9 (8%)	
Menstrual cycle duration	106				0.40 (c)
Regular		29 (74%)	56 (84%)	85 (80%)	
Long regular		6 (15%)	4 (6%)	10 (9%)	
Short regular		1 (3%)	3 (4%)	4 (4%)	
Amenorrhoea or spaniomenorrhea		3 (8%)	4 (6%)	7 (7%)	
Contraception	110				0.47 (c)
Oral contraceptive used or stopped <6 weeks prior		7 (17%)	16 (24%)	23 (21%)	
Other(*)		35 (83%)	52 (76%)	87 (79%)	

N is the number of non-missing values.
(a) Student's test assuming equal variances.
(b) Wilcoxon Mann-Whitney test.
(c) Fisher's exact test.
(*) Progestogen-only pills, subdermal contraceptive implants, levonorgestrel-releasing intrauterine system.

technique.

3.2. AMH

Overall, the mean AMH level was not significantly different between *pvBRCA1/2* carriers and control women (15.52 vs 17.82; p = 0.43). A deeper statistical analysis with GAM regression models taking into account the nonlinear effect of age on ovarian reserve is shown in Fig. 2 and Table 2 *pvBRCA1/2* women seemed to have a stronger drop in the AMH level after 30 years than women from the control group, despite large confidence intervals reflecting a small sample (Fig. 2). In a GAM model with age adjustment but unadjusted for known confounding factors, women with *pvBRCA1/2* were estimated to have a mean AMH level 1.33 pmol/l lower than that of the control group (95% CI = −2.05-1.16, p = 0.20) (Table 2). After adjustment for known confounding factors, the mean difference in AMH levels between the 2 groups remained similar (−1.30, 95% CI = −2.08-1.23, p = 0.5861). No confounding factor was found to have a sufficient impact on AMH levels to reach statistical significance (all p-values>0.05).

Analysis of extremely low AMH levels using logistic regression models (Table 3) showed that the risk of having an AMH level ≤ 5 pmol/l was 3.25-times higher in *pvBRCA1/2* carriers than in controls (odds ratio (OR) = 3.25, 95% CI = 1.04-10.14, p = 0.04). When adjusting for known confounding factors, the adjusted OR remained similar to the non-adjusted OR (adjusted OR = 3.57, 95% CI = 1.00-12.8, p = 0.05). Notably, 12 out of the 16 patients in our study with an AMH level below the 5th percentile had already been pregnant.

3.3. AFC

Overall, the mean AFC was not significantly different between *pvBRCA1/2* carriers and control women (19.08 vs 21.05, p = 0.4,

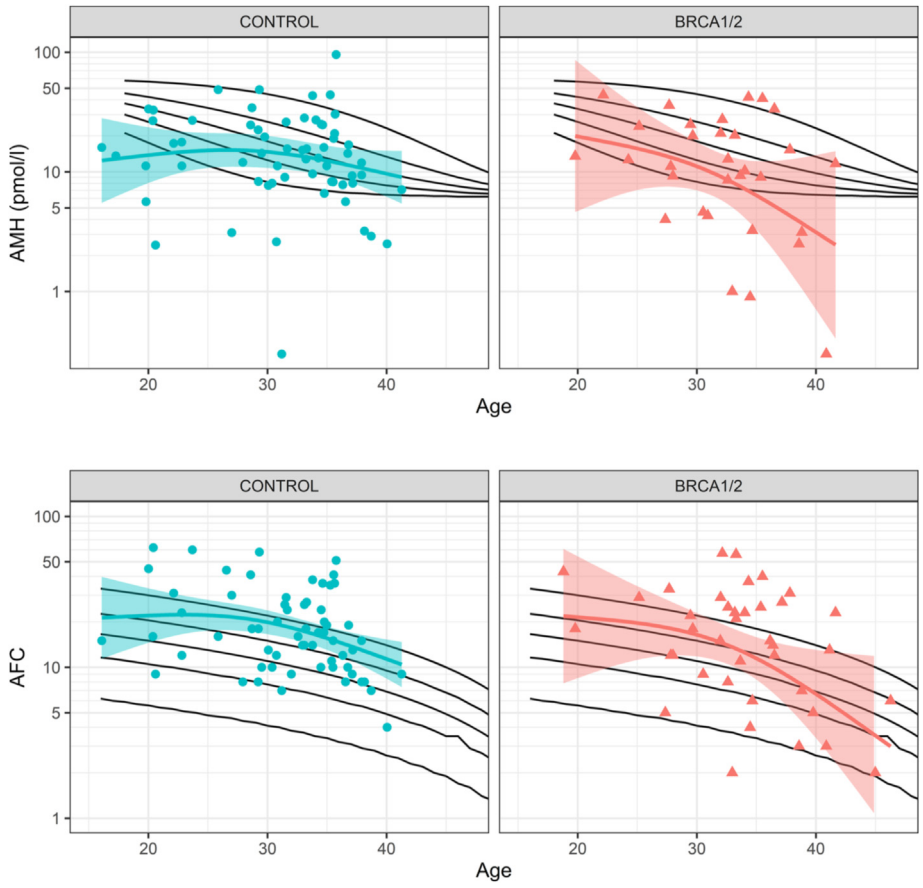

Fig. 2. AMH level (pmol/l) and antral follicle count (AFC) in *BRCA*1/2 pathogenic variant-positive women and in the control group. Note that ordinate axes were log-scaled for better data visualization. The mean AMH and AFC levels by age estimated from nonlinear regression models using restricted cubic spline with 3 degree of freedom are represented with their 95% CIs. The superimposed black curves represent the general population percentiles (5%, 25%, 50%, 75%, and 95%) estimated from the Tehrani et al. study for the AMH levels [22] and from the La Marca et al. study for the AFC assessment [23].

Table 2
GAM regression modelling of AMH and AFC levels.

Outcome: log(AMH pmol/l)	**exp(β) (95% CI)**	**p-value**
GAM model unadjusted for known confounding factors		
pvBRCA1/2 status (positive vs control)	−1.33 (−2.05-1.16)	0.20
Age	Non-linear effect[a]	0.026
GAM model adjusted for known confounding factors		
pvBRCA1/2 status (positive vs control)	−1.30 (−2.08-1.23)	0.28
Age	Non-linear effect[a]	0.11
Abnormal BMI	1.10 (−1.46-1.77)	0.70
Never smokers	1.13 (−1.89-2.40)	0.76
Cigarette smoking (pack-year)	1.02 (−1.11-1.14)	0.78
Oral contraceptive use or cessation < 6 weeks prior	1.54 (−1.12-2.66)	0.13
AMH assay technique (ECLIA Roche vs Gen II Beckman Coulter)	−1.08 (−1.70-1.47)	0.75
Outcome: log(AFC number)	**exp(β) (95% CI)**	**p-value**
GAM model unadjusted for known confounding factors		
pvBRCA1/2 status (positive vs control)	−1.10 (−1.46-1.20)	0.49
Age	Non-linear effect[a]	<0.001
GAM model adjusted for known confounding factors		
pvBRCA1/2 status (positive vs control)	−1.12 (−1.49-1.18)	0.42
Age	Non-linear effect[a]	<0.001
Abnormal BMI	−1.10 (−1.48-1.22)	0.51
Never smokers	1.13 (−1.46-1.85)	0.64
Cigarette smoking (pack-year)	1.04 (−1.04-1.12)	0.34
Oral contraceptive use or cessation < 6 weeks prior	1.13 (−1.26-)	0.50
Location of AFC assessment (Léon Bérard Center vs other location)	1.45 (1.06-1.98)	0.021

CI: confidence interval.
[a] Modelled with a restricted cubic spline with 3 degree of freedom.

Table 3
Logistic regression modelling of low levels of AMH and AFC.

Outcome: logit(AMH ≤ 5 pmol/l)	OR (95% CI)	p-value
Logistic model unadjusted for known confounding factors		
pvBRCA1/2 status (positive vs control)	3.25 (1.04-10.14)	0.04
Age	Non-linear effect[a]	0.19
Logistic model adjusted for known confounding factors		
pvBRCA1/2 status (positive vs control)	3.57 (1.00-12.8)	0.05
Age	Non-linear effect[a]	0.25
Abnormal BMI	0.33 (0.07-1.58)	0.17
Never smokers	0.76 (0.09-6.76)	0.81
Cigarette smoking (pack-year)	0.93 (0.64-1.33)	0.69
Oral contraceptive use or cessation < 6 weeks prior	0.32 (0.04-2.87)	0.31
AMH assay technique (ECLIA Roche vs Gen II Beckman Coulter)	2.15 (0.5-9.29)	0.31
Outcome: logit(AFC ≤ 7 follicles)	**OR (95% CI)**	**p-value**
Logistic model unadjusted for known confounding factors		
pvBRCA1/2 status (positive vs control)	4.88 (1.13-21.05)	0.03
Age	Non-linear effect[a]	0.008
Logistic model adjusted for known confounding factors		
pvBRCA1/2 status (positive vs control)	4.99 (1.10-22.62)	0.04
Age	Non-linear effect[a]	0.02
Abnormal BMI	0.67 (0.12-3.67)	0.64
Never smokers	0.08 (0-4.82)	0.23
Cigarette smoking (pack-year)	0.42 (0.11-1.63)	0.21
Oral contraceptive use or cessation < 6 weeks prior	*Variable removed*[b]	
Location of AFC assessment (Léon Bérard Center vs other location)	1.40 (0.23-8.44)	0.72

OR: odds ratio; CI: confidence interval; logit(p) = ln(p/1-p).
[a] Modelled with a restricted cubic spline with 3 degree of freedom.
[b] This variable was removed due to non-finite parameter estimates caused by small numbers.

Table 1).

Analysis with GAM regression models taking into account the nonlinear effect of age on ovarian reserve is shown in Fig. 2 and Table 2. In a GAM model with age adjustment but unadjusted for known confounding factors, women with *pvBRCA*1/2 were estimated to have a mean AFC 1.10 lower than that of the controls (95% CI: −1.46-1.20, p = 0.49) (Table 2). After adjustment for known confounding factors, the mean difference in AMH levels between the 2 groups remained similar (−1.12, 95% CI = −1.49-1.18, p = 0.42).

Analysis of low AFC (≤7 follicles) with logistic regression models showed that the risk of having an AFC ≤7 follicles was 4.88-times higher in *pvBRCA1/2* carriers than in controls (odds ratio (OR) = 4.88; 95% CI = 1.13-21.05, p = 0.03) (Table 3). After adjusting for known confounding factors, the adjusted OR remained similar to the non-adjusted OR (adjusted OR = 4.99; 95% CI: = 1.10-22.62, p = 0.05).

Patients with a pelvic ultrasound performed outside the Léon Bérard Center had a 1.45 mean follicle number lower AFC (Table 2, p = 0.021). Nevertheless, the location of AFC assessment was not associated with the probability of having a low AFC (≤7 follicles) (Table 3, p = 0.72).

3.4. Fertility

Among the whole population, 134 women previously tried to become pregnant before any chemotherapy (92 *pvBRCA* carriers and 42 controls). Among them, 26 (20%) reported fertility problems, 103 did not, and data were missing for 5 women. We did not find any statistical association between *pvBRCA* status and self-reported fertility problems (p-value = 0.81, Table 4). The mean ages at the first and second births and time to conception were comparable between the 2 groups.

4. Discussion

Our results suggest a tendency towards a premature decrease in the ovarian reserve, with a mean AMH level of −1.3 pmol/l and a 3.5-times higher risk of low AMH (≤5 pmol/l) in *pvBRCA1/2* carriers.

The AMH values found in our study were broadly comparable to those found by Tehrani et al. [22]. However, 26 values in our study were below the 5th percentile curve in the Tehrani study, which corresponds to 28% of the values (95% CI = 19%-38%). This result is significantly different from the expected percentage of values below the 5th percentile (p-value<0.001, exact binomial test). Our analyses showed that *pvBRCA1/2* carriers also had a 5-times higher risk of an AFC≤7.

AMH levels among *pvBRCA* carriers have been evaluated in the literature, with contradictory results. Some studies have found low AMH levels in only *pvBRCA1* carriers, especially after 35 years of age, and normal AMH levels among *pvBRCA2* carriers [4–7]. A recent meta-analysis confirmed these findings, with lower serum AMH levels in women with *pvBRCA1* (33% lower); but not with *pvBRCA2*, than in controls [25].

Other studies have shown opposite findings, such as a poor ovarian reserve in *pvBRCA2* carriers only or no difference between *pvBRCA*1 and *pvBRCA*2 carriers [26]. Some studies have failed to identify a significant difference in the AMH levels between *BRCA*1/2 carriers and the general population [27]. Recently, Grynberg et al. found similar mean AMH levels and AFCs between *pvBRCA1/2* carriers with breast cancer and non-carriers with breast cancer [28].

The link between a poor ovarian reserve and a low ovarian response to stimulation is not clear. Some authors have found a trend towards a decreased response to ovarian stimulation, especially in *pvBRCA1* carriers, either in the context of fertility preservation [30–32] or in the context of preimplantation genetic diagnosis [33]. Nevertheless, other authors have found no difference [28,34] or even a better response to ovarian stimulation in *pvBRCA1* carriers [29]. In this last study, cancer-free *pvBRCA* carriers were compared to women undergoing elective egg freezing. The latter group could have personal reasons for choosing this option that could be a cause of bias [29].

The discordant findings may stem from imprecise exclusion criteria, population bias, different indications for treatment, and small study cohort sizes. Differences in the choice of control groups

Table 4
Fertility and pregnancies among the 134 women who attempted to become pregnant.

	N	*pvBRCA1/2* Carriers (N = 92)	CONTROL group (N = 42)	ALL (N = 134)	p-value
History of infertility	129				0.81 (a)
No		71 (81%)	32 (78%)	103 (80%)	
Yes		17 (19%)	9 (22%)	26 (20%)	
Risk factors for fertility disorders	129				0.65 (a)
Yes		18 (20%)	10 (24%)	28 (22%)	
No		70 (80%)	31 (76%)	101 (78%)	
Regular menses	125				0.03 (a)
Regular		70 (83%)	35 (85%)	105 (84%)	
Short regular		1 (1%)	4 (10%)	5 (4%)	
Long regular		7 (8%)	0 (0%)	7 (6%)	
Amenorrhoea or spaniomenorrhea		6 (7%)	2 (5%)	8 (6%)	
Age at menarche	127				0.66 (a)
Physiological (10–15 years)		76 (88%)	39 (95%)	115 (91%)	
Early menarche(<10 years)		1 (1%)	0 (0%)	1 (1%)	
Late menarche (>15 years)		9 (10%)	2 (5%)	11 (9%)	
Age at first birth	114				0.57 (b)
Mean ± SD		27.4 ± 4.55	27.8 ± 3.03	27.5 ± 4.16	
[Min.–Max.]		[18–38]	[22–35]	[18–38]	
Time to conception for first birth	103				0.3 (a)
0–6 month		58 (81%)	23 (74%)	81 (79%)	
6 month–1 year		8 (11%)	7 (23%)	15 (15%)	
more than 1 year		6 (8%)	1 (3%)	7 (7%)	
Age at second birth	74				0.71 (c)
Mean ± SD		29.4 ± 4.43	29.9 ± 3.72	29.5 ± 4.28	
[Min.–Max.]		[20–39]	[22–35]	[20–39]	
Time to conception for second birth	67				0.73 (a)
0–6 month		43 (80%)	10 (77%)	53 (79%)	
6 month–1 year		4 (7%)	2 (15%)	6 (9%)	
more than 1 year		7 (13%)	1 (8%)	8 (12%)	

N is the number of non-missing values.
(a) Fisher's exact test.
(b) Student's test assuming unequal variances.
(c) Student's test assuming equal variances.

can also explain these results.

It seems that *pvBRCA*1/2 carriers are at higher risk of premature menopause than the general population [35–37]. However, this idea is contradicted by other studies [38,39]. Altogether, our data on spontaneous fertility are reassuring and are in accordance with the existing literature [38,40–42].

One explanation for a diminished ovarian reserve is a premature depletion of the primordial follicle stock as a result of a defect in double-stranded DNA repair [4,43]. Several susceptibility genes for premature ovarian insufficiency are linked to this particular DNA repair pathway [44]. Moreover, the *BRCA*1 and 2 genes play a role in maintaining the telomere length [45]. Finally, another explanation could be the presence of particular FMR1 genotypes in *pvBRCA1/2* carriers in comparison to noncarriers [46].

Some limitations of our study should be underlined. Our control population consisted of patients whose ovarian reserve evaluation may have been negatively impacted by cancer [47–50], while the study population comprised both affected and unaffected women. In addition, the control population included patients assume with good confidence, that the vast majority, if not all, of the women in the control group were noncarriers, as the 13 non-tested women had a low probability of genetic predisposition (older than 36 years at diagnosis, no family history, non-triple-negative type). Of note, these two limitations may have impacted our results by minimising the differences between the 2 groups, and thereby reducing the likelihood of false-positive conclusions. The small sample size may have reduced the power of our study and increased the margin of error. Given the limited number of women in our study, statistical analyses did not distinguish between *BRCA1* and *BRCA2* carriers. The retrospective nature of our study might have been responsible for recall bias in the spontaneous fertility analysis. We did not take into consideration the day of the menstrual cycle. However, AFC and AMH are considered stable throughout the menstrual cycle [23,51]. The main disadvantage of the AFC measure is the associated interobserver variability [52], which was partly avoided as ultrasound was performed by the same gynaecologist and on the same ultrasound machine in most patients, and this was included as a confounding factor in the multivariable regression models. Finally, the choice of the cut-offs for AMH and the AFC corresponded to values used by other authors [13], but not universally recognised.

In France, fertility preservation should be offered to “Every person (…) whose fertility is at risk of a premature alteration” [53]. The ovarian reserve evaluation criteria that we have previously described can indicate a possible premature alteration of fertility. In addition, considering the risk of breast cancer before the age of 40 [1] and the recommendation for prophylactic oophorectomy after 40 years of age, the question of systematic preservation can be discussed [28,54,55]. Although data are limited, the risk of breast or ovarian cancers may not be impacted by ovarian stimulation in *pvBRCA*1/2 carriers [56,57]. In addition, recent data have shown that pregnancy after breast cancer in patients with germline *BRCA* pathogenic variants is safe [58].

Although a decrease in the ovarian reserve is suspected, the clinical data on fertility are reassuring. Preservation of fertility techniques can be debated for these patients, who are already facing intense medical follow-up, prophylactic surgeries and a heavy psychological burden. Addressing the subject of a decrease in ovarian function with the patients may be a stressful factor in and of itself. Chan et al. [59] reported that 40% of *pvBRCA* carriers were ready to have their oocytes or embryos frozen. This study shows the need for information and education regarding reproduction for *pvBRCA1/2* carriers. It seems legitimate to explain to the patients that they should not delay their family planning, while reassuring them and avoiding making them feel guilty [60,61].

5. Conclusion

Attention should be paid to *pvBRCA*1/2 carriers' ovarian reserve, considering this potential risk of premature alteration. The question of systematic preservation of fertility is still debated today, but this study highlights the need for information and reproductive education.

Authors' roles

L.D.L: study design, data acquisition, manuscript drafting; Y.D.:data analysis, tables and figures, manuscript drafting; I.P.: AMH analysis, data acquisition, manuscript revision; N.C.: data acquisition, manuscript revision; V.B.: data acquisition, manuscript revision; J.L.: Interpretation of data, manuscript revision; B.S.: Interpretation of data and manuscript revision; C.L.: study design, data analysis; C.R.J: study design and supervision, data acquisition, manuscript drafting.

Declaration of competing interest

Authors have nothing to declare in relation with the study.

Acknowledgements

Elodie Pleynet, Center Léon Bérard, who elaborated the database.

Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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