



**HAL**  
open science

## Evolution of serum Anti-Müllerian Hormone (AMH) level in young women treated with chemotherapy for breast cancer according to basal AMH level

S. Loubersac, A. Dezellus, T. Lefebvre, A. Reignier, P Barriere, D. Masson, T. Freour

### ► To cite this version:

S. Loubersac, A. Dezellus, T. Lefebvre, A. Reignier, P Barriere, et al.. Evolution of serum Anti-Müllerian Hormone (AMH) level in young women treated with chemotherapy for breast cancer according to basal AMH level. *European Journal of Obstetrics & Gynecology and Reproductive Biology*, 2020, 254, pp.132 - 137. 10.1016/j.ejogrb.2020.09.016 . hal-03491568

**HAL Id: hal-03491568**

**<https://hal.science/hal-03491568>**

Submitted on 22 Sep 2022

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

1 **Running head:** AMH level during breast cancer treatment

2 **Title:** Evolution of serum Anti-Müllerian Hormone (AMH) level in young women  
3 treated with chemotherapy for breast cancer according to basal AMH level

4 **Authors**

5 *S Loubersac*<sup>1</sup>, *A Dezellus*<sup>1,2</sup>, , *T Lefebvre*<sup>1,3</sup>, *A Reignier*<sup>1,3,4</sup>, *P Barriere*<sup>1,3,4</sup>,  
6 *RESOVA Investigators Group*<sup>a</sup>, *D Masson*<sup>3,5,7</sup>, *T Freour*<sup>1,3,4,\*</sup>

7 <sup>1</sup>Service de médecine et biologie du développement et de la reproduction, CHU de  
8 Nantes, Bd Jean Monnet, 44000, Nantes, France

9 <sup>2</sup>Service d'oncologie, Institut de Cancérologie de l'Ouest, Bd Jacques Monod, 44805,  
10 Saint Herblain, France

11 <sup>3</sup>Faculté de médecine, Université de Nantes, rue Gaston Veil, 44035, Nantes, France

12 <sup>4</sup> Inserm, Université de Nantes, Centre de Recherche en Transplantation et  
13 Immunologie (ou CRTI), Nantes, France

14 <sup>5</sup>Laboratoire de biochimie, CHU de Nantes, place Alexis-Ricordeau, 44000, Nantes,  
15 France

16 <sup>7</sup>INSERM UMR 913, rue Gaston Veil, 44035, Nantes, France

17 *\*Corresponding author : Pr Thomas FREOUR, Service de médecine et biologie*  
18 *de la reproduction, Hôpital mère et Enfant, CHU de Nantes, 38 boulevard Jean*  
19 *Monnet, 44093 Nantes cedex 1 – France. Tel: +33 240083234 - Fax: +33*  
20 *240083228 - E-mail: thomas.freour@chu-nantes.fr*

21 <sup>a</sup>Group members are listed in the Appendix

22 **Funding**

23 This study was supported by a grant from the French Ministry of Health (PHRC  
24 2009, reference PHRC 2009 20-17)

25

## 26 **Abstract**

27 **Objective:** Serum AMH level has been shown to decrease in women treated for  
28 breast cancer in several studies. However, whether basal AMH status affects AMH  
29 dynamics during chemotherapy remains to be clarified. The objective of this study  
30 was to compare serum AMH dynamics in young women with either low, normal or  
31 high basal serum AMH level at diagnosis, during and after treatment with  
32 chemotherapy for breast cancer.

33 **Study Design:** In this secondary analysis of a prospective cohort study, serum AMH  
34 was measured during and after chemotherapy in 239 women of reproductive age  
35 diagnosed with breast cancer and treated with chemotherapy. The association  
36 between AMH dynamics throughout chemotherapy and during follow-up and basal  
37 AMH status, i.e. low AMH ( $<1\mu\text{g/l}$ ,  $<7\text{pmol/l}$ ), normal AMH ( $1\text{-}4.9\mu\text{g/l}$ ,  $7\text{-}36\text{pmol/l}$ )  
38 and high AMH ( $\geq 5\mu\text{g/l}$ ,  $>36\text{pmol/l}$ ), was evaluated. Menses occurrence was also  
39 recorded.

40 **Results:** A total of 21 women had low, 154 had normal and 64 had high basal AMH  
41 level. Serum AMH rapidly decreased during chemotherapy in all groups, and its  
42 variation during chemotherapy and follow-up was not significantly different between  
43 the 3 groups.

44 **Conclusion:** No association was found between AMH variation during chemotherapy  
45 and follow-up, and basal AMH level at diagnosis. However, women with high basal

46 AMH levels have significantly higher AMH levels throughout chemotherapy and  
47 follow-up than women with normal or low basal AMH levels at diagnosis.

48

49 ***Trial registration number:*** ClinicalTrial.gov identifier NCT01114464

50 ***Keywords:*** Breast cancer; fertility preservation; anti-Müllerian hormone; ovarian  
51 reserve

52 ***Word count:*** 2 306

53 **1. Introduction**

54 Breast cancer is the most common malignancy in women of reproductive age (1).  
55 The large majority of them are treated with adjuvant or neoadjuvant chemotherapy,  
56 based on gonadotoxic drugs such as anthracyclines and cyclophosphamide, and  
57 taxanes, potentially leading to impaired ovarian function, amenorrhea and infertility.  
58 We (2), and others (3),(4) , previously showed that serum Anti-Müllerian Hormone  
59 (AMH), also known as Müllerian-Inhibiting Substance (MIS), the most relevant  
60 hormonal marker of ovarian reserve, rapidly fell to low or even undetectable levels in  
61 young women with breast cancer treated with chemotherapy with a high prevalence  
62 of chemotherapy-related amenorrhea (2),(4), (5).

63 The modulation of AMH expression has recently been proposed as a  
64 promising therapeutic way to control ovarian physiology with clinical applications for  
65 ovulation induction, contraception or fertility preservation (6). Indeed, it was recently  
66 reported that AMH administration could protect ovarian reserve during chemotherapy  
67 in mice (7). Therefore, it can be postulated that basal AMH levels could account for  
68 intra-individual variability in terms of ovarian sensitivity to gonadotoxic treatment.  
69 However, and to the best of our knowledge no study on the potential association  
70 between AMH evolution during chemotherapy and post-chemotherapy follow-up and  
71 basal AMH level has been reported up to now.

72 The main objective of our study was to compare serum AMH dynamics in young  
73 women with either low, normal or high basal serum AMH level, during and until 24  
74 months after treatment with chemotherapy for breast cancer.

75

## 76        **2. Materials and Methods**

### 77        2.1 Patients

78        This is a subgroup analysis of our previously reported work conducted on the  
79        RESOVA cohort (2). Briefly, this observational and prospective study was conducted  
80        in 250 eligible patients, aged 18-39, diagnosed with breast cancer and about to be  
81        treated with chemotherapy in adjuvant/neoadjuvant setting. Patients were included at  
82        diagnosis in 11 French cancer centers, and followed up until 24 months after  
83        chemotherapy completion. A medical interview and blood sampling were performed  
84        for each patient at each visit, from diagnosis (sample S0), and before each of the 6  
85        chemotherapy cycles (samples S1 to S6) and 6, 12 and 24 months after completion  
86        of chemotherapy treatment (samples S7 to S9). Patients were also asked to register  
87        all menstruations occurrences throughout the study.

### 88        2.2 AMH assay

89        All assays were performed in the same centralized laboratory with 1<sup>st</sup> generation  
90        AMH/MIS EIA Immunotech Beckman Coulter™ method (Marseille, France) according  
91        to the manufacturer instructions. Repeatability (intra-assay precision) and  
92        reproducibility (inter-assay precision) coefficients were below or equal to 12.3% and  
93        14.2% respectively. The value of 0.42ng/mL was arbitrarily assigned to every values  
94        <0.42ng/mL (lower limit of quantification). Patients were grouped according to basal  
95        AMH level at diagnosis, i.e. low basal AMH level, defined as <1 ng/mL (<7 pmol/L),  
96        normal basal AMH level, defined as 1-4.9 ng/mL (7-36 pmol/L) and high basal AMH  
97        level, defined as ≥5 ng/mL (≥ 36 pmol/L). These thresholds were chosen arbitrarily  
98        but might reflect consensual values found in the existing literature on AMH reference

99 values (8), (9), (10). The variation of serum AMH level between 2 visits (S1 and S6  
100 for instance) was calculated (variation S1S6 =  $\Delta S6S1 / S1 - 0.42$ ).

101 The main endpoint of our study was the absolute (in ng/ml) and relative (in % of basal  
102 serum level) variation of serum AMH levels during chemotherapy and follow-up as  
103 compared to basal AMH level at diagnosis.

104 Subgroup analyses were carried out to evaluate the putative association between  
105 female age and AMH variation according to basal AMH levels, and between the  
106 prevalence of chemotherapy-related amenorrhea (CRA) and basal AMH level.

### 107 2.3 Statistical analysis

108 Mann –Whitney and Kruskal-Wallis tests were used for group comparison and  
109 Spearman coefficient for correlation studies. GraphPad Prism™ (version 5) software  
110 was used. A *p* value <.05 was considered statistically significant.

### 111 2.4 Ethical approval

112 This observational and prospective study (ClinicalTrials.gov identifier NCT01114464)  
113 has been approved by the local ethics committee (GNEDS). All patients provided  
114 written informed consent before starting study procedure.

115

## 116 3. Results

### 117 3.1 Demographic characteristics and basal serum AMH

118 AMH results could be analyzed in 238 women among the 250 initially included  
119 in the study. The demographic and treatment characteristics of these 238 women are  
120 presented in table 1. Basal AMH level at diagnosis was low in 21 patients (8.9%)  
121 (mean  $0.59 \pm 0.34$  ng/ mL; IC95% [0.45-0.72]; median 0.59 ng/ mL), normal in 153

122 patients (64.3%) (mean  $2.71 \pm 1.19$  ng/mL; IC95% [2.52-2.90]; median 2.46 ng/mL),  
123 and high in 64 patients (26.9%) (mean  $9.28 \pm 7.06$  ng/mL; IC95% [7.51-11.04]; median  
124 7.44 ng/mL) (Figure 1). No significant correlation between basal AMH level and age  
125 was found in high and low basal AMH groups, whereas a significant negative  
126 correlation was found between age and basal AMH in the normal basal AMH group  
127 ( $r = -0.076$ ,  $p = 0.0049$ ) (supplementary figure 1).

### 128 3.2 Comparison of AMH variation throughout study according to basal AMH level

129 Serum AMH level rapidly decreased during chemotherapy cycles, before slightly  
130 increasing during the 2-year follow-up in the low (Fig.2A), normal (Fig.2B) and high  
131 basal AMH group (Fig.2C). The calculated decrease in AMH level was -50.5% (-0.1  
132 ng/ml), -52.5% (-1.2 ng/mL) and -41.5% (-4.2 ng/mL) ( $p < 0.0001$ ) after the 1st  
133 chemotherapy cycle (sample S2), -82.6% (-0.28 ng/mL), -85.29% (-1.95 ng/mL) and -  
134 81% (-6.92 ng/mL) ( $p < 0.0001$ ) after 2 chemotherapy cycles (sample S3), and -97.7%  
135 (-0.35 ng/mL), -96.9% (-2.22 ng/mL), and -98.9% (-8.08 ng/mL), ( $n = 19$ ,  $n = 141$  and  
136  $n = 54$  respectively,  $p < 0.0001$ ) after chemotherapy completion (sample S6), in low (Fig  
137 2A), normal (Fig.2B) and high basal AMH groups respectively (Fig 2C).

138 The final AMH level at the end of the study (24 months after chemotherapy  
139 completion, sample S9) was  $0.35 \pm 0.23$  ng/mL,  $0.76 \pm 0.67$  ng/mL and  $1.83 \pm 2.11$  ng/mL  
140 in the low, normal and high basal AMH groups respectively ( $p < 0.0001$ ). The average  
141 increase in serum AMH level during the 2-year follow up after chemotherapy  
142 completion was not statistically different in the low, normal and high basal AMH  
143 groups (+11.9%, +10.3% and 16.1% respectively,  $p > 0.05$ ). However, the proportion  
144 of patients with increasing AMH level during the 24-month follow-up post-  
145 chemotherapy was significantly higher in the high basal AMH group (65%) than in the



146 normal (40%) or in the low basal AMH group (33%) ( $p=0.005$ ). The respective AMH  
147 decrease during chemotherapy and increase during follow-up did not differ according  
148 to age (supplementary figure 2).

149 Finally, the cumulative decrease of serum AMH during the whole study (i.e.  
150 chemotherapy and 24 month follow-up) was -89.8%, -86.2% and -83.7% in the low,  
151 normal and high basal AMH groups respectively ( $p>0.05$ ). It was significantly  
152 associated with age in women with normal basal AMH (fig. 3B), but not in the high  
153 and low basal AMH groups (fig.3A and 3C). The proportion of patients with  
154 undetectable AMH level after chemotherapy completion reached 95% in low basal  
155 AMH group vs 75% in normal basal AMH group and 81% in high basal AMH  
156 ( $p>0.05$ ). After 24-month follow-up, the proportion of patients with undetectable AMH  
157 was significantly lower in the high basal AMH group than in normal and low basal  
158 AMH groups (33% vs 58% and 76% respectively,  $p=0.0003$ ).

### 159 3.3 Prevalence of chemotherapy-related amenorrhea (CRA) and pregnancy

160 The prevalence of self-reported CRA according to basal AMH level is presented in  
161 figure 4. The prevalence of CRA was comparable in the 3 groups at the end of  
162 chemotherapy and after 12-month follow-up. However, it was significantly lower in the  
163 high basal AMH group than in the normal or low basal AMH groups after 6-month  
164 follow-up (75% vs 83 and 100% respectively,  $p=0.004$ ). Two patients with high basal  
165 serum AMH became pregnant during the study, aged 27.2 and 27.7 years. Basal  
166 AMH level was 39.70 and 6.38 ng/mL, falling to undetectable level after  
167 chemotherapy cycles in both cases and further increasing to 1.82 and 0.72 ng/mL  
168 respectively.

169

## 170 **4 Discussion**

171 In this prospective study, we compared serum AMH evolution in young women  
172 treated for breast cancer with chemotherapy, from diagnosis to 2-year follow-up after  
173 chemotherapy, according to basal AMH level. We found that AMH dynamics and  
174 variation throughout chemotherapy and follow-up were not significantly different in  
175 women with high, normal or low basal AMH level. These results suggest that ovarian  
176 reserve status does not condition the intensity of the chemotherapy-induced  
177 gonadotoxic effect.

178 We recently reported that serum AMH level decreased rapidly and significantly  
179 in young unselected women treated with chemotherapy for breast cancer, with a  
180 slight increase observed after 2-year follow-up in 45% of them (2). Although other  
181 recent studies have also evaluated serum AMH levels before and after chemotherapy  
182 in women with breast cancer, this subgroup analysis of the RESOVA cohort (2) is to  
183 our knowledge the first study focusing on the evolution of serum AMH in women  
184 treated for breast cancer according to basal ovarian reserve. As AMH expression  
185 modulates follicular recruitment and maturation, its pharmacologic control has  
186 recently been proposed as a potentially relevant strategy for fertility preservation in  
187 order to limit chemotherapy-induced gonadotoxic effects in young women treated for  
188 cancer (6), with promising results obtained in animal models (7). In this respect, the  
189 influence of basal AMH levels on individual ovarian sensitivity to gonadotoxic  
190 treatment can be questioned. More specifically, whether the pool of growing preantral  
191 and antral follicles in women with high basal AMH level are less sensitive to  
192 chemotherapy-induced degeneration than in women with normal or low basal AMH  
193 level is not known yet. Our results suggest that high basal AMH level is not  
194 associated with a lower follicular sensitivity to the gonadotoxic effect of

195 chemotherapy. Some further work should focus on the mechanisms involved in  
196 ovarian follicle toxicity, for example using animal models.

197         After 24-months follow-up post-chemotherapy, a significantly higher frequency  
198 of partial AMH recovery was observed in women with high basal AMH than in  
199 patients with normal and low basal AMH level. However, the average increase in  
200 serum AMH was comparable in both groups. This should obviously not lead to  
201 exclude women with high basal AMH levels from fertility preservation program.  
202 Moreover, the clinical relevance of serum AMH as a predictor of pregnancy, either  
203 spontaneous or after ART cycle, has been largely questioned (11), (12) (13). In our  
204 study, only 2 patients out of 64 with high basal AMH became spontaneously pregnant  
205 during follow-up. However, we do not know whether other women tried to become  
206 pregnant in the cohort, preventing from drawing conclusions on this topic.

207         Amenorrhea has been frequently reported as an indicator of chemotherapy  
208 toxicity on ovarian function (14). However, its relevance was largely questioned, as  
209 its association with ovarian reserve markers has been shown to be very weak (15). In  
210 our population, we observed a lower prevalence of Chemotherapy-related  
211 amenorrhea (CRA) after 6-month follow-up in the high basal AMH group than in the  
212 normal and low basal AMH groups, suggesting that higher residual AMH level after  
213 chemotherapy in the high basal AMH group might partly protect ovarian function,  
214 although AMH cumulative decrease was not significantly different than in the normal  
215 and low basal AMH groups. However, these results should be interpreted with care.  
216 Indeed, this difference in CRA between the groups was not significant any more after  
217 12-month follow-up. Moreover, the prevalence of CRA is difficult to evaluate in these  
218 women with high AMH owing to the suspected very high prevalence of PCOS, which  
219 is generally associate with menstrual cycle irregularity. Finally, tamoxifen use might

220 interfere with menstrual cyclicity in many patients (16), thus limiting the relevance of  
221 this marker.

222 Polycystic ovary syndrome (PCOS) is a common endocrine disorder, affecting  
223 up to 10-25% of the women of reproductive age (17). Numerous studies evaluated  
224 whether an association existed between PCOS and breast cancer, with conflicting  
225 results. While Kim et al reported a 3-fold increase in the risk of breast cancer in  
226 young patients with PCOS, this was not confirmed in a large case-control study  
227 conducted in more than 9000 women aged 20 to 54 (18), (19). Moreover, one meta-  
228 analysis did not retrieve any significantly higher risk of breast cancer in women with  
229 PCOS than in women without PCOS (20). The current diagnosis classification is  
230 based on hyperandrogenism, oligoanovulation, and polycystic ovarian morphology  
231 (PCOM) at ultrasound (21). Serum AMH level has been proposed as a surrogate for  
232 PCOS diagnosis (22). It could thus be postulated that most women with high basal  
233 AMH level in our study would have PCOS. However, we did not have access to all  
234 Rotterdam criteria. We could thus not determine precisely the proportion of women  
235 with PCOS and could not study a potential specific pattern for AMH evolution in these  
236 women.

237 BRCA status has been shown to be associated with ovarian reserve in some  
238 recent studies (23). However, the number of patients with BRCA mutation is too small  
239 in our population to yield relevant conclusion on the association between BRCA  
240 status and AMH dynamics during and after chemotherapy.

241 Although its prospective design, with long-term follow-up in a well-designed  
242 population can be considered as strengths for this study, we acknowledge a few  
243 limitations. First, the issue of AMH assay performance could eventually be raised. We  
244 used 1<sup>st</sup> generation AMH/MIS EIA Immunotech Beckman Coulter™ method, which is

245 being progressively replaced by automated AMH assays with better sensitivity.  
246 Second, the thresholds used for the definition of groups were chosen arbitrarily. We  
247 tried to choose the most consensual ones, but this might obviously be discussed.  
248 Finally, the real prevalence of self-reported chemotherapy-related amenorrhea was  
249 difficult to interpret.

250 In conclusion, this longitudinal study describes for the first time serum AMH  
251 kinetic during chemotherapy and 24-month follow up in young women of reproductive  
252 age treated with chemotherapy for breast cancer according to basal AMH levels at  
253 diagnosis. Although women with high basal AMH levels have significantly higher  
254 AMH levels throughout chemotherapy and follow-up than women with normal or low  
255 basal AMH level, AMH dynamics and cumulative variation is comparable in all the  
256 groups. Therefore, AMH variation during chemotherapy for breast cancer and follow  
257 up does not appear to be associated with basal AMH level at diagnosis. Pre-  
258 treatment fertility preservation and post-treatment ovarian insufficiency follow-up  
259 should obviously be offered to all women of reproductive age diagnosed with breast  
260 cancer, whatever their basal ovarian reserve.

261

## 262 **5 Acknowledgments**

263 None

## 264 **6 Conflict of interest**

265 All the authors confirm that they have no conflict of interest related to this work.

266

267 **References**

- 268 1. Fidler MM, Gupta S, Soerjomataram I, Ferlay J, Steliarova-Foucher E, Bray F.  
269 Cancer incidence and mortality among young adults aged 20-39 years  
270 worldwide in 2012: a population-based study. *Lancet Oncol.* 2017;18(12):1579–  
271 89.
- 272 2. Dezellus A, Barriere P, Campone M, Lemanski C, Vanlemmens L, Mignot L, et  
273 al. Prospective evaluation of serum anti-Müllerian hormone dynamics in 250  
274 women of reproductive age treated with chemotherapy for breast cancer. *Eur J*  
275 *Cancer Oxf Engl* 1990. 2017;79:72–80.
- 276 4. Fréour T, Barrière P, Masson D. Anti-müllerian hormone levels and evolution in  
277 women of reproductive age with breast cancer treated with chemotherapy. *Eur J*  
278 *Cancer.* 2017 Mar;74:1–8.
- 279 5. Lambertini M, Olympios N, Lequesne J, Calbrix C, Fontanilles M, Loeb A, et al.  
280 Impact of Taxanes, Endocrine Therapy, and Deleterious Germline BRCA  
281 Mutations on Anti-müllerian Hormone Levels in Early Breast Cancer Patients  
282 Treated With Anthracycline- and Cyclophosphamide-Based Chemotherapy.  
283 *Front Oncol.* 2019;9:575.
- 284 6. Kushnir VA, Seifer DB, Barad DH, Sen A, Gleicher N. Potential therapeutic  
285 applications of human anti-Müllerian hormone (AMH) analogues in reproductive  
286 medicine. *J Assist Reprod Genet.* 2017 Sep;34(9):1105–13.
- 287 7. Kano M, Sosulski AE, Zhang L, Saatcioglu HD, Wang D, Nagykerly N, et al.  
288 AMH/MIS as a contraceptive that protects the ovarian reserve during  
289 chemotherapy. *Proc Natl Acad Sci.* 2017 Feb 28;114(9):E1688–97.
- 290 9. Ferraretti AP, La Marca A, Fauser BCJM, Tarlatzis B, Nargund G, Gianaroli L, et  
291 al. ESHRE consensus on the definition of “poor response” to ovarian stimulation  
292 for in vitro fertilization: the Bologna criteria. *Hum Reprod.* 2011 Jul 1;26(7):1616–  
293 24.
- 294 10. Teede H, Misso M, Tassone EC, Dewailly D, Ng EH, Azziz R, et al. Anti-  
295 Müllerian Hormone in PCOS: A Review Informing International Guidelines.  
296 *Trends Endocrinol Metab.* 2019 Jul;30(7):467–78.
- 297 11. Streuli I, de Mouzon J, Paccolat C, Chapron C, Petignat P, Irion OP, et al. AMH  
298 concentration is not related to effective time to pregnancy in women who  
299 conceive naturally. *Reprod Biomed Online.* 2014 Feb;28(2):216–24.
- 300 12. Zarek SM, Mitchell EM, Sjaarda LA, Mumford SL, Silver RM, Stanford JB, et al.  
301 Is Anti-Müllerian Hormone Associated With Fecundability? Findings From the  
302 EAGeR Trial. *J Clin Endocrinol Metab.* 2015 Nov;100(11):4215–21.
- 303 13. Steiner AZ, Pritchard D, Stanczyk FZ, Kesner JS, Meadows JW, Herring AH, et  
304 al. Association Between Biomarkers of Ovarian Reserve and Infertility Among  
305 Older Women of Reproductive Age. *JAMA.* 2017 10;318(14):1367–76.

- 306 14. Spears N, Lopes F, Stefansdottir A, Rossi V, De Felici M, Anderson RA, et al.  
307 Ovarian damage from chemotherapy and current approaches to its protection.  
308 Hum Reprod Update. 2019 05;25(6):673–93.
- 309 16. Zhao J, Liu J, Chen K, Li S, Wang Y, Yang Y, et al. What lies behind  
310 chemotherapy-induced amenorrhea for breast cancer patients: a meta-analysis.  
311 Breast Cancer Res Treat. 2014 May;145(1):113–28.
- 312 17. Norman RJ, Dewailly D, Legro RS, Hickey TE. Polycystic ovary syndrome. The  
313 Lancet. 2007 Aug;370(9588):685–97.
- 314 18. Kim J, Mersereau JE, Khankari N, Bradshaw PT, McCullough LE, Cleveland R,  
315 et al. Polycystic ovarian syndrome (PCOS), related symptoms/sequelae, and  
316 breast cancer risk in a population-based case–control study. Cancer Causes  
317 Control. 2016 Mar;27(3):403–14.
- 318 19. Gammon MD, Thompson WD. Polycystic Ovaries and the Risk of Breast  
319 Cancer. Am J Epidemiol. 1991 Oct 15;134(8):818–24.
- 320 21. Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group.  
321 Revised 2003 consensus on diagnostic criteria and long-term health risks  
322 related to polycystic ovary syndrome. Fertil Steril. 2004 Jan;81(1):19–25.
- 323 22. Pigny P, Jonard S, Robert Y, Dewailly D. Serum Anti-Müllerian Hormone as a  
324 Surrogate for Antral Follicle Count for Definition of the Polycystic Ovary  
325 Syndrome. J Clin Endocrinol Metab. 2006 Mar;91(3):941–5.
- 326 23. Lambertini M, Goldrat O, Toss A, Azim HA, Peccatori FA, Ignatiadis M, et al.  
327 Fertility and pregnancy issues in BRCA-mutated breast cancer patients. Cancer  
328 Treat Rev. 2017 Sep;59:61–70.

329

330

## 331 **8. Appendix**

332 RESOVA Investigators Group members are:

333 M Campone, Service d'oncologie, Institut de Cancérologie de l'Ouest, Bd Jacques

334 Monod, 44805, Saint Herblain, France

335 C Lemanski, Service de radiothérapie oncologique, Institut du Cancer de Montpellier,

336 Avenue des Apothicaires, 34298, Montpellier, France

337 L Vanlemmens, Département de cancérologie sénologique, Centre Oscar Lambret,

338 rue Frédéric Combemale, 59020, Lille, France

339 L Mignot, Département de médecine oncologique, Institut Curie, rue d'Ulm, 75248,  
340 Paris, France

341 T Delozier & C Levy, Unité de pathologie mammaire, Centre François Baclesse,  
342 avenue du Général Harris, 14000, Caen, France

343 C Bendavid, Service de chirurgie, Centre Eugene Marquis, Avenue la Bataille  
344 Flandres-Dunkerque, 35042, Rennes, France

345 M Debled, Département d'oncologie médicale, Institut Bergonié, cours de l'Argonne,  
346 33076, Bordeaux, France

347 T Bachelot, Service de cancérologie médicale, Centre Léon Bérard, rue Laennec,  
348 69008, Lyon, France

349 C Jouannaud, Service d'oncologie médicale, Institut Jean Godinot,  
350 rue du Général Koenig, 51100, Reims, France

351 C Loustalot, Service de chirurgie, Centre Georges-François Leclerc, rue Professeur  
352 Marion, 21079, Dijon, France

353 MA Mouret-Reynier, Service d'oncologie médicale, Centre Jean Perrin, 58 rue  
354 Montalembert, 63011, Clermont-Ferrand, France

355



356 **9. Figure legends**

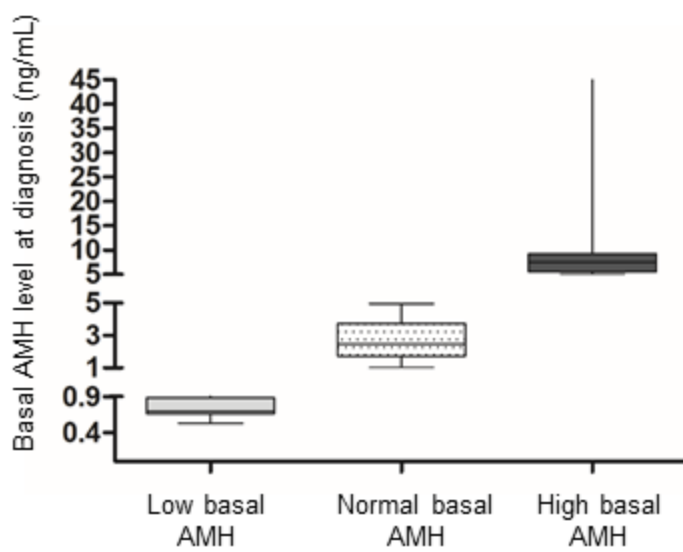
357

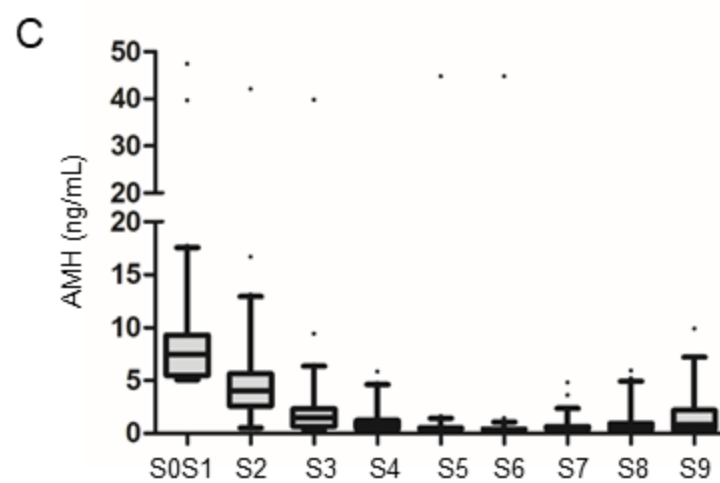
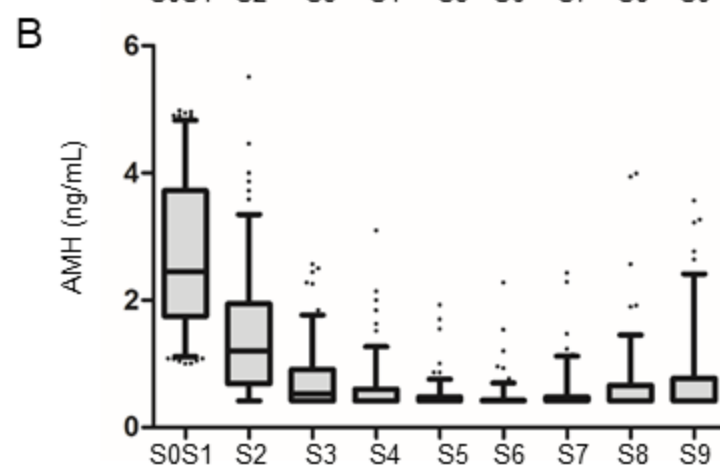
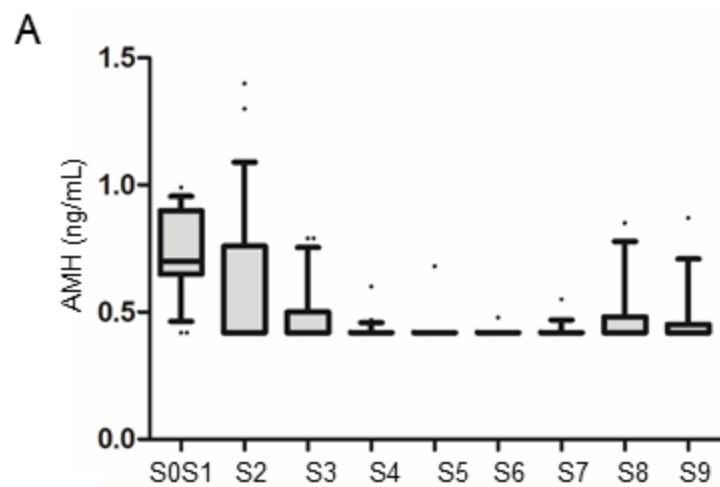
358 **Figure 1:** Basal AMH level at diagnosis in the group of patients with low basal AMH  
359 level ( $n=21$ ), normal AMH level ( $n=153$ ), and high basal AMH ( $n=64$ )

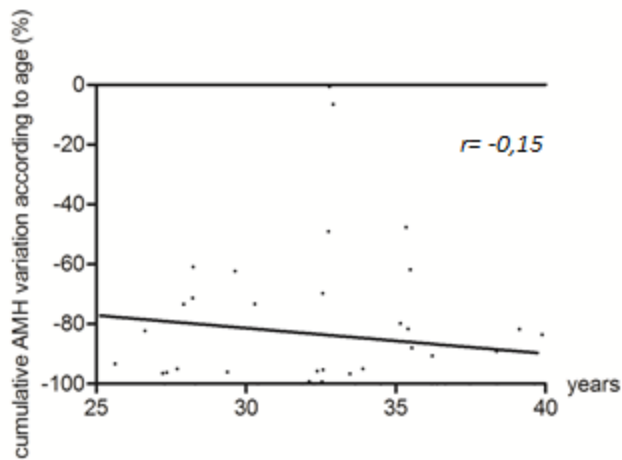
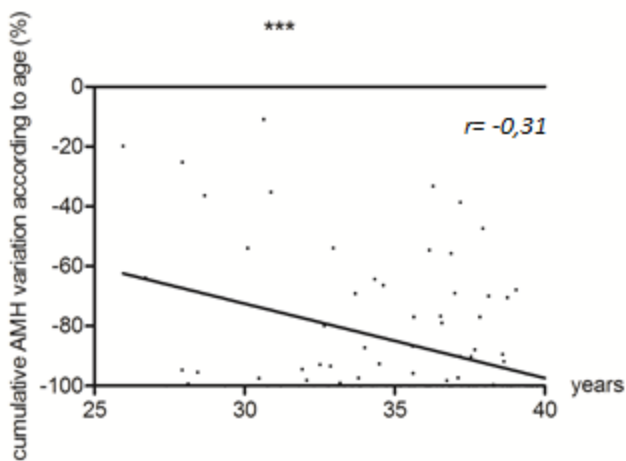
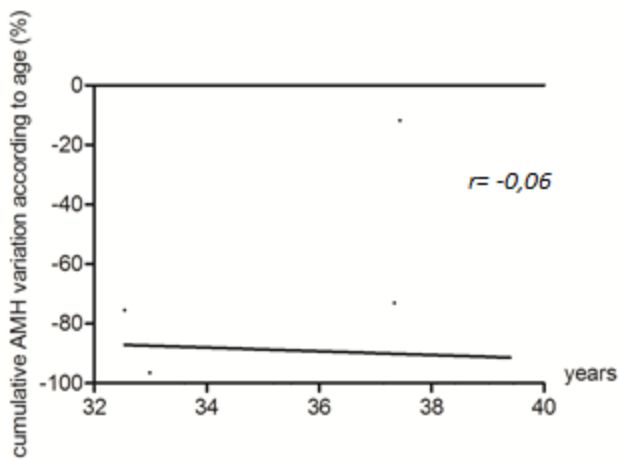
360 **Figure 2:** Evolution of serum AMH level during chemotherapy and during 24-month  
361 follow-up after chemotherapy completion in women with low (A), normal (B) and high  
362 basal AMH (C).

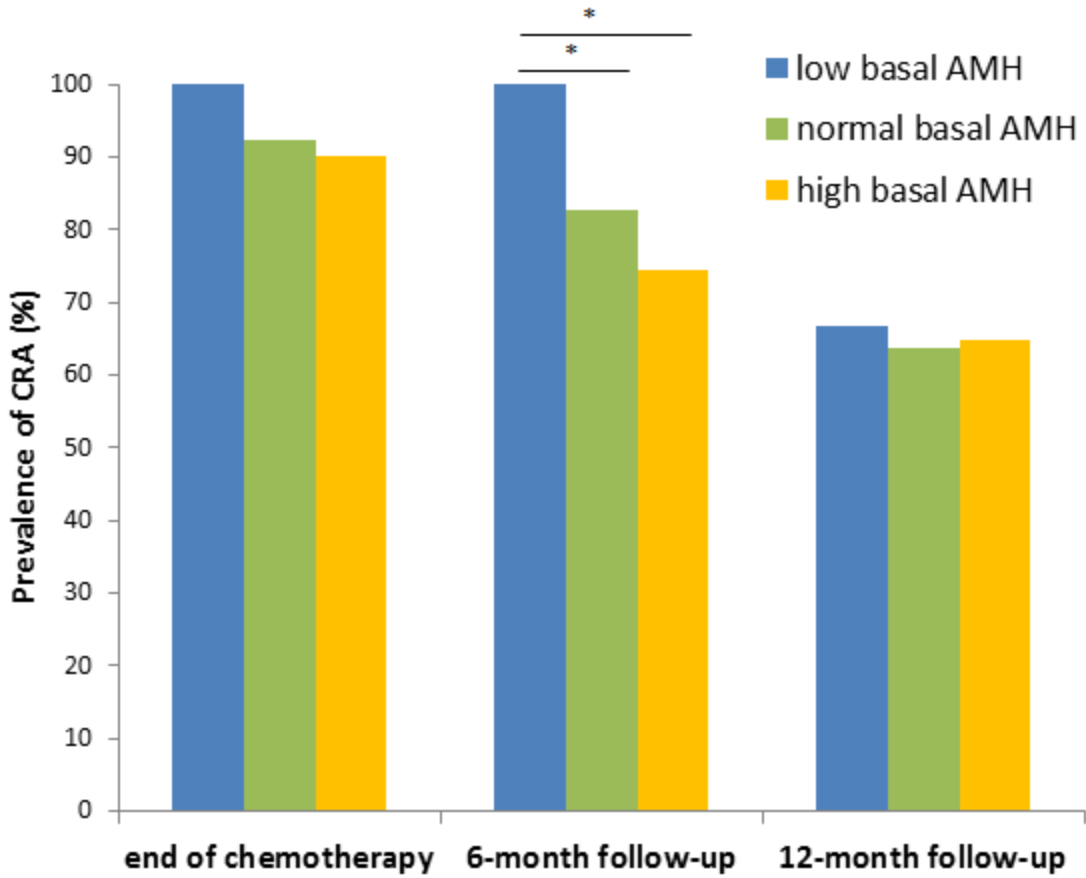
363 **Figure 3:** Cumulative variation of AMH between diagnosis and 24-month follow-up  
364 according to age at diagnosis in patients with high (A), normal (B) and low basal AMH  
365 level (C).

366 **Figure 4:** Prevalence of self-reported chemotherapy-related amenorrhea (CRA) in  
367 patients with high, normal and low basal AMH level at the end of chemotherapy  
368 cycles, after 6-month and 12-month follow-up.





**A****B****C**



|   | Patients with low basal AMH level (< 1ng/mL) (n=21) | Patients with normal AMH level (1-4.9 ng/mL) (n=153) | Patients with high basal AMH level (>5 ng/mL) (n=64) |
|---|---|--|--|
| <b>DEMOGRAPHIC CHARACTERISTICS</b>                        |   |  |  |
| Age (years)   | 37.1 ± 1.8*   | 35.4 ± 2.9*  | 32.4 ± 3.5*  |
| < 30 years, % (N)   | 0 (0)   | 8.5 (13)   | 31.2 (20)  |
| 30-34 years, % (N)  | 14.3 (3)  | 30.1 (46)  | 35.9 (23)  |
| 35-39 years, % (N)  | 85.7 (18)   | 61.4 (94)  | 32.8 (21)  |
| Women with children, % (N)                                | 85.7 (18)   | 77.1 (118)   | 68.7 (44)  |
| Number of child before diagnosis                          | 1.8 ± 0.6   | 2.6 ± 0.8  | 1.7 ± 0.7  |
| Time to have a child >6 months, % (N)                     | 5.5 (1/18)  | 15.3 (18/118)  | 25 (11/44)   |
| BMI (kg/m <sup>2</sup> )                                  | 23.6 ± 3.6  | 23.7 ± 4.8   | 23.8 ± 6.0   |
| Current smoker, % (N)                                     | 33.3 (7)  | 32.7 (50)  | 29.7 (19)  |
| Familial history of breast cancer, % (N)                  | 38 (8)  | 47.7 (73)  | 45.3 (29)  |
| BRCA 1/2 mutation carriers, % (N/number of women tested)  | 7.7 (1/13)  | 22.3 (17/74)   | 10.3 (4/39)  |
| <b>HISTOLOGICAL CHARACTERISTICS</b>                       |   |  |  |
| Invasive ductal carcinoma, % (N)                          | 95.2 (20)   | 91 (139)   | 93.7 (60)  |
| Invasive lobular carcinoma, % (N)                         | 4.7 (1)   | 8.4 (13)   | 1.6 (1)  |
| Other histological category, % (N)                        | 0 (0)   | 1.3 (2)  | 4.7 (3)  |
| Grade II-III, % (N)                                       | 95.2 (20)   | 94.1 (144)   | 93.7 (60)  |
| Positive for hormone receptors, % (N)                     | 61.2 (13)   | 71.2 (109)   | 59.4 (38)  |
| HER2 positive, % (N)                                      | 38 (8/21)   | 22.9 (35/153)  | 38.0 (24/63)   |
| Node-positive, % (N)                                      | 57.1 (12)   | 46.4 (71)  | 57.8 (37)  |
| Breast-conserving surgery, % (N)                          | 52.4 (11)   | 52.9 (81)  | 59.4 (38)  |
| <b>TREATMENT CHARACTERISTICS</b>                          |   |  |  |
| Neoadjuvant chemotherapy, % (N)                           | 33.3 (7)  | 28.1 (43)  | 35.9 (23)  |
| FEC, % (N)  | 4.8 (1)   | 0.6 (1)  | 3.1 (2)  |
| FEC-T, % (N)  | 76.2 (16)   | 89.5 (137)   | 85.9 (55)  |
| TAC, % (N)  | 0 (0)   | 0.6 (1)  | 0 (0)  |
| Epirubicin T, % (N)                                       | 0 (0)   | 0.6 (1)  | 1.6 (1)  |
| T, % (N)  | 9.5 (2)   | 5.8 (9)  | 7.8 (5)  |
| Taxotere cyclophosphamide, % (N)                          | 9.5 (2)   | 1.3 (2)  | 4.7 (3)  |
| Anthracycline doses (mg/m <sup>2</sup> )                  | 294 ± 43  | 277 ± 73   | 272 ± 64   |
| Taxanes doses (mg/m <sup>2</sup> )                        | 297.6 ± 66  | 304.8 ± 61.2   | 297 ± 43.2   |
| Number of chemotherapy cycles                             | 4.7 ± 1.4   | 4.3 ± 1.4  | 4.5 ± 1.5  |
| Trastuzumab co-treatment, % (N)                           | 9.5 (2)   | 5.8 (9)  | 9.3 (6)  |
| GnRH analog administration during chemotherapy, % (N)     | 4.7 (1/21)  | 1.9 (3/153)  | 6.4 (4/63)   |
| Ovarian cryopreservation before chemotherapy onset, % (N) | 0 (0/21)  | 0.65 (1/152)   | 6.4 (4/63)   |
| Tamoxifen administration, % (N)                           | 61.09 (13)  | 67 (103)   | 54.7 (35)  |

*Abbreviations: FEC: 5-Fluorouracil, epirubicin, cyclophosphamide – T: Taxanes – AC: Adriamycin, cyclophosphamide – BMI: Body Mass Index.*

**Table 1:** Demographic, histological and treatment characteristics in patients with low, normal or high basal AMH levels. Results are presented as mean  $\pm$  standard deviation or proportion (number) when appropriate. \* $p < 0.05$  when each group compared to the 2 other ones