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ARTICLE

# Ovarian reserve in breast cancer: assessment with anti-Müllerian hormone

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**Abstract** Anti-Müllerian hormone (AMH) levels fall during chemotherapy. Treatment-induced amenorrhoea is a reversible phenomenon, but few data are available on long-term AMH changes in breast cancer. The aim of the study was to describe serum AMH levels before, during and in the long term after chemotherapy, and to show a potential AMH recovery. Between May 2010 and June 2011, we selected 134 women aged 18–43 years at the time of breast cancer diagnosis who received chemotherapy between 2005 and 2011, and had not undergone an oophorectomy or had previous cytotoxic treatment. The AMH levels were assessed before, during and 4 months to 5.5 years after the end of chemotherapy. During chemotherapy, AMH was undetectable in 69% of women. After chemotherapy, a significant increase in AMH was found, with an average magnitude of +1.2% per month (95% credibility interval: 0.7 to 1.6). Older age and 12 months of amenorrhoea were found to be associated with a lower AMH recovery rate, whereas baseline AMH

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and number of chemotherapy cycles were not. The process of AMH changes during and after chemotherapy is dynamic, and shows recovery after ovarian injury. Caution should be exercised in interpreting individual AMH assessment in this context.


**KEYWORDS:** AMH, breast cancer, chemotherapy, ovarian reserve

## Introduction

Breast cancer in young women is a growing burden in developing countries. Treatment-induced ovarian damage is a frequent and detrimental adverse effect of chemotherapy, presenting as acute amenorrhoea sometimes followed by irreversible premature ovarian failure. To date, no predictive marker of ovarian function recovery has been validated, and quantification of chemotherapy damage remains a substantial challenge. An accurate and individual assessment of the risk of subfertility or infertility could help in counselling patients and in selecting those women eligible for fertility preservation. Reliable information could alleviate the burden for patients at a low risk for premature ovarian failure by reducing the additional emotional distress induced by the prospect of infertility that accompanies a cancer diagnosis. Amenorrhea and menstrual changes have long been the only variables reported in studies, but have weak predictive value. In recent years, anti-Müllerian hormone (AMH), a glycoprotein and member of the transforming growth factor superfamily of growth factors, has been extensively studied. It is produced exclusively in the somatic cells of the gonads. It plays a variety of roles in reproduction and in the processes of sexual development and differentiation, and it induces testicular differentiation and regression of the Müllerian ducts in men (Münsterberg and Lovell-Badge, 1991). Müllerian ducts evolve into the uterus, fallopian tubes and upper part of the vagina in the absence of AMH. In the human fetus, ovarian AMH expression is observed from 36 weeks' gestation and falls shortly after birth, with concentrations increasing at about 2 years of age and falling between the ages of 8 and 12 years. The relevance of AMH secretion is incompletely understood. After a prepubertal rise, AMH levels peak at 24.5 years and gradually decline throughout the reproductive years, becoming undetectable by menopause (Kelsey et al., 2011). Within the ovary, AMH expression is restricted predominantly to the granulosa cells of growing ovarian follicles (e.g. secondary, pre-antral and small antral follicles less than 4 mm in diameter) (Weenen et al., 2004). Although AMH expression is not observed in primordial follicles, serum AMH concentrations have been shown to be correlated with the size of the non-growing primordial follicle pool (Hansen et al., 2011). It has been reported that AMH reflects a marker of the so-called 'ovarian reserve' (i.e. the number of primordial follicles remaining in the ovaries) (Van Rooij et al., 2002). It has been known for its stability, and its blood concentrations have consistently been shown to have significantly low intra- and inter-cycle variability (Hehenkamp et al., 2006; La Marca et al., 2006; Van Disseldorp et al., 2010).

Several studies (La Marca et al., 2010; Van Rooij et al., 2002) have shown that, in assisted reproductive technology, AMH is a better marker of ovarian reserve than age or basal FSH, oestradiol and inhibin B (La Marca et al., 2010). To quantify chemotherapy-induced ovarian damage, many investigators (Anders et al., 2008; Anderson et al., 2006; Su et al., 2010) have recently measured serum AMH concentrations in women included in oncofertility studies. In women receiving chemotherapy, AMH rapidly declined. Most of the studies lacked long-term data (Anders et al., 2008; Yu et al., 2010), although it is well known that ovarian recovery can occur up to 2 or 3 years after the end of treatment (Sukumvanich et al., 2010). The aim of the present study was to evaluate AMH patterns of changes before, after and in the long term after chemotherapy in a population of women who received chemotherapy for breast cancer. This work is part of the *O.B.A.M.A study* (Ovarian reserve in Breast Cancer: AssessMent with Anti-Müllerian Hormone).

## Materials and methods

### Study population and participants

From May 1 2005 to January 31 2011, women aged between 18 and 43 years who received chemotherapy in our breast care unit (Saint Louis Hospital, Paris, France) were retrospectively identified from a computerized database. Women with a history of prior cytotoxic treatment or women who had undergone an oophorectomy were excluded from the study. Demographic data, detailed treatment characteristics and dates, type of surgery, chemotherapy doses and regimens, radiation and endocrine therapy were extracted from medical charts. One patient underwent fertility preservation (in vitro maturation). Clinical data (e.g. number of previous pregnancies and children, infertility, menstrual history and smoking habits) were retrieved in a follow-up consultation when possible or by a retrospective review of the medical charts of deceased patients. The study was approved by an institutional review board (IRB) (CPP Ile de France XI, 3 March 2010, study registered as 2009-A01225-52). No ultrasound antral follicle count was available owing to the retrospective design of the study.

### Blood collection

Blood samples were systematically retrieved for the monitoring of tumour marker levels on the day of the first chemotherapy (baseline time point, one sample) and during chemotherapy (two to four samples), The blood samples samples were centrifuged at 1000 g for 15 min and stored between –20°C and –30°C in freezers, in agreement with the manufacturer's instructions of the AMH assay, and in accordance with existing literature on the stability of AMH samples (Kumar et al., 2010). One post-treatment measurement was taken at least 4 months and to up to 5.5 years after the end

of chemotherapy. Patients were aged 44 years or younger at the time of the latest post-treatment analysis. For 34 patients, frozen blood samples were available for the post-treatment measurement (i.e. patients who received adjuvant trastuzumab therapy ($n = 8$ ) or who experienced relapse during the course of the study ($n = 26$). All samples were assessed for AMH levels, after patients' re-consent was obtained. In the other patients ($n = 100$), blood samples were collected specifically for the study, after informed consent was given by patients in a follow-up consultation. Other hormonal parameters were not assayed, because of their limited value as blood assays, and were sampled at any day of the menstrual cycle. Additionally, ultrasonographic antral follicle count was not available owing to the retrospective nature of the study.

### Assays

Analyses were carried out at Pitie Salpetriere Hormonal Biochemistry Unit, by a dedicated biologist in August 2011. eEnzyme-linked immunosorbent assay was carried out in duplicate in 26 assays, using Immunotech A11893 kits (Beckman Coulter, Marseille, France). For each patient, multi-time samples were assessed in the same assay. All AMH kits were purchased at once, and all the kits had the same batch number. The lower limit of detection for AMH was 0.14 ng/ml. The AMH concentrations, intra-assay and inter-assay coefficients of variation were as follows: 0.42 ng/ml (9.4% and 11.8%); 1.8 ng/ml (4.4% and 11.8%); 2.1 ng/mL (4.8% and 7.2%); 3.5 ng/ml (3.3% and 8.2%), respectively.

### Statistical analysis

The AMH values were log-transformed to stabilize their variance. Association of baseline (pre-chemotherapy) AMH with patients' characteristics (i.e. age, smoking status and previous pregnancy) was analysed using tobit regression models. The models account for the lower detection limit of AMH as detailed in the appendix. Briefly, AMH values under the 0.14 limit of detection were considered as left-censored at 0.14. The analysis of the evolution of AMH after chemotherapy used all repeated AMH measurements during follow up, beginning at the end of chemotherapy. Longitudinal tobit regression models were used to account for the lower detection limit and for the correlation between measurements carried out on the same participant using random effects for the intercept and the slope, as detailed in the **Appendix** (Twisk and Rijmen, 2009). As a result of these models, a slope of evolution of log-AMH during follow up was estimated. An interaction between time and variables, such as age, chemotherapy regimen, dose of cyclophosphamide, cycles of chemotherapy, baseline AMH and amenorrhoea were then added to the model to test whether the AMH recovery during follow up might be related to these parameters. The longitudinal tobit models were placed within a Bayesian framework using Markov chain Monte Carlo (MCMC) implemented in BRugs (Thomas et al., 2006). Briefly, MCMC is a simulation-based algorithm that allows solving numerically intractable or complex integrals. All tests were two-sided, and $P \leq 0.05$ was considered to indicate a significant association. Because Bayesian statistics do not provide *P*-values, the results of longitudinal tobit models were evaluated by examining the 95% credibility intervals (the Bayesian equivalent of confidence intervals). The analyses were performed using R statistical software version 2.15.0 (R Development Core Team, 2009, n.d).

## Results

### Patient characteristics

One hundred and forty-six women were initially included in the study. Twelve were excluded for various reasons: previous chemotherapy ($n = 1$), previous oophorectomy ($n = 1$), post-treatment measurement sampled less than 4 months after the end of treatment ($n = 6$), age over 43 years at inclusion ($n = 1$), missing blood samples before and during treatment ($n = 3$), and 134 patients were retained for the analysis. Patient characteristics are presented in **Table 1**. The median age at diagnosis was 35.5 years. At diagnosis, 77 women (57%) had children and 57 (43%) did not. The mean number of previous pregnancies was 1.4. Most women ($n = 89$ [66%]) received a standard polychemotherapy sequential regimen of anthracyclines and cyclophosphamide, followed by taxanes with ($n = 15$ [11%]) or without ($n = 74$ [55%]) trastuzumab. Thirty-seven women (28%) received anthracyclines and cyclophosphamide-based chemotherapy. The median total doses of epirubicin, taxanes and cyclophophamide were 536, 656 and 5410 mg, respectively. A total of 871 serum samples were assayed (baseline [$n = 135$]; during treatment [$n = 393$]; after the end of treatment [$n = 343$]).

### Baseline and post-treatment AMH assessment

The median time from last chemotherapy to the latest AMH assessment was 20 months (range: 4–65 months), and the median participant age at last assessment was 38 years. The mean baseline AMH was 1.95 ng/ml (median: 1.5 ng/ml; range: less than the limit of detection to 9.15). Four patients had undetectable AMH before treatment.

Older age was found to be significantly associated with a lower AMH rate, whereas smoking status or previous pregnancies were not (**Supplementary Table S1** and **Figure 1A**). Six months after starting chemotherapy, 80 women (60%) had amenorrhoea and 38 (28%) had persistant amenorrhoea at 12 months. The baseline AMH was non-significantly associated with 6 months of amenorrhea ($P = 0.16$) (**Figure 1B**), whereas it was 46% lower in women who remained amenorrheic at 12 months (95% CI 20 to 62%), ($P = 0.002$) (**Figure 1C**).

The patterns of change of AMH during and after chemotherapy are shown in **Figure 2**. During chemotherapy, AMH levels fell dramatically in all women, with 69% of the AMH values becoming undetectable at the end of treatment (87 out of 126 samples available in the last 3 weeks of chemotherapy). A significant increase in AMH was found after chemotherapy, with an average increase of +1.2% per month (95% credibility interval: 0.7 to 1.6). Older age was found to be associated with a lower AMH recovery rate (if any), as well as amenorrhoea at 12 months, and the rate of AMH decrease during chemotherapy (**Table 2**). Neither chemotherapy conditions (e.g.

**Table 1** Patient characteristics ($n = 134$).

| | |
|---|---|
| Age (mean ± SD [years]; range) | 35.5 ± 4.3; 26–43 |
| Number of previous pregnancies (mean ± SD; range) | 1.4 ± 1.4; 0–7 |
| Infertility *n* (%) | 9 (7) |
| Smoker *n* (%) | 39 (29) |
| Surgery | |
| Mastectomy *n* (%) | 70 (52) |
| Lumpectomy *n* (%) | 64 (48) |
| Axillary dissection *n* (%) | 109 (81) |
| Sentinel node biopsy *n* (%) | 24 (18) |
| No axillary procedure *n* (%) | 1 (1) |
| Anthracyclines and cyclophophamide-based regimen *n* (%) | 37 (28) |
| FEC 75 *n* (%) | 26 (19) |
| FEC 50 *n* (%) | 26 (19) |
| EC *n* (%) | 2 (1) |
| Taxanes containing regimen *n* (%) | 97 (72) |
| EC-T[a] *n* (%) | 74 (55%) |
| EC-TH[b] *n* (%) | 15 (11%) |
| ddEC-TC[c] *n* (%) | 3 (2%) |
| TH[d] *n* (%) | 4 (3%) |
| 5FU-docetaxel-bevacizumab[e] *n* (%) | 1 (1%) |
| Total dose received, median (range) mg | |
| Epirubicin | 536 (0 to 1094) |
| Taxanes | 656 (0 to 1658) |
| Cyclophosphamide | 5410 (0 to 16460) |
| Number of cycles of chemotherapy, median (range) | 8 (6 to 14) |
| Molecular targeted therapy[f] | |
| Trastuzumab *n* (%) | 37 (28) |
| Lapatinib *n* (%) | 2 (1) |
| Bevaciumab *n* (%) | 1 (1) |
| Radiation therapy | |
| No *n* (%) | 21 (16) |
| Yes *n* (%) | 113 (84) |
| Endocrine therapy | |
| No *n* (%) | 47 (35) |
| Tamoxifene *n* (%) | 85 (63) |
| Gonadotrophin-releasing hormone agonists *n* (%) | 2 (1) |

EC: epirubicin 75 mg/m$^2$ + cyclophosphamide 750 mg/m$^2$, every 21 days for eight cycles.
FEC 75: Fluorouracil 750 mg/m$^2$ + epirubicin 75 mg/m$^2$ + cyclophosphamide 1000 mg/m$^2$, every 21 days for six cycles.
FEC 50: Fluorouracil 750 mg/m$^2$ + epirubicin 50 mg/m$^2$ + cyclophosphamide 600 mg/m$^2$ D1, Fluorouracil mg/m$^2$+ cyclophosphamide 600 mg/m$^2$ day 8, every 28 days for eight cycles.
[a]EC-T: epirubicin 75 mg/m$^2$ + cyclophophamide 750 mg/m$^2$ 4 cycles every 21 days, followed by sequential docetaxel 100 mg/m$^2$ 1 cycle every 21 days. One patient received larotaxel instead of docetaxel in a clinical trial and two patients were re-switched to EC during docetaxel treatment.
[b]EC-TH : epirubicin 75 mg/m$^2$ + cyclophophamide 750 mg/m$^2$ 4 cycles every 21 days, followed by sequential docetaxel 100 mg/m$^2$ 1 cycle every 21 days 4 cycles with concomittant trastuzumab 8 mg/kg day 1, 6mg/kg for the following 18 injections. One patient received weekly paclitaxel associated with lapatinib in the clinical trial.
[c]ddEC-TC: epirubicine 75 mg/m$^2$ + cyclophosphamide 1200 mg/m$^2$ 6 cycles (dose dense EC), every 14 days, followed by surgery, and adjuvant docetaxel 600 mg/m$^2$ + cyclophosphamide 750 mg/m$^2$.
[d]TH: Docetaxel 100 mg/m$^2$ 1 cycle every 21 days 8 cycles with concomittant trastuzumab 8 mg/kg day 1, 6 mg/kg for the following injections.
[e]5FU Txt beva: 5 FU 750 mg/m$^2$ + docetaxel 75 mg/m$^2$ + bevacizumab 15 mg/kg every 21 days.
[f]Sum differs from 134 because one patient had trastuzumab in association with lapatinib.

receiving anthracyclines and taxanes versus anthracyclines-only based regimen, eight cycles or more compared with less than eight cycles, and total doses of cyclophosphamide), amenorrhoea at 6 months or baseline AMH were significantly associated with AMH recovery. In all but four patients, AMH variations slopes after treatment were positive, thus indicating that AMH recovery was almost likely to be constant after chemotherapy in our cohort (**Figure 3**).

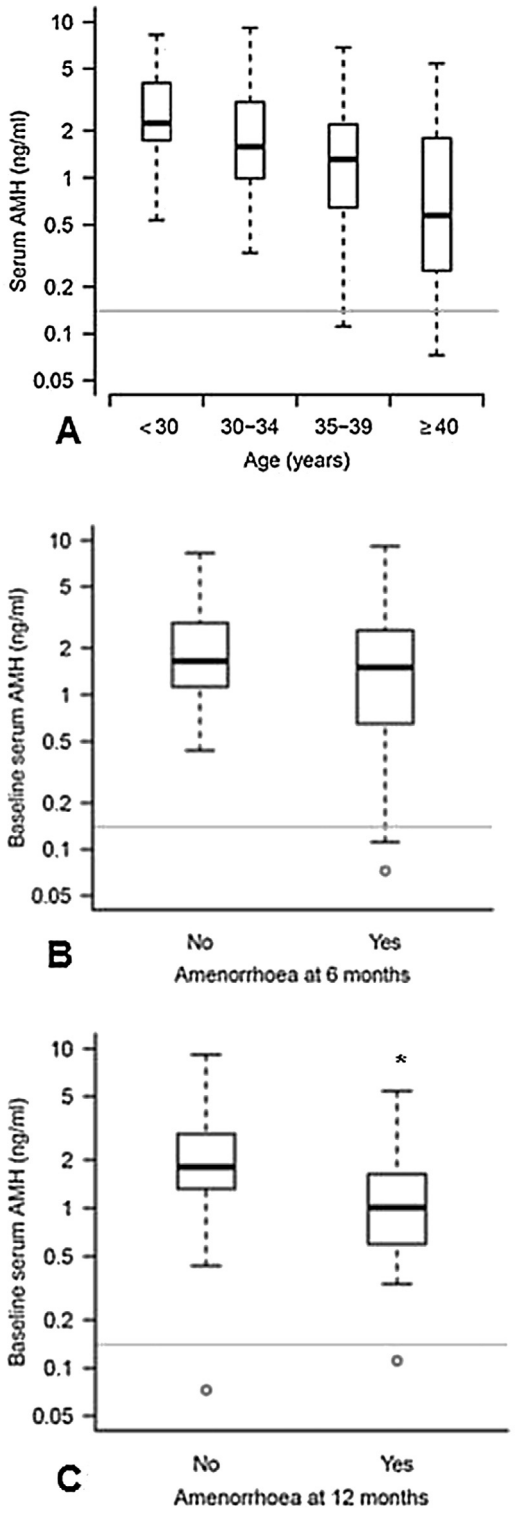


**Figure 1** Baseline Anti-Müllerian hormone (AMH) grouped by age cohort and by menstrual status after chemotherapy; (A) baseline AMH concentrations in four age groups (<30 years, 30–34 years, 35–39 years and > 40 years); (B and C) baseline AMH concentrations in women with (white bars) or without (grey bars) chemotherapy-related amenorrhoea for (B) 6 months ($n = 116$, missing data: $n = 18$); and (C) 12 months ($n = 96$, missing data: $n = 38$) after starting chemotherapy; *, $P = 0.002$ compared with no amenorrhea.

## Discussion

In women receiving chemotherapy for breast cancer, AMH dramatically falls, and is followed by a progressive recovery after treatment. As far as is known, we report one of the largest studies of AMH patterns of change during and after chemotherapy for breast cancer. As described by many investigtaors, patients undergoing chemotherapy experienced a fall in AMH levels (Anders et al., 2008; Anderson and Cameron, 2011; Anderson et al., 2006; Lutchman Singh et al., 2007; Partridge et al., 2010; Reh et al., 2008; Rosendahl et al., 2008; Su et al., 2010; Yu et al., 2010) during cytotoxic treatment (**Supplementary Table S2**). Anderson et al. (2006) first showed that AMH concentration during breast cancer chemotherapy showed a rapid and marked fall during chemotherapy, with undetectable concentrations in many women. These findings were confirmed by Lutchman Singh et al. (2007), who found significant differences between basal and post-chemotherapy AMH compared with controls. In case-control studies, it has also been reported that breast cancer survivors had a lower AMH than age-matched controls (Partridge et al., 2010; Su et al., 2010). In the study of Yu et al. (2010), AMH decreases rapidly and dramatically, but neither baseline nor change in AMH were predictive of the return of a menstrual function. Our study adds strength to previous studies because it included a large, young study population presenting concerns about reproductive needs. The baseline AMH correlation to 12 months amenorrhoea is consistent with the work of Su et al. (2010), who reported that pre-chemotherapy AMH and Inhibin B (Anders et al., 2008) were lower among women experiencing chemotherapy-related amenorrhoea. Similarly, Henry et al. (2013) recently showed that, in 26 women, a detectable serum AMH was predictive of recovery of ovarian function. Another study also showed that high pre-treatment AMH levels were predictive of a higher post-treatment AMH level (Anderson and Cameron, 2011).

The predominant finding of the current study is the long-term reincrease of AMH levels, described for the first time in a large cohort including only women with breast cancer. It was observed that the fall in AMH during chemotherapy was a reversible phenomenon, although AMH levels after chemotherapy did not return to pre-chemotherapy levels in any of the women during the course of the study. As AMH declines with advancing age, such a rise was not expected. This finding is consistent with previous reports in other malignancies. Rosendahl et al. (2010) reported on a cohort of 17 patients receiving chemotherapy (including eight for breast cancer), and observed an increase in AMH levels starting 8 months after chemotherapy. Decanter et al. (2010) showed similar results in 30 young women (median age 24 years) receiving chemotherapy for lymphoma. In women receiving the adriamycin, bleomycin, vinblastine and dacarbazine protocol, the AMH levels increased as early as 1 month after completing chemotherapy. Although uncertainties over the interpretation of AMH levels before puberty remain, Brougham et al. (2012) found that, in 22 pre-pubertal and pubertal girls, a progressive decrease in AMH during chemotherapy, followed by a recovery after completion of treatment, occurred in low to medium risk groups. In the group classified as high-risk of gonadotoxicity, AMH became undetectable in all patients and showed no recovery. In the present study, a lower AMH

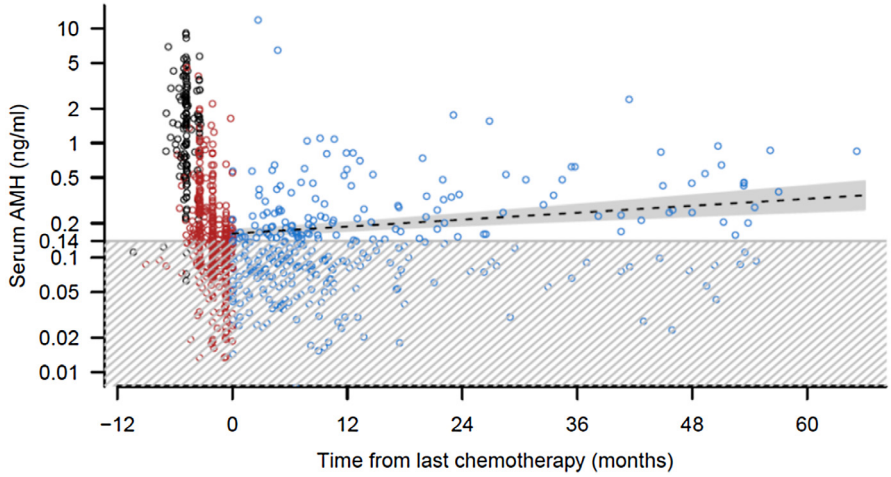


**Figure 2** The black points represent baseline values measured before chemotherapy; the red points are values measured during chemotherapy, and the blue ones are values measured after chemotherapy. The dashed line represents the average increase after chemotherapy and the grey area is the 95% credibility interval. The horizontal grey line shows the limit of detection of Anti-Müllerian hormone. The shaded area represents the values of an Anti-Müllerian hormone below the 0.14 threshold. AMH = Anti-Müllerian hormone.

recovery was associated with older age and 12 months of amenorrhoea, but not with any of the chemotherapy conditions, doses or regimen used in breast cancer.

A limitation of our work must be underlined. Menstrual status was documented retrospectively, and may therefore have been biaised. As the main outcome of the study was biological, and the sera were prospectively frozen, this point does not impair the main result of the study.

The clinical significance of AMH recovery remains unknown. To the best of our knowledge, the predictive values of AMH recovery on fertility after chemotherapy have not been reported. A large body of literature has demonstrated that AMH is a better marker of ovarian reserve than FSH, ostradiol, inhibin B in assisted reproductive technologies, predicting both over and poor response to ovarian stimulation (La Marca et al., 2010) (Nardo et al., 2009). Controversies over its widespread use in community practice for other indications (including the use of AMH to predict ovarian ageing and long-term fertility outside the IVF setting, its use in oncofertility and in polycystic ovary syndrome screening) have emerged (Loh and Maheshwari, 2011; Nelson et al., 2012). We believe that the yield of a single AMH assay during and after chemotherapy for breast cancer is little informative. As AMH may re-increase over time, this assessment does not accurately reflect the primordial follicle pool, but probably the follicles re-entering the growing pool on cessation of the toxic drug.

**Table 2** Association of factors with anti-Müllerian hormone recovery.

| *Effect* | *Mean coefficient* | *95% credibiltiy interval* |
|---|---|---|
| Overall time effect (per year) | 0.14 | 0.09 to 0.19 |
| Effect modifier (interaction) | | |
| Age (per year) | −0.02 | −0.05 to −0.001 |
| Taxane-containing regimen[a] | −0.06 | −0.17 to 0.05 |
| Total dose of cyclophosphamide (per g) | −0.01 | −0.07 to 0.05 |
| Number of chemotherapy cycles ≥8[b] | −0.06 | −0.17 to 0.06 |
| Amenorrhea at 6 months[c] | −0.12 | −0.25 to 0.01 |
| Amenorrhea at 12 months[d] | −0.13 | −0.25 to -0.01 |
| Baseline AMH ≥2 ng/ml[e] | 0.10 | −1.83 to 2.05 |
| Rate of AMH decrease during chemotherapy[f] | 0.07 | 0.03 to 1.32 |

Results are regression coefficients in longitudinal tobit models of Anti-Müllerian hormone (AMH) (as logarithm) with their 95% credibility interval.
Reference categories are as follows:
[a]Chemotherapy regimen without taxanes.
[b]Number of chemotherapy cycles less than eight.
[c,d]No amenorrhea.
[e]Baseline AMH <2 ng/ml.
[f]Rate of AMH decrease by month of chemotherapy.

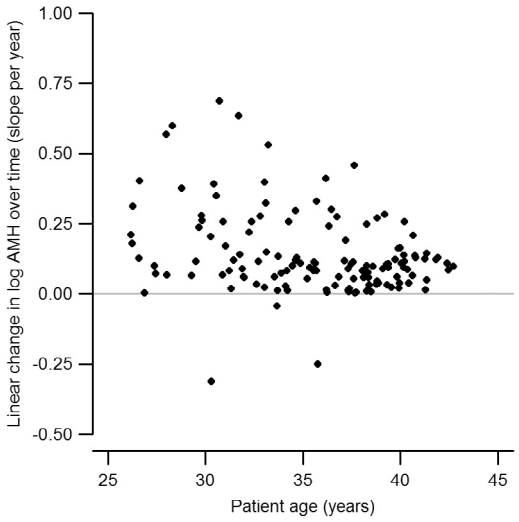


**Figure 3** Anti-Müllerian hormone (AMH) changes over time (log scale, slope per year), as a function of patient age.

Multiple longitudinal assessments could be of interest, but uncertainties remain about whether those assays should be performed and, if so, the time and frequency of when they should be performed and their interpretation and implication for patient counselling. Some investigators have established that, in oncology, AMH levels, even correlated to other hormonal parameters, do not accurately reflect ovarian activity (Dieudonné et al., 2011) and should not be used to assess menopausal status.

The precise mechanism by which ovarian damage occurs (detailed in a review by Morgan et al. (2012) is not clearly understood. The oocyte, or most likely the dividing granulosa cells, could be the target of chemotherapeutic agents because chemotherapy drugs act with particular cytotoxicity to dividing cells. The rapid fall of AMH during chemotherapy reflects the acute loss of growing follicles, translating clinically into acute amenorrhea. The restoration and recruitment of a new pool of primordial follicles unaffected by treatments (when it occurs) may explain the reincrease in the AMH level and the clinical ovarian function recovery. This phenomenon might not likely be a total recovery, as some of these women may experience premature ovarian failure (Partridge et al., 2007). It is also possible that a renewal of the primordial follicle pool exists after chemotherapy. Powell (2012) recently isolated oogonial stem cells in human ovaries. Further research is needed to improve both knowledge in cellular mechanisms and ovarian toxicity evaluation, before we can accurately prevent fertility chemotherapy induced-damages, and tailor fertility preservation options for patients requiring them. Prospective works assessing ovarian reserve markers before chemotherapy and during follow up and correlating them to menstrual patterns and reproductive outcomes should therefore be encouraged.

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## Appendix: Supplementary material

Supplementary data to this article can be found online at doi:10.1016/j.rbmo.2014.07.008.

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