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ARTICLE

Blood anti-Müllerian hormone is a possible determinant of recurrent early miscarriage, yet not conclusive in predicting a further miscarriage

BIOGRAPHY

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KEY MESSAGE

Although an altered ovarian reserve might contribute to unexplained recurrent early miscarriage, AMH measurement is less accurate than age or the number of previous losses in prediction of a further loss.

ABSTRACT

Research question: Is blood anti-Müllerian hormone (AMH) concentration a strong determinant of unexplained recurrent early miscarriage (REM)?

Design: In the first part of the study, AMH concentrations measured using an Immunotech ELISA Kit were compared between 188 unselected (mostly fertile) women consecutively referred for three or more miscarriages in the first trimester of pregnancy and 376 age-matched parous women without pregnancy loss. Cases and controls were previously enrolled in an incident case–control study on thrombophilic mutations. Blood samples were collected >2 months after any recognized obstetric event or hormonal treatment. In the second part of the study, a prospective 2-year follow-up of cases was performed.

Results: When considering all women irrespective of age, AMH concentration did not significantly differ between cases and controls. However, in the subgroup ≥25 years old (176 cases versus 358 controls of ~33.5 years), the cases had significantly lower AMH concentrations than the controls (median [interquartile range]: 2.8 [1.4–4.7] versus 3.25 [1.7–5.5], $P = 0.046$) and the proportion of cases with an AMH concentration <1 ng/ml was significantly higher (17.6% versus 10.6%; odds ratio 1.80; 95% confidence interval 1.07–3.00, $P = 0.028$). With regard to the subsequent pregnancy, AMH concentration was not correlated with either the conception delay or the miscarriage occurrence. However, increased age and number of previous miscarriages were significantly predictive of a subsequent miscarriage ($P = 0.046$ and 0.03, respectively).

Conclusion: An altered ovarian reserve is a possible determinant of unexplained REM. However, AMH blood concentration predicts neither the delay nor the outcome of a subsequent pregnancy.

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KEYWORDS

Anti-Müllerian hormone
Case–control study
Fertile women
Ovarian reserve
Prospective cohort study
Recurrent miscarriage

INTRODUCTION

Recurrent early miscarriage (REM) is currently defined by three or more consecutive losses occurring before 12 weeks' gestation (Delabaere *et al.*, *2014*). REM affects 1–2% of couples trying to have children. Standard investigations fail to reveal any well-recognized cause in more than 50% of these couples (*Branch et al., 2010*).

Ovarian dysfunction, through either oocyte quality or ovarian hormone production, has been proposed as a possible underlying mechanism of REM. In particular, a reduced ovarian follicle pool is suspected to be associated with REM. Increased maternal age is characterized by a quantitative alteration of the ovarian reserve and is also a well-known determinant of REM (*Brigham et al., 1999*) suggesting a combined quantitative and qualitative alteration of the ovarian reserve. Nevertheless, although chromosomal aberrations of the abortion product increase with maternal age (*Pellestor et al., 2003*), the exact relationship between those qualitative alterations and the quantitative ovarian reserve status still remains to be clarified (Jiang *et al.*, *2018; Plante et al., 2010*) especially in women aged <35 years old.

In addition to antral follicle count (AFC) by ultrasound, ovarian reserve can be quantitatively assessed by measurement of FSH, inhibin B and anti-Müllerian hormone (AMH) blood concentrations (*Broer et al., 2014*). AMH blood concentration is strongly correlated with AFC (*Hansen et al., 2011*), but discordance has been reported. Patient-specific features (e.g. polycystic ovary syndrome [PCOS], age, body mass index [BMI], altered folliculogenesis or steroidogenesis) may contribute to the differences between measured serum AMH concentrations and AMH values expected from the corresponding AFC, suggesting that AMH might also be a qualitative follicle marker (*Alebic et al., 2018*). AMH measurement can be easily obtained from a blood sample collected whatever the day in the menstrual cycle. In the female fetus, AMH is secreted by the ovarian granulosa from 36 weeks' gestation. Increase in plasma AMH concentration reaches a first peak between 7 and 10 years old and a second one between the ages of 20 and 25 (*Kelsey et al., 2011*).

Decrease in plasma AMH concentration begins around 25 years old and AMH becomes undetectable before menopause (*Freeman et al., 2012*). Thus, after 25 years old, the AMH blood concentration appears to be a more reliable marker of the ovarian pool size than age. Moreover, the decline in AMH concentration precedes the increase in FSH and inhibin concentrations and can be regarded as an early predictor for progressive reduction of the ovarian reserve. Nevertheless, a recent individual patient data meta-analysis (*Depmann et al., 2018*) reported only a slight added value of AMH combined with age as a predictor of menopause. Although the authors confirmed that AMH was a significant predictor of time to menopause (especially time to early menopause), an individual prediction of age at menopause was not accurate enough to be routinely used in clinical practice.

In infertile women, several studies (*Bishop et al., 2017; Sahu et al., 2010*; Tarasconi *et al.*, *2017*; *Tremellen and Kolo, 2010*) have assessed whether quantitative markers of the ovarian reserve would predict the occurrence of a miscarriage and a live birth after intrauterine insemination or IVF. They reported discrepant results. Although those markers, especially serum AMH concentrations, help to predict the intensity of ovarian response to ovarian stimulation, they do not seem to reflect oocyte quality.

Among 348 non-selected (mostly fertile) women, age-adjusted AMH concentrations were retrospectively correlated with reproductive outcomes, and no specific AMH-related pattern was suggested (*La Marca et al., 2012*). In the same way, *Zarek et al. (2016)* did not show any association between AMH concentration and further pregnancy loss in fertile women with a history of one or two previous losses. In contrast, Schumacher *et al.* (2018) found that fertile women with very low blood AMH concentrations (≤0.4 ng/ml) had a 2.3-fold increased risk of miscarriage compared with women with AMH concentrations ≥1 ng/ml. However, they did not observe an increased risk of miscarriage for women with AMH concentrations between 0.4 and 0.7 ng/ml (Schumacher *et al.*, 2018). This is consistent with the findings in infertile women and further supports the controversial role of blood AMH concentration as an accurate marker of oocyte quality in unselected fertile women.

What about women with REM? Lower plasma concentrations of oestradiol and higher plasma concentrations of FSH at Day 3 of the menstrual cycle were reported in women with previously unexplained REM, compared with women with well-identified causes of REM (*Trout and Seifer, 2000*), suggesting a reduced ovarian reserve. Before this study was designed, only one study had reported a comparison of AMH concentrations between 34 fertile women with REM and 10 controls. No significant difference was found (*Prakash et al., 2006*). However, in two recent studies, ~70 cases with unexplained REM displayed significantly lower AMH blood concentrations than those of 70 healthy controls seeking contraception (prospective Turkish cohort) (*Atasever et al., 2016*) or than those of 78 women with explained REM (retrospective study in Austria) (*Pils et al., 2016*).

Facing the above discrepant reports, this study raised the question of whether an altered ovarian reserve was a strong determinant of unexplained REM in unselected (mostly fertile) women. In the absence of an accurate qualitative marker of the ovarian reserve, it was decided to use blood AMH measurements in assessment of ovarian reserve among women with unexplained REM compared with age-matched parous controls. It was postulated that, unlike AFC, blood AMH could be used not only as a marker of ovarian reserve size but also of ovarian function/oocyte quality. All these women were previously enrolled in a master incident case–control study on thrombophilic mutations (*Pasquier et al., 2009*). The plan was also to study the prospective outcome of the subsequent pregnancy, according to the blood AMH concentration, in the cases.

MATERIALS AND METHODS

Study design

The study was organized in two parts: an incident case–control study and a prospective 2-year follow-up of cases (FIGURE 1).

Incident case–control study

A 1:2 paired incident case–control study was set up to compare blood AMH concentrations between women referred for unexplained REM, defined as three or

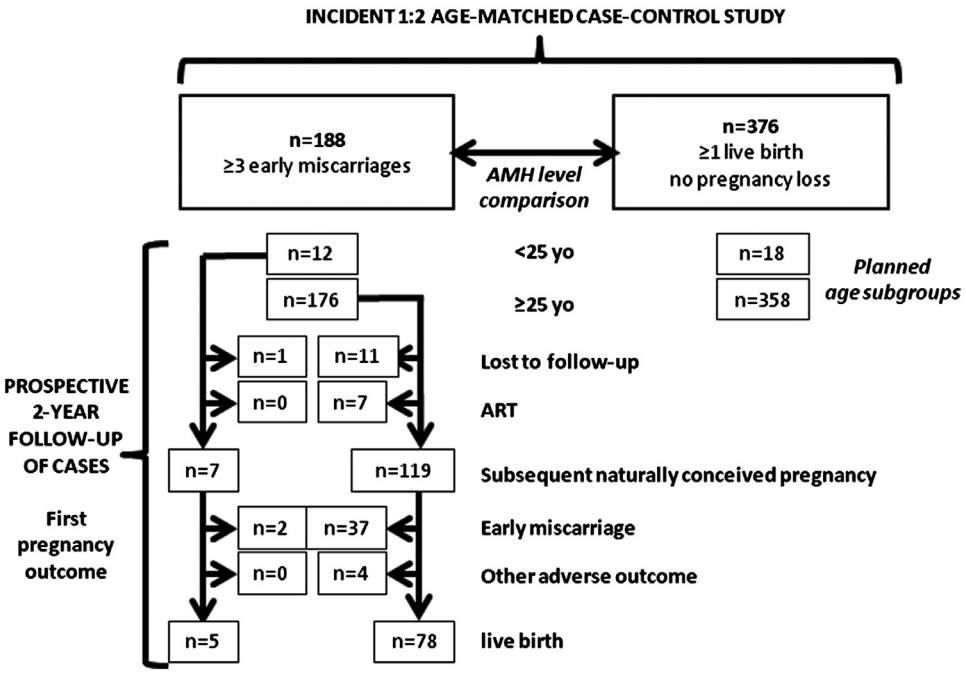

FIGURE 1 Flow chart of women included in the two parts of the study. Cases and controls enrolled in the incident 1:2 case–control study; subgroups according to age; cases enrolled in the prospective 2-year follow-up with outcome of the first further pregnancy. ART = assisted reproductive technology. Other adverse outcomes: late miscarriage (n = 2), ectopic pregnancy (n = 1), termination of pregnancy (n = 1).

more miscarriages in the first trimester of pregnancy (cases) and control women paired with respect to age, to within 1 year, with cases. The blood samples were collected randomly throughout the menstrual cycle, at least 2 months after any recognized obstetric event or hormonal treatment.

Both groups of women were enrolled from February 2003 to 2008 in the master study on thrombophilic mutations (*Pasquier et al., 2009*). In this previous incident case–control study on thrombophilic mutations, pregnancy loss was defined as two or more unexplained consecutive miscarriages with the same partner at or before 21 weeks of gestation, or at least one unexplained pregnancy loss after 21 weeks of gestation. Couples were seen in consultation by one of the physician investigators, who provided complete oral and written information. All couples gave their informed consent. Then the investigator conducted a standard questionnaire survey and venous blood sampling.

This ancillary study on AMH involves studying the whole subgroup of women with three or more previous miscarriages in the first trimester of pregnancy (REM). Two controls, the most comparable in regard to age, were selected for each case.

Prospective 2-year follow-up of cases

All the women who participated in the case–control study on thrombophilic mutations (incident case–control study) were secondly enrolled in a prospective cohort follow-up study. They were contacted by phone to collect new obstetric and medical events. In addition, all documents related to medical visits and hospitalizations were retrieved.

The plan was to prospectively seek a correlation between AMH concentrations and occurrence of a miscarriage in the subsequent pregnancy of REM cases (i.e. from the incident case–control study) during the first 2 years of follow-up.

Cases

All the cases were women from Brittany who were 18 to 45 years old. They were sent by their obstetricians and were consecutively seen in internal medicine consultation for a history of unexplained REM. Most cases were not followed up by a fertility care centre. REM was defined as three or more miscarriages in the first trimester of pregnancy. Exclusion criteria consisted of: a maternal or paternal carrier of a structural chromosomal rearrangement, maternal antiphospholipid antibodies, or any anatomical abnormality likely to be responsible for REM.

Controls

The controls were recruited during the same period, among women 18 to 45 years old registered on the local electoral lists. Women were potentially eligible if they had given birth to at least one living child. Exclusion criteria included pregnancy loss and preclinical miscarriages.

Use of assisted reproductive technology (ART) and PCOS were not considered as exclusion criteria for either cases or controls. Women were diagnosed as 'definite PCOS' when they met the internationally recommended criteria (*Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004*).

Samples

Five ml of blood was collected by venepuncture into a BD Vacutainer® SST gel tube (Becton-Dickinson, Franklin Lakes, NJ, USA) and then centrifuged at 2671*g* for 15 min at room temperature. The serum was then divided into aliquots of ~300 µl and stored at –80°C until use.

AMH measurements

In 2009, for both cases and controls, the measurement of AMH blood concentration was simultaneously carried out on 300 µl of serum bank using an

TABLE 1 BASELINE CHARACTERISTICS OF CASES AND CONTROLS

	Cases	Controls	*P*-value
n	**188**	**376**	
Age, years	32.9 ± 5.0	33.0 ± 4.6	NS
Median [min–max]	32.9 [18.8–44.8]	32.6 [18.5–44.7]	
≥25 years, *n* (%)	176 (93.6)	358 (95.2)	
Smoking, pack-years	4.8 ± 6.0	4.4 ± 6.0	NS
BMI, kg/m^2	23.0 ± 4.15	22.8 ± 4.5	NS
Menstrual cycle length, days	28.5 ± 3.0	28.8 ± 3.3	NS
Previous live birth, *n* (median [min–max])	0 [0–4]	2 [1–6]	<0.001
Age at the first obstetrical event, years	27.4 ± 5.2	26.1 ± 3.6	0.003
Assisted reproduction, *n* (%)	9 (4.8)	13 (3.5)	NS
Intrauterine inseminations, *n*	3	4	
IVF, *n*	6	9	
PCOS, *n* (%)	6 (3.2)	13 (3.5)	NS

Data are presented as mean ±SD unless otherwise stated.

BMI = body mass index; NS = not statistically significant; PCOS = polycystic ovary syndrome.

IMMUNOTECH® ELISA Kit (Beckman Coulter, Villepinte, France).

Statistical analysis

Statistical analysis was performed using SPSS Statistics for Windows, Version 19.0 (IBM Corp., Armonk, NY, USA).

Firstly, the plan was to compare AMH blood concentrations between cases and controls regardless of age. It is worth noting that no sample size calculation was performed: before this study was designed, only one study had reported similar AMH concentrations between 34 REM women and 10 controls (*Prakash et al., 2006*).

A model of AMH concentration from conception to menopause clearly demonstrated an AMH peak at ~25 years old, after a rise and before a decline in AMH concentrations (*Kelsey et al., 2011*). Consequently, the aim of this study was to check whether the distribution of AMH blood concentrations according to age also depicted a peak at ~25 years old. This would allow separate analyses on the subgroups of cases and controls <25 years old and ≥25 years old, when the sample size was sufficient (i.e. >30).

Student's *t*-test and chi-squared test were used for continuous variables and categorical variables, respectively. The odds ratio (OR) was calculated using logistical regression. $P < 0.05$ was considered statistically significant.

The OR were adjusted according to predefined variables (e.g. maternal age, smoking, BMI) and potential confounding variables ($P < 0.2$) as follows: number of previous live births, age at the first obstetrical event, use of ART and definite PCOS.

Lastly, for each case of natural conception, the delay and outcome of the subsequent pregnancy were studied (early miscarriage or not), occurring during the first 2 years of follow-up according to AMH concentration by Cox and logistic regression, respectively. Adjustment for age and number of previous losses was performed. Spline analyses and smaller subgroup analyses were also used to identify a possible clinical effect of AMH in specific age groups.

The study was approved by the local ethics committee, the CPP of Brest University Hospital (CPP Ouest-336) on 17 December 2002.

RESULTS

The AMH blood concentration was available for the 188 cases with REM and 376 age-matched controls, from the previous study on thrombophilic mutations (*Pasquier et al., 2009*). Except for parity and age at first obstetrical event, the baseline characteristics were similar between cases and controls (TABLE 1). It is worth noting that the age at inclusion, i.e. when blood was collected, was normally distributed and not statistically different between cases and controls (32.9 ± 5.0 vs 33 ± 4.6 years). The age at the first obstetrical event (loss or live birth) was slightly but significantly higher in cases compared with controls (27.4 ± 5.2 vs 26.1 ± 3.6 years, $P = 0.003$). The cases experienced between 3 and 17 early miscarriages. Of the 188 cases, 90 (47.9%) had a previous live birth. There was no significant difference in delivery term and weight at birth in comparison to the controls (data not shown).

Only 9 cases and 13 controls underwent ART. Indications for ART were male infertility (for 3 cases and 3 controls), Fallopian tube impairment (for 1 case and 2 controls), endometriosis (for 2 controls), and unknown or multifactorial (for 5 cases and 6 controls). At least one previous conception without ART was recorded for all the 9 cases versus only 4 controls. Furthermore, 6 cases and 13 controls had 'definite PCOS'.

The distribution of AMH concentrations in the serum of cases and parous control women was not normal, ranging from 0.1 to 18.8 ng/ml (median [IR]: 2.9 [1.5–4.9]) for the cases and from 0.1 to 21.5 ng/ml (median [IR]: 3.0 [1.7–5.5]) for the controls. AMH concentrations did not significantly differ between cases and controls. Among the 188 cases 31 (16.5%) had an AMH concentration <1 ng/ml in comparison to 40 (10.6%) controls among 376 (OR 1.66; 95% CI 1.00–2.75, $P = 0.059$), which failed to reach significance. Adjusting for maternal age, age at the first obstetrical event, smoking, BMI and parity did not change the results.

Exclusion of the women with 'definite PCOS' from the analysis did not change the results. Given that the estimated prevalence of PCOS is around 15%, and that the Beckman Coulter first-generation test is known to give higher values than the other tests available, the 85th percentile (>6.87 ng/ml) of the distribution of AMH concentrations was then looked for in the 574 women. Fifty-five controls and 28 cases had AMH concentrations above the 85th percentile. Although the ovarian morphology was often lacking, especially for controls, most of these women (45 controls and 21 cases) had no clinical evidence of PCOS. Exclusion of these

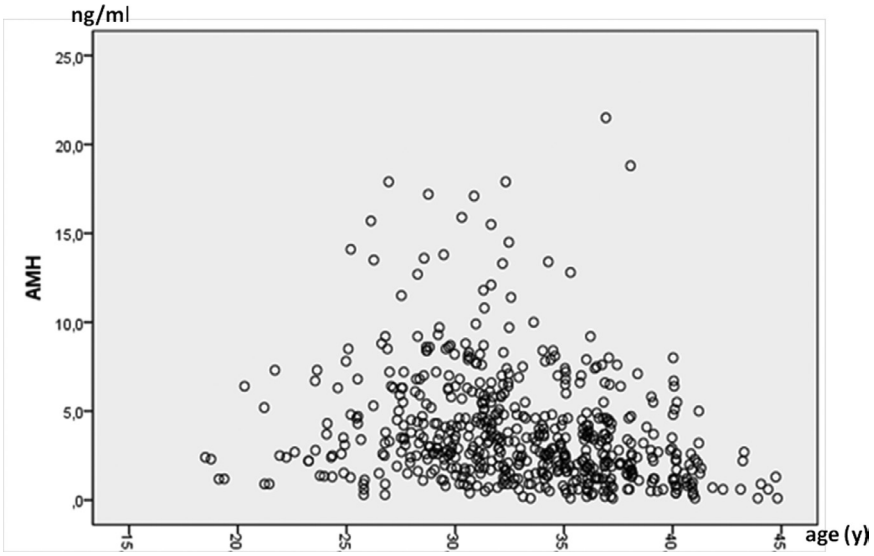

FIGURE 2 Distribution of anti-Müllerian hormone (AMH) blood concentrations (ng/ml) according to age (years) among cases and controls.

women from the analysis did not change the results.

Among cases, AMH concentrations were significantly lower in the 6 cases who underwent IVF compared with the other cases (P = 0.040). Among controls, the AMH concentrations were not different in women who underwent ART compared with the other controls. As expected, after exclusion of these ART women from analysis, AMH concentrations still did not differ between cases and controls.

Subgroup analyses

The distribution of AMH concentrations according to age is shown in FIGURE 2.

Subgroup of women <25 years old

Only 12 cases and 18 controls were <25 years old (TABLE 2).

Subgroup of women ≥25 years old

The analysis of the female subgroup ≥25 years old (176 cases of 33.5 ± 4.4 years vs 358 controls of 33.5 ± 4.1 years) showed significantly lower AMH concentrations in cases than in controls (P = 0.046, TABLE 2). Adjusting for maternal age, age at the first obstetrical event, smoking, BMI and parity did not change the results (P = 0.030). Moreover, 31 cases (17.6%) had an AMH concentration <1 ng/ml in comparison to 38 (10.6%) controls (OR 1.80; 95% CI 1.07–3.00, P = 0.028). Exclusion of 'definite PCOS' women or exclusion of cases (n = 25) and controls (n = 55) with AMH concentrations ≥85th percentile did not change the results (P = 0.030 and P = 0.021, respectively). Moreover, AMH concentrations were significantly lower in cases compared with controls (P = 0.039) when adjusting for 'definite PCOS'.

When excluding the 22 women who underwent ART, the AMH concentrations were 3.6 ± 3.0 and 4.1 ± 3.4 ng/ml for cases and controls, respectively. Although the absolute difference between cases and controls was similar to that before exclusion of this group, this difference was no longer statistically significant, thus suggesting a loss of statistical power. Indeed, when using another approach to assess the impact of previous ART, i.e. adjusting for this variable, AMH concentrations were significantly lower in cases compared with controls (P = 0.046).

Prospective follow-up

The prospective outcome of a subsequent pregnancy was assessed for the cases ≥25 years old (n = 176) during the first 2 years of follow-up. Eleven cases were lost to follow-up. A subsequent naturally conceived pregnancy was observed among 119 cases as follows: in 75, 108 and 115 cases, after 6, 12 and 18 months, respectively. Among these 119 cases, 37 suffered a further miscarriage in the first trimester of pregnancy whereas 78 had a live birth. Moreover, two women suffered a late miscarriage, one had an ectopic pregnancy and another had a termination of pregnancy (FIGURE 1).

AMH concentration regarded as a continuous or discrete variable, and set at different cut-offs (quartiles of the whole distribution and <1 ng/ml), was not correlated with the delay to conception through Cox regression analysis (FIGURE 3).

Adjusting for age, number of previous miscarriages, BMI, smoking or parity did not change the results.

AMH concentration regarded as a continuous variable was not correlated with the occurrence of a miscarriage (OR 1.01; 95% CI 0.88–1.15). Adjusting for maternal age and the number of previous miscarriages did not change the results, although both variables (i.e. age and number of previous miscarriages) were positively and significantly associated with a subsequent miscarriage (OR 1.11; 95% CI 1.002–1.22, P = 0.046 and OR 1.51; 95% CI 1.04–2.20, P = 0.03, respectively). Moreover, the association of a subsequent loss with blood AMH concentrations set at different cut-offs (quartiles of the whole distribution and <1 ng/ml) was not statistically significant (AMH <1 ng/ml: OR 0.94; 95% CI 0.32–2.76). In addition, using spline analyses and smaller subgroup analyses, the study did not identify a clinical effect of AMH in any specific age groups.

Lastly, some patients were given treatments (progesterone and/or antithrombotic) for pregnancy maintenance. Nevertheless, the occurrence of a subsequent miscarriage was not significantly associated with either progesterone intake or enoxaparin injections. It is worth noting that progesterone was never introduced during the periconceptional period but always after delayed menses.

TABLE 2 SUBGROUP RESULTS ACCORDING TO AGE ≥25 OR <25 YEARS

Women aged ≥25 years

	Cases	Controls	*P*-value
n	176	358	
Age, years	33.5 ± 4.4	33.5 ± 4.1	NS
Median [min–max]	33.3 [25.0–44.8]	33.3 [25.0–44.7]	
AMH, ng/ml	3.5 ± 3.0	4.1 ± 3.4	0.046
Median [IR]	2.8 [1.4–4.7]	3.25 [1.7–5.5]	0.028
<1 ng/ml, *n* (%)	31 (17.6)	38 (10.6)	

Women aged<25 years

	Cases	Controls
n	12	18
Age, years	23.0 ± 2.0	22.7 ± 2.0
Median [min–max]	23.7 [18.8–24.9]	23.3 [18.5–24.9]
AMH, ng/ml	5.0 ± 2.25	2.1 ± 0.9
Median [IR]	5.75 [2.75–7.15]	2.3 [1.3–2.6]

Data are presented as mean ±SD unless otherwise stated.

AMH = anti-Müllerian hormone; IR = interquartile range; NS = not statistically significant.

DISCUSSION

Taking into account all women regardless of age, AMH blood concentrations were not significantly different between the 188 REM cases and the 376 healthy controls.

The distribution of AMH concentrations according to age demonstrated a peak at ~25 years old, in agreement with the data reported by *Kelsey et al. (2011)*. Among the 534 women who were ≥25 years old (33.5 ± 4.0), statistically significantly lower AMH concentrations were found in cases compared with controls (median 2.8 vs 3.25 ng/ml, $P = 0.046$) and the proportion of cases with an AMH concentration <1 ng/ml was significantly higher than in controls (17.6% vs 10.6%; OR 1.80; 95% CI 1.07–3.00, $P = 0.028$). Exclusion of women with 'definite PCOS' or very high concentrations of AMH did not modify the results. In contrast, exclusion of the 22 ART women among cases and controls resulted in the loss of statistical significance of the difference in AMH concentrations. However, the difference in the values remained within the range of before exclusion results, suggesting a loss of statistical power. Indeed, when adjusting for a previous ART, AMH concentrations remained significantly lower in cases compared with controls ($P = 0.046$). The small sample size of the subgroup of women <25 years old did not allow data interpretation.

Regarding the prospective follow-up of cases, it is noteworthy that AMH concentrations were not correlated with the occurrence of a subsequent miscarriage in the first 2 years after blood collection, irrespective of age subgroups. However, consistent with the literature (*Brigham et al., 1999*), the two strong determinants of miscarriage, i.e. woman's

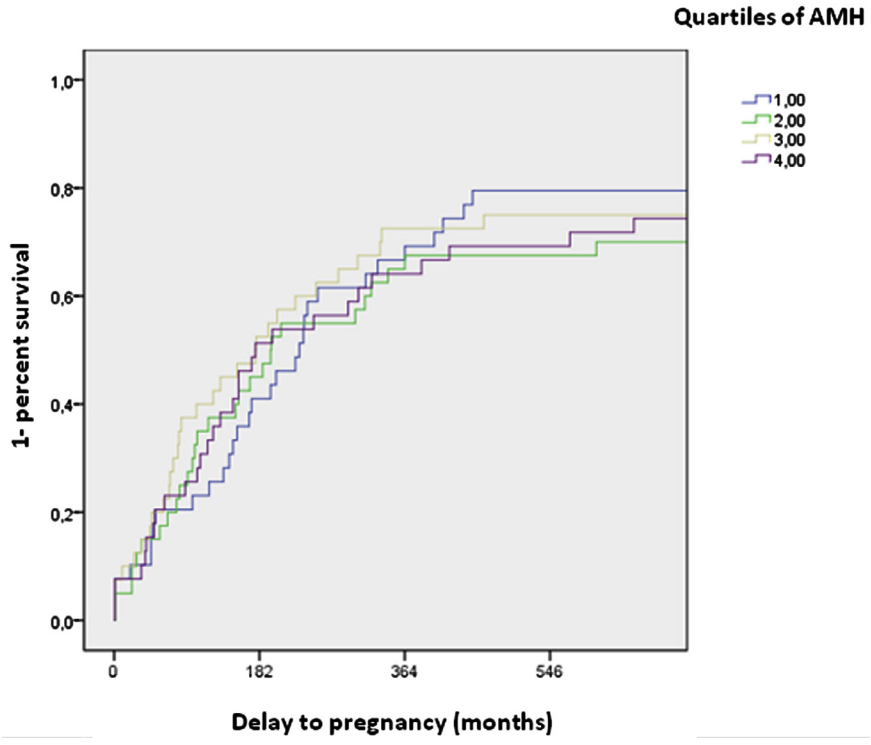

FIGURE 3 1–survival curves of the cases ($n = 176$) aged ≥25 years, according to quartiles of AMH distribution (quartile 1 ≤1.475, 1.475 < quartile 2 ≤ 2.85, 2.85 <quartile 3 ≤4.825 ng/ml, quartile 4 >4.825, unit: ng/ml). Cox regression, global *P* not significant.

age and number of previous miscarriages, were positively and significantly associated with a subsequent loss.

Thus, among the ≥25-year-old women, although clinically less meaningful, the results of this incident case–control study are in agreement with the two more recently reported case–control studies (*Atasever et al., 2016; Pils et al., 2016*), which showed significantly lower serum AMH concentrations in cases compared with controls despite smaller samples. In the prospective Turkish cohort (*Atasever et al., 2016*), the AFC, usually well correlated with AMH concentration (*Hansen et al., 2011*), was not different between cases and controls. A separate analysis of possible correlation between AFC and AMH according to age (<25 and ≥25 years old) would have been attractive in the study by *Atasever et al. (2016)*. In the other study (*Pils et al., 2016*), as the cases were slightly older, although not significantly, than the controls (median [IR]: 33 [28–38] vs 33 [28–36]), perhaps adjusting AMH concentration for age would have been appropriate.

In addition to the large sample of cases and controls, the other strength of this study was the bias-free recruitment of controls from the electoral lists in parallel with that of the cases, from the same geographic area and at the same time period. The controls had on average two healthy children, no history of pregnancy loss and were matched with cases by age within 1 year. Moreover, this is thought to be the first time that a correlation between blood AMH concentrations and subsequent pregnancy outcomes was sought in a prospective follow-up of fertile women with REM. One distinctive feature of this study was the characteristics of the enrolled cases: unselected women (mostly fertile: ~96%) referred to an internal medicine consultation and not to a fertility centre. The limitation of the study comes from the fact that infertile women or those with PCOS were not excluded from the master study on thrombophilic mutations (*Pasquier et al., 2009*). However, adjustments for these confounding variables and sub-analyses made it possible to avoid those possible biases. Moreover, this study was not designed to assess ovarian reserve using AFC due to ethics considerations for control women. Lastly, the results of AMH blood concentrations (measured using a first-generation Beckman Coulter ELISA Kit) cannot be compared with those of the literature. Indeed, the ELISA Kits used for AMH measurements were different across studies (i.e. a second-generation Beckman Coulter ELISA Kit [*Pils et al., 2016*] or an ELISA Kit from YH Biosearch [*Atasever et al., 2016*] vs a first-generation Beckman Coulter ELISA Kit [in this study]).

In brief, significantly lower AMH blood concentrations were observed among women ≥25 years old with unexplained REM, through comparison with age-matched well-selected controls. Nevertheless, the concentrations were most often within the normal range and, regarding the proportion of women with blood AMH<1 ng/ml, the OR was <2. Given that in this age subgroup the AMH concentration is well correlated with the ovarian reserve size, and consistent with other studies (*Atasever et al., 2016; Pils et al., 2016*) it cannot be ruled out that a relatively lower ovarian reserve could contribute to occurrence of unexplained REM among women ≥25 years old. However, if the sample size calculation had been based on those two recently reported studies, this study would have enrolled ~70 cases and controls and probably would not have found any significant association between AMH concentration and REM. Furthermore, the follow-up results demonstrated that unlike maternal age and number of previous miscarriages, AMH was not a predictor of REM. These findings were consistent with those reported by a very recent retrospective cohort study among women with idiopathic recurrent miscarriage (Pils *et al.*, *2019*). Nevertheless, the results of the Pils *et al.* (*2019*) study could be regarded as less conclusive due to its retrospective design.

In conclusion, this study suggests that an altered ovarian reserve should be regarded as one among other determinants of unexplained REM. However, AMH concentration measurement is not a useful tool in prediction of a subsequent pregnancy outcome in women with previously unexplained REM.

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