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Original article

Low ovarian reserve and risk of miscarriage in pregnancies derived from assisted reproductive technology

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ABSTRACT

STUDY QUESTION: Do low levels of anti-Müllerian hormone (AMH) or antral follicle count (AFC) properly predict miscarriage in young women conceiving with ART?

SUMMARY ANSWER: Low ovarian reserve, as indicated by AMH or AFC, is not associated with miscarriage in young women conceiving with ART.

WHAT IS KNOWN ALREADY: Presently, the impact of low ovarian reserve on the risk of miscarriage remains controversial. Some studies have reported an association between serum AMH levels and AFC and miscarriage, but others have failed to confirm these findings. The main limitation that undermines the reliability and consistency of the results is the confounding effect of female age. Indeed, after 35 years of age, on the one hand, the risk of miscarriage starts increasing because of impaired oocyte quality while, on the other, the physiological decline in AMH and AFC levels continues, thus hampering the possibility to properly explore the real effects of reduced ovarian reserve. Indeed, the two processes, i.e. the gradual loss of resting primordial follicles and the loss of oocyte quality, progress in parallel. In other words, the older the woman becomes, the higher is the risk of miscarriage, but one cannot distinguish between the effects of biological aging on oocyte quality and those mediated by a lower ovarian reserve.

STUDY DESIGN, SIZE, DURATION: The present retrospective monocentric cohort study was carried out at Fondazione IRCSS Ca Granda Ospedale Maggiore Policlinico, Milan. All women referred to the ART Unit between 2014 and 2021 and who underwent either conventional IVF (c-IVF), ICSI, or IUI were reviewed. Only women younger than 35 were eligible because, up to this age, the risk of miscarriage is steady and not strictly related to age.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Women younger than 35 who achieved a singleton clinical pregnancy with c-IVF, ICSI, or IUI were selected. Women with patent causes of recurrent miscarriage were excluded, as well as those undergoing pregnancy termination for fetal or medical causes. Women who did and did not have a pregnancy loss before 20 weeks' gestation were compared. Detailed information was obtained from charts of the consulting patients. ART procedures were performed according to the standardized policy of our Unit. All women underwent serum AMH measurement and a transvaginal assessment of AFC prior to initiation of treatment. AMH levels were measured by a commercially available ELISA assay. To assess AFC, all identifiable antral follicles 2–10 mm in diameter at ultrasound were recorded. The primary outcome was the risk of miscarriage for women with serum AMH levels below 5 pmol/l.

MAIN RESULTS AND THE ROLE OF CHANCE: There were 538 women were included, of whom 92 (17%) had a miscarriage. The areas under the ROC curves for prediction of miscarriage based on AMH levels and AFC were 0.51 (95% CI: 0.45–0.58) and 0.52 (95% CI: 0.45–0.59), respectively. The odds ratio (OR) of miscarriage for women with serum AMH levels below 5.0 pmol/l was 1.10 (95% CI: 0.51–2.36); the adjusted OR was 1.12 (95% CI: 0.51–2.45). Analyses were repeated considering other thresholds for AMH (2.9, 3.6 and 7.9 pmol/l) and for AFC (thresholds of 7 and 10). No associations emerged.

LIMITATIONS, REASONS FOR CAUTION: The retrospective design of the study hampered the collection of more precise but potentially relevant clinical information of the couples. We did not exclude women suffering from PCOS, a condition possibly associated with miscarriage. Moreover, the baseline characteristics of women who did and did not have a miscarriage differed in some characteristics. Thus, we adjusted the OR using a multivariate analysis, but we cannot fully exclude residual confounding effects. Finally, our results cannot be inferred to women older than 35. The mechanisms causing premature exhaustion of ovarian reserve may be different in younger and older women and this may lead to a different impact on the risk of miscarriage.

WIDER IMPLICATIONS OF THE FINDINGS: Women embarking on ART with low ovarian reserve should be informed of their likely poor response to ovarian stimulation but can be reassured that, if conception occurs, their risk of miscarriage is not increased.

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WHAT DOES THIS MEAN FOR PATIENTS?

The impact of low ovarian reserve on the risk of miscarriage remains controversial. Serum anti-Müllerian hormone (AMH) and ultrasound assessment of antral follicle count (AFC) are both biomarkers commonly used to assess ovarian reserve. Some studies have reported an association between AMH and/or AFC and miscarriage, but others failed to confirm these findings. In this regard, female age represents the most important limitation for the reliability and consistency of the results. Indeed, after 35 years of age, the risk of miscarriage starts increasing because of impaired oocyte quality but, at the same time, there is a physiological decline in AMH and AFC levels. In other words, the older the woman, the higher the risk of miscarriage. In assisted reproductive technology (ART), however, the effects of biological aging on oocyte quality and those mediated by a lower ovarian reserve cannot be distinguished during ART treatments. To address this issue, we retrospectively selected young women who had a clinical pregnancy through ART and evaluated whether low levels of AMH or AFC could predict miscarriage. All women under 35 who were referred to our ART Unit and who underwent ART procedures were recruitable. This study included 538 women, of whom 92 (17%) had a miscarriage. No associations emerged in terms of AMH levels and AFC between women who did and did not have a pregnancy loss before 20 weeks' gestation. Despite some limitations, we can conclude that serum AMH levels are not associated with miscarriage in young women conceiving with ART. Young women embarking on ART with low ovarian reserve should therefore be informed of their poorer response to ovarian stimulation but can be reassured that, if conception occurs, their risk of miscarriage is not increased.

Introduction

The term 'ovarian reserve' defines the quantity of the remnant ovarian primordial follicular pool of a woman at a certain time in her life (Practice Committee of the American Society for Reproductive Medicine, 2015; Steiner *et al.*, 2017; Tal and Seifer, 2017). In clinical practice, an estimation of the ovarian reserve cannot be made by direct histological evaluation and is routinely performed through indirect assessments. The serum anti-Mullerian hormone (AMH) concentration and transvaginal ultrasound (US) assessment of the antral follicle count (AFC) are considered the most accurate indicators of ovarian reserve (Dewailly *et al.*, 2014). Notably, robust evidence demonstrates their ability to predict oocyte yield as well as poor and high responsiveness to ovarian stimulation in ART cycles (Broer *et al.*, 2013; Iliodromiti *et al.*, 2014). On the other hand, both AMH and AFC assessments are not considered to be reliable in predicting the chance of natural pregnancy and have only a weak association with the most relevant ART outcomes, such as clinical pregnancy and live birth rates (Zarek *et al.*, 2015; Steiner *et al.*, 2017; Practice Committee of the American Society for Reproductive Medicine, 2020). The inability of ovarian reserve biomarkers to predict qualitative reproductive outcomes has been challenged, in recent years, by several studies investigating their association with the risk of miscarriage (Busnelli *et al.*, 2021). Two meta-analyses on this issue have been published (Busnelli *et al.*, 2021, Bunnewell *et al.*, 2020). Busnelli *et al.* (2021) showed, in both unassisted and assisted conception settings, an association between low serum AMH concentrations and a higher miscarriage rate. Data pooling performed by Bunnewell *et al.* (2020) highlighted a potential association between diminished ovarian reserve (i.e. AMH $\leq$ 7.14 pmol/l or AFC $\leq$ 7) and higher risk of recurrent pregnancy loss (RPL), particularly in women with unexplained RPL (Bunnewell *et al.* 2020). On the other hand, a recent large nationwide study focussing on women with idiopathic early ovarian aging questioned these results (Christensen *et al.* 2022).

Although the above-mentioned data generally suggest a correlation between low values of noninvasive biomarkers of ovarian reserve and miscarriage, the available evidence cannot be considered conclusive. The main limitation that undermines the reliability of the results is the possible confounding effect of female age. Indeed, in most of the original studies, the mean age of women was not reported separately for each AMH or AFC category, considerably limiting the possibility of controlling this variable in the data synthesis (Busnelli *et al.*, 2021). Furthermore, only a minority of studies have evaluated outcomes in women younger than 35 separately (Busnelli *et al.*, 2021), a group of women less exposed to the confounding effect of aging. Indeed, after 35 years of age, on the one hand, the risk of miscarriage starts increasing because of impaired oocyte quality and, on the other, the physiological decline in AMH and AFC levels progresses (Franasiak *et al.*, 2014; Magnus *et al.*, 2019). Thus, in women over 35, it seems that the two processes, i.e. the gradual loss of resting primordial follicles and the loss of oocytes quality, progress in parallel.

To further investigate this controversial issue, we retrospectively selected women younger than 35 who had a clinical pregnancy with ART and evaluated whether low levels of AMH or AFC could predict miscarriage. This study aimed to clarify whether low biomarkers of ovarian reserve could be associated with miscarriage in young women.

Materials and methods

Patient selection

The present retrospective monocentric cohort study was carried out at Fondazione IRCSS Ca Granda Ospedale Maggiore Policlinico, Milan. All women who were referred to the ART Unit between 2014 and 2021 to undergo either conventional IVF (c-IVF), ICSI, or IUI were reviewed. Women were initially selected using the software Meditex (Regensburg, Germany). Detailed information was then obtained from the charts of the consulting patients. Inclusion criteria were as follows: (i) age $\leq$35 at the time of the oocyte retrieval or the IUI, (ii) BMI <30 kg/m^2, and (iii) demonstration at transvaginal ultrasound of an intrauterine gestational sac (with or

without a viable embryo). Biochemical pregnancies, ectopic pregnancies, and twin pregnancies were excluded. The former was excluded because of the impossibility of assessing the localization of the pregnancy (as one cannot rule out an ectopic pregnancy ending very early). Twin pregnancies were excluded because the interruption of one of the two pregnancies could negatively influence the outcome of the remaining one (Batsry and Yinon, 2022). Women who underwent pregnancy termination because of fetal abnormalities (aneuploidy, genetic disorders, or malformations) or other reasons were also excluded. Women with conditions known to be associated to an increased risk of miscarriage were also excluded; specifically, we excluded women with antiphospholipid antibody syndrome, untreated thyroid disorders, uncontrolled diabetes, altered karyotype, untreated uterine anomalies (such as submucosal fibroids, severe adenomyosis, and uterine malformations) (Munro *et al.*, 2018), and severe male factor infertility (total sperm count below 5×10^6/ml). Moreover, we excluded women whose available ovarian reserve tests were performed more than 12 months before the oocyte retrieval or the IUI leading to the pregnancy. Finally, we excluded women for whom pregnancy outcome could not be assessed. If women had more than one pregnancy during the study period, only the first one was considered. The study was approved by the local Ethical Committee (Milano Area 2). Informed consent was not requested because this is a retrospective study. However, all women referring to our unit provide an informed consent for their data to be used for research purposes and those denying this consent were excluded.

Clinical procedures

Intrauterine insemination, ovarian stimulation, oocyte retrieval, embryo transfer, and progesterone supplementation were performed as described in detail elsewhere (Ragni *et al.*, 2006; 2009; Cardellicchio *et al.*, 2017; Somigliana *et al.*, 2021). Herein, it is worth highlighting that according to the policy of our unit, all women scheduled for ART undergo serum AMH measurement and a transvaginal assessment of AFC. The commercially available Beckman Coulter Gen II ELISA assay on the automated GEMINI platform (STRATEC Biomedical AG, Germany) after dilution with assay buffer was used to measure AMH concentrations (Somigliana *et al.*, 2015). To assess AFC, all identifiable antral follicles 2–10 mm in diameter were recorded, as previously described (Benaglia *et al.*, 2015).

Clinical pregnancy was defined as the presence of an intrauterine gestational sac at first ultrasound (generally performed at 6–7 weeks' gestation). Miscarriage was defined as the spontaneous loss of pregnancy before 20 weeks of gestation (Allison and Schust, 2009). Information regarding pregnancy outcome of ART pregnancies was routinely collected during the study period, as requested by the local Italian legislation (information on live births must be provided to the Ministry of Health at least annually). With this aim, charts at the hospital were regularly consulted. If the information was not available or was incomplete, women were directly contacted by phone. Annual loss to follow-up was <2%.

Data analysis

According to the pregnancy outcome, the entire population was divided into two groups: spontaneous miscarriage when pregnancy terminated before the 20 weeks' gestation versus ongoing when pregnancies progressed beyond this limit. The primary outcome of the study was the risk of miscarriage in women with serum AMH below 5 pmol/l (0.7 ng/ml) (Steiner *et al.*, 2017). Hypothesizing a frequency of this condition of 10%, setting type I and II errors at 0.05 and 0.20, and claiming a two folds increase in the risk of miscarriage (corresponding to an odds ratio (OR) of 2.4) as clinically relevant, the required sample size was about 530 women. We estimated that by retrospectively including women back to 2014 could allow us to achieve this sample size. Secondary outcomes included the risk of miscarriage using other thresholds for AMH (2.9, 3.6, and 7.9 pmol/l, corresponding to 0.4, 0.5, and 1.1 ng/ml) and the use of a different biomarker of ovarian reserve, i.e. AFC (with the threshold of 7 and 10) (Ferraretti *et al.*, 2011; van Tilborg *et al.*, 2017; Lyttle Schumacher *et al.*, 2018).

The software Statistical Package for Social Sciences (SPSS, Chicago, IL, USA), version 27.0, was used to analyze the data. Chi-square and Mann–Whitney tests were run to investigate any statistical difference between the two groups. The c statistics was used to obtain a receiver operating characteristic (ROC) curve and its area. As regards the ovarian reserve biomarkers, the OR, and the relative 95% CI was calculated for the different tested thresholds. A logistic multivariate regression model was used to calculate the adjusted OR. Variables included in the model were woman's age, man's age, and woman's BMI, previous delivery(ies) and cause of infertility, and other variables found to differ ($P < 0.10$) at univariate basal comparisons. Adjustment was not made for previous miscarriage to avoid over-correction.

Results

The study included 538 women, of whom 446 (83%) had a live birth and 92 (17%) had a miscarriage. Baseline clinical characteristics of the whole cohort and according to pregnancy outcome are shown in Table 1. Statistically significant differences between women who did and did not have miscarriage emerged for the indication to ART and for the history of previous miscarriage. Endometriosis was less common and unexplained infertility was more common among women who had a miscarriage. A history of miscarriage was reported by 36% of women who experienced a miscarriage in the study period compared to 14% in those who did not. Conversely, age did not significantly differ between the two groups.

Biomarkers of ovarian reserve did not differ between women who did and did not have a miscarriage. The median [IQR] serum AMH levels were 19.6 [10.5–35.2] and 18.6 [10.0–32.0], respectively ($P = 0.70$). The median [IQR] AFCs were 13 [9–18] and 13 [8–17], respectively ($P = 0.53$). The ROC curves for the prediction of miscarriage based on AMH levels and AFC are shown in Fig. 1. The AUCs were 0.51 (95% CI: 0.45–0.58) and 0.52 (95% CI: 0.45–0.59), respectively.

The analyses were repeated excluding 75 subjects with a history of ovarian surgery or previous radio- or chemo-therapy. The median [IQR] serum AMH levels in women who did and did not have a miscarriage were 20.0 [11.1–37.5] and 19.3 [10.7–33.6], respectively ($P = 0.61$). The median [IQR] AFCs were 13 [8–18] and 14 [9–20], respectively ($P = 0.53$). The AUCs for AMH and AFC were 0.52 (95% CI: 0.45–0.59) and 0.52 (95% CI: 0.45–0.59), respectively. Separate analyses focusing on previously operated women (n = 72) or to those previously exposed to radio- or chemo-therapy (n = 3) also did not produce statistically significant results (data not shown).

Table 2 illustrates the results of the analyses using prespecified thresholds for ovarian reserve, i.e. 2.9 (n = 18), 3.6 (n = 27), 5.0 (n = 49), and 7.9 (n = 98) pmol/l for AMH and 7 (n = 85) and 10 (n = 193) for AFC. None of the thresholds identified a subgroup at increased risk of miscarriage. In particular, the OR of miscarriage in women with serum AMH levels below 5.0 pmol/l was 1.10 (95% CI: 0.51–2.36). The OR adjusted for woman's age,

Table 1. Baseline characteristics of the whole cohort, and according to pregnancy outcome.

Characteristics	Whole cohort n = 538	Pregnancy outcome		P-value
		Live birth n = 446	Miscarriage n = 92	
Female age (years)	33 [31–34]	33 [31–34]	34 [32–35]	0.07
Male age (years)	37 [34–40]	36 [34–40]	37 [33–40]	0.77
BMI (kg/m^2)	21 [19.5–23.1]	21.0 [19.5–23.1]	21.3 [20.0–23.3]	0.23
AMH (pmol/l)	18.8 [10.0–32.1]	18.6 [10.0–32.0]	19.6 [10.5–35.2]	0.70
AFC	13 [9–18]	13 [9–18]	13 [8–17]	0.53
Previous chemo/radiotherapy	3 (1%)	3 (1%)	0 (0%)	1.00
Previous ovarian surgery				0.97
Monolateral	49 (9%)	40 (9%)	9 (10%)	
Bilateral	23 (4%)	19 (4%)	4 (4%)	
Indication to IVF				0.02
Endometriosis	89 (17%)	81 (18%)	8 (9%)	
Unexplained	176 (33%)	136 (31%)	40 (43%)	
Tubal factor	77 (14%)	66 (15%)	11 (12%)	
Ovulatory disorder	29 (5%)	20 (5%)	9 (10%)	
Male factor	46 (9%)	39 (9%)	7 (8%)	
Mixed	102 (19%)	90 (20%)	12 (13%)	
Genetic	19 (3%)	14 (3%)	5 (5%)	
IVF technique				0.13
IUI	32 (6%)	24 (5%)	8 (9%)	
Fresh embryo transfer	246 (46%)	212 (48%)	34 (37%)	
Frozen embryo transfer	260 (48%)	210 (47%)	50 (54%)	
Previous miscarriages	95 (18%)	62 (14%)	33 (36%)	< 0.001
Previous live births	63 (12%)	55 (12%)	8 (9%)	0.38

Data are reported as median [interquartile range] or number (percentage).
Data were compared using Mann–Whitney or Chi-square tests.
BMI: body mass index; AMH: anti-Müllerian hormone; AFC: antral follicle count; IVF: *in vitro* fertilization; IUI: intrauterine insemination.

man's age, woman BMI, previous delivery and the cause of infertility was 1.12 (95% CI: 0.51–2.45).

All the analyses were repeated separately considering those undergoing IUI and c-IVF/ICSI, and the results were substantially similar (data not shown).

Discussion

The present study failed to show any relation between biomarkers of ovarian reserve and miscarriage in women younger than 35 conceiving with ART. The 95% CI of the OR of pregnancy loss in women showing serum AMH below 5 pmol/l (the primary aim of the study) included the unity, even when adjusting for woman's age, man's age, woman's BMI, previous delivery, and cause of infertility. Noteworthy, the use of other thresholds for the definition of low ovarian reserve (2.9, 3.6, and 7.9 pmol/l) or the use of AFC (threshold of 7 and 10) led to similar results. These negative findings are corroborated by the c-statistics. The ROC curves grossly overlap with the bisectrix for both biomarkers and the AUC was close to 0.5. These latter results tend also to rule out the possibility that other non-tested thresholds could be of some value.

Our results are in contrast with previous meta-analytic findings (Bunnewell *et al.*, 2020; Busnelli *et al.*, 2021; Tan *et al.*, 2022). Reasons to explain this inconsistency can only be hypothesized. We speculate a crucial role of the baseline characteristics of the women and of the thresholds used. To note, even if statistically significant, the association emerging from the meta-analysis in women younger than 35 was obtained based on just five studies, of whom only two showed a statistically significant association (Busnelli *et al.*, 2021). Moreover, none of them was specifically designed to investigate the relation between low AMH levels and miscarriage. Interestingly, two studies were excluded from the meta-analysis because they used different thresholds for age (Tarasconi *et al.*, 2017; Cornille *et al.*, 2022). They both failed to highlight a relation between low ovarian reserve and miscarriage. Tarasconi *et al.* (2017) did not show any association when focusing on women younger than 33. Cornille *et al.* (2022) showed that, in women <37 years, low serum AMH level was not associated with an increase in the miscarriage rate after fresh blastocyst transfer. Two additional studies not considered in the meta-analyses as they were published later also merit consideration (Tan *et al.*, 2022; Christensen *et al.*, 2022). Tan *et al.* (2022) showed significantly lower serum levels of AMH in women who had three miscarriages compared to those who did not have any. However, the authors focused on RPL, and they did not perform an analysis per threshold, limiting the possibility of comparing their findings with ours. Moreover, even if women with PCOS were stated to be excluded, in that population, serum AMH concentrations were markedly higher (median 34.3, IQR: 21.4–60.0 pmol/l) compared to those observed in our study, suggesting again that our two cohorts were markedly different. Finally, Christensen *et al.* (2022) used Danish registers to identify women ≤ 37 years with idiopathic low response (retrieval of ≤ 5 oocytes) (2734 cycles), comparing the risk of miscarriage in 22,573 women with ≥8 oocytes harvested. The adjusted hazard ratio of pregnancy loss in the poor responders was 1.04 (95% CI: 0.86–1.26), in line with our findings.

It is well known that oocyte competence normally decreases with aging leading to a higher number of pregnancies with aneuploidy (Franasiak *et al.*, 2014; Magnus *et al.*, 2019). Although some articles found correlations between low AMH levels and higher risk of aneuploid embryos at pre-implantation genetic testing (Katz-Jaffe *et al.*, 2013), a more recent article did not find this association (Morin *et al.*, 2018). Of relevance here is that we generally assumed in our study that miscarriage was consequent to impaired oocyte quality and used this outcome as a surrogate assessment of aneuploidy. This may not be entirely true. Miscarriage

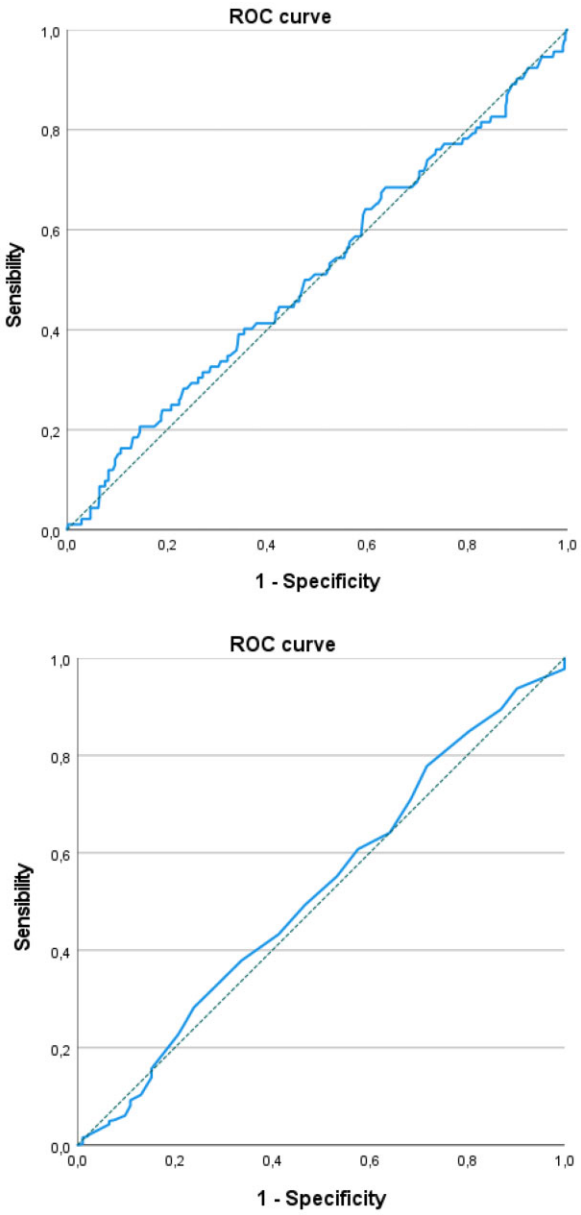

Figure 1. Receiver operating characteristic (ROC) curves. ROC curves for the prediction of miscarriage for anti-Müllerian hormone (AMH; upper panel) and antral follicle count (AFC; lower panel). The area under the curves (AUCs) were 0.51 (95% CI: 0.45–0.58) and 0.52 (95% CI: 0.45–0.59), respectively.

Table 2. Univariate and multivariate analysis according to pre-specified thresholds of AMH and AFC.

	OR (95% CI)	*P*-value	Adjusted OR* (95% CI)	*P*-value
AMH (pmol/l)				
≤2.9	1.64 (0.52–5.22)	0.40	1.81 (0.54–6.00)	0.34
≤3.6	1.11 (0.41–3.00)	0.84	1.22 (0.44–3.42)	0.71
≤5.0	1.10 (0.51–2.36)	0.81	1.12 (0.51–2.45)	0.79
≤7.9	1.13 (0.64–2.00)	0.67	1.05 (0.58–1.91)	0.87
AFC				
≤7	1.38 (0.77–2.45)	0.28	1.39 (0.76–2.55)	0.28
≤10	1.00 (0.63–1.60)	1.00	0.99 (0.60–1.63)	0.97

AMH: anti-Müllerian hormone; AFC: antral follicle count; OR: odds ratio of miscarriage.
* OR was adjusted for woman's age, man's age, woman BMI, previous delivery, and the cause of infertility.

may also be due to non-chromosomal causes. For instance, hormonal fluctuations during the cycle and cycle length differ in women with low ovarian reserve (Harris *et al.*, 2021) and one cannot exclude that these variables may impact on early pregnancy loss. On the other hand, our study was pragmatic, and the clinical emerging conclusion (i.e. that young women with low ovarian reserve can be reassured on the risk of miscarriage) remains valid.

Disentangling whether serum AMH can predict miscarriage is clinically relevant. It may allow more precise counseling to women. This information would be of value not only in the context of ART but also for fertile women conceiving naturally and in case of recurrent miscarriages. To note, the recent meta-analysis evaluating women with recurrent miscarriages showed a significant association with low AMH (OR = 3.23, 95% CI: 1.81–5.76) (Bunnewell *et al.*, 2020). If AMH is associated with early pregnancy loss independently of age, one could foresee to include this test in the diagnostic work-up of women with recurrent miscarriage. Even if our results argue against this possibility, it must be recognized that we did not study recurrent miscarriages, but only episodic miscarriages (as only a very small proportion of women have three or more miscarriage events). Moreover, we exclusively selected women conceiving with ART. Overall, inferences to the general population of fertile women should be made with caution. On the other hand, it must be emphasized that the meta-analysis of Bunnewell *et al.* included only two studies for AMH (and the same two for AFC) and failed to show any association with FSH (Bunnewell *et al.*, 2020). The results therefore cannot be considered conclusive. Noteworthily, only one previous study has investigated the role of AMH levels in predicting episodic miscarriage in women conceiving naturally (Lyttle Schumacher *et al.*, 2018). These authors showed a 2-fold increased risk for women with serum levels below 2.9 pmol/l; the same risk was confirmed after stratifying between women above and below 35 years of age. However, some methodological caveats deserve to be mentioned. First, this study was a secondary analysis of a cohort study aimed at testing the capacity of AMH to predict conception (Steiner *et al.*, 2017). Second, the authors used a threshold of 2.9 pmol/l, a value that was different from that (5 pmol/l) used for the primary outcome of the original study (i.e. natural conception) (Steiner *et al.*, 2017).

Our decision to exclusively focus on a group of young women with a steady risk of miscarriage overcame the confounding effect of age. In women older than 35, the loss of ovarian reserve and the impairment of oocytes quality progress in parallel, hampering the possibility of distinguishing between the detrimental effects of the two. Up to age 35, the risk of aneuploidy was conversely shown to be steady (Franasiak *et al.*, 2014; Magnus *et al.*, 2019), allowing selective investigation of the effects of remnant ovarian reserve from those of aging. On the other hand, this choice hindered inferences of our results to older women. One cannot exclude that the mechanisms behind earlier impairment of ovarian reserve may differ from those regulating normal exhaustion of the pool of primordial follicles or that the decreases in oocyte quality and ovarian reserve are due two independent mechanisms (Christensen *et al.*, 2022). In addition, our study did not distinguish between women with spontaneous early exhaustion and those who had an anticipated exhaustion due to iatrogenic treatments such as ovarian surgery, chemotherapy, or radiotherapy. Pathogenetic mechanisms leading to premature exhaustion radically differ in these two groups. To overcome this limit, we performed a subgroup analysis excluding the 75 women exposed to these harmful interventions. The study power remained high (we excluded only 14% of our cohort) and the results were similar. On the other hand, the number of women

previously exposed to surgery or radio and chemotherapy was too small to allow any robust conclusion for this group. Further studies are needed.

Some additional strengths and limitations of our study need to be discussed. Strengths include the large sample size and the homogeneity of the population. Apart from the exclusion of women older than 35, we also excluded women with BMI above 30 kg/m^2. This choice was aimed at protecting our results from the confounding effects of obesity (excessive weight may both impact on serum concentration of AMH and risk of miscarriage). Additional strengths comprise the setting, i.e. an academic research hospital that uses well-established and standardized diagnostic work-up and hormonal stimulation protocols, the exclusion of women with clinical conditions known to be associated with high miscarriage risk, and the use of a centralized and unique methods for AMH measurement. To note, one should take into consideration that not all methods for AMH testing provide similar results (Punchoo and Bhoora, 2021) and this must be taken into consideration in the interpretation of our findings. Limitations of the study first include the retrospective design that hampered the collection of more precise but potentially relevant clinical information of the couples. Second, we did not exclude women suffering from PCOS (of whom all inevitably displayed AMH and AFC above the used thresholds), a condition that some, but not all, authors claimed to be associated with miscarriage (Tarasconi *et al.*, 2017). Third, baseline characteristics of women who did and did not have miscarriage differed in some characteristics, i.e. indication to treatment and a history of previous miscarriages. These differences could be expected (Coomarasamy *et al.*, 2021; Quenby *et al.*, 2021), but we did not deem it necessary to match for indication or to exclude those with previous miscarriages. To overcome these baseline differences, we adjusted the OR using a multivariate analysis, but we cannot fully exclude residual confounding effects. Fourth, the decision to exclude legal abortions could be arguable because these pregnancies could be informative up to the time of interruption (using a Cox regression analysis). On the other hand, a different choice could also be arguable and, most importantly, only four cases were excluded because of this reason. The impact of this choice is therefore unremarkable. Fifth, we referred to thresholds of AMH and AFC previously reported in the literature (Ferraretti *et al.*, 2011; van Tilborg *et al.*, 2017; Lyttle Schumacher *et al.*, 2018) and we cannot exclude that other values could reveal different findings. Results of the ROC curves, however, argue against this limitation.

In conclusion, serum AMH levels are not associated with miscarriage in young women conceiving with ART. Women embarking on ART with low ovarian reserve should be informed of their likely poor response to ovarian stimulation but can be reassured that, if conception occurs, their risk of miscarriage is not increased. Further evidence is needed to disentangle the role of AMH as predictor of miscarriage in natural conceptions, in RPL, and in older women.

Data availability

The data underlying this article will be shared upon reasonable requests to the corresponding author.

Authors' roles

A.C.: implemented the study, data collection and corrected the draft. M.R.: collaborated in data collection and statistical analyses. G.F.: collaborated in data collection and corrected the draft. L.B.: collaborated in data collection and corrected the draft. A.B.: implemented the study, provided supervision and corrected the draft. P.V.: implemented the study, provided supervision and corrected the draft. L.M.: designed the study, provided discussion and corrected the draft. E.S.: designed the study, wrote the first draft and did the statistical analyses.

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Conflict of interest

E.S. reports grants from Ferring and honoraria for lectures from Merck-Serono and Gedeon-Richter. All the other authors do not have any competing interest to declare.

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