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

Short title: Blood anti-müllerian hormone level and unexplained recurrent early miscarriage

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Abstract

Research Question: Is blood anti-müllerian hormone (AMH) level a strong determinant of unexplained recurrent early miscarriage?

Design: In the first part of the study, AMH levels measured using the ELISA kit of Immunotech* (Beckman-Coutler, France) were compared between 188 all-comer (mostly fertile) women consecutively referred for ≥ 3 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 whatever their age, AMH level did not significantly differ between cases and controls ($P=0.152$). However, in the subgroup ≥ 25 years old (176 cases vs 358 controls of ~ 33.5 years), the cases had significantly lower AMH levels than the controls (median[IR]: 2.8[1.4-4.7] vs 3.25[1.7-5.5], $P=0.046$) and the proportion of cases with an AMH level <1 ng/ml was significantly higher (17.6% vs 10.6%; OR, 1.80; 95% CI, [1.07–3.00], $P=0.028$). In regard with the subsequent pregnancy, AMH level was not correlated with either the conception delay or the miscarriage occurrence ($P=0.88$). However increased age and number of previous miscarriages were significantly predictive of a subsequent miscarriage ($P=0.046$ and $P=0.03$, respectively).

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

Key, words: Recurrent miscarriage, anti-müllerian hormone, ovarian reserve, case-control study, prospective cohort study, fertile women

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

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 one to two percent of couples trying to have children. Standard investigations fail to reveal any well-recognized cause in more than 50% of the couples (Branch et al, 2010).

Ovarian dysfunction, through either oocyte quality or ovarian hormone production, has been put forward 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 increases with maternal age (Pellestor et al, 2003), the exact correlation between those qualitative alterations and the quantitative ovarian reserve status still remains to be clarified (Plante et al, 2010; Jiang et al, 2018) especially in women aged <35 years old (yo).

In addition to antral follicle count (AFC) by ultrasound, ovarian reserve can be quantitatively assessed by measurement of FSH, inhibin B and AMH blood levels (Broer et al, 2014). AMH blood level is highly correlated with AFC (Hansen et al, 2011). However, discordance was reported. Patient-specific features (e.g. PCOS, age, 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 foetus, AMH is secreted by the ovarian granulosa since 36 weeks' gestation. Increase in plasma AMH concentration reaches a first peak between 7 and 10 yo and a second one between age 20 and 25 yo (Kelsey et al, 2011). Decrease in plasma AMH concentration

begins around 25 yo and AMH becomes undetectable before menopause (Freeman et al, 2012). Thus, after 25 yo, the AMH blood level appears to be a more reliable marker of the ovarian pool size than the civil age. Moreover, the decline in AMH level precedes the increase in FSH and inhibin levels 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, studies (Sahu et al, 2010; Tremellen et al, 2010; Tarasconi et al, 2017; Bishop et al, 2017) have assessed whether quantitative markers of the ovarian reserve would predict the occurrence of a miscarriage and a live-birth after intra-uterine insemination or in vitro-fertilization. They reported discrepant results. Although those markers, especially serum AMH levels, help to predict the intensity of ovarian response to a controlled stimulation, they do not seem to reflect oocyte quality.

Among 348 non selected (mostly fertile) women, age-adjusted AMH levels were retrospectively correlated with reproductive outcomes, no specific AMH-related pattern was suggested (La Marca et al, 2012). In the same way, Zarek et al. did not show any association between AMH level and further pregnancy loss in fertile women with a history of one to two previous losses (Zarek et al, 2016). In contrast, Schumacher et al found that fertile women with very low blood AMH levels (≤ 0.4 ng/mL) had a 2.3-fold increased risk of miscarriage compared with women with AMH levels ≥ 1 ng/mL. However, they did not observe an increased risk of miscarriage for women with AMH levels 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 level as an accurate marker of oocyte quality in all-comer fertile women.

What about women with REM? Lower plasma levels of oestradiol and higher plasma levels of FSH at day 3 of the menstrual cycle were reported in women with previous unexplained REM, compared to women with well-identified causes of REM (Trout et al, 2000), suggesting a reduced ovarian reserve. Before our study was designed, only one study had reported the comparison of AMH levels 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 levels 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, we raised the question of whether an altered ovarian reserve was a strong determinant of unexplained REM in all-comer (mostly fertile) women. In the absence of an accurate qualitative marker of the ovarian reserve, we chose to use blood AMH measurements in assessment of ovarian reserve among women with unexplained REM compared with age-matched parous controls. We hypothesized 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 our master incident case-control study on thrombophilic mutations (Pasquier et al, 2009). Additionally, we planned to study the prospective outcome of the subsequent pregnancy, according to the blood AMH level, in the cases.

Materials and methods

Study design

The study was organized in two parts: an incident cases-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 levels between women referred for unexplained REM defined as 3 or 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 women groups were enrolled from February 2003 to 2008 in our 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 both 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 with age, were selected for each case.

Prospective 2-year follow-up of cases

All the women, who participated in our 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.

We planned to seek prospectively a correlation between AMH levels 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 women cases, 18 to 45 yo, were from Brittany. 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 center. REM was defined as 3 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 yo 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 polycystic ovary syndrome (PCOS) were not considered as exclusion criteria for both cases and controls. Women were diagnosed as “definite PCOS” when they met the internationally recommended criteria (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004).

164 *Samples*

165 5 ml of blood was collected by venipuncture into a SST gel tube (Vacutainer, Becton-
166 Dickinson) and then centrifuged at 3500 rpm for 15 minutes at room temperature. Then the
167 serum was divided into aliquots of ~300 µl and stored at -80°C until use.

169 *AMH measurements*

170 In 2009, for both cases and controls, the measurement of AMH blood level was
171 simultaneously carried out on 300 µl of serum bank using the ELISA kit of Immunotech*
172 (first generation test, Beckman-Coutler, France).

174 *Statistical Analysis*

175 Statistical analysis was performed using SPSS Statistics v.19.

176 Firstly, we planned to compare AMH blood levels between cases and controls regardless of
177 age. Noteworthy, no sample size calculation was done: before our study was designed, only
178 one study had reported similar AMH levels between 34 REM women and 10 controls
179 (Prakash et al, 2006).

180 A model of AMH concentration from conception to menopause clearly demonstrated an
181 AMH peak at ~25 yo, after a rise and before a decline in AMH concentrations (Kelsey et al,
182 2011). Consequently, we planned to check whether the distribution of AMH blood levels
183 according to age also depicted a peak ~25 yo. This would allow to do separate analyses in the
184 subgroups of cases and controls <25 yo and ≥25 yo, when the sample size was sufficient (i.e.
185 >30).

Student's t-test and chi-square 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 ORs 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 to undergo natural conception, we studied, the delay and outcome of the subsequent pregnancy (early miscarriage or not) occurring during the first 2 years of follow-up according to AMH level by cox and logistic regression, respectively. Adjustment for age and number of previous losses was performed.

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

Results

The AMH blood level was available for the 188 cases with REM and 376 age-matched controls, from our previous study on thrombophilic mutations (Pasquier et al, 2009). Except for parity, the baseline characteristics were similar between cases and controls (Table 1). Noteworthy, the age at inclusion, i.e. when blood was collected, was normally distributed and not statistically different between cases and controls (32.9 ± 5 vs. 33 ± 4.6 years, $P=0.8$). The age at the first obstetrical event (loss or live birth) was slightly but significantly higher in cases compared to 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 (47.8%) had previously given a live birth. There was no significant difference in delivery term and weight at birth in comparison to the controls.

Only 9 cases and 13 controls underwent ART. Main indications for ART were male infertility (for 3 cases and 3 controls), fallopian tube impairment (for 1 case and 2 controls), and endometriosis (for 2 controls). Noteworthy, at least one previous conception without ART was recorded for all the 9 cases vs. only 4 controls. Furthermore, 6 cases and 13 controls had a “definite PCOS”.

The distribution of AMH levels 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[1.7-5.5]) for the controls. AMH levels did not significantly differ between cases and controls ($P=0.152$). Among the 188 cases 31 (16.5%) had an AMH level <1 ng/ml in comparison to 40 (10.6%) controls among 376 (OR, 1.66; 95% CI, [1.00–2.75], $p=0.059$). 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 first generation test of Beckman-Coutler is known to give higher values than the other tests available then, we looked for the 85 percentile (>6.87 ng/ml) of the distribution of AMH levels in our 574 women. 55 controls and 28 cases had AMH levels above the 85 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 women from the analysis did not change the results.

Among cases, AMH levels were significantly lower in the 6 cases who underwent ART compared to the other cases ($P=0.040$). Among controls, the AMH levels were not different in women who underwent ART compared to the other controls ($P=0.32$). As expected, after exclusion of these ART women from analysis, AMH levels did not still differ between cases and controls ($P=0.34$).

Subgroup analyses

The distribution of AMH levels according to age is shown in Figure 2 and demonstrated a peak at ~25 yo in agreement with the data reported by Kelsey et al (Kelsey et al, 2011).

Subgroup of women <25 yo.

Only 12 cases and 18 controls were <25 yo (Table 2).

Subgroup of women ≥ 25 yo.

The analysis of the female subgroup ≥ 25 yo (176 cases of 33.5 ± 4.4 years vs 358 controls of 33.5 ± 4.1 years) showed significantly lower AMH levels 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 level <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 levels ≥ 85 percentile, did not change the results ($P=0.030$ and $P=0.021$, respectively). Moreover, AMH levels were significantly lower in cases compared to controls ($P=0.039$) when adjusting for “definite PCOS”. When excluding the 24 women who underwent ART, the AMH levels remained lower in cases compared to controls and the absolute difference between cases and controls was similar. However, this difference was no longer statistically significant ($P=0.091$), 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 levels were significantly lower in cases compared to controls ($P=0.046$).

Prospective follow-up

The prospective outcome of a subsequent pregnancy was assessed for the cases ≥ 25 yo ($n = 176$) 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 month-6, -12 and -18 months, respectively. Among these 119 cases, 37 suffered a further miscarriage in the first trimester of pregnancy (Figure 1).

AMH level regarded as a continuous or discrete variable, and set at different cut-offs (quartiles of the whole distribution and $<1\text{ng/ml}$) was not correlated with the delay to conception through cox regression analysis (Figure 3)).

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

AMH level, regarded as a continuous variable, was not correlated to the occurrence of a miscarriage (OR, 1.01; 95% CI, [0.88–1.15], $P=0.88$). 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 levels set at different cut-offs (quartiles of the whole distribution and $<1\text{ng/ml}$) was not statistically significant (AMH $<1\text{ng/ml}$: OR, 0.94; 95% CI, [0.32–2.76], $P=0.91$).

In addition, using spline analyses and smaller subgroup analyses, we 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 ($P=1$ and $P=0.42$, respectively). Noteworthy, progesterone was never introduced during the periconceptional period but always after delayed menses.

Discussion

Taking into account all women regardless of age, AMH blood levels were not significantly different between the 188 REM cases and the 376 healthy controls ($P=0.152$).

Among the 534 ≥ 25 yo (33.5 $\pm$ 4) women, we found statistically significantly lower AMH levels in cases compared to controls (median 2.8 vs 3.25 ng/ml, $P=0.046$) and, the proportion of cases with an AMH level <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 levels of AMH did not modify the results. In contrast, exclusion of the 22 ART women among cases and controls had an impact on the statistical significance of the difference in AMH levels. However, this difference remained within the range of before exclusion results suggesting, rather, a loss of statistical power. Indeed, when adjusting for a previous ART, AMH levels remained significantly lower in cases compared to controls ($P=0.046$). The small sample size of the subgroup of women <25 yo did not allow data interpretation.

Regarding the prospective follow-up of cases, it is noteworthy, that AMH levels were not correlated with the occurrence of a subsequent miscarriage in the first 2 years after blood collection, irrespective of the age subgroups (25–35 and ≥ 35 yo). However, consistent with the literature (Brigham et al, 1999), the two strong determinants of miscarriage, i.e. woman’s age and number of previous miscarriages, were positively and significantly associated with a subsequent loss.

Thus, among the ≥ 25 yo women, though clinically less meaningful, the results of our 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 levels in cases compared to controls despite smaller samples. In the prospective Turkish cohort

(Atasever et al, 2016), the AFC, usually well-correlated with AMH level (Hansen et al, 2011), was not different between cases and controls. This raises the following question: how the AMH level was regarded in their study ? Was the AMH level regarded as a marker of ovarian follicular activity or follicular pool size in those women 29.5 ± 4.5 yo (i.e. <25 yo for 16% of them)? A separate analysis of possible correlation between AFC and AMH according to age (<25 and ≥ 25 yo) would have been attractive in the study by Atasever et al. In the other study (Pils et al, 2016), as the cases were slightly older than the controls (median [IR]: 33[28-38] vs. 33[28-36], $P=0.124$) perhaps, adjusting AMH level for age would have been appropriate.

In addition to the large sample of cases and controls, the other strength of our 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 in average two healthy children, no history of pregnancy loss and were matched with cases by age within 1 year. Moreover, to our knowledge, this is the first time that a correlation between blood AMH levels and subsequent pregnancy outcomes was sought in a prospective follow-up of fertile women with REM. One distinctive feature of our study was the characteristics of the enrolled cases: all-comer women (mostly fertile:~96%) referred to an internal medicine consultation and not to a fertility center. Some limitations of our study come from the design of our master study on thrombophilic mutations. That said, the present work did not exclude infertile women or those with PCOS. 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 consideration for control women. Lastly, the results of AMH blood levels (measured using first generation ELISA Beckman Coulter 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 ELISA Beckman

Coulter kit (Pils et al, 2016) or an ELISA kit from YH Biosearch (Atasever et al, 2016) vs. a first generation ELISA Beckman Coulter kit in our study).

In brief, significant lower AMH blood levels were observed among women ≥ 25 yo 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 less than 2. Given that in this age subgroup the AMH level is well correlated with the ovarian reserve size, and consistent with other studies (Atasever et al, 2016; Pils et al, 2016) we cannot rule out that a relative lower ovarian reserve could contribute to occurrence of unexplained REM among women ≥ 25 yo. However, if we had based a sample size calculation on those two recently reported studies we would have enrolled ~70 cases and controls and probably would not have found any significant association between AMH level and REM. Furthermore, our 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 Pils et al study's results could be regarded as less conclusive due to its retrospective design.

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

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	CASES	CONTROLS	<i>P</i> value
N	188	376	
Age, y (mean±SD) Median [min-max] ≥25 y, n (%)	32.9±5.0 32.9[18.8-44.8] 176 (93.6)	33.0±4.6 32.6[18.5-44.7] 358 (95.2)	0.788
Smoking, pack-y (mean±SD)	4.8±6.0	4.4±6.0	0.551
Body mass index, kg/m2 (mean±SD)	23.0±4.15	22.8±4.5	0.603
Menstrual cycle, d (mean±SD)	28.5±3.0	28.8±3.3	0.342
Previous live birth, n (median [min-max])	0 [0-4]	2 [1-6]	<0.001
Age at the first obstetrical event, y (mean±SD)	27.4±5.2	26.1±3.6	0.003
Assisted Procreation Technology, n (%) <i>Intrauterine inseminations, n</i> <i>In vitro fertilization, n</i>	9 (4.8) 3 6	13 (3.5) 4 9	0.442
Polycystic Ovarian Syndrome, n (%)	6 (3.2)	13 (3.5)	0.869

447 **Table 1.** Baseline characteristics of cases and controls.

Women aged ≥ 25 years			
	CASES	CONTROLS	P value
N	176	358	
Age, y (mean$\pm$SD) Median [min-max]	33.5 $\pm$ 4.4 33.3[25- 44.8]	33.5 $\pm$ 4.1 33.3[25- 44.7]	0.914
AMH, ng/ml (mean$\pm$SD) Median [IR]	3.5 $\pm$ 3.0 2.8[1.4-4.7]	4.1 $\pm$ 3.4 ng/ml 3.25[1.7-5.5]	0.046
<1ng/ml, n (%)	31 (17.6%)	38 (10.6%)	0.028
Women aged <25 years			
	CASES	CONTROLS	
N	12	18	
Age, y (mean$\pm$SD) Median [min-max]	23.0 $\pm$ 2.0 23.7[18.8- 24.9]	22.7 $\pm$ 2.0 23.3[18.5-24.9]	
AMH, ng/ml (mean$\pm$SD) Median [IR]	5.0 $\pm$ 2.25 5.75[2.75-7.15]	2.1 $\pm$ 0.9 2.3[1.3-2.6]	

Table 2. Subgroup results according to age ≥ 25 or <25 years

Interquartile Ranges: IR

454 **Figure 1. Flowchart.**

455 Cases and controls enrolled in the incident 1:2 case-control study; subgroups according to
456 age; cases enrolled in the prospective 2-year follow-up with outcome of the first further
457 pregnancy.

458 W: Woman, ART: assisted reproductive technology

459

460 **Figure 2: Distribution of AMH blood levels (ng/ml) according to age (y) among cases and**
461 **controls**

462

463 **Figure 3:** 1-survival curves of the cases aged ≥ 25 years, according to quartiles of AMH
464 distribution (quartile 1: ≤ 1.475 , $1.475 < \text{quartile 2} \leq 2.85$, $2.85 < \text{quartile 3} \leq 4.825$ ng/ml, quartile
465 $4 > 4.825$, unit: ng/ml). Cox regression, global $P=0,954$

466

INCIDENT 1:2 AGE-MATCHED CASE-CONTROL STUDY