Low anti-Müllerian hormone level is not a risk factor for early pregnancy loss in IVF/ICSI treatment

Running title: Low AMH and early pregnancy loss in IVF/ICSI

P. Peuranpää*[1], H. Hautamäki[2], M. Halttunen-Nieminen[2], C. Hydén-Granskog[2], A. Tiitinen[2]

[1] The Department of Obstetrics and Gynecology, University of Helsinki and HUS Hyvinkää Hospital, Sairaalankatu 1, FI-05850 Hyvinkää, Finland [2]The Department of Obstetrics and Gynecology, University of Helsinki and Helsinki University Hospital, PO Box 140, FI-00029 HUS, Helsinki, Finland

* Correspondence address: Department of Obstetrics and Gynecology, HUS Hyvinkää Hospital, FI-05850 Hyvinkää, Finland. Tel: +358-50-427-0431; Email: pirkko-liisa.peuranpaa@hus.fi

Abstract

Study question: Is a low (<1.0μg/L) or moderately low (1.0–1.9μg/L) serum anti-Müllerian hormone (AMH) level a risk factor for early pregnancy loss in IVF/ICSI with a fresh or frozen-thawed embryo transfer (ET)?

Summary answer: A low or moderately low serum AMH level does not associate with miscarriage, non-visualized pregnancy loss, or overall early pregnancy loss rate in the IVF/ICSI treatment.

What is known already: Low AMH predicts poor ovarian response and small oocyte yield in the IVF/ICSI treatment, but its value in the evaluation of live birth rate (LBR) is modest. Little is known about the risk of early pregnancy loss in ART among women with low AMH.

Study design, size, duration: A retrospective cohort study on 1383 women undergoing their first oocyte retrieval for IVF/ICSI in Helsinki University Hospital in Helsinki, Finland, between 2012 and 2016, with all connected fresh (n=1315) and frozen-thawed (n=1418) ET cycles finished by August 2018. AMH was measured within twelve months before the IVF/ICSI stimulation.

Participants/materials, setting, methods: Of all women, 235 (17.0%) had low (<1.0μg/L), 278 (20.1%) had moderately low (1.0–1.9μg/L) and 870 (62.9%) had normal (≥2.0μg/L) AMH. The primary outcomes were miscarriage, non-visualized pregnancy loss, and early pregnancy loss (miscarriage and non-visualized pregnancy loss combined) after fresh or frozen-thawed ET. The impact of AMH on these outcomes was calculated in three populations: among all women who became pregnant, among women with AMH ≤6.0μg/L and in a population weighted by the inverse probability of becoming pregnant (inverse probability weighting, IPW). The impact of AMH was also assessed on the secondary outcomes, cumulative pregnancy rate (cPR), and cumulative live birth rate (cLBR) across all ET cycles in the woman's first IVF/ICSI. Potential confounders (the woman's age, overweight, smoking, history of endometriosis, and underlying medical conditions) adjusted the final results.

Main results and the role of chance: Of 1123 pregnancies, 285 (25.4%) ended in non-visualized pregnancy loss and 143 (12.7%) in miscarriage. The LBR was 24.6% per ET (673/2733). Low or moderately low AMH, compared with normal AMH, did not associate with miscarriage or non-visualized pregnancy loss in analyses among all women who became pregnant (adjusted RR for miscarriage vs. live birth 0.70, 95% CI 0.42–1.17 in low AMH and 1.00, 95% CI 0.68–1.49 in moderately low AMH; adjusted RR for non-visualized pregnancy loss vs. live birth 0.90, 95% CI 0.65–1.23 in low AMH and 1.09, 95% CI 0.85–1.41 in moderately low AMH), nor did low or moderately low AMH associate with the overall early pregnancy loss rate (adjusted RR for early pregnancy loss vs. live birth 0.86, 95% CI 0.68–1.10 in low AMH and 1.01, 95% CI 0.86–1.27 in

moderately low AMH). Results remained similar after restricting the analysis to women with AMH ≤6.0 ug/L. The women with low or moderately low AMH had fewer pregnancies and live births than the women with normal AMH in their first IVF/ICSI (cPR/cLBR in the women with low AMH 50.6/34.0%, moderately low AMH 59.0/36.3% and normal AMH 68.3/49.2%). When the lower probability for pregnancy was considered by using IPW, the women with low or moderately low AMH did not have a higher risk for miscarriage, non-visualized pregnancy loss, or overall early pregnancy loss compared to women with normal AMH.

Limitations, reasons for caution: The number of miscarriages in women with low AMH was moderately small, limiting the power of the study. The real-world clinical setting of the study restricted the ability to control for all factors causing selection bias.

Wider implications of the findings: The cLBR was higher among women with normal AMH than among women with low or moderately low AMH in their first IVF/ICSI treatment because these women had more oocytes and embryos. Women with low or moderately low AMH did not have an increased risk for early pregnancy loss. This information is reassuring for couples and useful in counseling. These results are also valuable when assessing the overall effectiveness of IVF/ICSI treatment.

Study funding/competing interest(s): The research funds from Helsinki University Hospital (No. TYH2018232), Hyvinkää Hospital (No. M3080TUT18) and the Emil Aaltonen Foundation for P.P., the grants from the Paulo Foundation and the Finnish Medical Foundation for H.H.. The authors report no conflicts of interest.

Trial registration number: HUS/138/2017

Key words

Anti-Müllerian hormone, early pregnancy loss, miscarriage, ovarian reserve, IVF/ICSI

Introduction

Today, for various reasons, many women delay childbearing. As women age, their fecundity decreases, and the risk for miscarriage increases (Menken *et al.*, 1986; Magnus *et al.,* 2019). Such an age-related increase in the miscarriage rate has been reported in assisted reproduction as well (Farr *et al.,* 2007). About 20% of IVF pregnancies end in a pregnancy loss, and half of the losses are biochemical (Farr *et al.,* 2007).

Advanced age associates with a diminished number and quality of the remaining oocytes, described as ovarian reserve. There is, however, individual variation in the size of ovarian follicle pool at birth and rate of its decline thereafter (te Velde and Pearson, 2002). Genes largely explain this variability, but other factors, such as ovarian surgery, endometriosis, cancer treatments, smoking, and infections, may also have an impact. Whether the quantitative decrease in the oocytes, independent of the women's age, associates with poor oocyte quality as well, has been widely discussed (Broekmans *et al.*, 2006; Zamah and Stephenson, 2018).

Anti-Müllerian hormone (AMH) predicts the ovarian response and the oocyte yield in ovarian stimulation (La Marca *et al.*, 2010), but studies have shown a limited value of AMH to predict live birth rate (LBR) (Broer *et al*., 2013; Iliodromiti *et al*. 2014). These studies have rarely reported on early pregnancy loss rate, although it might reflect the oocyte quality better than the LBR does. Early pregnancy loss is often a result of fetal aneuploidy (Hassold and Hunt, 2001), but research on other etiologies is much needed. Low AMH level as an etiological factor has been suggested, but literature on this subject, especially regarding ART, is sparse. The few previous works in this area have studied miscarriage only after IVF/ICSI fresh embryo transfer (ET) (Tarasconi *et al.*, 2017), whereas taking the outcome of the whole IVF/ICSI treatment into consideration is what in real life matters to the couples as well as the clinicians taking care of them.

Therefore, our study aimed to clarify the impact of low AMH on the risk of early pregnancy loss and overall pregnancy outcome in the woman's first IVF/ICSI treatment. As pregnancy loss is possible only after becoming pregnant, we considered the lower probability of pregnancy in women with low AMH in our analyses. Finally, we aimed to deepen the understanding of AMH as a potential biomarker of the oocyte quality.

Materials and Methods

Study population and design

The study population comprised of women who underwent their first oocyte retrieval for IVF or ICSI treatment in the Reproductive Medicine Unit of Helsinki University Hospital (Helsinki, Finland) between January 1st, 2012 and December 31st, 2016. Figure 1 shows the flowchart of participant selection and an overview of the treatment. We included women who had their serum AMH measured within the preceding twelve months of their ovarian stimulation and who had had at least one subsequent ET cycle (fresh or frozen-thawed). The exclusion criteria included treatment with preimplantation genetic testing or for fertility preservation. Couples' own gametes were used in all treatment cycles. We compared the early pregnancy loss rates, including the miscarriage rates and the non-visualized pregnancy loss rates, between the women with low (<1.0μg/L), moderately low (1.0–1.9μg/L) and normal (≥2.0μg/L) AMH and calculated the cumulative pregnancy rates (cPRs) and the cumulative live birth rates (cLBRs).

The data were collected from medical databases. The baseline characteristics were the woman's age at the oocyte retrieval, AMH, BMI, smoking history, the woman's underlying medical conditions, previous pregnancies, previous ovarian surgeries, and the diagnosis, the duration and the type

(primary vs. secondary) of the infertility. The treatment characteristics included the stimulation protocol (GnRH agonist or antagonist), the total gonadotropin dose, the number of retrieved, mature and fertilized oocytes, the treatment type (IVF or ICSI) and the number of frozen embryos. The data on each separate ET cycle included the number of transferred embryos, the cycle type (fresh or frozen-thawed ET), the pregnancy test result, and the pregnancy outcome. The analysis included the subsequent frozen-thawed embryo transfers (FETs) before August 31st, 2018.

AMH measurement

The laboratory quantified AMH with an ELISA assay (AMH Gen II ELISA, Beckman Coulter, Brea, CA, USA). The limit of detection (LoD) was 0.08µg/L, and the limit of quantitation (LoQ) 0.16 µg/L. The intra-assay and inter-assay coefficient of the variation (CV%) was <6% in the range of 3.8–16.5µg/L. The total CV% was <8%. The statistical analyses used AMH value of 0.1µg/L for those women who had their AMH level below the limit of quantification (n=28).

Treatment protocol

The women underwent ovarian stimulation by either the long agonist (midluteal GnRH agonist suppression) or the short antagonist protocol (antagonist administration starting on stimulation day five or six). The initial dose of recombinant FSH or human menopausal gonadotropin was 100–375 IU/day depending on the woman's age, BMI, AMH, and antral follicle count. When the diameter of three or more follicles reached ≥17mm, the women received a 250µg recombinant hCG or 5000IU hCG injection subcutaneously, and the oocyte retrieval was scheduled for 36–40 hours later. One embryo was transferred 2–5 days after the oocyte retrieval. Vaginal micronized progesterone initiated on the third day after the oocyte retrieval, and it continued on for 12 days. In the cases of possible severe ovarian hyperstimulation syndrome, all embryos were frozen. The frozen-thawed embryos

were transferred as single ETs either in a natural cycle with luteal support or in a hormonal substitution cycle (oral estradiol valerate 4–8mg/day and vaginal progesterone 600mg/day).

Pregnancy assessment

Pregnancy was detected either by a serum hCG concentration >5.3IU/L 10–12 days after the ET (n=2655) or by a positive urine hCG test 14 days after the ET (n=78). An intrauterine gestational sac on the ultrasound examination five weeks after the ET confirmed the clinical pregnancy. The pregnancy losses were classified according to the European Society of Human Reproduction and Embryology's (ESHRE) early pregnancy special interest group's consensus statement (Kolte *et al*., 2015). The definition of a miscarriage is the spontaneous demise of an ultrasonically confirmed intrauterine pregnancy before viability. Non-visualized pregnancy loss, which comprises of biochemical pregnancies and pregnancies of unknown location (PULs), is defined as decreasing serum or urinary hCG without the ultrasonic confirmation of the pregnancy. Here, the definition of early pregnancy loss includes the miscarriages and the non-visualized pregnancy losses; we excluded the ectopic pregnancies from the pregnancy loss analyses because of their different etiology.

We diagnosed miscarriages ultrasonically as an intrauterine gestational sac and absent fetal heartbeats. When a woman had a low serum hCG concentration (<20.0IU/L) 10 – 12 days after ET, or symptoms of pregnancy loss, serum hCG was measured once a week. When the pregnancy was diagnosed only by serum or urine hCG, and the serial measurements of hCG decreased to negative, a biochemical pregnancy was diagnosed. When the ultrasound examination, histology, or surgery failed to confirm the location of the pregnancy, a diagnosis of PUL was set. HCG measurements continued once a week until hCG was ≤5.3IU/L. Histologic examination was used for differential diagnosis on demand (e.g., suspected molar or ectopic pregnancy). Live birth was defined as an infant born alive after 22 gestational weeks.

Statistical analyses

The differences in the categorial explanatory variables between the women with low (<1.0μg/L), moderately low (1.0–1.9μg/L) and normal (≥2.0μg/L) AMH were analyzed by the Chi-square test for independence. The differences in the continuous variables were analyzed by the Kruskal-Wallis test with a *post hoc* test of Mann-Whitney U with Bonferroni's adjustment. The differences in the PRs per ET and the pregnancy outcomes after a positive pregnancy test between the women with various AMH levels were analyzed with the Chi-square test.

The primary outcomes were a miscarriage, non-visualized pregnancy loss, and these variables combined as early pregnancy loss after a fresh or frozen-thawed ET cycle. To assess the impact of AMH level on these outcomes, we estimated the relative risk (RR) using the log-binomial regression among women, who became pregnant (excluding ectopic and terminations of pregnancies). We calculated RRs in three populations. First, we analyzed all women who became pregnant; second, we analyzed women with AMH ≤6.0μg/L to exclude PCOS patients, and third, we used inverse probability weighting (IPW) to better estimate the pregnancy loss risk among women with low probability for pregnancy. To account for the repeated ET cycles by individual patients, we performed modeling with the generalized estimating equation (GEE) analysis (Missmer *et al*., 2011; Yland *et al*., 2019). AMH was tested both as a categorical and as a continuous variable. The selection of the potential confounders was based on a directed acyclic graph (DAG), which describes the relationship between the exposure (AMH) and the outcome (early pregnancy loss) (Greenland *et al.*, 1999) (Supplementary Figure S1). Based on the DAG, the results were adjusted by the woman's age on the oocyte retrieval day in the age groups <35, 35–37 and ≥38, smoking during IVF/ICSI (yes/no), overweight (BMI >25kg/m^2) and a diagnosis of endometriosis (yes/no). We adjusted the results also by those underlying medical conditions, which may influence both AMH and the risk of early

pregnancy loss, such as diabetes, rheumatic disease, celiac or inflammatory bowel disease, multiple sclerosis, or a previous cancer treatment (medical condition, yes/no). We were not able to adjust the results by woman's ethnic origin, a potential confounder, because no information on it exists in the medical databases. Since women with PCOS may be more likely to experience pregnancy loss, we conducted sub-analyses among women with AMH ≤6.0μg/L. Currently, no consensus exists of an AMH cutoff value, which discriminates PCOS patients from non-PCOS patients (Teede *et al.*, 2018). Based on a recent publication in Finnish population (Sova H *et al.,* 2019), we chose the AMH limit of >6.0μg/L to exclude PCOS patients.

Because pregnancy loss is possible only after becoming pregnant, women with low probability for pregnancy in IVF/ICSI may be underrepresented in the pregnancy loss study population. It is possible to address this selection bias through weighting the data by inverse probability of pregnancy. IPW is a method, which is widely used in epidemiological studies (Crowson *et al.,*2013) and which has been used in IVF studies as well (Modest *et al.*, 2018). Here, we used IPW to better estimate pregnancy loss risk among women with low probability for pregnancy, such as women with low or moderately low AMH. IPW created a pseudopopulation with heavier weight on women, who became pregnant despite of their low pre-treatment probability for pregnancy and lighter weight on women who had a high probability of becoming pregnant.

We used a binary logistic regression to create women's predictive probability for becoming pregnant (p) and not becoming pregnant (1-p). As the dependent variable, we used cumulative pregnancy (whether a woman became pregnant at least once after any ET cycle in the woman's first IVF/ICSI). As the independent variables, we used ten baseline covariates: woman's age at oocyte retrieval, AMH, primary/secondary infertility, being healthy (yes/no), smoking during IVF, BMI, the total gonadotropin dose, treatment type (IVF/ICSI), the number of mature oocytes produced by IVF/ICSI

and the embryo/mature oocyte ratio. We then created stabilized weights for women with cumulative pregnancy (P/p) and women without cumulative pregnancy [(1-P)/(1-p)], where P was the overall probability for cumulative pregnancy in the study population. The mean of the stabilized weights was 1,00. We truncated the final weights at 99th percentiles. Stabilized weights were then used in the log-binomial regression analysis with GEE to estimate RRs for miscarriage and non-visualized pregnancy loss vs. live birth. We restricted this sub-analysis to women with AMH ≤6.0µg/L. The model had fairly good discrimination between those who became pregnant and those who did not (c-statistics 0.71). As a limitation of the IPW analysis, the GEE model allowed only a single weight for each woman. Thus, we were not able to use cycle-specific variables in creating the weights.

The secondary outcomes were cumulative pregnancy (at least one positive pregnancy test result in the woman's first IVF/ICSI, including all consecutive ETs) and cumulative live birth (at least one live birth in the woman's first IVF/ICSI). We calculated the cPR (and the cLBR) in a "conservative" manner, which assumes that women who did not return to the next ET had a zero probability of live birth (Maheshwari et al., 2015). We used the number of women with their first pregnancy (first live birth) after consecutive ETs as a numerator and the number of all women as a denominator. The impact of AMH on the cumulative pregnancy (vs. no pregnancy) and on the cumulative live birth (vs. no live birth) in the couple's first IVF/ICSI were analyzed by using the log-binomial generalized linear model in all women and in women with AMH ≤6.0µg/L. The results were then adjusted for age, smoking, overweight, endometriosis, and underlying medical conditions — the selection of adjusting variables was based on a DAG (Supplementary figure S2). Finally, we stratified the results by the number of mature oocytes (1-4, 5-9, and ≥10).

The data were analyzed by using Microsoft's Statistical Package for Social Sciences (SPSS), version 25.0. A *P*-value of less than 0.05 was considered statistically significant.

262

263 Missing data

264 Data on BMI was missing for five (0.4%,) and smoking for nine (0.6%,) of the 1383 women. The
265 outcome of one pregnancy remained unknown. Since the number of missing data was few, they were
266 omitted from the corresponding analyses.

267

268 Ethical approval

269 This study received research permissions from Helsinki University Hospital and Hyvinkää Hospital;
270 these hospitals do not require ethical approval for register-based studies.

271

272

273 **Results**

274

275 The study population comprised of 1383 women having their first IVF/ICSI treatment with at least
276 one ET (fresh or frozen-thawed). The women's median age was 33.8 years and ranged from 21 to 40
277 years. AMH ranged from <0.2μg/L to 43.6μg/L. Of all women, 235 (17.0%) had low (<1.0μg/L),
278 278 (20.1%) had moderately low (1.0–1.9μg/L) and 870 (62.9%) had normal AMH (≥2.0μg/L). The
279 women with low and moderately low AMH were older than the women with normal AMH, as
280 presumed (Table I). This led to differences in the IVF/ICSI protocols and outcomes as the women
281 with low AMH had the highest gonadotropin dose, the smallest number of retrieved, mature and
282 fertilized oocytes, and frozen embryos (Table II). The mean number of retrieved oocytes were 6.9,
283 9.5 and 13.4, and the mean number of frozen embryos were 1.4, 1.9 and 3.2 in women with low,
284 moderately low and normal AMH, respectively.

285

The women underwent 2733 ET cycles (median 2, range 1−11 per woman), of which 1315 were fresh and 1418 frozen-thawed ETs. A single embryo was transferred in 2719 (99.5%) of all ET cycles. The PR per ET cycle in the whole study population was 41.1% (40.8% per transferred embryo) and the clinical PR 30.2% (30.0% per embryo). Of all 1123 pregnancies, 285 (25.4%) ended in a non-visualized pregnancy loss (of which 272 were biochemical pregnancies and 13 PULs), 143 (12.7%) ended in a miscarriage, 7 (0.6%) in a termination of the pregnancy and 14 (1.2%) were ectopic pregnancies. The LBR was 24.6% per ET cycle (673/2733) and 23.5% per transferred embryo (673/2861). The outcome of one pregnancy remained unknow. There were no stillbirths.

The PRs per ET varied between the women with different AMH levels, but the women with low or moderately low AMH were not more likely to undergo early pregnancy loss than those with normal AMH (Figure 2).

Low or moderately low AMH, compared with normal AMH, did not associate with miscarriage or non-visualized pregnancy loss, not even after adjusting for age and the other confounders (Table III). Results remained similar after restricting the analysis to women with AMH ≤6.0μg/L and considering the lower probability of pregnancy in women with low AMH by IPW analysis. The women with very low AMH (<0.5μg/L vs. ≥2.0μg/L) showed no increase in the miscarriage or non-visualized pregnancy loss risk either (unadjusted RR for miscarriage vs. live birth 0.89, 95% CI 0.40 – 1,94 and for non-visualized pregnancy loss vs. live birth 1.11, 95% CI 0.68 – 1.70). When AMH was tested as a continuous variable, the unadjusted RR for miscarriage was 0.99, 95% CI 0.90 – 1.09, and for non-visualized pregnancy loss 0.95, 95% CI 0.88 – 1.02, women with AMH >6.0μg/L omitted. Compared to women who were <35 years of age, women aged ≥38 had higher miscarriage risk, and women aged ≥35 had higher non-visualized pregnancy loss risk. Smoking, overweight, history of

endometriosis or medical conditions were not associated with miscarriage or non-visualized pregnancy loss.

Compared with live birth, neither low nor moderately low AMH increased the risk for early pregnancy loss (RR 0.86, 95% CI 0.68–1.10 for low and RR 1.01, 95% CI 0.86–1.27 for moderately low AMH after adjustment for age, smoking, overweight, endometriosis and medical conditions). The results were similar even though women with AMH >6.0μg/L were omitted from analyses.

Figure 3 shows the cPRs and the cLBRs across all ETs connected with the first IVF/ICSI in women with the different AMH levels. The univariable log-binomial regression analysis showed lower RRs for cumulative pregnancy and cumulative live birth for the women with low or moderately low AMH when compared with the women with normal AMH (Table IV). Adjustment for age and the other confounders and the omission of women with AMH >6.0 did not have an impact on the results. Compared to women <35 years of age, women ≥38 years showed lower cPR and cLBR, whereas smoking, overweight, endometriosis, or underlying medical conditions showed no effect. After stratifying the results by the number of mature oocytes, and adjusting by women's age, the differences in cPR or cLBR between AMH groups were no more evident, although women with moderately low AMH and mature oocyte number of ≥10 had lower cPR and cLBRs than women with normal AMH having the same amount of oocytes (table V). Also, women with 1-4 oocytes and low or moderately low AMH had a tendency to a lower cLBR compared with women with normal AMH. The proportion of women who went through their first IVF treatment without becoming pregnant during the follow-up period (drop-out) was 49.4% in low AMH, 41.0% in moderately low and 31.7% in normal AMH.

Discussion

This study showed that the women with low (<1.0μg/L) or moderately low (1.0–1.9μg/L) serum AMH levels had fewer pregnancies than the women with normal AMH (≥2.0μg/L) in their first IVF/ICSI treatment. When the pregnancy began, however, the women with low or moderately low AMH did not have an increased early pregnancy loss rate.

In the light of previous studies, the lack of an association between low AMH and pregnancy loss was somewhat unexpected. A very similar study design as ours showed an association between low AMH and an increased miscarriage rate in a population of 1060 IVF-ET cycles, although the difference in the women younger than 34 years was non-significant (Tarasconi *et al*., 2017). They included mostly double ETs and fewer possible confounding variables than we did. They studied miscarriage after fresh ETs only whereas we also included the subsequent FETs in our analyses. This is what matters in the clinical practice context and to the infertile couples. Their study population might differ from ours in the women's ethnicity, a known factor to influence both AMH (Seifer *et al*., 2009) and the miscarriage rate after IVF (Seifer *et al.,* 2008). Additionally, we calculated the risk for early pregnancy loss in three different populations, with consistent results, which strengthens our findings of no association between AMH and early pregnancy loss.

Studies on the AMH level and miscarriage after a natural conception have shown confounding results. The largest prospective cohort study on 533 women (aged 30 to 44 years, who conceived naturally) reported that those with very low AMH (≤0.4ng/mL) had an over two-fold increased risk for miscarriage when compared to the women with AMH ≥1.0ng/mL (Lyttle Schumacher *et al*., 2018), while we did not find an increased risk among women with very low AMH (<0,5μg/L). Lyttle Schumacher's study population differs markedly from ours since women with infertility, PCOS or endometriosis were excluded from their study. PCOS and endometriosis are both common reasons

for infertility and seem to alter ovarian reserve and the odds of a pregnancy loss. Moreover, they define pregnancy loss differently than we and apply different AMH cut-off levels.

Some previous studies have suggested recurrent miscarriage to be associated with low AMH. Atasever *et al.* (2016) found lower AMH in the women with recurrent miscarriages when compared with the age-matched general population (2.9ng/mL vs. 3.6ng/mL) and another cohort of 144 women reported lower AMH in the women with idiopathic recurrent miscarriage when compared with the women with explained recurrent miscarriage (1.2ng/mL vs. 2.0ng/mL) (Pils *et al*., 2016). On the other hand, AMH was not associated with pregnancy loss in the women with one or two previous pregnancy losses (Zarek *et al*., 2016), nor did it predict the outcome of further pregnancies in the women with recurrent miscarriage (Pils *et al*., 2019). In Zarek's study, women conceived without ART and received 400µg folic acid with either placebo or 81mg aspirin with the primary outcome of live birth, and in Pils'es study, women received combination therapy (aspirin, dydrogesterone, prednisone, and folic acid) for the prevention of miscarriage. Study populations in the recurrent pregnancy loss studies differ from ours, as the women were fertile and younger. In our study, the number of women with recurrent pregnancy loss was too small for sub-analyses. Hence, our study does not add knowledge of the role of AMH on recurrent miscarriage. In general, recurrent miscarriage has multiple etiologies, and the role of AMH remains controversial.

The strengths of our study are a homogenous population of women undergoing the their first IVF/ICSI treatment in one university hospital unit with very uniform treatment practices. Well-documented data allowed us to control the results with several confounding factors. The lack of association between AMH level and early pregnancy loss was shown even after exclusion of women with high AMH and considering the lower pregnancy rate connected with low or moderately low AMH by IPW. These sub-analyses are key strengths of our study. The vast majority of the ETs were

385 single ETs, which enabled us to identify each embryo's individual risk for loss, not confounded by
386 other transferred embryos. We observed not only the fresh ET, but also the entire IVF/ICSI treatment,
387 including all subsequent FETs, which increased the power of the study, and allowed the calculation
388 of the cPR and the cLBR. The cumulative live birth rate was higher among women with normal AMH
389 compared to women with low or moderately low AMH. About half of the women with normal AMH
390 had at least one child during their first IVF/ICSI treatment, while only one-third of women with low
391 AMH did. This finding is useful in everyday practice when clinicians counsel couples before their
392 first IVF/ICSI treatment. This information is also useful when assessing the overall effectiveness of
393 the services of an IVF clinic.
394
395 Only a few previous studies have reported associations between AMH and non-visualized pregnancy
396 loss. An elevated AMH level associated with a biochemical loss in Lyttle Schumacher's study (2018),
397 but the number of biochemical losses (n=9) was too small for conclusions. An earlier study reported
398 a biochemical PR of 13.8% after IVF-ET, which was comparable with the biochemical PR of the
399 fertile population with natural conceptions (Zeanda *et al*., 2015). The non-visualized pregnancy loss
400 rate in our study, including the biochemical pregnancies and the PULs, was high; 25.4% of all
401 pregnancies. Such a high rate may have two explanations. First, hCG was measured mostly from
402 serum, a sensitive method to detect even minor elevations. Second, ESHRE's definition for non-
403 visualized pregnancy loss is broad, including all pregnancies not confirmed by ultrasound, histology,
404 or surgery, irrespective of the gestational age. Thus, a proportion of the pregnancies, which are in
405 everyday clinical practice classified as miscarriages, were classified as non-visualized pregnancy
406 losses in our study.
407
408 The lack of association between low AMH and early pregnancy loss does not support the idea of
409 AMH as a biomarker for the oocyte quality. Poor oocyte quality is thought to associate with the

aneuploidy of the oocytes. Although some evidence exists that low AMH might relate to a higher rate of aneuploid embryos detected by preimplantation genetic testing (Katz-Jaffe *et al*., 2013), a more recent report did not find such an association (Morin *et al*. 2018). We showed lower cPRs and cLBRs for the women with low or moderately low AMH when compared to those with normal AMH. The smaller oocyte yield and higher age largely explained these differences, however. Also Li *et al.* (2013) found that after adjusting for age and the number of available embryos, AMH was not a significant predictor for the cLBR in the woman's first IVF. The results of our study and this previous study indicate that women with low or moderately low AMH have a smaller number of retrieved oocytes, less embryos to select from for the ET, and less ETs, leading to lower cPR and cLBR, but these women do not have a higher pregnancy loss rate. As a conclusion, AMH seems to be a biomarker of oocyte quantity rather than oocyte quality.

Pregnancy loss is conditional upon becoming pregnant, and AMH has an impact on pregnancy rate. In our study, women with low or moderately low AMH went through their first IVF/ICSI treatment without having a pregnancy (dropped out) more often than women with normal AMH, which may cause selection bias because these women were underrepresented in the pregnancy loss study population. In order to assess the possible selection bias, we used the IPW method and found no differences in early pregnancy loss risk between AMH levels. Unfortunately, we were not able to assess the individual reasons (the depletion of the frozen embryos, spontaneous pregnancy, other personal reasons, or the end of the follow-up period) for stopping the treatment. Our observational study reflects a real-world setting, where the couple's preferences as well as the IVF doctor's clinical view affect the decision of starting or continuing the IVF/ICSI treatment. Taken together, our results indicate that if embryo transfer was carried out, the risk of early pregnancy loss was not increased in women with low or moderately low AMH.

Finally, as limitations of our study, we did not have information on women's ethnicity, which is one potential confounder affecting both AMH and the miscarriage rate. Since nearly all women were Finnish, ethnicity should not be a source of residual confounding. AMH was measured within 12 months before the IVF stimulation since AMH is considered rather stable over this time period (decline of 5,6% per year) (Bentzen J *et al.,* 2013). Furthermore, the same time frame for AMH assessment was used in a previous study with similar aims (Tarasconi *et al.,* 2017). AMH has less inter- and intracycle variability than FSH or antral follicle count, and therefore, is the most appropriate measurement for ovarian reserve. Although the number of miscarriages was limited, especially in the women with low AMH, our data included many non-visualized pregnancy losses and the combination of these outcomes increased the power to detect possible associations. Thus, this study is, to the best of our knowledge, the largest one testing the association between AMH and early pregnancy loss. However, even after combining miscarriage and non-visualized pregnancy loss, the confidence intervals for the RRs were quite wide and one might argue that a larger study population is required. Therefore, research with an even larger number of pregnancy losses would give more information on the role of AMH in early pregnancy loss.

Conclusions

The women with low (<1.0μg/L) or moderately low (1.0–1.9μg/L) AMH had fewer pregnancies in the their first IVF/ICSI treatment than the women with normal AMH (≥2.0μg/L), but a higher age and smaller number of oocytes mainly explained the differences. When the pregnancy began, the women with low or moderately low AMH had as a good chance for a live birth as the women with normal AMH since the pregnancy loss rates were similar. These results suggest that AMH is a biomarker for the oocyte quantity, not for the oocyte quality. Our results have clinical value on counseling and ultimately give comfort and hope to the patients with low AMH.

460

461

462 **Authors' roles**

463 All authors participated on the design of the study. P.P. and H.H. collected the data. P.P. conducted
464 the statistical analyses with biostatistician Anna But and drafted the initial version of the manuscript.
465 H.H. provided important guidance throughout the study process and the manuscript preparation. All
466 authors contributed in the interpretation of the data, critically revised the manuscript and approved
467 the final article.

468

469

470 **Acknowledgements**

471 We thank biostatistician Anna But (MSc, PhD) from the Biostatistics Unit of the University of
472 Helsinki for her professional assistance on the design and implementation of the statistical analyses
473 and Mikko Anttonen (MD, PhD) from HUSLAB clinical chemistry, Helsinki University Hospital,
474 for his aid with the laboratory analysis methods.

475

476

477 **Funding**

478 The funds of Helsinki University Hospital, Helsinki, Finland (No. TYH2018232) , Hyvinkää
479 Hospital, Hyvinkää, Finland (No. M3080TUT18) and the Emil Aaltonen Foundation supported P.P.
480 and the grants from the Paulo Foundation and the Finnish Medical Foundation supported H.H.
481 throughout the study period.

482

483

484 **Conflict of interest**

485 None.

486

487

488

489 **References**

490

491 Atasever M, Soyman Z, Demirel E, Gencdal S, Kelekci S. Diminished ovarian reserve: is it a
492 neglected cause in the assessment of recurrent miscarriage? A cohort study. *Fertil*
493 *Steril* 2016;**105**:1236-1240.

494 Bentzen JG, Forman JL, Johannsen TH, Pinborg A, Larsen EC, Andersen AN. Ovarian antral follicle
495 subclasses and anti-mullerian hormone during normal reproductive aging. *J Clin Endocrinol*
496 *Metab* 2013;**98**:1602-1611.

497 Broekmans FJ, Kwee J, Hendriks DJ, Mol BW, Lambalk CB. A systematic review of tests predicting
498 ovarian reserve and IVF outcome. *Hum Reprod Update* 2006;**12**:685-718.

499 Broer SL, van Disseldorp J, Broeze KA, Dolleman M, Opmeer BC, Bossuyt P, Eijkemans MJ, Mol BW,
500 Broekmans FJ, IMPORT study group. Added value of ovarian reserve testing on patient
501 characteristics in the prediction of ovarian response and ongoing pregnancy: an individual patient
502 data approach. *Hum Reprod Update* 2013;**19**:26-36.

503 Crowson CS, Schenck LA, Green AG, Atkinson EJ, Therneau TM. The basics of propensity scoring
504 and marginal structural models. Technical report #84, August 1, 2013, Department of Health
505 Sciences Research, Mayo Clinic, Rochester, Minnesota

506 Farr SL, Schieve LA, Jamieson DJ. Pregnancy loss among pregnancies conceived through assisted
507 reproductive technology, United States, 1999-2002. *Am J Epidemiol* 2007;**165**:1380-1388.

508 Greenland S, Pearl J, Robins JM. Causal diagrams for epidemiologic
509 research. *Epidemiology* 1999;**10**:37-48.

510 Hassold T, Hunt P. To err (meiotically) is human: the genesis of human aneuploidy. *Nat Rev*
511 *Genet* 2001;**2**:280-291.

512 Iliodromiti S, Kelsey TW, Wu O, Anderson RA, Nelson SM. The predictive accuracy of anti-Mullerian
513 hormone for live birth after assisted conception: a systematic review and meta-analysis of the
514 literature. *Hum Reprod Update* 2014;**20**:560-570.

515 Katz-Jaffe MG, Surrey ES, Minjarez DA, Gustofson RL, Stevens JM, Schoolcraft WB. Association of
516 abnormal ovarian reserve parameters with a higher incidence of aneuploid blastocysts. *Obstet*
517 *Gynecol* 2013;**121**:71-77.

518 Kolte AM, Bernardi LA, Christiansen OB, Quenby S, Farquharson RG, Goddijn M, Stephenson MD,
519 ESHRE Special Interest Group, Early Pregnancy. Terminology for pregnancy loss prior to viability: a
520 consensus statement from the ESHRE early pregnancy special interest group. *Hum*
521 *Reprod* 2015;**30**:495-498.

522 La Marca A, Sighinolfi G, Radi D, Argento C, Baraldi E, Artenisio AC, Stabile G, Volpe A. Anti-
523 Mullerian hormone (AMH) as a predictive marker in assisted reproductive technology (ART). *Hum*
524 *Reprod Update* 2010;**16**:113-130.

525 Li HW, Lee VC, Lau EY, Yeung WS, Ho PC, Ng EH. Role of baseline antral follicle count and anti-
526 Mullerian hormone in prediction of cumulative live birth in the first in vitro fertilisation cycle: a
527 retrospective cohort analysis. *PLoS One* 2013;**8**:e61095.

528 Lyttle Schumacher BM, Jukic AMZ, Steiner AZ. Antimullerian hormone as a risk factor for
529 miscarriage in naturally conceived pregnancies. *Fertil Steril* 2018;**109**:106-1071.e1.

530 Magnus MC, Wilcox AJ, Morken NH, Weinberg CR, Haberg SE. Role of maternal age and pregnancy
531 history in risk of miscarriage: prospective register based study. *BMJ* 2019;**364**:l869.

532 Maheshwari A, McLernon D, Bhattacharya S. Cumulative live birth rate: time for a
533 consensus? *Hum Reprod* 2015;**30**:2703-2707.

534 Menken J, Trussell J, Larsen U. Age and infertility. *Science* 1986;**233**:1389-1394.

535 Missmer SA, Pearson KR, Ryan LM, Meeker JD, Cramer DW, Hauser R. Analysis of multiple-cycle
536 data from couples undergoing in vitro fertilization: methodologic issues and statistical
537 approaches. *Epidemiology* 2011;**22**:497-504.

538 Modest AM, Wise LA, Fox MP, Weuve J, Penzias AS, Hacker MR. IVF success corrected for drop-
539 out: use of inverse probability weighting. *Hum Reprod* 2018;**33**:2295-2301

540 Morin SJ, Patounakis G, Juneau CR, Neal SA, Scott RT, Seli E. Diminished ovarian reserve and poor
541 response to stimulation in patients <38 years old: a quantitative but not qualitative reduction in
542 performance. *Hum Reprod* 2018;**33**:1489-1498.

543 Pils S, Promberger R, Springer S, Joura E, Ott J. Decreased Ovarian Reserve Predicts Inexplicability
544 of Recurrent Miscarriage? A Retrospective Analysis. *PLoS One* 2016;**11**:e0161606.

545 Pils S, Stepien N, Kurz C, Nouri K, Springer S, Hager M, Promberger R, Ott J. Does anti-Mullerian
546 hormone predict the outcome of further pregnancies in idiopathic recurrent miscarriage? A
547 retrospective cohort study. *Arch Gynecol Obstet* 2019;**299**:259-265.

548 Seifer DB, Frazier LM, Grainger DA. Disparity in assisted reproductive technologies outcomes in
549 black women compared with white women. *Fertil Steril* 2008;**90**:1701-1710.

550 Seifer DB, Golub ET, Lambert-Messerlian G, Benning L, Anastos K, Watts DH, Cohen MH, Karim R,
551 Young MA, Minkoff H *et al.* Variations in serum mullerian inhibiting substance between white,
552 black, and Hispanic women. *Fertil Steril* 2009;**92**:1674-1678.

553 Sova H, Unkila-Kallio L, Tiitinen A, Hippeläinen M, Perheentupa A, Tinkanen H, Puukka K, Bloigu R,
554 Piltonen T, Tapanainen JS *et al.* Hormone profiling, including anti-Müllerian hormone (AMH), for
555 the diagnosis of polycystic ovary syndrome (PCOS) and characterization of PCOS
556 phenotypes. *Gynecol Endocrinol* 2019;**35**:595-600.

557 Tarasconi B, Tadros T, Ayoubi J, Belloc S, de Ziegler D, Fanchin R. Serum antimüllerian hormone
558 levels are independently related to miscarriage rates after in vitro fertilization-embryo
559 transfer. *Fertil Steril* 2017;**108**:518-524.

560 Teede HJ, Misso ML, Costello MF, Dokras A, Laven J, Moran L, Piltonen T, Norman RJ, International
561 PCOS Network. Recommendations from the international evidence-based guideline for the
562 assessment and management of polycystic ovary syndrome. *Hum Reprod* 2018c;**33**:1602-1618.

563 te Velde ER, Pearson PL. The variability of female reproductive ageing. *Hum Reprod*
564 *Update* 2002;**8**:141-154.

565 Yland J, Messerlian C, Minguez-Alarcon L, Ford JB, Hauser R, Williams PL, EARTH Study Team.
566 Methodological approaches to analyzing IVF data with multiple cycles. *Hum Reprod* 2019;**34**:549-
567 557.

568 Zamah AM, Stephenson MD. Antimullerian hormone and miscarriage: fifty shades of gray... *Fertil*
569 *Steril* 2018;**109**:1008-1009.

570 Zarek SM, Mitchell EM, Sjaarda LA, Mumford SL, Silver RM, Stanford JB, Galai N, Schliep KC, Radin
571 RG, Plowden TC *et al.* Antimullerian hormone and pregnancy loss from the Effects of Aspirin in
572 Gestation and Reproduction trial. *Fertil Steril* 2016;**105**:94-952.e2.

573 Zeadna A, Son WY, Moon JH, Dahan MH. A comparison of biochemical pregnancy rates between
574 women who underwent IVF and fertile controls who conceived spontaneously. *Hum*
575 *Reprod* 2015;**30**:783-788.

576

577

578

Table I The basic characteristics of the women with low, moderately low and normal AMH.

Characteristics	**AMH**			***P*-value**
	Low (<1.0 μg/L)	**Moderately low (1.0–1.9 μg/L)**	**Normal (≥2.0 μg/L)**	
Number of patients	235	278	870	
Age (years)	36.3 (23.8–40.7)[a,b]	34.6 (22.4–40.7)[a,c]	33.1 (21.4–40.8)[b,c]	**<0.0001**
BMI (kg/m^2)	23.0 (16.6–36.1)	23.1 (16.9–36.6)	22.7 (16.3–37.4)	0.38
Smoking in general	54/233 (23.2%)	78/278 (28.1%)	188/867 (21.7%)	0.09
Smoking during IVF/ICSI	11/233 (4.7%)	11/275 (4.0%)	44/866 (5.1%)	0.76
Healthy	161 (68.5%)	204 (73.4%)	635 (73.0%)	0.36
One or several previous miscarriage(s)	28 (11.9%)	36 (12.9%)	117 (13.4%)	0.82
Duration of infertility (years)	3.0 (0.3–15.0)	3.2 (0.9–18.0)	3.3 (0.7–17.0)	0.06
Primary infertility	154 (65.5%)	174 (62.6%)	586 (67.4%)	0.34
Main diagnosis				**<0.0001**
Ovulation disorder	8 (3.4%)	10 (3.6%)	104 (12.0%)	
Tubal factor	13 (5.5%)	33 (11.9%)	82 (9.4%)	
Male factor	37 (15.7%)	46 (16.5%)	209 (24.0%)	
Endometriosis	57 (24.3%)	43 (15.5%)	97 (11.1%)	
Unexplained	120 (51.1%)	146 (52.5%)	378 (43.4%)	
Prior ovarian surgery	40 (17.0%)	36 (12.9%)	62 (7.1%)	**<0.0001**

The continuous variables are presented as medians (range) and analyzed by using the Kruskal-Wallis test.
The same superscripts show significant differences between the women with different AMH levels in the Mann-Whitney U-test with Bonferroni's adjustment, $P^{a\text{-}c} < 0.0001$.
The categorial variables are presented as numbers (percentages within each AMH level) and analyzed by using the χ^2 test.
AMH, anti-Müllerian hormone

Table II The characteristics of the ovarian stimulation and the embryo transfer cycles in the women with low, moderately low and normal AMH.

Characteristics	**AMH**			***P*-value**
	Low (<1.0μg/L)	**Moderately low (1.0–1.9μg/L)**	**Normal (≥2.0μg/L)**	
Number of stimulation cycles	235	278	870	
Stimulation protocol				**<0.0001**
Long agonist	221(94.0%)	262 (94.2%)	641 (73.7%)	
Antagonist	14 (6.0%)	16 (5.8%)	229 (26.3%)	
Fertilization				**0.004**
IVF	177 (75.3%)	203 (73.0%)	568 (65.3%)	
ICSI	57 (24.3%)	67 (24.1%)	275 (31.6%)	
IVF and ICSI	1 (0.4%)	8 (2.9%)	27 (3.1%)	
Total dose of gonadotropin (IU)	3000 (1250–6600)^{a,b}	2000 (800–5100)^{a,c}	1375 (440–3875)^{b,c}	**<0.0001**
Number of oocytes retrieved	6 (1–24)^{a,b}	9 (1–32)^{a,c}	12 (1–51)^{b,c}	**<0.0001**
Number of mature oocytes	5 (1–24)^{a,b}	8 (1–24)^{a,c}	10 (1–45)^{b,c}	**<0.0001**
Number of fertilized oocytes	3 (1–21)^{a,b}	5 (1–17)^{a,c}	6 (1–30)^{b,c}	**<0.0001**
Number of frozen embryos	1 (0–15)^{d,b}	1 (0–16)^{d,b}	2 (0–25)^{b,c}	**<0.0001**
Women with ≥1 frozen embryos	128 (54.5%)	186 (66.9%)	665 (76.4%)	**<0.0001**
Total number of embryo transfer cycles	373	505	1855	
Fresh embryo transfer cycles	232 (62.2%)	277 (54.9%)	806 (43.5%)	
FET, spontaneous cycles with luteal support	79 (21.3%)	169 (33.5%)	614 (33.1%)	
FET, hormonal substitution cycles	62 (16.6%)	59 (11.7%)	435 (23.5%)	

The categorial variables are presented as numbers (percentages within each AMH level) and analyzed by using the χ^2 test.
The continuous variables are presented as medians (range) and analyzed by using the Kruskal-Wallis test.
The same superscripts show significant differences between the women with different AMH levels in the Mann-Whitney U-test with Bonferroni's adjustment, $P^{a,b,c}$ <0.0001, P^{d}=0.006
AMH, anti-Müllerian hormone; FET, frozen-thawed embryo transfer.

Table III The risk of miscarriage and non-visualized pregnancy loss according to woman's AMH level in three study populations among women who became pregnant in their first IVF/ICSI treatment, including all fresh and frozen-thawed embryo transfers. The log-binomial regression analysis with the GEE.

	Miscarriage vs. live birth				**Non-visualized pregnancy loss vs. live birth**			
	Unadjusted RR (95% CI)	***P*-value**	**Adjusted[a] RR (95% CI)**	***P*-value**	**Unadjusted RR (95% CI)**	***P*-value**	**Adjusted[a] RR (95% CI)**	***P*-value**
All women								
AMH								
Normal (≥ 2.0μg/L)	Reference		Reference		Reference		Reference	
Moderately low (1.0–1.9μg/L)	1.08 (0.73 – 1.61)	0.69	1.00 (0.68 – 1.49)	0.99	1.18 (0.91 – 1.52)	0.21	1.09 (0.85 – 1.41)	0.51
Low (< 1.0μg/L)	0.88 (0.54 – 1.42)	0.60	0.70 (0.42 – 1.17)	0.17	1.02 (0.74 – 1.40)	0.90	0.90 (0.65 – 1.23)	0.49
Women with AMH ≤ 6.0μg/L								
AMH								
Normal (2.0–6.0μg/L)	Reference		Reference		Reference		Reference	
Moderately low (1.0–1.9μg/L)	1.04 (0.69 – 1.55)	0.87	0.99 (0.67 – 1.48)	0.97	1.17 (0.90 – 1.52)	0.25	1.10 (0.85 – 1.43)	0.48
Low (< 1.0μg/L)	0.85 (0.52 – 1.38)	0.51	0.69 (0.41 – 1.16)	0.16	1.01 (0.73 – 1.40)	0.94	0.90 (0.65 – 1.24)	0.51
IPW[b], women with AMH ≤ 6.0μg/L								
AMH								
Normal (2.0–6.0μg/L)	Reference		Reference		Reference		Reference	
Moderately low (1.0–1.9μg/L)	1.18 (0.82 – 1.71)	0.37	1.13 (0.78 – 1.64)	0.51	1.16 (0.90 – 1.50)	0.21	1.09 (0.85 – 1.40)	0.43
Low (< 1.0μg/L)	0.88 (0.55 – 1.38)	0.57	0.70 (0.43 – 1.15)	0.16	1.05 (0.79 – 1.41)	0.72	0.89 (0.66 – 1.19)	0.43

AMH, anti-Müllerian hormone; GEE, generalized estimating equation; RR relative risk

[a]RRs adjusted for age, smoking, overweight, history of endometriosis and medical conditions (diabetes, rheumatic or celiac disease, inflammatory bowel disease, multiple sclerosis, and previous cancer)

[b]Data weighted by the inverse of the probability of becoming pregnant (IPW), based on the characteristics of the woman and the IVF/ICSI treatment.

Table IV The RRs for having at least one pregnancy (cumulative pregnancy) vs. no pregnancy or at least one live birth (cumulative live birth) vs. no live birth in the woman's first IVF/ICSI, including all embryo transfer cycles among all women and women with AMH ≤6.0μg/L according to AMH level. The log-binomial regression analysis.

	Cumulative pregnancy				**Cumulative live birth**			
	Unadjusted RR (95% CI)	***P*-value**	**Adjusted[a] RR (95% CI)**	***P*-value**	**Unadjusted RR (95% CI)**	***P*-value**	**Adjusted[a] RR (95% CI)**	***P*-value**
All women	**N=1383**				**N=1382**			
AMH								
Normal (≥ 2.0μg/L)	Reference		Reference		Reference		Reference	
Moderately low (1.0–1.9μg/L)	0.86 (0.78 – 0.96)	**0.008**	0.87 (0.78 – 0.97)	**0.012**	0.74 (0.63 – 0.88)	**0.001**	0.76 (0.64 – 0.90)	**0.002**
Low (< 1.0μg/L)	0.74 (0.65 – 0.85)	**<0.001**	0.78 (0.68 – 0.89)	**<0.001**	0.70 (0.58 – 0.84)	**<0.001**	0.79 (0.65 – 0.96)	**0.02**
Women with AMH ≤6.0μg/L	**N=1193**				**N=1192**			
AMH								
Normal (2.0–6.0μg/L)	Reference							
Moderately low (1.0–1.9μg/L)	0.89 (0.80 – 0.99)	**0.048**	0.90 (0.80 – 1.01)	0.06	0.77 (0.65 – 0.92)	**0.003**	0.79 (0.66 – 0.94)	**0.007**
Low (< 1.0μg/L)	0.77 (0.67– 0.88)	**<0.001**	0.80 (0.69 – 0.92)	**0.002**	0.72 (0.59 – 0.88)	**0.001**	0.81 (0.66 – 0.99)	**0.04**

AMH, anti-Müllerian hormone; RR, relative risk

[a]RRs adjusted for age, smoking, overweight, history of endometriosis and medical conditions (diabetes, rheumatic or celiac disease, inflammatory bowel disease, multiple sclerosis, and previous cancer)

Table IV The RRs for having at least one pregnancy (cumulative pregnancy) vs. no pregnancy or at least one live birth (cumulative live birth) vs. no live birth in the woman's first IVF/ICSI, including all embryo transfer cycles among all women and women with AMH ≤6.0μg/L according to AMH level. The log-binomial regression analysis.

	Cumulative pregnancy				**Cumulative live birth**			
	Unadjusted RR (95% CI)	***P*-value**	**Adjusted[a] RR (95% CI)**	***P*-value**	**Unadjusted RR (95% CI)**	***P*-value**	**Adjusted[a] RR (95% CI)**	***P*-value**
All women	**N=1383**				**N=1382**			
AMH								
Normal (≥ 2.0μg/L)	Reference		Reference		Reference		Reference	
Moderately low (1.0–1.9μg/L)	0.86 (0.78 – 0.96)	**0.008**	0.87 (0.78 – 0.97)	**0.012**	0.74 (0.63 – 0.88)	**0.001**	0.76 (0.64 – 0.90)	**0.002**
Low (< 1.0μg/L)	0.74 (0.65 – 0.85)	**<0.001**	0.78 (0.68 – 0.89)	**<0.001**	0.70 (0.58 – 0.84)	**<0.001**	0.79 (0.65 – 0.96)	**0.02**
Women with AMH ≤6.0μg/L	**N=1193**				**N=1192**			
AMH								
Normal (2.0–6.0μg/L)	Reference							
Moderately low (1.0–1.9μg/L)	0.89 (0.80 – 0.99)	**0.048**	0.90 (0.80 – 1.01)	0.06	0.77 (0.65 – 0.92)	**0.003**	0.79 (0.66 – 0.94)	**0.007**
Low (< 1.0μg/L)	0.77 (0.67– 0.88)	**<0.001**	0.80 (0.69 – 0.92)	**0.002**	0.72 (0.59 – 0.88)	**0.001**	0.81 (0.66 – 0.99)	**0.04**

AMH, anti-Müllerian hormone; RR, relative risk

[a]RRs adjusted for age, smoking, overweight, history of endometriosis and medical conditions (diabetes, rheumatic or celiac disease, inflammatory bowel disease, multiple sclerosis, and previous cancer)

Table V The RRs for having at least one pregnancy (cumulative pregnancy) vs. no pregnancy or at least one live birth (cumulative live birth) vs. no live birth in the woman's first IVF/ICSI in women with normal, moderately low and low AMH stratified by the number of mature oocytes. A log-binomial regression analysis, women with AMH >6.0μg/L excluded.

Mature oocytes (number of women)	**AMH**	**Cumulative pregnancy**				**Cumulative live birth**			
		Unadjusted RR (95% CI)	***P*-value**	**Adjusted[a] RR (95% CI)**	***P*-value**	**Unadjusted RR (95% CI)**	***P*-value**	**Adjusted[a] RR (95%CI)**	***P*-value**
1-4 (245)	Normal (2.0–6.0μg/L)	Reference		Reference		Reference		Reference	
	Moderately low (1.0–1.9μg/L)	1.05 (0.74 – 1.50)	0.77	1.04 (0.73 – 1.50)	0.81	0.70 (0.39 – 1.25)	0.28	0.67 (0.38 – 1.17)	0.16
	Low (<1.0μg/L)	0.91 (0.65 – 1.27)	0.59	0.92 (0.65 – 1.31)	0.63	0.77 (0.48 – 1.25)	0.16	0.78 (0.48 – 1.26)	0.31
5-9 (484)	Normal	Reference		Reference		Reference		Reference	
	Moderately low	1.08 (0.91 – 1.28)	0.40	1.09 (0.92 – 1.29)	0.34	1.00 (0.77 – 1.30)	0.99	1.02 (0.79 – 1.32)	0.89
	Low	0.93 (0.75 – 1.14)	0.47	0.94 (0.76 – 1.16)	0.54	0.95 (0.71 – 1.28)	0.75	1.01 (0.75 – 1.35)	0.96
>10 (463)	Normal	Reference		Reference		Reference		Reference	
	Moderately low	0.81 (0.68 – 0.97)	**0.02**	0.81 (0.68 – 0.96)	**0.02**	0.75 (0.57 – 0.97)	**0.03**	0.79 (0.61 – 1.03)	0.08
	Low	0.92 (0.73 – 1.16)	0.40	0.92 (0.73 – 1.16)	0.49	0.97 (0.69 – 1.35)	0.71	1.09 (0.79 – 1.51)	0.57

AMH, anti-Müllerian hormone; RR, relative risk; CI, confidence interval.

[a]RRs adjusted for age, smoking, overweight, history of endometriosis and medical conditions (diabetes, rheumatic or celiac disease, inflammatory bowel disease, multiple sclerosis, and previous cancer)

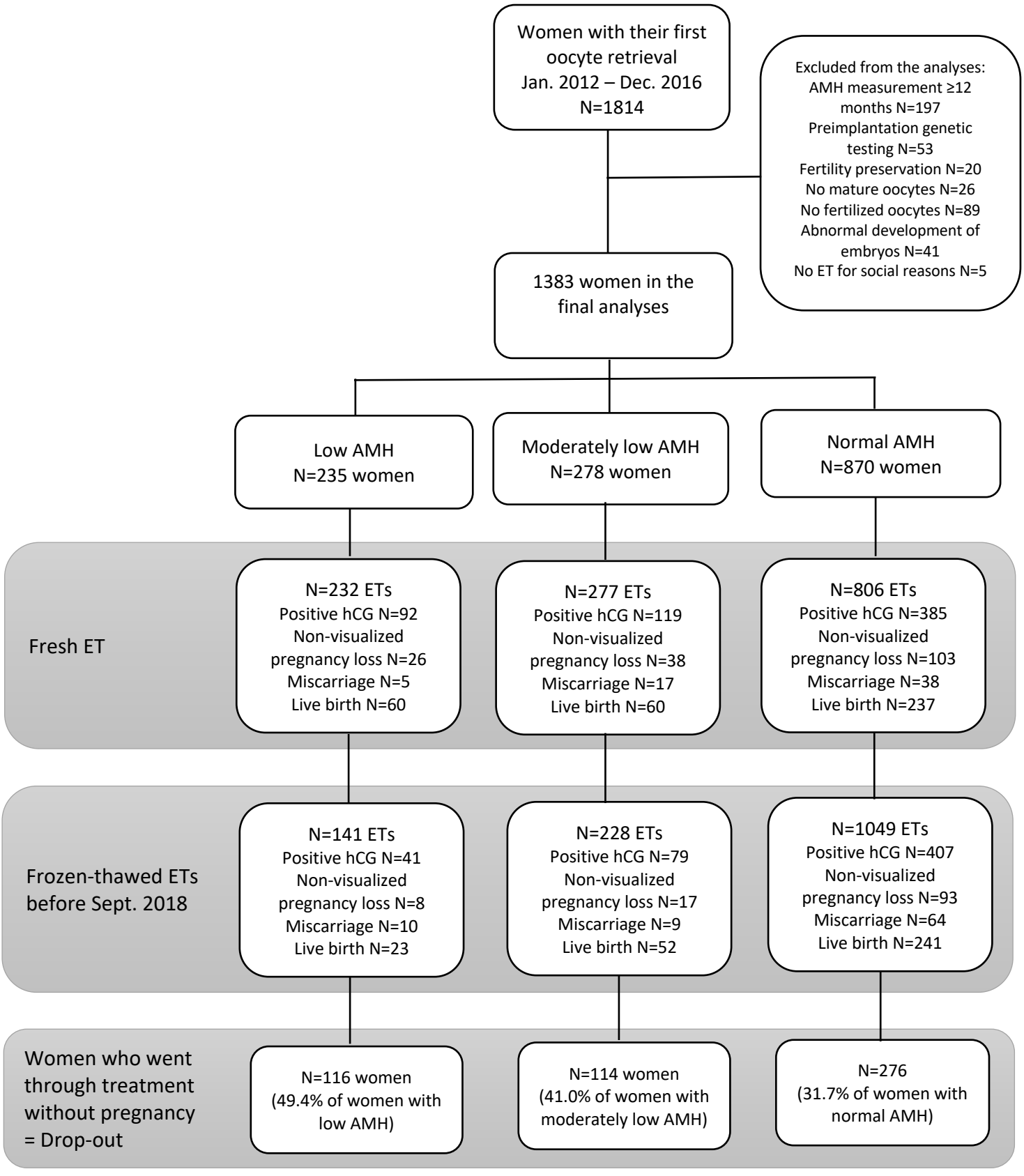
Table V The RRs for having at least one pregnancy (cumulative pregnancy) vs. no pregnancy or at least one live birth (cumulative live birth) vs. no live birth in the woman's first IVF/ICSI in women with normal, moderately low and low AMH stratified by the number of mature oocytes. A log-binomial regression analysis, women with AMH >6.0μg/L excluded.

Mature oocytes (number of women)	**AMH**	**Cumulative pregnancy**				**Cumulative live birth**			
		Unadjusted RR (95% CI)	***P*-value**	**Adjusted[a] RR (95% CI)**	***P*-value**	**Unadjusted RR (95% CI)**	***P*-value**	**Adjusted[a] RR (95%CI)**	***P*-value**
1-4 (245)	Normal (2.0–6.0μg/L)	Reference		Reference		Reference		Reference	
	Moderately low (1.0–1.9μg/L)	1.05 (0.74 – 1.50)	0.77	1.04 (0.73 – 1.50)	0.81	0.70 (0.39 – 1.25)	0.28	0.67 (0.38 – 1.17)	0.16
	Low (<1.0μg/L)	0.91 (0.65 – 1.27)	0.59	0.92 (0.65 – 1.31)	0.63	0.77 (0.48 – 1.25)	0.16	0.78 (0.48 – 1.26)	0.31
5-9 (484)	Normal	Reference		Reference		Reference		Reference	
	Moderately low	1.08 (0.91 – 1.28)	0.40	1.09 (0.92 – 1.29)	0.34	1.00 (0.77 – 1.30)	0.99	1.02 (0.79 – 1.32)	0.89
	Low	0.93 (0.75 – 1.14)	0.47	0.94 (0.76 – 1.16)	0.54	0.95 (0.71 – 1.28)	0.75	1.01 (0.75 – 1.35)	0.96
>10 (463)	Normal	Reference		Reference		Reference		Reference	
	Moderately low	0.81 (0.68 – 0.97)	**0.02**	0.81 (0.68 – 0.96)	**0.02**	0.75 (0.57 – 0.97)	**0.03**	0.79 (0.61 – 1.03)	0.08
	Low	0.92 (0.73 – 1.16)	0.40	0.92 (0.73 – 1.16)	0.49	0.97 (0.69 – 1.35)	0.71	1.09 (0.79 – 1.51)	0.57

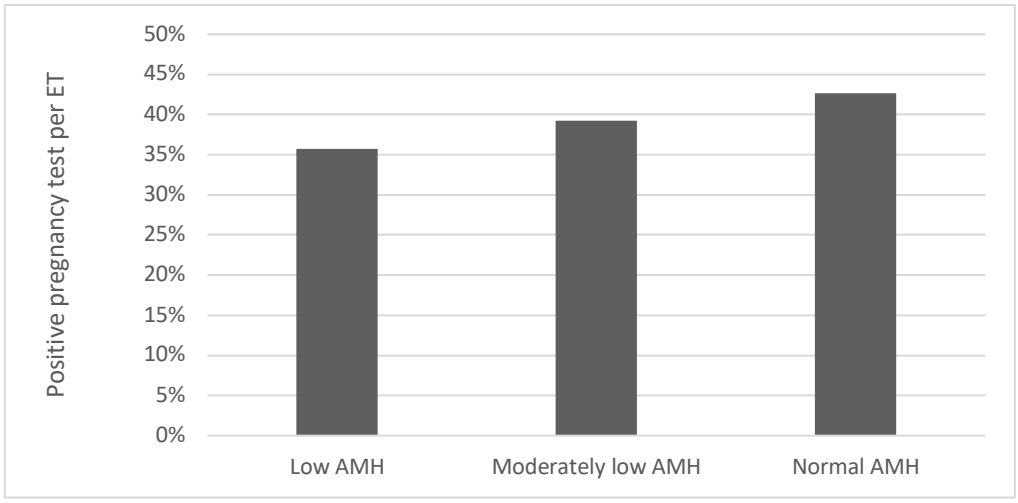
AMH, anti-Müllerian hormone; RR, relative risk; CI, confidence interval.

[a]RRs adjusted for age, smoking, overweight, history of endometriosis and medical conditions (diabetes, rheumatic or celiac disease, inflammatory bowel disease, multiple sclerosis, and previous cancer)

Figure 1. A flowchart of the patient selection and an overview of the IVF/ICSI treatments with all connected fresh and frozen-thawed embryo transfer (ET) cycles with the reproductive outcomes in women with low (<1.0µg/L), moderately low (1.0 – 1.9µg/L) and normal (≥2.0µg/L) anti-Müllerian hormone (AMH) level.



A



B

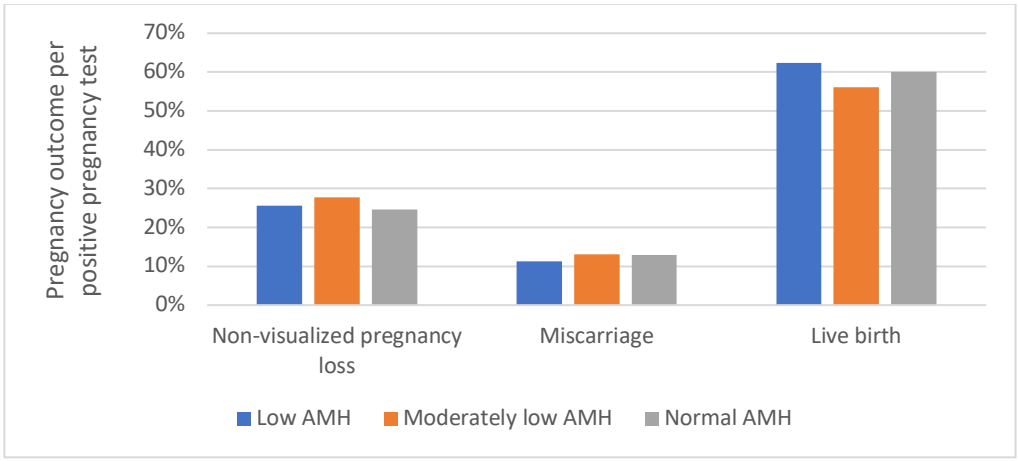
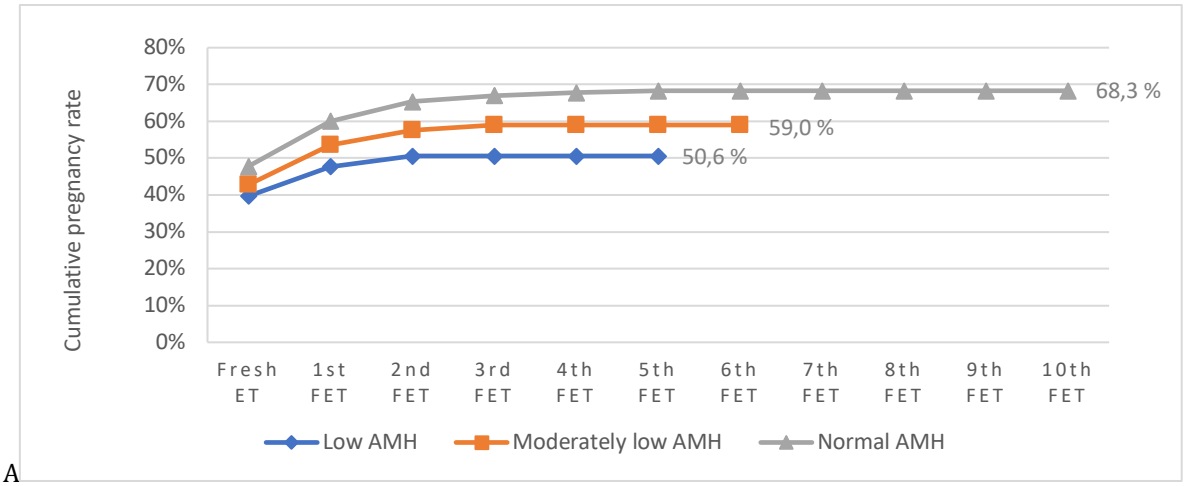
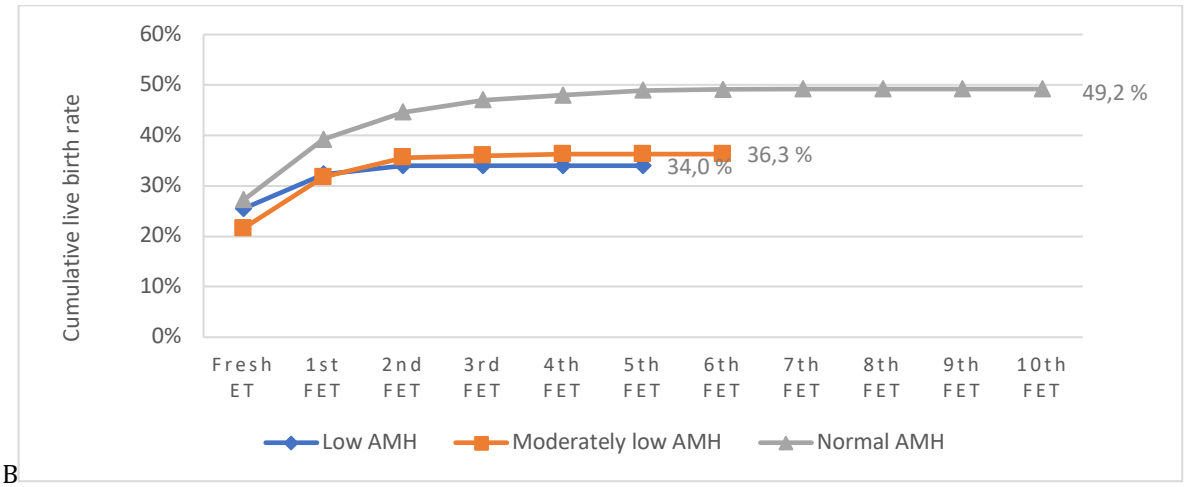


Figure 2 (A) The unadjusted pregnancy rates (positive pregnancy test per ET cycle) among the women with low (<1.0μg/L), moderately low (1.0–1.9μg/L), and normal (≥2.0μg/L) AMH level. (B) The unadjusted frequencies of non-visualized pregnancy losses, miscarriages, and live births after a positive pregnancy test according to the AMH levels. A Chi-square test showed a relationship between the AMH level and the positive pregnancy test rate (P<0.001), but not between AMH and the pregnancy outcome (P=0.63).



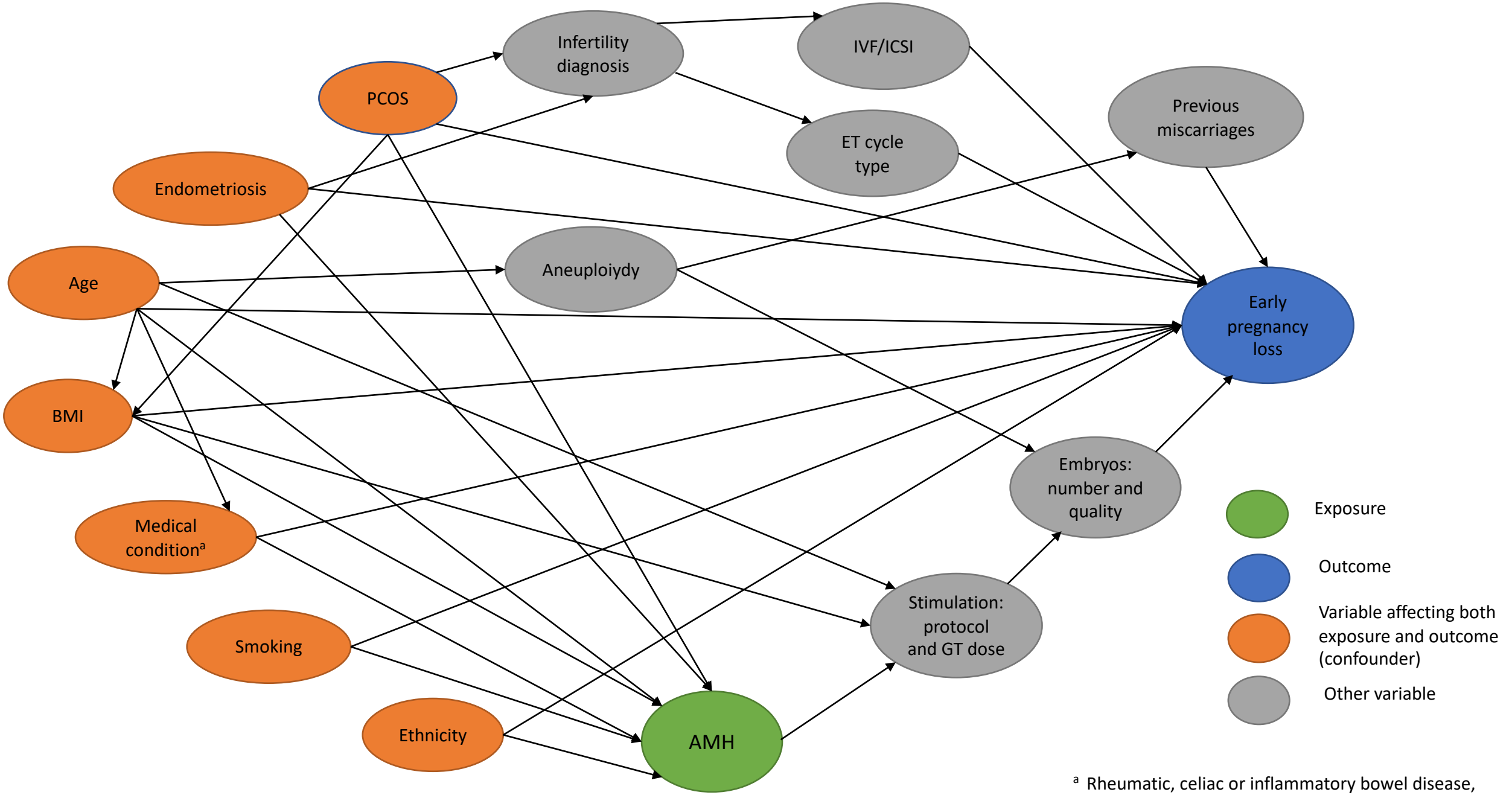
A



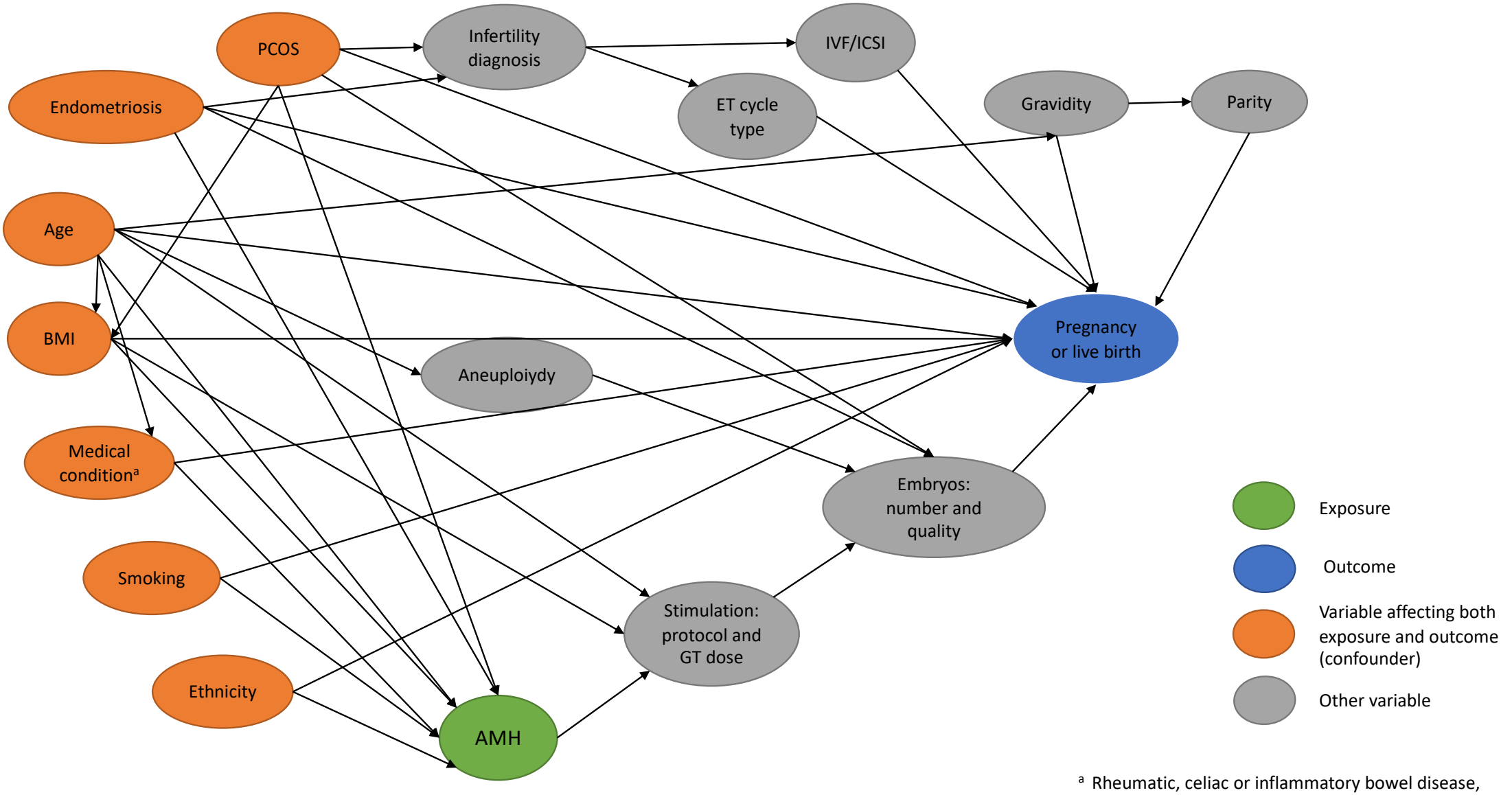
B

Figure 3 The unadjusted cumulative pregnancy rates (A) and the unadjusted cumulative live birth rates (B) in the women with low (<1.0μg/L), moderately low (1.0 – 1.9μg/L), and normal (≥2.0μg/L) AMH after consecutive ET cycles connected with couple's first IVF/ICSI stimulation.

AMH, anti-Müllerian hormone; ET, embryo transfer; FET, frozen embryo transfer



[a] Rheumatic, celiac or inflammatory bowel disease, diabetes, multiple sclerosis or previous cancer



[a] Rheumatic, celiac or inflammatory bowel disease, diabetes, multiple sclerosis or previous cancer