Original Article

Is There a Relationship between Serum Anti-Mullerian Hormone Levels and Abortion Rates in Patients Who Received *In vitro* Fertilisation-Embryo Transfer Cycles?

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ABSTRACT

Background: The studies which investigated the relationship between anti-Mullerian hormone (AMH) level and abortion rate have conflicting results. **Aims:** This retrospective study aimed to evaluate the relationship between AMH levels and abortion in women who achieved pregnancy with *in vitro* fertilisation (IVF) treatment. **Settings and Design:** This retrospective study was conducted in the Department of Gynecology and Obstetrics, Etlik Zubeyde Hanim Women's Health Training and Research Hospital, between January 2014 and January 2020. **Materials and Methods:** Patients below 40 years of age who conceived after IVF-embryo transfer treatment during a 6-year period and had a serum AMH level measurement were included. The patients were divided into three groups according to the serum AMH levels as low AMH (L-AMH, ≤1.6 ng/mL), intermediate AMH (I-AMH, 1.61–5.6 ng/mL) and high AMH (H-AMH, >5.6 ng/mL). The groups were compared in terms of obstetric, treatment cycle characteristics and abortion rates. **Statistical Analysis Used:** The Mann–Whitney *U*-test was used in comparison of non-parametric data of two groups; the Kruskal–Wallis test was used to compare the data of more than two groups. When a statistically significant difference was found in the Kruskal–Wallis test result, the groups were compared in pairs using the Mann–Whitney *U*-test, and the groups that made a statistical difference were determined. The Pearson's Chi-square and Fisher's exact tests were used to compare the independent categorical variables. **Results:** L-AMH (n = 164), I-AMH (n = 153) and H-AMH (n = 59) groups were similar in terms of obstetric histories and number of cycles applied, with an abortion rate of 23.8%, 19.6% and 16.9%, respectively (P = 0.466). The same analyses were repeated in two subgroups under 34 years of age and above, and no difference was found in terms of miscarriage rates. The number of oocytes retrieved and the number of mature oocytes were higher in H-AMH group compared to intermediate and low groups. **Conclusion:** No relationship was found between serum AMH level and abortion rate in women who achieved clinical pregnancy with IVF treatment.

Keywords: *Abortion, anti-Mullerian hormone, in vitro fertilisation, miscarriage, pregnancy*

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Introduction

Assisted reproductive technology (ART) is used in infertile patients when other treatment modalities fail; however, in some cases such as bilateral tubal occlusion or severe male factor, *in vitro* fertilisation and embryo transfer (IVF-ET) is the first choice. Although a significant progress has been made in ART over the years, the success rates have not still reached to the expected level.[1] Abortion is the most common complication of the first-trimester pregnancy, and 26% of all pregnancies and 10% of clinically proven pregnancies result in abortion, whereas this rate is approximately 15%–25% in IVF-ET pregnancies.[2]

Anti-Mullerian hormone (AMH) plays a crucial role in folliculogenesis.[3] AMH, used as one of the biomarkers of ovarian reserve, progressively declines over the years and becomes undetectable near menopause.[3] Serum AMH level is measured for evaluation of the ovarian reserve besides other ovarian reserve tests.[4,5] Many studies reported serum AMH level as superior to other commonly measured markers in predicting ovarian stimulation response in ART cycles.[4] Previous studies have shown that women with normal AMH levels respond better to ovarian stimulation and have lower cycle cancellation rate, higher retrieved oocyte count and higher pregnancy rate per cycle in IVF treatment.[6,7] However, the results from a meta-analysis are controversial as a weak association was reported between AMH level and clinical pregnancy rates.[8] In a recent study, Sun *et al*. reported that AMH to be a predictor of clinical pregnancy besides age and the number of retrieved oocytes.[9] The impact of AMH levels on oocyte and, subsequently, embryo quality have been investigated.[10] The number of studies evaluating the relationship between the pregnancy outcome and early pregnancy loss in women who achieved pregnancy with IVF treatment and serum AMH level is limited. The results of the two studies evaluating the relationship between AMH level and abortion rates are conflicting.[11,12] One study showed that the abortion risk increased, especially in pregnant women aged 34 and above in patients with low AMH (L-AMH) (<1.6 ng/mL) level.[11] On the other hand, in Peuranpaa's study, investigating the relationship between L-AMH levels and IVF results, no relationship was found between L-AMH and abortion rates.[12]

We aimed to evaluate the relationship between serum AMH levels and abortion rate in women who conceived by IVF-ET treatment.

Materials and Methods

The study participants were women who achieved pregnancy with IVF-fresh ET treatment between January 2014 and January 2020 at our hospital. The Ethics Committee approval was obtained from the Ethics Comittee of …… Hospital (Approval number: 2020/76, Date: 17 June 2020).

Of the 748 evaluated patients, 376 women who met the study criteria were included in the study [Figure 1]. Inclusion criteria were: being aged between 18 and 40 years, having clinical pregnancy detected after the IVF-ET cycle, having no history of polycystic ovary syndrome (PCOS), reccurent pregnancy loss, genetic disorder, or giving birth to an offspring with congenital anomaly and having serum AMH level measured within the past 12 months before the IVF treatment at our institution. Women aged <18 and >40 years of age, who had a family history of a genetic disorder or a congenital malformation, with detected uterine anomaly or malformation, PCOS and had a history of recurrent pregnancy loss were excluded [Figure 1]. As a part of the policy of the institution, all the patients who received treatment at our institution are required to give consent to the utilization of their medical data anonymously.

Patients' age, aetiology of infertility, body mass index (BMI), serum AMH levels, number of oocytes retrieved, number of mature oocytes, number of fertilised oocytes, number of embryos transferred and abortion rates were obtained from the patient's files

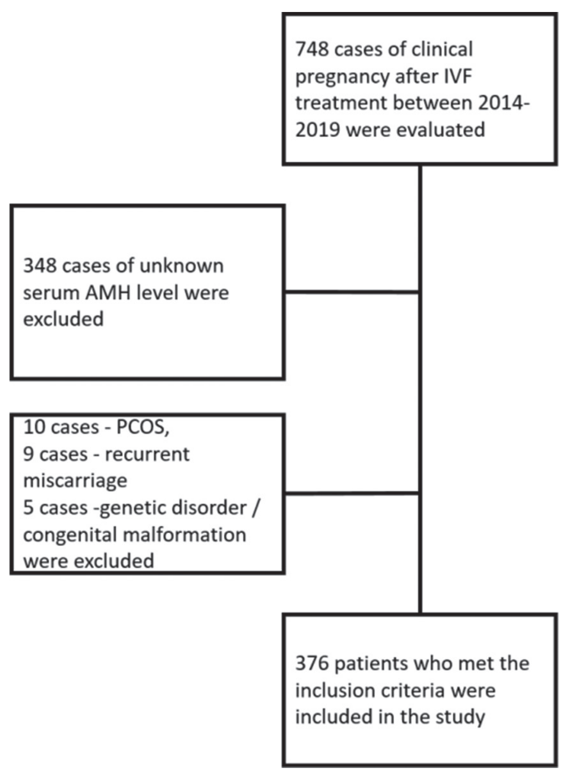

Figure 1: Flow chart of the study. IVF = *In vitro* fertilisation, AMH = Anti-Mullerian hormone, PCOS = Polycystic ovary syndrome

and hospital's electronical records. The presence of an embryo with a positive heartbeat on transvaginal ultrasonography performed 6 weeks after ET was defined as clinical pregnancy. The gestational age was calculated according to the last menstrual date, and it was confirmed by the measurement of crown-rump length with transvaginal ultrasonography. Abortion was defined as 'the termination of a pregnancy within 20 weeks of pregnancy after accompanied by, resulting in or closely followed by the death of the embryo or foetus' as defined by Merriam-Webster's Medical Dictionary.[13]

Based on a study by Tarasconi *et al.*,[11] the patients were classified into three groups according to the serum AMH levels as (L-AMH; ≤1.6 ng/mL), intermediate AMH (I-AMH, 1.61–5.6 ng/mL) and high AMH (H-AMH >5.6 ng/mL). The groups were compared in terms of demographic, clinical, obstetric, cycle characteristics and abortion. In addition, the same analysis was performed in two subgroups, defined by the age of the women (a) under 34 years and (b) 34 and above.

The study of Tarasconi *et al.*[11] was taken as a reference, and the G*Power software determined that the sample number should consist of a minimum of 75 abortion cases.

Statistical analysis

The independent samples *t*-test was used to compare the data of two groups showing normal distribution between independent groups; ANOVA test was used to compare the data of more than two groups. When a statistically significant difference was detected as a result of the ANOVA test, *Post hoc* Tukey analysis determined which groups made this difference. The Mann–Whitney *U*-test was used in comparison of non-parametric data of two groups; the Kruskal–Wallis test was used to compare the data of more than two groups. When a statistically significant difference was found in the Kruskal–Wallis test result, the groups were compared in pairs using the Mann–Whitney *U*-test, and the groups that made a statistical difference were determined. The Pearson's Chi-square and Fisher's exact tests were used to compare the independent categorical variables. Receiver operator characteristic (ROC) analysis was performed to determine the optimum cut-off value of serum AMH level that could predict abortion. Statistical analysis of the study was done in two ways, and $P < 0.05$ was considered statistically significant.

Results

The mean age of 376 patients included in the study was 31 ± 4.3 years (median: 30 years, range: 19–39 years). Unexplained infertility was the most common cause of infertility among the patients (36.5%), followed by male factor (31.6%) and diminished ovarian reserve (20.5%) [Table 1]. Abortion occured in 21% of women who had a clinical pregnancy after IVF-ET cycle. The patients were evaluated in three groups according to their serum AMH levels, and a comparison of age, BMI, number of stimulation cycle and IVF-ET data of the three groups is shown in Table 1. The L-AMH group's median age was higher than the I-AMH and H-AMH groups ($P < 0.001$ and $P = 0.002$, respectively). The most common cause of infertility in the L-AMH group was diminished ovarian reserve (40.9%), whereas it was male factor (44.4%) in the I-AMH group and unexplained infertility in the H-AMH group (69.5%). There was no significant difference between the groups in terms of BMI and the number of stimulation cycles ($P = 0.768$ and $P = 0.391$). The number of retrieved, mature and fertilised oocytes in the L-AMH group was significantly lower than in the I-AMH and H-AMH groups ($P = 0.001$). In addition, the number of retrieved oocytes, mature oocytes and fertilised oocytes in the H-AMH group was higher than the I-AMH group ($P = 0.001$, $P = 0.002$ and $P = 0.003$).

The data are compared in patients aged <34 and ≥34 years as shown in Table 2. The abortion rates in the low, I-AMH and H-AMH groups were 23.8%, 19.6% and 16.9% for all patients, 24.7%, 18.2% and 18.0% for patients <34 years of age and it was 22.4%, 25.0% and 11.1% for patients ≥34 years of age ($P = 0.466$, $P = 0.436$ and $P = 0.675$, respectively) [Figure 2]. These numerically different abortion rates of the subgroups were not statistically significant.

The patients were evaluated in two groups according to the pregnancy outcome: Group A included the patients whose pregnancy resulted with abortion, and Group B included those resulted with live birth. Age, IVF

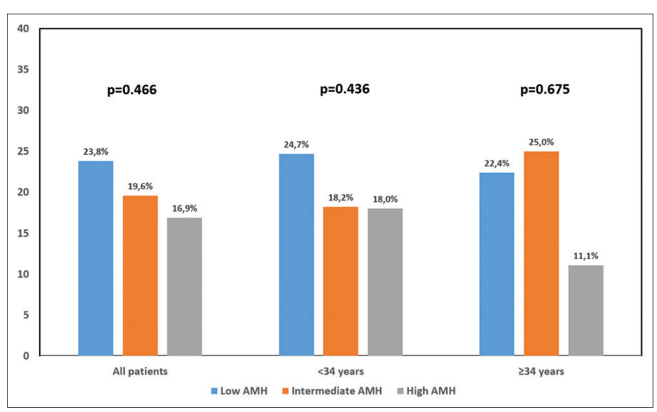

Figure 2: Abortion rates according to AMH groups. AMH = Anti-Mullerian hormone

Table 1: Comparison of the groups determined according to serum anti-Mullerian hormone levels in terms of age, *in vitro* fertilisation indications, body mass index, number of cycles and oocyte counts

	All patients	Groups according to serum AMH levels			*P*
		L-AMH ≤1.6 ng/mL (*n*=164)	I-AMH 1.61-5.6 ng/mL (*n*=153)	H-AMH >5.6 ng/mL (*n*=59)	
Age (year), median (IQR)	30 (19–39)	32 (28–36)	29 (26–33)	30 (27–32)	<0.001"
Indications for IVF-ET, *n* (%)					
Male factor	119 (31.6)	42 (25.6)	68 (44.4)	9 (15.2)	NA
Tubal factor	28 (7.4)	9 (5.5)	16 (10.5)	3 (5.1)	
Diminished ovarian reserve	77 (20.5)	67 (40.9)	7 (4.6)	3 (5.1)	
Endometriosis	15 (4.0)	8 (4.9)	4 (2.6)	3 (5.1)	
Unexplained infertility	137 (36.5)	38 (23.1)	58 (37.9)	41 (69.5)	
BMI (kg/m^2), mean±SD	26.8±5.3	26.7±5.7	26.7±4.9	27.2±5.1	0.768
Treatment cycle, *n* (%)					
1st	207 (55.1)	89 (54.3)	87 (56.8)	31 (52.5)	0.391
2nd	99 (26.3)	42 (25.6)	44 (28.8)	13 (22.0)	
≥3rd	70 (18.6)	33 (20.1)	22 (14.4)	15 (25.5)	
Stimulation protocol, *n* (%)					
Long agonist	124 (33.0)	60 (36.6)	52 (34.0)	12 (20.3)	0.071
Antagonist	252 (67.0)	104 (63.4)	101 (66.0)	47 (79.7)	
Number of oocytes retrieved (IQR)	10 (6–14)	7 (5–9)	11 (8–15)	15 (12–21)	<0.001"
Number of mature oocytes (IQR)	7 (5–11)	6 (4–8)	9 (5–12)	12 (9–15)	<0.001"
Number of fertilised oocytes (IQR)	4 (3–7)	3 (2–5)	5 (3–7)	6 (5–10)	<0.001"
Oocyte quality index (IQR)	5.2 (2.0–6.1)	5.3 (4.9–5.7)	5.1 (4.5–5.6)	5.1 (4.7–5.5)	0.107
Number of embryo transfer cycles, mean±SD	1.3±0.5	1.3±0.5	1.2±0.4	1.3±0.5	0.126

AMH=Anti-Mullerian hormone, IVF=*In vitro* fertilisation, BMI=Body mass index, IQR=Interquartile range, SD=Standard deviation, NA=Not applicable, ET=Embryo transfer, L-AMH=Low AMH, I-AMH=Intermediate AMH, H-AMH=High-AMH

indications, BMI and the number of stimulation cycles of the groups were compared. The number of oocytes retrieved, the number of mature and fertilised oocytes and the oocyte quality index and AMH levels were similar in both groups [*P* = 0.839, *P* = 0.581, *P* = 0.573 and *P* = 0.283 and *P* = 0.450, respectively, Table 3]. Whether the serum AMH level predicted abortion rate was evaluated by ROC analysis [Figure 3].

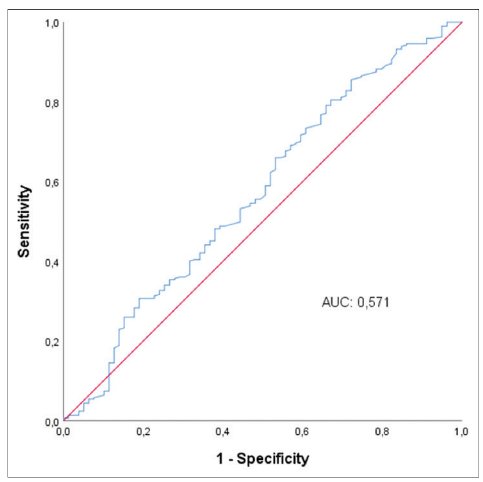

Figure 3: ROC analysis to determine the optimum AMH value that can predict abortion. AMH = Anti-Mullerian hormone, ROC = Receiver operator characteristic, AUC = Area under curve

Discussion

The live birth rate in fresh and frozen-thawed ET cycles still needs improvement as even the most recent studies present live birth rate values below 50%.[14] There has been ongoing research to identfy the risk factors for pregnancy loss in pregnancies achieved after intracytoplasmic sperm injection-ET (IVF/ICSI-ET) treatment cycles to obtain higher live birth rates. In a large series presented by Yang *et al*., the rate of pregnancy loss was 19.7%, and age and BMI were shown as the risk factors.[15] In the presented study, 376 women achieved pregnancy with fresh IVF/ET cycles, and the abortion rate was 21%. Peuranpää *et al*. analysed 1323 pregnancies conceived by IVF/ICSI cycles with fresh and frozen-thawed ET and reported miscarriage in 12.7% and non-visualised pregnancy loss in 25.4%.[12] Peuranpää reported a lower cumulative live birth rate in women with L-AMH (<2 μg/L) compared to the ones with an AMH level ≥2.0 μg/L during the first treatment cycle; however, the risk of early pregnancy loss was not found to be related with AMH levels. In the presented study, we evaluated the clinical pregnancies that ended in abortion, and non-visualised pregnancy loss was not investigated. Cornille *et al*. investigated the miscarriage rate in women aged under 37 years with L-AMH and normal AMH levels and reported a miscarriage rate of

Table 2: Comparison of the age, *in vitro* fertilisation indications, body mass index, number of cycles and oocyte counts of the patients under and above 34 years of age

	<34 years of age				≥34 years of age			
	Low ≤1.6 (*n*=164)	Intermediate 1.61–5.6 (*n*=153)	High >5.6 (*n*=59)	*P*	Low ≤1.6 (*n*=164)	Intermediate 1.61–5.6 (*n*=153)	High >5.6 (*n*=59)	*P*
Age (year), median (IQR)	29 (27–31)	28 (26–30)	29 (27–30)	0.110	36 (35–37)	36 (34–37)	35 (34–37)	0.300
IVF indications, *n* (%)								
Male factor	27 (27.8)	60 (49.6)	7 (14.0)	NA	15 (22.4)	8 (25.0)	2 (22.2)	NA
Tubal factor	6 (6.2)	10 (8.3)	2 (4.0)		3 (4.5)	6 (18.8)	1 (11.1)	
Diminished ovarian reserve	43 (44.4)	5 (4.1)	1 (2.0)		24 (35.7)	2 (6.3)	2 (22.2)	
Endometriosis	5 (5.2)	3 (2.5)	3 (6.0)		3 (4.5)	1 (3.1)	0 (0)	
Unexplained	16 (16.4)	43 (45.5)	37 (74.0)		22 (32.9)	15 (46.8)	4 (44.4)	
BMI (kg/m^2), mean±SD	26.6±5.8	26.5±4.5	27.0±5.0	0.829	26.9±5.5	27.4±6.0	28.7±5.6	0.674
Cycles, *n* (%)								
1st	52 (53.6)	67 (55.4)	25 (50.0)	0.279	37 (55.2)	20 (62.5)	6 (66.7)	0.882
2nd	27 (27.8)	39 (32.2)	12 (24.0)		15 (22.4)	5 (15.6)	1 (11.1)	
≥3rd	18 (18.6)	15 (12.4)	13 (26.0)		15 (22.4)	7 (21.9)	2 (22.2)	
Stimulation protocol, *n* (%)								
Long agonist	37 (38.1)	38 (31.4)	9 (18.0)	0.045	23 (34.3)	14 (43.8)	3 (33.3)	0.643
Antagonist	60 (61.9)	83 (68.6)	41 (82.0)		44 (65.7)	18 (56.3)	6 (66.7)	
Number of oocytes retrieved (IQR)	7 (5–10)	12 (8–16)	15 (12–23)	<0.001	6 (5–8)	10 (7–12)	14 (12–20)	<0.001”
Number of mature oocytes (IQR)	6 (4–8)	9 (5–13)	12 (9–15)	<0.001	5 (4–7)	8 (5–11)	9 (8–12)	<0.001”
Number of fertilised oocytes (IQR)	3 (2–5)	5 (3–7)	6 (5–10)	<0.001	3 (2–5)	4 (3–6)	5 (5–9)	0.002”
Oocyte quality index (IQR)	5.2 (4.8–5.7)	5.1 (4.5–5.5)	5.2 (4.7–5.5)	0.320	5.3 (5.0–5.7)	5.2 (4.8–5.7)	4.9 (4.6–5.2)	0.215
Number of embryo transfer cycles, mean±SD	1.1±0.3	1.1±0.3	1.2±0.4	0.167	1.7±0.4	1.7±0.4	1.7±0.5	0.986

AMH=Anti-Mullerian hormone, IVF=*In vitro* fertilisation, BMI=Body mass index, IQR=Interquartile range, SD=Standard deviation, NA=Not applicable

9.5% and 6.8%, respectively, in fresh cycles.[16] L-AMH level was defined as <10th percentile by Cornille *et al*., and these low levels did not alter the miscarriage rate in young women.

L-AMH levels were proposed to be related with pre-term birth, pre-eclampsia and recurrent miscarriage.[17-19] The relationship between serum AMH level and abortion in women getting pregnant through natural conception was also evaluated in studies, and conflicting results were obtained.[20-22] Lyttle Schumacher *et al*. reported that low serum AMH level (0.4 ng/mL) could predict abortion in their prospective cohort study, which included 533 women aged 30–44 years.[20] Atasever *et al*. showed that women with recurrent pregnancy loss were three times more likely to have L-AMH levels (<1 ng/mL) than the control group.[21] However, Zarek *et al*. did not find any relationship between serum AMH level and abortion.[22] Similarly, Cornille *et al*. failed to find any relationship between L-AMH levels and miscarriage rate in young women in fresh IVF-ET cycles.[16] Peuranpää *et al*. did not detect a higher miscarriage rate in fresh and frozen-thawed ET cycles in women with moderately low and L-AMH levels. In the other hand, they reported higher cumulative live birth rate among women with normal AMH levels than women with L-AMH, related to the higher number of oocytes and embryos obtained.[12] However, Szafarowska *et al*. found a negative correlation between H-AMH levels and abortion rates when AMH rate was >2.5 ng/mL.[23] In the same study, the clinical pregnancy rates in the three groups with AMH levels <1 ng/mL, 1–2.5 ng/mL and >2.5 ng/ml were 42.3%, 41.1% and 38.9%, respectively ($P > 0.05$). In the presented study, the relationship between serum AMH level and abortion was evaluated in women who achieved clinical pregnancy with fresh IVF-ET treatment, and the abortion rates were 23.8%, 19.6% and 16.9%, respectively. The abortion rate was slightly higher, but not statistically significant in women with L-AMH.

The predictive value of various clinical and biochemical markers in IVF/ICSI treatment cycle success has been investigated widely; however, using these makers in prediction of abortion rate is a new field of research. Increasing maternal age has been related with a lower pregnancy and higher abortion rate in IVF-ET cycles.[24] Tan *et al*. reported a miscarriage rate of 15.1% in women aged below 30 years, while the rates increased to 30% and 47.7% at 38 and 39 years of age, respectively.[24]

Table 3: Comparison of the patients who experienced abortion with the patients with ongoing pregnancy after *in vivo* exposure therapy treatment

	State of pregnancy		*P*
	Group A (abortion group) (*n*=79)	Group B (ongoing pregnancy) (*n*=297)	
Age (year), median (IQR)	31 (29–34)	30 (27–34)	0.062
Age (year), *n* (%)			
<34	55 (69.6)	213 (71.7)	0.714
≥34	24 (30.4)	84 (28.3)	
IVF indications, *n* (%)			
Male factor	31 (39.2)	88 (29.6)	NA
Tubal factor	4 (5.1)	24 (8.1)	
Diminished ovarian reserve	4 (5.1)	73 (24.6)	
Endometriosis	3 (3.8)	12 (4.0)	
Unexplained infertility	37 (46.8)	100 (33.7)	
BMI (kg/m^2), mean±SD	27.3±5.8	26.6±5.1	0.294
Cycles, *n* (%)			
1st	38 (48.1)	169 (56.9)	0.351
2nd	25 (31.6)	74 (24.9)	
≥3rd	16 (20.3)	54 (18.2)	
Stimulation protocol, *n* (%)			
Long agonist	25 (31.6)	99 (33.3)	0.777
Antagonist	54 (68.4)	198 (66.7)	
Number of oocytes retrieved (IQR)	9 (7–14)	10 (6–14)	0.839
Number of mature oocytes (IQR)	7 (5–12)	7 (5–11)	0.581
Number of fertilised oocytes (IQR)	4 (3–7)	4 (3–6)	0.573
Oocyte quality index (IQR)	5.3 (4.8–5.7)	5.2 (4.7–5.6)	0.283
Number of embryo transfer cycles, mean±SD	1.3±0.5	1.3±0.4	0.266
AMH level (ng/mL), mean±SD	2.74±3.43	3.04±2.94	0.450

AMH=Anti-Mullerian hormone, IVF=*In vitro* fertilisation, BMI=Body mass index, IQR=Interquartile range, SD=Standard deviation, NA=Not applicable

AMH together with age is investigated for prediction of IVF treatment cycles,[25] and pregnancies and live births were achieved in young patients with lower AMH levels.[26] Tarasconi *et al*. evaluated the relationship between AMH and abortion rates in 1060 patients who achieved clinical pregnancy after IVF-ET, and showed that the risk of abortion was doubled in women aged 34 years and above who had low serum AMH levels (≤1.60 ng/mL). In the young patient group (<34 years of age), the risk of abortion was not increased in patients with L-AMH.[11] The researchers speculated that this result was due to the lower incidence of abortion in the younger patient group compared to the older patient group.[11] However, when the study details were examined, there were 467 patients in the young age patient group, and the abortion rate was 15%.

There are several limitations arising from the retrospective nature of the study. However, the abortion rate of 21% in the patient population included in the study, and its compatibility with the literature suggested that our sample size could represent the study population. The study groups based on AMH levels are not homogenous in the published studies as different researchers used different values for defining low, I-AMH and H-AMH levels. For this reason, the studies failed to report a standard cut-off value for serum AMH levels in terms of predicting abortion and live birth rate. This limitation is valid for almost all cited studies. Therefore, the cut-off values used in the study of Tarasconi *et al*. were taken as a reference while creating AMH groups in our study. On the other hand, the exclusion of patients with PCOS from the study provided an objective comparison of mean AMH values, which was one of the strengths of our study. Many factors affecting abortion may have affected the results. Another study limitation was that these factors could not be matched between groups.

Conclusion

In women who achieved pregnancy with IVF treatment, there was no relationship between serum AMH levels and abortion rates. The conflicting results from previous studies evaluating the relationship between serum AMH level and abortion suggest that comprehensive prospective randomised studies are needed.

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Nil.

Conflicts of interest

There are no conflicts of interest.

Data availability

Our data are available for the journal if requested by the editor(s). Data can be shared as per requirement.

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