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ORIGINAL ARTICLE

High Anti-Müllerian Hormone Strongly Correlates with Reproductive Outcomes in Women Undergoing Assisted Reproduction

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SUMMARY

Background: **In a retrospective cohort of 881 women with gynecologic and unexplained infertility, we aimed to study the relationship between serum AMH levels and ART outcomes. This retrospective cohort includes 881 infertile women aged 20 - 45 who underwent their first fresh autologous non–preimplantation genetic diagnosis ART cycles between 2012 and 2020.**

Methods: **We assessed the correlation between AMH levels and reproductive outcomes among infertile women with different causes of infertility (including endometriosis, polycystic ovary syndrome (PCOS), and unexplained infertility).**

Results: **We found a strong correlation between high AMH levels and reproductive outcomes independent of age and the cause of infertility in women undergoing ART. In all patients with gynecologic and unexplained infertility, higher AMH correlated with the improved number of oocytes ($p < 0.001$), MII oocytes ($p < 0.001$), good-quality embryos ($p < 0.001$), chemical pregnancy rate ($p < 0.001$ in women < 37; and $p = 0.002$ in women over 37), clinical pregnancy rate ($p < 0.05$), and live birth rate ($p = 0.05$).**

Conclusions: **Serum AMH concentrations can be invaluable for predicting ovarian reserve and reproductive outcomes in young and advanced-age infertile patients undergoing ART. However, it should not be used as the sole predictive marker for disqualifying infertile women from ART treatment. Further large cohort studies are warranted to determine an exact cutoff point for AMH to provide an accurate ART success prediction.**

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KEYWORDS

anti-müllerian hormone, reproductive techniques, assisted, fertilization *in vitro*, reproductive outcomes, pregnancy outcomes

LIST OF ABBREVIATIONS

AMH - Anti mullerian hormone
ART - Assisted reproductive technique
PCOS - Polycystic ovary syndrome
OHSS - Ovarian hyperstimulation syndrome

AFC - Antral follicle count
FSH - Follicle stimulating hormone
LH - Luteinizing hormone
IVF - *In vitro* fertilization
ICSI - Intracytoplasmic sperm injection
POR - Poor ovarian reserve
BMI - Body mass index
PGD - Pre-implantation genetic diagnosis
FET - Frozen embryo transfer
DOR - Diminished ovarian reserve
ET - Embryo transfer

INTRODUCTION

The anti-müllerian hormone (AMH) has long been used in reproductive medicine practice to assess the ovarian follicle pool size, estimate ovarian reserve, predict ovarian lifespan, forecast the onset of menopause, support the diagnosis of polycystic ovary syndrome (PCOS), predict the ovarian response to stimulation, identify patients with a higher risk of ovarian hyperstimulation syndrome (OHSS), and assist in gonadotropin dosing for women undergoing assisted reproductive techniques (ART) [1].

AMH is a superior clinical predictor of ovarian reserve and cycle success in ART patients compared to age, AFC, FSH, estradiol, and inhibin B [2-4]. Many studies have reported a positive correlation between AMH levels and ART outcomes and reduced time to live birth [5-7]. However, using AMH to estimate ART outcomes has been a subject of much controversy. Although some studies consider serum AMH as an independent predictor of ovarian reserve and ovarian stimulation outcome in infertile women [8,9], other researchers present it as a weak independent predictor of live birth following ART after controlling for potential confounding variables [10-12].

Another essential matter with regard to the use of AMH in ART practice is that AMH is considered a reliable predictor of quantitative ART outcomes, though there is still controversy surrounding the effect of AMH concentrations on the qualitative ART outcomes [13]. AMH positively correlated with the number of good-quality embryos in different age groups of infertile women who received their first IVF/intracytoplasmic sperm injection (ICSI) cycle [14]. The association was also reported in patients with poor ovarian reserve (POR) [15]. Nevertheless, some authors [16] found AMH levels to correlate with blastocyst quality, but they observed no association between AMH concentration and cleavage embryo quality or pregnancy rate. At the same time, very high AMH levels negatively correlated with fertilization and embryo development rates in women undergoing ART [17].

On the other hand, gynecological diseases may affect AMH levels and ovarian reserve. Thus, it would be helpful to consider AMH levels when treating infertility in women with different causes [1]. Most women with elevated AMH levels have PCOS. Increased AMH levels correlate with PCOS severity (hyperandrogenism) in PCOS patients and are associated with a greater risk of OHSS, improved gender hormone profile, and higher clinical pregnancy rates following ART [18]. Although, some studies have found elevated serum AMH levels to be associated with lower live birth [19] and a higher risk of preterm delivery in PCOS patients undergoing ART [20].

Endometriosis patients show worse ovarian reserve biomarkers (lower AMH and higher FSH) than the normal population, regardless of a history of ovarian surgery [21]. In endometriosis patients undergoing ART, oocytes retrieved and live birth rates decrease compared to women with unexplained infertility, especially when the disease is severe [22]. Endometriosis also correlates with lower cumulative live birth rates by decreasing the number of available embryos (but not their quality) [23]. Reduced follicular output rate and oocytes retrieved have also been observed in endometriosis patients undergoing ICSI independent of AMH, AFC, and age [24]. Nevertheless, the patient's age may play a key role in the pregnancy rate since young severe endometriosis patients with a diminished ovarian reserve showed a reduced oocyte yield but not a reduced embryo quality or pregnancy outcome [25].

In women with unexplained infertility, serum AMH concentration is not a strong independent predictive factor for ART outcomes [26]. Although, a positive correlation has been observed between the serum levels of AMH and the ongoing pregnancy rate [27].

Hence, the variations mentioned above between studies regarding the clinical utility of AMH concentrations in ART practice led us to design a retrospective cohort study of infertile women presenting to our infertility clinic from 2012 to 2020 to investigate the relationship between serum AMH levels and ART outcomes.

MATERIALS AND METHODS

In this hospital-based retrospective cohort study, including 6,682 infertile women, we investigated reproductive outcomes in 881 patients aged 20 - 45 who underwent their first fresh autologous non-preimplantation genetic diagnosis (PGD) ICSI cycles at the referral infertility clinic of Dr. Shariati hospital affiliated with Theran University of Medical Sciences (TUMS), Theran, Iran, between 2012 and 2020. Patients with severe male factors, multiple diagnoses, and those with donation oocytes, *in vitro*-matured (IVM) oocytes, and oocytes from cancer patients or hepatitis patients were excluded.

Medical records of infertile women undergoing ART cycles were reviewed and collected. Demographic data (age and BMI) and baseline reproductive data, including serum FSH, LH, and AMH levels, gynecological causes of infertility (PCOS, endometriosis, unexplained infertility), and the stimulation protocol used (gonadotro-

Table 1. Baseline characteristics and stimulation parameters of the patients.

Characteristic	n = 881[1]
Age	34 (30, 37)
BMI	23.50 (21.80, 25.40)
AMH	3.70 (2.30, 5.50)
FSH	5.60 (4.30, 7.00)
LH	6.4 (4.6, 8.4)
Disease	
Endometriosis	150 (17%)
PCOS	501 (57%)
Unexplained	230 (26%)
Stimulation protocol	
Agonist	101 (11%)
Antagonist	780 (89%)
Total oocytes retrieved	12 (9, 17)
MII	10 (7, 13)
Oocyte maturity	80 (73, 86)
Fertilization rate	82 (75, 89)
High-quality embryos	6 (5, 8)
Number of transferred embryos	3 (3, 3)
Cleavage	772 (88%)
Blastocyst	106 (12%)
Chemical pregnancy rate	346 (39%)
Clinical pregnancy rate (CPR)	276 (31%)
Miscarriage rate (MR)	58 (6.7%)
Live birth rate (LBR)	174 (20%)
Age Categories	
Under 37	646 (73%)
37 and more	235 (27%)

[1] - Median (IQR); n (%).
AMH levels - Low [48 (5.4%)], Normal [284 (32%)], High [549 (62%)].

phin-releasing hormone (GnRH) agonist or antagonist protocol) were collected.

Controlled ovarian stimulation (COS) was performed based on the patient's parameters using gonadotropin-releasing hormone (GnRH) antagonists or GnRH agonists protocol. Recombination follicle stimulating hormone (Gonal-F; Merck Serono Europe, London, UK; or Puregon; MSD, Kenilworth, NJ, USA) was initiated at the beginning of the cycle and continued until trigger. Ovarian response was evaluated through serial transvaginal ultrasound and serum hormone levels. Human chorionic gonadotropin (hCG; Pregnyl; MSD, Brussels, Belgium) was administered to trigger ovulation when three or more follicles were at least 17 mm. About 34 - 38 hours after hCG injection, oocyte pick-up (OPU) was performed. Luteal phase support was started on day 1 after OPU. Fresh embryo transfer was carried out 72 hours after OPU.

Reproductive outcomes, including the number of oocytes retrieved, the number of metaphase II (MII) oocytes, oocyte maturity rate, fertilization rate, the number of good-quality embryos, the number of embryos transferred per cycle, chemical pregnancy rate, clinical pregnancy rate, miscarriage rate, live birth rate, cleavage embryo rate, and blastocyst embryo rate were also collected. Oocyte maturity rate was defined as the number of normal MII oocytes per total number of normal oocytes retrieved [28]. The number of oocytes with 2 pronuclei and 2 polar bodies (2PN/2PB) (16 - 20 hours post-insemination) per number of MII oocytes injected was considered as fertilization rate, expressed as a percentage [29]. Chemical pregnancy was defined as serum β-hCG level > 50 IU/L two weeks after embryo transfer (ET) [30]. Grade A and B cleavage embryos based on the ASEBIR criteria (considering the number of cells/ blastomeres, fragmentation, and the presence of multiple nuclei, pits, and vacuoles) [31] were classified as high-quality. Clinical pregnancy was defined as the visualization of one or more gestational sacs on ultrasound or a clinical sign of pregnancy [32]. Live birth rate was defined as the number of pregnancies contributing to at least one live birth (≥ 24 weeks of gestation) per transfer cycle, expressed as a percentage [32]. Cleavage rate was defined as the number of cleaved embryos on Day 3 per number of 2PN/2PB oocytes on Day 1, expressed as a percentage. Blastocyst development rate was defined as the number of blastocysts on Day 5 per number of normally fertilized oocytes, expressed as a percentage [29].

A cutoff point of 37 years for fertility decline was used to categorize patients into two age strata (< 37 and ≥ 37). AMH levels were stratified into low (≤ 0.3 - 0.9 ng/mL), normal (≥ 1.0 ng/mL), and high (≥ 3.0 ng/mL). Kruskal-Wallis rank sum test was used to assess differences among independent categories. Fisher's exact test and Pearson's chi-squared test were applied to compare proportions of categorical outcomes between independent groups. Wilcoxon rank sum test was used to compare dependent samples. Data analysis was conducted using SPSS v22.0 software (IBM SPSS Statistics for Windows; IBM Corp., Armonk, NY, USA). All data are presented as median (IQR) and n (%), and a p-value of less than 0.05 was considered significant. Data entry and analysis were performed using a unique code for each patient to ensure confidentiality.

Ethics approval

The study was approved by the Deputy of Research and Ethics Committee of TUMS (code: IR.TUMS.MEDICINE.REC.1401. 221).

Table 2. Association between serum hormone levels and ART outcomes.

Outcome	AMH (n = 881)			FSH (n = 881)			LH (n = 880)		
	p-value [2]			p-value [2]			p-value [2]		
Chemical pregnancy	negative (n = 534 [1])	3.30 (2.00, 5.30)	< 0.001	negative (n = 534	5.60 (4.30, 7.10)	0.14	negative (n = 534 [1])	6.4 (4.6, 8.6)	0.5
	positive (n = 347 [1])	4.10 (2.85, 5.70)		positive (n = 347	5.40 (4.20, 6.90)		positive (n = 347 [1])	6.3 (4.7, 8.3)	
Clinical pregnancy	negative (n = 604 [1])	3.40 (2.00, 5.30)	< 0.001	negative (n = 604	5.60 (4.30, 7.10)	0.034	negative (n = 604 [1])	6.5 (4.7, 8.7)	0.11
	positive (n = 277 [1])	4.20 (3.00, 5.80)		positive (n = 277	5.30 (4.20, 6.90)		positive (n = 277 [1])	6.2 (4.6, 8.2)	
Development at embryo transfer									
Cleavage stage	negative (n = 108 [1])	4.40 (3.20, 5.73)	< 0.001	negative (n = 108	6.00 (4.60, 7.60)	0.092	negative (n = 108 [1])	6.4 (4.6, 8.9)	0.8
	positive (n = 773 [1])	3.50 (2.20, 5.50)		positive (n = 773	5.60 (4.30, 7.00)		positive (n = 773 [1])	6.4 (4.6, 8.3)	
Blastocyst stage	negative (n = 774 [1])	3.50 (2.20, 5.50)	< 0.001	negative (n =774 [1])	5.60 (4.30, 7.00)	0.2	negative (n = 774 [1])	6.4 (4.6, 8.3)	0.7
	positive (n = 107 [1])	4.40 (3.30, 5.75)		positive (n = 107	5.90 (4.60, 7.35)		positive (n = 107 [1])	6.6 (4.6, 9.0)	
Live birth rate	negative (n = 706 [1])	3.50 (2.10, 5.50)	< 0.001	negative (n = 706	5.60 (4.30, 7.00)	0.14	negative (n = 706 [1])	6.3 (4.6, 8.4)	0.9
	positive (n = 175 [1])	4.30 (3.10, 5.50)		positive (n = 175	5.30 (4.20, 7.00)		positive (n = 175 [1])	6.5 (4.9, 8.3)	
Miscarriage rate	negative (n = 822 [1])	3.65 (2.30, 5.50)	0.7	negative (n = 822	5.60 (4.30, 7.00)	0.6	negative (n = 822 [1])	6.4 (4.7, 8.5)	0.2
	positive (n = 59 [1])	3.80 (2.50, 5.50)		positive (n = 59 [1])	5.20 (4.40, 6.90)		positive (n = 59 [1])	6.0 (4.1, 8.1)	

[1] - Median (IQR).
[2] - Wilcoxon rank sum test.

RESULTS

Of 6,682 infertile women who presented to our infertility clinic between 2012 and 2020, 881 met the inclusion criteria (Figure 1). The patients' mean ages and BMI were 34 ± 7 and 23.50 ± 3.6. FSH (5.60 ± 2.5), LH (6.4 ± 3.8), and AMH (3.70 ± 3.2) levels are also shown in Table 1. AMH levels were high (≥ 3.0 ng/mL) in the majority of patients (62%; mostly PCOS), normal (≥ 1.0 ng/mL) in 32%, and low (≤ 0.3 - 0.9 ng/mL) in 5.4%. PCOS accounted for 57% of all infertility cases, while unexplained infertility was responsible for 26%, and endometriosis was diagnosed in 17% of the patients. In most cases (89%), GnRH antagonistic protocols were used. Reproductive outcomes, including the number of oocytes (12 ± 8.0), the number of MII oocytes (10 ± 6.0), oocyte maturity rate (80 ± 13), fertilization rate (82 ± 14), the number of good-quality embryos (6 ± 3.0), the number of embryo transfers per cycle (2.0), chemical pregnancy rate (346; 39%), clinical pregnancy rate (276; 31%), miscarriage rate (58; 6.7%), live birth rate (174; 20%), cleavage embryo rate (772; 88%), and blastocyst embryo rate (106; 12%) are presented in Table 1 as well.

We assessed the associations between serum levels of FSH, LH, and AMH and ART outcomes (Table 2). AMH concentration was strongly correlated with chemical pregnancy ($p < 0.001$), clinical pregnancy ($p < 0.001$), cleavage embryo rate ($p < 0.001$), blastocyst rate ($p < 0.001$), and live birth rate ($p < 0.001$). A positive correlation was also observed between serum FSH levels and clinical pregnancy ($p = 0.034$).

In endometriosis patients (both age groups), there were significant increases in the number of oocytes ($p < 0.001$), the number of MII oocytes ($p < 0.001$), the number of good-quality embryos ($p < 0.001$), chemical pregnancy rate ($p < 0.001$ in women < 37; and $p = 0.023$ in women ≥ 37), and clinical pregnancy rate ($p = 0.001$ in women < 37; and $p = 0.009$ in women ≥ 37) with an increase in AMH levels. However, the live birth rate increased remarkably only in women over 37 ($p = 0.044$) (Table 3).

In PCOS patients (Table 4), there was a correlation between a higher AMH with a remarkably improved num-

Table 3. Reproductive outcomes in endometriosis patients of two age groups (< 37 and ≥ 37).

	Under 37					37 and more				
Outcome	**n**	**Low n = 13 [1]**	**Normal n = 47 [1]**	**High n = 33 [1]**	**p-value [2]**	**n**	**Low n = 15 [1]**	**Normal n = 33 [1]**	**High n = 9 [1]**	**p-value [3]**
Total oocytes retrieved	**93**	**4 (2, 5)**	**9 (6, 10)**	**14 (10, 16)**	**< 0.001**	**57**	**3 (2, 4)**	**9 (7, 11)**	**12 (10, 15)**	**< 0.001**
Number of MII oocytes	**93**	**3 (2, 4)**	**6 (5, 8)**	**10 (8, 13)**	**< 0.001**	**57**	**2 (1, 2)**	**6 (5, 8)**	**9 (8, 11)**	**< 0.001**
Oocyte maturity	**93**	**75 (70, 83)**	**78 (67, 87)**	**81 (73, 86)**	**0.8**	**57**	**60 (45, 76)**	**73 (64, 83)**	**69 (60, 73)**	**0.2**
Fertilization rate	**93**	**100 (71, 100)**	**90 (78, 100)**	**83 (80, 91)**	**0.5**	**57**	**100 (17, 100)**	**80 (70, 83)**	**78 (73, 83)**	**0.8**
Number of transferred embryos	**93**	**2 (1, 2)**	**5 (4, 6)**	**7 (5, 10)**	**< 0.001**	**57**	**1 (0, 2)**	**4 (3, 5)**	**5 (4, 6)**	**< 0.001**
Chemical pregnancy	**93**	**3 (23%)**	**13 (28%)**	**22 (67%)**	**< 0.001**	**57**	**2 (13%)**	**15 (45%)**	**6 (67%)**	**0.023**
Clinical pregnancy	**93**	**2 (15%)**	**10 (21%)**	**10 (21%)**	**0.001**	**57**	**1 (6.7%)**	**10 (30%)**	**6 (67%)**	**0.009**
Miscarriage rate	**93**	**1 (7.7%)**	**1 (2.1%)**	**1 (3.0%)**	**0.5**	**57**	**1 (6.7%)**	**4 (12%)**	**1 (11%)**	**> 0.9**
Live birth rate	**93**	**1 (7.7%)**	**7 (15%)**	**10 (30%)**	**0.2**	**57**	**0 (0%)**	**3 (12%)**	**3 (33%)**	**0.044**

[1] - Median (IQR); n (%).
[2] - Kruskal-Wallis rank sum test; Fisher's exact test; Pearson's Chi-squared test.
[3] - Kruskal-Wallis rank sum test; Fisher's exact test.

Table 4. Reproductive outcomes in PCOS patients of two age groups (< 37 and ≥ 37).

	Under 37					37 and more				
Outcome	**n**	**Low n = 10 [1]**	**Normal n = 46 [1]**	**High n = 348 [1]**	**p-value[2]**	**n**	**Low n = 9 [1]**	**Normal n = 33 [1]**	**High n = 55 [1]**	**p-value [2]**
Total oocytes retrieved	**404**	**3 (2, 4)**	**10 (8, 12)**	**17 (14, 21)**	**< 0.001**	**97**	**3 (3, 4)**	**10 (8, 11)**	**15 (13, 18)**	**< 0.001**
Number of MII oocytes	**404**	**2 (1, 3)**	**8 (6, 9)**	**13 (10, 17)**	**< 0.001**	**97**	**2 (2, 3)**	**8 (6, 9)**	**11 (9, 13)**	**< 0.001**
Oocyte maturity	**404**	**71 (52, 100)**	**79 (75, 87)**	**80 (73, 86)**	**0.7**	**97**	**80 (50, 100)**	**80 (71, 86)**	**77 (69, 85)**	**0.6**
Fertilization rate	**404**	**100 (75, 100)**	**86 (80, 100)**	**80 (74, 88)**	**< 0.001**	**97**	**75 (50, 100)**	**83 (75, 89)**	**82 (75, 88)**	**0.8**
Number of transferred embryos	**404**	**2 (1, 3)**	**5 (4, 7)**	**8 (6, 10)**	**< 0.001**	**97**	**1 (1, 1)**	**5 (4, 6)**	**8 (6, 9)**	**< 0.001**
Chemical pregnancy	**404**	**2 (20%)**	**17 (37%)**	**154 (44%)**	**0.2**	**97**	**0 (0%)**	**11 (33%)**	**24 (44%)**	**0.034**
Clinical pregnancy	**404**	**2 (20%)**	**12 (26%)**	**126 (36%)**	**0.3**	**97**	**0 (0%)**	**10 (30%)**	**19 (35%)**	**0.1**
Miscarriage rate	**404**	**0 (0%)**	**4 (8.7%)**	**23 (6.6%)**	**0.8**	**97**	**0 (0%)**	**2 (6.1%)**	**2 (3.6%)**	**0.8**
Live birth rate	**404**	**1 (10%)**	**7 (15%)**	**81 (23%)**	**0.4**	**97**	**0 (0%)**	**6 (18%)**	**14 (25%)**	**0.2**

[1] - Median (IQR); n (%).
[2] - Kruskal-Wallis rank sum test; Fisher's exact test.

Table 5. Reproductive outcomes in patients with unexplained infertility of two age groups (< 37 and ≥ 37).

	Under 37					37 and more				
Outcome	**n**	**Low n = 1 [1]**	**Normal n = 79 [1]**	**High n = 69 [1]**	**p-value [2]**	**n**	**Low n = 0 [1]**	**Normal n = 46 [1]**	**High n = 35 [1]**	**p-value[3]**
Total oocytes retrieved	**149**	**4 (4, 4)**	**9 (7, 12)**	**13 (10, 16)**	**< 0.001**	**81**	**NA (NA, NA)**	**8 (6, 9)**	**11 (9, 12)**	**< 0.001**
Number of MII oocytes	**149**	**4 (4, 4)**	**7 (6, 9)**	**10 (8, 13)**	**< 0.001**	**81**	**NA (NA, NA)**	**6 (5, 8)**	**9 (7, 10)**	**< 0.001**
Oocyte maturity	**149**	**100 (100, 100)**	**82 (75, 89)**	**82 (77, 86)**	**0.3**	**81**	**NA (NA, NA)**	**83 (75, 90)**	**83 (80, 88)**	**0.9**
Fertilization rate	**149**	**100 (100, 100)**	**83 (78, 91)**	**83 (77, 88)**	**0.3**	**81**	**NA (NA, NA)**	**81 (75, 88)**	**82 (78, 89)**	**0.4**
Number of transferred embryos	**149**	**4 (4, 4)**	**5 (4, 6)**	**7 (5, 8)**	**< 0.001**	**81**	**NA (NA, NA)**	**4 (3, 5)**	**6 (4, 7)**	**< 0.001**
Chemical pregnancy	**149**	**0 (0%)**	**18 (23%)**	**35 (51%)**	**< 0.001**	**81**	**0 (NA%)**	**10 (22%)**	**15 (43%)**	**0.054**
Clinical pregnancy	**149**	**0 (0%)**	**13 (16%)**	**27 (39%)**	**0.003**	**81**	**0 (NA%)**	**8 (17%)**	**12 (34%)**	**0.12**
Miscarriage rate	**149**	**0 (0%)**	**4 (5.1%)**	**8 (12%)**	**0.3**	**81**	**0 (NA%)**	**5 (11%)**	**2 (5.7%)**	**0.7**
Live birth rate	**149**	**0 (0%)**	**9 (11%)**	**19 (28%)**	**0.032**	**81**	**0 (NA%)**	**3 (6.5%)**	**10 (29%)**	**0.013**

[1] - Median (IQR); n (%).
[2] - Kruskal-Wallis rank sum test; Fisher's exact test.
[3] - Wilcoxon rank sum test; Fisher's exact test.

Table 6. Reproductive outcomes in all patients (irrespective of the disease) of two age groups (< 37 and ≥ 37).

	Under 37					37 and more				
Outcome	**n**	**Low n = 24 [1]**	**Normal n = 172 [1]**	**High n = 450 [1]**	**p-value [2]**	**n**	**Low n = 24 [1]**	**Normal n = 112 [1]**	**High n = 99 [1]**	**p-value [2]**
Total oocytes retrieved	**646**	**4 (2, 4)**	**9 (7, 11)**	**16 (12, 20)**	**< 0.001**	**235**	**3 (2, 4)**	**8 (7, 10)**	**13 (10, 16)**	**< 0.001**
Number of MII oocytes	**646**	**2 (2, 3)**	**7 (6, 9)**	**12 (10, 16)**	**< 0.001**	**235**	**2 (1, 2)**	**7 (5, 8)**	**10 (8, 12)**	**< 0.001**
Oocyte maturity	**646**	**75 (58, 100)**	**80 (73, 89)**	**80 (73, 86)**	**0.7**	**235**	**63 (48, 100)**	**80 (71, 88)**	**80 (71, 87)**	**0.054**
Fertilization rate	**646**	**100 (70, 100)**	**86 (78, 100)**	**81 (75, 88)**	**< 0.001**	**235**	**92 (46, 100)**	**80 (72, 88)**	**82 (75, 88)**	**0.8**
Number of transferred embryos	**646**	**2 (1, 3)**	**5 (4, 6)**	**8 (6, 10)**	**< 0.001**	**235**	**1 (1, 1)**	**4 (3, 6)**	**7 (5, 8)**	**< 0.001**
Chemical pregnancy	**646**	**5 (21%)**	**48 (28%)**	**211 (47%)**	**< 0.001**	**235**	**2 (8.3%)**	**36 (32%)**	**45 (45%)**	**0.002**
Clinical pregnancy	**646**	**4 (17%)**	**35 (20%)**	**172 (38%)**	**< 0.001**	**235**	**1 (4.2%)**	**28 (25%)**	**37 (37%)**	**0.003**
Miscarriage rate	**646**	**1 (4.2%)**	**9 (5.2%)**	**32 (7.1%)**	**0.7**	**235**	**1 (4.2%)**	**11 (9.8%)**	**5 (5.1%)**	**0.4**
Live birth rate	**646**	**2 (8.3%)**	**23 (13%)**	**110 (24%)**	**0.003**	**235**	**0 (0%)**	**13 (12%)**	**27 (27%)**	**< 0.001**

[1] - Median (IQR); n (%).
[2] - Kruskal-Wallis rank sum test; Fisher's exact test; Pearson's chi-squared test.

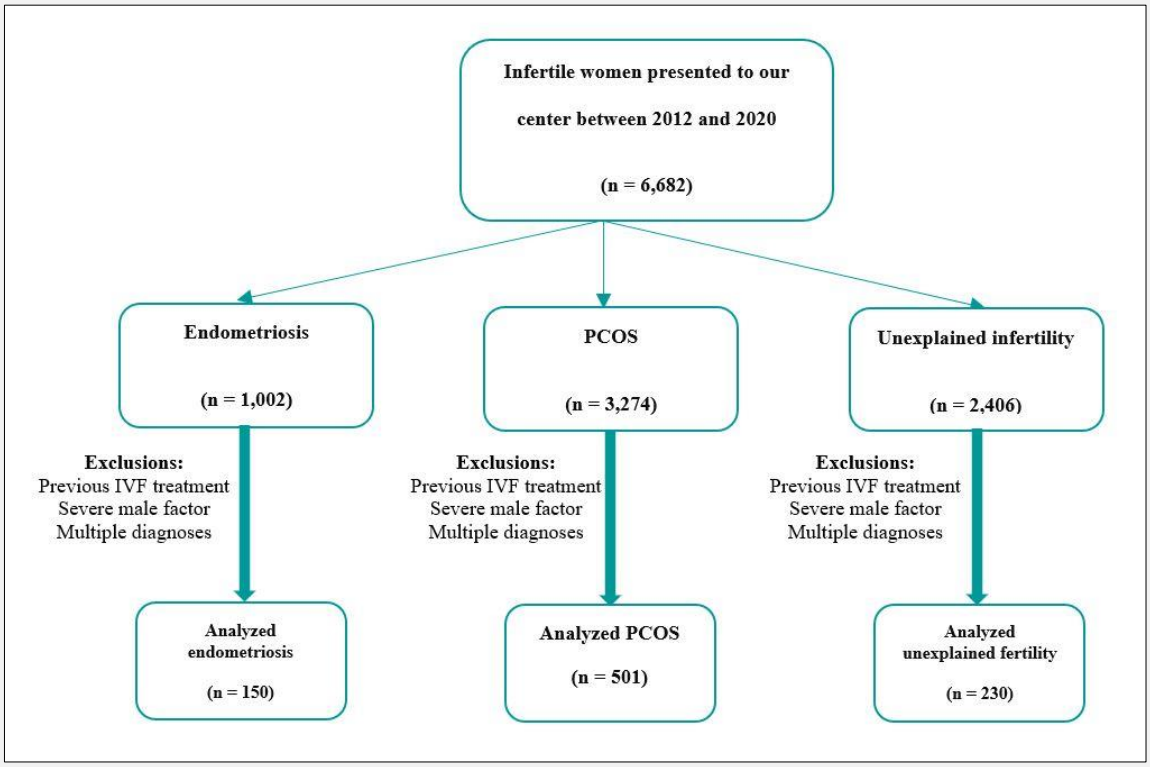

Figure 1. An illustration of the data collection, verification, and selection of the cases (endometriosis, PCOS, and unexplained infertility).

Diagrammatic representation of the data collection, verification, and selection of the cases (endometriosis, PCOS, and unexplained infertility) within an eight-year study duration. In this hospital-based retrospective cohort study, including 6,682 infertile women, we investigated reproductive outcomes in 881 patients aged 20 - 45 who underwent their first fresh autologous non-preimplantation genetic diagnosis (PGD) ICSI cycles at the referral infertility clinic of Dr. Shariati hospital affiliated with Tehran University of Medical Sciences (TUMS), Tehran, Iran, between 2012 and 2020.

ber of oocytes ($p < 0.001$), number of MII oocytes ($p < 0.001$), and number of good-quality embryos ($p < 0.001$) in both age groups. Although, a higher fertilization rate was only observed in women under 37 ($p < 0.001$ in women < 37; and $p = 0.8$ in women ≥ 37); while chemical pregnancy rate showed a notable increase only in women over 37 ($p < 0.2$ in women < 37; and $p = 0.034$ in women ≥ 37).

As shown in Table 5, in patients with unexplained infertility (both age groups), higher AMH levels were correlated with a significantly improved number of oocytes ($p < 0.001$), number of MII oocytes ($p < 0.001$), number of good-quality embryos ($p < 0.001$), and live birth rate ($p = 0.032$ in women < 37; and $p = 0.013$ in women ≥ 37). A higher chemical pregnancy rate was only observed in women under 37 ($p < 0.001$ in women under 37; and $p = 0.054$ in women ≥ 37).

In all patients (irrespective of age and the disease), higher AMH was correlated with the improved number of oocytes ($p < 0.001$), the number of MII oocytes ($p < 0.001$), the number of good-quality embryos ($p < 0.001$), chemical pregnancy rate ($p < 0.001$ in women < 37; and $p = 0.002$ in women over 37), clinical pregnancy rate ($p < 0.001$ in women < 37; and $p = 0.003$ in women over 37), and live birth rate ($p = 0.003$ in women < 37; and $p < 0.001$ in women over 37). In addition, high AMH levels were associated with improved oocyte maturity in women over 37, though this was not statistically significant ($p = 0.054$). The fertilization rate significantly increased among women under 37 ($p < 0.001$); however, those over 37 did not experience the same trend ($p = 0.8$) (Table 6).

DISCUSSION

This retrospective cohort study on 881 fresh autologous non-PGD cycles found a strong correlation between high AMH levels and reproductive outcomes independent of age and the cause of infertility in women sub-

jected to ART. High AMH levels were associated with improved oocyte maturity rate in women over 37, though this was not statistically significant. The fertilization rate significantly improved among younger women; however, those over 37 did not experience the same trend.

Using data from a large hospital-based retrospective cohort over 8 years, we studied the correlation between AMH levels and ART outcomes among women with common causes of female infertility, separately and collectively. The cohort nature of the study enabled us to evaluate several outcomes. This study would represent a reasonable sample from the whole country since patients from all over the country visit Dr. Shariati hospital, a large educational, research, and medical center which provides comprehensive specialized and subspecialized health care services. A homogenous study population undergoing uniform treatment practices was included in our referral fertility center. Our findings may be therefore generalizable to infertile patients in the I.R. Iran. Furthermore, considering 37 years as a cutoff point for fertility decline, we studied the ART outcomes in both age groups (< 37 and ≥ 37). It is important to note, however, that our study had some limitations. Embryo ploidy and, more importantly, live birth outcomes are significantly influenced by age. However, due to high costs, preimplantation genetic testing for embryo aneuploidy was not conducted in the higher age group. In addition, our analysis was limited to fresh IVF cycles without assessing pregnancy outcomes in FET cycles. Multicenter larger population studies might be warranted to increase the study's power to confirm the causal inference. Moreover, in this manuscript, we evaluated the ART performance quantitatively and qualitatively. However, differences in the number of transferred embryos may affect clinical outcomes. In addition, high AMH levels, leading to a higher number of MII oocytes, are associated with a higher number of developing follicles and elevated estradiol levels at the time of OPU. Since estradiol level affects endometrial receptivity, it may also affect the pregnancy rate. To evaluate this effect, it is necessary to compare AMH levels with clinical outcomes within a group with the same number of oocytes picked up and the same number of embryos transferred. However, not enough data was available to perform such an analysis.

In our study, high AMH levels strongly correlate with favorable ART outcomes in all age- and cause-stratified groups. This is consistent with a systematic review and meta-analysis including 28 studies of infertile patients subjected to ART treatment, which revealed that AMH is a superior predictor of poor response to ovarian stimulation and ongoing pregnancy than age, and no improvement in accuracy was seen by combining the two markers [33]. Our findings further support those of Tal et al. [9], who reported AMH as a strong independent predictor of cumulative live birth rate in women with DOR. Although, in a largescale database including 85,062 cycles, AMH was found to be a weak independent predictor of live birth in young women undergoing ART [11]. Pacheco et al. [34] and Zhang et al. [35] also confirmed the significance of age in predicting embryo quality and pregnancy rate in their more recently published papers. This study showed significant increases in stimulation parameters in endometriosis patients (both age groups), including live birth rate with increased AMH levels. However, the live birth rate improved remarkably only in women with advanced age. It is encouraging to compare these findings with those of Pacchiarotti et al. [25]. They found that the low value of AMH had no effect on oocyte quality and pregnancy outcomes in younger patients with severe endometriosis undergoing IVF. The contradictory findings concerning AMH accuracy in predicting pregnancy outcomes may result from the small sample size used in this study. Although, in a multicenter study on 228 colorectal endometriosis patients, Ballester et al. [36] showed that an AMH level < 2 ng/mL was remarkably more common in patients who did not achieve pregnancy than in patients who achieved pregnancy following IVF-ICSI. Sahmay et al. [37], who studied the ability of AMH concentrations in predicting clinical pregnancy in women of advanced age, also found AMH to be a valuable biomarker of quantitative ovarian reserve and clinical pregnancy rate among patients over 35.

In PCOS patients, there was a strong correlation between a higher AMH with a remarkably improved number of oocytes, the number of MII oocytes, and the number of good-quality embryos in both age groups. A higher fertilization rate was only observed in younger women, while the chemical pregnancy rate showed a notable increase only in women over 37. These findings seem consistent with Du and Cao [38] and Tal et al. [18], who documented a strong correlation between AMH and clinical pregnancy in PCOS infertile patients undergoing IVF-ET. However, these results differ from a recently published study by Liu et al. [39], a cohort study of 2,973 infertile women, including 418 women with PCOS, which found no association between AMH and clinical pregnancy rate or live birth rate after controlling by age and other confounding factors. This contradictory finding might be caused by the similar number of oocytes in PCOS patients with different serum AMH concentrations. In a more recent retrospective cohort of 184 PCOS women, Tal et al. [19] [19] reported that extremely high serum AMH levels (> 8.27 ng/mL) were associated with PCOS severity (hyperandrogenism) and thus are associated with a greater risk of OHSS, improved sex hormone profile, and lower clinical pregnancy and live birth rates in women with PCOS who underwent ART. The different PCOS phenotypes and AMH concentration groupings across studies could explain the inconsistent results.

We observed a strong correlation between AMH levels and improved number of oocytes, MII oocytes, good-quality embryos, and live birth rate in women with unexplained infertility. A higher chemical pregnancy rate was only shown in women under 37. This corroborates

the findings of Moro et al. [27], who found serum AMH concentration as an independent predictor of ongoing pregnancy rate in 276 women with unexplained infertility subjected to their first intrauterine insemination (IUI) cycle. In addition, our results refute those of Hansen et al. [26], who did not find significant associations between AMH and pregnancy outcomes in women with unexplained infertility. This discrepancy could be attributed to differences in study populations, ovarian stimulating protocols, and AMH cutoff points.

In conclusion, the evidence from this study suggests that serum AMH concentrations can be an invaluable tool for predicting ovarian reserve and reproductive outcomes in young and advanced-age infertile patients undergoing ART. Nevertheless, we found no level below which no pregnancies occurred, indicating that AMH should not be used as the sole predictive marker for disqualifying infertile women from ART treatment. Thus, patients should not be excluded from treatment due to low serum AMH levels. Further large cohort studies are warranted to confirm these findings and determine an exact cutoff point to predict treatment outcomes in infertile women undergoing ART.

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