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Original article

The Role of Anti-Müllerian Hormone in Oocyte and Embryo Quantity and Quality in IVF

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Abstract
The aim of this study was to explore the association between AMH and the number and quality of oocytes and embryos in IVF. This was a retrospective study in Benghazi Infertility Teaching Hospital, Libya, and included women being treated for infertility by in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) during the period January-September 2021. The study population consisted of women 18-40 years of age with infertility, who received controlled ovarian stimulation with either antagonist or agonist protocols. Inclusion criteria included an initial antral follicle count (AFC) ≥5 and complete records of hormonal levels, stimulation modality, and embryology reports. The analytical reliability of commercially available ELISA kits was confirmed with the intra- and inter-assay coefficients of variation being less than 10% and 5%, respectively, in the present study. Oocyte maturity was determined on the basis of standard criteria, and embryos were graded on day 3 of development using the Istanbul consensus scoring system. Out of the 156 women included, 48.7% were between 31-40 years, and 42.9% were over 40 years of age. An inverse correlation between age and AMH levels was found to be statistically significant (P = 0.002). AMH values significantly correlated with the required dose of HMG during ovarian stimulation (P < 0.001), with women presenting with higher AMH levels requiring lower doses of gonadotropins. The average number of oocytes retrieved differed significantly between AMH groups (p<0.001), with increasing AMH associated with greater numbers of oocytes retrieved. The number of MII oocytes decreased significantly with decreasing levels of AMH (p<0.001). The numbers of grade I embryos were significantly decreased with a decline in the AMH levels (p<0.001), while the fertilization rate did not differ between the groups. The probability of reaching embryo transfer was strongly affected by the value of AMH (p<0.001). AMH is an optimal test for predicting ovarian reserve; it correlates well with age and FSH level and is a good predictive model for IVF outcomes, which require quantitatively large numbers of oocytes and embryos. This biomarker is unmissable for personalized gonadotropin stimulation protocols and powerfully predicts the likelihood of patients reaching embryo transfer and is therefore an essential parameter for patient counseling and treatment individualization in today's fertility centers.
Keywords. Anti-Müllerian Hormone, In Vitro Fertilization, Oocyte Quality, Infertility, Embryo Quality, Ovarian Reserve.

Introduction

Anti-Müllerian hormone (AMH) is a protein made of two parts that is similar to transforming growth factor β. It is produced by Sertoli cells during the development of male embryos and by granulosa cells in developing ovarian follicles [1]. Male internal sexual differentiation is controlled by AMH, a hormone from Sertoli cells that causes Müllerian ducts to shrink and disappear during early development [2]. Within the ovary, AMH stands as a barrier for primordial follicles, preventing their activation and reducing the sensitivity of existing follicles to FSH, which manages how many and which follicles grow [3, 4]. Serum AMH shows the number of small developing follicles and is considered the strongest biomarker for both a woman's ovarian function and her reaction to stimulation treatment [5]. Humans have the gene for AMH on the short arm of chromosome 19, at locus 19p13.3. The anti-Müllerian hormone receptor type 2 is the cognate form of this receptor. It is encoded by the AMHR2 gene at 12q13.13 on chromosome 12 [6].

Results from both animal and human tissue studies suggest that AMH helps to maintain the resting state of primordial follicles. For that reason, different groups are studying ways to use recombinant AMH or to boost AMH production with genetic means so that mature follicles remain inactive, which may help preserve the ovarian reserve while patients are treated with chemotherapy or radiation for cancer [7]. In addition to what it does to the primordial follicle pool, AMH strongly affects the reaction of growing preantral and small antral follicles to FSH. It does this at least in part by keeping the number of Follicle Stimulating Hormone (FSH) receptors (FSHR) and how much aromatase is active inside granulosa cells lower [8].

Varied amounts of AMH in women of similar ages can be linked to different counts of antral follicles and cause the ovaries to age and lose function differently. Medically, AMH results help adjust gonadotropin doses for controlled ovarian stimulation, estimate the chance of excessive or poor ovarian response, inform the threat of ovarian hyperstimulation syndrome, support diagnoses of polycystic ovary syndrome and disorders of sex development, and supply details on when menopause and ovarian damage from cytotoxic treatment may take place [5, 9].

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New studies indicate that AMH may control the reproductive axis from regions besides the ovary. It seems that AMH serves to act on GnRH neurons, within the hypothalamus, to possibly change how Gonadotropin-Releasing Hormone (GnRH) is secreted and influence LH levels [10]. AMH is seen by experts as a highly accurate sign of ovarian reserve because it indirectly measures the remaining pool of primordial follicles by reflecting the number of small antral (growing) follicles. The number of antral follicles seen on ultrasonography agrees very well with Antral Follicle Count (AFC) results [1]. In the field of Assisted Reproductive Technology (ART), Doctors in ART use AMH levels to estimate how the ovaries will react to Controlled Ovarian Stimulation (COS). Low levels of AMH are a sign that the ovaries may not respond well, and very high levels might suggest a risk of Ovarian Hyperstimulation Syndrome (OHSS) [11]. AMH predicts how many eggs will be retrieved, how those eggs will mature, and how well the embryos will develop, so it is vital for planning gonadotropin treatment and estimating whether the outcome will be low or high [12]. On the other hand, AFC performs better than AMH when looking for patients with a low response, which is important in clinical work because it helps to recognize them [13, 14].
Even though AMH is a good predictor, its effects on embryonic development are not definite. AMH correlates with how well the embryo can be fertilized and if it reaches the blastocyst stage, yet it does not control the quality of cleavage-stage embryos, so morphological observation should be added [15]. The purpose of this study was to analyze the link between AMH and the number and quality of oocytes and embryos in in vitro fertilization (IVF) cycles.

Methods

Study Design and Setting

The study, conducted at the Benghazi Infertility Teaching Hospital in Libya, used data from women with infertility treated by IVF or Intracytoplasmic sperm injection (ICSI) cycles between January and September 2021. We were given permission to use the anonymized data from patients who had given written informed consent and whose protocol was approved by our institution's Research and Ethics Committee.

Participant Selection

Eligible for the study were women ages 18 to 40 with infertility who used either the antagonist or agonist protocol for their controlled ovarian stimulation. Both boundary requirements were maintained for inclusion: an initial AFC of five or higher and total records of hormone levels, stimulation details, and embryology write-ups. A patient was excluded if they had had surgery on the ovaries, if they had endocrine problems such as Polycystic ovary syndrome (PCOS), hypothyroidism, or hyperprolactinemia, or if they were using donor oocytes or preimplantation genetic testing.

Variables and Data Collection

Data from both clinical areas and laboratory studies were obtained from electronic medical records by trained clinicians. Age, how long the patient has been infertile, and the cause were all considered demographic variables. To get baselines, we measured the hormones FSH, Luteinizing Hormone (LH), Estradiol (E2), and AMH in blood drawn during the first phase of the cycle. Ovarian response data were the kind and amount of gonadotropin given, how long stimulation lasted, the number of follicles present (AFC), the number of oocytes collected, the number of mature (metaphase II) oocytes, and the endometrial thickness when triggering occurred. Measures of fertilization and the quality of the embryos were recorded in the embryology data.

Laboratory Methods

To determine AMH serum concentrations, the investigators used an ELISA kit from a commercial manufacturer as instructed. The percentage differences found in running the same assays and different assays remained below 10% and 5%, respectively, which indicates great analytical reliability.

Oocyte/embryo assessment

Oocytes were given a maturity status based on the criteria: GV, MI, or MII, with MII identified when the first polar body appeared. Embryos were categorized into Grades I, II, or III using the Istanbul consensus scoring system performed on day 3 of their development.

Statistical Analysis

All data were analyzed with SPSS v28 (IBM). The results for continuous variables are presented as mean±SD. This assessment was modified for age, BMI, and gonadotropin dose. The research was considered statistically significant at a p-value less than 0.05.

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Results

The study's population is described in (Table 1) by its demographic characteristics. More than half of the women (76, 48.7%) were ages 31 to 40, and just under half (61, 42.9%) were more than 40 years old. Thirteen participants, or 8.3% of the whole group, were from the youngest age range (20-30 years).

Table 1. Distribution of women according to age/years

Age/years	No.	%
20-30	13	8.3
31-40	76	48.7
> 40	61	42.9
Total	156	100.0

Data represented as number (No.) and percentage (%).

A proper statistical analysis found that there is an inverse, or negative, connection between age and the level of AMH (P = 0.002). The vast majority of AMH values below 0.3 ng/mL belonged to women over 40, although just 6.1% of women this age had AMH levels in the range of 3–6 ng/mL. By contrast, most of the 31–40-year cohort, or 57.8% (n = 37/64), had serum AMH levels within a normal range (1–2.9 ng/mL). Among those aged 20–30 years (n = 13), 8 cases (61.5%) had an AMH level within normal limits, and there were no very low AMH levels.

Table 2. The relation of patient age /years and AMH level

Age group	AMH level classification					
	High (3 – 6)	Normal (1- 2.9)	Normal low (0.7- 0.9)	Low (0.3 - 0.6)	Very low (< 0.3)	Total
20-30	1	8	3	1	0	13
31-40	16	37	9	10	3	75
> 40	4	19	17	17	9	66
Total	21	64	29	28	12	154

Data represented as a number, AMH: anti-Müllerian hormone.

The breakdown of participants by their type of infertility is shown in (Figure 1). The largest group in the study was women with primary infertility, totaling 108 (69.2%) of the cohort. About 48 women in the group (30.8%) developed secondary infertility.

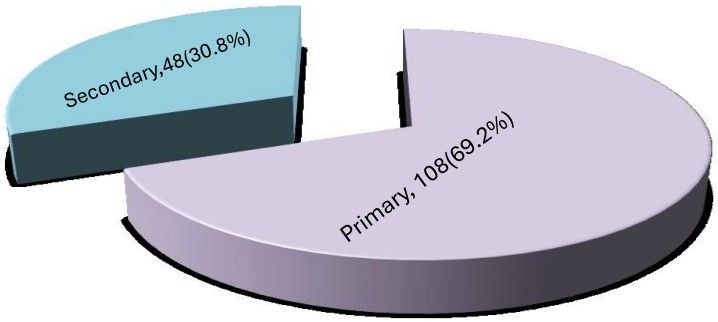

Figure 1. Distribution of women according to type of infertility

(Figure 2) showed how infertility is divided among women by its known causes. Of all the cases, 48.7% (n=76) were caused by female factor infertility. In the remaining cases, male factor infertility and combined factor infertility were equally responsible, and each made up 20.5% (n = 32) of the people studied. Unexplained infertility was found in just 1.9% (3) of the participants.

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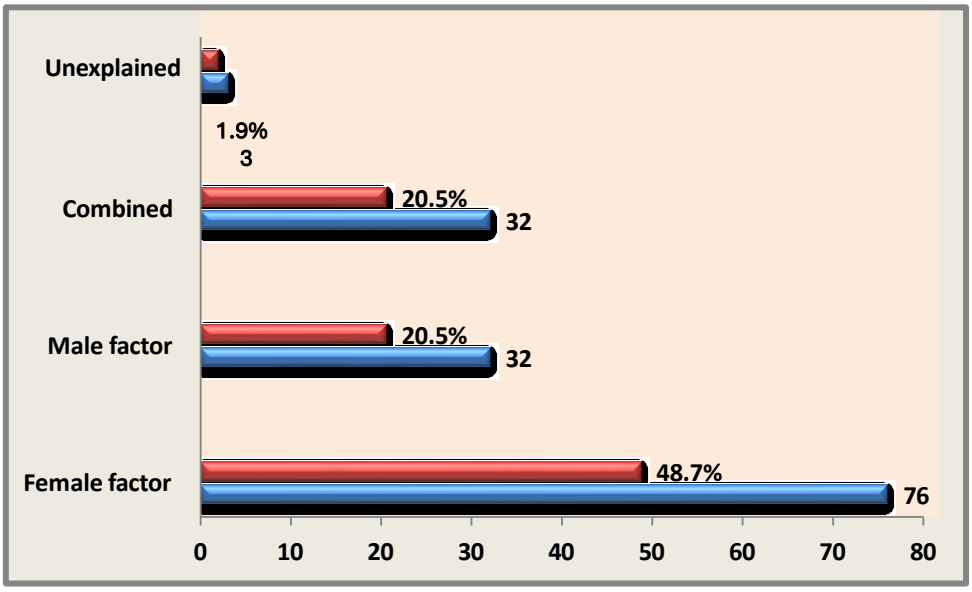

Figure 2. Distribution of women according to type of factor
Data represented as a number and percentage (%).

Participants' experience with infertility is shared in (Table 3). More than eight out of ten women (89.7% total) said they had struggled with infertility for 1 year or more. The groups showing the largest numbers were those who were infertile for 1–5 years (59.0%, n = 92) and fertility challenges for 6–10 years (26.9%, n = 42). Only 4 cases (2.6%) extended beyond 11–15 years, and 2 (1.3%) cases ran for more than 15 years.

Table 3. Distribution of women according to infertility duration/ years

Infertility duration (years)	No.	%
1-5	92	59.0
6 – 10	42	26.9
11 – 15	4	2.6
> 15	2	1.3
Total	140	89.7

Data represented as a number (No.) and percentage (%).

A connection between basal FSH amounts and the AMH classifications was identified as statistically significant (P = 0.008). Of 147 women with full hormone results, 73.5% (or 108) had FSH within the 6–10 IU/L range that corresponded with several AMH results. Raised levels of FSH (11–15 IU/L) were present in 6.1% of participants (n = 9/147), and every person with these FSH levels had low or very low AMH. Women whose FSH levels were considered normal (6–10 IU/L) mostly showed either average values or less AMH than expected. There was not one participant with FSH >10 IU/L who retained AMH values ≥1 ng/mL, indicating that an increased FSH level triggers significant loss of ovarian reserve (Table 4).

Table 4. The relation between basic FSH level and AMH level

	AMH level classification					
FSH level	**High (3 – 6)**	**Normal (1-2.9)**	**Normal low (0.7- 0.9)**	**Low (0.3 - 0.6)**	**Very low < 0.3**	Total
1- 5	9	16	2	1	2	30
6- 10	12	44	23	22	7	108
11- 15	0	2	3	4	0	9
Total	21	62	28	27	9	147

Data represented as a number, FSH: follicle-stimulating hormone, AMH: anti-Müllerian hormone.

Participants' AMH levels were strongly associated with the dose of HMG during treatment (P < 0.001). Analysis of 138 cycles indicated that each increase in HMG use caused a unique pattern of AMH distribution. Women who got smaller doses of HMG (between 14 and 24 IU) most often had higher AMH (3–6 ng/mL), representing 42.9% (n = 9/21), and just 4.8% (n = 1/21) were very low (<0.3 ng/mL). By comparison, using greater doses of HMG (range 47–57 IU) often resulted in fewer eggs, as 30.6% of cycles had low-normal AMH (0.7–0.9 ng/mL) and 22.2% had low AMH (0.3–0.6 ng/mL). The divide between HMG dose and AMH

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categories was easier to notice as stimulation increased (at 58–68 IU). Of the sixty cycles reviewed, about forty percent (n = 6/15) screened with a low AMH, while only thirty-three and two-thirds percent (n = 5/15) kept normal AMH levels. In particular, HMG at the highest dose range (69–79 IU) was related only to AMH levels below 0.3 ng/mL, as examined in the single analyzed cycle, shown in (Table 5).

Table 5. The relation between the patient dose of Human Menopausal Gonadotropin (HMG) and AMH level

HMG dose (IU)	AMH level classification					
	High 3 – 6	**Normal 1- 2.9**	**Normal low 0.7- 0.9**	**Low 0.3 - 0.6**	**Very low < 0.3**	Total
14 - 24	9	8	2	1	1	21
25 - 35	8	26	2	3	2	41
36 - 46	0	8	8	7	1	24
47 - 57	1	12	11	8	4	36
58 - 68	0	5	2	6	2	15
69 - 79	0	0	1	0	0	1
Total	18	59	26	25	10	138

Data represented as a number, HMG: Human Menopausal Gonadotropin, AMH: anti-Müllerian hormone.

A relationship between induction duration and AMH levels was not seen (P = 0.687) (Table 6). Ninety-six percent of 150 women with complete data had induction cycles that lasted 7–12 days, and the group had nearly equal AMH distribution. In only 4.0% (n = 6/150) of the results, the treatment was extended (13–18 days), yet all were among the middle range of AMH levels.

Table 6. The relation between patient duration of induction and AMH level

	AMH level classification					
Induction duration/ days	**High (3 – 6)**	**Normal (1- 2.9)**	**Normal low (0.7- 0.9)**	**Low (0.3 -0.6)**	**Very low (< 0.3)**	**Total**
7-12	20	59	28	25	12	144
13 – 18	0	4	1	1	0	6
Total	20	63	29	26	12	150

Data represented as a number, AMH: anti-Müllerian hormone.

(Table 7) shows how oocyte and embryo variables are divided by different levels of serum AMH. Statistical testing showed that the mean number of oocytes collected was not the same among the four groups ($p<0.001$). Those with high AMH (3 to 6 ng/mL) produced the highest number of oocytes, and the amount declined with lower levels of AMH. There was also a big change in the number of Metaphase I (MI) oocytes among the various groups ($p<0.001$). These fertility markers suggest that the group with the most AMH generally had more mature oocytes, and this amount diminished in those with lower AMH levels.

Fertilization-important Metaphase II (M II) oocyte yield declined as AMH levels fell ($p<0.001$). The highest mean M II counts were found in women with high AMH levels, and the lowest counts were found in those with low AMH. By contrast, the mean number of missing oocytes (empty follicles) was remarkably similar across the various AMH groups.

The number of embryos retrieved was very different between the AMH ranges ($p=0.001$). Women in the high AMH group had more embryos on average, and the number dropped as AMH levels went down.

A decline in Grade I embryos was statistically significant when AMH levels were lower ($p<0.001$). In the high AMH group, there were 1.56±0.86 GI embryos, whereas the very low AMH group only had 0.50±0.58. The mean total of Grade II and Grade III embryos did not vary significantly among the AMH groups ($p>0.05$). A total of 91 patients completed embryo transfer, but for 59 patients, an embryo transfer wasn't performed. There is a significant link between serum AMH levels and being offered embryo transfer ($p<0.001$).

The largest out of the 91 patients who had an embryo transfer was the group with normal AMH (47 and 51.6%). A total of twenty patients (22.0%) in our group had high levels of AMH. Patients treated with Embryo Transfer (ET) represented a much smaller percentage of AMH Group 2 and 3.

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Table 7. Distribution of oocyte and embryo (Number) (Mean ±SD) according to AMH level

AMH level	High 3 - 6	Normal 1 – 2.9	Normal low 0.7 – 0.9	Low 0.3 – 0.6	Very low < 0.3	P value
Number of Oocytes	(21)7.8±3.6	(63)3.8±1.9	(26)2.8±1.4	(23)2.1±1.2	(9)1.4±1.2	0.0001
Empty Oocyte	(20)0.45±.88	(51)0.61±.75	(20)0.55±.69	(18)1.0±.49	(8)0.88±0.84	0.155
M I	(21)2.4±1.9	(58)1.2±.89	(26)1.23±1.1	(21)0.86±.79	(9).56±0.73	0.0001
M II	(21)4.9±3.2	(57)2.4±1.5	(25)1±0.88	(17)0.65±0.79	(7)1.00±0.0	0.0001
ET number	(19)1.9±.74	(48)1.5±.65	(17)0.94±.89	(11)1.1±0.70	(4)0.75±0.96	0.001
G I	(18)1.56±0.86	(45)0.80±0.55	(15)0.73±0.88	(8)0.50±0.4	(4)0.50±0.58	0.0001
G II	(17)0.18±0.4	(38)0.61±0.68	(13)0.38±0.65	(10)0.60±0.52	(4)0.25±0.50	0.135
G III	(17)1.00±0.03	(37)0.27±0.51	(11)1.00±0.06	(7)0.29±0.48	(3)1.00±0.05	0.077

Data represented as number (No.), and mean ± SD, AMH: anti-Müllerian hormone, ET: Embryo Transfer.

When looking at the 59 patients who had not received ET, their disease distribution was different. Patients who did not have ET were most often identified in the normal-low, normal, and low AMH subgroups. The very low AMH group had fewer women, and only a very small number of women were seen in the high AMH group, as (Table 8) shows.

Table 8. Distribution of Embryo Transfer (ET) according to AMH level

	High 3 – 6		Normal 1 – 2.9		Normal low 0.7-0.9		Low 0.3 - 0.6		Very low < 0.3		P value
ET	No.	%	No.	%	No.	%	No.	%	No.	%	
Yes	20	22	47	51.6	11	12.1	9	9.9	4	4.4	0.001
No	1	1.7	16	27.1	18	30.5	16	27.1	8	13.6	

Data represented as number (No.) and percentage (%), AMH: anti-Müllerian hormone, ET: Embryo Transfer.

No significant differences were seen in the fertilization rate for those with different AMH levels, as shown in the analysis (Table 9). Though the number of fertilized eggs was similar for everyone, having high or normal AMH improved the likelihood of a patient going on to embryo transfer compared to those with low AMH ($p<0.001$).

Table 9. Distribution of fertilization rate according to AMH level

AMH Level	High (3 – 6		Normal (1 – 2.9)		Normal low (0.7- 0.9)		Low (0.3 - 0.6)		Very low < 0.3		P value
Cleavage	No.	%	No.	%	No.	%	No.	%	No.	%	
Yes	1	1.7	16	26.7	19	31.7	15	25	9	15	
No	20	22.2	47	52.2	10	11.1	10	11.1	3	3.3	0.0001

Data represented as number (No.) and percentage (%), AMH: anti-Müllerian hormone.

Discussion

Women over 40 were more likely to have very low levels of AMH, as shown by our data. This observation matches up with recent long-term studies that found this decline to be a basic feature of aging ovaries. In 171,595 Indian women, researchers found that AMH levels were highest in those aged under 25 years (4.42 ng/mL) and the lowest in women over 50 years (0.24 ng/mL) [16]. As in the West, the UAE's research discovered a significant decrease in AMH among people of all ages during the 2022-2023 period, with the most significant decline seen in younger women [17].

AMH in our youngest group (20-30 years old) varied widely; normal values were seen in most, with none showing extremely low, which suggests that age is not enough to predict what a woman's ovarian reserve really is. The study result lines up with evidence from wider groups of healthy women showing significant differences in AMH levels within the same age group [18, 19].

Our results indicate that FSH and AMH levels are inversely connected, as has been documented before. Intensive investigation in recent literature found that a high FSH and a low AMH ratio are related to a lower chance of successful fertility treatment [(20].

Human menopausal gonadotropin (HMG) dose is strongly linked to AMH levels, and this key discovery supports the use of personalized ovarian stimulation therapies. Our findings show that a normal response

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was achieved in women with high AMH if they received a lower dose of HMG. The same relationship has been noted in studies, where researchers advise personalizing gonadotropin administration based on women's AMH to increase success and reduce the danger of ovarian hyperstimulation syndrome [(21, 22].
Reductions in mature oocyte (MII) numbers and the total number of embryos with declining AMH confirm that AMH plays a major role in ART outcomes. The results agree with recent research in which the relationships between AMH and the number of retrieved oocytes are found to be strong, with correlation coefficients from 0.624 to 0.659 [23]. A different study of 12,588 patients found that AMH levels are strongly related to embryo quality, with better outcomes in women with higher AMH groups [21].
Our findings demonstrated that there was no big difference in fertilization rates among AMH groups backs up recent findings that AMH mostly reflects the number of available oocytes rather than their quality. It is very important for patient counseling that low AMH levels might not stop women from getting pregnant when they receive ample oocytes [23].
Since AMH levels relate to whether or not an embryo transfer will be performed, this has a big impact on doctors' therapy choices and their counseling of patients. The finding shows that 51.6% of patients who went through embryo transfer had normal AMH, whereas many patients with lower AMH did not make it to transfer. Current studies of cumulative live birth rates have found that patients with higher AMH are much more likely to succeed, setting the cumulative live birth rate at 65.80% as compared to 43.95% in normal versus low AMH groups [24].
What we found out helps fill the gaps in our knowledge about different AMH levels in different places and times. New studies reveal a trend of lower AMH levels in many populations, and in 2022-2023, the number of patients with low AMH increased by 5-7% globally [17]. The predominance of female factor infertility (48.7%) in our cohort aligns with global epidemiological data, indicating that female factors account for approximately 25% of infertility cases, with ovulatory disorders being the most common etiology [25]. In conclusion, our findings strongly reinforce the clinical utility of serum AMH as a robust biomarker in the management of female infertility. It serves as an excellent predictor of ovarian reserve, correlating significantly with age and FSH levels.

Conclusion

Strong prediction of the quantitative outcomes of IVF, including the number of recovered oocytes (total, MI, and M II) and the number of top-quality (Grade I) embryos, AMH is indispensable for customizing gonadotropin stimulation regimes. Most importantly, it greatly forecasts a patient's chances of getting to the embryo transfer point. Although it might not exactly forecast fertilization rates per oocyte, in the modern fertility clinic, its impact on the total number of accessible oocytes and embryos makes it a great tool for advising patients, controlling expectations, and improving therapy tactics. More extensive, prospective research is justified to validate these conclusions and investigate the relationship between AMH and live birth rates among several patient groups.

Conflicts of Interest

The authors declared no conflict of interest.

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