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The Relation between Follicular Fluid Anti-Müllerian Hormone (AMH), Oocyte Maturation and Embryo Development in Intracytoplasmic Sperm Injection (ICSI) Cycles

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ORIGINAL ARTICLE

The Relationship Between Follicular Fluid Anti-Müllerian Hormone (AMH), Oocyte Maturation, and Embryo Development in Intracytoplasmic Sperm Injection (ICSI) Cycles

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Abstract

Background: **A comparatively recent and rapidly developing method of resolving human reproductive issues involves externalizing the human ovum with spermatozoa and transporting the generated embryo to the woman's uterus. The 1970s saw the publication of the first findings of implantations and pregnancy after the application of this technology in humans. Since then, the procedures recognized as in vitro fertilization (IVF) and embryo transfer (ET) have led to multiple pregnancies, new knowledge about gamete interactions, and earlier embryogenesis progress. Unfortunately, compared with the therapy of tubal factor infertility, IVF is typically less effective for treating male factor infertility.**

Aim of the work: **To assess the relationship between follicular fluid AMH, oocyte maturation, fertilization, and embryo grading.**

Patients and methods: **The current prospective cohort study was carried out on 85 infertile women with unexplained infertility recruited from Ain Shams University Maternity Hospitals (ART unit)-assisted reproductive technology unit, undergoing ICSI (intracytoplasmic sperm injection) during the period between December 2018 and December 2021.**

Results: **FF AMH with a cutoff value > 1.2 ng/ml could be used as a single independent factor for the prediction of successful fertilization. FF AMH associated with the MII oocyte was significantly higher compared with GV and MI oocytes, with a *P* value < 0.001. FF AMH with a cutoff value > 3.4 ng/ml could be used as a single independent factor for the prediction of the production of MII oocytes. FF AMH level associate grade I embryo was significantly higher compared with grades II–IV, with a *P* value of 0.006. FF AMH with a cutoff value > 4 ng/ml could be used as a single independent factor for the prediction of grade I embryo.**

Conclusion: **Our findings suggest that FF AMH density and fertilization, oocyte maturation, and embryo quality are positively correlated. As a result, we can use the FF AMH value as a predictor of the success of IVF.**

Keywords: Follicular fluid anti-Müllerian hormone, Intracytoplasmic sperm injection, Oocyte maturation

1. Introduction

Nowadays, intracytoplasmic sperm injection (ICSI) is one of the most effective and practical methods for assisted reproduction.[1]

In those who have no identifiable or correctable causes, ICSI provides new hope for infertility. ICSI can improve previous fertilization limitations on conventional IVF.[2]

The fertilization rate in assisted reproductive technology instances has continuously grown as a result of recent advancements in the knowledge of ovarian stimulation, procedures of oocyte retrieval, management of gametes, procedures of assisted fertilization, and higher living standards of culture media. When

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doing intracytoplasmic sperm injection (ICSI) or traditional insemination, fertility rates of 70–80% may now be anticipated. Unfortunately, there has not been a comparable rise in implantation ratios, which for a very long time have been stable at 10–15%.[3]

The major purpose of the mammalian ovarian follicle, known as folliculogenesis, is the production of an egg that can be fertilized by a sperm. This entails the development and maturation of the follicles in addition to its development, ovulation, and restart of the egg's meiotic division.[4]

Human oocyte maturing is defined as the restart and accomplishment of the first meiotic division from the germinal vesicle phase (prophase I) to metaphase II, and the cytoplasm maturity that occurs along with it. These processes are required for fertilization and early embryogenesis progression. For an egg to mature, its cytoplasm is crucially important. Enhancing oocyte quality is the strongest strategy to increase embryo quality.[3]

Oocyte quality has an impact on fetal growth, pregnancy formation, and maintenance, and early embryogenesis survivability. Quality, or developmental competence, is developed both during the oocyte's growth and maturity processes, or folliculogenesis.[5]

Among the main objectives of embryologists in human IVF is to assess oocytes and embryo quality. There are various techniques used to assess the quality of oocytes and embryos.[6]

2. Patients and methods

In all, 85 infertile women participated in this prospective trial. All individuals who underwent assisted reproductive technology procedures in the ART unit of Ain Shams University Maternity Hospitals between December 2018 and December 2021 were included in the research after providing signed consent.

Using EpiInfo® version 6.0, the sample size was computed with the power (β) set to 80% and the significance value (α) set to 0.05. A prior study's data showed that 83 instances should be the minimum sample size. Consequently, there were 85 individuals in the overall sample.

Women must be between the ages of 25 and 35 years, have both ovaries observed<AQ: Is it absorbed instead of observed>, be free of morphological malformations, be able to see both ovaries clearly on a TV U/S ultrasound, have menstrual cycles that last from 25 to 35 days, have Day 3 FSH values below 10 IU/ml, and be free of any ocular diseases that might affect the production or clearance of gonadotropins or sex hormones as well as the absence of any symptoms of hyperandrogenism.

We disqualified anyone older than 35 years, any growing lesions seen on a TV U/S scanning of the ovaries and the uterus, abnormal menstrual cycles, and any present or previous disorders (medical or surgical) impacting the ovaries and its functioning (e.g., an inappropriate synthesis of hormones), indications of a disordered endocrinological system (such as hyperprolactinemia, hypo- or hyperthyroidism, and hyperandrogenism), and male element infertility, where individuals with immotile sperms, azospermia, and frozen thawing sperm were candidates for ICSI.

2.1. Methods

Each participant in this research underwent.

Recording a history that includes previous medical and surgical history as well as evidence of ovulation inducement, such as ovarian hyperstimulation.

For indications of compromised endocrinological functioning, perform a general and abdominal assessment (e.g.: hyperandrogenism).

All subjects had an ovarian reserve assessment on day 3 of spontaneous cycles using hormones FSH, LH, E2, TSH, and prolactin.

Any participant with ovarian, uterine, or tubal pathologies was omitted from the trial based on transvaginal ultrasound performed by a transvaginal probing on day 3 of non-stimulated phases.

Daily subcutaneous injections of triptorelin acetic were administered as part of an extensive GnRh agonist program for ovarian hyperstimulation beginning in the middle of the luteal phase (Decapeptyl 0.01 mg daily, Ferring Pharmaceuticals, Kiel, Germany); thereafter, on day 3 of the subsequent cycle, HMG injections were given daily to begin ovarian hyperstimulation (Menogon 75 IU/ampule (Ferring Pharmaceutical, Kid<Kiel?>, Germany) Gonadotropins were first administered based on the individuals' ages and body types, and then their doses were changed in accordance to the ovary reaction as determined by TV folliculometry on cycling days 7 or 9. Daily TV U/S was carried out in accordance with ovarian responses, starting when the leading follicles reached a diameter of 16 mm and continuing every day until the biggest follicles had a diameter of >18 mm. It was given HCG. On the day that the HCG injection was given, TV U/S was done to count any follicles larger than 10 mm. The ART unit at the Ain Shams University Maternity Hospital accepted this procedure.

A second-generation ELISA was used to assess the AMH value in each follicular fluid, and traditional IVF was done particularly on the oocyte isolated from the follicular fluids in a different cultured dish. Directly upon retrieval, oocytes were assessed for nuclear maturity and scored as being in metaphase II, metaphase I, or prophase I.

2.2. Statistical methods

Version 13 of MedCalc© was used for the data analysis (MedCalc© Software bvba, Ostend, Belgium). Categorical data were reported as numbers and percentages, whereas numerical values were shown as median and interquartile range. The Kruskal-Wallis analysis or the Mann–Whitney test was used to evaluate numeric data for distinctions between two populations (for differences among multiple groups). Whenever the Kruskal-Wallis analysis revealed statistically substantial disparities between the groups; the Conover test was used for post hoc assessments. When applicable, Fisher's exact test or the Pearson chi-squared analysis was used to contrast numeric values. Using the chi-squared tests for trending, ordinal data were analyzed.

Evaluation of the receiver-operating characteristic (ROC) curve was used to assess the usefulness of quantitative factors for binary outcome predictions. The areas below each ROC curve were compared using the DeLong technique. To identify independent variables for binary outcomes, multivariate binary logistic regression was used. To add pertinent indicators to the models, the simultaneous technique was applied. Statistical significance was defined as a two-tailed P value < 0.05.

3. Results

A sample of the follicular fluid from one follicle larger than 18 mm in diameter was taken from every subject. The viability of the embryos was examined before embryo transfer by embryo scoring; the fertilization of every oocyte was examined using a microscope 18–20 h after IVF, and the AMH level of each follicular fluid was quantified using a second-generation ELISA.

This table shows that the number of retrieved oocytes ranged from 0 to 22 oocytes per patient, with a mean number of 8.4 ± 5 oocytes, while the number of produced embryos per patient ranged from 0 to 12 embryos with a mean number of 4.7 ± 3.2 embryos.

In this table most of the patients represents (56 patients, 65.9%) had primary infertility, while 29 patients (34.1%) had secondary infertility. The majority of the produced oocytes were MII maturation, 53 oocytes representing 63.9%, while GV and MI were (12, 18) oocytes, representing (14.5, 21.7%) respectively. Also, 88.7% of the produced oocytes were fertilized and 11.3% were not fertilized, most of the produced embryos (58.7%) were of grade 1 maturation while grade 2, 3, and 4 represented (15.9, 14.3, and 11.1%) respectively.

This table shows a positive correlation between FF AMH, number of retrieved oocytes, oocyte maturation, fertilization, number of produced embryos, and embryo grading.

This table shows no statistically significant relation between FF AMH and type of infertility; however, the level of FF AMH associated with MII oocyte was significantly higher compared with oocyte of GV, MI maturation. Also there was a statistically significant relation between FF AMH and occurrence of fertilization. As regards embryo grading, the level of FF AMH associated with grade I embryos was significantly higher compared with grade II and grade III embryos, respectively.

This table shows that there was no statistically significant difference between the AUC associated with the ROC curve derived from FF AMH alone and that from a multivariable logistic regression model.

4. Discussion

This investigation sought to determine how follicular fluids AMH correlated with oocyte maturation, fertilization, and embryo grade.

A sample of follicular fluid from one follicle larger than 18 mm in diameter was taken out of each individual, and the AMH concentration of each specimen was determined using a second-generation ELISA. Oocytes were analyzed and scored, every oocyte's fertilization was checked under a microscope 18–20 h after IVF, and the embryo scoring was used to determine the quality of the embryo before embryo transfer.

In this study, no oocytes were obtained from two patients during oocyte retrieval.

Our study found that the age of the studied population ranged from 22 to 35 years. The mean age was 31.2 ± 3.3 years, while the duration of infertility ranged from 1.5 to 18 years, with a mean duration of 7.8 ± 4.4 years. Also the body mass index ranged from 22 to 43; the mean BMI was 28.8 ± 4.3.

In this study, the FF AMH (follicular fluid antimullerian hormone) level per follicle ranged from 1.1 to 5.8 ng/ml. The mean level was 3.1 ± 1.3 ng/ml as shown in Tables 1 and 2.

This result is consistent with *Tolikas et al.*,[7] who reported that the mean FF AMH was 3.6 ± 2.2 ng/ml.

Table 1. Descriptive statistics for the whole study population as regards embryological data.

Variable	*N*	Min.	Max.	Mean	SD	Median	IQR
Number of retrieved oocytes	85	0.0	22	8.4	5	7	5–12
Number of embryos in women given fertilization trial	71	0.0	12	4.7	3.2	4	2.3–7

Mean, median, IQR (interquartile range).

Table 2. Descriptive statistics for the whole study population: Qualitative variables.

	Number valid (percent)
Type of infertility	
Primary	56 (65.9)
Secondary	29 (34.1)
Oocyte maturation	
GV	12 (14.5)
MI	18 (21.7)
MII	53 (63.9)
Fertilization	
Not fertilized	8 (11.3)
Fertilized	63 (88.7)
Embryo grade	
Grade 1	37 (58.7)
Grade 2	10 (15.9)
Grade 3	9 (14.3)
Grade 4	7 (11.1)

GV (germinal vesicle), MI (metaphase I).

However, *Lee et al.*[8] found that the mean FF AMH was 1.8 ± 0.4 ng/ml.

In addition, we discovered that the antral follicle count (AFC) varied from 3 to 20 follicles, with an average of 9.9 ± 3.4. Ovarian stimulation lasted between 8 and 21 days; the average time was 12.1/2 days. According to the table, the average HMG dosage was 49.5 ± 13.4 ampoules, and the mean duration to oocyte extraction was 14.1 ± 2 days (3).

These results were similar to *Aflatoonian et al.,*[9] who found that the mean duration of ovarian stimulation was 11.5 ± 2.6 days; however, he found that the mean dose of gonadotropins was 27.2 ± 7.8 ampoules.

In this study, the number of retrieved oocytes ranged from 0 to 22 oocyte per patient, with a mean number of 8.4 ± 5 oocytes, which were retrieved, while the number of produced embryos per patient ranged from 0 to 12 embryos with a mean number of 4.7 ± 3.2 embryos, as shown in Tables 3 and 4.

Table 3. Relationship between follicular fluid AMH level and other quantitative variables as regards embryological data.

Variable	Follicular fluid AMH level	
Number of retrieved oocytes	rho	0.503
	P value	<0.0001[a]
	n	85
Oocyte maturation	rho	0.555
	P value	<0.0001[a]
	n	83
Number of embryos in women given fertilization trial	rho	0.438
	P value	0.0001[a]
	n	71
Number of embryos in women with fertilized oocytes	rho	0.342
	P value	0.005[a]
	n	65
Embryo grade	rho	−0.313
	P value	0.013[a]
	n	63

Mann–Whitney *U* test.
[a] Significant.

Table 4. Relationship between follicular AMH level and other qualitative variables.

Variable	Follicular fluid AMH level, ng/ml		*P* value
Type of infertility	1ry ($n = 56$)	3.4 (2.03–4.38)	0.127[a]
	2ry ($n = 29$)	2.5 (1.58–3.80)	
Oocyte maturation	GV ($n = 12$)	1.5 (1.30–2.35)	<0.00001[b,c]
	MI ($n = 18$)	2.15 (1.50–2.90)	
	MII ($n = 53$)	3.8 (2.50–4.50)[c]	
Fertilization	Not fertilized ($n = 8$)	1.2 (1.10–3.15)	0.004[a,c]
	Fertilized ($n = 63$)	3.7 (2.43–4.40)	
Embryo grade	Grade I ($n = 37$)	4.2 (2.78–4.50)[d]	0.041[b,c]
	Grade II ($n = 10$)	2.9 (1.60–3.80)	
	Grade III ($n = 9$)	2.1 (1.85–3.73)	
	Grade IV ($n = 7$)	3.1 (2.83–3.85)	

*Significant.
[a] Mann–Whitney *U* test.
[b] Kruskal-Wallis test.
[c] *P* value < 0.05 vs. GV and MI (Conover test).
[d] *P* value < 0.05 vs. Grade II and Grade III (Conover test).

Most of the patients represent (56 patients, 65.9%) had primary infertility while 29 patients (34.1%) had secondary infertility.

The majority of the produced oocytes were of grade MII maturation, 53 oocytes, which represent 63.9%, while GV and MI were 12 and 18 oocytes, which represent 14.5 and 21.7%, respectively, as shown in Table 5.

Also, 88.7% of the produced oocyte were fertilized while 11.3% were not fertilized; most of the produced embryos (58.7%) were of grade 1 maturation, while grade 2, 3, and 4 represent 15.9%, 14.3%, and 11.1% respectively, as shown in Table 5.

We found no significant correlation between FF AMH and age, duration of infertility, body mass index (BMI), and other hormonal profiles, but *Lee et al.*[8] found a negative correlation between FF AMH with age.

As regards fertilization, we divided the population into two groups (fertilized and nonfertilized) and found no significant correlation between the two groups as regards age, duration of infertility, and BMI.

However, there was a significant correlation between successful fertilization and AFC (antral follicular count) with a P value of 0.040 and the number of retrieved oocytes with a P value < 0.001.

Also, there was a significant correlation between the production of MII oocytes and successful fertilization with a P value of 0.021.

When follicles are capable of producing large levels of AMH in follicular fluids, oocytes have a higher chance of becoming fertilized. AMH may therefore serve as a predictor of conception.[10]

In our study, there was a highly significant correlation between FF AMH and successful fertilization with a P value of 0.0001.

In addition, the median FF AMH content in the fertilized sample was 3.7 ng/ml and in the nonfertilized group, it was 1.2 ng/ml with a P value of 0.0004; the FF AMH rate in the fertilized sample was substantially greater than that in the nonfertilized group.

These results are consistent with *Aflatoonian et al.*,[9] who found that the median FF AMH value in the fertilized sample was 5.7 ng/ml and in the nonfertilized sample, it was 2.7 ng/ml. Group I had a considerably greater level of AMH than group II ($P < 0.001$).

Also, a retrospective study was conducted on 31 women undergoing IVF cycles. Depending on whether fertilization was successful or unsuccessful, women were separated into two groups. Samples of follicular fluid were taken from just one follicle in each participant. Evaluation of the level of FF AMH was conducted on the day of the IVF procedure's oocyte extraction. Fertilized individuals (group 1) had follicular fluid AMH levels that were 2.40 ± 3.48 ng/ml, which were 3.42 times more than nonfertilized patients' values of 0.70 ± 1.01 ng/ml ($P = 0–045$).[10]

To anticipate successful fertilization using FF AMH, we used the receiver-operating characteristic (ROC) curve analysis. We discovered that the FF AMH value had a very strong predictive value as indicated by an area under the curve (AUC) of 0.813 (95% CI, 0.–0.896, P value 0.0002).

The best cutoff value was an AMH level >1.2 ng/ml, this had a sensitivity of 98.4%, specificity of 62.5%, +PV 95.4%, and −PV of 83.3%.

As regards oocyte maturation, the resulting oocytes were grades as germinal vesicle (GV), metaphase I (MI), and metaphase II (MII).

This study shows that no significant correlation between oocyte maturation, age duration of infertility, and BMI.

There was a positive correlation between proper oocyte maturation and the number of retrieved oocytes and the occurrence of fertilization.

Also, the FF AMH associated with MII oocyte was significantly higher compared with GV and MI oocyte, with a P value < 0.001.

The median FF AMH was 3.8 ng/ml in the MII oocyte and 2.1 ng/ml in GV and MI oocytes; the level was significantly higher in the MII oocyte, compared with GV, MI oocyte, with a P value < 0.001.

We compared the receiver-operating characteristic (ROC) curve analysis for the prediction of MII oocyte using FF AMH level or the multivariable logistic regression model (MVLR) and found that there was no statistically significant difference between AUC (area under the curve) derived from FF AMH alone (0.831) and that from MVLR (0.862), with a P value of 0.305.

Table 5. Comparison of the receiver-operating characteristic (ROC) curves for the prediction of successful fertilization using follicular AMH level or the multivariable logistic regression model (MVLR).

Source of ROC	AUC	95% CI	ΔAUC	95% CI	P value
Follicular AMH	0.813	0.703 to 0.896	0.117	−0.009 to 0.243	0.069
MVLR model	0.931	0.845 to 0.977			

AUC (area under the curve); ROC (receiver-operating characteristic); ΔAUC (delta AUC).

So, FF AMH with a cutoff value > 3.4 ng/ml could be used as a single independent factor for the prediction of production of MII oocytes.

As regards embryo grading, before embryo transference, embryo scoring was used to evaluate the quality of the embryo. A quality scoring for each embryo on days 2 and 3 (48 and 72 h following oocyte retrieval) was calculated by multiplying the embryo's morphological grading by the quantity of blastomeres. Embryos were grouped morphologically as follows: grade 1, symmetrical blastomeres of equal size; grade 2, uneven blastomeres with extracellular fragmentations of greater than 10%; grade 3, uneven blastomeres with extracellular fragmentations of between 10 and 50%; and grade 4, 50% blastomeric fragmentations with unequal-sized blastomeres, ***Hardarson et al.***[11]

In this study, there was no significant correlation between embryo grading and age, duration of infertility, and BMI.

In addition, there was no significant correlation between embryo grading and AFC, duration of ovarian stimulation, HMG doses, and time to oocyte retrieval.

We also found that there was a positive correlation between the production of grade I embryos and the number of retrieved oocytes, the number of produced embryos, and fertilization rate compared with grade II–IV embryos, with *P* values of 0.009, 0.016, 0.016, respectively.

The estradiol level associate grade I embryo was significantly lower compared with embryos grade II–IV with a *P* value of 0.042.

However, the FF AMH level associate grade I embryo was significantly higher compared with embryo grades II–IV with a *P* value of 0.006.

The median FF AMH was 4.2 ng/ml in grade I embryos and 3 ng/ml in grades II–IV embryos; the level was significantly higher in grade I embryos compared with II–IV embryos, with a *P* value of 0.006.

This result was similar to ***Aflatoonian et al.***,[9] who found that the discrepancy between FF AMH level and embryo scoring was statistically significant ($P < 0.001$). The medians of FF AMH levels were 6.7 ng/ml in embryos of better quality and 3.80 ng/ml in embryos of fair quality.

In addition, we used the receiver-operating characteristic (ROC) curve analysis for the prediction of grade I embryo production using FF AMH level and found that FF AMH level had a good predictive value as evidenced by an AUC (area under the curve) of 0.705 (95% CI, 0.576 to 0.813, *P*- value 0.002).

The best cutoff value was an AMH level >4 ng/ml, this had a sensitivity of 54.05%, specificity of 88.46%, +PV 87%, and −PV57.5%.

However, using multivariable binary logistic regression for prediction of grade I embryo production, we found that production of MII oocyte is independent of reduction of grade I embryo production.

We compared receiver-operating characteristic (ROC) curve analysis for the prediction of grade I embryo using FF AMH level or the multivariable logistic regression model (MVLR), and found that there was no statistically significant difference between AUC (area under the curve) derived from FF AMH alone (0.831) and that from MVLR (0.862) with a *P* value of 0.305, table (33).

So, FF AMH with a cutoff value > 4 ng/ml could be used as a single independent factor for the prediction of production of grade I embryo.

4.1. Conclusion

Our findings suggest that FF AMH levels and fertilization, oocyte maturation, and embryo quality are positively correlated. As a result, we can use the FF AMH levels as a predictor of the success of IVF.

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Conflicts of interest

The authors declared that there were no conflicts of interest.

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