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RESEARCH ARTICLE

# The Correlation between Follicular Fluid Antimullerian Hormone Levels and Fertilization and Embryo Quality in ART Cycles

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## ABSTRACT

**Background:** Determination of oocyte and embryo quality are one of the most important goals in *in vitro* fertilization (IVF). Antimullerian hormone (AMH) is secreted by the ovarian granulosa cells into blood flow and follicular fluid. Follicular fluid (FF) AMH level is probably a marker of activity of granulosa cells.

**Objective:** To evaluate whether high level of FF AMH level is related to success of fertilization and better embryo quality.

**Materials and methods:** Sixty-two women, whose FF sample was obtained from a single follicle in each patient, underwent IVF with GnRH-agonist long protocol. Based on oocyte fertilization, the patients were divided into fertilized group (n = 42) and nonfertilized group (n = 20). FF AMH levels were measured in both groups and the quality of embryos was determined in fertilized group.

**Results:** Median of FF AMH level in fertilized group was higher than that in nonfertilized group (5.7 *vs* 2.7 ng/ml) and a statistically significant difference was observed between the two groups. There was a significant difference between FF AMH level and scores of embryos ($p < 0.001$). The medians levels of FF AMH were 6.7 ng/ml in good quality embryos and 3.80 ng/ml in fair quality embryos.

**Conclusion:** Our results indicate that FF AMH level has positive correlation with fertilization and embryo quality; therefore, it can be considered as a marker of IVF outcome.

**Keywords:** Antimullerian hormone, Follicular fluid, Fertilization, Embryo quality, *In vitro* fertilization.

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## INTRODUCTION

Recognition and selection of the best oocyte and embryo can improve *in vitro* fertilization (IVF) outcome. Follicular fluid (FF) is composed of substances which are secreted by granulosa cells, theca cells and blood flow. It is a suitable environment for oocyte growth and development. Biochemical characteristics of the FF play an important role in the prediction of oocyte quality, fertilization and ultimately the embryo quality in noninvasive methods.[1,2] Antimullerian hormone (AMH) is one member of transforming growth factor-β (TGF-β).[2-5] AMH plays a fundamental role in gonadal differentiation during fetal period and inhibits the formation of mullerian ducts in male fetus.[2,6-8] AMH is secreted by the ovarian granulosa cells into blood flow and follicular fluid in adult female, although its concentration is much higher in the follicular fluid.[2,4,6] AMH production is independent of FSH and inhibits FSH-induced follicular growth.[9-11] It also has a direct autocrine-paracrine effect on the granulosa cells, oocyte function and embryo quality.[5,12-14] Determination of oocyte and embryo quality are one of the most important goals of embryologists in human IVF. Several methods are employed for determining oocyte and embryo quality.[1] FF AMH level is probably a marker of the qualitative and quantitative activity of granulose cells.[13] Although some studies have showed the relationship between the level of serum AMH, and quality of oocyte and embryo,[9,11,12] there are few studies about the relationship existing between these factors and FF AMH level.[13] Our study was designed to investigate the association between FF AMH level and successful fertilization and embryo quality in IVF cycles. Based on FF AMH level, we can choose one or two embryos of high viability and thus improve IVF outcome.

## MATERIALS AND METHODS

The study was conducted at a reproduction center (Kinder IVF) between May and November 2012. A total of 62 infertile patients, who underwent IVF, participated in this prospective study. The inclusion criteria were: Female age <35 years old, presence of both ovaries in ultra-sonography, BMI <25 kg/m$^2$, day 3 FSH <10 IU/ml, and the duration of menstrual cycle between 25 and 35 days. Patients with PCOD, history of pelvic surgery, endometriosis, endocrine disorders and couples with male factor who were candidate for ICSI (patients with immotile sperm, azoospermia, normal morphology of sperm <4% and frozen-thawed sperms)[15] were not enrolled in this study. Also, patients whose follicular fluid was bloody during the oocyte retrieval were excluded from the study. Before initiating the treatment, venipuncture for assay of FSH,

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estradiol ($E_2$), and transvaginal ultrasound scan were also performed on the third day of the menstrual cycle.

## ASSAY

All transvaginal ultrasonographic evaluations were performed by a single investigator, using a conventional two-dimensional ultrasound (HS-400, Honda, Japan) equipped with a 7.5 MHz vaginal transducer. FSH concentrations were measured by competitive immunoassay (IDCS, Korbach, Germany), intraassay and interassay coefficients of variation were 6% and 6.8% respectively. $E_2$ concentrations were measured using an enzyme-immunoassay kit (DRG, Marburg, Germany), intraassay and interassay coefficients of variation proved to be 6.3% and 6.4% respectively. Measurement of FF AMH levels was performed using AMH/MIS enzyme-linked immunosorbent assay kit (Beckman Coulter Immunotech Com, Fullerton, CA).

## TREATMENT PROTOCOL

All of the patients were treated with a long protocol for ovarian stimulation. For pituitary suppression, the patients were treated with daily administration of 0.5 mg/day buserelin SC (Superfact, Aventis, Frankfurt, Germany) which started in the luteal phase of menstrual cycle. When desensitization was occurred, as evidenced by plasma $E_2$ levels of ≤50 pg/ml and the absence of ovarian cyst on transvaginal ultrasound examination, buserelin was reduced to 0.25 mg/day and continued until the day of hCG administration. The controlled ovarian hyperstimulation (COH) was initiated with recombinant FSH (Gonal F, Serono, Aubnne, Switzerland) or HMG (Menogon, Ferring, Pharmaceuticals, Germany) 150 IU/day on the day 2 of menstrual cycle. Ovarian response was monitored by serial ultrasound examinations and the evaluation of serum $E_2$ levels, then gonadotropin doses adjustment was done as required. Urinary human chorionic gonadotropin (Pregnyl, Organon, Oss, the Netherlands) 10000 IU was administered when at least three follicles reached a mean diameter of 18 mm.

Oocyte retrieval was performed by the transvaginal ultrasound-guided approach, 34 to 36 hours after the hCG injection. Each patient's follicular fluid sample was collected from one follicle with a diameter greater than 17 mm. Only the first follicle of ovary was selected and the needle was washed before the puncture of the remaining follicles. Follicular fluid of the first selected follicle was separated from the cumulus-oocyte complex and then was centrifuged.

Each of the centrifuged follicular fluid was freezed at 80°C until the samples were all completed. Then, the AMH level of every respective FF was measured and conventional IVF was performed specifically on the oocyte obtained from the follicular fluid in a separate culture dish. The fertilization of each oocyte was assessed using a microscope 18 to 20 hours following IVF and the fertilization was confirmed through pronuclei detection. Also the quality of embryo was determined in fertilized oocyte 48 hours after IVF and was scored based on the shape, number and fragmentation of blastomers.[16] Score ≤18 was decided to indicate good quality, 15-17 fair quality and <15 poor quality in our center. Based upon fertilization of oocytes, the patients were divided into two groups. Patients with fertilized oocytes were defined as group I and nonfertilized oocytes as group II. FF AMH level and quality of embryos were compared in both groups.

## STATISTICAL ANALYSIS

The parameters relevant to demographic and COH characteristics were presented based on mean ± SD, median and range. The statistical package for social sciences (SPSS, version 15.0 for windows, SPPS Inc, Chicago, IL) was utilized for data analysis. Normality was assessed using Kolmogorov-Smirnov test.

T-test, Mann-Whitney and Chi-square test were used for analysis as needed. To determine the relationship between the score of embryos and the concentration of FF AMH, Kruskal-Wallis test was employed, p-value of less than 0.05 was considered to be statistically significant.

## RESULTS

Sixty-two infertile patients underwent IVF and 62 oocytes retrieved, of which 42 oocytes were fertilized (group I) and 20 oocytes were not fertilized (group II). There was no significant difference between fertilized and nonfertilized groups regarding age, infertility duration, BMI, basal FSH, basal E2, duration of stimulation, doses of the administrated gonadotropin, and the E2 level on the day of hCG administration (Table 1). Infertility etiology distribution was similar in both groups (Table 2). Medians of FF AMH level were 5.7 ng/ml (IQ = 3) in fertilized group and 2.7 ng/ml (IQ = 1.7) in nonfertilized group. The level of AMH in group I was significantly higher than that in group II ($p < 0.001$). There was a significant difference between FF AMH level and embryo scores ($p < 0.001$). The medians of FF AMH level were 6.7 ng/ml (IQ = 5.05) in good quality embryos and 3.80 ng/ml (IQ = 3.32) in fair quality embryos. No poor quality embryos were detected in this study.

## DISCUSSION

In the present study, the correlation between FF AMH level and fertilization, and embryo quality was investigated.

**Table 1:** Comparison of the patient's characteristics in fertilized and nonfertilized groups

| | *Group I (n = 42) | **Group II (n = 20) | p-value |
|---|---|---|---|
| Age (years) | 29.60 ± 4.30 | 28.70 ± 4.2 | 0.481 |
| BMI (kg/m$^2$) | 22.20 ± 2 | 21.30 ± 1.0 | 0.085 |
| Day 3 FSH (mIU/ml) | 5.80 ± 2.10 | 5.90 ± 2.20 | 0.988 |
| Day 3 $E_2$ (pg/ml) | 39.60 ± 17.50 | 39.90 ± 22 | 0.967 |
| Infertility duration (years) | 7.90 ± 4.40 | 7.20 ± 3.90 | 0.548 |
| Duration of stimulation (days) | 11.20 ± 1.88 | 11.50 ± 2.67 | 0.678 |
| Dose of gonadotropin (no AMP) | 24.18 ± 6.80 | 27.26 ± 7.80 | 0.123 |
| $E_2$ on day hCG (pg/ml) | 1712 | 1082 | 0.058 |

*Fertilized group; **Nonfertilized group

**Table 2:** Etiology of infertility in fertilized and nonfertilized groups

| *Etiology* | *Group I (n = 42)* | **Group II (n = 20)* |
|---|---|---|
| Male factor | 44.2% | 52.6% |
| Tubal factor | 27.9% | 21.1% |
| Unexplained | 20.9% | 26.3% |
| Mixed | 7% | 0% |

p = 0.586; *fertilized group; **Nonfertilized group

According to the findings of this study, FF AMH level in fertilized group was higher than that in nonfertilized group and there was a significant difference between the two groups in this regard. Also, the FF AMH level was significantly higher in good quality embryos.

Similarly, Takahashi et al investigated FF AMH level in patients who had undergone IVF. Their study indicated that the concentration of FF AMH in fertilized group was higher than that in nonfertilized group. They concluded that FF AMH level proves to be an important indicator of fertilization.[17] Mashiach et al reported a positive relationship between FF AMH level and embryo quality in women who had undergone IVF.

Mashiach's study was performed on PCOD women while our study was done on women with normal menstrual cycles.[18] Jancar et al also showed that FF AMH level in modified natural cycles were higher than those in COH cycles. In contrast with our results, they found no significant difference between AMH level and embryo quality in modified natural cycles.[19] One reason for the differences in correlation between FF AMH level and embryo quality in different studies is probably the variety of embryo scorings in IVF centers. In two studies, FF AMH level was significantly higher in women who were conceived with IVF.[20,21] Fanchin's investigation failed to find any significant difference between FF AMH level and embryo scores. However, they showed that higher FF AMH level was associated with higher implantation rate.[20] Cupisti and Lee's studies found no correlation between the number of follicles and retrieved oocytes with FF AMH level.[22,23] We were not able to prove the existence of any such relationship because we had selected only one follicle in each patient.

## CONCLUSION

Our results indicate that there exists a positive correlation between FF AMH level and fertilization, and embryo quality. Therefore, we can make use of FF AMH level as an indicator in IVF outcome.

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