Relation of Antimüllerian, Follicular Stimulating Hormone and Antral Follicle Count on Intracytoplasmic Sperm Injection Outcome in Infertile Patients

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Abstract

Background Studying some of fertility-related hormones is of major benefit to identify the causative factors and to search for an appropriate treatment. Anti-müllerian hormone regarded as quantitative markers for ovarian reserve. Basal follicular stimulating hormone provides a picture of how well the hypothalamic-pituitary-gonadal axis is functioning and is the most commonly used tests for predicting success in intracytoplasmic sperm injection (ICSI) treatment.

Objective To evaluate the level of serum and follicular fluid antimüllerian hormone, serum follicular stimulating hormone and antral follicle count and its relation to ICSI outcome in infertile patients.

Methods Seventy four infertile women were selected randomly from those attending the Fertility Centre, Al-Sader Teaching Hospital, Al-Najaf /Iraq. Ultrasound was performed for antral follicle count and their measurement at cycle day 2. Hormonal analysis is done for serum follicular stimulating hormone at cycle day 2 and for serum and follicle fluid antimüllerian hormone at day of ovum pickup.

Result The fertilization rate was positively correlated with follicular fluid antimüllerian hormone \((r = 0.303; P = 0.048)\) but not with serum follicular stimulating hormone, serum antimüllerian hormone and antral follicle count.

Conclusion Follicular fluid antimüllerian hormone level was positively correlated with fertilization rate, while serum antimüllerian hormone level does not affect the fertilization rate in ICSI cycle. The basal follicular stimulating hormone level do not relate to fertilization rate, and the same thing regarding antral follicle count.

Keywords Anti-müllerian hormone, follicular stimulating hormone, antral follicle count, intracytoplasmic sperm injection.

List of Abbreviation: AMH = antimüllerian hormone, FSH = follicle stimulating hormone, AFC = antral follicle count, CD2 = cycle day two, FR = fertilization rate, ICSI = intracytoplasmic sperm injection, ART = Assisted reproductive technology, IVF = in vitro fertilization, OR = ovarian reserve, 3D = three dimension, 2D = two dimension.

Introduction Infertility affects approximately 13-14% of reproductive-aged couples and it might be due to ovulatory disorder in 27%, abnormal semen in 25%, tubal occlusion in 22%, endometriosis in 5% and unexplained in 21% of cases \(^{(1,2)}\).

Intracytoplasmic sperm injection (ICSI) is an assisted reproductive technology (ART) used to treat sperm-related infertility problems. Many fertility programs routinely do ICSI on some of the eggs even if everything is normal \(^{(3)}\). The use of early follicular-phase follicular stimulating hormone (FSH) as a marker of ovarian reserve (OR) and fertility outcome was proposed many years ago, where basal FSH levels were predictive of estradiol response, oocyte yield, and pregnancy rates \(^{(4)}\).
The earliest and most consistent reproductive endocrine finding associated with reproductive aging in women is a “monotropic” elevation in FSH, which reflects poor hormone production from an aging pool of ovarian follicles and disinhibition of FSH production \(^6\). Since FSH levels are co-regulated by inhibin, it has been suggested that decreased secretion of ovarian inhibin by the decreasing follicular pool, may be primarily responsible for the monotropic rise in FSH \(^6\). Currently, women with raised FSH levels (> 10 IU/ml) in the early follicular phase are counseled against in vitro fertilization (IVF) treatment due to their probable poor response to stimulation \(^6\).

Basal FSH, through the feedback of inhibin B and estradiol, will represent cohort size but mostly at the extremes and therefore give a more thorough indication of quality aspects. This is in contrast to the more direct quantitative tests using antral follicle count (AFC), antimüllerian hormone (AMH) and ovarian volume that are capable of describing a more complete range of OR states \(^7,8\).

AMH level is not affected by classical endocrine fluctuations of the menstrual cycle, and it plays a role in the regulation of ovarian function during both early and late follicle development. It can be considered a factor that reflects the depletion rate of the primordial follicle pool and affects the maintenance of the pool of growing follicles \(^9,10\). Women with higher AMH values will tend to have better response to ovarian stimulation for ICSI; more eggs retrieved and give a higher success rate \(^11\).

The objective of this study is to evaluate the level of serum and follicular fluid AMH, serum FSH and antral follicle count and its relation to ICSI outcome in infertile patients.

**Methods**

A hospital-based cohort study was conducted to determine the levels of AMH, FSH and AFC with fertilization rate in ICSI cycle. Seventy four infertile women agreed to participate in this research were selected randomly from those attending the Fertility Centre, Al-Sader Teaching Hospital, Al-Najaf Holly City during the period from March 2013 to November 2013. The study was approved by the Institute Review Board of the College of Medicine, Al-Nahrain University. The mean age for infertile female patients was 31.41±5.45 years. Infertility due to a female cause was present in 39 (52.7%) and to a male cause in 35 (47.3%) of the cases. Primary infertility was present in 57 (77%) whereas only 17 (23%) patients have secondary infertility. The duration of infertility for the entire patients group (8.11±3.94 years).

Full history and general examination, pelvic examination and transvaginal ultrasound examination at cycle day two (CD2) were performed for each infertile women for antral follicle count measurement. Those women with visible ovaries on ultrasound, no uterine fibroid, uterine anomaly or ovarian cyst measuring ≥20 mm in diameter, negative screening tests for hepatitis B and C, as well as for human immune deficiency viruses, inability to achieve pregnancy in a period of ≥ 12 months despite regular unprotected intercourse, no history of heart, liver, or kidney disease and no matter the cause of infertility was female or male factor were selected for the study.

Once the couple has been selected according to our selection criteria, they were randomized to go on with the designed program. The dependent variable for this study was the FR. The independent variables of this study were the FSH, AMH and antral follicular counts. All the participants were asked to come back on CD2 for complete medical evaluation. They were subjected to the routine steps of infertility assessment, usually performed by their own primary physician, to evaluate their fitness for the ICSI program; by the following:

**Transvaginal sonography**: performed by a specialist using ultrasound device with a vaginal probe (5-7 MHZ) aiming for antral follicle count and their measurement.

**Hormonal analysis**: Ten ml of venous blood samples were collected in plain tubes at CD 2 between 08:00-10:00 am and left at least 15 minutes at room temperature before
centrifugation at 3000 rpm for 10 minutes. Serum aliquots were obtained to measure FSH by miniVIDAS technique 2-3 hours following blood aspiration.

Another blood and follicular fluid samples were taken from the patients at the day of ovum pick up for later measurement of AMH by sensitive Enzyme Linked ImmunoSorbant Assay technique (ELISA). Under general or local anesthesia, ovum pick up through transvaginal aspiration usually timed 34-36 hours following human chorionic gonadotrophine injection and carried out via ultrasound guidance. Those patients eligible for ICSI cycle were scheduled for oocyte pick up after programmed ovulation induction.

During the ICSI procedure, the head of a single sperm is injected into the egg, eliminating the need of the sperm to penetrate the egg for fertilization. A full ICSI cycle includes a number of steps

Step 1: Ovulation stimulation and egg retrieval
Step 2: Sperm retrieval
Step 3: Fertilization
Step 4: Embryo transfer

To check for fertilization of oocytes, the fertilized oocytes must be examined 16-20 hours after insemination for the presence of two round nuclear structures, the male and female pronuclei (PN). The cells surrounding the eggs are carefully dissected away to allow clear visualization of the egg. Pronuclei must be scored within the appropriate time span, before they merge and are no longer visible. This ensures only normal zygotes with two pronuclei (2PN's) are cultured for embryo transfer.

Data Analysis

Statistical analysis was carried out using Statistical Package for Social Sciences (SPSS) version 18. Categorical variables were presented as frequencies and percentages. Continuous variables were presented as mean and standard deviation 95% confidence interval. Pearson’s correlation coefficient was used to compare between two continuous variables. A P value of ≤ 0.05 was considered as significant \(^{(12)}\).

Results

Table 1 shows that the majority of infertile patients had FSH level < 9 mIU/mL, serum AMH level > 1 ng/ml, and follicular fluid AMH level > 1 ng/ml. Similarly, the AFC was < 10 and FR was ≥ 50% in the majority of our patients (Table 1).

Table 1. Illustrates the hormonal status, antral follicular counts and fertilization rate of infertile patients

<table>
<thead>
<tr>
<th>Reproductive Parameters</th>
<th>(%)</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSH</td>
<td>&lt; 9 mIU/mL</td>
<td>97.3</td>
</tr>
<tr>
<td></td>
<td>≥ 9 mIU/mL</td>
<td>2.7</td>
</tr>
<tr>
<td>Serum AMH</td>
<td>≤ 1 ng/ml</td>
<td>20.9</td>
</tr>
<tr>
<td></td>
<td>&gt; 1 ng/ml</td>
<td>79.1</td>
</tr>
<tr>
<td>Follicular Fluid AMH</td>
<td>≤ 1 ng/ml</td>
<td>23.3</td>
</tr>
<tr>
<td></td>
<td>&gt; 1 ng/ml</td>
<td>76.7</td>
</tr>
<tr>
<td>Antral follicular count</td>
<td>≥ 10</td>
<td>39.2</td>
</tr>
<tr>
<td></td>
<td>&lt; 10</td>
<td>60.8</td>
</tr>
<tr>
<td>Fertilization rate</td>
<td>≥ 50%</td>
<td>64.9</td>
</tr>
<tr>
<td></td>
<td>&lt; 50%</td>
<td>35.1</td>
</tr>
</tbody>
</table>

FSH = follicular stimulating hormone, AMH = antimüllerian hormone, Level of FSH and AMH represent the cutoff levels of those hormones according to the kit used. Antral follicle count:10 represent median number of all follicle counted. Fertilization rate:50% represent median number of fertilization rate.
The fertilization rate was positively correlated with follicular fluid AMH hormone ($r = 0.303; P = 0.048$) (Fig. 1) but not with serum FSH, serum AMH and AFC (Table 2).

**Table 2. Correlation of fertilization rate with antral follicle count, serum FSH, serum AMH and follicular fluid AMH hormone levels**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean± S.D</th>
<th>$r$</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFC</td>
<td>11.24 ± 5.13</td>
<td>0.063</td>
<td>0.596</td>
</tr>
<tr>
<td>FSH (mIU/ml)</td>
<td>4.40 ± 2.23</td>
<td>-0.032</td>
<td>0.788</td>
</tr>
<tr>
<td>Serum AMH (ng/ml)</td>
<td>1.88 ± 1.17</td>
<td>0.103</td>
<td>0.512</td>
</tr>
<tr>
<td>FF AMH (ng/ml)</td>
<td>2.06 ± 1.51</td>
<td>0.303</td>
<td>0.048*</td>
</tr>
</tbody>
</table>

FSH = Follicular Stimulating hormone, AMH = antimüllerian hormone, FF = follicular fluid, AFC = antral follicle count.

**Discussion**

The AMH level in serum and follicular fluid of the infertile women lies within the normal standard levels and were comparable to values noticed by Lee et al and Freour et al who demonstrated follicular fluid AMH level to be approximately 1 ng/ml ($^{13,14}$). Low ICSI outcome and poor response reported to be associated with serum AMH levels < 1 ng/mL, normal response with levels from 1-4 ng/mL, and high response with levels > 4 ng/mL ($^{15-17}$).

In the present study, follicular fluid AMH hormone level was positively correlated with FR. This influence could be related to that AMH is produced in females by the granulosa cells from the pre-antral and antral follicles in the human fetus after 36 weeks of gestation ($^{18}$). The levels of AMH reflect the number of preantral follicles and thus are a marker of oocyte pool - a germlinal reserve of the ovary for reproduction.

AMH also has a direct autocrine-paracrine effect on the granulosa cells, oocyte function and embryo quality. It seems to be a promising parameter for early detection of reduced OR as well as ovarian dysfunction, thus AMH, which indicate ovarian aging and OR can become a critical factor in infertility ($^{19}$). Several studies have demonstrated that AMH is a better marker of OR than age, basal FSH, estradiol and inhibin ($^{20}$).

Furthermore, basal serum AMH levels <1.1 ng/ml were associated with IVF failure and this finding support that AMH levels are believed to be a reflection of the number of growing follicles, which is also related to the number of small antral follicles ($^{21}$).

Likewise, AMH levels have also been shown to be 10-fold lower in the cancelled cycles compared with patients who had a complete IVF cycle. In ∼75% of cancelled cycles, AMH levels were below the detection limit (0.098 ng/ml) ($^{22}$). Besides women with higher AMH values tend to have better response to ovarian stimulation for IVF/ICSI, have more eggs retrieved, gives a higher fertilization and pregnancy rate ($^{14}$).

The data of the present study strongly support the previously published reports dealing with the prognostic value of the AMH on its relation to fertility and ICSI outcome.
The basal FSH level in the current study was comparable to that reported by Vladimirov et al and Göksedef et al. Our study showed that the basal FSH do not relate to FR. FSH levels vary during the menstrual cycle and will peak prior to ovulation. FSH blood tests are generally performed on the second or third day of menstrual cycle. Basal FSH levels provides a picture of how well the hypothalamic-pituitary-gonadal axis is functioning, as it measures pituitary production of FSH in response to feedback from ovarian hormones and it is the most commonly used tests for predicting success in ICSI treatment.

A normal FSH level probably indicates a good OR. However, elevated FSH levels may suggest impaired OR.

The result of the present study was in accordance with the findings of Smotrich et al and Evers et al who declared that normal FSH, have been associated with improved stimulation response, higher correlation with fertilization and hence pregnancy rates and lower cycle cancellation rates.

The AFC in the present study does not correlate with FR, which disagrees with the finding of Muttukrishna et al that demonstrate clear association between AFC and the number of eggs collected and the likelihood of good fertilization and clinical pregnancy. This discrepancy might be due to the sample size being smaller in the current study, or to the stimulation protocol used in the Fertility Center; since the antagonist analogue have not been introduced yet, rather short or long protocol was used. Alternatively this difference could be related to the limited types of gonadotropins accessible in our country.

In conclusion, Follicular fluid AMH hormone level was positively correlated with FR while serum AMH, basal FSH and antral follicle count do not relate to FR.

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Author Contribution
The authors share the responsibility in preparing and completing this work.

Conflict of interest
The authors declare no conflict of interest.

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