The role of FSH to AMH ratio in poor prognosis patients undergoing ICSI cycle

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Abstract

Objective: The objective of this study was to estimate the number of oocyte retrieval and cycle cancellation using follicle stimulating hormone (FSH) to anti-Mullerian hormone (AMH) ratio in poor prognosis patients undergoing intracytoplasmic sperm injection treatment.

Material and Methods: This retrospective study including fresh cycles was conducted in Zekai Tahir Burak Women’s Health Training and Research Hospital, between January 2015 and October 2018. Women aged between 24 and 44 years were recruited and the baseline serum hormone levels, FSH/AMH ratio, and the antral follicle count were recorded. Number of retrieved oocytes, metaphase-II oocytes, fertilised oocytes, and the number and grade of the embryos were also recorded.

Results: A total of 108 cycles, corresponding to 92 women with poor prognosis were eligible for analysis. The use of FSH/AMH ratio performed well in predicting retrieved oocyte count <5 with an area under the curve (AUC) of 0.82 [95% confidence interval (CI): 0.71-0.92]. A FSH/AMH ratio cut-off of 11.36 was set for the retrieval of <5 oocyte at oocyte pick-up (OPU) with 80% sensitivity and 87% specificity. The FSH/AMH cut-off value was 14.22 to differentiate cycle cancellation and no oocyte retrieval at OPU, with a sensitivity of 91% and a specificity of 44% (AUC of 0.71; 95% CI: 0.59-0.83). There was no correlation between FSH/AMH ratio and clinical pregnancy.

Conclusion: The assessment of this simple ratio at the beginning of the cycle may help clinicians better anticipate gonadotropin stimulation treatment and better counsel patients about cycle cancellation and the expected oocyte yield. (J Turk Ger Gynecol Assoc 2022; 23: 184-9)

Keywords: FSH to AMH ratio, cycle cancellation, ICSI, poor responder, oocyte retrieval

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Introduction

The management of patients with poor ovarian response (POR) to exogenous gonadotropin stimulation is a challenging problem in in-vitro-fertilization (IVF) cycles. Since POR may be relevant to the decreased number of retrieved oocytes, together with extremely low pregnancy rates, and some patients cannot achieve oocyte pick-up (OPU) due to a cancelled cycle (1). Therefore, the prediction of ovarian response before treatment is fundamental for counselling patients including the management of expectations, especially about their chances of success. The incidence of poor response to ovarian stimulation is estimated to be 9-24%. Several tests have been postulated in an attempt to best assess POR in low prognosis patients (2,3). Currently, the markers most often used by physicians are the age, early follicular phase follicle stimulating hormone (FSH), estradiol (E2), antral follicle count (AFC), and anti-Mullerian hormone (AMH) levels (4). Among these markers, FSH provides indirect assessment of ovarian reserve through suppression of hypophyseal production of FSH by ovarian E2. The elevation
of FSH at an early phase of the menstrual cycle indicates a decrease in secretion of ovarian hormones due to a failure in the ovarian follicular cohort (5). Although the specificity of basal FSH level >10 IU/L (10-20) is high (45-100%) when POR to ovarian stimulation is predicted, its sensitivity is low (11-86%) (6). Additionally, the intercycle and intracycle variability of basal FSH reduce its reliability (7).

Another predictive marker, AMH, is a glycoprotein that is a member of the transforming growth factor beta superfamily. AMH is secreted from the granulosa cells of preantral and antral follicles. AMH and AFC are currently used as the most reliable biomarkers for the estimation of ovarian reserve (8,9). AMH is reported to be as valid as AFC, but has primacy due to less interobserver variability (10). Many authors have reported that AMH concentrations simply reflect the total developing follicular cohort and POR to stimulation in ART cycles (11-13). Low AMH levels indicate a decrease in the number of selectable follicles and are correlated with decreased yield of oocytes, cycle cancellation, and low chances of achieving pregnancy in ART cycles (14,15). Therefore, FSH and AMH are, respectively, in positive and negative correlation with POR. There are already many studies showing the relationship between the use of variable derived markers, such as LH/FSH ratio, glucose-insulin ratio, and neutrophil-to-lymphocyte ratio (16-18). We hypothesised that the predictive effect of FSH and AMH can be used in the same way as a ratio. The aim of this retrospective study was to estimate the number of retrieved oocyte and cycle cancellations with FSH/AMH ratio in poor prognosis patients.

Material and Methods

This retrospective, monocentric study was conducted in Ankara Zekai Tahir Burak Women's Health Training and Research Hospital, between January 2015 and October 2018. The study protocol was approved by the Ankara Zekai Tahir Burak Women's Health Training and Research Hospital Institutional Ethics Committee (approval number: 9, date: 31.10.2018). All subjects gave informed consent for the utilization of their clinical data and were included as “low prognosis patients” in assisted reproductive technology according to the POSEIDON stratification (19). Only fresh IVF-intracytoplasmic sperm injection (IVF-ICSI) cycles were included. Patients who underwent frozen-thawed embryo transfer and with an element of oligo-azoospermia were excluded.

Women between 24 and 44 years were recruited, and baseline demographics and fertility characteristics were obtained from archive file records. Basal serum E₂, FSH levels, AFC and AMH levels were determined and FSH/AMH ratio was calculated. The serum levels of E₂ and FSH were measured with an electrochemiluminescence immunoassay (Roche, E170. ELECSYS, Mannheim, Germany) on Elecsys and Cobas immunoassay analysers. AMH values were determined with AMH Gen II enzyme-linked immunosorbent assay (Beckman Coulter, Brea, USA). The number of retrieved oocytes, metaphase II oocytes, fertilised oocytes, and number and grade of the embryos were also recorded. Controlled ovarian hyperstimulation was performed by either a gonadotrophin-releasing hormon (GnRH)-antagonist or microdose GnRH-agonist protocol. In the antagonist protocol, a daily GnRH antagonist dose of 0.25 mg was started based on a flexible protocol once a follicle reached ≥14 mm in diameter and continued up to the trigger day. Patients in the flare-up protocol were started on 50 µg of leuprolide acetate (Lucrin; Abbott, Turkey) subcutaneously twice daily on cycle day 1 and 2, and high dose gonadotropin was started on cycle day 3.

Human menopausal gonadotropin was used for controlled ovarian stimulation (Menagon; Ferring, Istanbul, Turkey) in different doses. Patients were monitored with serum E₂ and progesterone levels, and serial transvaginal ultrasonographic examinations. Ovulation was triggered with 250 mg recombinant-choriogonadotropin alpha (Ovitrelle; Merck-Serono, Istanbul, Turkey) when the leading follicle reached 18 mm in diameter or there were at least three follicles ≥17 mm in diameter. Oocyte retrieval was performed 36 hours later. Cycles were cancelled when the follicles persisted at <10 mm after 14 days of stimulation. OPU was performed even with the existence of a single dominant follicle. Luteal phase support was maintained by vaginal progesterone gel (Crinone 8% gel, Serono, Istanbul, Turkey). All eligible oocytes were fertilized by ICSI and embryos were cultured individually according to standard procedures. No more than two embryos were transferred. A serum pregnancy test was performed 14 days after embryo transfer. Clinical pregnancy was confirmed 10-14 days later by the presence of a gestational sac on transvaginal ultrasound scan. Patients were designated as clinically pregnant, non-pregnant, cycle cancellation, no oocyte retrieved at OPU, and fertilization failure.

Statistical analysis

Statistical analysis was performed using Statistical Package for Social Sciences (SPSS), version 21 (SPSS Inc. Chicago, IL, USA) and the distribution of the groups was analyzed with one sample Kolmogorov-Smirnov test. Continuous variables were not normally distributed and expressed as median and range (minimum-maximum). Spearman rank R test was used for correlation analyses. All p-values were two-sided, and 5% was chosen to denote significance (p<0.05). Receiver operating characteristic (ROC) curves were generated for FSH/AMH ratio to predict outcomes. All the data were evaluated within 95% confidence interval (CI) in both directions. Non-parametric
Mann-Whitney U test was used for testing differences between groups that were based on FSH/AMH ratio.

**Results**

A total of 108 fresh IVF-ICSI cycles, corresponding to 92 women with poor prognosis were eligible for analysis. According to the Poseidon criteria categories, 8 (8.7%) were type 1, 8 (8.7%) were type 2, 40 (43.5%) were type 3 and 36 (39.1%) were type 4. Median (range) age and BMI were 35 (24-44) years and 24 (18-35) kg/m², respectively. Patient characteristics involving FSH/AMH ratio are presented in Table 1. Eighty-three patients with GnRH antagonist protocol and 25 patients with flare-up protocol were identified.

Embryo transfer was successfully carried out in 65 cycles and 18 clinical pregnancies were achieved. The pregnancy rate was 16.7% per initiated cycle and 27.7% per transfer cycle. Among patients whose cycles has no embryo transfer, there were eight patients with cancelled cycle, 20 patients with fertilization failure and 15 patients with no oocyte retrieved at OPU. Correlation analysis between FSH/AMH ratio and other parameters are presented in Table 2.

As a result, FSH/AMH ratio was moderately negatively correlated with the number of oocytes retrieved (p<0.0001, r=-0.4) and weakly positively correlated with cycle cancellation or no retrieval of oocyte at OPU (p=0.002, r=0.3) (Figure 1). The use of this ratio performed well with an AUC of 0.82 (95% CI: 0.71-0.92). A cut-off value of 11.36 was set for the retrieval of <5 oocytes at OPU with 80% sensitivity and 87% specificity. In addition, ROC curves were drawn separately for AMH, bFSH, and age to evaluate the prediction of oocyte yield less then 5. The AUC was below 0.5 for age and bFSH, whereas the AUC value for AMH was 0.80. A cut-off value of 1.2 AMH was predicted for the retrieval of less than 5 oocytes at OPU with 88% sensitivity and 40% specificity (Figure 2). The optimal FSH/AMH cut-off value was 14.22 to predict the cycle cancellation or no retrieval of oocyte at OPU, with a sensitivity of 91% and a specificity of 44% (AUC of 0.71; 95% CI: 0.59-0.83) (Figure 3). There was no-correlation between FSH/AMH ratio and clinical pregnancy.

**Discussion**

This is the first report to describe the prediction of POR to gonadotropin stimulation with the use of FSH/AMH ratio. We found that FSH/AMH ratio at a certain cut-off value of 11.36 may provide guidance for the estimation of the number of oocytes retrieved <5 with acceptable sensitivity and specificity (AUC 0.82 with 95% CI: 0.71-0.92, sensitivity 80% and specificity 87%). Although AMH alone had a predictive value with similar sensitivity for oocyte yield, it was not as specific as the FSH/AMH ratio. Furthermore, this study emphasized the significant role of this ratio at higher cut-off value to anticipate cancelled cycles and pointless OPU. A cut-off value of FSH/AMH ratio of >14.22 has been shown to be predictive of the cycle cancellation or no retrieval of oocyte at OPU (AUC 0.71, sensitivity 91%, specificity 44%). However, this ratio had low specificity and therefore clinical use may not be as valuable as the former pregnancy ratio.

POR was determined with reduced pregnancy rate during appropriate gonadotropin treatment (20). Advisable prediction of poor response could have clinical value because if the pregnancy chance is inconclusive, patients may want to avoid treatment. FSH, AFC, and AMH have all been used as markers for this purpose. Firstly, AMH inhibits primordial follicle recruitment and restrains follicle growth under the influence of FSH. Plasma AMH concentrations have been positively correlated with the size of the primordial follicle pool and AFC (4,21). Outstanding

**Table 1. Clinical and laboratory findings of all patients**

| Parameters                      | Median (mean ± SD) |
|---------------------------------|--------------------|
| Age (year)                      | 35 (33.97±4.5)     |
| BMI (kg/m²)                     | 24 (25.23±4.4)     |
| AMH (ng/mL)                     | 0.59 (0.66±0.5)    |
| Basal FSH (IU/L)                | 10 (10.47±4)       |
| Basal E₂ (pg/mL)                | 36 (43.92±26.2)    |
| FSH/AMH ratio                   | 17.75 (55.02±136.62)|
| Antral follicle counts          | 5 (5.4±2.5)        |
| Infertility duration (year)     | 4 (4.9±4)          |
| Initial gonadotropine dose (IU) | 300 (290.97±36.57) |
| Total gonadotropine dose (IU)   | 2700 (2707.59±804.73)|
| Peak E₂ (pg/mL)                 | 815.5 (917.26±713.48)|
| Total stimulation day           | 9 (9.3±2.1)        |
| Endometrial thickness (mm)      | 9 (8.8±2.6)        |
| Patients with embryo transferred (n, %) | 65 (60.2%) |
| Pregnant                        | 18 (16.7%)         |
| Non-pregnant                    | 47 (43.5%)         |
| Patients without embryo transferred (n, %) | 43 (39.8%) |
| Fertilisation failure           | 20 (18.5%)         |
| Cancelled cycle                 | 8 (7.4%)           |
| No oocyte retrieval at OPU*     | 15 (13.9%)         |
| No. of total oocytes            | 3 (3.4±2.8)        |
| No. of metaphase II oocytes     | 3 (3.2±2.4)        |
| No. of fertilized oocytes       | 2 (2.4±1.7)        |
| No. of embryo                   | 2 (2.2±1.6)        |
| The day of transferred embryo   | 3 (3±0.8)          |
| Embryo quality (grade)          | 2 (1.8±0.6)        |

*OPU: Oocyte pick up, SD: Standard deviation, FSH: Follicle stimulating hormone, E₂: Estradiol, No.: Number, AMH: Anti-Mullerian hormone, BMI: Body mass index
**Table 2. Correlation analysis between FSH/AMH ratio and other parameters based on ICSI cycles**

| Parameters                                      | Correlation coefficient | P       |
|-------------------------------------------------|-------------------------|---------|
| Antral follicle count                           | -0.4                    | 0.001   |
| AMH (ng/mL)                                     | -0.93                   | 0.001   |
| Basal FSH (IU/L)                                | 0.52                    | 0.001   |
| Basal E2 (pg/mL)                                | -0.36                   | 0.001   |
| Peak E2 (pg/mL)                                 | -0.19                   | 0.04    |
| Total stimulation day                           | 0.005                   | 0.9     |
| Initial gonadotropine dose (IU)                 | 0.33                    | 0.001   |
| Total gonadotropine dose (IU)                   | 0.15                    | 0.12    |
| Endometrial thickness (mm)                      | -0.15                   | 0.13    |
| Clinical pregnancy                              | -0.06                   | 0.5     |
| No. of total oocytes                            | -0.4                    | 0.001   |
| No. of metaphase II oocytes                     | -0.28                   | 0.01    |
| No. of fertilized oocytes                       | -0.21                   | 0.09    |
| No. of embryo                                   | -0.23                   | 0.06    |
| No. of transferred embryo                       | 0.1                     | 0.4     |
| The day of transferred embryo                   | -0.1                    | 0.4     |
| Embryo quality                                  | 0.04                    | 0.7     |
| Cancelled cycle or no oocyte retrieval at OPU   | 0.3                     | 0.002   |

*OPU: Oocyte pick-up, FSH: Follicle stimulating hormone, AMH: Anti-Mullerian hormone, ICSI: Intracytoplasmic sperm injection, E2: Estradiol, No.: Number

**Figure 1. ROC curve for prediction of retrieved oocyte in all patients.** ROC curve for FSH/AMH ratio (area below the curve 0.82; 95% confidence interval, 0.71-0.92) cut-off point, 11.36; sensitivity, 80%; specificity, 87%  
**ROC:** Receiver operating characteristic, **FSH:** Follicle stimulating hormone, **AMH:** Anti-Mullerian hormone

**Figure 2. ROC curves of AMH, bFSH and age for prediction of retrieved oocyte in all patients.** ROC curve for AMH (area below the curve 0.80; 95% confidence interval, 0.68-0.92) cut-off point, 1.2; sensitivity, 88%; specificity, 40%  
**ROC:** Receiver operating characteristic, **FSH:** Follicle stimulating hormone, **AMH:** Anti-Mullerian hormone

**Figure 3. ROC curve for prediction of cancelled cycle and absence of oocyte after OPU.** ROC curve for FSH/AMH ratio (area below the curve 0.71; 95% confidence interval, 0.59-0.83) cut-off point, 14.22; sensitivity, 91%; specificity, 44%  
**ROC:** Receiver operating characteristic, **OPU:** Oocyte pick up, **FSH:** Follicle stimulating hormone, **AMH:** Anti-Mullerian hormone
correlation between AMH concentrations and the number of retrieved oocytes has been documented in previous studies (11,22). In one review including patients undergoing controlled ovarian stimulation, low AMH cut-off values (0.1-1.66 ng/mL) have been reported to have 44-97% sensitivity and 41-100% specificity to predict POR (23). In the present study, AMH at 1.2 cut-off value was predictive of oocyte yield with high sensitivity but low specificity. In a meta-analysis consisting of 28 studies, AMH was demonstrated as a decent predictor for POR, with an AUC of 0.78 (10). This dependence was substantially stronger than the associations reported with other ovarian reserve tests, including serum FSH and E$_2$ (24). However, AMH levels show interassay and intra-assay variability (9). In contrast, a more precise prediction with basal FSH levels rather than AFC has been reported in some patients (25). Secondly, FSH has been demonstrated to have a high specificity for prediction of POR but a low sensitivity. In our study, bFSH and age alone were not found as a predictive marker for oocyte yield. Lastly, AFC, measured by transvaginal ultrasonography on the first days of menstrual cycle, quickly estimates and provides results for prediction of POR (26). However, AFC has limitation due to high interobserver and intracycle variability (21,27). Additionally, AFC can cause misjudgement of FSH-sensitive follicle count and oocytes retrieved because of atretic follicles with similar size (28,29). Therefore, each of these well-known methods has some advantages and disadvantages. We hypothesized that the logical combination of the first two tests in one parameter may provide a new assessment method in POR patients and this is supported by our findings.

There was a negative correlation between AFC and FSH/AMH ratio in the present study. This outcome favored the aforementioned findings and the assessment of ovarian reserve condition in POR with this new ratio. A negative correlation between basal E$_2$ and FSH/AMH ratio was also found. However, the explanation of negative E$_2$ relevance can be troublesome because real E$_2$ levels may show reciprocal interference with FSH. High FSH levels can be easily masked by high E$_2$ levels. On the other hand, peak E$_2$ was negatively correlated with FSH/AMH ratio that favoured our other findings.

When the comparison was done based on the number of retrieved oocytes, there were no difference regarding the number of transferred embryos, the day of transferred embryo, and the total motile sperm count. So, this similarity in two groups favored our findings that were not affected by these variables. However, the day of transferred embryos was significantly higher in patients with FSH/AMH <11.36. This may indicate a possible relationship between this ratio and embryo quality which was not found when this correlation was tested statistically (p=0.7) Majumder et al. (22) demonstrated that serum AMH and AFC were significantly associated with the number of high-quality embryos and the number of embryos frozen. Some authors also found an association between AMH and the number of embryos (11,30), yet some did not (31,32). Unfortunately, neither AMH nor AFC independently predict pregnancy rates (33). Similarly, there was no correlation between FSH/AMH ratio and clinical pregnancy in our study. Due to the inclusion of fresh embryo transfer, a possible negative effect of gonadotropin on endometrial receptivity cannot be excluded. This could have prevented the reflection of our findings on clinical pregnancy.

**Study Limitations**

The retrospective design and small sample size were major limitations of our study. Furthermore, heterogeneous gonadotropin treatment protocols used, including flare-up and antagonist protocols, was also a limitation. Another limitation was that the clinical situation for frozen transfer patients was not known, since mostly fresh transfers are made in our clinic. To our knowledge this is the first study to suggest the utility of FSH/AMH ratio in IVF cycles. In addition, performing the study in a highly specific study group, that of poor responders who had been freshly transferred, was another strength of our study.

**Conclusion**

The FSH/AMH ratio can easily be calculated without bringing extra cost, since FSH and AMH are already evaluated in almost every infertile case. Assessment of this simple ratio at the beginning of the cycle may help clinicians better anticipate the gonadotropin-stimulation treatment and better counsel patients about cycle cancellation and expectations for the number of retrieved oocytes.

**Ethical Committee Approval:** The study protocol was approved by the Ankara Zekai Tahir Burak Women's Health Training and Research Hospital Institutional Ethics Committee (approval number: 9, date: 31.10.2018).

**Informed Consent:** All subjects gave informed consent for the utilization of their clinical data.

**Peer-review:** Externally peer-reviewed.

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