Original Investigations

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

FSH to AMH ratio

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Abstract
Objective: The objective of this study was to estimate the number of oocyte retrieval and cycle cancellation via FSH/AMH ratio in low prognosis patients undergoing ICSI treatment.
Material and Methods: This retrospective study including fresh cycles was conducted in 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 low prognosis were eligible for analysis. The use of FSH/AMH ratio performed well in predicting retrieved oocyte lesser than 5 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 less than 5 oocyte at OPU with 80% sensitivity and 87% specificity. The FSH/AMH cut-off value was 14.22 to differentiate the cycle cancellation and no oocyte retrieval at OPU, providing a sensitivity of 91%; 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 the gonadotropin-stimulation treatment and better counsel patients about the cycle cancellation and the expected oocyte.
Keywords: FSH to AMH ratio, cycle cancellation, icsi, poor responder, oocyte retrieval

Introduction

The management of patients with poor ovarian response (POR) to exogenous gonadotropin stimulation is a challenging problem in vitro fertilization (IVF) cycles. Since POR may be relevant to the decreased number of retrieved oocyte together with extremely low pregnancy rates and some patients can not reach up to 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 on their chances of success. The incidence of poor response to ovarian stimulation is approximated to be 9- 24%. Several tests have been postulated in an attempt to best assess POR in low prognosis patients (2, 3). Nowadays, 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-Müllerian Hormone (AMH) (4). Among these markers FSH provide indirect assessment of ovarian reserve concerning the suppression of it's hypophyseal production 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 the member of transforming growth factor beta superfamily and 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 marked as valid as antral follicle count (AFC), but has primacy due to less interobserver variability (10). Many authors reported that AMH concentrations just reflect the total developing follicular cohort and POR to stimulation in ART cycles(11-13). Low AMH levels indicate a decrease in 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 ratios and diseases such as LH/FSH ratio, glucose-insuline ratio, and neutrophil-to-lymphocyte ratio (16-18). We hypothesised that the predictive effect of FSH and AMH can be used in the same variable as a ratio. The aim of this retrospective study is to estimate the number of retrieved oocyte and cycle cancellations with FSH/AMH ratio in low prognosis patients.

Material and Methods

This retrospective monocentric study was conducted in Hospital, between January 2015 and October 2018. The study's protocol was approved by the institution's ethics committee. All subjects have been given an informed consent form for the utilization of their clinical data and were included as 'low prognosis patients' in assisted reproductive technology according to the POSEIDON's stratification(19). Only fresh IVF-ICSI cycles were included. Patients who underwent frozen-thawed embryo transfer and oligo- azospermia 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 electrochemiluminescence immunoassay (Roche, E170. ELECSYS, Mannheim, Germany) on Elecsys and cobas immunoassay analysers. AMH values were determined with AMH Gen II enzymelinked immunosorbent assay (Beckman Coulter, Brea, USA). Number of retrieved oocytes, metaphase II (MII) oocytes, fertilised oocytes, and number and grade of the embryos were also recorded. Controlled ovarian hyperstimulation was performed by either a GnRH-antagonist or microdose GnRH-agonist protocol. In antagonist protocol 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 SC of leuprolide acetate (Lucrin; Abbott, Turkey) 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, İstanbul, Türkiye) in different doses. Patients were monitored with serum E2, 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 h later. Cycles were cancelled when the follicles persisted in <10 mm after 14 days of stimulation. Oocyte pick-up was performed even in the existence of single dominant follicle. Luteal phase support was maintained by vaginal progesterone gel (Crinone 8 % gel, Serono, Istanbul, Turkey). All eligible oocytes were fertilized by intracytoplasmic sperm injection (ICSI) and embryos were cultured individually according to standard procedures. No more than 2 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 gestational sac in trasvaginal ultrasound scan. Patiens were named as clinical 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 (SPPS) 21 software (SPSS Inc. IL, Chicago, 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, (mean± standart deviation). 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 in both directions. Nonparametric 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 low prognosis were eligible for analysis. According to Poseidon criteria, 8 (8.7%) patients were in type 1, 8(8.7%) patients were in type 2, 40 (43.5%) patients were in type 3 and 36 (39.1%) patients were in type 4 category. Median age and BMI were 35 (min 24- max 44) years and 24 (min18-max35) kg/m^2 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 involved.

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 8 patients with cancelled cycle, 20 patients with fertilization failure and 15 patients with no ocyte 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 correlated with the number of oocytes retrieved ($P < 0.0001$, $r = -0.4$) and weakly 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% confidence interval 0.71–0.92). A cut-off value of 11.36 was set for the retrieval of less than 5 oocyte 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 oocyte 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, providing sensitivity of 91%; specificity of 44% (AUC of 0.71; 95% confidence interval, 0.59-0.83) (Figure 3). There was no-correlation between FSH/AMH ratio and clinical pregnancy ($p > 0.05$).

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 in certain cut-off value may provide guidance for the estimation of the number of oocytes retrieved. The AUC for FSH/AMH ratio of 11.36 in predicting retrieval of less then 5 oocytes was 0.82 (95% confidence interval 0.71–0.92, sensitivity 80% and specificity 87%.). Although AMH alone had predictive value with similar sensitivity in oocyte yield, it was not as specific as the FSH /AMH ratio. Further, 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>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 has low spesificity and therefore clinical use of it may not be valuable as the former ratio.

Poor ovarian response has been determined with reduced pregnacy 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 be dissent from treatment. FSH, AFC, and AMH have all been currently used as markers for this purpose. Firstly, AMH prevents primordial follicle recruitment and restrains follicle growing 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 correlation between AMH concentrations and the number of retrieved oocytes has been documented in the previous studies(11, 22). In one review including patients undergoing controlled ovarian stimulation with 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 predicitive of oocyte yield with high sensitivity but low spesificity. 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 dependance was substantially stronger than the associations reported with other ovarian reserve tests like serum FSH and estradiol (24). However, AMH levels show interassay and intraassay variability (9). On the other hand, a more precise prediction with basal FSH levels 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, by the way of transvaginal ultrasonography on the first days of menstrual cycle, quickly estimates and provides results for prediction of POR (26). But it 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). Anyway, each of these well-known methods has some advantages and disadvantages. Logical combination of the first two tests in one parameter seemed to provide a new assesment method in POR patients according to our findings.

There was also a negative correlation between AFC and FSH/AMH ratio in the present study ($r=-0.4$ $p=0.001$). This outcome favored the forementioned findings and the assessment of ovarian reserve condition in POR with this new ratio. A negative correlation between basal E2 and FSH/AMH ratio was also shown. However, the explanation of negative E2 relevance can be troublesome because real E2 levels may show reciprocal interference with FSH. High

FSH levels can be easily masked by high E2 levels. On the other side the peak E2 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 to 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 from 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. But there was no any correlation between this ratio and embryo quality (p 0.7) Majumder et al. demonstrated that serum AMH and AFC were significantly associated with the number of high-quality embryos and the number of embryos frozen (22). Some authors found also an association between AMH and the number of embryos (11, 30), yet some did not (31, 32).
Unfortunately, both AMH and AFC independently do not predict pregnancy rates(33). Similarly, there was no correlation between FSH/AMH ratio and clinical pregnancy in our study. Becuase of fresh embriyo transfer, possible negative effect of gonadotropine on endometrial receptivity may not 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. Nonhomogenous state regarding to gonadotropin treatment protocols including flare-up and antagonist ones was also a limitation of our study. Another limitation is that the clinical situation in frozen transfer patients is not known, since mostly fresh transfers are made in our clinic.
To our knowledge this is the first study to bring the idea of FSH/AMH ratio useage in IVF cycle. Besides, performing the study in a highly specific study group, poor responders who are freshly transferred, is another strength of our study.

Conclusion

FSH/AMH ratio can easily be calculated without bringing extra cost, since FSH and AMH are already evaluated in almost every infertile cases. 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 the cycle cancellation and the expectation of the number of retrieved oocytes.

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Table 1. Clinical and laboratory findings of all patients	
Parameters	**Median (Mean ± SD)**
Age (year)	35 (33.97± 4.5)
BMI (kg/m^2)	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 8ransferred (n, %)	65 (60.2%)
Pregnant	18 (16.7%)
Non-pregnant	47 (43.5%)
Patients without Embryo 8ransferred (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	

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
*Oocyte pick-up		

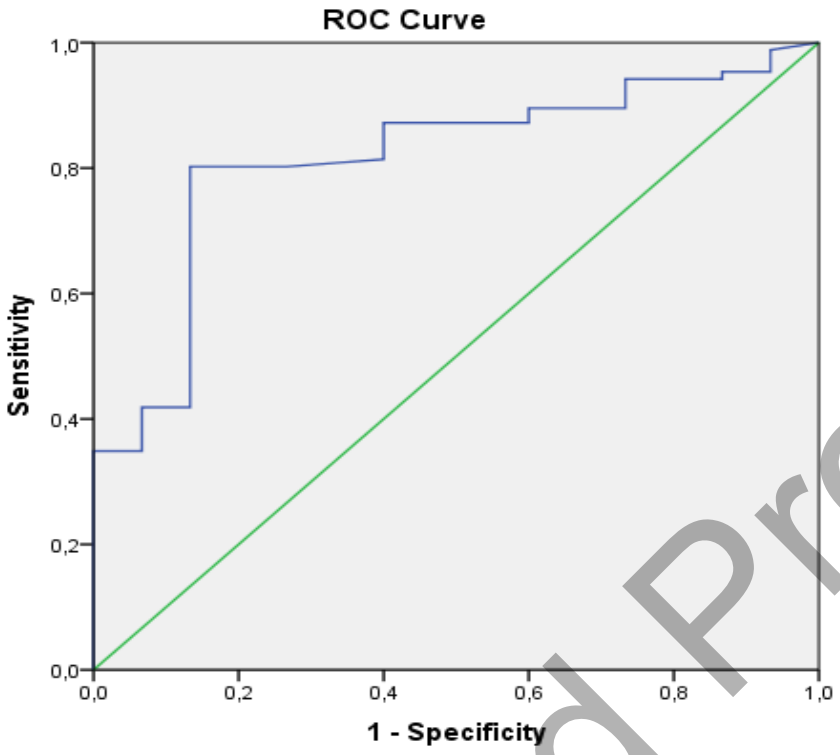

Figure 1. Receiver operating characteristic (ROC) curve for prediction of retrieved oocyte in all patients. ROC curve for FSH/AMH ratio (Area below the curve 0.82; 95% confidence interval, 0.71-0.92) cut-off point, 11.36; sensitivity, 80%; specificity, 87%

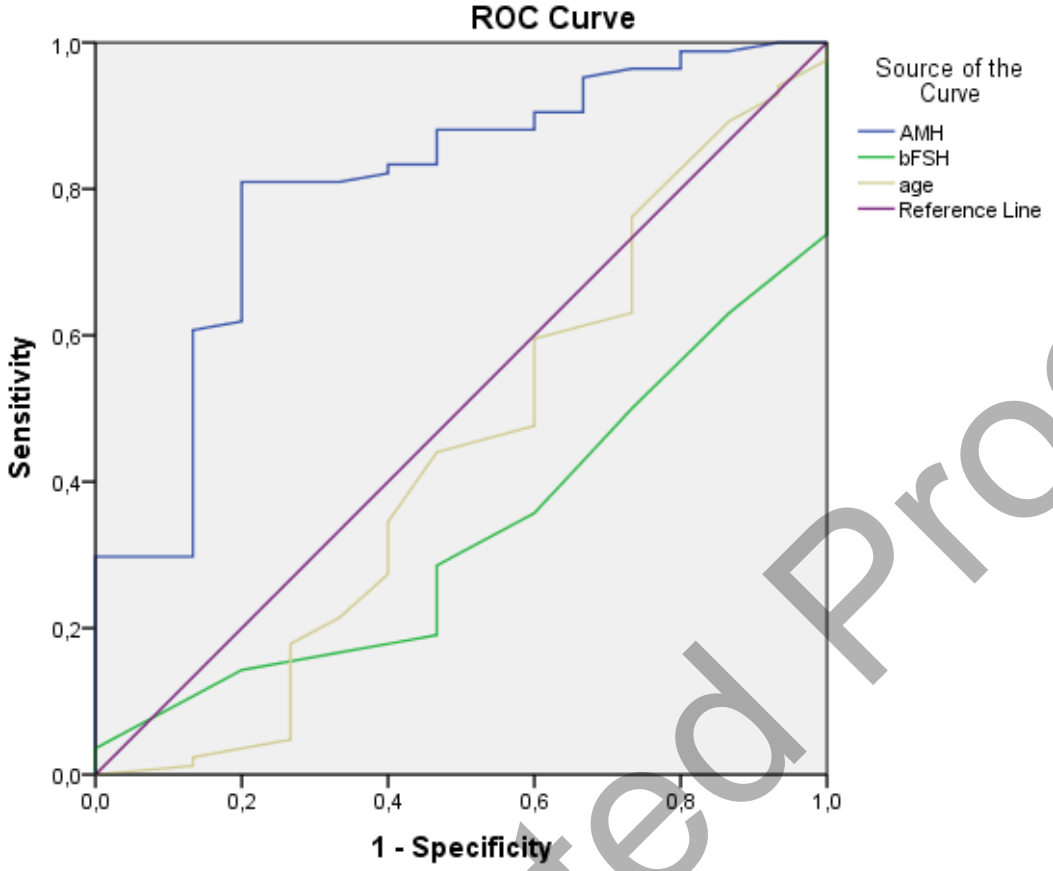

Figure 2. Receiver operating characteristic (ROC) curves of AMH, bFSH and age for prediction of retrieved oocyte in all patients. ROC curve for AMH (Area below the curve 0.80; 95% confidence interval, 0.68-0.92) cut-off point, 1.2; sensitivity, 88%; specificity, 40%

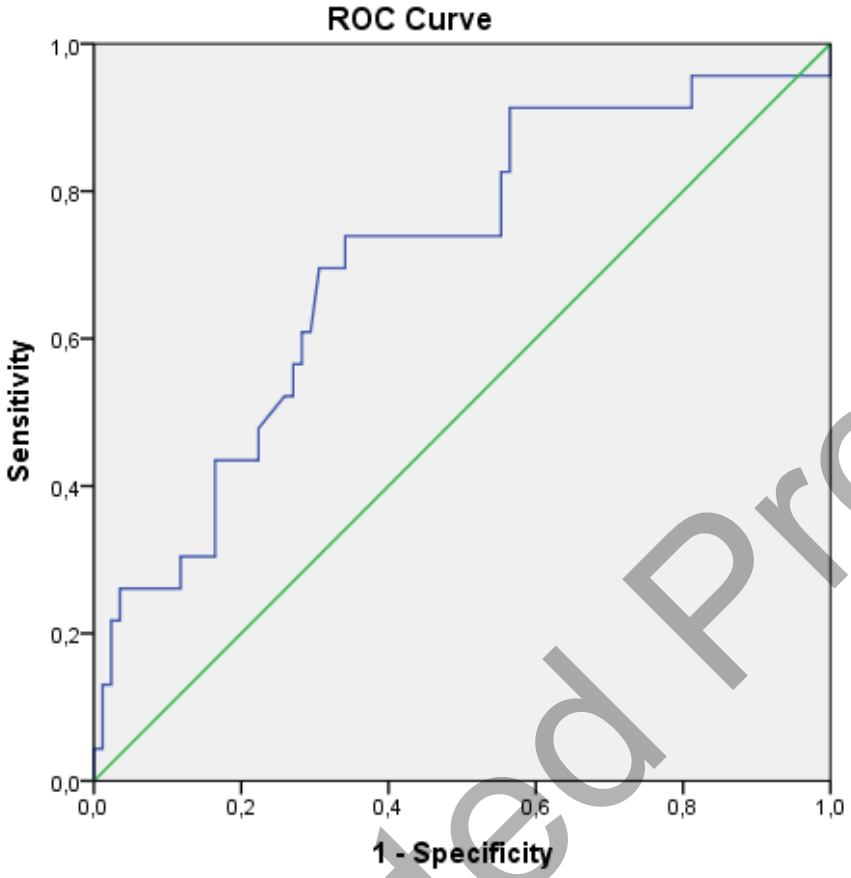

Figure 3. Receiver operating characteristic (ROC) curve for prediction of cancelled cycle and absence of oocyte after OPU. ROC curve for FSH/AMH ratio (Area below the curve 0.71; 95% confidence interval, 0.59-0.83) cut-off point, 14.22; sensitivity, 91%; specificity, 44%