ROLE OF THE ANTI MULLERIAN HORMONE AND THE FOLLICULAR STIMULATING HORMONE RECEPTORS GENOTYPES IN PREDICTION OF THE OVARIAN RESPONSE PRIOR TO ASSISTED REPRODUCTIVE TECHNIQUES

By

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ABSTRACT:

Objective(s): To evaluate the role of anti Mullerian hormone (AMH) and the follicular stimulating hormone receptors (FSHR) genotypes in prediction of the ovarian response and pregnancy rate during ICSI.

Setting: Minia infertility research unit, Department of Obstetrics and Gynecology, and Biochemistry Department, Faculty of Medicine, Minia University, Minia, Egypt.

Design: Prospective study.

Patients: 124 patients undergoing their 1st ICSI cycle.

Methods: Basal serum levels of FSH, AMH and E2 (estradiol) were measured, and antral follicle count (AFC) calculated on day 3 of the cycle prior to stimulation. FSHR polymorphism at position 680 was determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis. The long protocol for gonadotrophin stimulation was used in all patients. Patients were divided into 2 groups according to the number of oocytes retrieved; good responders (≥ 5 oocytes) and poor responder (< 5 oocytes).

Outcome measure(s): The primary outcome measure was ovarian response. Secondary outcome measures included the number of HMG ampoules and duration of stimulation, the quality of the retrieved eggs, the rate of fertilization, and the number and quality of the embryos and the chemical pregnancy rate.

Results: Patients were classified into two groups; good responders including 90 patients (72.6%) and poor responders including 34 patients (27.4%). Receiver operating characteristic (ROC) curve analysis has shown that basal serum E2 and AMH level, and AFC were good predictors of poor response to gonadotrophin stimulation. At cut of levels of 45 pg/ml for E2, 2.3 ng/ml for AMH and 5 for AFC, the area under the ROC curve (AUC) was 83%, 98% and 98% respectively. Basal serum FSH was a bad predictor of poor response with AUC of 38% at a cut off level of 8.2 IU/L. ROC curve analysis revealed that none of them was a good predictor of the occurrence of pregnancy with AUC of 43%, 34%, 53% and 48% for FSH, E2, AFC and AMH respectively. There were no statistically significant differences in the distribution of the Asn/Asn and the Asn/Ser variants of the FSHR genotypes between both groups. While the Ser/Ser variant was significantly higher in poor responders than in good responders (47.1% vs 29.8% respectively, $P = 0.03$). The sensitivity and specificity of FSHR genotypes in prediction of poor response were 47.1% and 71.1% respectively. There were no statistically significant differences in the distribution of the FSHR genotypes among patients who achieved pregnancy in both groups

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Conclusion: Basal serum AMH level is an ideal promising test in predicting the ovarian response prior to ARTs techniques. Though the Ser/Ser genotype was significantly more frequent among poor responders, determining the FSHR genotype was of little value in predicting poor response. No parameter was effective in predicting pregnancy.

KEY WORDS:

AMH – FSH – FSHR genotypes – ICSI – Ovarian reserve.

INTRODUCTION:

Ovarian reserve is an important test to predict the success rates with assisted reproductive techniques (ARTs). Accurate ovarian response tests would be very useful for counseling patients about their response to and starting dose of injectable gonadotrophins (HMG or FSH) in individual women[1].These tests include static tests (age, basal serum FSH, basal serum estradiol (E2), basal LH/FSH ratio, basal serum inhibin-B level, basal serum anti-Mullerian hormone level (AMH), basal ovarian volume, basal antral follicle count (AFC), ovarian stromal blood flow and ovarian biopsy) and dynamic tests (clomiphene citrate challenge test, GnRH agonist stimulation test, exogenous FSH ovarian reserve test)[2].

An age-related decline in the AFC as visualized by trans-vaginal ultrasound scan has been observed[1,3]. A meta-analysis has demonstrated the superiority of AFC over basal FSH in the prediction of poor ovarian response [4]. The precise definition of what constitutes an antral follicle is variable, with cited diameters ranging between 2–10 and 2–5 mm[5,6]. Inter-cycle variability has been investigated in women with proven fertility[7], those undergoing IVF[5] and in general sub-fertile women[8]. Inter-cycle variability appears to be more significant in young women and in women with high AFC. Hence, a low AFC in young, infertile but ovulatory women should be interpreted cautiously, as this may not indicate poor ovarian reserve[9].

In women, AMH is expressed uniquely by the ovary in the granulosa cells. AMH expression begins in the primary follicles, gradually increasing then peaking in preantral and antral follicles of maximum of 4 mm. After this threshold, the expression decreases, becoming undetectable when the follicles reach a diameter of 8 mm[10]. AMH is therefore expressed during the two critical regulatory steps of folliculogenesis: the initial recruitment and the cyclic selection for follicular dominance[11].

To assess an individual's ovarian reserve, early follicular phase serum levels of FSH, inhibin B and E2 have been measured. With the decline of the follicle pool, serum levels of inhibin B and E2 decrease and subsequently serum FSH levels rise[12]. Because these factors are part of a feedback system, their serum levels are not independent of each other. A serum marker that reflects the number of follicles that have made the transition from the primordial pool into the growing follicle pool, and that is not controlled by gonadotropins, would benefit both patients and clinicians. Accumulated data indicate that AMH may fulfill this role[13].

During screening for mutations of the follicle-stimulating hormone receptor (FSHR) gene, two polymorpolymer-phisms were identified:

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one located in the extracellular domain at position 307, occupied by either alanine (Ala) or threonine (Thr); and the other one, located in the intracellular domain at position 680, occupied either by asparagines (Asn) or serine (Ser). Both polymorphic sites are within exon 10 and give rise to two discrete allelic variants of the FSHR, i.e. Thr307/ Asn680 and Ala 307/Ser 680[14]. Simoni et al.,[15] reported that there was no difference regarding frequencies of these polymorphisms between infertile couples and normal population, however, Sudo et al.,[16] found differences in the genotype distribution between the control group and the anovulatory infertile group or polycystic ovary syndrome (PCOS) woman population.

In assisted reproduction programs, the standard stimulation protocol can result in either poor response (requiring adjustment of the FSH doses) or in ovarian hyper-stimulation syndrome. The latter is a serious, potentially life-threatening complication of IVF characterized by enlarged ovaries and extravasation of fluid to the abdominal cavity, resulting in ascites, hypovolemia, and hemoconcentration [17]. Advance identification of patients who will elicit a poor response or hyper-response to standard treatment would be of great clinical advantage.

The aim of this study was to study the association between AMH and FSHR gene polymorphism at position 680 and the outcomes of controlled ovarian hyper-stimulation for ICSI in infertile women, in comparison with reliable and well established parameters in prediction of the ovarian response: AFC, and day 3 serum levels of FSH and E2.

PATIENTS AND METHODS:

This prospective study was conducted at Minia Infertility Research Unit (MIRU) in coordination with Biochemistry Department, Faculty of Medicine, Minia University. The study approved by scientific ethical committee of the department of Obstetrics and Gynecology, in October 2008, and the Institutional Review Board of the University Hospital-Quality control unit of the Faculty of Medicine, Minia University in the same month.

The study included 124 infertile patients scheduled for ICSI. Patients were less than 40yrs, with regular menstrual cycles, first ART trial indicated by infertility due to male factor, tubal factor and/or unexplained infertility for more than one year, basal serum FSH level less 15 mIU\ml, body mass index (BMI) $\leq$ 39.99 kg/m^2. Patients with ovarian mass, previous ovarian surgery, PCO, endometriosis and presence of uterine factor were excluded from the study. The study was explained to all patients and a written informed consent was taken from each prior to enrollment.

All patients were subjected to the following on the first 3 days of the cycle prior to the start of induction program: full history taking, systematic and local pelvic examination and routine laboratory investigations as CBC, liver & kidney functions. Trans-vaginal ultrasound scan (using 7.5 MHz intracavitary probe, Sonoace 9900, Medison, Seoul, Korea) to assess the uterus, ovaries and to estimate the AFC on day 3 of the cycle (follicles smaller than 10 mm, counted from lateral to medial margin of the ovary)[6]. Venous blood samples were taken for quantitative assessment of basal serum levels of FSH, AMH and E2 on day 3 of the cycle (all samples were centrifuged within 2 hours after withdrawal and serum was stored at -

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20° C until time of assay). 10 ml blood was drawn from each patient with EDTA added as anticoagulant for isolation of genomic DNA from peripheral blood leukocytes.

Outcome measures:

The primary outcome measure was ovarian response based upon the number of oocytes retrieved, and accordingly, all participants were classified into either poor responder (less than 5 oocytes) or good responder (5 or more oocytes)[18]. Secondary outcome measures included the number of human menopausal gonadotrophin (HMG) ampoules, duration of stimulation, the quality of the retrieved eggs, the rate of fertilization, the number and quality of the embryos and the chemical pregnancy rate.

Detection of FSHR genotype:

DNA isolation

A volume of 300 µl of blood was drawn from each subject with EDTA added as an anticoagulant. Genomic DNA was obtained from peripheral blood leukocytes with the WizardTM Genomic DNA Purification Kit according to the manufacturer's instructions (Promega, Madison, WI, USA).

DNA analysis

The FSHR polymorphism at Position 680 was determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis. The region of nucleotide number 1624 to 2143 in the FSH receptor gene was amplified by PCR reaction mixture contained 0.1 µg of genomic DNA, 0.4 µM of each primer: (upstream 5'TTTGTGGTCAT CTGTGGCTGC3', downstream 5'CAAAGGCAAGGACTGAATTAT CATT3'), 1.25u of Taq polymerase, 1.5 mm of MgCl2, and 200 µm of dNTP. Following an initial denaturation step at 94°C for 5 min, samples were subjected to 30 rounds of PCR. Products were digested with 2 IU of restriction enzyme BSrI followed by electrophoresis on 2% agarose gel, and the digested products were identified using ethidium bromide staining. Uncleaved band indicated the asparagines homozygote (Asn/Asn), cleaved bands the serine homozygote (Ser/Ser), and simultaneous cleaved and uncleaved bands the heterozygote (Asn/Ser).

Controlled ovarian hyperstimulation (COH) protocol:

At MIRU, the induction protocol was the long luteal phase agonist protocol. Participants received subcutaneous injection of a highly purified Gonadotrophin releasing hormone agonist (GnRH) (0.1 mg of Decapeptyl, TAP Pharmaceutics Products Inc., North Chicago, IL) daily, starting at mid luteal phase (on day 21 of the preceding cycle of stimulation cycle), and continued until down regulation of pituitary ovarian axis occurred detected by ultrasonography in the form of endometrial thickness of <7 mm, without ovarian activity (no follicles >10 mm in diameter), by occurrence of menstruation or by low serum E2 level (less than 30 pg\ml). On the 2nd day of menstruation, 150 -300 IU of HMG was administered by intramuscular injection in daily doses. The given dose depended on the patient's anticipated response guided by age, basal FSH, antral follicle count and body mass index. Repeated sonographic measurements of ovarian follicle growth were carefully monitored every other day starting on day 5 of stimulation. The patient received human chorionic gonadotrophin (HCG) when the largest follicle had a mean diameter of 18 mm and there were at least two other follicles with a diameter of 16 mm. Oocyte retrieval

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was performed 24–36 hours after HCG injection by guided trans-vaginal ultrasound follicle aspiration with double way aspiration needle connected to ultra quite William A Cook aspiration machine (model number K-MAR-5100; Australia) under mild sedation and analgesia. Cycles were cancelled if the follicles remained < 10 mm after 14 days of stimulation.

Retrieved oocytes were classified after enzymatic and mechanical removal of the cumulus and corona cells prior to intra-cytoplasmic sperm injection (ICSI) into mature metaphase II eggs or immature, either at the metaphase-I (absence of both a germinal vesicle and a first polar body) or at the germinal vesicle (GV) stage [19] and embryos were classified into 4 grades [20]. Embryo transfer was done on day 3 after oocyte retrieval using the COOK catheter (Number of embryos transferred was reported for each patient). Luteal support was provided with progesterone initiated after oocyte aspiration and extended until the 8th week of pregnancy or until the initiation of menses.

Biochemical pregnancies were defined as a positive pregnancy test on the 18th day after oocyte retrieval.

Statistical analysis

All statistical calculations were done using computer programs Microsoft Excel version 7 (Microsoft Corporation, NY, USA), SPSS 16 (Statistical Package for the Social Science; SPSS; Inc., Chicago, IL, USA) and Arcus Quick Stat (Biomedical version, Addison Wesley Longman Ltd, USA) statistical-program. Data were statistically described in terms of range, mean ± standard deviation (±SD), median, frequencies (number of cases) and relative frequencies (percentages) when appropriate. Comparison of quantitative variables between different groups in the present study was done using Mann Whitney U test for independent samples. For comparing categorical data, Chi square test was performed. Exact test was used instead when the expected frequency is less than 5. Accuracy was represented using the terms sensitivity and specificity.

Receiver operator characteristic (ROC) analysis was used to determine the optimum cut off value for the studied diagnostic markers. Correlation between various variables were done using Pearson moment correlation and Spearman rank correlation equations. A probability value (p value) less than 0.05 was considered statistically significant.

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RESULTS:

This study included 124 patients undergoing their first ICSI trial. There was no cycle cancellation due to poor response. The independent variables included basal day 3 AMH level and the FSH receptor genotypes at position 680, which were studied for their effectiveness in predicting the res-ponse to ovarian stimulation with HMG in addition to their value in predicting pregnancy, in comparison with other well established predictor variables including basal serum level of FSH, E2 in addition to AFC on day 3.

Patients were classified according to their response to COH into 2 groups; good responders including 90 patients (72.6%) and poor responders including 34 patients (27.4%).

Regarding patients' characteristics, table (1) showed that there were no statistically significant differences between both groups as regards type of infertility, cause of infertility, and basal serum FSH level. On the other hand, both groups showed statistically significant differences in age, duration of infertility, BMI, basal E2 level, basal AMH level, and AFC.

As regard the distribution of the FSH receptors genotypes among the studied patients, there was no statistically significant difference in the Asn/Asn and the Asn/Ser variants between both groups. While the Ser/Ser variant was significantly higher (P = 0.03) in poor responders than in good responders (47.1% vs 29.8% respectively)(table 1). The sensitivity and specificity of FSHR genotypes in prediction of poor response were 47.1% and 71.1% respectively.

Table (2) shows the outcomes of controlled ovarian hyper-sti-mulation. There were statistically significant differences between both groups regarding duration of stimu-lation, number of HMG ampoules, number of eggs retrieved, number of metaphase II eggs, fertilization rate, number of grade I and II embryos, and chemical pregnancy rate.

The results of receiver-operating characteristic (ROC) curves analysis of the ability of day 3 serum FSH, serum E2, AFC and serum AMH in prediction of poor ovarian response and the occurrence of pregnancy are shown in table3 and figures1-4.

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Table 1: Patients' characteristics.

	Good responders N. = 90	Poor responders N. = 34	Total N. = 124	P val
Age (years)¥	21-37 (28.7 ± 4.2)	27-40 (35.1 ± 4.3)	21-40 (30.5 ± 5.1)	0.0
Type of infertility				
• **1ry**	62 (68.9%)	24 (70.6%)	86 (69.4%)	0.
• **2ry**	28 (31.1%)	10 (29.4%)	38 (30.6%)	
Duration of infertility (years)¥	2-10 (5.7 ± 1.5)	5-20 (11.9 ± 4.5)	2-20 (7.4 ± 3.8)	0.0
Cause of infertility≠				
• **Tubal**	32 (35.5%)	14 (41.2%)	46 (37.1%)	
• **Male**	24 (26.7%)	10 (29.4%)	34 (27.4%)	0.
• **Unexplained**	34 (37.8%)	10 (29.4%)	44 (35.5%)	
BMI (kg/m^2)¥	20-38 (28.6 ± 4.8)	22.4-38 (30.9 ± 5.1)	20-38 (29.2 ± 5.2)	0.0
Basal FSH (IU/L)¥	4-11 (7.7 ± 1.8)	4-12 (8.6 ± 2.2)	4-12 (8.03 ± 1.9)	0.0
Basal E2 (pg/ml)¥	16-38 (25.9 ± 5.5)	36-75 (56.1 ± 11.4)	16-75 (34.2 ± 15.4)	0.0
AMH (µg/L)¥	2.3-8.1 (5.1 ± 1.6)	0.4-3 (1.5 ± 0.9)	0.4-8.1 (4.1 ± 2.1)	0.0
AFC (2-5 mm)¥	4-12 (8.2 ± 1.8)	2-6 (4.1 ± 0.9)	2-12 (7.09 ± 2.04)	0.0
FSHR polymorphism 680≠				
• **Asn/Asn**	30 (33.3%)	10 (29.4%)	40 (32.3%)	0.
• **Asn/Ser**	34 (37.8%)	8 (23.5%)	42 (33.9%)	0.
• **Ser/Ser**	26 (28.9%)	16 (47.1%)	42 (33.9%)	0.0

¥Range (mean±SD), ≠number (percent), *statistically significant.

Table 2: Outcomes of controlled ovarian hyper-stimulation.

	Good responders N. = 90	Poor responders N. = 34	P value
Duration of stimulation (days)[¥]	6-12 (9.7 ± 1.7)	11-17 (12.7 ± 1.5)	0.001*
Number of HMG ampoules[¥]	14-48 (30.9 ± 8.8)	36-68 (47.8 ± 8.06)	0.001*
Number of eggs retrieved[¥]	5-20 (10.9 ± 4.3)	1-4 (2.6 ± 0.9)	0.001*
Number of metaphase II eggs[¥]	0-6 (2.9 ± 1.9)	0-2 (0.7 ± 0.6)	0.001*
Fertilization rate (%)[¥]	58-100 (74.1 ± 14.6)	25-100 (58.3 ± 24.1)	0.04*
Number of grade I & II embryos[¥]	1-8 (4.06 ± 1.7)	0-2 (0.8 ± 0.6)	0.001*
Pregnancy rate[≠]	32 (35.6%)	6 (17.6%)	0.04*

[¥]Range (mean±SD), [≠]number (percent), *statistically significant.

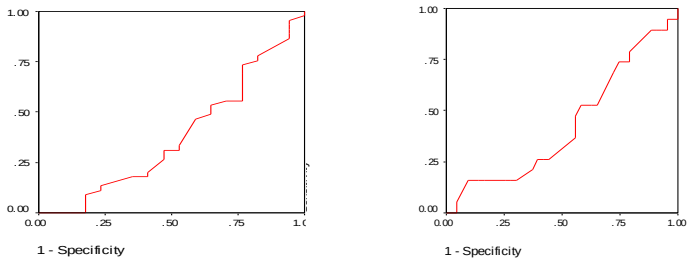
Table 3: The area under the ROC curve, the optimal cut-off level as well as the sensitivity and specificity of FSH, E2, AFC and AMH for the prediction of poor ovarian response and the occurrence of pregnancy.

	Poor response					Pregnancy			
	Cut off	**Sens.**	**Spec.**	**AUROC**	***P***	**Cut off**	**Sens.**	**Spec.**	**AUROC**
FSH	8.2 IU/L	52.9%	70%	38%	0.06	7.5 IU/L	47.3%	62.7%	43%
E2	45 pg/ml	82.3%	88.8%	83%	0.001*	29 pg/ml	63.1%	55.8%	34%
AFC	5	94.1%	93.3%	98%	0.001*	8	52.6%	55.9%	52%
AMH	2.3 ng/ml	70.5%	97.7%	98%	0.001*	4.7 ng/ml	44.2%	68.4%	48%

Sens. = sensitivity, spec. = specificity, AUROC = area under ROC curve, *statistically significant

Figure 1: ROC curve of basal serum FSH in prediction of:

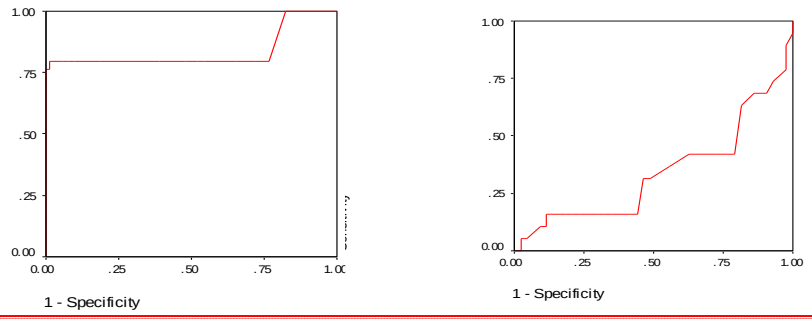
a) Poor response b) Pregnancy



Receiver operating characteristic (ROC) curve showing the sensitivity on the y-axis and the 1-specificity (false-positive rate) on the x-axis of basal serum FSH to predict a) poor ovarian response and b) the occurrence of pregnancy.

Figure 2: ROC curve of basal serum E2 in prediction of:

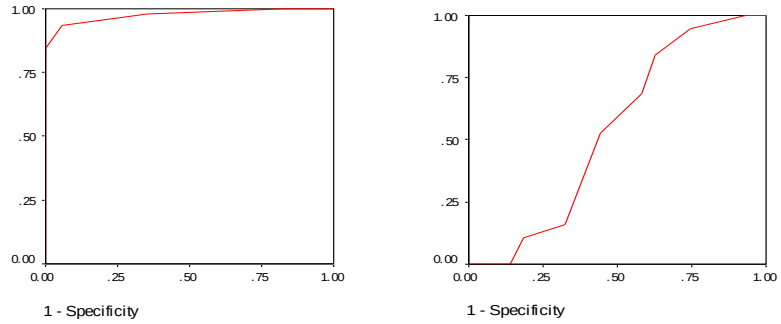
a) Poor response b) Pregnancy



Receiver operating characteristic (ROC) curve showing the sensitivity on the y-axis and the 1-specificity (false-positive rate) on the x-axis of basal serum E2 to predict a) poor ovarian response and b) the occurrence of pregnancy.

Figure 3: ROC curve of AFC in prediction of:

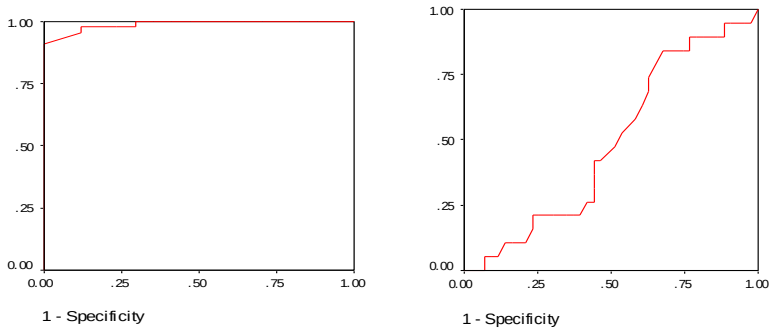
a) Poor response b) Pregnancy



Receiver operating characteristic (ROC) curve showing the sensitivity on the y-axis and the 1-specificity (false-positive rate) on the x-axis of AFC to predict a) poor ovarian response and b) the occurrence of pregnancy.

Figure 4: ROC curve of basal serum AMH in prediction of:

a) Poor response b) Pregnancy



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Receiver operating characteristic (ROC) curve showing the sensitivity on the y-axis and the 1-specificity (false-positive rate) on the x-axis of basal serum AMH to predict a) poor ovarian response and b) the occurrence of pregnancy.

There were no statistically significant differences in the distribution of the FSH receptors genotypes among patients who achieved pregnancy in both groups (table 4).

Table 4: Findings of FSHR genotypes among pregnant and non-pregnant -patients.

FSHR genotype	Good responders N. = 90		Poor responders N. = 34	
	Pregnancy positive N. = 32	Pregnancy negative N. = 58	Pregnancy positive N. = 6	Pregnancy negative N. = 28
Asn/Asn[≠]	8 (25%)	22 (37.9%)	2 (33.3%)	8 (28.6%)
Asn/Ser[≠]	10 (31.3%)	24 (41.4%)	2 (33.3%)	6 (21.4%)
Ser/Ser[≠]	14 (43.8%)	12 (20.7%)	2 (33.3%)	14 (50%)
P	0.06		0.7	

[≠]number (percent)

Table 5: Correlation of age and AFC with AMH, FSH and E2.

	Good responders r (p)		Poor responders r (p)	
	AGE	AFC	AGE	AFC
AMH	0.12 (0.2)	0.53 (0.001*)	-0.35 (0.04*)	-0.43 (0.04*)
FSH	-0.02 (0.8)	0.03 (0.7)	0.24 (0.1)	-0.19 (0.2)
E2	-0.07 (0.7)	0.02 (0.8)	0.07 (0.6)	0.11 (0.5)

r = correlation coefficient, *statistically significant, Grades of r: 0.00 - 0.24 = weak or no correlation, 0.25 - 0.49 = fair correlation, 0.50 - 0.74 = moderate correlation, ≥ 0.75= strong correlation

Table5 showed weak or no correlation between basal serum level of FSH & E2 with maternal age and AFC neither in good nor poor responders, while the basal level of AMH had a significant positive correlation with AFC in good responders and significant fair negative correlation with age & AFC in poor responders.

Our results (table 6) showed no significant relation between different FSH receptor genotypes as regard age either in both good and poor responders, while the genotypes Asn/Asn & Asn/Ser related significantly to higher AFC than Ser/Ser genotype in good responders.

There was a significant moderate positive correlation of basal

level of AMH with good quality eggs (metaphase II) in both good and poor responders (r = 0.64 and 0.56 respectively, and $p = 0.001$ for both).

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Table 6: Relation of age and AFC with FSHR genotypes.

FSHR genotype	Good responders		Poor responders	
	AGE (Mean±SD)	AFC (Mean±SD)	AGE (Mean±SD)	AFC (Mean±SD)
Asn/Asn	28.3±4.2	8.5±1.7	34.2±6.2	4.6±1.7
Asn/Ser	28.1±4.7	8.8±1.7	35.2±2.4	4.2±1.3
Ser/Ser	30±3.5	7±1.6	35.6±3.7	3.8±0.3
***P* value**	0.2	0.001*	0.7	0.1

*Statistically significant

The number of good quality eggs retrieved was significantly higher among the Ser/Ser genotype in both good and poor responders (p = 0.01 and 0.04 respectively). The mean±SD number of good quality eggs in good and poor responders was 7.1 ± 3.1 vs 1.3 ± 0.4 in the Asn/Asn genotype, 6.1 ± 1.6 vs 1.2 ± 0.4 in the Asn/Ser genotype, and 8.2 ± 3.04 vs 1.8 ± 0.8 in the Ser/Ser genotype respectively.

DISCUSSION:

Our results showed that day 3 serum AMH was useful in predicting poor response; however it was less significant in predicting pregnancy. Several studies have shown that AMH levels do not fluctuate across and between cycles making it a cycle independent marker[21,22]. In another study, AMH cut-off of ≤1.26 ng/ml had a 97% sensitivity for predicting poor responses (< 4 oocytes retrieved) and a 98% accuracy in predicting a normal COS response[23]. These findings agreed with our study and indicated that circulating AMH levels might be a good indicator of ovarian reserve, and are highly correlated with ovarian response to COS[23].

Our study showed that AMH had a significant positive correlation with AFC in good responders and significant negative correlation with age & AFC in poor responders. While there was weak or no correlation between basal serum levels of FSH & E2 with maternal age and AFC neither in good nor poor responders. Likewise, Anckaert et al.,[24] reported that AMH had a significant negative correlation with age and a significant positive correlation with AFC.

Our results are comparable with Eldar-Geva et al.,[25], who concluded that the number of oocytes retrieved showed highly significant positive correlations with AFC, AMH and follicular phase inhibin B. Negative correlations were found with age and basal FSH[25].

In the present study there was a significant positive correlation between AMH and good quality eggs (metaphase II) in both good and poor responders. Ebner et al.,[26] showed that, basal level of AMH was associated with oocyte quality in stimulated cycles in comparing the following groups; AMH groups 1 (<1.66 ng/ml) and 3 (>4.52 ng/ml) showed oocytes of lower quality (dark central granulation, aggregation of smooth endoplasmic reticulum) compared with the median group 2 (1.66–4.52 ng/ml). Basal serum FSH did not allow for adequate prognosis in terms of gamete appearance. AMH levels did not affect fertilization and further cleavage up to blastocyst stage. They concluded that, AMH seems to

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be superior to FSH in predicting both oocyte number and quality [26].

Silberstein et al., [27] found embryos of better morphology and cleavage behavior in patients with AMH levels ≥ 2.7 ng/ml as compared with patients with values below this threshold. This may in part be explained by the fact that these authors performed AMH measurement on the day of ovulation induction (not on cycle day 3), a time when AMH values are usually declining because of the presence of growing follicles [28] and may thus fail to reflect the actual competence of the oocyte or embryo. Guerif et al., [29] stated that at the moment, serum AMH is a relatively predictive indicator of the ovarian reserve, in terms of quantity but not in terms of quality. Moreover, it is still not possible to determine serum AMH cut-off value to predict clinical pregnancy in IVF programs.

As regard the distribution of the FSH receptors genotypes among the studied patients, Mayorga et al., [30] reported comparable results with our study. The distribution of FSHR isoforms was 29% for the Asn/Asn, 45% for the Asn/Ser, and 26% for the Ser/Ser FSHR variant. They reported that treatment was equally successful, independent of the FSHR isoform. However, the number of FSH ampoules required for achieving this effect was significantly different among the groups (31.8 ± 2.4, 40.7 ± 2.3, and 46.8 ± 5.0 for the Asn/Asn, Asn/Ser, and Ser/Ser groups, respectively; *P* < 0.01). Multiple linear regression analysis revealed that the number of ampoules could be predicted from a linear combination of type of polymorphism and basal FSH level (P < 0.001) [30]. Likewise, data obtained from 58 patients undergoing ovulation induction for IVF and embryo transfer showed that women with the Ser/Ser genotype require significantly more HMG before HCG administration [16]. de Castro et al., [31] in a retrospective study in IVF patients have shown an association between the presence of serin in position 680 and poor responses to gonadotrophins.

Behre et al., [32] looked at FSH-induced E2 levels in women who were homozygous for the Ser 680 variant compared with women with the Asn/Asn genotype. Ser/Ser carriers were randomly allocated to two subgroups to receive a daily FSH dose of 150 IU or 225 IU, respectively. Age and BMI matched Asn/Asn carriers, receiving a daily dose of 150 IU, constituted a control group. Even though the treatment details, number of oocytes retrieved and fertilization rates were similar, the Asn/Asn group had higher E2 production after treatment with 150 IU/day of FSH compared with the Ser680 group given the same dose. Conversely, when Ser/Ser carriers were treated with 225 IU/day, this difference disappeared [32]. These findings were consistent with carriers of the Ser/Ser variant experiencing lower biological activity of both endogenous and exogenous FSH due to lower sensitivity of the FSH receptor to FSH [33]. Alviggi et al., [33] reported that in at least one out of four patients with normal AFC (which reflects the frequency of Ser/Ser variant), the optimal FSH dose should be higher, thus AFC and genetic approaches should be integrated to determine COS treatment protocols.

Klinkert et al., [34] reported that the frequencies of polymorphism 680 in their study population of 105 patients were 38% Asn/Asn, 45% Asn/Ser and 17% Ser/Ser. The basal FSH level and the ovarian response did not differ between the three groups.

Therefore, they were unable to confirm a relationship between the FSH receptor polymorphism and ovarian response to stimulation with gonado-trophins [34]. Most patients included in their study had already experienced ovarian hyper-stimulation in the past (only 19 patients were undergoing their first treatment cycle and the remaining 86 patients had had one or more unsuccessful cycles), so the starting dose was adjusted according to the results of prior stimulation, while in our study, the starting dose was not based on a previous trials as we included patients undergoing their first ART trial. Several hypotheses have been postulated for the possible mechanisms of different receptor activity, depending on the genotype [30,35]. However, all these hypotheses need to be tested in an appropriate in vitro system [30].

As regard the distribution of the FSH receptors genotypes among patients who achieved pregnancy, the effect of the Ser680 single nucleotide polymorphism has been studied in the general population receiving sub-fertility treatment and results have been reviewed previously [36]. Studies have reported conflicting results with some demonstrating worse outcomes with the mutation and others demon-strating positive outcomes. Jun et al. [37] reported a significantly higher clinical pregnancy rate in the Asn/Asn genotype compared to the others (45.7 vs 30.5%, P = 0.013). They concluded that the homozygous Ser/Ser genotype of FSHR polymorphism at position 680 might be associated with a reduced ovarian response to COH for IVF-ET, while Asn/Asn genotypes showed a higher pregnancy rate. On the other hand, Klinkert et al. [34] reported that in spite the ovarian response was compa-rable between patients with different FSH receptor genotypes, patients with polymorphism Ser/Ser had an implantation rate and pregnancy rate that was three times higher compared to patients with poly-morphism Asn/ Asn. And so concluded that FSH receptor genotype was not associated with a poor response in IVF, but showed a positive correlation with pregnancy, which was independent of age. However, Sheikhha et al. [14] showed that regarding different FSHR genotypes, there were no significant differences in the dosage of gonado-trophins used, peak E2 level and clinical pregnancy rate in women who underwent IVF-ET, while there was a significant difference in the number of oocytes retrieved and the response to ovarian stimulation among different genotypic groups.

Our findings that the FSH receptor genotype Ser/Ser related significantly higher to good quality eggs (metaphase II) than genotypes Asn/Asn and Asn/Ser in both good and poor responders could be explained in the light of the results of Klinkert et al. [34]. The disagreement regarding differences in the ongoing pregnancies with the Ser/Ser genotype should thus be attributed to differences in one of the two factors determining the process of implantation: endometrial receptivity and the viability of the embryo [34]. As there are no FSH receptors in the endometrium [38], the FSH receptor subtype acts at the endometrium level indirectly through the corpus luteum, as well as direct effects at the level of the granulosa-oocyte complex [34]. Add to that, the use of HCG as luteal support in most of their patients, in contrary to the use of progesterone for luteal support in our study.

In conclusion, measuring the basal serum level of AMH represents an ideal promising test in the

prediction of the ovarian response prior to ARTs techniques, with results comparable to AFC results. Also being a laboratory test with no inter-personal variations makes AMH superior to AFC. Despite that our study has shown that the Ser/Ser genotype is more associated with poor responders and at the same time more associated with higher quality eggs than other genotypes, inconsistent results in the literature on role of FSHR ~~polymor~~polymer-phism in prediction of outcome prior to ART procedures makes it of limited value. Therefore, future larger studies are required before using FSHR genotypes in counseling infertile couples and adjusting stimulation protocol.

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