Anti-Mullerian Hormone versus Basal FSH in Prediction of Ovarian Function, Quality of the Embryos, and Pregnancy Rate in Infertile Patients Undergoing Intracytoplasmic Sperm Injection (ICSI) Cycles.

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Abstract: Objective: The aim of this study was to evaluate the predictive value of AMH versus basal FSH measurement for prediction of poor ovarian response, quality of the embryos, and pregnancy rate in infertile patients undergoing intracytoplasmic sperm injection (ICSI) cycles. **Study Design:** This prospective study included 118 patients, 8 of them were cancelled due to inadequate ovarian response and the remaining 110 continued the study. All patients were subjected to the following on the first 3 days of the cycle prior to starting induction program; full history taken, systematic and local pelvic examination, routine investigations as CBC, liver and kidney function tests, transvaginal ultrasound scan to assess the uterus, ovaries and to exclude any pelvic pathology. Venous blood samples were taken for quantitative FSH, AMH on day 3 of the cycle. All samples centrifuged within 2 hours after withdrawal and serum was stored at – 70°C until time of assay. A standard long step-down protocol was used for controlled ovarian hyperstimulation. Embryo transfer of maximum three good embryos was done on day three after oocyte retrieval using the Cook catheter. Vaginal progesterone pessaries (Cyclogest® 400 mg twice daily) were used to support the luteal phase until the day of β-hCG assay. **Results:** This study included 118 patients undergoing their first intracytoplasmic sperm injection (ICSI). Eight cycles (6.8%) were cancelled due to inadequate response and the remaining 110 patients (93.2%) continued the study. Patients were classified according to their response to controlled ovarian hyperstimulation into 2 main groups; good responders group (group 1) including 75 patients (68.2%), and poor responders group (group 2) including 35 patients (31.8%). There were statistically significant differences between good and poor responding groups as regards age, types of infertility, duration and causes of infertility, BMI and serum levels of AMH, however, there was no significant differences in the basal levels of FSH between the two groups. There were statistically significant differences between good and poor responding groups regarding duration of stimulation, number of HMG ampoules, number of eggs retrieved, number of metaphase II eggs, fertilization rate, quality of the embryos, and both chemical and clinical pregnancy rate. Serum AMH levels at cut-off levels of 2.8 ng/ml is more sensitive, specific, and more predictive of poor ovarian response, quality of the embryos and occurrence of pregnancy either chemical or clinical pregnancy. **Conclusion:** Simple measuring the basal serum AMH level represents an ideal promising test in prediction of ovarian reserve, good quality of retrieved eggs and fertilized embryos, and for the first time in our knowledge in prediction of occurrence of pregnancy as well. However, measurement of basal FSH serum levels carries no predictive value to predict poor ovarian reserve, quality of retrieved eggs or fertilized embryos or pregnancy rate

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1.Introduction:

It is very important to determine the state of ovarian reserve before starting assisted reproductive treatment (ART), as it carries prognostic information about the chances of success as well as helping in determine the optimal protocol suitable for each patient [(2)]. Recently, many women tried to postpone the age of marriage and childbirth, the process that led to increased rates of age-related female subfertility and infertility. As a result of this trend fertility clinicians have been faced with the challenge of determining the degree of ovarian reserve to better tailor assisted reproductive technology treatment. The cost-effect of the fertility drug regimens [(21)], patient's discomfort [(9)], and the significant risk of complications associated with ovarian stimulation [(5)]. All of these issues justify the need for obtaining clinically relevant information before starting the conception cycles. Moreover, unexpected excessive and poor responses to ovarian controlled hyperstimulation are no longer acceptable. Anti-Mullerian hormone (AMH) is a member of the transforming growth factor-β (TGF- β), synthesized exclusively by the gonads of both sexes [(28)]. Several studies have examined the clinical usefulness of serum AMH levels as a predictor of ovarian response and pregnancy in ART cycles. AMH is produced by the granulosa cells from the pre-antral and small antral follicles together with two other factors (growth and

differentiation factor-9), that inhibit the initiation of premature follicle growth and decreases the sensitivity of follicles to FSH [11]. AMH levels decline with age from adulthood toward menopause reflecting the size of ovarian follicle pool [26]. Some studies have shown that serum AMH measurement is more accurate than serum FSH, inhibin-B, or estradiol in predicting ovarian response[14,22]. Furthermore, AMH levels seem to be remained constant throughout the menstrual cycle and thus can be reliably measured at any time unlike other hormone markers that must be measured in the early follicular phase [19]. Many investigators have attempted to define poor ovarian response using varying criteria depending on the number of mature follicles developed or the number of mature oocytes retrieved. Kailasam *et al.* [16] suggested that the definition of poor response should take into account the degree of stimulation used. The absolute number and functional capacity of follicles and germ cells comprise what is termed ovarian reserve or ovarian age, which affects a given patient's response to stimulation with gonadotropins and their chances for success. The most important aspect of ovarian reserve is that it declines with age, but it is a biological and not just a chronological function. Therefore, a major challenge is the assessment of ovarian reserve for prediction of oocyte retrieval. AMH and antral follicle count (AFC), both seem to be the most reliable predictors of ovarian ageing and reserve and they are equivalent in terms of their accuracy in predicting ovarian reserve, but none of the currently employed tests of ovarian reserve can reliably predict pregnancy success [3] [3, 18, 29]. Moreover, it has been shown that serum AMH levels are increased in women with polycystic ovarian syndrome (PCOS) compared with non-ovulatory women, corresponding to the follicle excess seen on ultrsonographic examination [20]. The aim of this study was to evaluate the predictive value of AMH versus basal FSH measurement for prediction of poor ovarian response, quality of the embryos, and pregnancy rate in infertile patients undergoing intracytoplasmic sperm injection (ICSI) cycles.

2.Patients and Methods:

This prospective study included 118 patients, 8 of them were cancelled due to inadequate ovarian response and the remaining 110 continued the study. It was conducted at El-Minia Infertility Research Unit and Clinical Pathology Department, Faculty of Medicine, El Minia University from November 2010 to October 2012. This study approved by scientific ethical committee of the Department of Obstetric and Gynecology and it was explained to all patients and a written consent obtained from each participant prior to enrollment. The criteria for inclusion in the study included; age ≤ 42 years, BMI < 39 kg/m^2, no evidence of polycystic ovarian syndrome according to Rotterdam criteria [13]. No evidence of endocrine disorders, no history of ovarian surgery, no exposure to cytotoxic drugs or pelvic radiation therapy. All patients were subjected to the following on the first 3 days of the cycle prior to starting induction program; full history taken, systematic and local pelvic examination, routine investigations as CBC, liver and kidney function tests, transvaginal ultrasound scan (7.5 MHZ intracavitary probe, sonoace 9900, Medison, Seol, Korea) to assess the uterus, ovaries and to exclude any pelvic pathology. Venous blood samples were taken for quantitative FSH, AMH on day 3 of the cycle. All samples centrifuged within 2 hours after withdrawal and serum was stored at – 70°C until time of assay. The primary outcome measure was ovarian response based on the number of oocytes retrieved. Second outcome measures included the number of human menopausal gonadotropin (HMG) ampoules, duration of stimulation, the quality of the retrieved eggs, fertilization rate, the number and quality of the embryos and pregnancy rate. Pregnancy was defined as a positive pregnancy test on the 18th day after oocyte retrieval. A standard long step-down protocol was used for controlled ovarian hyperstimulation. The GnRH-analogue (Buserelin acetate; Suprecur, Aventis Pharma LTD., West Malling, Kent, UK) was administered at a dose of 500 µg subcutaneously starting in the mid luteal phase of the previous cycle. We used recombinant follicle stimulating hormone (rFSH) as exogenous purified gonadotropin on the daily bases depending on the age of the patient and the basal levels of FSH. The GnRH agonist was reduced to 250 µg from the first day of stimulation. Serum estradiol (E2) concentrations were measured on day 3 of stimulation and then daily from day 8 until the administration of hCG. Trans-vaginal ultrasound scan was done on days 8 and 10 of stimulation and daily thereafter, as required. 5000 IU of human chorionic gonadotropin (hCG) was given IM (Pregnyl®, Organon Laboratories) provided that, there was at least 2 follicles of ≥ 18 mm and another 2 follicles of ≥ 16 mm.Transvaginal ultrasound-guided oocyte retrieval was undertaken 24-36 hours after hCG injection. Retrieved oocytes were classified after enzymatic and mechanical removal of the cumulus and corona cells prior to ICSI into mature metaphase II eggs or immature, either at metaphase I, (absence of both germinal vesicle and first polar body) or at germinal vesicle (GV) stage [7]. After fertilization, embryos were classified into 4 grades according to degree of cell fragmentation and cytoplasmic integrity [1]. Embryos of Veeck grades 1 or 2 were considered high quality and thus suitable for transfer [27]. Embryo transfer of maximum three good embryos was done on day three after oocyte retrieval using the Cook catheter.

Vaginal progesterone pessaries (Cyclogest® 400 mg twice daily, Alpharma Barnstaple, UK) were used to support the luteal phase until the day of β-hCG assay, approximately 2 weeks after embryo transfer. If pregnancy test was positive, the progesterone support will continued until 12 weeks gestation.

Statistical Analysis:

All statistical calculations were done using computer programs Microsoft Excel version 7 (Microsoft Corporation, NY, USA, SPSS 16). Data were described in terms of mean ± SD, frequencies and percentage. Comparison of quantitative variables between different groups were done using Mann Whitney U test for inadequate samples. For comparing categorical data, Chi Square test was used. Accuracy was represented using the term sensitivity, and specificity. Receiver Operator Characteristic (ROC) analysis was used to determine the optimum cut-off levels for the studied markers. Correlation between various variables was done using Pearson and Spearman rank correlation. *P*-value of ≤ 0.05 was considered significant.

3.Result:

This study included 118 patients undergoing their first intracytoplasmic sperm injection (ICSI). Eight cycles (6.8%) were cancelled due to inadequate response and the remaining 110 patients (93.2%) continued the study. Patients were classified according to their response to controlled ovarian hyperstimulation into 2 main groups; good responders group (group 1) including 75 patients (68.2%), and poor responders group (group 2) including 35 patients (31.8%). Regarding patient's characteristics, our results have shown that, there were statistically significant differences between good and poor responding groups as regards age, types of infertility, duration and causes of infertility, BMI and serum levels of AMH, however, there was no significant differences in the basal levels of FSH between the two groups as shown in table 1. Meanwhile, there were statistically significant differences between good and poor responding groups regarding duration of stimulation, number of HMG ampoules, number of eggs retrieved, number of metaphase II eggs, fertilization rate (%), quality of the embryos, and both chemical and clinical pregnancy rate as shown in table 2. This study also shown that, serum AMH levels at cut-off levels of 2.8 ng/ml is more sensitive, specific, and more predictive of poor ovarian response, quality of the embryos and occurrence of pregnancy rate either chemical or clinical pregnancy as shown in table 3&4. Receiver operating characteristic (ROC) curve of basal serum AMH and serum FSH in prediction of poor response and pregnancy rate are shown in figures 1 & 2.

Table 1: Patient's characteristics in both good and poor responder groups.

	Group 1 (N = 75) Good responder	Group 2 (N = 35) Poor responder	*P*- value
Age (years)	29.5 ± 5.3	36.4 ± 6.2	0.001*
Type of infertility			
Primary	56 (73.7%)	28 (82.4%)	0.001*
Secondary	20 (26.3%	6 (17.6)	0.01*
Duration of infertility (years)	3.5 ± 2.1	5.8 ± 2.7	0.01*
Causes of infertility			
Female causes	31 (40.8%)	16 (47.0%)	
Male causes	29 (38.2%)	9 (26.5%)	0.01*
Unexplained	16 (21.0%)	9 (26.5%)	
BMI (kg/m2)	29.3 ± 4.6	31.7 ± 5.2	0.02*
Basal FSH (IU/L)	6.8 ± 2.6	7.9 ± 2.8	0.09
AMH (µg/L)	6.3 ± 2.3	2.1 ± 0.9	0.001*

BMI = Body mass index * means significant FSH = Follicle stimulating hormone AMH = Anti-Mullerian hormone

Table 2:

	Group 1 (N = 75) Good responder	Group 2 (N = 35) Poor responder	*P*- value
Duration of stimulation (days)	8.6 ± 2.1	13.8 ± 2.3	0.001*
Number of HMG ampoules	28.6 ± 7.8	48.3 ± 7.9	0.001*
Number of eggs retrieved	11.4 ± 3.8	2.9 ± 0.7	0.0001**
Number of metaphase II eggs	4.2 ± 1.7	1.3 ± 0.8	0.001*
Fertilization rate (%)	86.4%	56.7%	0.01*
Number of grade I & II embryos (High quality embryos)	5.3 ± 1.9	1.1 ± 0.04	0.001*
Pregnancy rate (%)			
Chemical pregnancy rate (%)	52.4%	38.9%	0.001*
Clinical pregnancy rate (%)	34.7%	18.5%	0.0001**

HMG = Human menopausal gonadotropin * means significant ** means highly significant

Table 3: Cut-off levels, the area under ROC, sensitivity and specificity of basal FSH and serum AMH for the prediction of poor ovarian reserve in poor responder patients.

	Cut-off	Sensitivity	Specificity	AUROC	*P*-value
Basal FSH	7.2 mIU/L	51.7%	68.9%	39%	0.09
Serum AMH	2.8 ng/ml	84.6%	98.9%	99%	0.0001**

FSH = Follicle stimulating hormone ** means highly significant AMH = Anti-Mullerian hormone

Table 4: Cut-off levels, the area under ROC, sensitivity and specificity of basal FSH and serum AMH for the prediction of occurrence of pregnancy in poor responder patients.

	Cut-off	Sensitivity	Specificity	AUROC	*P*-value
Basal FSH	7.2 mIU/L	41.7%	64.9%	45%	0.09
Serum AMH	2.8 ng/ml	82.8%	94.7%	97%	0.001*

FSH = Follicle stimulating hormone AMH = Anti-Mullerian hormone * means significant

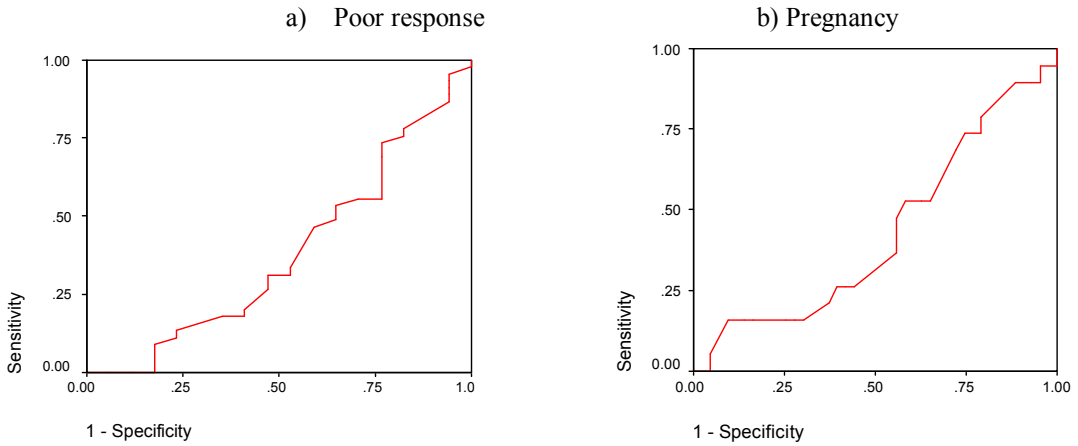

Figure 1: ROC curve of basal serum FSH in prediction of poor response and occurrence of pregnancy.
Receiver operating characteristic (ROC) curve showing the sensitivity on the y-axis and the 1-specificity (false-positive rate) on the x-axis of basal serum FSH to predict a) poor ovarian response and b)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

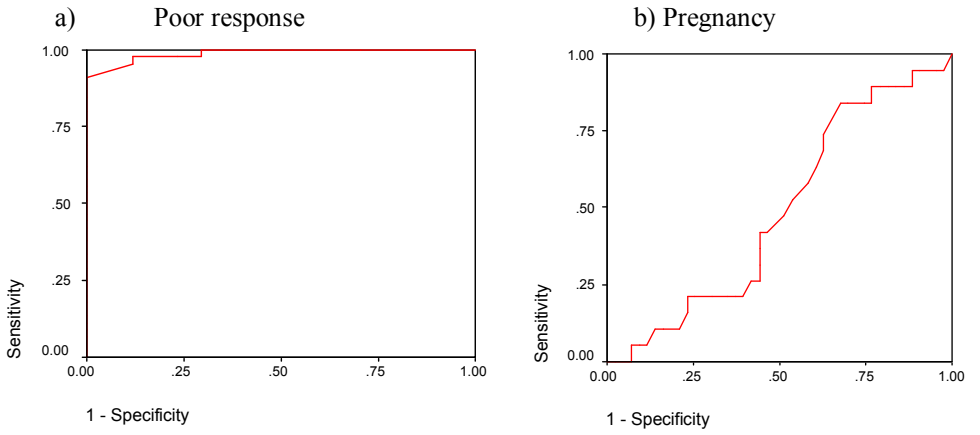

Figure 2: ROC curve of basal serum AMH in prediction of poor response and occurrence of pregnancy.
Receiver operating characteristic (ROC) curve showing the sensitivity on the y-axis and the 1-specificity (false-positive rate) on the x-axis of basal serum AMH to predict a) poor ovarian response and b) the occurrence of pregnancy.

4. Discussion:

We have excluded patients with polycystic ovarian disease who have been shown to exhibit elevated serum AMH levels to avoid falsely elevating the serum value of measured AMH [4]. Our results have shown that the day 3 serum AMH level was good

prediction predicting patients with poor ovarian response, good quality of the eggs retrieved, high grading of the embryos, and pregnancy rate as well. In another reported study, AMH at cut-off level of ≤ 1.26 ng/ml had a 97% sensitivity for predicting poor ovarian responses (< 4 oocytes retrieved) and a 98% accuracy in predicting a normal controlled ovarian stimulation response [10]. These findings agreed with our study and indicated that circulating AMH serum levels might be good indicator of ovarian reserve, but in contrast with our study in that serum AMH levels were not correlated with pregnancy rate. In the present study, there was a good significant positive correlation between AMH and good quality of the retrieved eggs (metaphase II) in poor responders and these results are also in accordance with other study done by Ebner *et al.* [6]. However, their findings were related to normal ovarian responder not poor ovarian responders. Moreover, basal serum FSH did not allow for adequate prognosis in terms of gamete quality. AMH serum levels did not affect fertilization, and they concluded that AMH seems to be superior to FSH in predicting both oocyte number and quality [6]. Nelson *et al.* [23] in a study evaluating the usefulness of serum measurement in a routine IVF program, also found a significant positive correlation between serum AMH levels and ovarian response. They also found that poor responders were significantly older and had a significantly lower clinical pregnancy rate. In another study Kunt *et al.* [17] compared AMH with other ovarian reserve markers such as antral follicles count (AFC), basal serum FSH, E2, inhibin-B for predicting ovarian reserve in an *in vitro* fertilization (IVF) program. They found that, AMH is the best marker of those tested in predicting the outcome of the in vitro fertilization. Moreover, Guerif *et al.* [12] 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. In addition, it is still not possible to determine serum AMH cut-off value to predict clinical pregnancy in IVF program. However our results have shown that serum AMH at cut-off levels of 2.8 ng/ml is a good predictor of the occurrence of pregnancy rate in contrast with what reported before. On the other hand, our results have shown that basal FSH at cut-off levels of 7.2 mIU, had no predictive role for prediction of poor ovarian reserve, eggs and embryos quality or pregnancy outcome. These findings were in accordance with what reported recently by Negm *et al.* [24]. However, they also failed to find any predictive value or role for AMH to predict the occurrence of pregnancy in their study either in normal or low responder infertile patients. In conclusion, simple measuring the basal serum AMH level represents an ideal promising test in prediction of ovarian reserve, good quality of retrieved eggs and fertilized embryos, and for the first time in our knowledge in prediction of occurrence of pregnancy rate as well. However, measurement of basal FSH serum levels carries no predictive value to predict poor ovarian reserve, quality of retrieved eggs or fertilized embryos or pregnancy rate. More researches are needed to support our finding.

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