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Anti-Mullerian hormone and antral follicle count as predictors of ovarian reserve and successful IVF

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ABSTRACT

Objective: To investigate the role of Anti-Mullerian hormone and antral follicle count in predicting the ovarian reserve, and success of IVF. **Methods:** Ninety two infertile couples complaining of infertility due to male or tubal or unexplained factors were included in this comparative prospective study for IVF/ICSI. Day-3 basal hormonal level of FSH, LH, E2, and AMH were measured, followed by Transvaginal ultrasound (TVS) to evaluate the AFC. Controlled ovarian hyperstimulation was done using the long protocol for ovarian hyperstimulation. **Results:** According to the number of retrieved oocytes women included in this study were classified into two groups; good responders (≥ 4 retrieved oocytes) and poor responders (< 4 retrieved oocytes). Ovarian reserve in this study was assessed by day-3 basal hormonal levels and AFC. The mean Day-3 AMH and mean AFC were significantly high (4.93±1.22) ng/mL, and (12.72±5.70) ng/mL; respectively) in good responders compared with poor responders, also, the number of retrieved oocytes were significantly high in the good responders group compared with poor responders (13.52±9.70) versus (3.91±1.20) (P<0.05). The numbers of chemical and clinical pregnancies were significantly high (6 cases (75%) and 13 cases (72.2%); respectively) in the good responders compared with poor responders (2 cases (25%) and 5 cases (27.8%); respectively). **Conclusions:** Day-3 AMH and AFC are good predictors for ovarian reserve, there were positively correlated with the number of retrieved oocytes and numbers of chemical and clinical pregnancies.

1. Introduction

The determination of ovarian reserve is important in the assessment and treatment of infertility. Ovarian reserve also refers to the number and quality of oocytes available to produce a dominant follicle late in the follicular phase of the menstrual cycle at any given age[1]. The prediction of IVF success is possible after better estimation of the ovarian reserve[2].

The antral follicle count (AFC) is a minimally invasive, easily performed test provides a representation of remaining follicular pool levels to assess the probability of a positive response to controlled ovarian hyperstimulation (COH) and success of IVF[3]. The AFC is defined as the number of follicles smaller than 10 mm in diameter detected by TVS in the early follicular phase. The AFC is a good predictor of the number of retrieved oocytes and rate of cancellation in IVF after COH[3,4].

Anti-mullerian hormone (AMH) is a member of the TGF-β superfamily and is produced by the granulosa cells of pre-antral and small antral follicles. Follicular growth is modulated by AMH, which inhibits recruitment of follicles from the primordial pool by modifying the FSH sensitivity of those follicles[5,6]. AMH reflects the non-FSH dependant follicular growth. As a follicle matures, AMH production disappears allowing the follicle to complete the development process during the FSH-dependant stages of growth[6]. As a woman approaches menopause, there is a linear decline of AMH levels over time[5,6]. The AMH is not influenced by the gonadotropic status and reflects only the follicle population[7]. Treatment of IVF patients with a single high dose of gonadotropin-releasing hormone (GnRH) agonist resulting in a rise of endogenous FSH and LH, without affecting the serum AMH suggesting that AMH acts as a paracrine rather than a systemic factor and thus is not part of a negative feedback loop[8]. The expression of

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AMH at a constant level independent of cycle day and the secretion of AMH without dependence on other hormones, particularly the gonadotropins make AMH very attractive as a direct measurement of ovarian reserve[9-11]. Since AMH is solely produced by the growing ovarian follicles, the serum levels may be used as a marker for ovarian reserve representing the quantity and quality of the ovarian follicle pool[12-14]. Logistic regression analysis for prediction of poor response showed that the serum AMH levels had a better predictive value than the serum levels of FSH, inhibin B, E2 and the predictive values of AMH, and AFC were almost identical, also, Feyereisen et al, demonstrated that the AMH had the strongest relationship with AFC than the other typical biomarkers[9]. So, this study was designed to investigate the role of AMH, and AFC in predicting the ovarian reserve and the success of IVF.

2. Patients and methods

Ninety two (92) infertile couples due to male or tubal or unexplained factors were included in this comparative study for IVF/ICSI over two year from May 2009 to May 2011. Women < 40 years were included in this study after informed consent and approval of the study protocol by the institute ethical committee of Al-Rashid Maternity Hospital. Women with irregular menses or received hormonal therapy in the last 3 months before ICSI or endometriosis or ovarian abnormalities (inadequate visualization of both ovaries by TVS or previous oophorectomy) were excluded from this study. Women with BMI >27 kg/m^2 or endocrine disturbance (thyroid, prolactin, testosterone, androstandione) were also excluded from this study. Hysteroscopy was done as routine procedure for uterine cavity assessment for the women scheduled for the first attempt of IVF/ICSI to exclude uterine cavity abnormalities.

Hormonal assay and transvaginal ultrasound: On day-3 of the cycle 5 mL of blood was drawn, the blood was centrifuged at the 3 500 rpm for 10 min and the serum was stored in polypropylene tubes at 2-8 °C for hormonal assay, which was performed within 24 h by technician who was blinded to the patients' data, followed by TVS to evaluate the number of antral follicles. AFC is defined as the number of follicles smaller than 10 mm in diameter detected by TVS in the early follicular phase. Serum AMH level was measured using a "second generation" enzyme-linked immunosorbent assays (ELISA) (Immunotech, Beckman, Coulter Laboratories). The immunoassay was specific for AMH and the analytical sensitivity was 0.7 pmol. Serum FSH, LH and E2 levels were measured by the same laboratory technician for all women on day-3 of the cycle by ELISA technique. TVS examinations were done by an expert sonographer, who was also blinded to the patients' criteria using Philips HD9 with 2D convex probe 4-9 MHz.

Ovarian stimulation protocol: COH was done using the long protocol for ovarian hyperstimulation. In the long protocol, pituitary down-regulation was achieved by administering Buserelin acetate (Suprefact, Hoechst AG, Germany) (0.5 mg SC) starting from day-21 of the menstrual cycle and the dose was decreased to 0.25 mL/d with the first day of menses. Then stimulation was started using human menopausal gonadotropin (HMG) (Menogon, Ferring, Germany) from the day-2 of the menstrual cycle with a dose of 225-300 IU/day[14].

Monitoring was carried out by TVS on day-7 of HMG stimulation. When more than 3 follicles larger than 18 mm in diameter were detected 10 000 IU of human chorionic gonadotropin (HCG) (Organon) was administered intramuscularly. Thirty-six hours later, follicles were retrieved under general anaesthesia by TVS-guided aspiration. Mature oocytes were retrieved from follicular fluid, and fertilized immediately by mature sperms using intra-cytoplasmic injection technique (ICSI), after fertilization; 2PN zygote was transferred to G-1 media (G-1 TM version 3, Vitrolife, Goteborg, Sweden). All embryo transfers were performed two days after retrieval using Labotect catheters (Labor-Technik, Germany). Before the transfer, the embryos were evaluated microscopically, and the best-quality embryos were selected for the transfer. A maximum of three embryos were transferred. Luteal phase was supported with Cyclogest (Alpharma, Barnstaple, UK) 800 mg vaginally/day was started from the day of oocytes retrieval.

The study population was divided into two groups according to the number of retrieved oocytes; good responders (≥ 4 retrieved oocytes), and poor responders (< 4 retrieved oocytes). Poor ovarian response (POR) to ovarian stimulation usually indicates a reduction in follicular response, resulting in a reduced number of retrieved oocytes. Ferraretti and colleagues, concluded that in order to define the poor response in IVF, at least two of the following three features must be present; (1) advanced maternal age, (2) a previous POR, and (3) an abnormal ovarian reserve test (ORT)[15]. In this study, the outcomes measure; the number of retrieved oocytes, ovarian reserve (POR)15 and numbers of chemical (serum β-HCG >50 mIU/mL two weeks after IVF/ICSI) and clinical pregnancies (detection of fetal heart beats 8 weeks after the initiation of ART cycles).

3. Results

Ninety two (92) infertile couples were included in this comparative study for IVF/ICSI; 68 couples (73.9%) due to male factors, 16 couples (17.4%) due to tubal factors and 8 couples (8.7%) due to unexplained factors. According the number of the retrieved oocytes women included in this study were classified into groups; good responders (≥ 4 retrieved oocytes) = 52 women, and poor responders (< 4 retrieved oocytes) = 40 women. The good and the poor responders were matched with no significant difference regarding mean age, mean duration of infertility and mean BMI (Table 1).

Ovarian reserve in this study was assessed by day-3 basal hormonal levels, and AFC. There was no significant difference between the good and poor responders regarding mean Day-3 FSH (7.62±2.50) mIU/mL versus (8.15±1.90) mIU/mL respectively, mean Day-3 LH (7.25±3.40) mIU/mL versus (6.82±5.20) mIU/mL; respectively and mean Day-3 E2 (42.76±9.60) pg/mL versus (45.21±8.80) pg/mL respectively, while, the mean Day-3 AMH was significantly high (4.93±1.22) ng/mL in good responders compared with poor responders (2.18±0.49) ng/mL (Table 2).

The AFC was significantly high in good responders compared with the poor responders (12.72±5.70) versus

(6.31±2.80) respectively) and the numbers of retrieved oocytes were also significantly high in the good responders compared with poor responders (13.52±9.70) versus (3.91±1.20), also, the numbers of chemical and clinical pregnancies were significantly high (6 cases (75%) and 13 cases (72.2%); respectively) in the good responders compared with poor responders (2 cases (25%) and 5 cases (27.8%); respectively) (Table 3). In this study, the Day-3 AMH and the AFC were positively correlated with the number of the retrieved oocytes and numbers of chemical and clinical pregnancies.

4. Discussion

The AFC is a minimally invasive, easily performed test provides a representation of remaining follicular pool levels to assess the probability of a positive response to COH[3]. The expression of AMH at a constant level independent of cycle day without dependence on other hormones make AMH very attractive as a direct measurement of ovarian reserve[9-11]. So, this study was designed to investigate the role of AMH and AFC in predicting the ovarian reserve and success of IVF.

Ninety two (92) infertile couples were included in this comparative study for IVF/ICSI, and were classified into two groups; good responders and poor responders. The good and the poor responders were matched with no significant difference regarding, mean age, mean duration of infertility and mean BMI, also, there was no significant difference between the two groups regarding, mean Day-3 FSH, LH, and E2, while, the mean Day-3 AMH was significantly high (4.93±1.22) ng/mL in good responders compared with poor responders (2.18±0.49) ng/mL. Feyereisen et al, found that AMH was a marker of ovarian function and the relationship between AFC and serum AMH was stronger than that observed with FSH and E2, also, Nelson and colleagues found that the levels of baseline FSH were significantly higher and the baseline AMH level was significantly lower in the cancelled group compared to the completed cycle group and they concluded that the plasma AMH was a better predictor of live birth and oocyte retrieved compared with FSH[9,16].

In this study, the AFC was significantly high in good responders compared with poor responders (12.72±5.70) versus (6.31±2.80) respectively and the number of retrieved oocytes were significantly high in good responders compared with poor responders (13.52±9.70) versus (3.91±1.20). The Day-3 AMH and the AFC in this study were positively correlated with the number of the retrieved oocytes. In good responders, the high AMH levels and the increased number of the antral follicle were associated with increased number of retrieved oocytes.

Ficicioglu and colleagues, found that the serum AMH levels were more strongly correlated with antral follicle counts than did the serum levels of FSH and E2 and they found a positive association between AMH levels, AFC and number of retrieved oocyte[17].

Seifer and colleagues, found that a higher day-3 serum

Table 1
The characteristics of the studied population.

Variables	Good responders (*n*=52)	Poor responders (*n*=40)	*P* value
Significance age (Years) Mean±SD	28.72±3.80	29.51±2.50	0.56 (NS*)
Duration of infertility (Years) Mean±SD	4.56±2.61	5.32±3.30	0.87 (NS*)
BMI (kg/m^2) Mean±SD	24.78±0.72	25.41±1.57	0.41 (NS*)
Causes of infertility			
Male factor *n*(%)	37 (54.4%)	31 (45.6%)	0.82 (NS*)
Tubal factor *n*(%)	10 (62.5%)	6 (37.5%)	0.76 (NS*)
Unexplained *n*(%)	5 (62.5%)	3 (37.5%)	0.38 (NS*)

NS* = Non significant.

Table 2
Day-3 basal hormonal level of the studied population.

Variables	Good responders (*n*=52)	Poor responders (*n*=40)	*P* value
Significance Day-3 FSH (mIU/mL)	7.62±2.50	8.15±1.90	0.92 (NS*)
Day-3 LH (mIU/mL)	7.25±3.40	6.82±5.20	0.71 (NS*)
Day-3 E2 (pg/mL)	42.76±9.60	45.21±8.80	0.54 (NS*)
Day-3 AMH (ng/mL)	4.93±1.22	2.18±0.49	0.02 (S**)

Data are expressed as Mean±SD. NS* = Non Significant; S** = Significant.

Table 3
The AFC, retrieved oocytes, chemical and clinical pregnancies of the studied population.

Variables	Good responders (*n*=52)	Poor responders (*n*=40)	*P* value
Antral follicles Count (AFC) Mean±SD	12.72±5.70	6.31±2.80	0.001 (S**)
Retrieved oocytes Mean±SD	13.52±9.70	3.91±1.20	0.003 (S**)
Chemical pregnancies *n*(%)	6 (75.00%)	2 (25.00%)	0.010 (S**)
Clinical pregnancies *n*(%)	13 (72.20%)	5 (27.80%)	0.020 (S**)
Multiple pregnancies *n*(%)	3 (3.26%)	1 (1.09%)	0.460 (NS)

AFC = Antral follicles count; S** = Significant; NS = Non significant.

AMH concentrations were associated with greater number of retrieved oocytes and they concluded that the mean serum AMH concentration was more than two fold in the group with <11 retrieved oocytes compared to the group with ≤ 6 retrieved oocytes,18 also, in this study the mean day-3 AMH was more than two folds (4.93±1.22) ng/mL in good responders in them the mean number of oocytes retrieved was (13.52±9.70) ng/mL than poor responders (2.18±0.49) ng/mL in them the mean number of oocytes retrieved was (3.91±1.20).

In this study, the higher serum AMH level and the increased number AFC were associated with increased numbers of chemical and clinical pregnancies, also, Razieh *et al.*, found that the AFC had a significant relation with the number of retrieved oocytes and number of clinical pregnancies[14].

Broer *et al*. found that day-3 AMH levels was related to the number of retrieved oocytes and was able to predict extremes in ovarian response to controlled ovarian hyperstimulation but cannot predict pregnancy after ART treatment[19] also, Ficicioglu and colleagues, concluded that levels of AMH could predict the number of retrieved oocytes with a positive predictive value of 96% and it has little value for predicting pregnancy[17].

In this study, the patients with <4 retrieved oocytes had lower day-3 AMH levels and fewer antral follicles, thus, day-3 AMH and AFC were good predictors for ovarian reserve before IVF and were positively correlated with the number of the retrieved oocytes and numbers of chemical and clinical pregnancies.

Conflict of interest statement

No actual or potential conflict of interest in relation to this article exists.

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