The Role of Follicular Fluid Anti-Mullerian Hormone in Success Rate of Intracytoplasmic Sperm Injection

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Abstract

BACKGROUND: The role of anti-Mullerian hormone (AMH) in the ovary is to participate in the regulation of ovarian function, especially in follicle development and selection. It inhibits the initiation of human primordial follicle growth and prevents multiple selection of a dominant follicle by reducing the sensitivity of follicles to follicle stimulating hormone.

MATERIALS AND METHODS: In this prospective clinical trial, outcomes were followed in 60 women undergoing cycles of *in vitro* fertilization (IVF)/intracytoplasmic sperm injection (ICSI) within El-Minia University Hospital. AMH concentration was estimated in pooled follicle fluid (FF) on day of oocyte pickup. Cycles were sorted into low and high groups according to median (50th centile) values of measurement. The fertilization rate (FR), implantation rate, blastocyst development, embryo quality, chemical pregnancy, clinical pregnancy, and ongoing pregnancy after ICSI were counted as the main outcomes.

RESULTS: Low FF AMH group shows significantly higher percentage of top-quality oocytes (67.1 ± 24.3 vs. 49.6 ± 30.3 %, $p = 0.014$), FR (83.9 ± 20.9 vs. 72.4 ± 21.4%, $p = 0.021$), clinical pregnancy (57.57 vs. 16.67%, $p > 0.0001$), and embryo implantation rates (57.7 vs. 16.7%, $p = 0.001$) compared to high FF AMH group. FF AMH shares an inverse correlation with FF E2 (Pearson $r = -0.409$, $p < 0.001$) and clinical pregnancy (Pearson $r = -0.618$, $p < 0.001$). Threshold value of FF AMH for pregnancy is >1.75 ng/mg protein.

CONCLUSION: FF AMH is a plausible specific indicator of functional viability and quality of oocyte in IVF cycles.

Introduction

Anti-Mullerian hormone (AMH), also referred to as Mullerian inhibiting substance, may be a dimeric glycoprotein member of the reworking growth factor-β family. AMH is secreted by granulosa cells within preantral and early antral follicles, <4 mm in diameter. Its secretion decreases because the antral follicles begin to grow and stops when the follicles are larger 8 mm in diameter or when atresia occurs [1]. A previous study has showed that the performance of AMH as a predictor of poor ovarian response was very almost like that achieved with antral follicle counts (AFCs) [2]. However, AFC was tested within the early stage of the cycle and was evaluated using ultrasound [3].

Thus, the accuracy and stability of AFC testing are inferior thereto achieved with serum AMH. The previous studies have found associations between AMHs (including serum AMH and follicle fluid [FF] AMH), fertilization rate (FR), blastocyst development, embryo quality, pregnancy outcome, and birth rate live birth rates. Some studies showed that prime serum AMH levels on day 3 were correlated with high numbers of mature oocytes, leading to more embryos and ultimately a better clinical pregnancy rate [4]. Other workers found no associations between basal serum AMH levels and embryo quality [5]. An association has also been found between FF AMH (FF AMH) levels and therefore the quality of embryos in patients with polycystic ovary syndrome (PCOS) [6]. However, during this study population, there was no correlation between FF AMH and therefore the degree of maturation of retrieved oocytes or the success of fertilization.

The aim of this work is to know the predictive value of AMH on FR, implantation rate, blastocyst development, embryo quality, chemical pregnancy, clinical pregnancy, and ongoing pregnancy after intracytoplasmic sperm injection (ICSI).

Materials and Methods

This quasi-experimental study was conducted in the Department of Obstetrics and Gynecology, El-Minia Infertility Center Faculty of Medicine, El-Minia University and two private centers during the period

from June 2016 to June 2018 after being approved by the Department of Ethical Committee. The study population included 60 women who had their first cycles of ICSI treatment.

Inclusion criteria

The women with age ≤38 years, the body mass indexes (BMIs between 18 and 29 kg/m^2), day 3 serum follicle-stimulating hormone (FSH) levels <12 IU/L, the women with a history of regular, ovulatory menstrual cycles (every 24–35 days), and the women with no previous history of ovarian surgery were included in the study.

Exclusion criteria

Women with ovarian cyst (>3 cm in diameter), women with PCOS, women with endometriosis, and women with a history of ovarian surgery or endocrine disorders were excluded from the study.

Methods

- All patients were subjected on day 3 of the menstrual cycle and before treatment, blood samples for assay of AMH, FSH, E2, and luteinizing hormone (LH) were collected by venipuncture
- Ultrasound scanning with a 6.5 MHz transvaginal probe was used to count the number of antral follicles in each ovary that had a mean diameter of 3–10 mm
- AMH was measured using the immunotech enzyme immune assay kit consistent with the handbook. On the day of ovum pick-up, under transvaginal ultrasound guidance, fluid from 3 to 5 dominant follicles was gently and thoroughly aspirated employing a 10 mL syringe. The fluid was maintained at 37°C until the oocyte was found and isolated
- The level of AMH in FF was measured as described above
- All patients received standard ovarian stimulation protocol with recombinant FSH under pituitary suppression with a GnRH agonist
- Briefly, the GnRH agonist (Decapeptyl, 3.75 mg Ferring, Kiel, Germany) was administered subcutaneously in the mid-luteal phase of the previous menstrual cycle
- Stimulation commenced 2 weeks later, when the circulating E2 level was <150 pmol/L, the thickness of endometrium was <5 mm, serum LH was <5 IU/L, and a vaginal ultrasonographic scan showed an absence of follicles more than 10 mm in diameter
- The criteria for human chorionic gonadotropin (HCG) administration are the presence of 3 or

more follicles ≥17–18 mm in diameter with a consistent rise in serum estradiol concentration
- Oocyte aspiration was performed using vaginal ultrasound, 34–36 h after HCG injection
- Egg quality: Metaphase II oocyte collected from the patient varying in qualities, both nuclear and cytoplasmic maturation have to be completed in a coordinate mode to ensure optimal condition for subsequent fertilization. Disturbances of these processes may result in different morphological abnormalities, depending on whether nuclear or cytoplasmic maturation has been affected
- ICSI was performed using standard procedures and the embryos were transferred 2 or 3 days later. The luteal phase was supported with 40 mg progesterone administration by daily injection
- A pregnancy test was carried out on day 14 after embryo transfer
- After 2 weeks, a transvaginal ultrasound was performed to confirm pregnancy
- Study endpoints were FR, the good quality embryos, clinical pregnancy, and biochemical pregnancy
- Then, follow-up of the pregnant women by serial ultrasound was done.

Ethical consent

The nature of the study was clearly explained to each patient. An informed written consent was obtained. Furthermore, an approval from the local committee was taken.

Results

Results were analyzed using SPSS (ver. 25.0; IBM, Chicago, IL, USA). Quantitative data were displayed in the form of mean ± standard deviation (SD). Qualitative data were demonstrated through figures of frequency and percentage. Table 1: This table showed that age, BMI, baseline AMH, and E2d HCG showed statistical insignificant difference between both groups, while FF E2 was significantly higher among low than high group (p = 0.001). Table 2: This table showed significantly higher rates of fertilization, more number of

Table 1: Hormone data in low versus high FF AMH groups

Variables	Low FF AMH (≤1.720 ng/mg protein) (n=30)	High FF AMH (>1.720 ng/mg protein) (n=30)	Test value	p-value
Age (years)	32.6 ± 1.09	32.5 ± 1.04	0.482	0.631[1]
BMI (kg/m^2)	27.6 ± 1.4	28.03 ± 1.4	1.196	0.231[1]
Baseline (d3) serum AMH (ng/mL)	1.73 ± 0.21	2.24 ± 0.30	2.120	0.195[1]
E2 d hCG (pg/mL)	1221.9 ± 21.11	1185.3 ± 17.8	0.921	0.420[1]
FF E2 (pg/mL)	262628.5 ± 155320	164853.8 ± 52411	29.04	0.001*[1]

[1]Independent *t*-test used. *Statistical significant when p<0.05. AHM: Anti-Mullerian hormone, FF: Follicle fluid, BMI: Body mass index.

Table 2: Embryology data in low versus high FF AMH groups

Variables	Low FF AMH (≤1.720 ng/mg protein) (n=30)	High FF AMH (>1.720 ng/mg protein) (n=30)	Test value	p-value
No. of oocytes retrieved	416	402	1.23	0.731[1]
Topquality oocytes (%)	67.1 ± 24.3	49.6 ± 30.3	23.81	**0.014*[1]**
Fertilization rate (%)	83.9 ± 20.9	72.4 ± 21.4	18.56	**0.021*[1]**
Total no. of embryos transferred (mean)	140 (1.98 ± 0.87)	159 (2.29 ± 0.91)	31.34	**0.016*[1]**
No. of clinical pregnancies (%)	17 (56.7)	5 (16.7)	10.34	**0.001*[2]**
No. of twin pregnancies (%)	3 (10)	2 (6.7)	0.220	0.884[3]
Embryo implantation rate (%)	10 (33.3)	5 (16.7)	17.89	**<0.001*[2]**

[1]Mann–Whitney U- test, [2]Chi-square test, [3]Fisher's exact test. [4]Statistically significant when p<0.05.
AHM: Anti-Mullerian hormone, FF: Follicle fluid, BMI: Body mass index.

top-quality oocytes, and higher clinical pregnancy and embryo implantation rates than high FF AMH group. However, the twin pregnancy rates were comparable and did not differ significantly between the two groups. Figure 1: This figure showed that clinical pregnancy had significant indirect correlations with AMH. Figure 2: ROC curve showed that AMH had 73.1% sensitivity and 85.3% specificity for predication of clinical pregnancy.
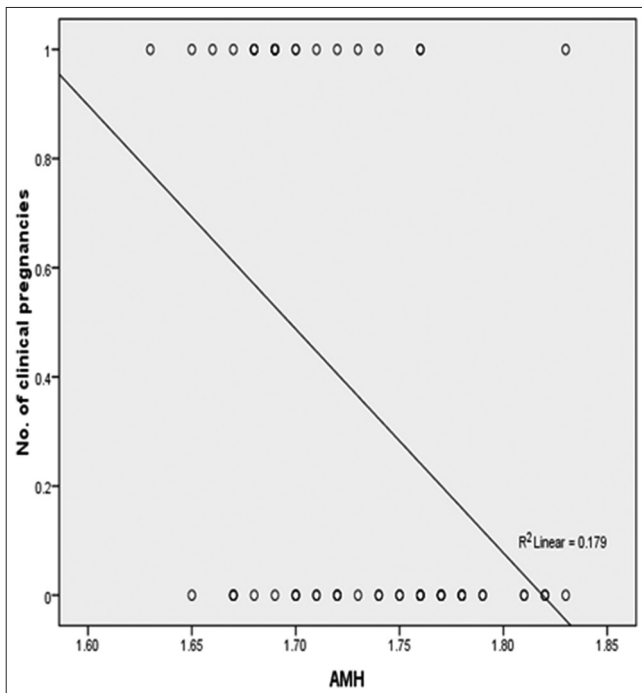

Figure 1: Correlation clinical pregnancy and anti-Mullerian hormone

Discussion

The role of AMH within the ovary is to participate within the regulation of ovarian function, especially in follicle development and selection. It inhibits the initiation of human primordial follicle growth and prevents multiple selection of a dominant follicle by reducing the sensitivity of follicles to FSH [7]. Several reports suggest that AMH could be a far better predictor of ovarian responses to controlled ovarian hyperstimulation than traditional parameters such as age, FSH, estradiol (E2), and inhibin B (INH-B) [8]. Our results, completely conform to and are in conjunction with earlier reports of a progressive decline in AMH levels during ovarian stimulation, hence confirming the decreased ability of maturing follicles to supply AMH [9].

Similar to Mehta *et al*. [10] study in which FF AMH shares an inverse correlation with FF E2 (Pearson $r = -0.43$, $r^2 = 0.18$) and clinical pregnancy (Pearson $r = -0.46$, $r^2 = 0.21$). Another study administered in monofollicular fluid (FF obtained from each individual follicle) of stimulated cycles by Takahashi *et al*. [11] reported correlation of upper FF AMH levels with higher rates of fertilization. However, they might not associate it with pregnancy outcome. Moreover, their study involved comparison of FF AMH between two broad groups, namely, fertilization success versus fertilization failure. Wunder *et al*. [12] correlated higher FF AMH with reproductive outcome in *in vitro* fertilization (IVF)-ICSI cycles. Some other observe done using Fanchin *et al*. [13] in monodominant follicles (single lead follicle) of unstimulated cycles, mentioned correlation of FF AMH with implantation rates, and pregnancy outcome but not with fertilization costs. (have a look at, Aflatoonian *et al*. [14] correlated FF AMH with fertilization and embryo excellent). Concerning AMH validity, our take a look at consequences determined that AMH >1.75 ng/mg had 73.1% sensitivity and 85.3% specificity for predication of medical being pregnant.

Furthermore, Mehta *et al*. [10] have a look at located that AMH >1.75 ng/mg had 80% sensitivity and 63.1% specificity for predication of medical being pregnant. Regarding bad reaction, the AMH cutoff price is 1.26 ng/mL with a sensitivity of 72.0% and a specificity of 86.4%. Patients with AMH underneath
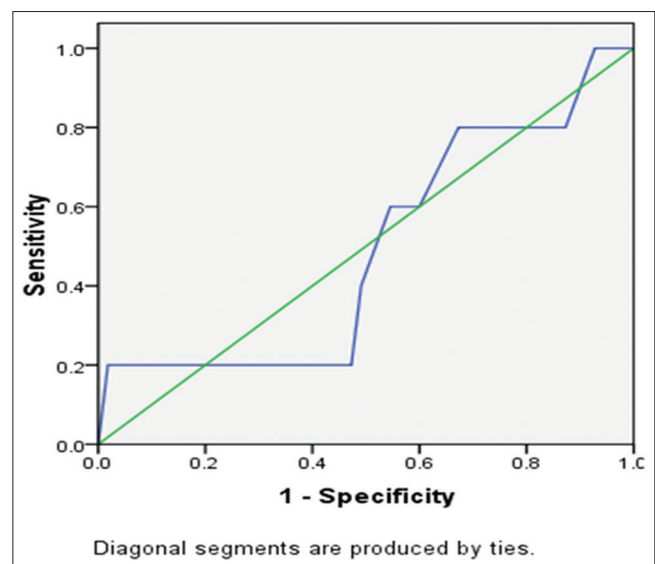

Figure 2: Receiver operating characteristic curve for anti-Mullerian hormone

this threshold have to be knowledgeable earlier in their fantastically low opportunity of reaching being pregnant because of a substantially higher rate of no available embryos (36.9 vs. 7.3%, $p < 0.001$). Nevertheless, it must now not be utilized in isolation as the criterion for withholding fertility treatment [15]. A major strength of the current study stems from its prospective and randomized design.

Conclusion

Our study demonstrates that FF AMH is an adequate predictor of clinical pregnancy after ICSI. Further studies are urgently needed to investigate the efficiency, safety, and cost-effectiveness of individualized gonadotropin dosing based on the AMH level before IVF.

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