

## Hormonal Profile in Intracytoplasmic Sperm Injection Cycles may Predict Poor Responders: A Comparative Study

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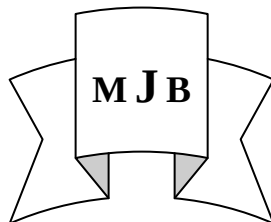
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### Abstract

**Background:** Ovarian response varies considerably among individuals and depends on various factors. Poor response in In Vitro Fertilization (IVF) yields lesser oocytes and is associated with poorer pregnancy perspective. Cycle cancellation due to poor response is frustrating for both clinicians and the patient.

**Objective:** This paper studies some ovarian reserve hormonal profiles which may be a contributing factor for poor ovarian response in Intra Cytoplasmic Injection (ICSI) cycles.

**Design:** A prospective controlled trial.

**Setting:** Fertility unit in Al- Sadar medical city, Najaf province.

**Subjects and Methods:** Eighteen poor responders to controlled ovarian hyperstimulation with gonadotropin have been withdrawn from 67 initially selected participants intended to undergo intracytoplasmic sperm injection as a treatment option for their infertility. Basal cycle day 2 hormonal levels for follicular stimulating hormone (FSH), Anti mullerian hormone (AMH) and estradiol (E2) as well as antral follicular count (AFC) were measured and compared for both groups.

**Results:** The poor responder group found to be of higher age and basal serum FSH and lower basal serum AMH.

**Conclusions:** some markers of ovarian reserve may be used as a predictor of poor responders in assisted reproduction programme.

**Key words:** Anti mullerian hormone (AMH), Follicle stimulating hormone (FSH), Estradiol (e2), antral follicular count, Intracytoplasmic sperm injection (ICSI), Poor responders.

### الخلاصة

تختلف استجابة المبيض للمحضرات الخارجية أختلافا كبيرا بين النساء و تعتمد على عوامل مختلفة . ان ضعف استجابة المبيض عند اجراء التقنيات المساعدة على الإنجاب يؤدي الى قلة عدد البويضات المستحصلة وضعف منظور الحمل. ان الغاء الدورة العلاجية بسبب ضعف استجابة البويض امر يؤثر الأحياط لدى الأطباء والمريض. الهدف من هذه الدراسة : تحديد ملامح احتياطي المبيض الهرموني والتي يمكن ان تكون عاملا مساهما في هذا الضعف عند اجراء عمليات الحقن المجهرية للحيوانات المنوية داخل البويضة . تمت هذه الدراسة في وحدة الخصوبة في مدينة الصدر الطبية في مدينة النجف الأشرف . تم أستحصل ثمانية عشر ضعيفة استجابة من اصل سبعة و ستون يخضعون لعلاجات تحريض الإباضة في برنامج الحقن المجهرية كخيار علاجي لحالة عدم الإنجاب التي يعانون منها. تم قياس مستويات مصل الدم المستحصل ثاني يوم الدورة الشهرية لكل من هرمون المحرض للجريبات ،انتي موليرين هرمون وهرمون الأسترايول و تم ايضا قياس عدد الجريبات الغارية ، وتم مقارنة نسب هذه الهرمونات لكلا الفريقين. تم استنتاج ان عمر فريق ضعيفي الاستجابة وهرمون المحرض للجريب أعلى من الفريق الأخرولكن الأنتي موليرين هرمون اوطأ في فريق الضعيفي الاستجابة . من هذه النتائج يمكن توقع الضعيفي الاستجابة في برامج المساعدة على الإنجاب من خلال اجراء فحوصات احتياطي المبيض.

## Introduction

Ovarian response to exogenous stimulus varies considerably among individuals and in the same individual as well [1]. Ovulation induction and controlled ovarian stimulation aim at achieving a distinct ovarian response, which predicts the success of such treatment [2]. To select best embryos, it is important to obtain several embryos [3,4] Poor response in IVF yields lesser oocytes and is associated with poorer pregnancy perspective. Older patients and patients with elevated basal FSH are known to respond poorly owing to the poor ovarian reserve [5]. Younger patients with no obvious cause are also found to respond poorly in IVF. In fact, poor responders can be those with low ovarian reserve and those with normal ovarian reserve but inherent low response to gonadotrophin [1]. Although no definite cause could be attributed, differences in metabolism of exogenous FSH could be responsible for the varying ovarian response [6]. Hence, age cannot be taken as a sole predictor of cumulative pregnancy to identify poor prognosis cases [7, there is a need for predictors of poor response which could be used to reduce cycle cancellation rate. Antral follicle count may be considered as the best single predictor for poor ovarian response than age and endocrine markers [8]. Apart from basal FSH and AFC, several other factors like basal inhibin and AMH are currently being investigated as predictors of ovarian response. There are various female and male lifestyles habits, which could possibly affect success rates in ART, smoking has been proved to affect IVF outcome negatively whereas role of stress, caffeine and alcohol is inconclusive [9]. Cycle cancellation due to poor response is frustrating for both clinician and the patient and demands meticulous follow up. Studies

have shown that women conceiving after poor ovarian response have more pregnancy complications like Pregnancy induced hypertension and preeclampsia than women with normal ovarian response [10]. In addition, poor ovarian response could be a predictor of early menopause [11]. This paper studies various demographic and hormonal profiles of poor responders which could contribute to poor ovarian response in ART.

## Subjects and Methods

This is a prospective comparative analytic study of 67 women underwent ICSI treatment at the fertility unit of Al Sadder medical city of Al Najnaf province, from December 2010-April 2011. Patients, medical history were recorded. All patients underwent general physical and gynecological examination height and weight measurement, in addition to serological tests for hepatitis B&C viruses as well as for Human Immunodeficiency virus antibodies. Hysterosalpingiography was done for tubal assessment, thyroid function test and serum prolactin was evaluated. Patients' partners were requested for seminal fluid analysis.

### Selection Criteria:

1. Male partner with normal semen analysis according to World Health Organization(W.H.O) guideline, (concentration> 15 million /ml, motility > 32% and strict morphology > 4, [12].
2. Regular menstrual cycle of 21-35 days.
3. Female partner have normal both ovaries; visible on US not having poly cystic ovaries (PCO ) defined by The Rotterdam ESHRE / ASRM Criteria (2004) [3]; since woman with ( PCO) may have high serum hormonal levels during ovarian hyper stimulation

[14], and have lower fertilization rate [15].

4. The cause of infertility is either unexplained (n=43) and the couple underwent at least three trials of intrauterine insemination; or tubal factor infertility (n= 24) but not hydrosalpinx.

5. Vaginal U\S show no uterine fibroid; anomaly or ovarian cyst measuring 20 mm or more in diameter.

6. No endocrine cause for their infertility such as hyperprolactinaemia or hyperandrogenism.

7. Screening tests for hepatitis B and C as well as for human immune deficiency viruses (HIV) prove to be negative.

8. The participants have their first or previous trials of assisted reproduction technique.

9. Informed written consent was obtained from the patients.

#### **Hormonal Assay:**

Five mL of blood was drawn on cycle day 2. The blood was centrifuged at 3500 rpm for 10 minutes and sera were stored at -20 °C; subsequently AMH sera levels were measured with AMH\Gen II analysis kit (Beckman coulter, USA) using Enzyme Linked ImmunoSorbant Analysis(ELISA). The interassay Coefficient of Variance (CV) % was 4.5 and the intra-assay CV% was 5.6, the lower limit of detection was .08 ng/ml within 95% probability. Kits for measurement of FSH was (bioMérieux® France) using Mini VIDAS analysis. Interassay CV% was 4.7 and intraassay CV% was 5.9, lower limit of detection was ≤0.1mIU/ml, within 95% probability. The kit for E2 measurement was ( bioMérieux® France) using Mini VIDAS analysis, interassay CV% was 4.6, interassay CV% was 3.2 minimal detection limit was 9 pg/ml Within 95% probability. Ultrasound was performed cycle day 2

by the radiology specialist of the center using real time ultrasound device (Philips 11\*E), using vaginal probe (7 MHZ), Follicles measuring 2-8 mm were counted from the lateral to medial margin of each ovary to determine the antral follicle cohort. The total number of the follicles per patient counted in both ovaries

was used for calculation.

In all patients treated for ICSI, the protocol for pituitary down-regulation was by short protocol the patient receives 0.1 mg/day of GnRH analogue as a morning dose and follicular stimulating hormone FSH as an evening dose. These are given subcutaneously . Decapeptyl in the form of Diphereline 0.1 mg (triptoreline Beaufour pharma, France) and FSH in the form of (Gonal-f, Follitrop alfa 75 IU /ampul ,serono .Switzerland). The dose can be administered either via step up or step down protocol where the dosage of FSH is maintained or gradually increased or initial high dose are tailored down. Participants were monitored for follicular recruitment and growth by serial transvaginal ultrasound and serum E2 from the 6th day of stimulation with gonadotropins. Titration of FSH upward or downward is based on response of

consecutive cycles which were cancelled because of a poor follicular response were initially selected. Those were participant that did develop less than 3 follicles measuring 18 mm after 14 days of FSH treatment ,or E2 level < 100 Pg/ml, have their treatment cycle cancelled or was converted to have intra uterine insemination depending on their clinical factor of infertility (poor responder, n= 18); those considered to have an oocyte retrieval = zero, [16] The remaining 49 women having a completed ICSI cycle and have been analyzed as a control to those in the cancelled group. When at least 2 dominant follicles of 17 mm in

diameter in each ovary is ready, ovulation is triggered by HCG, pregnyl 5000- 10000 IU ( HCG, Prygnyl, Organon, Holland) intramuscular. Thirty six hours after HCG trigger, follicular aspiration is done by transvaginal guidance. Transvaginal ovum pick up were done guided by transvaginal ultrasound. The oocytes were incubated and evaluated for maturity after their denudation. ICSI was done under inverted microscope with manipulators (Bickland industrial, UK). Embryo transfesr were done 48-72 hours after ICSI. Embryos were graded under the microscope, and then transferred to the uterus with Labotec

catheter. Luteal phase was supported by Progesterone vaginal cream (Crinon 8%, Serono, U.K), Duphastone tablets (10 mg, Solvay pharmaceutical, Holland), Aspirin tablet (Aspin-100mg/enteric coated tab. SDI. Iraq), and Folic acid tablet (5 mg/tab. SDI- Iraq). On the fourteenth day of embryo transfer serum B- HCG was performed to confirm pregnancy.

Statistical analysis: descriptive statistics were expressed as mean and standard deviation. Student's t- test was used to compare groups. P- Values < 0.05 were considered to be significant. Statistical analysis was performed using SPSS version 17.

**Table 1** Demographic characteristic of the studied population. Values are mean  $\pm$  standard deviation. (n= 67).

| Base line characteristic       |                   |
|--------------------------------|-------------------|
| Aga(years)                     | 31.76 $\pm$ 6.85  |
| Duration of infertility(years) | 8.09 $\pm$ 4.79   |
| BMI(Kg/m <sup>2</sup> )        | 28.23 $\pm$ 5.08  |
| Type of infertility            |                   |
| Primary (no)%                  | (54) 80.59        |
| Secondary                      | (13) 19.41        |
| Etiology                       |                   |
| Unexplained (no)%              | (43) 64.2         |
| Blocked tube (no)%             | (24) 35.8         |
| Cycle day 2                    |                   |
| Basal FSH(ml IU/ml)            | 5.75 $\pm$ 2.61   |
| Basal AMH(ng/ml)               | 2.90 $\pm$ 3.44   |
| Basal E2(pg/ml)                | 39.64 $\pm$ 19.67 |
| Total AFC                      | 7.42 $\pm$ 2.87   |

**Table 2** Comparison of ovarian reserve tests in the control and poor responder group.

| Parameter                | Normoresponders<br>n=49 | Poor responders<br>n= 18 | p- value |
|--------------------------|-------------------------|--------------------------|----------|
| Age (years)              | 29.75 ± 6.34            | 37.22 ± 4.94             | <.0005   |
| BMI (Kg/m <sup>2</sup> ) | 27.73 ± 4.79            | 32.16 ± 12.19            | NS       |
| FSH(mIU/ml)              | 5.37 ± 2.13             | 6.78 ± 3.49              | <0.05    |
| E2 (Pg/ml)               | 39.72 ± 17.61           | 39.43 ± 25.03            | NS       |
| AMH(ng/ml)               | 3.38 ± 3.32             | 1.58 ± 3.49              | <0.05    |
| AFC                      | 7.79 ± 2.62             | 6.39 ± 3.31              | NS       |
| Total no. of follicles   | 14.16 ± 6.36            | 1.11 ± 00.96             | <0.0005  |

**Results**

Table (I): shows patients characteristics, basal AMH, FSH, estradiol serum concentrations, and total antral follicles count (AFC) in the whole study population.

Table (2) compares the mean age of the control group and the poor responders. The subjects in the poor responder group are significantly older than the controls ( $p < 0.0005$ ) ( $29.75 \pm 6.34$  vs  $37.22 \pm 4.94$  years). The BMI (mean  $\pm$  SD) in the control group was lower than that of the poor responder group ( $27.73 \pm 4.79$  kg/m<sup>2</sup> vs.  $32.16 \pm 12.19$  kg/m<sup>2</sup>) but this difference haven't reach significant level. The difference in serum levels of E2 and AFC was insignificant between the two groups. ( $39.72 \pm 17.61$  pg/ml vs  $39.43 \pm 25.03$  pg/ml) and ( $7.79 \pm 2.62$  vs.  $6.39 \pm 3.31$ ) respectively.

Serum levels measurement of FSH in the poor responders group were significantly higher than those of the

control group ( $6.78 \pm 3.49$  mIU/ml v s  $5.37 \pm 2.13$  mIU/ml),  $p < 0.05$  AMH measurement in the two groups reveals significant difference ( $p > 0.05$ ) between them, ( $3.38 \pm 3.32$  ng/ml) in the control group and ( $1.58 \pm 3.49$  ng/ml) in the poor responder group.

As expected the number of follicles recruited was significantly higher in the control group.

All control patients underwent ICSI and embryo transfer and the total number of ICSI and ET was 44 cycles. Fertilization rate was 63.18%, cleavage rate 70.97% and pregnancy rate was 23.25%.

**Discussion**

Poor ovarian reserve to ovarian stimulation usually indicates a reduction in follicular response, resulting in a reduced number of retrieved oocytes [17]. Poor ovarian response after

IVF stimulation requires thorough counseling prior to ovum pick up (OPU), regardless of the woman's age. In poor responders >37 years of age, especially those who require high FSH doses, the decision whether or not to proceed to OPU should include the couple after

thorough counseling, even though the chance of successful outcome is extremely low [18,19].

The concept of female poor responders are represented by normovulatory women showing a 'gonadal failure' in term of inadequate number of recruited follicles under conventional controlled ovarian hyperstimulation (COH) for assisted reproductive technologies (ART) is applied to our poor responders group. Age is an established indicator of poor response as with advancing age there is depletion of primary oocytes quantity and quality [20]. The significantly higher mean age of poor responders group compared to good responders group in our study ( $37.22 \pm 4.94$  vs  $29.75 \pm 6.34$  year) ( $p < 0.0005$ ) may be added to and confirmed by several studies [21, 22]. It seems that the age-related decline in fertility may be due more to degenerative oocytes than to aneuploidy. A decline in the number of oocytes retrieved with age may be of less importance than the decline in oocyte quality. Women in the older age group have a higher chance of achieving pregnancy from ovum-donation programs than by persisting in using their own aged oocytes, which have a very poor prognosis for success [23], or they may benefit from the use of IVF modified natural cycle (mini IVF) [21]. The insignificant difference in BMI in the two groups is in accordance with Moini et al who find that BMI as an independent factor had no effect on ovarian reserve markers and ovarian response [24]. On the contrary some

other researchers found an association between BMI and poor response to gonadotropins stimulation since obesity may have negative impact on hypothalamic pituitary ovarian axis [25], which is applicable to our poor responders group where they are considered to be as obese ( $BMI 32.16 \pm 12.19$  Kg/m<sup>2</sup>). In contrary to the control where they can be considered as over weight ( $BMI 27.73 \pm 4.79$  Kg/m<sup>2</sup>) according to WHO classification of obesity [26]. AMH is a glycoprotein that belongs to the transforming growth factor-B (TGF-B)—a member of the superfamily of growth and differentiation factors. AMH is solely produced by the granulosa cells of the growing ovarian follicles, serum levels may be used as a marker for ovarian reserve, representing the quantity and quality of the ovarian follicle pool [27], and that the initial AMH level is associated with ovarian response in In vitro fertilization (IVF) patients with normal FSH levels. [28]. Recent preliminary reports indeed indicate that AMH levels decline with increasing female age [29,30]. The mean serum AMH levels of poor responders group were found to be significantly lower than that of good responders ( $1.58 \pm 3.49$  vs.  $3.38 \pm 3.32$  ng/mL),  $p < 0.05$ , a finding which is concluded by other researchers [21, 30, 31].

Muttukrishna and colleagues found that the levels of basal FSH were significantly higher in the cancelled group and the mean AMH level was significantly lower in the cancelled group compared to the completed cycle group, a findings that confirm our results [32]. Van Rooij et al. demonstrated that the basal levels of FSH, but not E2, were higher in poor responders and that AMH levels were lower in the poor responders compared to normal responders results which consolidate ours [33].

In our study, we observed that patients with <3 recruited follicles had higher age and day 3 FSH level, but lower day three AMH levels. Thus, these markers may be good predictors used incounseling patients attending infertility clinic [34, 35]. The insignificant difference in the AFC in the two groups in spite of the highly difference in thenumber of the recruited follicles in the controls ( $14.16 \pm 6.3$  Vs  $1.11 \pm 0.96$ )  $p < 0.0005$ , may be due to the fact that the number of FSH-sensitive follicles is limited in the poor responder group which may be hereditary in nature [20]. In such patients aggressive stimulation is not expected to increase the number of follicles because there are simply too few. Several alternative stimulation protocols, such as the flare-up protocol, have been suggested for the treatment of poor responders in IVF. However, the efficacy of such alternative regimes has never been established in large-scale prospective randomized trials and it seems that any strategy in patients with a diminished ovarian reserve is bound to fail [36, 37] Basal FSH values obtained during the infertility work-up were significantly higher in the poor responders.

Inspite of these studies Peñarrubia et al., concluded that the history of an IVF/ICSI cancelled cycle due to poor follicular response in a standard stimulation protocol is a better predictor ofcancellation in subsequent treatment cycles than age or FSH [38].

### **Conclusion**

Some ovarian markers such as age, AMH and FSH may predict the response to gonadotropins stimulation in ART and expecting poor responders.

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