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Antral Follicle Count versus Basal Follicle Stimulating Hormone as Predictors of Ovarian Response in Women Undergoing Superovulation with Long Protocol for Assisted Reproduction

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ABSTRACT

Background: the prediction of ovarian response before undertaking the expensive IVF treatment is quite important; it seemed that Patient characteristics, rather than the stimulation protocol are the determinant of the individual response. Advance identification of patient who will elicit a poor response or hyper response to standard treatment would be of great clinical advantage. Several parameters have been postulated as predictors of the ovarian response. **Aim of work:** to compare between bFSH and AFC in predicting the ovarian response in women undergoing super-ovulation with long protocol for assisted reproduction.

Patients and Methods: this is an observational cohort study that included 80 infertile women who performed IVF/ICSI. It took place at Assisted Reproductive Technology (ART) Unit. Ain shams University Maternity hospital. No extra interventional measures were taken in the study apart from routine investigations and procedures done during ART therapy, being already approved in signed consent to undergo treatment. Patients who are fulfilling the inclusion and exclusion criteria were enrolled in the study. They all underwent superovulation with long GnRH agonist. They were followed up through the stimulation protocol steps, then data were analysed. **Results:** all basal measured data such as Age *p. value* =0.035, AFC *p. value* =0.000, basal FSH *p. value* =0.000 and basal E2 p. value =0.020 showed statistical significant difference between the good responders and poor responders when both groups were compared, When we apply ROC curve to compare between AFC and basal FSH as regards both (good and poor responders) to detect which one of them is better, there was no statistical significance difference between them with p. value = 0.371, the best cut off point for AFC as a predictor for good responders was found > 3 follicles with sensitivity of 90.5%, specificity of 94.1% and area under curve (AUC) of 97.5%, while while the best cut off point for basal FSH level was found ≤ 8 with sensitivity of 77.78%, specificity of 100% and AUC of 93.7%.

Conclusion: AFC and basal FSH are good predictors of ovarian response in women undergoing superovulation with long protocol. There was no absolute superiority of AFC on basal FSH in predicting ovarian response. Age and basal E2 are also considered good predictors of ovarian response.

Keywords: Antral Follicle Count, Basal Follicle Stimulating Hormone, Ovarian Response, Superovulation, Long Protocol for Assisted Reproduction.

INTRODUCTION

The prediction of ovarian response before undertaking the expensive IVF treatment is quite important [(1)] especially with assisted reproduction program in which the response of ovulating woman to exogenous gonadotropin therapy is often inconsistent[(2)]. Patient characteristics, rather than the stimulation protocol seem to determine the individual response [(3)]. In young ovulating women undergoing in vitro fertilization treatment, the standard stimulation protocol can result in either poor response or ovarian hyper stimulation syndrome [(4)], the later, however, is one of the challenging complications [(5)].

Poor ovarian response to gonadotropin stimulation results in small number of oocytes collected and thus a smaller number of embryos available for transfer, which therefore reduces the success rate of IVF [(6)]. Advance identification of patient who will elicit a poor response or hyper response to standard treatment would be of great clinical advantage [(7)].

Several parameters have been postulated as predictors of the ovarian response [(8)] all of which stripe to assist ovarian reserve [(9)]. Ideal ovarian reserve parameter should be easily measurable, minimally invasive, inexpensive, and should have a good predictive value [(10)].

Ultrasonographic measurements of antral follicle count (AFC) and ovarian volume have also been explored as predictors of response to ovulation induction [(11)]. 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 hyper stimulation (COH) and success of IVF [(12)].

Antral Follicle Count is the sum of antral follicle in both ovaries, as observed with transvaginal ultrasonography during the early follicular phase. Most studies have defined antral follicles as those measuring 2-10 mm in mean diameter in the greatest 2-dimensional (2-D) plane; some have defined antral follicles as those

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measuring 3-8 mm in mean diameter [13]. The AFC is a good predictor of the number of retrieved oocytes and rate of cancellation in IVF after COH [14]. It was also clear that women with fewer antral follicle needed longer duration and higher dosage of gonadotropin during the stimulation [15].

AIM OF WORK

To compare between bFSH and AFC in predicting the ovarian response in women undergoing super-ovulation with long protocol for assisted reproduction.

PATIENTS AND METHODS

Study Design: This is an observational cohort study that included infertile women who were involved in Assisted Reproduction Technique (ART).

Study Site: was carried out at Assisted Reproductive Technology (ART) Unit. Ain shams University Maternity hospital.

Population: 80 infertile women who performed IVF/ICSI were enrolled in this study after obtaining oral consent as there are no extra interventional measures were taken in the study apart from routine investigations and procedures done during ART therapy, being already approved in signed consent to undergo treatment

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Patient Selection and Management

Patients within our trust who were attending the infertility clinic and Assisted Reproductive Technology Unit (ART) of the hospital complaining of infertility were approached. They were invited to take part in the study by the investigator. At first visit, general data were obtained for criteria of eligibility including full history taking and general examination. Then Base-line Transvaginal ultrasound scanning (TV/US) was performed to detect morphological changes in ovary and uterus.

Patients who are fulfilling the inclusion and exclusion criteria were enrolled in the study, the nature of the study was explained and signed written consent was obtained. All the participants were assured that the information gathered through the study would be kept confidential, being collected anonymously. The data of shared women were filled in a case record form (CRF I). This was stored in independent premises far from routine files held by authorized people.

Inclusion Criteria

1. Age between: 18-40 years.
2. Body mass index (BMI) between 20 and 30.
3. Regular menstrual cycles ranges 25-35 day.
4. Both ovaries present deprived of morphological abnormalities and adequately visualized in TVS.
5. Long-GnRH agonist /HMG regimen is to be used.

Able to communicate well with the investigator & to comply with the requirements of the entire study.

Signed informed consent obtained from subject and husband / witness before undergoing ART and study entry (with the understanding that consent may be withdrawn by the patient at any time without prejudice).

Exclusion Criteria

1. Current or past ovarian diseases such as polycystic ovary syndrome (PCOs), previous ovarian cystectomy, previous endometriosis symptoms or concurrent endometriosis.
2. Signs or symptoms of disturbed endocrinological functions (as hyperandrogenism, hypo- or hyperthyroidism, hyperprolactinemia).
3. Gross lesion found in the ovaries, tubes and uterus as visualized on TVS.

Known allergic reaction against one of the ingredient of the medications used during the study.

Study Method

On day 2-3 of spontaneous cycles all planned women to have super-ovulation had basal hormonal profile FSH, LH and E2. TVS on day 2-3 of un-stimulated cycles was done by transvaginal probe 5-9 MHZ (MINDRAY DP 8800 plus) and antral follicle was counted as the number of 2-10 mm diameter follicles measured in both ovaries. Any patient discovered to have uterine, tubal and ovarian pathology and was excluded from the study. Ovarian hyper-stimulation protocol was performed according to long protocol, on day 18 of the preceding cycle to superovulation, Gn-RH agonist injection of (Decapeptyl 0.1mg, triptorelin, Ferring and Switzerland) was started daily till the day of HCG, on day 2-3 of the cycle ovarian hyper stimulation was started by daily injection of gonadotrophins (Mengon 75mg, menotrophin, Ferring and Switzerland) after confirming the suppression of the ovaries by measuring E2 and endometrial thickness.

The starting dose of gonadotropin was prescribed according to age, BMI of the subjects and FSH, and subsequently, the dose was adjusted according to sonographic response of the ovaries.

Ovarian response was assessed by TV/US folliculometry to be done on cycle day 6. According to the ovarian response, TV/US was performed every other day and at the moment where the leading follicle reach 15 mm daily TVS was performed daily till the largest follicle reach a diameter > 18 mm with good count of secondary follicles when HCG (Pregnyl 10,000 IU/amp Organon, DSS,

Netherlands) was administered. Serum estradiol (E2) was measured on HCG day. The number and sizes of developed follicles on HCG day were recorded. Thirty six hours after HCG injection, ovum pick up was done, good response was defined as ≥4 oocytes at the ovum pickup day. The quantity and quality of retrieved oocyte was obtained in the CRF.

We then follow up of oocytes and formed embryos through different stages in the incubator, till the specified days of embryos transfer for each patient, 14 days later pregnancy test in blood (quantitative βHCG) to detect biochemical pregnancy which is defined as the pregnancy being too early to confirm except through biochemical means, but the baby and gestational sac will not develop enough to be visible on an ultrasound. Whereas clinical pregnancy is a pregnancy that is confirmed by both high levels of hCG and ultrasound confirmation of a gestational sac or heartbeat (fetal pole).

RESULTS

We observed 80 infertile women referred to Assisted Reproductive Technology Unit (ART) at Ain shams University Maternity hospital, who underwent super-ovulation with long protocol for assisted reproduction. Our study was to compare between the prediction power of AFC and basal FSH.

It was noted that the mean age of the studied group was about 32 years and more than half of them suffered from 1ry infertility mostly due to male type infertility as shown in table (1).

Table (1): Demographic and basal measures of included women

Age (years)	31.61 ± 4.51
BMI	27.16 ± 2.74
Infertility	
Primary	49 (61.2%)
Secondary	31 (38.8%)
Duration of infertility	5.53±3.6
Type and etiology of infertility	
Male	43 (53.8%)
Combined	4 (5%)
Unexplained	18 (22.5%)
Female tubal	13(16.3%)
Female uterine	2 (2.5%)
Female ovulatory	0 (0%)
(AFC) ovary	5.00 ± 2.85
Basal FSH level (mIU/ml)	7.54 ± 2.65
Basal E2 level (Pg/ml)	1.37 ± 25.37

Data are presented as mean ± SD, number (Percentage)

Table (2): Number of oocytes retrieved and embryos transferred after stimulation protocol and clinical pregnancy rate

No of oocytes retrieved on ovum pickup day	8.74 ± 5.91
No. of MII oocytes	7.70 ± 5.25
No. of embryos transferred	2.34 ± 0.98
Clinical Pregnancy (+ve)	21 (26.2%)

Data are presented as mean ± SD, number (Percentage). The good responders were defined as the number of retrieved oocyte at the ovum pickup to be ≥ 4 oocytes. Good responders were about 63 patients.

Table (3): Comparison between poor and good responders as regards demographic and basal measures of included women

	Poor responders N=17	**Good responders N=63**	**P-value**
Age (years)	33.65 ± 4.49	31.06 ± 4.39	0.035 [1]
BMI	27.71 ± 2.69	27.01 ± 2.76	0.359 [1]
Infertility			
Primary	11 (64.7%)	38 (60.3%)	0.742 [2]
Secondary	6 (35.3%)	25 (39.7%)	
Duration of infertility	5.59±3.45	5.51±3.67	0.936 [1]
Type and etiology of infertility			
Male	9 (52.9%)	34 (54.0%)	0.132 [2]
Combined	2 (11.8%)	2 (3.2%)	
Unexplained	5 (29.4%)	13 (20.6%)	
Female tubal	0 (0.0%)	13 (20.6%)	
Female uterine	1 (5.9%)	1 (1.6%)	
Ovulatory	0 (0.0%)	0 (0.0%)	
US(AFC)	2.12±0.60	5.75±2.35	0.000 [1]
Basal FSH level (mIU/ml)	10.61 ± 1.59	6.71 ± 2.23	0.000 [1]
Basal E2 level (Pg/ml)	38.75 ± 11.14	54.77 ± 27.07	0.020 [1]

Data are presented as mean ± SD, number (Percentage)

[1]Independent student's t-test was used [2]Chi-square (x2) test was used

All basal measured data such as Age, AFC, basal FSH and basal E2 showed statistical significant difference between the good responders and poor responders when both groups were compared as shown in table (3).

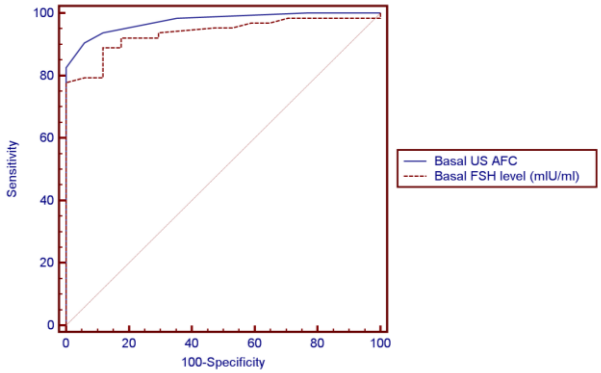
Table (4): Comparison between poor and good responders as regard ovarian response parameters and clinical pregnancy

	oor response N = 17	**od response** N = 63	**-value**
No of oocytes retrieved on ovum pickup day	4.12 ± 3.04	9.98 ± 5.89	0.000 [1]
No. of MII oocytes	3.82 ± 2.81	8.75 ± 5.28	0.000 [1]
No. of embryos transferred	1.53 ± 0.94	2.56 ± 0.88	0.000 [1]
Clinical pregnancy (+ ve)	0 (0.0%)	21 (33.3%)	0.006 [2]

Data are presented as mean ± SD, number (Percentage(

[1]Independent student's t-test was used [2]Chi-square (x2) test was used

Also when comparing both groups regarding No. of oocytes retrieved on ovum pickup day, No. of embryos transferred and clinical pregnancy there were a highly significant difference between both groups.



	AUC	Cut off point	Sensitivity	Specificity	NPV	PPV	P-value
Basal US AFC	0.975	>3	90.5	94.1	98.3	72.7	0.371
Basal FSH level (mIU/ml)	0.937	≤8	77.78	100.00	100.0	54.8	

Fig. (1): Receiver operating characteristic curve (ROC) for predictors of good response

The previous ROC curve shows that the best cut off point for AFC as a predictor for good responders was found > 3 with sensitivity of 90.5%, specificity of 94.1% and area under curve (AUC) of 97.5% while the best cut off point for basal FSH level was found ≤ 8 with sensitivity of 77.78%, specificity of 100% and AUC of 93.7%. Also the p-value (0.371) shows that there was no statistically significant difference between basal FSH and AFC but still AFC have a higher area under curve (0.975) than basal FSH (0.937) which means that AFC was found to be superior to FSH in prediction of patients with good response.

DISCUSSION

Reproductive aging is associated with a reduction of the primordial follicle pool and loss of oocyte quality [(16)]. The number of follicles leaving the pool of resting follicles to enter the growth phase toward the antral stages of development decreases with increasing age [(17)].

Correct identification of patients at risk of poor ovarian response by assessment of ovarian reserve before entering an IVF program is important [(18)]. It can help physicians to tailor their advice to individual couples and help patients to decide whether to proceed with a costly and often demanding and disappointing IVF treatment, or not [(19)].

The current study compares the accuracy of AFC with that of basal FSH in predicting of ovarian response to stimulation (using GnRH-a long protocol) and clinical pregnancy in IVF. The results show that both parameters are good in predicting ovarian response, there was no statistically significant

difference between basal FSH and AFC but still AFC have a higher area under curve (0.975) than basal FSH (0.937) which means that AFC was found to be superior to FSH in prediction of patients with good response.

In 2005 a meta-analysis carried by Hendriks ***et al.*** [20] identified 11 studies on AFC and an updated total of 32 studies on basal FSH from the literature on the basis of preset criteria. The estimated summary receiver operating characteristic (ROC) curves showed AFC to perform well in the prediction of poor ovarian response. Also, prediction of poor ovarian response seemed to be more accurate with AFC compared with basal FSH. The estimated summary ROC curves for the prediction of non-pregnancy indicated a poor performance for both AFC and basal FSH [20].

Also, two studies ***Nahum et al.*** [21]; ***Bancsi et al.*** [6] concluded that AFC was a better predictor of IVF outcome than was basal FSH.

Assessment of basal FSH, introduced by ***Scott et al.*** [22], is generally accepted as a test to assess ovarian reserve [8]. However, basal FSH concentrations might vary from cycle to another [13], and the reported threshold values can range from 10 IU/L to 25 IU/L [23]. This might lead to variable results in the prediction of ovarian response; hence the clinical application of basal FSH refers to only a minority of patients with extremely high basal FSH levels [23].

In a study by ***van Rooij et al.*** it was found that younger patients (<41 years of age) with elevated basal FSH levels have quite acceptable ongoing pregnancy rates, despite a considerable probability of cycle cancellation due to poor ovarian response [24].

The declining cohort of antral follicles with age first results in gradually elevated FSH levels, followed by subsequent stages of overt cycle irregularity. The gradual decline in the size of the antral follicle cohort is best represented by decreasing levels of anti-Mullerian hormone [25]. In the current study it was shown that age as a clinical marker was a good ovarian response predictor, apparently elder patients were mostly bad responders while younger patients appeared to be good responders. In a study done by ***Fang et al.*** detected that basal FSH levels combined with age (age-specific FSH levels) can be used as a more accurate marker for the ovarian response in women with normal ovarian reserves undergoing IVF-ET [26].

Another study by ***Jayaprakasan et al.*** demonstrated that AFC thresholds are useful to predict live birth rates and risks of poor ovarian response and OHSS during IVF treatment [27].

Another study done by ***Islam et al.*** estimated that both AFC and AMH are effective in predicting the ovarian reserve as well as the response to induction, and both are accurate for the assessment of ovarian reserve. On the other hand, Basal FSH and ovarian volume are non-significant predictors of ovarian reserve or response. FSH was not a good predictor of ovarian reserve and response. This may be attributed to the emergence of other novel markers as AMH which proved to be a better indicator [28].

Also in a recent study done by ***Tehraninezhad et al.*** it was found that in different age groups when measuring AFC, AMH and basal FSH levels they all assist prediction of outcome variables including oocytes number, frozen\fresh embryo, chemical and clinical pregnancy but, AFC was more accurate [29].

Ovarian response in IVF is clearly dependent on the genotype of the FSH receptor [30]. Different variants in receptor genotype have been related to different basal FSH levels and to different numbers of FSH ampoules needed to achieve adequate ovarian response in IVF [31].

Because AFC is a rather new ovarian reserve test, and most studies reported that AFC is one of the best predictors of response to ovarian stimulation with exogenous gonadotropins, significant questions remain regarding factors that might influence AFC [32]. As already stated, young patients with a low AFC might be considered as having diminished ovarian reserve, but oocyte quality might still be very acceptable and therefore young patients should not be restricted from treatment [33].

Recently, it was concluded that an AFC can be reliably performed before or after pituitary down regulation [34].

The finding that AFC performed better than basal FSH seems not to be surprising, because basal FSH levels are influenced in many ways and are potentially susceptible to large variations [(35)]. Therefore, basal FSH can only act as an indirect measure for the actual cohort size [(36)]. Antral follicle count should instead be considered a more direct reflection of ovarian reserve and is found to be stable in terms of inter-cycle variability [(33)].

In conclusion, current study showed that when we compare between AFC and basal FSH as regards both groups (good and poor responders) to detect which is better in predicting the ovarian response and clinical pregnancy, there was no statistical significance difference between them, whereas AFC have a higher area under curve (0.975) than basal FSH (0.937) which means that AFC was found to be superior to basal FSH in prediction of ovarian response in patients undergoing superovulation with long protocol for assisted reproduction.

CONCLUSIONS

AFC and basal FSH are good predictors of ovarian response in women undergoing superovulation with long protocol.

There was no absolute superiority of AFC on basal FSH in predicting ovarian response.

Age and basal E2 are also considered good predictors of ovarian response.

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