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The relationship between serum FSH level and ovarian response during controlled ovarian stimulation

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ABSTRACT

Objectives: To evaluate whether serum follicle stimulating hormone (FSH) level during the early controlled ovarian stimulation can be used as a predictor of the ovarian response in the in vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI) cycles.

Material and methods: The participants of this retrospective study were chosen from Reproductive Medicine Center, Weifang People's Hospital between January 2015 and December 2020.The participants of this study met the age of 20~43 years old, anti-Müllerian hormone (AMH) ≥ 1.2 ng/mL, antral follicle count (AFC) ≥ 5, and the data was complete and no cancellation cycle. Each participant was given GnRH agonist protocol and given a fixed dose of recombinant FSH in the first four days during the controlled ovarian stimulation (COS). According to the number of oocytes retrieved, the participants were divided into two different ovarian response groups. Serum FSH level after the fourth recombinant follicle stimulating hormone (rFSH) injection were compared during the different ovarian responders.

Results: The number of participants who met both the inclusion criteria and exclusion criteria was 235. Serum sFSH levels (mean: 11.76 ± 3.10 IU/L) in the inappropriate responders was significantly higher than serum sFSH levels (mean: 10.79 ± 2.52 IU/L) in the superior responders($p = 0.029$). There was a weak correlation between serum sFSH levels and the number of oocytes retrieved ($r = -0.134$, $p = 0.041$). Serum sFSH levels had significant clinical valuable ($p = 0.0346$) in predicting the number of oocytes retrieved.

Conclusions: Serum sFSH levels may be a potential marker to predict the ovarian response during the early COS in the IVF/ICSI cycles, which can guide the adjustment of the exogenous rFSH dose.

Key words: IVF/ICSI; follicle stimulating hormone; controlled ovarian stimulation; sFSH; ovarian response

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INTRODUCTION

Controlled ovarian stimulation (COS) is a crucial for optimizing IVF/ICSI success. The major element which may be responsible for COS is ovarian response, that is the sensitivity of ovary to exogenous gonadotrophins. Poor ovarian response may have negative consequences, leading to adverse outcomes. Now multiple factors have been proposed as predictors of ovarian response. Anti-Müllerian hormone (AMH) is synthesized by granulose cells located on ovarian follicles and is proved to be a good predict marker [1–3]. Antral follicle count (AFC), which range in size 2~10 mm can be counted by transvaginal sonography [4], is related to the number of growing follicles and thereby predict ovarian response [5]. Basal follicle stimulating hormone (FSH) is better than female age in predicting the number of the oocytes retrieved [6]. AMH and AFC have been proved to be the best demonstration in predicting ovarian response to exogenous gonadotrophins [7–9]. Although these markers are widely used to predict the ovarian response, they could not really reflect ovarian response to exogenous gonadotrophins during the COS. Some young women with normal ovarian reserve, as indexed by AMH, basal serum FSH levels and AFC did present with a poor ovarian response. It is very necessary to find a predictor that can identify ovarian response to exogenous gonadotrophins during the early COS.

Currently, the most used exogenous gonadotrophin during the COS is recombinant follicle stimulating hormone (rFSH). When administered with a fixed dose daily, serum FSH concentration reaches steady state within

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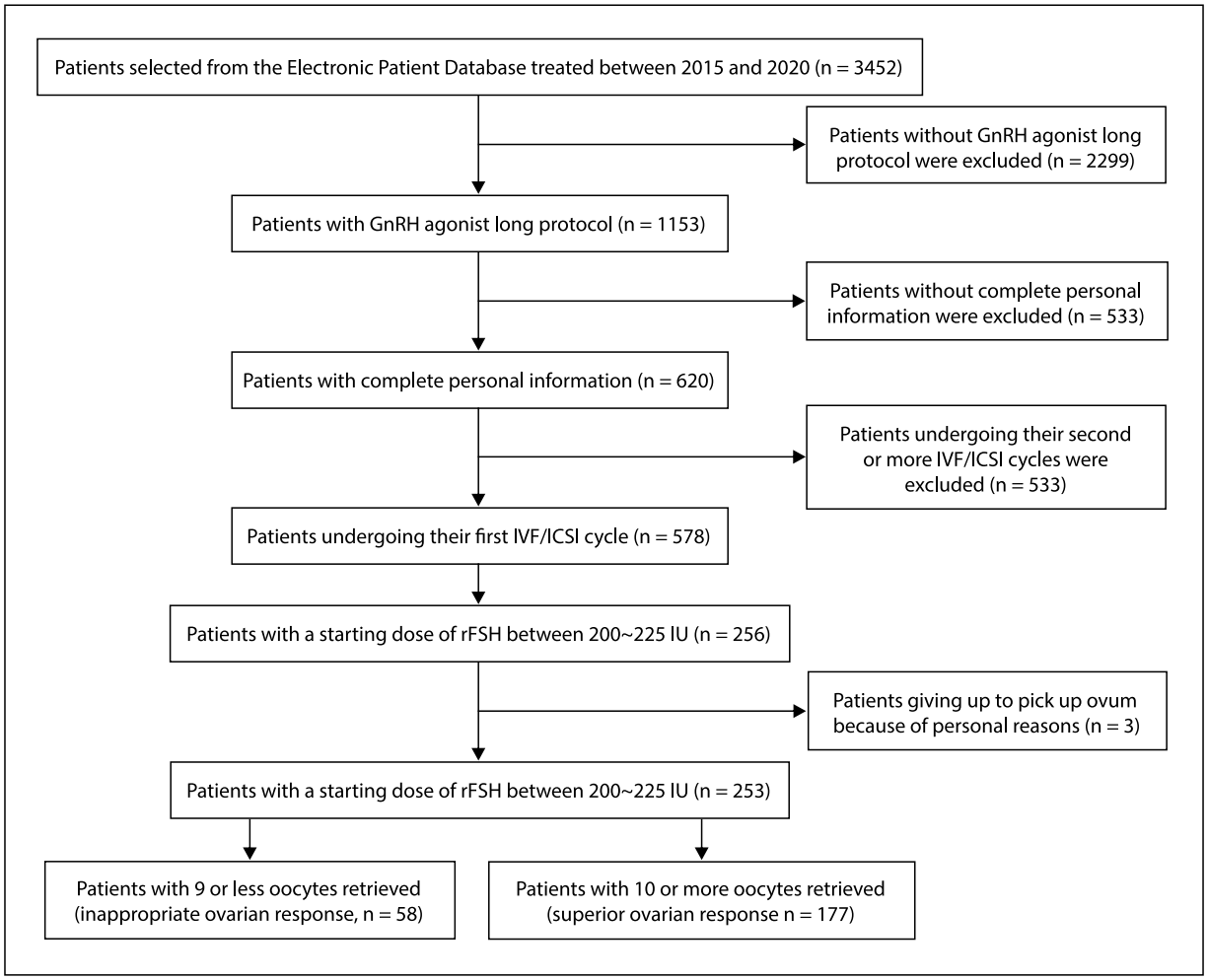

Figure 1. Patient selection flowchart

4~5 days [10]. The measured serum steady state FSH (sFSH) levels represent the balance between the rate of absorption and the rate of elimination of FSH [11]. One of the mechanisms by which FSH is cleared from the circulation is that it is consumed after binding to FSH receptor (FSHR) [12]. When the expression or activity of FSHR reduces, the consumption of FSH in the circulation decreases, and then serum FSH levels would increase. Poor ovarian response to gonadotropin stimulation is associated with low expression of FSHR in granulosa cells [13]. So, we hypothesized that serum sFSH levels would be increased during the COS in the poor ovarian responders.

Purpose

The primary aim of the present study is to assess whether serum sFSH levels differ significantly between different ovarian responders. The secondary aim is to estimate whether serum sFSH levels can be used as a potential predictor of the ovarian response.

MATERIAL AND METHODS

We retrospectively analyzed our database including all the patients who were subjected to a first cycle of COS for IVF/ICSI at Weifang People's Hospital Reproductive Medicine Center between January 2015 and December 2020. All the patients included had given at the time of the procedure a written informed consent to the analysis of their data for research purposes. The study was approved by the Ethical Committee of Weifang People's Hospital (Jan 13th, 2021).

Patients

According to the inclusion criteria and exclusion criteria, 235 patients with an indication for IVF/ICSI were included in this retrospective study (Fig. 1). Inclusion criteria were as follows: women aged between 20 and 43 years, AMH ≥ 1.2 ng/mL, AFC ≥ 5; mid luteal GnRH agonist long protocol; only recombinant FSH was used for ovarian stimulation. Cycles that were cancelled prior to oocytes retrieval or data incomplete were all excluded. It has been shown that

Table 1. Patient characteristics and the comparision of the steady-state follicle stimulating hormone (FSH), estradiol (E_2) in different ovarian response

Parameters	Inappropriate responders	Superior responders	h value	p value
n	58	177		
Age [year]	32 (30, 33)	31 (29, 33)	1.70	0.193
BMI [kg/m^2]	22.9 (21.0, 24.58)	23.4 (21.05, 25.65)	0.69	0.407
AMH [ng/mL]	2.19 (1.52, 3.53)	4.59 (3.05, 6.8)	37.19	0.000*
Basal AFC [N]	12 (9, 16)	17 (14, 20)	29.89	0.000*
Basal FSH [IU/L]	6.78 (5.69, 7.70)	6.08 (5.27, 6.92)	9.69	0.002*
Basal LH [IU/L]	3.86 (2.88, 5.09)	4.82 (3.45, 6.33)	6.56	0.010*
Basal E_2 [pmol/L]	146.55 (92.34, 216.30)	119.00 (84.48, 164.06)	4.34	0.037*
Basal PRL [IU/L]	273.85 (190.72, 418.77)	329.60 (219.64, 468.52)	1.88	0.170
Basal T [nmol/L]	0.79 (0.56, 1.13)	0.89 (0.62, 1.16)	1.28	0.259
Basal P [nmol/L]	1.00 (0.47, 1.94)	0.89 (0.51, 1.50)	0.80	0.371
AFC on day of the Gn [N]	12.5 (10, 15)	18 (14, 23)	46.19	0.000*
FSH on day of the Gn [IU/L]	3.65 (3.22, 4.39)	3.48 (3.00, 4.08)	4.19	0.051
Starting dose of Gn [IU]	225 (218.75, 225)	225 (200, 225)	3.09	0.079
Days of Gn [day]	9 (8, 10)	9 (8, 10)	0.65	0.420
Total doses of Gn [IU]	2025.00 (1800.00, 2356.25)	1950.00 (1725.00, 2162.50)	4.15	0.042*
sFSH [IU/L]	11.76 ± 3.10	10.79 ± 2.52	4.85	0.029*
sE_2 (pmol/L)	624.90 (436.50, 1326.25)	1360.00 (820.95, 2278.00)	28.46	0.000*
FSH on day of hCG [IU/L]	14.91 (11.21, 17.74)	12.10 (10.5, 14.88)	6.91	0.009*
E_2 on day of hCG [pmol/L]	6252.50 (5083.75, 8122.50)	13542.00 (10329.25, 19577.00)	84.42	0.000*

*$p < 0.05$; AFC — antral follicle count; AMH — anti-Müllerian hormone; BMI — body mass index; E_2 — estradiol; FSH — follicle stimulating hormone; Gn — gonadotropin; hCG — human chorionic gonadotropin; LH — luteinizing hormone; T — testosterone

women with less than nine oocytes have poorer outcomes than women with more than ten oocytes, without considering the ovarian hyperstimulation syndrome, suggesting that the number of oocytes retrieved reflect the ovarian response during the COS [14, 15]. Based on this, the patients were divided into two groups as follows:

— inappropriate responders, that is ovarian inappropriate response group, the numbers of oocytes retrieved were ≤ 9, which include the poor ovarian response and suboptimal ovarian response;
— superior responders, that is ovarian superior response group, the numbers of oocytes retrieved were ≥ 10, which include the normal ovarian response and hyper-ovarian response.

Treatment procedures

Patients were subjected to pituitary downregulation with daily administration GnRH agonists 0.05~0.1 mg/mL (triptorelin, Ferring Pharmaceuticals) during the mid-luteal phase of the preceding cycle. About after 14 days, when reaching the standard of the pituitary regulation, rFSH (Gonal-F; Merck Serono) was used for ovarian stimulation. According to age, weight, and ovarian reserve, the starting dose of the rFSH was decided between 200 and 225 international units (IU). The patient returned for the first visit to record the development of follicles and the serum FSH and estradiol (E_2) levels after the fourth day of rFSH. Follicular monitoring was performed by transvaginal ultrasonography. Hormones were measured using automated chemiluminescent immunoassays (Roche automatic biochemical immunoassay analyzer Cobas 8000, Switzerland). Limits of detectability for each assay were as follows: luteinizing hormone (LH) 0.07 mIU/mL; FSH 0.3 mIU/mL; estradiol 18.36 pmol/L. Anti-Müllerian hormone (AMH) was measured using an enzyme linked immunosorbent assay (Beckman Coulter Inc, USA). The lower limit of detection was 0.01 ng/mL.

Ovulation was triggered with Recombinant Human Choriogonadotropin 250 μg (rhCG; Merck Serono) or 10,000 IU Urinary Chorionic Gonadotropin (hCG) when at least one follicle 18 mm in diameter or two follicles 17 mm in diameter, combining with an appropriate E_2 levels. Oocyte retrieval was undertaken 36 h after the trigger injection.

Statistical analysis

Statistical Package for the Social Sciences version 22.0 and GraphPsd Prism 7.0 were used for statistical analyses and graphing. Kolmogorov-Smirnov test was used to determine whether the clinical data were normally distrib-

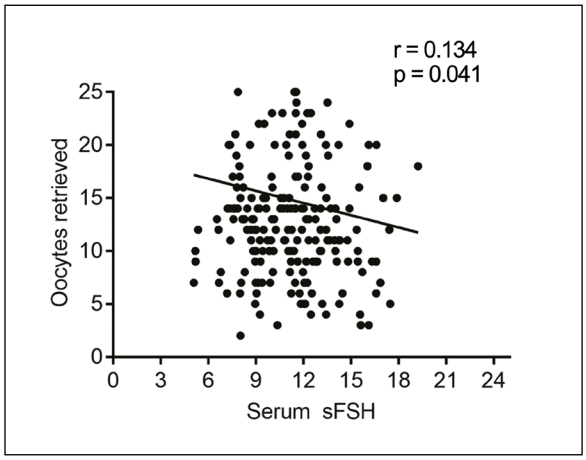

Figure 2. Serum sFSH level in relation to the number of oocytes retrieved. XY graph showing the number of oocytes retrieved according to the serum FSH concentration on the 4th day of the rFSH injection

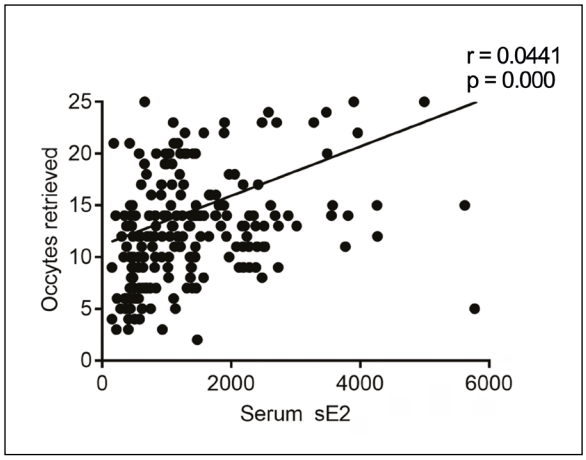

Figure 3. Serum sE_2 level in relation to the number of oocytes retrieved. XY graph showing the number of oocytes retrieved according to the serum E_2 concentration on the 4th day of the rFSH injection

Table 2. Multiple regression analysis evaluating the values of different parameters in predicting the number of oocytes obtained

	B	β	p value
Constant	15.274	–	0.000*
FSH on the Gn4th	–0.370	–0.125	0.036*
E_2 on the Gn4th	0.002	0.415	0.000 *

Adjusted R^2 = 0.189; Total number of oocytes =15.274 – 0.370 × Gn4th FSH + 0.002 × Gn4th E_2; * p < 0.05; E_2 — estradiol; FSH — follicle stimulating hormone; Gn — gonadotropin

uted. Only serum sFSH levels was normal distribution and was expressed as mean ± standard deviation, using one way ANOVA test to compare the significance.

Non-normal distribution data was expressed as means (25th percentile, 75th percentile) [M (P25, P75)] and was tested the significance of continuous parameters by the Kruskal-Wallis test. Correlation was assessed by the Spearman rank method. Multiple regression analysis was applied to evaluate the predictive values of serum sFSH levels on the ovarian response. P < 0.05 was considered statistically significant.

RESULTS

According to the inclusion criteria and exclusion criteria, 235 patients were included in the data analysis. Of these women, 58 were inappropriate responders, and 177 were superior responders. The baseline characteristics for the two response groups are listed in Table 1. As shown, the markers standing for the ovarian response differed significantly between two groups. Superior responders had a significantly higher AMH compared to inappropriate responders [4.59 (3.05, 6.8) vs 2.19 (1.52, 3.53), p = 0.000], a significantly higher AFC [17 (14, 20) vs 12 (9, 16), p = 0.000], but a significantly lower basal FSH [6.08 (5.27, 6.92) vs 6.78 (5.69, 7.70), p = 0.002]. There was no difference in serum FSH levels on the gonadotrophin starting day between two groups. In addition, superior responders had a significantly higher serum sE_2 levels compared to inappropriate responders [1360.00 (820.95, 2278.00) vs 624.90 (436.50, 1326.25), p = 0.000] but a significantly lower serum sFSH levels (10.79 ± 2.52 vs 11.76 ± 3.10, p = 0.029), total gonadotrophin doses [1950.00 (1725.00, 2162.50) vs 2025.00 (1800.00, 2356.25), p = 0.042].

We found serum sFSH levels were negatively correlated with the number of oocytes retrieved (r = –0.134, p = 0.041) (Fig. 2). On the contrary, serum sE_2 levels were positively correlated with the number of oocytes retrieved (r = 0.441, p = 0.000) (Fig. 3).

Serum sFSH and sE_2 levels were entered in a stepwise fashion in the multiple regression analysis using the number of oocytes retrieved as the dependent variable with a constant included in the equation. As shown in Table 2, serum sFSH and sE_2 levels can be the markers in predicting the ovarian response (p < 0.05).

DISCUSSION

In this study, we identified that serum sFSH levels had significantly distinction between different ovarian responders undergoing the IVF/ICSI cycles in GnRH agonist cycles. A recent similar study [16] concluded that there was a weak relationship between ovarian response and serum delta FSH levels (the difference between serum FSH level on D6 of gonadotropin (Gn) use and basal serum FSH level) in the rFSH fixed dose treatment protocol. Because the study only focused on the comparison between the discrepancy of FSH level and ovarian response, it is difficult to directly compare these results to our study. But this study

indirectly proved that there was a correlation between the serum FSH concentration in the early stage of COS and ovarian reactivity.

Our findings suggested a possibility of predicting ovarian response with serum sFSH levels. According to the number of oocytes retrieved, we divided the patients into inappropriate responders and superior responders. We found there was a significant correlation between serum sFSH levels and and ovarian response. It was like the reported by Bentov et al. [17], serum sFSH levels showed a significant negative correlation with oocytes, the higher serum sFSH level was, the less the number of oocytes retrieved, suggesting the poorer the ovarian response.

There were two main factors that affected serum sFSH levels. One was the daily dose of exogenous rFSH, and the other was the balance between the rate of absorption and rate of elimination of exogenous rFSH [11]. After reaching downregulation criteria, all patients were given similar rFSH starting dose, which was constant for the initial four days of cycles during ovulation induction. Therefore, the change of serum sFSH levels did not due to the daily dose of FSH. It seemed that the change of serum FSH levels were related to the imbalance of FSH metabolism. FSH plays a pivotal role in the control of female reproduction through binding to its specific G-protein-coupled transmembrane receptor, which is located in the granulosa cells in the ovary [18]. When the FSHR expression was not enough, exogenous rFSH could not eliminated because of lacking binding to sufficient FSHR, which led to the elevated of the serum FSH concentration, accompanying the lower serum sE_2 levels. It had been reported poor ovarian response to gonadotropin stimulation was associated with low expression of FSHR in granulosa cells [13]. Some novel, specific mutations or density dysregulation of FSHR dramatically reduce receptor expression and impair proper signal transduction [19–21]. Thus, impaired FSHR activity led to decrease sensitivity of follicles to FSH and reduce the combination to the exogenous FSH, eventually leading to increase of serum sFSH levels and decrease FSH-dependent estradiol production and dominant follicle selection and development. This also explained the unexpected low ovarian response in some young women with good ovarian reserve. In the past, clinicians used to judge the ovarian response based on the follicular development through transvaginal ultrasound after fourth day of gonadotropin injection. Now the result of this study indicated that serum sFSH levels with serum sE_2 levels can predict the ovarian response. Mechanistically, it can be concluded that there was no use to increase the dose of exogenous rFSH at this time since the FSH administered was not being completely used yet.

The study had several limitations. On account of the sample size of the poor responders was too small to be classified separately, it was not possible to compare serum sFSH levels from the poor responders, normal responders and ovarian hyper-responders, which was more in line with the clinical standard. Second, while we excluded women with a different FSH starting dose, a dose adjustment during the treatment cycle could possibly affect the number of oocytes retrieved. Additionally, there are fewer independent variables included in the regression analysis due to insufficient sample size, which cannot better reflect the relationship between the dependent variable and the independent variables.

In conclusion, this research suggested that serum sFSH levels can be as a potential predictor of ovarian response during the COS in the IVF/ICSI cycles. Further research will be designed to calculate cut-off of serum sFSH levels through enlarging the variables, and then to evaluate the relationship between serum sFSH levels and the pregnancy outcome of IVF/ICSI.

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Ethics approval

This study was approved by the Ethical Committee of Weifang People's Hospital.

Availability of data and materials

All data generated or analyzed during this study are available from the corresponding author upon reasonable request.

Authors' contributions

All authors designed the study, interpreted the results, reviewed the manuscript and approved the final version. PP Sun took the lead in writing the manuscript and conducted the statistical analyses. All authors have read and approved the final manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

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