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Research Article

Serum FSH Levels during IVF Cycles: A New Paradigm for Clinical Management of Ovarian Stimulation

Barry Perlman[1]*, Kulak D[1,2,3], Oh C[4] and McGovern PG[1,2]

[1]*Division of Reproductive Endocrinology and Infertility, Department of Obstetrics, Gynecology and Women's Health, Rutgers New Jersey Medical School, Newark, NJ, USA*

[2]*University Reproductive Associates, Hasbrouck Heights, NJ, USA*

[3]*Genesis Fertility, Brooklyn, NY, USA*

[4]*New York University School of Medicine, New York, NY, USA*

***Corresponding Author:** Barry Perlman, Division of Reproductive Endocrinology and Infertility, Department of Obstetrics, Gynecology and Women's Health, Rutgers New Jersey Medical School, Newark, NJ, USA.

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Abstract

Exogenous FSH is the catalyst controlling controlled ovarian stimulation (COS), with high dosing being associated with negative outcomes. A retrospective cohort study of COS cycles was performed to determine the effect dosing and BMI has on serum FSH levels and to determine if serum FSH levels are associated with changes in live birth rates. In this study, 397 GnRH suppressive cycles were analyzed. Serum FSH levels were measured every 48-72 hours throughout COS and the change (delta) in serum FSH levels from baseline was calculated. Elevated day 3 serum FSH levels were significantly associated with decreased live births (OR: 0.86; 95%CI: 0.78 - 0.94, p = 0.002), while antral follicle count (OR: 01.077; 95%CI: 1.028 - 1.128, p = 0.002), number of oocytes retrieved (OR: 1.053; 95%CI: 1.002 - 1.107, p = 0.04), and number of embryos transferred (OR: 1.292; 95%CI: 1.284 - 1.301, p < 0.001) were significantly associated with an increased birth rate. Elevated delta FSH and maximum serum FSH significantly (p < 0.001) correlated with a decrease in live births. Increased BMI significantly reduced serum FSH levels, suggesting BMI should be considered when calculating FSH dose. Overall, maximum serum FSH and elevated serum delta FSH levels were associated with lower live birth rates supporting a rationale for measuring serum FSH during COS.

Keywords: *IVF; Serum FSH; BMI; Live Birth Rate*

Introduction

Exogenous FSH administration to achieve supraphysiologic serum levels for multifollicular recruitment is a mainstay of modern *in-vitro* fertilization (IVF). Initial dosing and subsequent titration of these medications to optimize ovarian response is one of the most onerous and expensive parts of IVF for both the patient and the physician. Yet, it is still considered part of the art of assisted reproductive technology (ART), meaning there are no definitive data or clear guidelines on how to dose and titrate these medications. In addition, patient response to these medications can be unpredictable. While there are some data on excessive use of gonadotropins and detrimental

impact on live birth rates [1,2], doses exceeding 450 IU of gonadotropins daily are still used by providers based on their training and anecdotal evidence. While FSH dose regimens are often based on known predictors of response, including age and ovarian reserve testing [3-5], it remains unclear how each of these should be weighted and if other positive and negative prognostic factors (such as body mass index [BMI]) [6-10] should be considered when establishing a dosing regimen.

Adjustment of FSH dosing also remains a debated topic, with serial measurements of follicle size and serum estradiol levels being used as the major factors for dose titrations. Suboptimal ovarian response to gonadotropins is generally treated by administering higher doses of gonadotropins, although there is often no evidence that serum FSH levels are low or that follicular recruitment would be improved with higher doses [1].

Direct measurements of serum FSH levels are not typically assessed during an IVF cycle. While prior data has shown that live birth rates significantly decrease with increasing FSH dose, these studies did not assess the serum FSH levels [1,2]. Serum gonadotropin levels achieved will be affected by tissue blood flow, volume of drug distribution, BMI, along with metabolism and clearance rates, all of which may be highly variable between individuals.

Although considered standard care for IVF, gonadotropin administration brings with it a significant risk of ovarian hyperstimulation syndrome, which may be life threatening. Early studies using pituitary-derived gonadotropin preparations showed a clinical response to a particular gonadotropin dose after at least 5 days of injections [11]. Using the pharmacologic therapeutic index (TI), which refers to the ratio between the average toxic dose of a drug over the average needed dose to produce the desired effect, these investigators noted that there was a remarkably narrow therapeutic index of only 1.3 - 1.5 for gonadotropins. Typically, when using drugs with a small therapeutic index, such as digoxin (TI = 2.5) and gentamicin (TI = 3), providers are guided by drug serum levels for maximum safety with minimum toxicity. Given this background information, coupled with the ready access to serum FSH levels and relative frequency of the complications of under-dosing (poor ovarian response, cancelled cycles) and overdosing (cycle cancellation, OHSS), we postulated that measuring serum FSH levels during IVF stimulation might be useful to help guide exogenous FSH dosing decisions.

Since the serum levels better reflect the amount of FSH actually available to the ovary during superovulation, we hypothesized that the change of serum FSH level from baseline/suppression to average level achieved during COS would be more predictive of pregnancy outcomes than the dose administered. As BMI has already been shown to affect cycle outcomes [6-8,10] and is likely to reduce tissue blood flow and volume of distribution of a drug [12], we further hypothesize that BMI is a significant modifier of serum FSH levels achieved during superovulation.

Objective of the Study

The primary objective of this study was to assess the association between serum FSH levels following exogenous administration and clinical outcomes in women undergoing IVF. A secondary objective was to determine the interaction of BMI in this relationship.

Materials and Methods

This was an IRB approved retrospective cohort study (IRB# Pro2013003410) performed at a university-affiliated reproductive endocrinology and infertility clinic. Inclusion criteria included all patients aged 30 to 40 years, undergoing GnRH agonist down-regulated IVF cycles over a three-year period (1/2011-12/2013) regardless of infertility diagnosis. Exclusion criteria included age outside of accepted range, other COS cycle types. After applying the exclusion criteria, there were 326 unique women with 397 cycles. Data for each cycle was assessed separately.

Transvaginal sonograms, FSH and E2 serum levels were obtained on day 3 of the menstrual cycle preceding the start of an IVF cycle to determine ovarian reserve. Suppression of endogenous gonadotropins was confirmed prior to gonadotropin stimulation by measuring serum FSH and estradiol levels on cycle day 3 following initiation of GnRH agonist. Gonadotropins (recombinant or urinary) were used for controlled ovarian stimulation (COS). Serum FSH and E2 levels were measured every 24 - 48 hours using Immulite® 2000 immunoassay after FSH steady state was reached on day 5 of stimulation, throughout the entire cycle until day of oocyte retrieval. Transvaginal sonograms were performed at the same time as serum level assessment for monitoring ovarian follicular growth. Oocyte maturation was triggered with 10,000 IU of human chorionic gonadotropin (hCG) when at least two follicles reached a mean diameter of 18 mm. Oocyte retrieval was performed approximately 34 hours after the hCG injection. Oocyte maturity was evaluated using standard criteria. Evidence for fertilization was assessed approximately 18 hours post insemination. The presence of two pronuclei was used as evidence of normal fertilization, whereas the presence of ≤ 1PN or > 2PN was consistent with abnormal fertilization. Trans-cervical embryo transfer (ET) was performed under transabdominal ultrasound guidance on day 3 post insemination. Selection of embryos for fresh ET was based on optimal cleavage status and morphological parameters using standard criteria. Luteal support was provided by intramuscular progesterone (P) supplementation (50 mg daily) until the first pregnancy test 12 days post ET. P supplementation was continued in all cycles with a positive serum βhCG until 8 weeks from egg retrieval (Figure 1).

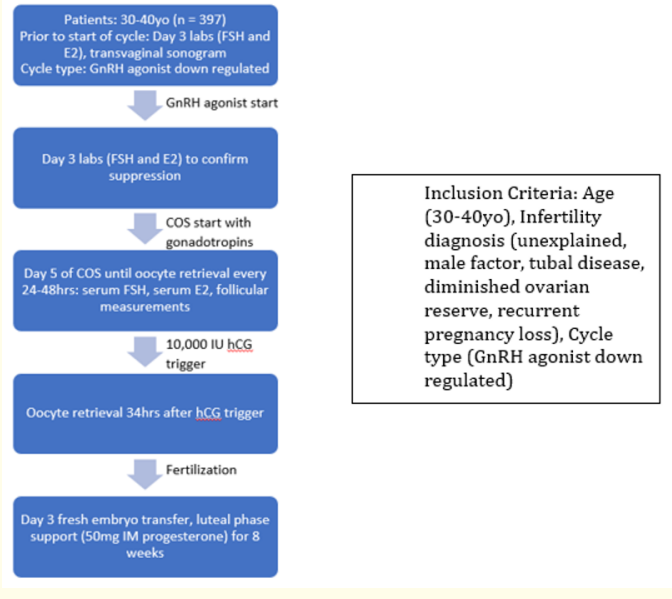

Figure 1: *Schematic of study design protocol.*

Statistical analysis

The subject demographics were summarized using descriptive statistics in mean ± SD for continuous variables or number (%) for categorical variables. All the continuous outcome variables were assessed for normality using the Shapiro-Wilk test. Correlations between continuous variables were evaluated by means of Pearson's correlation coefficient or Spearman's rank correlation coefficients where deviated from normality. When assessing for effects of volume of distribution we used BMI as our surrogate measurement. As per CDC guidelines (https://www.cdc.gov/healthyweight/assessing/bmi/adult_bmi/index.html), patients were categorized into groupings of normal weight if < 25 kg/m^2, overweight if between 25-30 kg/m^2, or obese if BMI >30 kg/m^2. In investigating the association of predictors with

pregnancy outcomes, the prediction model was fitted by using a non-linear mixed model featuring a logistic model in which the patient was considered as a random factor to account for more than one cycle for the same patient. Similarly, we evaluated the association of the outcome with variables in a univariate manner. Multivariable logistic regression models were also fit to the data using a stepwise variable selection approach to identify independent predictors of pregnancy outcome. All variables with $p < 0.1$ for the bivariate association with outcome were considered for initial inclusion in the multivariate model, but only those which remained significant to $p < 0.05$ were retained in the final model. Results are reported as odds ratio (OR) with 95% confidence intervals (CI). Two-sided p-values < 0.05 were considered to be statistically significant. All the statistical analyses were carried out using the R statistical software package (R, version 2.12.2). Unless otherwise noted, all analyses used mixed-effects models (package lme4: Bates, Maechler and Bolker 2011) in R 2.14.2 (R Development Core Team 2012).

Results

Over the course of the study period (1/2011-12/2013), 397 IVF cycles met criteria for inclusion and were included for the final analysis. The 397 cycles were from 326 individual patients, 19.3% of whom underwent more than 1 cycle. Demographics and characteristics are shown in table 1. The mean ± SD age was 35.8 ± 5.7 years. The mean ± SD BMI was 26.5 kg/m^2 ± 6.6. Of all cycles, 33.7% resulted in a live birth.

Variables	Value
Number of cycles patients underwent	
1 cycle	263 (80.7%)
2 cycles	55 (16.9%)
3 cycles	8 (2.4%)
Pregnancy outcome	
No-pregnancy	233 (58.7%)
Live birth	134 (33.7%)
Spontaneous abortion	26 (9.9%)
Termination of pregnancy	4 (1.5%)
Age (years)	35.8 ± 5.07
BMI (kg/m^2)	26.5 ± 6.6
< 25	208 (52.5%)
25 - 30	100 (25.3%)
> 30	88 (22.2%)
Antral follicle count	13.5 ± 7.6
Day 3 FSH: Historical max (mIU/mL)	7.1 ± 2.8
Cycle Baseline FSH: From day 3 while suppressed with GnRH agonist (mIU/mL)	3.5 ± 1.7
Total FSH administered: Combined purified and recombinant over COS cycle dosage (IU)	3446 ± 1488
Average Daily FSH dose (purified and recombinant) (IU)	336.2 ± 102.8
FSH serum level: Mean over COS starting on Day 5 of stimulation (mIU/mL)	20.36 ± 9.441

Delta FSH (ΔFSH): Change in serum level from suppression check to mean COS level	16.85 ± 9.8
Maximum serum FSH level (mIU/mL)	23.22 ± 10.75
Complications: Severe hyperstimulation (hospitalization)	 1 (0.3%)
Oocytes Retrieved	11.95 ± 7.329
Embryos transferred	
0	16 (6.1%)
1	20 (7.6%)
2	185 (70.3%)
3 or more	42 (16%)

Table 1: *Clinical characteristics of women undergoing IVF.*

Data are expressed as mean ± SD or number (%).

FSH serum levels were assessed throughout COS. Day 3 FSH levels were measured for diagnostic purposes prior to start of any medications (mean 7.1 mIU/mL). To assess adequate ovarian suppression following GnRH agonist, FSH level was remeasured at baseline. Mean cycle baseline FSH was 3.5 mIU/mL. The daily FSH dose administered over the course of the cycle was measured (mean ± SD 336.2 ± 102.8 IU). The change in serum FSH levels from cycle baseline to average cycle serum level was denoted as delta FSH (mean ± SD 16.85 ± 9.8 mIU/L). Delta FSH was used as an assessment of the increase in the serum FSH level, secondary to the amount of exogenously administered FSH. The mean ± SD maximum serum FSH was 23.22 ± 10.75 mIU/mL.

Logistic regression models were used to assess the effect of each exposure on the outcomes and adjusted for age, BMI, antral follicle count, day 3 FSH, cycle baseline FSH, total FSH administered, average serum FSH, delta FSH, maximum serum FSH, number of oocytes retrieved and number of embryos transferred. As expected, elevated historical day 3 FSH levels were significantly associated with decreased live births (OR: 0.86; 95%CI: 0.78 - 0.94, p = 0.002), while antral follicle count, number of oocytes retrieved and number of embryos transferred were more likely to increase the rate of live births (ORs>1) (Table 2). These results confirmed our data to be comparable to expected outcomes following any IVF cycle. Interestingly, elevated delta FSH and maximum serum FSH significantly (p < 0.001) correlated with a decrease in live births (Table 2). However, the total amount of FSH administered did not significantly correlate with live birth rates (Table 2).

Variable	Unadjusted OR[1] [95% CI]/p-value	p-value by multivariate[2]
Age	0.892 [0.826 0.964]/0.004	< 0.001
BMI < 25 25 - 30 > 30	 Ref 1.155 [0.575 2.316]/0.686 1.314 [0.661 2.612]/0.436	NA
Antral follicle count	1.077 [1.028 1.128]/0.002	< 0.001
Day 3 FSH	0.861 [0.783 0.947]/ 0.002	< 0.001
Cycle baseline FSH	0.868 [0.761 0.989]/0.033	< 0.001
Average total FSH administered (by 75 unit change)	0.658 [0.514 0.842]/0.001	0.316
Average FSH serum level	0.963 [0.957 0.969]/ < 0.001	0.604
Delta FSH	0.965 [0.933 0.998]/0.039	< 0.001
Maximum serum FSH level	0.970 [0.964 0.976]/ < 0.001	< 0.001
Oocytes Retrieved	1.053 [1.002 1.107]/ 0.04	< 0.001
Embryos transferred	1.292 [1.284 1.301]/ < 0.001	< 0.001

Table 2: *Mixed effect logistic regression for pregnancy outcomes.*

Mixed effect logistic regression results showing (un) adjusted odds ratios [95% CI]/p-value and adjusted p-values for predictor factor for pregnancy outcomes. [1]Univariate analysis. [2]Multivariate analysis. Ref, reference category, has an odds ratio of 1.0. NA excluded from the multivariate analysis.

The average daily FSH dose administered correlated with a change in serum delta FSH levels ($r = 0.5303$, $p < 0.0001$) (Figure 2). However, the representative strength of that correlation only accounts for approximately 25% of the variation in delta FSH, meaning that there must be other factors affecting the change in serum FSH levels other than the daily dosage. Our data suggest that BMI also plays a significant role in FSH serum changes (Figure 3). As BMI increased, changes in FSH dose had less impact on serum FSH levels. In patients with a BMI < 25 kg/m^2 (n = 208), the change in serum FSH level increased by a factor of 0.07170, while BMI of 25 - 30 kg/m^2 (n = 100) saw an increase of only 0.05508 and patients with a BMI >30 kg/m^2 (n = 88) saw a much lower increase of only 0.03658. These data suggest that increased BMI reduces the effect of serum FSH changes per exogenous FSH. Ultimately, obese patients had about 50% the serum change in FSH level compared to normal weight women per ampule of FSH administered.

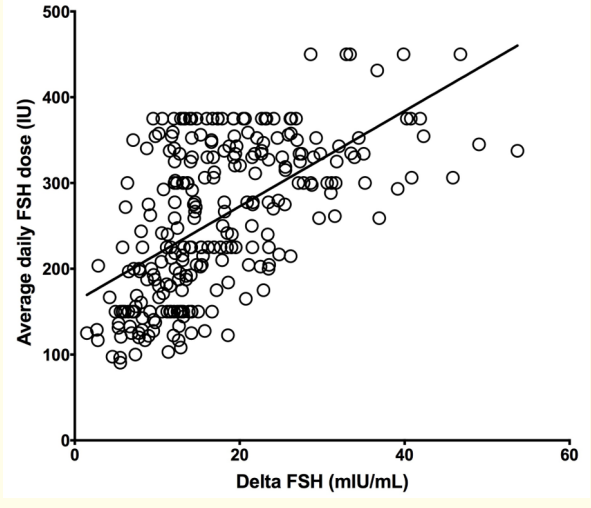

Figure 2: *Serum delta FSH in comparison to daily FSH dose.*

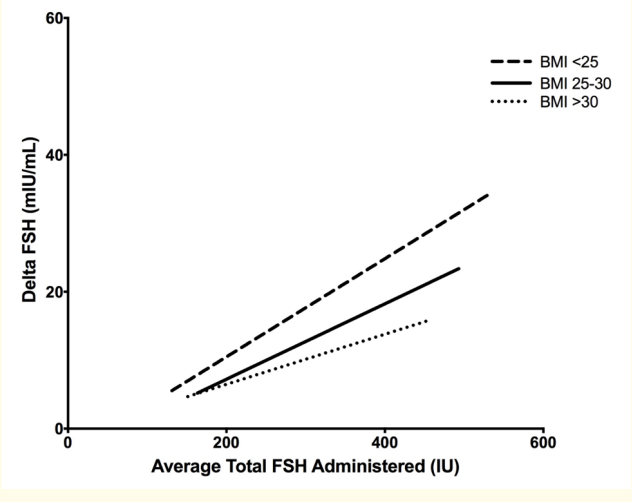

Figure 3: *Delta FSH in relation to average total FSH dose based on BMI.*

Discussion

Serum FSH levels are often measured at baseline prior to the start of an IVF cycle but are usually not measured thereafter during stimulation. Prior studies have shown a negative correlation between higher dosages of FSH and live birth rates [1,2] but have failed to interpret the relationship. The purpose of our study is to assess if elevated serum FSH rather than FSH dose correlated with live birth rates. In our review of the literature, we found only a single case report and no previous large studies looking at serum FSH levels during COS [13]. In addition to well-accepted predictors of successful cycle outcome (age, basal FSH, AFC, number of oocytes retrieved, and number of embryos transferred), our study provides two novel factors which are also significant predictors of IVF live birth success: maximum FSH level and delta FSH. Surprisingly, contrary to other studies, total dose of administered FSH and BMI were not predictive factors for live births in our study [14,15]. The differences seen in our data may be due to the cycle type since these studies included all types of cycle while our data was limited to GnRH long down regulation cycles. Our data demonstrates that the FSH level achieved in the patient's bloodstream in response to exogenous FSH, rather than the gonadotropin dose administered, was the more important factor in predicting outcomes.

Whether supraphysiologic serum FSH level negatively affects the oocyte, the endometrium, or both, is still in question [16]. A recent study reported live birth rate to be negatively impacted by a high dose of total FSH used in fresh transfers, as compared to the live birth rates in subsequent paired frozen embryo transfer cycles, suggesting that the endometrium may be adversely affected by high doses of gonadotropins [17]. The effect of elevated serum FSH on the endometrium may be both indirect, due to subsequent elevated levels of estrogen and progesterone [18], or directly on endometrial cells [19].

Our data suggest that measuring serum FSH throughout COS may be beneficial to adjust dosing and determine success. However, it cannot be assumed that increasing the dose of FSH by a single ampule will have the same effect in each individual. Our data show that while delta FSH was associated with dose administered; the relationship involved such variability, that some patients who received a mean dose of 250IU had a higher delta FSH than patients who received 500IU. Our findings suggest that this variability may be at least partially attributable to BMI and may be the reason why BMI has shown variability in studies regarding its effect on IVF success rates.

Previous studies have shown that BMI can play a major role in how the ovaries respond to gonadotropin stimulation [20,21]. Our data provide a potential explanation by showing that the decreased response in obese patients might be secondary to a reduced change in serum FSH levels. Our data show with strong statistical significance that every 75IU of exogenous FSH increases serum FSH levels by 4.4 mIU/mL in normal weight individuals. Obesity reduced this rate of change so that overweight patients (BMI = 25 - 30) only had an increase of 3.1 mIU/ml and obese patients (BMI >30) only had an increase of 1.8 mIU/ml per 75 IU of FSH administered. How BMI alters uptake of exogenous gonadotropins remains unknown but its effect on plasma volume and metabolism may play a role. Significant variability exists among patients, so monitoring serum FSH levels during stimulation for IVF may help to predict the marked variability in ovarian response. Understanding the role of BMI in the serum FSH levels achieved after a dose change will also help better guide therapeutic decisions.

As this study was conducted between 2012 and 2013, some IVF practices have changed. As more GnRH antagonist cycles are currently used, the delta FSH may be different for different types of GnRH down regulation cycles (the lack of down-regulation at the base line FSH measurement in GnRH antagonist cycles). Hence the data in this paper is only applicable for GnRH long down regulation cycles. Additionally, new forms of long-acting recombinant FSH, follitropin delta, have been developed that specifically recommend calculating dose based on BMI (REKOVELLE®), but this unfortunately has not become common practice for current use of short-acting gonadotropins.

Limitation of the Study

Some of the limitations of the study include the retrospective nature of the study which may be associated with selection bias and the use of both recombinant and urinary FSH. Due to the retrospective nature, this study was not able to assess the correlation between FSH

levels and the final progesterone concentrations or to baseline AMH levels which would be of interest. In regards to the type of FSH used, differences in the carbohydrate moieties of the hormone may affect metabolism and clearance of FSH altering serum levels. Further large-scale prospective studies could help to support the findings in this study.

Conclusion

In our analysis of down-regulated IVF patients, we report for the first time that larger quantitative changes in serum FSH levels during COS were significant negative predictors of live birth after IVF, while high doses of exogenous FSH were not. Serum FSH levels correlate only moderately with the dose of FSH administered. This correlation is directly affected by BMI, thus when choosing FSH dose BMI should be considered. Further work will be needed to determine whether an ideal serum FSH goal, which will optimize live birth rates during IVF, exists.

Disclosure of Interest

The authors report no conflict of interest.

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