Correlation of the Day 3 FSH/LH Ratio and LH Concentration in Predicting IVF Outcome

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Abstract
Background: This study was undertaken to evaluate the role of day 3 FSH/LH ratio and day 3 LH level as predictors of IVF cycle outcomes.

Methods: This prospective observational study was undertaken in the IVF and Reproductive Biology Centre and Lok Nayak Hospital, affiliated to Maulana Azad Medical College, in New Delhi, India. The study included 105 women who underwent controlled ovarian hyperstimulation for in vitro fertilization. Characteristics of IVF cycles and outcomes were studied in patient subgroups based on day 3 FSH/LH ratio (<2 and ≥2) and day 3 LH levels (>3 and ≤3 mIU/ml). The student t-test, Bartlett's test, chi-squared (χ²) and Fisher's exact test, and linear regression model were used for data analysis. A p-value less than 0.05 was considered as statistically significant.

Results: Women with an elevated FSH/LH ratio ≥2 (n=31) required higher doses of gonadotrophins (3019.34 vs. 2482.43 IU). The outcome of IVF was poor in these patients and they had fewer number of mature follicles (>16 mm) (5.44 vs. 6.09), less E2/mature follicle ratio (4.65 vs. 6.36), fewer retrieved oocytes (6.67 vs. 7.91) and fewer pregnancy rates (11.1% vs. 33.8%). On the other hand, patients with low basal LH levels (≤3 mIU/ml) did not differ significantly in terms of response to controlled ovarian hyperstimulation except for fewer number of retrieved oocytes (7.33 vs. 7.91) but there was a trend towards poor pregnancy rates (7.33 vs. 7.91) but there was a trend towards poor pregnancy rates as compared to subgroup with LH levels >3 mIU/ml.

Conclusion: Elevated day 3 FSH/LH ratio is associated with inferior outcome in IVF treatment cycles and it could be used as an additional predictor of decreased ovarian reserve.

Keywords: FSH, IVF outcome, LH, Ovarian hyperstimulation, Ovarian reserve, FSH/LH ratio.


Introduction
Progressive increase of reproductive aging has increased the number of couples seeking help for assisted reproductive techniques. Futile infertility treatment is associated with obvious psychological and financial burdens. Lenton et al. demonstrated that an increase in follicle stimulating hormone (FSH) occurs several years before elevation in luteinizing hormone (LH) and concluded that the first intimation of decreased ovarian reserve may be due to an increased FSH/LH ratio (1). Though, FSH and LH act together in stimulating follicular growth and maturation, the
relative importance of LH in follicle stimulation is still debatable in the clinical settings (2).

Liu et al. demonstrated that day 3 FSH/LH≥2 is associated with higher rates of cancellation of IVF cycles (3). Previous studies suggest that day 3 LH values ≤3 mIU/ml may also predict poor response to ovarian stimulation (4, 5).

Thus, the present study was conducted with the endeavor to search for an accurate marker which would allow suitable selection of stimulation protocols and appropriate counseling before pursuing the stressful and expensive course of IVF. This is particularly important in our set-up we encounter, frequently as the patients belong to the lower and middle socioeconomic classes and cannot afford multiple cycles of IVF.

Therefore, the objective of the present study was to evaluate whether elevated day 3 FSH/LH ratio ≥2 and LH values ≤3 mIU/ml, in the presence of normal FSH, are predictive of poor responses to ovarian stimulation.

**Methods**

This prospective study was carried out at the IVF and Reproductive Biology Centre and Lok Nayak Hospital, affiliated to Maulana Azad Medical College, New Delhi, India from March 2008 to October 2009 after obtaining ethical clearance from Institutional Ethical committee. All the 105 patients who underwent controlled ovarian hyperstimulation (COH) for IVF were included in the study. Women with the following characteristics were excluded from the study: (i) age >40 years, (ii) more than three IVF cycles failures, (iii) donor oocytes recipients, (iv) frozen-thaw embryo recipients, (v) endocrinal abnormalities such as hyperprolactinemia or hypothyroidism, (vi) ovarian cysts or follicular cyst, (vii) polycystic ovarian syndrome or similar features like high LH/FSH ratio and (viii) high FSH and estradiol (E2) concentrations (day 3 FSH≥11 mIU/ml and E2≥50 pg/ml).

Fasting blood samples were obtained for baseline assays of FSH, LH, E2, and progesterone (P4) on day 2 of a spontaneous menstrual cycle prior to ovarian stimulation.

FSH and LH measurements were standardized against the second IRP WHO Reference standard 78/549 and the second international standard (NIB SC) 80/552, respectively. The measuring range for FSH and LH was 0.1 to 200 mIU/ml while for E2 and P4 it was 5 to 4300 and 67 pg/ml and 0.03 to 60 ng/ml, respectively. E2 and P4 were measured by electrochemiluminescence immunoassay using competition principle. All the women were divided into two groups based on (i) day 3 FSH/LH ratio; with subgroup I consisting of women with FSH/LH<2 and subgroup II with FSH/LH ratio ≥2 and (ii) day 3 LH concentrations; with subgroup A consisting of women with LH>3 mIU/ml and subgroup B with LH≤3 mIU/ml. Parameters were compiled and statistically compared between the two subgroups.

A standard long step-down protocol was used for controlled ovarian stimulation. The GnRH analogue (0.5 mg) (Leuprolide acetate and Lupride; Sun Pharmaceuticals, India) was administered subcutaneously during the last five days of a low dose oral contraceptive (OCs). Ovarian stimulation was started after confirmation of down-regulation by E2<50 pg/ml, and ruling out any follicular cyst>10 mm in diameter on baseline transvaginal sonogram. Controlled ovarian hyperstimulation (COH) was initiated with the administration of 225 IU recombinant FSH (Gonal-F; Serono SA, Switzerland) with the same dose of leuprolide acetate (0.5 mg) till the day of final oocyte maturation. After the sixth day, the gonadotropin dose was adjusted according to the ultrasonographic and endocrinal parameters. The cycle was cancelled if ovaries were resistant to stimulation or peak E2 was <500 pg/ml. Final oocytes maturation was induced by 10000 IU injection of hCG (Fertigyn; Sun Pharmaceuticals) intramuscularly which provided three or more follicles at least 16 mm in diameter.

Transvaginal oocytes retrieval was scheduled 34 to 36 hr after hCG injection. The maturation status of the oocytes and the embryo grading was recorded as per the published criteria (6). On day 2, three or fewer embryos were transferred under trans-abdominal ultrasonographic guidance. Luteal phase was supported by 300 mg micronized vaginal progesterone pessaries (Susten; Sun Pharmaceuticals) twice daily and 10 mg oral dydrogesterone (Duphaston; Solvay Pharma India Ltd., India) three times a day until day 14 post-embryo transfer.

Pregnancy was confirmed on 14th day post-embryo transfer by estimating β-hCG serum concentrations.

Clinical pregnancy was confirmed by ultrasound scans after four weeks of embryo transfer. Clinical pregnancy was defined as the presence of a gestational sac showing a positive heart beat.

**Statistical analysis:** Results are expressed as
mean±SD. Continuous variables were assessed by Student’s t-test and Bartlett’s test, as appropriate. Categorical variables were assessed by Chi-squared ($\chi^2$) test with Yate’s correction or Fisher’s exact test in case of small cell frequencies. Correlation of FSH/LH ratio with day 3 FSH and LH was performed using linear regression analysis. A p-value <0.05 was considered statistically significant. The statistical analysis was performed using Epi info software (version 3.4.3). All patients were demographically matched.

### Results

The study included 105 women who underwent 112 IVF cycles with long luteal phase GnRH agonist down-regulation protocol. Out of 112 cycles, COH was completed in 100 cycles for IVF procedures and 12 cycles (10.71%) were cancelled due to inadequate ovarian response.

The data is presented in Table 1. Subgroup I (Day 3 FSH/LH ratio <2) included 81 clients and subgroup II (Day 3 FSH/LH ratio ≥2) had 31 clients. Subgroup A (Day 3 LH>3 mIU/ml) included 80 and Subgroup B (Day 3 LH≤3 mIU/ml) included 32 clients.

Cancellation rate was higher in subgroup II than subgroup I but the difference was not significant (19.23% vs. 9.45%, p=0.30). Four out of 5 (80%) cancelled cycles in subgroup II were because of under-stimulation while in subgroup I only 2 out of 7 cycles (28.57%) were cancelled due to this reason. The remaining 3 out of 7 cycles were cancelled due to unwanted risks of hyperstimulation and the remaining 2 because of failure to down-regulate. Similarly, cancellation rate was not significant in two LH value subgroups (subgroup A vs. subgroup B, 8.75% vs. 15.6%, p=0.31).

While comparing IVF cycle outcomes between subgroup I and II, day 3 LH was found to be lower in subgroup II (Table 2) compared to subgroup I (2.28±1.58 mIU/ml vs. 5.14±2.35 mIU/ml, respectively) and this difference was statistically significant (Table 1, p=0.02). Subgroup II also required higher FSH doses for stimulation in comparison with subgroup I.

On follow-up, we observed that subgroup II had significantly poorer outcome in their IVF cycles which included fewer number of follicles >16 mm in size (p=0.03), less E2/follicle>16 mm ratio (p=0.02), fewer retrieved oocytes (p=0.04) and poorer pregnancy rates (p=0.02) in spite of similar number and grade of transferred embryos.

Overall, the total pregnancy rate was 28% with a clinical pregnancy rate of 21%. Twenty-five (33.8%) pregnancies were achieved in subgroup I (FSH/LH<2) and three (11.1%) in subgroup II (FSH/LH≥2) which was found to be statistically significant (p=0.05). No significant differences were observed in pregnancy rates between subgroups A and B (Table 1, 30.6 vs. 18.5%; p=0.17).

Using linear regression analysis, we found that day 3 LH showed a strong negative correlation with FSH/LH ratio (coefficient -0.775, SE 0.164; p-value 0.000008) while day 3 FSH showed a less significant correlation (coefficient 0.38, SE 0.169; p=0.06).

### Table 1. Demographic characteristics of subgroups based on day 3 FSH/LH ratio

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>Subgroup I Day 3 FSH/LH ratio &lt;2 (n=81)</th>
<th>Subgroup II Day 3 FSH/LH ratio ≥2 (n=31)</th>
<th>p-value</th>
<th>Subgroup A Day 3 LH&gt;3 (n=80)</th>
<th>Subgroup B Day 3 LH≤3 (n=32)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participant’s age (years)</td>
<td>31.45±4.46</td>
<td>31.07±4.08</td>
<td>0.70 a</td>
<td>31.24±4.55</td>
<td>31.66±3.82</td>
<td>0.67 a</td>
</tr>
<tr>
<td>Type of infertility</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary infertility</td>
<td>51 (62.9%)</td>
<td>17 (54.8%)</td>
<td>0.56 b</td>
<td>42 (65%)</td>
<td>16 (50%)</td>
<td>0.20 b</td>
</tr>
<tr>
<td>Secondary infertility</td>
<td>30 (37.1%)</td>
<td>14 (45.2%)</td>
<td></td>
<td>28 (35%)</td>
<td>16 (50%)</td>
<td></td>
</tr>
<tr>
<td>Duration of infertility (years)</td>
<td>7.17±4.19</td>
<td>5.99±4.02</td>
<td>0.21 a</td>
<td>6.80±3.82</td>
<td>7.03±5.04</td>
<td>0.81 a</td>
</tr>
<tr>
<td>Cycle length (days)</td>
<td>30.55±3.01</td>
<td>30.06±3.21</td>
<td>0.43 a</td>
<td>30.57±3.22</td>
<td>29.96±2.57</td>
<td>0.37 a</td>
</tr>
<tr>
<td>BMI</td>
<td>24.54±3.08</td>
<td>23.83±2.93</td>
<td>0.31 a</td>
<td>24.26±3.23</td>
<td>24.60±2.50</td>
<td>0.63 a</td>
</tr>
<tr>
<td>Etiology a %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male factor</td>
<td>15 (20.2%)</td>
<td>4 (15.38%)</td>
<td>0.77 c</td>
<td>14 (9.1%)</td>
<td>5 (18.5%)</td>
<td>0.83 b</td>
</tr>
<tr>
<td>Tubal factor</td>
<td>40 (54%)</td>
<td>17 (65.4%)</td>
<td>0.43 b</td>
<td>38 (52%)</td>
<td>14 (51.9%)</td>
<td>0.83 b</td>
</tr>
<tr>
<td>Ovulatory factor</td>
<td>15 (20.2%)</td>
<td>4 (15.3%)</td>
<td>0.77 c</td>
<td>17 (23.2%)</td>
<td>2 (7.4%)</td>
<td>0.13 b</td>
</tr>
<tr>
<td>Endometriosis</td>
<td>10 (13.5%)</td>
<td>3 (11.5%)</td>
<td>1 c</td>
<td>8 (11%)</td>
<td>4 (14.8%)</td>
<td>0.72 c</td>
</tr>
<tr>
<td>Unexplained</td>
<td>12 (16.2%)</td>
<td>3 (11.5%)</td>
<td>0.75 c</td>
<td>15 (20.5%)</td>
<td>8 (29.6%)</td>
<td>0.48 b</td>
</tr>
<tr>
<td>Others</td>
<td>2 (2.7%)</td>
<td>1 (3.8%)</td>
<td>1 c</td>
<td>1 (1.4%)</td>
<td>1 (3.7%)</td>
<td>0.46 c</td>
</tr>
</tbody>
</table>

a) Independent t test; b) Chi-square test; c) Fisher’s exact test; d) The total percentage is >100% as some patients had more than one etiology
**Table 2.** IVF cycle stimulation characteristics and outcome in subgroups based on day 3 FSH/LH ratio and day 3 LH

<table>
<thead>
<tr>
<th>Day 3 FSH</th>
<th>Subgroup I (&lt;2 mIU/ml, n=81)</th>
<th>Subgroup II (≥3 mIU/ml, n=31)</th>
<th>p-value</th>
<th>Subgroup A (≥3 mIU/ml, n=80)</th>
<th>Subgroup B (≤3 mIU/ml, n=32)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 3 LH</td>
<td>5.45±2.28</td>
<td>6.94±2.65</td>
<td>0.35&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.33±2.53</td>
<td>5.05±1.89</td>
<td>0.08&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Day 3 E&lt;sub&gt;2&lt;/sub&gt; (pg/ml)</td>
<td>42.73±15.72</td>
<td>39.70±18.73</td>
<td>0.42&lt;sup&gt;a&lt;/sup&gt;</td>
<td>42.73±15.72</td>
<td>39.70±18.73</td>
<td>0.42&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Recombinant FSH dose (IU)</td>
<td>2015.20±633.19</td>
<td>2317.42±412.02</td>
<td>0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2041.78±650.12</td>
<td>2108.44±494.761</td>
<td>0.10&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total Gonadotropins dose (IU)</td>
<td>2482.43±550.77</td>
<td>3019.34±1090.79</td>
<td>0.001&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2515.75±582.25</td>
<td>2667.70±571.82</td>
<td>0.38&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Day 6 E&lt;sub&gt;2&lt;/sub&gt; (pg/ml)</td>
<td>415.62±334.54</td>
<td>345.18±259.57</td>
<td>0.10&lt;sup&gt;c&lt;/sup&gt;</td>
<td>412.03±324.62</td>
<td>342.67±303.16</td>
<td>0.67&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Day 6 P&lt;sub&gt;4&lt;/sub&gt; (ng/ml)</td>
<td>0.35±0.27</td>
<td>0.32±0.21</td>
<td>0.73&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.35±0.28</td>
<td>0.34±0.18</td>
<td>0.81&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>E&lt;sub&gt;2&lt;/sub&gt; on day of hCG trigger (pg/ml)</td>
<td>3064.61±4093.80</td>
<td>1880.01±3378.34</td>
<td>0.26&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2841.16±4101.84</td>
<td>2528.05±3521.43</td>
<td>0.36&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>P&lt;sub&gt;4&lt;/sub&gt; on day of hCG trigger (ng/ml)</td>
<td>0.79±0.34</td>
<td>0.81±0.44</td>
<td>0.11&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.78±0.33</td>
<td>0.84±0.45</td>
<td>0.06&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>No of follicles &gt;16 mm</td>
<td>6.36±3.51</td>
<td>4.65±3.50</td>
<td>0.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.09±3.61</td>
<td>5.44±3.50</td>
<td>0.84&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>E&lt;sub&gt;2&lt;/sub&gt; follicle &gt;16 mm ratio</td>
<td>545.36±971.67</td>
<td>515.81±555.80</td>
<td>0.002&lt;sup&gt;c&lt;/sup&gt;</td>
<td>532.40±978.78</td>
<td>501.32±503.45</td>
<td>0.06&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>No of retrieved oocytes</td>
<td>9.09±5.59</td>
<td>6.67±3.94</td>
<td>0.04&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.91±5.71</td>
<td>7.33±3.80</td>
<td>0.01&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>No of embryos transferred</td>
<td>2.79±0.46</td>
<td>2.61±0.57</td>
<td>0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.76±0.51</td>
<td>2.70±0.46</td>
<td>0.57&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cancellation rate</td>
<td>7 (9.45%)</td>
<td>5 (19.23%)</td>
<td>0.30&lt;sup&gt;d&lt;/sup&gt;</td>
<td>7 (8.75%)</td>
<td>5 (15.6%)</td>
<td>0.31&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pregnancy rate</td>
<td>25 (33.8%)</td>
<td>3 (11.1%)</td>
<td>0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>23 (31.5%)</td>
<td>5 (18.5%)</td>
<td>0.30&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Clinical pregnancy rate</td>
<td>19 (25.6%)</td>
<td>2 (7.6%)</td>
<td>0.72&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17 (23.28%)</td>
<td>4 (14.81%)</td>
<td>0.79&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

a) Independent t test; b) Chi Square test; c) Bartlett’s test; d) Fisher’s test

**Discussion**

A number of screening tests have been introduced to predict poor ovarian responses (7–9). However, none of these tests have been found to be sufficiently accurate for predicting the ovarian response (10–14).

Here, we studied a homogeneous group of clients undergoing IVF where high responders (PCO and PCO-like) and low responders (high basal FSH or E<sub>2</sub> concentrations) were excluded from the analysis. Our findings showed that patients with an elevated FSH/LH ratio has reduced ovarian response to stimulation and pregnancy rates in IVF treatment while low basal day 3 LH concentrations alone did not have a statistically significant negative impact.

We found poorer IVF cycle outcomes in clients undergoing IVF with long protocols even with a FSH/LH ratio ≥2. The cut-off point for FSH/LH ratio ≥2 was also studied by Liu et al. (3). They observed that FSH/LH ratio showed best correlation with clinical pregnancy over other measures of ovarian reserve. However, the differences in pregnancy rate did not reach statistical significance. Patients with elevated FSH/LH ratios were more likely to have been placed on microdose flare protocol. Our patients did not receive microdose flare or antagonist protocol.

Barosso et al. (15) and Shrim et al. (16) reported similar results in patients with elevated FSH/LH ratios >3. Mukherjee et al. (8) reported decreased ovarian response and lower pregnancy rates in 14 patients with FSH/LH ratios >3.6. But high cancellation rate in the group with elevated FSH/LH ratio made it difficult to draw conclusion regarding differences in pregnancy rate. Moreover, high responders were not excluded from the analysis in their study.

In our study, FSH/LH ratio strongly correlated with the LH concentration. Various studies have shown that FSH/LH ratio is elevated secondary to low LH and not to higher FSH concentrations (3, 15, 16). However, Mukherjee et al. reported that 11 out of 14 patients had elevated FSH/LH ratio in their study (5). FSH concentrations remained >15 mIU/ml in their first 6 months of follow-up. This indicates that elevation in FSH/ LH concentrations may have been simply forecasting an imminent rise in FSH levels.

Our findings were consistent with several other studies which do not support the predictive significance of low day 3 LH levels alone in ovarian stimulation outcome (17). Noci et al. (4) found that low day 3 LH values were predictive of a reduced response to ovarian stimulation. However, their study was based on a population subjected to
ovulation stimulation other than IVF management; hence, oocyte number and quality was not included.

From experimental and clinical evidence, it seems that there is a "threshold" for LH requirements during folliculogenesis which together with the intricate auto and paracrine actions of the intra-ovarian regulators, it influences follicular development (4, 18, 19). Whether the exogenous addition of LH may be of benefit to enhance oocyte quality is controversial (20, 21).

The present study specifically examined peak $E_2$/follicle$>16$ mm ratio and this difference was statistically significant between subgroups I and II. Since the pregnancy rate reflects the implantation potential of each embryo, it may be speculated that a higher FSH/LH ratio negatively impacts oocyte quality leading to a poor embryonic development. We also examined serum $E_2$ and $P_4$ concentrations on day 6 of COH and those of serum $P_4$ on the day of $\beta$-hCG measurement which was not done in previous studies. However, these differences were not statistically significant in either of the subgroups.

**Conclusion**

The present study demonstrated that an elevated FSH/LH ratio is associated with an inferior outcome in IVF. The day 3 FSH/LH ratio adds more predictive power over day 3 FSH alone, especially in younger patients with a normal FSH concentration $\leq 11$ mIU/ml. Additional prospective randomized controlled trials on different regimens are warranted in order to clarify the physiological significance of an elevated FSH/LH ratio and decreased LH concentration in relation to ovarian function and outcome in IVF treatments. Since Day 3 serum FSH and LH concentrations are usually readily available, their ratio could be used as an additional important predictor for compromised ovarian reserve, in refining the treatment protocol accordingly and avoiding potential reticulations.

**Acknowledgement**

The authors deeply acknowledge the patients for giving consent for the study. Technical assistance from staff of IVF and Reproductive Biology Centre is highly acknowledged.

**Conflict of Interest**

The authors declare no conflict of interest.

**References**


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Correlation of FSH/LH Ratio and IVF Outcomes


