JMSCR Volume||03||Issue||04||Page 5105-5114||April 2015

www.jmscr.igmpublication.org
Impact Factor 3.79
ISSN (e)-2347-176x

IGM Publication
Journal Of Medical Science And Clinical Research
An Official Publication Of IGM Publication

Ratio of Follicle Stimulating and Luteinizing Hormone with Hormones of Implantation in Intracytoplasmic Sperm Injection

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ABSTRACT

Objective: *To assess relationship of ratio of follicle stimulating (FSH) and luteinizing hormone (LH) with hormones of implantation in females down-regulated for Intracytoplasmic sperm injection (ICSI).*

Material & Methods: *Retrospective study of 564 primary infertile females booked for ICSI from June 2012 till August 2014 was carried. They were 20-40-years old with infertility of more than two years and body mass index 18-35kg/m^2. FSH and LH were assessed before and Estradiol (E2) and Progesterone (P) after down regulation of ovaries by enzyme linked Immunosorbent assay. Logistic regression was applied for clinical pregnancy (CP) with FSH, LH and its ratio. Median value of variables with best results after regression was used for stratification of groups. Hormones of implantation; E2, P and their ratios were compared in the derived groups.*

Results: *On the basis of highest Beta error, groups was stratified as;group I ≤ 1.26 and group II > 1.26 median value of FSH/LH ratio. The CP rate was 36%* with higher number of pregnancies *158 /286 (55 %)* in Group I as *compared to 44/278 (16%) in group II. Group I females had high peak and mid luteal E2 and low P as compared to other group (p<0.001). Linear correlation of FSH/LH was observed with E2/P (r=-0.250, p<0.001).*

Conclusion: *High E2/P ratio required for CP in stimulated cycles was acquired by females with low FSH/LH ratio measured before down regulation of ovaries.*

Key Words: *Infertility, Intracytoplasmic sperm injection, follicle stimulating hormone, luteinizing hormone, FSH/LH ratio*

INTRODUCTION

The hypothalamus hypophyseal –ovarian axis is an essential component for regulation of natural menstrual cycle. The gonadotropic releasing hormone (GnRH) from hypothalamus stimulates the synthesis and release of two gonadotropins; luteinizing hormone (LH) and follicle stimulating hormone (FSH) from the anterior pituitary gland. Gonadotropins stimulate estradiol (E2) and progesterone (P) secretion from the ovaries that causes folliculogenesis and formation of a corpus luteum in follicular and luteal phase of ovarian cycle respectively. GnRH and gonadotropins arerigorously regulated by ovarian hormones through feedback communication between the ovaries and the hypothalamic-pituitary unit in a normal cycle.[(1)]

FSH is responsible for proliferation of granulosa cells and development of LH receptors during the follicular phase. This is made possible by activation of aromatase and p450 enzymes which causes increased E2secretion into the follicles. This up regulates FSH receptors on granulosa cells and increases its sensitivity to E2 by a positive feedback effect. The pituitary FSH and increased E2 promote the LH receptors on the original granulosa cells, thus allowing LH stimulation to occur. The increasing estrogens from the follicle plus the increasing LH from the anterior pituitary gland, act synergistically causing proliferation of the follicular and thecal cells and increasing their own secretions.

Decline in LH concentration in the mid-follicular phase fails to produce sufficient E2 to support further normal development. In luteal phase greater concentrations of P has an inhibitory effect on gonadotropin secretion mainly on LH. [(2)]Then at the end of luteal phase of natural menstrual cycle estrogen and P level declineand menstruation occurs. It has been observed that besides role of FSH & LH in normal cycles, optimal levels of FSH and LH are required for maturation of the ovarian follicles in females of intracytoplasmic sperm injection (ICSI). [(3)] Low concentration of LH follicular fluid is associated with impaired fetal development and hence high FSH: LH ratio has been associated with poor morphology embryo and failure rate of pregnancy. [(4)] This is supported by the evidences that follicles having fertilized oocytes contain higher concentration of LH. Persisting LH production and secretion have key role in oocyte maturation because it is responsible for synthesis of aromatizable substrate by theca interna cells like P plus E2 which are essential factors for implantation of the embryo. [(5)]

There are evidences that elevated E2 and E2/P ratio are good predictors of oocyte maturation and have been associated with a greater chance of pregnancy. [(6)]Alarming increase in infertility rate has increased the number of couples in quest of help for assisted reproductive techniques. [(7)]The role of the hypothalamus hypophyseal–ovarian axis well established in the regulation of the normal menstrual cycle is however, disrupted in females who are down regulated for treatment of ICSI. The present study was carried out to assess if the levels of FSH and LH measured before down regulation of patients has an effect on the levels of E2 and P during the cycle. Present study was thus conducted with the endeavor to explore for an accurate relationship of FSH/LH with hormones (E2, P) in females down regulated for ICSI.

MATERIAL & METHODS

Participants:

It was a retrospective study in which data was collected from females who underwent ICSI from June 2012 till August 2014. The treatment protocol was followed after ethical approval from review board of "Islamabad Clinic Serving Infertile Couples". The inclusion and exclusion criteria and treatment protocol is mentioned in a previous study. [(7)]

Apparatus:

Serum samples were analyzed for FSH and LH by Enzyme linked Immuno Sorbent (ELISA) on day three of unstimulated cycle and the FSH/LH ratio calculated. The treatment protocol was followed as given in Fig.1.Estimation of E2 and P by ELISA was measured (peak levels) on the day of ovulation induction (OI) and Embryo transfer (mid luteal levels).

Procedure:

The ratio of E2/P on day of OI was considered whereas ratio on day of ET was ignored because of P supplementation by Cyclogest pessaries started after oocyte pick up. A clinical pregnancy (CP) was confirmed by the presence of one or more gestation sacs with cardiac activity on TVS, 2 weeks after positive pregnancy test by serum beta hCG measurement. [(8)]Data was analyzed by SPSS version 20. Continuous variables were represented by mean ± SD and categorical variables by frequencies and percentages. The hormones and ratio to be compared in groups stratified on the basis of median level of FSH, LH or FSH/LH ratio identified to be the best predictor of logistic linear regression. The characteristics of groups then to be compared by Mann-Whitney U test. Results considered significant with p value less than 0.05.

RESULTS

Out of a total number of 564 females, CPwas 202with a CP rate of 36%. The mean levels in all these females were; FSH 6.68 6.26 ± 1.08 (IU/l), LH 5.19± 1.42 (IU/l) and FSH/LH ratio 1.40± 0.51 (mean ± SD). The comparison of these variables declared significant low FSH, high LH and low FSH/LH ratio in pregnant as compared to non-pregnant females as given in Figure 2a, b,c. On the basis of highest Beta error of FSH/LH ratio by logistic regression (Table1) it was selected for categorization of females to assess the association with hormones of implantation.

Group I included 286 (51%) females with FSH/LH ratio ≤ 1.26 and Group II included 278 (49%) females with FSH/LH ratio > 1.26.In group I, 45% (128/286) females were non-pregnant and 55% (158/286) were pregnant. In group II 84% (234/278) were non pregnant and 16% (44/278) had conceived. CP rate was 78% (158 /202) in group I and 22% (44/202) in group II, respectively. The comparison of peak and mid luteal hormonesin both groups is given in Table 2.Significant linear correlation of FSH/LH ratio was observed with E2/P (r= - 0.250, p<0.001) as shown in Figure 3.

Table1: Logistic Linear regression of Clinical Pregnancy with variables

Variable	Beta	Standard error	Sig.	Odd ratio	95.0% C.I. for EXP(B)	
					Lower	**Upper**
FSH/LH ratio	-2.40826	0.418813	0.000	0.090	0.040	0.204
FSH (IU/l)	-0.72697	0.157042	0.000	0.483	0.355	0.658
LH (IU/l)	0.601176	0.107524	0.000	1.824	1.478	2.252

FSH stands for Follicle stimulating hormone

LH stands for luteinizing hormone

Table2: Assessment of Hormones of Implantation by FSH/LH Ratio

Variable	**Group I (n= 286) FSH/LH ≤ 1.26**	**Group II n = 278 FSH/LH > 1.26**	**P value**
FSH (IU/l)	6.20 ± 0.69	7.17 ±1.19	0.0001
LH (IU/l)	6.16 ± 1.41	4.2 ± 1.44	0.0001
Peak Progesterone (ng/ml)	1.23 ± 0.67	1.73 ± 0.73	0.0001
Mid luteal Progesterone (ng/ml)	129.47 ± 56.28	168.75 ± 44.10	0.0004
Peak Estradiol (pg/ml)	2379.99 ± 305.82	2264.29 ± 279.31	0.0001
Mid luteal Estradiol (pg/ml)	1019. 85 ± 161.85	928.23 ± 149.77	0.0001
Peak E2/P	2765.26 ± 1918±82	1798.47 ± 1471±20	0.0001

FSH stands for Follicle stimulating hormone

LH stands for luteinizing hormone

Values are expressed in Mean ± SD

Figure 1: Treatment Protocol for Intracytoplasmic Sperm Injection

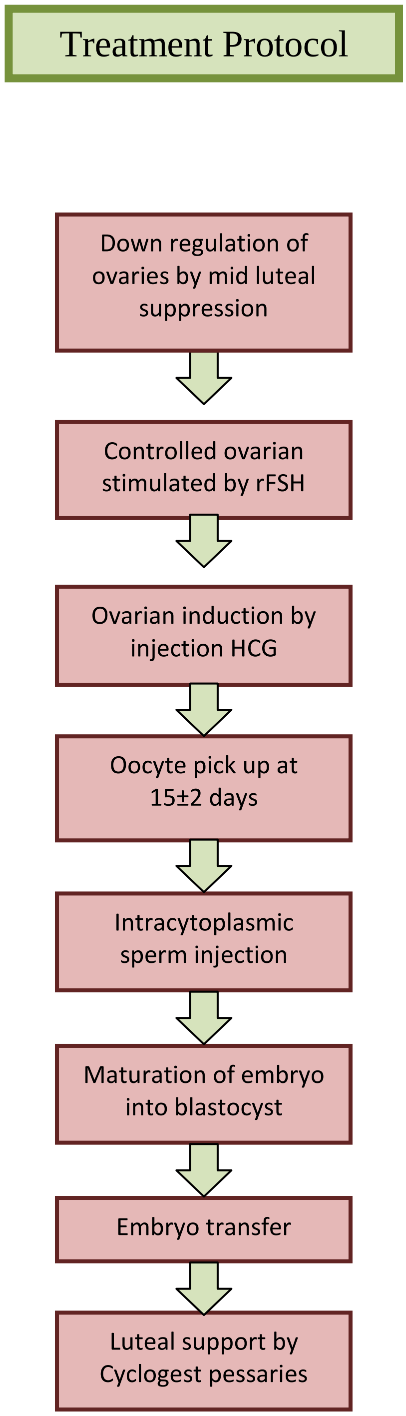
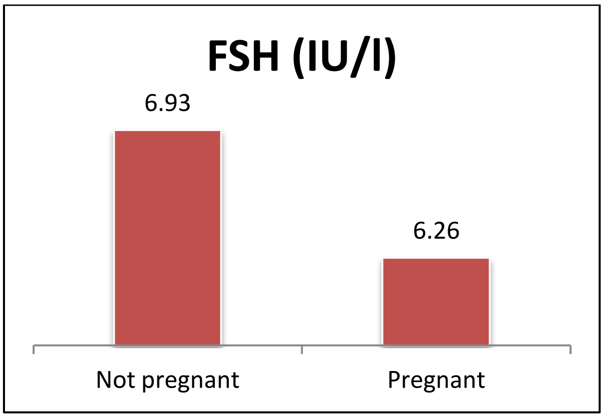
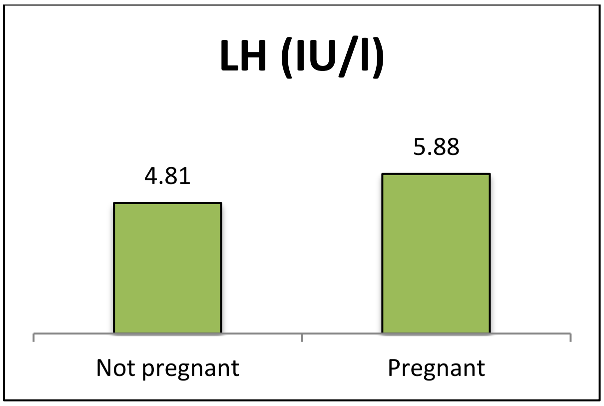


Figure 2 (a-c): Comparison of FSH (a), LH (a) and FSH/LH ratio (c) in pregnant and non- pregnant females.

a.


b.


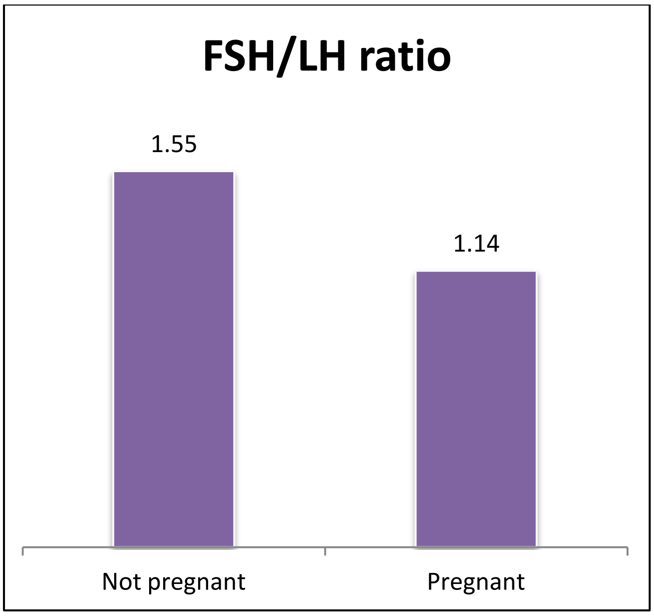
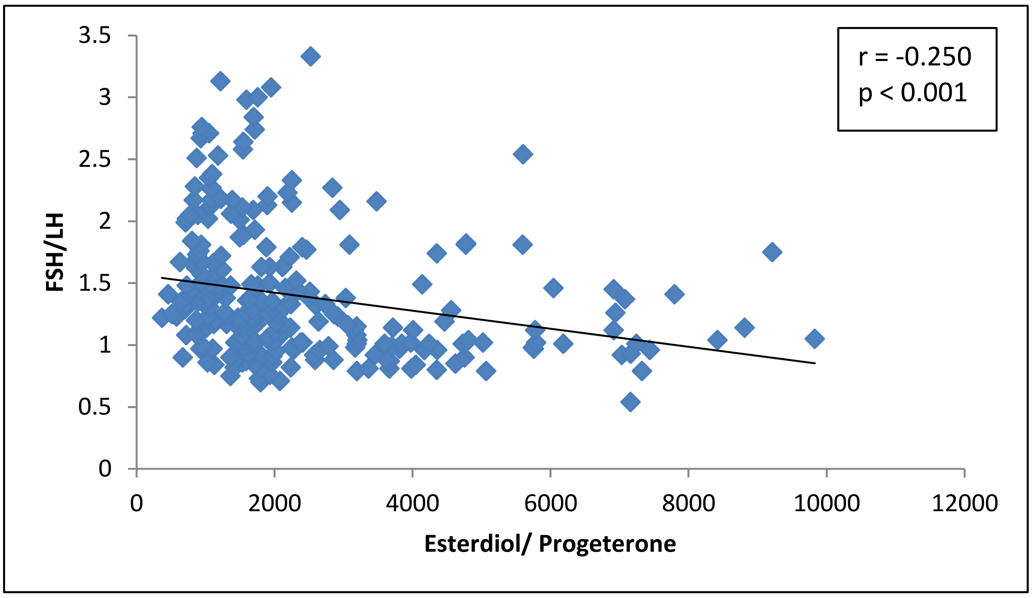
c.


Figure 3: Correlation of FSH/LH and estrogen/Progesterone



DISCUSSION

E2 is necessary for endometrial preparation, blastocysts implantation and P for priming of the endometrium and thus are substantial for pregnancy. [9] In our study, the levels of peakE2 and P were measured to find out the relation between the FSH/LH ratio and E2, P concentrations. The results of our study showed that the group of patients with FSH/LH ratio less than 1.26 had a higher peak and mid luteal E2 levels. Many studies have evaluated its effect on IVF outcomes and have shown a positive relation. However, no study has investigated the effect of FSH/LH ratio on E2 levels.

Our results also showed those females with FSH/LH ratios less than 1.26 had a higher peak E2.Kara et al [10] in their study noticed that patients with a high peak E2 level had a better response to the ICSI procedure. Similar findings were reported by Rehman et al [11] in women undergoing ICSI as a result of increased endometrial thickness due to high peak E2. This is supported by use of Estradiol valerate for improvement in endometrial thickness in females onclomiphene citrate therapy.[12] There are evidences that the administration of E2 can stimulate the proliferation of endometrium, up regulate the P receptor for continuation of pregnancy. However, a study by Chiasson et al [13] showed no significant difference on the ICSI outcomes between the different groups of patients with high to low peak E2 levels. A study by Ektan [8] on women undergoing ICSI-ET showed high mid-luteal E2 levels in the pregnant patients. These results agree with our findings as the patients in group I of our study, i.e. those with a FSH/LH ratio less than 1.26 had significantly higher mid-luteal E2 levels.

Pis another essential hormone for pregnancy, but an elevation in its level on the day of hCG administration has been described as "premature luteinization" [14] and is considered harmful. A large retrospective study by BeiXu et al [15] showed an inverse relation between the peak serum P levels and the pregnancy rates in over 10,000 patients. Another study by Bosch et al [16] showed that higher peak P levels were associated with decreased ongoing pregnancy rates. The results of our study are in accordance with these researches, as a higher peak P was observed in the poor responders group (FSH/LH ratio >1.26). This shows that the patients who had a higher peak P also had a high FSH/LH ratio and thus had unsuccessful ICSI outcomes.

Another parameter that could be used to predict the outcome of an ICSI procedure is the E2/P ratio. In a study by Irmhild Gruber et al [17] a high E2/P ratio was used as a prognostic tool for successful IVF outcome. Two previous studies by Rehana Rehman et al [7,18] also investigated the effects of E2/P ratios on the clinical pregnancy rates and proved that a higher ratio conceded with a higher pregnancy rate. Females with FSH/LH ratios less than 1.26 had a higher E2 /P ratio compared to the group with high FSH/LH ratios. All these results support our hypotheses that FSH/LH ratio can be used to predict the value of E2/P ratio and thus predict the outcome of the ICSI procedure.

Our previous study regarding FSH/LH ratio's effect on ICSI outcome showed that a low FSH/LH ratio was needed for a successful outcome and ratio higher than 1.26 corresponded to poor outcomes. The results of the present study showed a relationship between FSH/LH ratio and the levels of P and E2in the patients of ICSI. Since a high FSH/LH ratio is present in patients with low peak and mid-luteal E2, high peak P, low estradiol/progesterone ratio, these patients respond poorly to the ICSI procedure and showed significantly less pregnancy rates.

It has been observed that serum FSH levels are raised in early reproductive age and LH at a later reproductive age. [19]Elevated FSH/LH ratio even with normal basal FSH has been proved to be a sign of diminished ovarian reserve and poor IVF outcome.[20, 21] Some recent studies documented that raised basal FSH/LH ratio in the presence of a normal day 3 FSH level is predictive of diminished pregnancy rate in older women but not in younger women. [22] Studies have also shown a positive relation between a low FSH/LH ratio and positive IVF outcomes, we have identified that a relation between the FSH/LH and hormones of implantation might be responsible for successful pregnancy results after ICSI.

With the help of this study, we can prove that FSH/LH ratio can be used not only to predict poor and good responders but also the values of all hormones of implantation which foretell about ICSI outcome. This will spare our patients from the pain of repeated needle pricks and also save the extra money spent on estimation of these tests in the procedure.

CONCLUSION

Females with low FSH/LH ratio had high E2 and low P levels in peak and mid luteal phases of ovarian cycle. Association of high E2/P ratio with clinical pregnancyis well known which supports association of low FSH /LH ratio in unstimulated cycles with high E2 and P after stimulation in ICSI patients.

ACKNOWLEDGMENT

All authors strongly acknowledge Dr. Najeeha Talat Iqbal for providing us the statistical support.

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