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FSH/LH ratio in females and intracytoplasmic sperm injection

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ORIGINAL ARTICLE

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Abstract

Objective: To observe the effect of follicle stimulating hormone-luteinizing hormone ratio on oocyte parameters, quality of embryo, implantation and clinical pregnancy rate after intracytoplasmic sperm injection.

Methods: The retrospective study was conducted at a fertility clinic in Islamabad, Pakistan, and comprised data of primary infertile females who underwent intracytoplasmic sperm injection from June 2011 to March 2013. All subjects had duration of infertility more than two years, and age range was 20-40 years. Follicle stimulating hormone and luteinizing hormone were estimated by enzyme-linked immunosorbent assay on day 3 of the cycle and the ratio was calculated. Groups were stratified on the basis of median ratio into groups I ≤1.26 and group II >1.26. SPSS 20 was used for data analysis.

Results: Of the 282 females, 143(51%) were in group I and 139(49%) in group II. Pregnancy was acquired by 79(55%) and 22(16%) females in group I and II respectively. The number of retrieved, metaphase, fertilised oocytes, cleaved embryos and endometrial thickness was significantly larger in group I ($p< 0.0001$).

Conclusion: Follicle stimulating hormone-luteinizing hormone ratio less than 1.26 was associated with good oocyte parameters, top quality embryo and implantation after intracytoplasmic sperm injection.

Keywords: Infertility, Intracytoplasmic sperm injection, follicle stimulating hormone, luteinizing hormone, FSH/LH ratio. (JPMA 65: 1330; 2015)

Introduction

Infertility is the inability of a sexually active, non-contracepting couple to achieve pregnancy in one year.[1] It is found to be highly prevalent in South Asia, with an estimated rate of 3% and above cases of primary infertility in Pakistan.[2] Advancement in medical expertise and technology has made way for effective treatment modalities of infertility, available for individuals of suboptimal reproductive health.[3] One of the artificial reproductive techniques (ART) is intra-cytoplasmic sperm injection (ICSI) in which single sperm is introduced by a micropipette into the centre of the egg.[4]

ICSI is a procedure which is indicated in anatomical and physiological aberration in the male reproductive tract or abnormality in sperm count, morphology and motility. Besides, ICSI has been opted for in cases of failure with respect to in-vitro fertilisation (IVF) cycles irrespective of cause of infertility. Among the many factors involved in female infertility are the hormonal abnormalities, especially of follicle stimulating hormone (FSH), luteinizing hormone (LH), progesterone, estradiol, prolactin and thyroid hormones. Out of these hormones, FSH and LH have a central role in regulation of delicate endocrine mechanisms of the ovary.[5]

FSH and LH belong to the human gonadotropins family; they stimulate the ovaries and are essential for reproduction.[6] FSH secreted from the anterior pituitary gland not only stimulates growth of ovarian follicles, but also promotes estradiol (E2) production. Basal FSH is an established endocrine marker of ovarian reserve (OR), along with inhibin B, E2,anti-Mullerian hormone (AMH) and antral follicle count (AFC).[7] FSH initiates follicular growth and stimulates the ovaries to produce the steroid hormones, LH surge causes ovulation and regulates the release of reproductive hormones.[8] High FSH levels are associated with premature ovarian failure.[9] So, the right balance between FSH and LH in women is essential for reproduction.[10]

With age, fertility in women decreases. An increase in FSH occurs before elevation in LH so the first intimation of decreased OR may be due to an increased FSH-LH ratio.[11,12] Adequate OR is a prerequisite for successful IVF or ICSI treatment; thus a measurement of FSH/LH ratio is important to determine which patients have a high outcome possibility.

The current study was planned to observe the effect of FSH-LH ratio on oocyte parameters, quality of embryo, implantation and clinical pregnancy rate after ICSI.

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Subjects and Methods

The retrospective study was conducted at Islamabad Clinic, Islamabad, and comprised data related to females who underwent ICSI between June 2011 and March 2013. After approval from the institutional ethics review board, women records of women aged 18-40 years, who had primary infertility for 2 years, whose existent both ovaries were without any morphological abnormalities, had normal ovulatory cycle (25-35 days), had body mass index (BMI) 18-27 kg/m^2, and whose basal FSH levels on day 2 was <10 ml U/mL. Infertile cases with cause in the male partner were excluded.

FSH and LH levels had been estimated by enzyme-linked immunosorbent assay (ELISA) on day three of the cycle and the ratio was calculated. These patients underwent long down-regulation of the pituitary gland by use of gonadotrophin releasing hormone (GnRH) agonist. Development of the follicular cohort was induced with higher doses of rRecombinantFSH (rFSH) (Puregon). Follicular growth was monitored by transvaginal scan (TVS). When the leading follicles were mature, ovulation was triggered with human chorionic gonadotropin (hCG; injection Pregnyl). Endometrial thickness was measured on this day with the help of TVS. The oocytes were retrieved from the follicles 36 hours after injection by ultrasound-guided transvaginal aspiration of the follicular fluid. Microinjected mature oocytes were grown in culture medium till blastocysts stage. On average 1 to 3blastocysts were transferred into the uterine cavity on day 5 after retrieval of the oocytes.

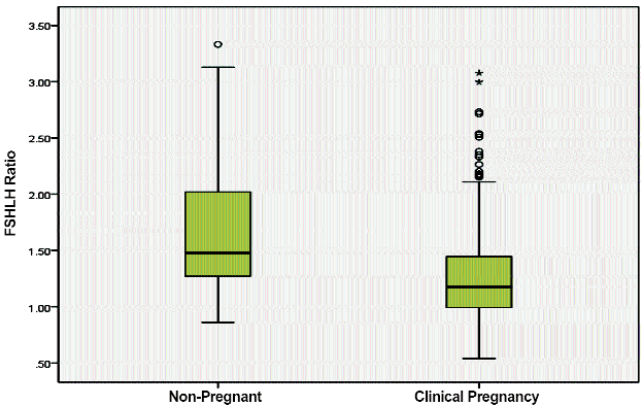
A clinical pregnancy (CP) was confirmed by the presence of one or more gestation sacs with cardiac activity on TVS, 2 weeks after positive pregnancy test by serum beta hCG measurement.[13] Mean implantation rate was the proportion of embryos transferred resulting in an intrauterine gestational sac.[4,13]

The cycle characteristics, oocyte and embryo parameters, implantation and CP rate were compared in groups stratified on median level of FSH/LH ratio 1.26. Group I included women with FSH/LH ratio $\leq$1.26 and group II comprised those with >1.26 FSH/LH ratio.

Data was entered into MS Excel and analysed using SPSS 20. Frequencies and percentages for categorical variables and mean ± standard deviation were taken for continuous variables. Shapiro-Wilk test identified non-normal variables in the data, hence Mann-whitney U test was used to assess difference between variables on the basis of median value of FSH/LH ratio. Results were considered significant at p<0.05.

Results

Of the 282 females whose records were reviewed, 143(51%) and 139(49%) comprised group I and II respectively. Overall, mean FSH level was 6.68±1.08, mean LH was 5.19±1.42, and the mean FSH/LH ratio was



FSH: Follicle stimulating hormone
LH: Luteinizing hormone.

Figure: Comparison of FSH/LH ratio with clinical pregnancy. The ratio of median, 25th and 75th percentiles are shown in box plots for both groups.

Table: Comparison of cycle characteristics.

Variables	Group I FSH/LH ratio ≤ 1.26 n= 143	Group II FSH/LH ratio > 1.26 n= 139	P value
Age at marriage	24.55 ± 4.36	25.47 ± 4.62	0.08
Cause of Infertility			
Polycystic	83(58%)	43 (31%)	0.0001
Tubal	3 (3%)	0	
Unexplained	57 (39%	96 (69%)	0.0001
Duration of infertility	7.13± 4.14	7.09 ±3.59	NS
Body mass index (Kg/m2)	24.03 ±3.59	24.47 ± 3.78	NS
Pre ovulatory follicle count	7.98 ±1.91	7.63 ±1.81	0.08
Number of oocytes/patient	7.87 ± 1.70	7.50 ± 1.63	0.0001
Metaphase II	7.65 ± 1.65	6.59 ± 2.10	0.00005
Fertilised oocytes	6.34 ± 1.29	5.54 ± 1.70	0.0001
Number of cleaved embryos	6.24 ± 1.25	5.46 ± 1.65	0.001
Grade 1	2.66 ± 1.64	1.43 ± 1.26	0.001
Grade 2	2.38 ± 1.26	2.75 ± 1.33	0.004
Grade 3	1.20 ± 0.47	1.29 ± 0.51	NS
Blastocysts formed	2.27 ± 0.53	1.93 ± 0.52	0.0001
Number of transferred embryos	1.71 ± 0.57	1.53 ± 0.58	0.007
Endometrial thickness	9.51 ± 3.47	7.65 ± 3.09	0.0001
Implantation rate (%)	49.29	14.23	0.0001
Total number of gestational sacs/ Total number of embryos transferred × 100			

p<0.0001.

1.40±0.51.

Age, BMI and duration of infertility did not show significant difference in the groups (Table). The number of retrieved, mature (metaphase) and fertilised oocytes, cleaved embryos and endometrial thickness was significantly larger in group I (p<0.0001). Total number of pregnancies in the study group was 101/282 with a CP rate of 36%. In group I, non-pregnant were 64/143 (45%) and 79/143 (55%) were pregnant. In group II, 117/139 (84%) were non-pregnant and 22/139 (16%) had conceptions. CP rate in group I was 79/101 (78%) and 22/101 (22%) in group II. The implantation rate in group I was significantly higher than in group II (p<0.00-1).

The box plots of FSH-LH ratio with respect to non-pregnant (181) and CP (n=101) groups showed greater number of CPs in group I (Figure).

Discussion

Several earlier studies have investigated the effects of FSH-LH ratio on IVF outcomes or ovarian response. This study was carried out to identify and confirm the importance of FSH-LH ratio as an indicator of outcomes after ICSI procedures in Pakistan. Women with LH and FSH concentrations within the normal limits and those with low FSH/LH ratio are typically defined as 'normal' and 'high' responders respectively. High responders have been identified by infertility specialists as females with high peak E2 and better oocyte recovery and embryo quality.[14]

We have observed significantly lower number of oocytes in group II which exhibited higher FSH/LH ratio. It is reported that higher levels of FHS/LH ratio greater than 2 is associated with lower number of retrieved oocytes.[15] Similar results were presented by other researchers who observed a decreased oocyte retrieval in patients with a high FSH/LH ratio (< or = 2).[16]

The number of oocytes in metaphase II indicates the number of oocytes that reach maturation and is thus a sign of positive outcome of ICSI. We observed that number of oocytes in metaphase II were significantly lower in group II. In a study on mature oocyte yield in pituitary down-regulated women, it was observed that the number of mature oocytes was inversely related to the FSH/LH ratio.[17] A similar study on patients undergoing IVF cycles showed that elevated FSH-LH ratio was associated with fewer mature oocytes.[18] On the contrary, a study showed no significant difference in the number of mature oocytes in groups demarcated by 3 FSH-LH ratio.[19] The results may also be different due to a large difference between the two group sizes, 477 and 198 respectively, and difference in median value of both research studies.

The results of our study also showed a significant difference between FSH-LH ratio and the number of cleaved and grading of embryos. The results are similar to the findings of a previous study which showed an inverse relation between FSH-LH ratio and the number of day 3 embryos.[20] Our study size was almost similar to theirs, with the difference of a higher FSH-LH ratio in their study females. Literature search did not reveal many other studies to highlight relation between cleaved embryos and FSH-LH levels.

The number of blastocysts formed were also significantly different in the two groups, with more blastocysts formed in group I. In a similar study, there was no significant difference observed in the number of frozen blastocyst between the two groups stratified on the basis of FSH-LH ratio 3.[19] Implantation of embryo depends on their quality and endometrial receptivity usually estimated by measurement of endometrial thickness.[21,22] Females with low FSH/LH ratio had thick endometrial lining required for implantation of embryo. Similar findings were reported in a study that showed a significantly decreased endometrial thickness with 3 FSH-LH ratio.[23] The implantation rates in group I was high which is supported by researchers who observed aspiration of fewer mature oocytes and poorer implantation rates in those undergoing IVF treatment.[24] On the contrary, a study showed no significance difference on the implantation rate between the two groups, FSH/LH > or =2 and FSH/LH <2.[25] This difference may be due to the lower sample size, or because the women included all had polycystic ovary syndrome (PCOS).

Our study had limitations as it was a uni-centre study which involved non-randomised sampling technique with unequal proportion of cases. Receiver operating curve (ROC) was not used in this study to determine the cutoff values of FSH-LH ratio for demarcation of the groups.

We recommend that hormone assays, including FSH and LH levels, should be a prerequisite before starting ART cycles. FSH-LH ratio could be considered as a standard screening test to categorise patients who can best benefit from ART treatment. If maximum accuracy in counselling is warranted, endocrine testing, especially of FSH and LH, may be used for predictive information along with other parameters such as AFC and AMH.

Conclusion

Patients with an elevated FSH/LH ratio (>1.26) had

decreased number of oocytes, mature oocytes, cleaved embryos, endometrial thickness, implantation rate and blastocysts formed; all implying poor response to ICSI. FSH-LH ratio can be used to assess response of treatment in ART.

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