Journal Homepage: - www.journalijar.com
INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)
ISSN NO. 2320-5407
Article DOI: 10.21474/IJAR01/1593
DOI URL: http://dx.doi.org/10.21474/IJAR01/1593

RESEARCH ARTICLE

Early Follicular Phase LH concentration and LH to FSH ratio as Predictor of IVF/ICSI Outcome.

Lubna Amer Al-Anbari.
High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University, Baghdad-IRAQ.

Manuscript Info

Manuscript History

Received: 13 July 2016
Final Accepted: 18 August 2016
Published: September 2016

Key words:-
FSH, LH, IVF/ICSI outcome, ovarian reserve, LH: FSH ratio.

Abstract

Background: This study was undertaken to evaluate the effect of early follicular phase LH level and LH: FSH ratio on number of mature follicles, retrieved oocytes, oocytes maturity, quality of embryo, and pregnancy rate.
Methods: This retrospective observational study was undertaken in the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies /Al-Nahrain University. The study included 115 women who underwent IVF/ICSI treatment cycles from February 2015 to may 2016. Prior to the first IVF/ICSI treatment all women included in the study had blood samples analyzed on menstrual cycle Day 2, 3 or 4 for basal levels of Follicle stimulating hormone and Luteinizing hormone. Patients was sub grouped according to early follicular phase LH to FSH ratio into group A ≤0.5 and group B>0.5, and regarding early follicular phase LH into Group I (≤3 *mIU/ml*) and Group II (>3 *mIU/ml*)
Results: Women with elevated early follicular phase LH to FSH ratio (LH: FSH>0.5) required significantly (p<0.001) lower doses of gonadotrophins. The outcome of IVF/ICSI was significantly (p<0.001) better in those patients they had larger number of mature follicles (≥*17mm*), retrieved oocytes, mature oocytes (metaphase II), fertilized oocytes, cleaved embryos, and pregnancy rates.
On the other hand, patients with early follicular phase LH >3 *mIU/ml* did not differ significantly than patients with early follicular phase (≤3 *mIU/ml*).
Conclusion: Elevated early follicular phase LH/FSH ratio rather than LH concentration alone is associated with the highest success rates in IVF/ICSI, perhaps reflecting a well-preserved ovarian reserve.

Introduction:-

Infertility is defined as inability to conceive after one to two years of unprotected intercourse [1, 2]. Infertility affects one in seven couples most of them need medical help [3, 4]. Assisted reproductive technologies (ARTs) are being used progressively more in infertility treatments [5]. Around 1.5 million ART cycles are performed each year, with an estimated 350,000 babies born annually worldwide [6]. ICSI is the most common technique used accounting for around two-thirds of all IVF treatments Cycles [7]. Infertility treatment is associated with evident psychological

Corresponding Author:- Lubna Amer Al-Anbari.
Address:- High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University, Baghdad-IRAQ.

and financial burdens [8]. Improving the outcome of IVF/ ICSI by achieving the highest pregnancy rate is continues challenge for reproductive physicians [9]. Success of IVF/ICSI depends on the ability to engage adequate numbers of mature follicles, yielding good number of MII oocytes, so ovarian response is a key factor during in vitro fertilization cycles [10, 11].
Although ovarian response can be predicted by many factors including maternal age, antral follicle count (AFC), basal serum follicle-stimulating hormone (FSH), serum inhibin B, and serum anti-Müllerian hormone (AMH) [12–13-14], so many patients who have normal parameters for ovarian reserve response poorly to ovulation induction protocols producing low-quality oocytes and poor embryos [15,16,17]. Follicle stimulating hormone (FSH), and Lutinizing hormone(LH) are related to the human gonadotropins family; stimulating the ovaries and playing vital role in reproduction[18]. Both secreted from the anterior pituitary gland [19]. FSH stimulates ovarian follicular growth, and promotes production of ovarian steroid hormones [18, 20]. LH surge initiate final oocyte maturation, induce ovulation and regulates the reproductive hormones release [21]. Elevation in Follicular stimulating hormone (FSH) occurs several years before rise in luteinizing hormone (LH) [22, 23], giving a suggestion that the first sign of decreased ovarian reserve may be an increased FSH: LH ratio [24]. High FSH levels are associated with premature ovarian failure [25]. So, the accurate balance between FSH and LH in women is critical for successful reproduction [26].
The objective of the present study was to evaluate whether elevated early follicular phase LH: FSH ratio and relatively high LH level, in the presence of normal FSH, are predictive of good responses to ovarian stimulation before entering the stressful and expensive course of IVF.

Subjects and Methods:-

Study population:-
This retrospective observational study was undertaken in the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies/ Al-Nahrain University. Data related to one hundred fifteen women underwent ICSI from February 2015 to May 2016. The study was approved by the Local Medical Ethical Committee of the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies.

Patients were enrolled regardless the duration and cause of infertility, but excluded if older than 40 years, history of ovarian surgery or presence of single ovary, endometriosis (other than minimal), endocrinal abnormalities such as hypogonadal –hypogonadism, hyperprolactinemia, thyroid disorders, and protocols rather than a standard long GnRH agonist down-regulation protocol. In our institution all patients undergoing ART treatment receive intracytoplasmic sperm injection (ICSI).

Hormone assays:-
All women included in the study had Fasting blood samples analyzed for basal levels of Follicle stimulating hormone and luteinizing hormone, were taken on Day 2, 3 or 4 of a spontaneous or induced menstrual cycle prior to long GnRH agonist down-regulation protocol. Serum E2 concentrations were estimated for follicular development fallow up. Serum β-hCG titer was done for patients to confirm pregnancy 14 day post embryo transfers. All hormones were measured using fluorescence assay system (mini VIDAS, bioMerieux, France).

Study protocol:-
The long GnRH agonist down-regulation protocol were used in all patients, by daily administration of gonadotropin releasing hormone agonist (0.1 mg/ampoule Tryptorelin, Ferring, Germany), started in the mid-luteal phase of the previous cycle until the day of HCG administration. Satisfactory pituitary desensitization was achieved when serum E2 levels lower than 50 pg/ml, FSH <5 IU/L, LH <5 IU/L, antral follicles <10 mm, and endometrial thickness <5 mm). Indivualized starting dose of daily gonadotropin stimulation using recombinant FSH (75 U/ampoule,
Gonal F, Merck Serono.Ltd, United Kingdom), gonadotropin dose was adjusted according to follicular development observed by transvaginal ultrasonography and serum E2 concentrations. When at least two follicles reached18 mm 6500 IU injection of recombinant human chorionic gonadotrophin (Ovitrelle;Merck Serono, UK), was administered for final oocyte maturation, 36 h later oocytes were retrieved by transvaginal ultrasound-guided follicular aspiration. The maturation status of the oocytes was recorded as the published criteria [27]. Up to three Best quality embryos per patient were transferred into the uterine cavity under trans-abdominal ultrasonographic guidance on day3 post oocytes retrieval or day 5 post ova pick up for blastocysts. The embryos were considered good quality if no fragmentation or no more than 1/3, absence of multinucleation, 3~5 blastomeres after 48 hours and at least 7 blastomeres by 72 after ova pick up [28]. Blastocysts assessment done according to Gardner grading system [29]. Luteal phase was supported by 400 *mg* vaginal progesterone pessaries (Cyclogest;Actavis, UK) twice daily and 10

mg oral dydrogesterone (Duphaston; Abbott, Netherlands) three times a day until day 14 post-embryo transfer. Clinical pregnancy was confirmed by the presence of one or more gestational sacs with cardiac activity on transvaginal ultrasound examination, two weeks after positive pregnancy test by serum beta hCG measurement.
Patients sub grouped according to early follicular phase LH into Group I (≤3 *mIU/ml*) and Group II (>3 *mIU/ml*) and regarding early follicular phase LH: FSH ratio into group A ≤0.5 and group B>0.5.Compartion between each two groups regarding the number of gonadotropin ampoules, number of mature follicles, number of retrieved oocytes, Metaphase II oocytes, number of fertilized oocytes, number of embryos, blastocysts and pregnancy rate was done.

Statistical Analysis:-
Data were analyzed using the Statistical Package for Social Science (SPSS), version 20. Mean ± standard deviation were taken for continuous variables. The statistical significance difference between data of each two groups were compared by Student's t-test Categorical variables were assessed by Fisher's exact test. A p-value <0.01 was considered statistically significant.

Result:-
A total of 115 women ICSI treatment cycles were retrospectively reviewed. Demographic characteristics of the participants are shown in (table 1). Body mass index of all participants was between 18-27 kg / m^2. Forty nine(42.6%) patients included in group A(LH : FSH ratio ≤0.5), sixty six (57.4%) in group B (LH : FSH ratio >0.5), the mean LH: FSH ratios of group A was 0.4, while in group B was 0.8.This study found that patients in group B used significantly lower number of gonadotropin ampoules(p=0.0003), and their treatment revealed significantly higher number of mature follicles(≥*17mm*) (p= 0.004), more retrieved oocytes (p=0.0001), more Metaphase II oocytes (p=0.0001), larger number of fertilized oocytes (p=0.0001), higher number of embryos (p=0.0001), and number of blastocysts were higher but not significant in group B than group A. Pregnancy rate was significantly superior in group B than group A (54.5% versus30.6%) (p=0.0043) (figure1, and table2).

Regarding LH level, group I (LH ≤3 *mIU/l*) 46 (40%) patients, group II 69 (60%) patients. Mean LH level were 2.1±0.62 mUI/ml in Group I, and 5.5±1.6m UI/ml in group II. This study found Pregnancy rate higher but not significant in group II (47%) than group I (39%), all other study parameters showed no significant difference between the two groups (figure2, and table2).

As regards causes of infertility polycystic ovarian syndrome PCOS was significantly higher in group B than group A (p=0.0006), and in group II than group I (p=0.0004). Other causes of infertility showed no significant differences between each two groups (table 1).

Table (1) Demographic Characteristics of patients.

	Group A n49	**Group B n66**	**P value**	**Group I n46**	**Group II n69**	**P value**
Patients age	30.46±5.5	29.05±7.2	NS	29.37±6.218	32.57±7.3	NS
Duration of infertility	5.6±1.92	5.2±1.92	NS	5.3±1.6	6.5±2.9	NS
Causes of Infertility						
PCOS	13	39	0.0006	11	40	0.0004
Male factor	15	11	NS	15	11	NS
Tubal factor	16	13	NS	15	14	NS
Unexplained	5	3	NS	5	4	NS

The values are expressed as mean± S.D, PCOS: polycystic ovarian syndrome, NS=Not significant.

Table (2) : Comparison of treatment data between each two groups.

Parameters	group A LH:FSH ≤0.5 n49	group B LH :FSH >0.5 n66	P value	Group I LH ≤3 *mIU/l* n46	Group II LH>3 *mIU/m*l n69	P value
Number of gonadotropin ampules	**30.5±2.77**	**24 ±2.82**	**0.0003**	**27±±2.39**	**27.7±±5. 0**	**NS**
Number of follicles ≥*17mm*	**9.1±2.18**	**14±4.16**	**0.004**	**9.1±1.52**	**11±5.09**	**NS**
Number of Oocytes	**5.9±1.372**	**9.76±2.05**	**0.0001**	**8±0.94**	**7.6±*2.633***	**NS**
Number of MII oocytes	**4.9±1.37**	**8.6±2.06**	**0.0001**	**5±0.81**	**5.6±1.95**	**NS**
Number of fertilized oocytes	**3.9±1.37**	**7.6±2.06**	**0.0001**	**4.2±0.63**	**4.8±1.22**	**NS**
Numbers of embryos	**3±1.24**	**6.6±2.06**	**0.0001**	**3.7±0.67**	**4±0.66**	**NS**
Numbers of blastocysts	**1.5±0.53**	**2.14±0.6**	**NS**	**1.4±0.84**	**2±1.15**	**NS**
Numbers of transferred embryos	**2.6±0.96**	**2.7±0.82**	**NS**	**2.2±0.63**	**2.8±0.78**	**NS**
Pregnancy rate	**30.6% (14/49)**	**54.5% (37/66)**	**0.0043**	**39% (18/46)**	**47% (33/69)**	**NS**

The values are expressed as mean± S.D, FSH: Follicle stimulating hormone, LH: Luteinizing hormone.

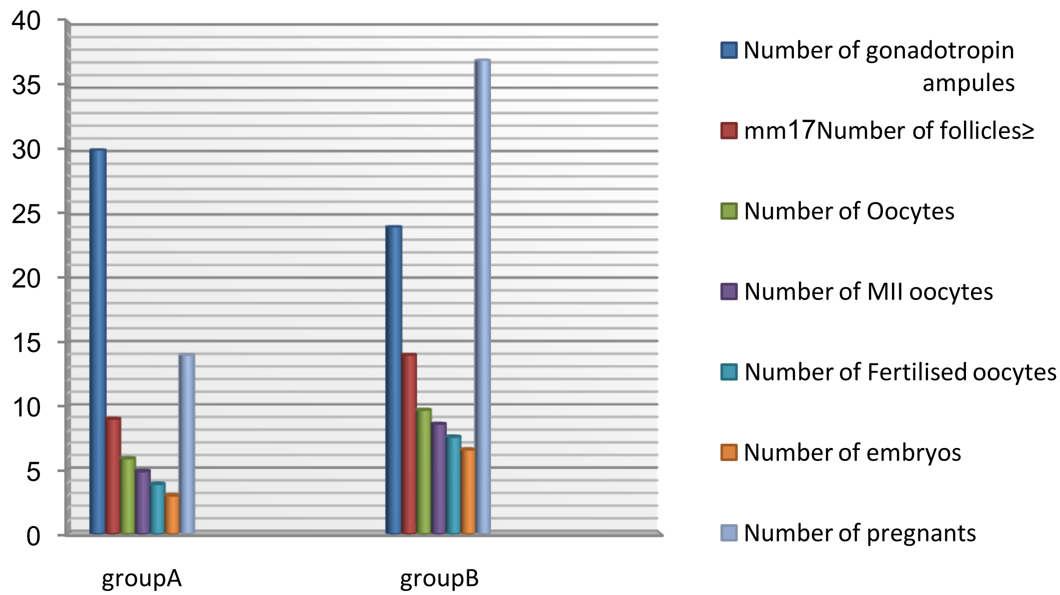

Figure (1): Comparison between group A and group B.

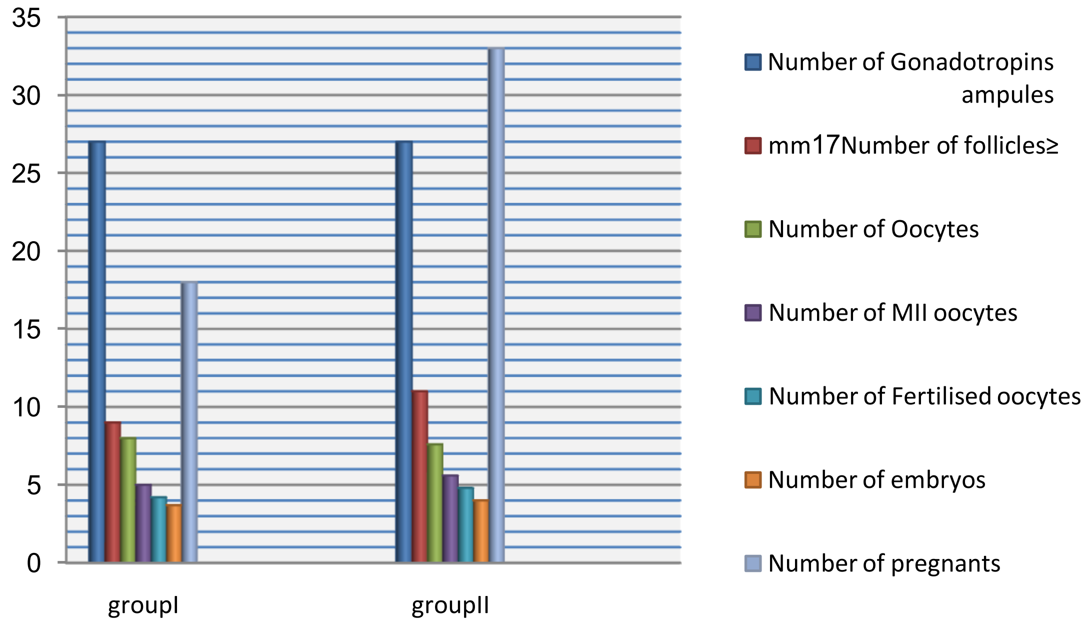

Figure (2): Comparison between group I and group II.

Discussion:-

Numerous previous studies have investigated the effects of FSH, LH level on IVF/ ICSI outcomes or ovarian response, but few studies investigate the prediction power of LH: FSH ratio in predicting IVF/ ICSI outcomes. This study was carried out to recognize and validate the importance of early follicular phase LH concentration and LH to FSH ratio as predictor of IVF/ICSI outcome. Women with LH and FSH within the normal concentrations limits, but with different LH: FSH ratio was studied.

We have observed significantly better IVF/ ICSI cycles characteristics in group B which exhibited higher LH: FSH ratio. During ovarian stimulation group of higher LH: FSH ratio used lower number of gtonadotropin ampoules than other groups, this agree considerably with Brodin *et al*. as they found patients with high LH and relatively low FSH used lower total gonadotropin dosage than patients with opposite combination[30]. Larger number of mature follicles stimulated in patients with higher LH: FSH ratio, this finding be in agreement to some extent with Rehman *et al*. who found that patients with lower FSH: LH ratio produced higher number of preovulatory follicles [31].
It is reported that patients with high FSH levels or high FHS: LH ratio greater than 2 is associated with lower number of retrieved oocytes, poorer IVF outcome, and higher risk of cycle cancellation as shown by Prasad *et al*., and Kofinas *et al*. respectively, these finding be very in line with our results[32,33].

Otsuki *et al*. considered metaphase II oocytes a positive sign of IVF outcome because they represent the number of oocytes that reach maturation [34]. Schoot *et al*. showed that lutenizing hormone is essential for oocytes maturation in gonadotropin-deficient women [35]. Drakakis *et al*. and Ho *et al*. found that mature oocytes production in pituitary down-regulated women, were inversely related to the FSH/LH ratio [36, 37].
Our results also showed a significant reduction in the number of embryos in group A who showed lower LH: FSH ratio, Seckin *et al*. finding was near to our results, but he classified the patients according to their age as he found that women ≥35 years old with elevated FSH/LH ratio had significantly lower number of retrieved oocytes, lower transferred good grade embryos count, and lower pregnancy rate versus women at the same age but with lower FSH/LH ratio. [38]. Liu *et al*., and Rehman *et al*. found an inverse relationship between FSH-LH ratio and the number of day 3 embryos, and number of blastocysts, while in our study the count of blastocysts were lower in patients with lower LH: FSH ratio but not significant [24,31].

Clinical pregnancy rate were higher in patients with elevated LH: FSH ratio in our study, Brodin *et al* found highest pregnancy rate in women with low FSH and with high LH levels; while pregnancy rate was lowest in women with opposite combination. Pregnancy rates were intermediary if FSH and LH were either both high or both low [30].

Conclusions:-

There was a significant positive correlation between the serum LH/FSH ratio and IVF/ICSI outcome. This suggests that LH: FSH ratio can be used before starting IVF/ICSI cycles, as helpful marker for ovarian reserve, and to sort out patients who can achieve the best success rate of treatment.

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