doi.org/10.36721/PJPS.2022.35.6.SP.1761-1765.1

Follicular sensitivity index and insulin growth factor-1: Predictor of success after intracytoplasmic sperm injection

Rehana Rehman, Huma Salahuddin, Amara Zafar, Sadia Rehman, Nargis Anjum and Zara Sami
Department of Biochemistry, Bahria University of Health Science, Pakistan

Abstract: To compare follicular sensitivity index (FSI) and Insulin Growth Factor **(**IGF) -1 for prediction of oocyte yield, embryo quality and the pregnancy consequence in infertile females booked for Intracytoplasmic Sperm Injection (ICSI). Study design was cross sectional where in 133 infertile females enrolled for ICSI were included. Antral follicle count (AFC), Pre-ovulatory follicle count (PFC), FSI, total dosages of follicle stimulating hormone (FSH); designed as pre-ovulatory follicle count × 100,000/ (antral follicle count × total administered stimulation doses) were estimated. IGF was measured by Enzyme Linked Immuno Sorbent Assay. Efficacious pregnancy conception after Intracytoplasmic Sperm Injection (ICSI) was established by means of intrauterine gestational sac with cardiac activity after transfer of embryo. Odds ratio was determined for clinical pregnancy by means of FSI and IGF-I, p-values <0.05 were considered significant. FSI was found to be a stronger predictor of pregnancy than IGF-I. Both IGF-I and FSI contributed positive association with clinical pregnancy consequences but FSI was found to be a more reliable predictor of clinical pregnancy. The advantage of using FSI over IGF-I is that FSI is a noninvasive test while IGF-I needs blood sampling. We recommend calculation of FSI for prediction of pregnancy outcomes.

Keywords: Follicular sensitivity index, intracytoplasmic sperm injection, insulin growth factor-1, infertility.

INTRODUCTION

Infertility is the inability of females to naturally conceive for a baby in a period of one year or more of having consistent unprotected sexual intercourse (Surcel *et al.,* 2022). Globally about 8-12% of pairs are affected by infertility (Ryzhov *et al.,* 2021), approximately; however 21.9% of prevalence in Pakistan was reported (Man *et al.,* 2022). Chiefly there are two major types of infertility, includes primary infertility where in after consistent regular unguarded intercourse, couples are still unable to conceive a baby (Yang *et al.,* 2022), while secondary infertility outlines that couples have conceived earlier at one time in their lives but are unable to conceive again (Wang *et al.,* 2020).

Female related causes of infertility are the most common (40-55%) of entire cases of infertility, followed by male linked causes (30-40%), including both partners (10%) and unexplained (10%) (Puri *et al.,* 2015). The causes of male related cases of infertility consist of variations in the sperm parameters, specifically the motility, the total count or the morphological configuration (Kumar *et al.,* 2015), causes of female infertility comprise numerous etiology like polycystic ovarian syndrome (PCOS), endometriosis, (uterine fibroids), pelvic inflammatory disease (PID), ovulation complications, tubal obstruction, age-linked issues, uterine obstacles, prior tubal ligation and hormonal disorders (Yang *et al.,* 2022).

Antral Follicle count (AFC) stated as the number of follicles that measures (3-10 mm), estimated in both the ovaries. It signifies count of residual primordial pool in females, establishes reaction of follicles of ovary to stimulus and associates to the count of oocytes recovered; however it does not impact the oocyte and quality of embryo (Rehman *et al.,* 2016).

Pre-ovulatory follicle count (PFC) is defined as number of follicles measures greater than or equal to 16 mm, appraised at the end of ovarian stimulus and in turn depends on number of minor antral follicles available for stimulation management (Gunasheela *et al.,* 2021).

Follicular Sensitivity index (FSI) is a relatively new tool introduced to evaluate follicular responsiveness to external gonadotropins. It is used to express the clinical pregnancy proportion in females with inexplicable infertility undergoing *In vitro* Fertilization (IVF)/ Intracytoplasmic Sperm Injection (ICSI). Previous studies by Hassan *et al.,* 2017 observed that increased FSI levels had significantly greater oocyte production, fertilization and clinical pregnancy rate.

$$\text{FSI} = \frac{\text{Pre-ovulatory folliclecount (PFC)}}{\text{Antral folliclecount x Total recived FSH doses}} \times 100$$

Hassan *et al.,* (2017) in their study suggested FSI as a novel tool for future guide of IVF cycle management for couples by the calculation of FSI by estimation of their ovarian follicles and by being an excellent and absolute criterion of good/poor ovarian reaction in IVF/ICSI phases and can be a worthy guide cycle termination

**Corresponding author:* dr.sadia89@hotmail.com

criteria for deprived ovarian reaction (Hassan *et al.,* 2017). ICSI is microscopic technique that aids in conception of an oocyte by injecting a solitary spermatozoon directly (Lawrenz *et al.,* 2022). Insulin like growth factor-1 (IGF-1), 'an ovarian IGF system's member, has expected to show immediate outcomes on function of granulosa cell in human. A vital function of IGF-I in controlling of human follicles and embryo's growth by regulation of the cell cycle has been noted and observed at various levels (Lawrenz *et al.,* 2022). It has also been seen to be mediating production of estrogen and aromatase activity by the emerging follicles (Yang *et al.,* 2022). IGF-1 has been proved as a prospective biochemical indicator of embryo's quality and is likewise thought to be liable for efficacious implantation rates in cycles of *In vitro* Fertilization (IVF).

Entire treatment using ART procedure carries different challenges, utmost would be the recovery of healthy eggs from females for treatment with the aim of getting the desired results. Majority of female cases suffer from inability to conceive and miscarriages occur because of bad eggs' quality. Hereafter, it is extremely important and ideal to assess the state of embryo and oocyte at start of implantation. Since, ART is an expensive technique and need a lot of proficiency it is relevant and can be utilized to evaluate the procedure of securing subjects from unwanted exposure and exertions. This study will be very beneficial and gives an idea of ART outcome along with itscost effectiveness. It relieves subjects from the hazards of redundant interventions.

The purpose of this study is toe compare the oocyte yield, embryo's quality and pregnancy consequence between FSI and IGF-I. which can be used to evaluate and influence the pregnancy consequence in women who are opting for ART. We wanted to explore which of these is better predictor of successful pregnancy outcome in terms of the yield of oocyte, the quality of embryo and the outcome of pregnancy.

MATERIALS AND METHODS

It is a cross sectional design study conducted in Australian Concept Infertility Clinic Karachi in teamwork with Basic Medical Sciences Institute (JPMC). Sample size was computed by formula by using online software (epi.com) that gives 133 sample size. Selection of sample was done by convenient random sampling technique. Grouping of subjects was done on the basis of causes and types of infertility. Infertile females (133) were used in the study. Evaluation of results was done by dividing the sample into two groups. Clinically pregnant group 1 (β hCG >25m IU/mL) with presence of cardiac activity and non-pregnant group 2 (β hCG 5-25m IU/mL). Written informed consent was filled and signed by the couple (mutually by both partners) at the stage of induction.

Subjects affected by primary and secondary infertility were inducted in the study. Other inclusion criteria include females who are infertile for more than 2 years, 20-45 years of age females, normal ovulation cycle (duration 25-35 days), no deviation in morphology of any of the two ovaries.

Exclusion criteria include females aged >45 years, existence of ovarian cysts, females with earlier failed ICSI/IVF, endometriosis with ovarian abnormalities. Complete family history and general physical examination was evaluated to rule out the cause of infertility in the couple (both partners).

Pre-ovulatory follicle count (PFC), the total doses of FSI, IGF-1 and FSH and Antral Follicle Count (AFC) were calculated and associated with quality of embryo and oocytes yield. ELISA kit for IGF-I was utilized in the analysis. It is based on sandwich technique (ELISA) for quantitative *in vitro* measurement of IGF-I. Number of Kit was SEA050Hu with detection range of '0.19-12 ng/mL'.

FSI calculation

$$FSI = \frac{PFC \times 100000}{AFC \times \text{Total dose of FSH}}$$

$$FSI = \frac{\text{Pre-ovulatory follicle count (PFC)}}{\text{Antral follicle count x Total recived FSH doses}} \times 1000$$

Data analysis

Data was analyzed by IBM SPSS version 23.0. Mean and standard deviation (SD) were evaluated for quantitative analysis. Percentages and count were analyzed by qualitative measurements. Comparison of mean values of primary and secondary infertility subjects was analyzed by Mann Whitney U test. Pearson coefficient of correlation was utilized to assess the strength of association between FSI, IGF-I, amount of oocytes recovered, number of oocytes present in Metaphase II, number of oocytes fertilized and amount of cleaved embryos.

Binary logistic regression analysis was utilized to estimate the odds ratio with 95% CI for clinical pregnancy by means of FSI and IGF-I. To rule out Area Under the Curve (AUC) for clinically pregnant females by means of FSI and IGF-I, ROC analysis was done, all p-values <0.05 were considered significant.

RESULTS

Table 1 gives mean comparison of studied samples between types of infertility. IGF-I of secondary infertility patients was significantly higher in comparison to primary infertility (p value= 0.02), where as all other parameters were statistically insignificant.

Table 1: Descriptive characteristics on the basis of types of infertility

Variables	'Primary Infertility' (Mean ± SD)	'Secondary Infertility' (Mean ± SD)	p-value
Female age	31.51±4.45	32.59 ±4.41	0.37
BMI (kg/m^2)	25.88 ±2.72	25.35 ±3.14	0.57
Duration of infertility (years)	5.21 ±3.92	6.76 ±5.03	0.12
Preovulatory Follicle count	7.62 ±2.09	7.76 ±2.03	0.50
Antral Follicle count	14.82 ±3.24	14.26 ±3.01	0.39
Follicle Stimulating Index	9.95 ±2.84	11.09 ±3.13	0.06
Insulin like Growth fator-1(IGF-1)	239.11 ±74.55	279.40 ±85.89	0.02*
Follicle Stimulating Hormone(FSH)	3204.68 ±755.79	3126.35 ±721.17	0.50

p value < 0.05 was considered significant using Mann Whitney U Test.

Table 2: Pearson Correlation

Parameters		FSI		IGF-I	
		r-value	p-value	r-value	p-value
FSI	r-value	1		0.729	<0.01*
	p-value				
IGF-I	r-value				
	p-value				
No of oocytes fertilized	r-value	0.535	<0.01*	0.398	<0.01*
	p-value	<0.01*			
No of oocytes retreived /patient	r-value	0.483	<0.01*	0.326	<0.01*
	p-value	<0.01*			
No of oocytes Metaphase II	r-value	0.518	<0.01*	0.386	<0.01*
	p-value	<0.01*			
No of cleaved embryos	r-value	0.516	<0.01*	0.369	<0.01*
	p-value	<0.01*			

Table 3: Odds ratio for clinical pregnancy using Binary Logistic Regression

Variables	Odds Ratio (95% C.I)	p-value
FSI	2.07 (1.51- 2.84)	<0.01*
IGF-I	1.01 (1.0- 1.01)	<0.01*

Nagelkerke R Square for FSI, 43.5%, Nagelkerke R Square for IGF-I 15.6%, Dependent: Clinical Pregnancy, Independent: FSI, IGF-I, *p<0.05 was considered significant for Odds ratio, 'FSI- Follicle stimulating Index' 'IGF-I- Insulin like growth factor-I'

Receiver operator curve for predicting clinical pregnancy

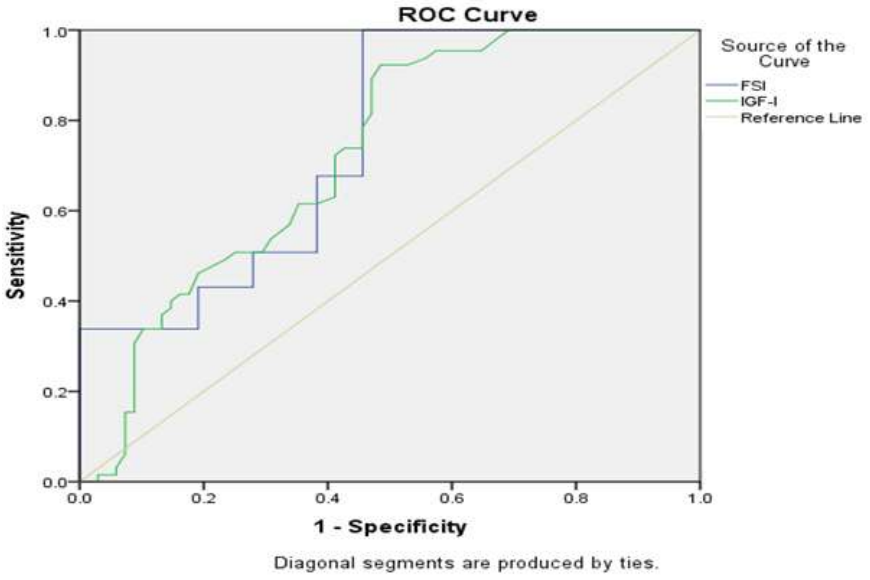

Table 2 gives the correlation coefficients with the significant p-values, it showed that correlation of FSI and IGF-I has the (r^2 value of 0.729) and (p-value of less than 0.01), showing that FSI is better predictor of clinical pregnancy as compared to IGF-I. Similarly the number of cleaved embryos correlation with FSI showed r-value 0.516 and with IGF-I showed r-value of 0.369.

Table 3 reports the results of odds ratio for clinical pregnancy using binary logistic regression .It was found that increased FSI samples were 2.07 times more likely to be found with clinical pregnancy while increased IGF-I samples were 1.01 times more likely to be found with clinical pregnancy. Both(FSI) and (IGF-I) gave positive association with clinical pregnancy outcome but FSI was more reliable predictor of clinical pregnancy as compared to IGF-I. Based on Nagelkeke R square, FSI explained 43.5% variation in clinical pregnancy outcome whereas IGF-I explained only 15.6% variation, both predictors were statistically significant with $p<0.01$.

DISCUSSION

The growth and development in the field of Assisted Reproductive Techniques (ART), particularly the ICSI/IVF have paved the way for couples intended for infertility treatment. In the past, numerous researchers have identified various markers that are needed to evaluate ovarian and follicular response to extrinsic stimulus that can escort upcoming treatment procedure for the couples and also assist them in attaining better therapeutic outcome with negligible complications.

Two of the previous tools that are used to predict the consequences of ART include Ovarian Sensitivity Index (OSI) (Jones *et al.,* 2019) and Follicular Output Rate (FORT) (Dyer *et al.,* 2016) In this study we have used FSI (a biophysical method) and IGF-I (biochemical marker) which functions as an intra-ovarian regulator to analyze the follicular role as possible and probable indicators of embryo's quality and predictors of the effective and fruitful result of Assisted Reproductive Techniques (ART).

Comparison of values between primary and secondary infertile females was evaluated that comprises of age, Body Mass index (BMI), extent of infertility in years, (PFC), (AFC), (FSI), (IGF-I) and (FSH). IGF-1 levels are significantly increased in females with secondary infertility in comparison with females affected with primary infertility ($p<0.05$). While the differences in other parameters were comparable in between primary and secondary infertile females. Likewise, a study reported that differences in age and BMI of primary and secondary infertile females were insignificant respectively ($p=0.08$, $p=0.14$) (Ly *et al.,* 2022). Similar studies revealed significant differences in extent of infertility in years in between primary and secondary infertile females ($p<0.01$) that are contrary to our findings (Palma *et al.,* 2011)(Zhou *et al.,* 2013).

FSI had a 72.9% positive correlation with IGF-I. On comparison of FSI and IGF-1 we found that FSI had a stronger positive relationship with the amount of oocytes fertilized (53.5% and 39.8% respectively), number of oocytes retrieved (48.3% and 32.6% respectively), number of oocytes in Metaphase II (51.8% and 38.6% respectively) and the numeral of cleaved embryos (51.6% and 36.9% respectively) as compared to IGF-1.

In the study by Hassan *et al.,* (2017) the first novel study to use (FSI), a relative increase in clinical pregnancy rates was seen with increasing (FSI) values and (PFC), (rate of fertilization), (number of fertilized oocytes) and the (total amount of embryos) all increased with increasing FSI values (Zamah *et al.,* 2015). The number of oocytes retrieved, the number of oocytes fertilized and the amount of cleaved embryos all were significantly related to FSI in present study which was in conjunction with the study of Hassan *et al.,* (18).

On the basis of odds ratio and Negelkerke r square, comparison of two models' statistics showed FSI as a good predictor for clinical pregnancy as compared to IGF-I. To evaluate the role of FSI and IGF-I we used ROC curve which likewise displayed FSI as a best predictor of pregnancy than IGF-I, with Area Under the Curve (AUC) 0.749 for FSI. Another advantage of using FSI over IGF-I is that FSI is a noninvasive test while IGF-I needs blood sampling. The ROC and multivariate analysis model showed three times positive association of FSI with clinical pregnancy.

Comparing the outcomes of current study that increased values of IGF-I and FSI predicts enhanced and higher rates of pregnancy in females with normal ovulation who are going through IVF treatment. Higher levels of IGF-I were linked to clinical pregnancy. It is consequently a superior interpreter of viable pregnancy outcome. Increased retrieved oocytes are associated with increased FSI values in females along with this advanced rates in clinical pregnancy reported. FSI calculation is very advantageous in cases of unsuccessful ART cycle. Women with decrease FSI values should be given increased dosages of FSH in future whereas females with increased values of FSI should be advised similar doses of FSH. Therefore, FSI is considered as a more reliable predictor for clinical pregnancy in comparison to IGF-I.

On comparing the results of (FSI) and (IGF-I), we found out that FSI is considered as a better monitor for the future management of ICSI/IVF treatment cycles and a better predictor of response to follicles to hormones. To the best of our knowledge it is the first novel study till

now that is done locally. The consequences of the current study will definitely benefit and assist the infertile pairs looking for advantage by means of ART. FSI which is a non-invasive and a less expensive detection of the outcome of infertility treatment which will be of great importance in the future. The results of our study signify that FSI's predictive value cannot be substituted by any other component. It is therefore suggested that FSI calculation should be made mandatory in the fertility clinics as it is non-invasive and not much expertise is required to calculate it. This is a vital parameter that is not been utilized locally and globally.

Subsequently we verified the significance of IGF-I values in the positive consequence of pregnancy thus it is also be utilized as a progressive biomarker to evaluate the result of fertility management. The inadequacy of the current study is that sample size is small. Consequently, study with a bigger sample size may be valuable and beneficial for further studies.

CONCLUSION

Out of the two markers (FSI and IGF-I), FSI became evident to be superior and inexpensive choice, as it does not require much expertise and finances. It also gives us the idea of success rate of future ART cycles, therefore if much research is done on FSI it can prove to be an important biophysical test in the field of infertility.

REFERENCES

Dyer AH, Vahdatpour C, Sanfeliu A and Tropea D (2016). The role of Insulin-Like Growth Factor 1 (IGF-1) in brain development, maturation and neuroplasticity. *Neurosci. J.,* **325**(12): 89-99.

Fortin CS, Leader A, Mahutte N, Hamilton S, Leveille MC, Villeneuve M and Sirard MA (2019). Gene expression analysis of follicular cells revealed inflammation as a potential IVF failure cause. *J. Assist. Reprod. Genet*. **36**(6), 1195-1210.

Hassan A, Kotb MM, AwadAllah A, Shehata NA and Wahba A (2017). Follicular sensitivity index (FSI): A novel tool to predict clinical pregnancy rate in IVF/ICSI cycles. *J. Assist. Reprod. Genet.*, **34**(10): 1317-1324.

Gunasheela D, Murali R, Appaneravanda LC, Gerstl B, Kumar A, Sengeetha N and Chandrikadevi PM (2021). Age-specific distribution of serum anti-mullerian hormone and antral follicle count in Indian infertile women. *J. Hum. Reprod. Sci.,* **14**(4): 372.

Lawrenz B, Melado L, Del Gallego R, Loja R, Coughlan, C, Ruiz F and Fatemi H (2022). P-569 Reduction of gonadotropin-dosage towards the end of ovarian stimulation for IVF/ICSI improves ART-outcome in a subgroup of patients. *Hum. Reprod.*, **37**(Supp 1): deac107-525.

Ly, Nathalie (2022). Sophie Dubreuil and Philippe Touraine. Normal-high IGF-1 level improves pregnancy rate after ovarian stimulation in women treated with growth hormone replacement therapy. *Endocr. Connect.* **11**(5): 12.

Jones AS and Shikanov A (2019). Follicle development as an orchestrated signaling network in a 3D organoid. *J. Biol. Eng*., **13**(1): 1-12.

Kumar N and Singh AK (2015). Trends of male factor infertility, an important cause of infertility: A review of literature. *J. Hum. Reprod. Sci.,* **8**(4): 191.

Palma GA, Arganaraz ME, Barrera AD, Rodler D, Mutto, AA and Sinowatz F (2012). Biology and biotechnology of follicle development. *Sci. World J.,* **2012**: Article ID 938138.

Puri S, Jain D, Puri S, Kaushal S and Deol SK (2015). Laparohysteroscopy in female infertility: A diagnostic cum therapeutic tool in Indian setting. *Int. J. Appl. Basic Med.,* **5**(1): 46.

Rehman R, Mustafa R, Baig M, Arif S and Hashmi MF (2016). Use of follicular output rate to predict intracytoplasmic sperm injection outcome. *Int. J. Fertil. Steril.,* **10**(2): 169.

Ryzhov JR, Shpakov AO, Tkachenko NN, Mahmadalieva MR, Kogan IY and Gzgzyan AM (2021). The follicular levels of adipokines and their ratio as the prognostic markers of in vitro fertilization outcomes. *Gynecol. Endocrinol.,* **37**(sup1): 31-34.

Surcel M, Doroftei B, Neamtiu IA, Muresan D, Caracostea G, Goidescu I and Zlatescu-Marton C (2022). Impact of follicle stimulating hormone receptor (FSHR) polymorphism on the efficiency of co-treatment with growth hormone in a group of infertile women from Romania. Diagnostics, **12**(10): 2371.

Yang J, Zhang X, Jing G, Chen Q and Huang G (2022). Growth hormone supplementation during ovarian stimulation does not alter outcomes in non-poor ovarian response patients with multiple IVF failures. **2**(1): 59.

Yucel GS, Ahn S, Ecemis T, Keskin M, Arslanca T and Atik A (2022). P-655 Insulin like growth factor-1 levels in follicular fluids of patients with poor ovarian response undergoing in vitro fertilization. *Hum. Reprod.*, **37**(Supplement-1): deac107-604.

Wang L, Lv S, Mao W, Pei M and Yang X (2020). Assessment of endometrial receptivity during implantation window in women with unexplained infertility. *Gynecol. Endocrinol.,* **36**(10): 917-921.

Zamah AM, Hassis ME, Albertolle ME and Williams KE (2015). Proteomic analysis of human follicular fluid from fertile women. *Clin. Proteomics.,* **12**(1): 1-12.

Zhou P, Baumgarten SC, Wu Y, Bennett J, Winston N, Hirshfeld-Cytron J and Stocco C (2013). IGF-I signaling is essential for FSH stimulation of AKT and steroidogenic genes in granulosa cells. *J. Mol. Endocrinol.,* **27**(3): 511-523.