ROLE OF FIBRILLIN-3 ON FERTILIZATION CAPACITY OF INFERTILE WOMEN IN ICSI CYCLE

Saad Al-Dujaily*[1], Rasha Abas[1], Wim Decleer[2], Bushra Al-Musawi[3], Abdul-Razak Al-Nakash[4]

[1]High Institute for Infertility Diagnosis and ART, Al-Nahrian University. Baghdad-Iraq.

[2] IVF Centrum, Jan Palfijn Hospital,9000 Ghent-Belgium

[3]Kamal Al-Samarie IVF Center Baghdad-Iraq[4]

Al-Ilwiya Hospital.Ibn-Sinna University .Baghdad-Iraq.

ABSTRACT

Background: The growth and maturation of the oocyte and follicle are influenced by many paracrine and growth factors like fibrillin-3 hormone. However, there is lack of understanding its function and how interact in IVF programs. **Objectives**: The aims of this study were to measure the level of Fibrillin-3 hormone at different phases of the menstrual cycle in controlled ovarian stimulation program and to elucidate the correlation between the levels of this hormone with the number of the oocytes, the number of metaphase II oocytes, and the pregnancy rate following ICSI cycle. **Materials and Methods:** Two hundred women undergoing ICSI treatment were chosen randomly. Controlled ovarian stimulation was done with two different protocols, measurements of serum Fibrillin-3 (ng/ml) were done on different phases of the cycle and also in follicular fluid. Intra cytoplasmic sperm injection was done, the oocyte, embryo development, and pregnancy rate were determined in relation to the levels of this hormone. **Results:** No statistical significant (P>0.05) difference was observed in the fibrillin-3 levels for any combination of stimulation protocol and cycle phase. The AUC values for fibrillin-3 are maximum 0.6, indicating that this hormone has little predictive use for the pregnancy prediction. **Conclusion:** This hormone has little predictive use for the pregnancy prediction in ICSI cycle.

KEYWORDS: Fibrillin-3, ICSI, Controlled ovarian stimulation.

INTRODUCTION

Fibrillins are a glycoproteins constitute the major backbone of multi-functional microfibrils in elastic and non-elastic extracellular matrix.[1] Fibrillins are secreted into the extracellular matrix by the fibroblast, they are a group of three proteins in human;fibrillin-1,-2,and -3 that their microfibrils have a role in maintaining the structural integrity of the matrix and in controlling the growth and differentiation of the cells they surround, by regulating the signaling of growth factors.[2] Proper functions of the extra-cellular matrix depend on proper organization and assembly of monomeric molecules into polymeric molecules by the cell.[3,4] Thus any defect or mutation in assembly or organization can lead to pathological situation such as Marfan syndrome which is the result of mutation in fibrillin-1[2] ,and the reduced expression of fibrillin-3 in the perifollicular stroma of the ovary has an association with the development of polycystic ovary.[5] Fibrillins have an overlapping roles in tissue which raise the possibility that they can substitute for each other in some circumstances leading to some resilience to inactivating mutations in family members.[6] It is unknown to what extent the functions of the fibrillins are overlapping or specific in the ovary.

Fibrillin-1 is expressed throughout life, whereas fibrillin-2 and-3 are primarily present during fetal development .In adult fibrillin-3 is present mainly in brain but also in gonads and ovary.[7] The fibrillins and latent transforming growth factor binding proteins (LTBPs) have a unique domain, the 8-cyctein transforming growth factor beta binding domain. Thus they interact functionally in the sequestering and hence inactivation of TGF-ß family members[8] which are cytokines involved in cell proliferation, differentiation and apoptosis.[9] Also fibrillins interact with the prodomains of BMP and growth differentiation factors (GDFs) which play a role in folliculogenesis and atresia of follicles.[10,11] Mutations in BMP-15 is associated with hypergonadotropic ovarian failure.[12]

The restricted localization of fibrillin-3 to the perifollicular stroma of a subset of follicles in morphological transition from primordial to primary type, suggests a role for fibrillin-3 in the regulation of TGF-β-related growth factors in the perifollicular stroma.[13] In this context, abnormalities in the structure or quantity of fibrillin-3 are hypothesized to alter the transition of primordial to primary follicles and subsequently influence the growth and maturation of follicles and competent oocytes.[14]

In this study the role of fibrillin-3 on the quantity and quality of oocytes ,embryos ,and pregnancy rate in ICSI cycle was studied.

PATIENTS, MATERIALS AND METHODS

A prospective study which involves two hundred infertile females selected from the consultant clinic in Kamal Al-Samaria IVF center in Baghdad-Iraq and Janpalfijn IVF center in Ghent-Belgium through the period from August 2014 to July 2015.

The females age ranged between 20-45 years. Exclusion criteria were patients with elevated FSH levels, polycystic ovary syndrome (PCOS),and those with endocrinological pathology such as elevated prolactin, thyroid dysfunction and diabetes.

Two protocols were used for controlled ovarian hyperstimulation: The long protocol GnRH agonist (GnRH-a) and the short GnRH antagonist (GnRH-ant) protocol .The hCG (Ovitrelle®, Merck Serono -USP) 6500-13000 IU or (Pregnyl® , Merck Serono Company USP)5,000–10,000 IU were given ~30 hours after the last FSH or HMG injection.HCG injection has been administered when the following criteria had been met: at least 3 follicles equal or greater than 17 mm in diameter were present with 17ß estradiol levels of at least 3500 pmol/L (920 picogram/ml).[15] Then oocyte retrieval was carried out 34 - 36 hours after the hCG injection. Oocytes were harvested by needle aspiration through the posterior fornix with TVU guidance. Fresh semen were collected at time of oocytes pick up by masturbation, sperm aspiration from the testes by FNA or from testicular biopsy . Frozen sperm had been used also.[16]

The procedure of ICSI was performed 3-5h after oocyte aspiration by choosing mature MII oocytes according to [17]. Embryo transfer was done on day 2 or 3 of embryonic development and rarely on blastocyst stage. Mostly not more than two or three embryos were transferred depending on the legislations of the country, recommendation of the couple and the quality of the embryos.

Support of the luteal phase was performed by injecting 1500 IU hCG immediately after oocyte retrieval and repeated seven days later for those who are not at risk of ovarian hyperstimulation syndrome(OHSS).In addition vaginal administration of 200mg of micronized progesterone, three times a day ,was started in the evening after oocyte pickup.[18]

For each patient measurement of serum Fibrillin-3 (ng/ml) was done on cycle day (CD) 2-3; at the day of ovum pickup; and 14 days after embryo transfer(ET).Also the concentration of this hormone was determined in follicular fluid after ovum pick up. The numbers of oocytes

retrieved and the number of metaphase II oocyte for each patient were recorded. The samples were stored at -20º C. Then the levels of these hormone were measured using quantitative sandwich enzyme immunoassay technique.

Statistical Analysis

Data analyzed using R project for statistical computing version 3.2.1 with R Studio interface. Numeric variables were expressed as mean±SE where as nominal variables were expressed as numbers and percentages. Comparison of variables was done using Fisher exact test and two sample t-test with Satterthwaite approximation of the degrees of freedom which allows separates variance estimations per group. Receiver Operating Characteristic (ROC) curve analysis was done to calculate the cutoff values of numeric variables. The non-parametric Spearman rank correlation coefficient is calculated for each combination of a hormone level (mid-cycle in serum or follicular fluid) and pregnancy rate. The level of significance was p value< 0.05.

RESULTS

Table -1 shows the age distribution of all studied groups. The mean age of the pregnant women was 31.5 ±0.82 and the mean age for the non-pregnant women was 31.0 ±0.49.There was insignificant statistical difference (p = 0.572) between the pregnant and non-pregnant women's age included in this study in relation to pregnancy rate (PR).

The number and percentage of women with primary infertility was 125 (62.5%) and women with secondary infertility 75 (37.5%).The pregnancy rate was 18.4% for primary infertility and 26.6 % for secondary infertility. The association between infertility (primary or secondary) and pregnancy was tested with a Fisher exact test. There was no statistically significant association between infertility primary/secondary and pregnancy after IVF program ,the OR=1.609 (0.765, 3.370), p= 0.213 as shown in table 1.

The mean of BMI for the pregnant women was 27.2 ±0.52 and for the non-pregnant was 27.4 ±0.294.There was insignificant statistical difference between the BMI of the women and the pregnancy rate in this study (p= 0.762).

ICSI outcome in relation to the type of COS protocol, oocytes and embryos

The number and percentage of women who were pregnant or not is shown in table -2 in relation to stimulation protocol. Two types of COS protocols were used :the long GnRH-a

used for 114 patients (57%) it resulted in a PR of 21.1%.The second protocol was the short GnRH-ant protocol ,used for 86 patients (43%) with a PR of 22.1%. There was no statistically significant association between stimulation protocol and pregnancy in this study , and the long and the short protocol resulted in very similar pregnancy frequencies (long: 21.1%, short: 22.1%).The average of sum of the PR in long and short ovulation induction protocols following ICSI cycle for all the patients in the study was 21.5%. Pregnancy rate in Janpalpijn IVF center was 40% while in Kamal Al-Samarei IVF center it was 19.4%.

In table 2, tabulated the number of total oocytes, MII oocytes, total embryos and embryos per grade was compared between the pregnant versus the non- pregnant women. There was a significant difference (p=0.045) between the MII oocytes of pregnant and non-pregnant women, with the pregnant women having a mean number of 7.07±0.6 MII oocytes and the non-pregnant women having a mean number of 5.68±0.33 MII oocytes. There was a significant difference (p=0.041) between the G1 embryos of pregnant and non-pregnant women, with the pregnant women having a mean number of 2.07±0.22 G1 embryos and the non-pregnant women having a mean number of 1.56±0.11 G1 embryos. There were no significant differences between pregnant and non pregnant women regarding the total number of oocytes(P=0.131), the total embryo numbers (P=0.190) and the G2 embryo numbers(P=0.099).

Fibrillin-3 hormone levels

Table-3 shows the concentration of fibrillin-3 hormone measured in different three phases of the ICSI cycle. For the female who used the long GnRH-a protocol ,fibrillin-3 concentration on CD2-3 in the pregnant group was 0.83 ±0.18 ng/ml then reduced in the midcycle to 0.49 ±0.06 ng/ml, and then increased in the late luteal phase reaching to 0.86 ±0.13 ng/ml.For the non-pregnant females on the same COS protocol ,the level of fibrillin-3 at CD2-3 was 0.64 ±0.07 ng/ml, and at mid-cycle 0.59 ±0.05 ng/ml whereas at late luteal phase was 0.64 ±0.05 ng/ml.

For the pregnant women on the short GnRH-ant,Fibrillin-3 level was 1.65 ±0.7 ng/ml ,then reduced to 0.7 ±0.11 ng/ml on the day of egg retrieval and then increased again to 0.89±0.18 in the late luteal phase while for the non pregnant it was 0.87 ±0.08 ng/ml on CD2-3 then decreased in the mid-cycle phase to 0.57±0.05 ng/ml and then increased to 0.74±0.08 ng/ml. No statistical significant (P>0.05)difference was observed in the fibrillin-3 levels for any combination of stimulation protocol and cycle phase.

Table 1: Characteristics overview of patients showing the age and the type of infertility as well as BMI for the pregnant and non-pregnant that involved in IVF program.

Variable		Non-Pregnant	Pregnant	All subjects	Statistical Comparison Status
Age of female (Y)		31.0 ±0.49	31.5 ±0.82	31.1±0.42	p = 0.572
Infertility	1° No (%)	102 (51%)	23 (11.5%)	125 (62.5%)	OR=1.609 (0.765, 3.370), p= 0.213
	2° No (%)	55 (27.5%)	20 (10%)	75 (37.5%)	
BMI (kg/m2)		27.4 ±0.294	27.2 ±0.52	27.4±0.26	p= 0.762
Total N&PR		157	43(21.5%)	200	

-Two sample t-test with Satterthwaite approximation of the degrees of freedom which allows separates variance estimations per group.

--The values of age and body mass index(BMI) were expressed as mean±SE

Table 2: Number of pregnant and non-pregnant women per ovulation stimulation protocol with the total oocytes , MII oocytes ,total embryos ,G1 and G2 embryos following ICSI procedure.

| Variable		Non-pregnant	Pregnant	Statistical Comparison Status
Ovarian Stimulation	Long	90/114 (78.9%)	24/114 (21.1%)	OR=1.063 (0.506, 2.213), p=0.864
	Short	67/86 (77.9%)	19/86 (22.1%)	
Total Oocytes		8.77 ±0.41	10.26±0.88	p=0.131
MII Oocytes		5.68±0.33	7.07±0.6	p=0.045
Total embryos		2.58±0.1	2.81±0.17	p=0.190
G1 embryos		1.56±0.11	2.07±0.22	p=0.041
G2 embryos		1.03±0.1	0.74±0.14	p=0.099

Values are expressed as mean + SE .

Fisher exact test and Satterthwaite t-test were used.

Table -3: Fibrillin-3 hormone level in the serum at different phases of the ICSI cycle of the studied groups in relation to the type of ovarian stimulation protocol

	status	Early- Follicular phase	Mid-Cycle phase	Late-Luteal Phase
Long GnRH agonist	Non-pregnant	0.64±0.07	0.59 ±0.05	0.64±0.05
	Pregnant	0.83±0.18	0.49±0.06	0.86±0.13
	P-value	0.340	0.219	0.142
Short GnRH antagonist	Non-pregnant	0.87±0.08	0.57±0.05	0.74±0.08
	Pregnant	1.65±0.7	0.7 ±0.11	0.89±0.18
	P-value	0.297	0.290	0.445

ROC of Fibrillin-3 hormone: Figure 1, revealed that the AUC values for fibrillin-3 are maximum 0.6, indicating that this hormone has little predictive use for the pregnancy

prediction. Thus the ROC in different phases of menstrual cycle of women stimulating with long or short ovarian protocol have no prediction on pregnancy. This corresponds to the results of the t-test (Table 3) where no significant differences were observed.
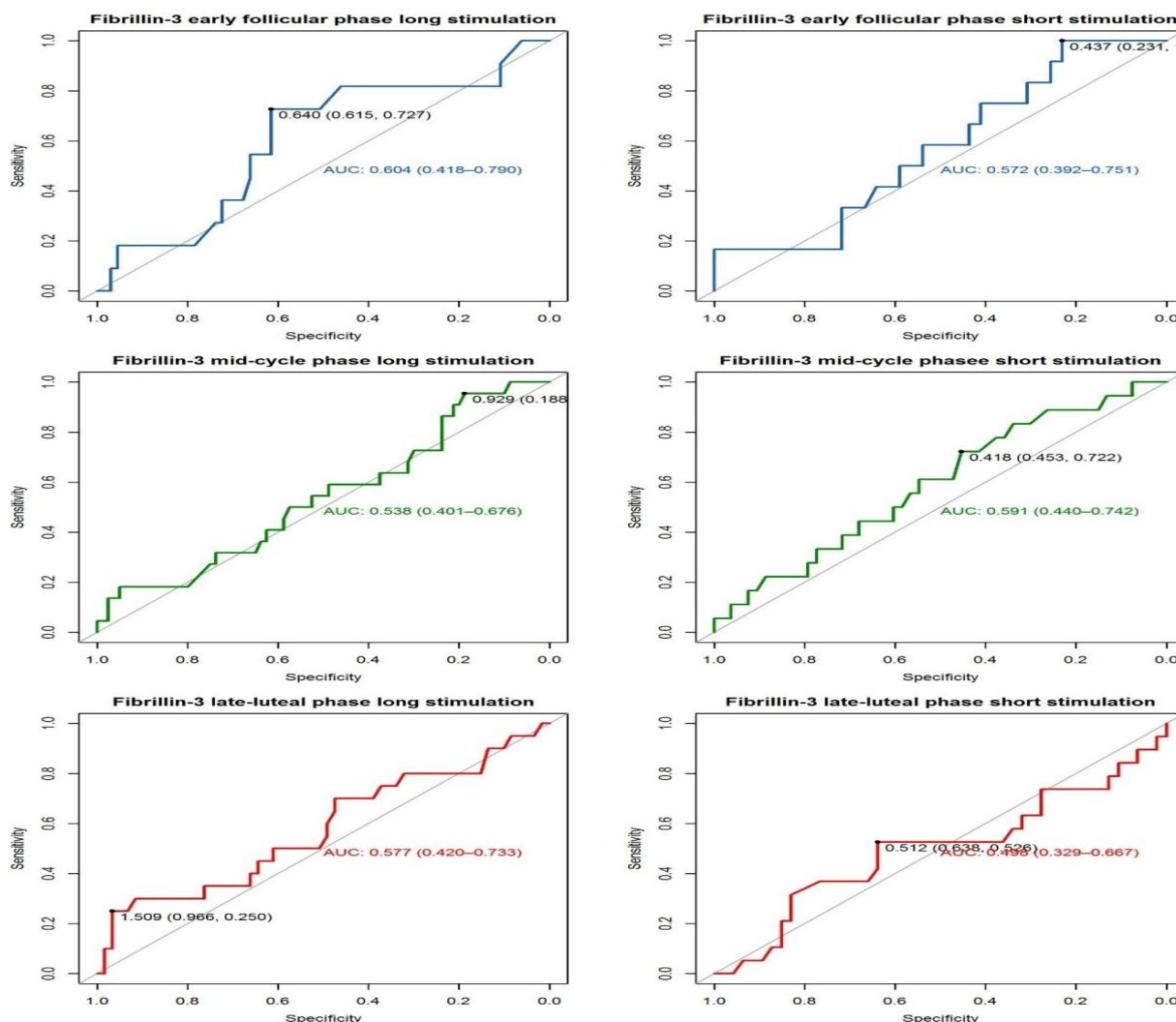
Figure 1. ROC curve of the Fibrillin-3 hormone levels as predictors for pregnancy(at different menstrual phases). The AUC together with the 95%confidence interval is provided. The most optimal cut-off for positivity (predicted pregnancy) is shown tighter with the specificity and sensitivity for this point in the ROC.

The number of oocytes retrieved, the number of metaphase II oocytes, and quality of embryo transferred with the pregnancy rate and the level of each hormone on the day of ovum pick up

In order to find the relation between, fibrillin-3 levels at mid-cycle in serum and follicular fluid on one hand and on the other hand the number of oocytes, metaphase II oocytes, and embryos. The hormone levels together with the oocytes and embryo counts are shown in Table 4. Per stimulation protocol and pregnancy status the mean (standard error) hormone

levels are provided, together with the mean total oocytes, the mean MII oocytes and the mean embryos.

Table 4 shows the total number of the retrieved oocytes, total MII, good MII ,the total number of embryos and G1 embryos in relation to the serum and follicular fliud levels of Fibrillin-3 hormone.The total oocytes number was higher in pregnant than non-pregnant women for both the long and short protocols. The number of total oocyte nearly similar in both protocols. While the total number of metaphase II oocytes and the morphologically good MII are higher in pregnant than in non-pregnant women in both the short and long protocols which reached statistical significant with PR.The total and G1 embryos number are higher in pregnant than non-pregnant for both types of COS protocols.

Table 4:The number of total oocyte, total metaphase II oocytes, good metaphase II oocytes retrieved, number of total embryos ,G 1 embryos, and the mid-cycle fibrillin-3 hormone levels (in serum and FF) shown per stimulation method and pregnancy status.

Ovarian stimulation method	Pregnancy state	Total oocytes retrieved	Total metaphase II oocytes	Good metaphase II oocytes	Total embryos	Grade 1 Embryos	Mid-cycle serum Fibrillin-3	Fibrillin-3 follicular fluid
Long	**Non pregnant**	8.9 (0.54)	5.61 (0.4)	3.73 (0.42)	2.6 (0.13)	1.88 (0.15)	0.59 (0.05)	4.17 (0.48)
	Pregnant	10.04(0.98)	7.08 (0.86)	5.33 (1.02)	3.08 (0.22)	2.25 (0.29)	0.49 (0.06)	4.64 (0.8)
Short	**Non pregnant**	8.6 (0.63)	5.77 (0.56)	4.89 (0.59)	2.54 (0.16)	1.14 (0.15)	0.57 (0.05)	2.93 (0.42)
	Pregnant	10.53(1.58)	7.05 (0.83)	6.37 (0.85)	2.47 (0.23)	1.84 (0.34)	0.7 (0.11)	1.91 (0.32)

Correlation and linear regression of the number of oocytes, number of metaphase II oocytes, and embryos versus mid-cycle serum and follicular fluid fibrillin-3 levels.

In table -5 the non-parametric Spearman rank correlation coefficient is calculated for each combination of fibrillin-3 level (mid-cycle in serum or follicular fluid) and the oocyte, MII oocyte or embryo numbers. Correlation coefficients are between -1 and 1, with positive numbers indicating a positive correlation.

Overall the correlation coefficients between fibrillin-3 levels and the oocytes, MII oocytes and embryo numbers were quite low,most correlation coefficients were very close to zero, indicating no correlation.

The follicular fluid fibrillin was positively correlated (0.24) with the G2 embryos numbers, and very little (0.16) correlated with the total embryo numbers.However the interpretation of

the correlation coefficient should be considered as exploratory as the correlation observed were rather low, and could be due to chance alone.

In table-6 ,spearman correlation coefficient shows that fibrilin-3 in mid cycle serum and FF has a high or relatively high correlation within the pregnant subject and low till very low correlation in the non-pregnant subjects, independent of the stimulation.

Table-5: Spearman rank correlation coefficients for the relation of the hormone levels and the oocyte, MII oocyte and embryo numbers.

	Oocytes total	MII Oocytes	MII Oocytes good	Total embryos	G1 embryos	G2 embryos	Mid-cycle Fibrillin-3	Fibrillin follicular fluid
Oocytes total		0.80	0.56	0.38	0.27	-0.02	-0.08	0.08
MII Oocytes	0.80		0.67	0.51	0.32	0.06	-0.04	0.09
MII Oocytes good	0.56	0.67		0.30	0.19	0.04	0.03	0.02
Total embryos	0.38	0.51	0.30		0.62	0.21	-0.13	0.16
G1 embryos	0.27	0.32	0.19	0.62		-0.60	-0.03	-0.04
G2 embryos	-0.02	0.06	0.04	0.21	-0.60		-0.08	0.24
Mid-cycle Fibrillin-3	-0.08	-0.04	0.03	-0.13	-0.03	-0.08		-0.17
Fibrillin follicular fluid	0.08	0.09	0.02	0.16	-0.04	0.24	-0.17	

Table 6: Correlation (Spearman rank) between the mid-cycle phase and the follicular fluid fibrillin-3 levels provided per combination of stimulation and pregnancy status.

Stimulation	Pregnancy status	Hormone	Fibrillin follicular fluid
Long GnRH agonist	**Pregnant**	**Mid-cycle Fibrillin-3**	**-0.60**
	Non-pregnant		**-0.04**
Short GnRH antagonist	**Pregnant**	**Mid-cycle Fibrillin-3**	**0.50**
	Non-pregnant		**-0.09**

DISCUSSION

The correlation between stimulation protocol and pregnancy was tested in this study and it shows no statistical significant difference in PR between the women who were subjected for the long GnRH-a and the short GnRH-ant protocol (OR=1.063 (0.506, 2.213), p=0.864). The same findings were reported by [19] who failed to detect a significant difference between two protocols for the difference in duration of ovarian stimulation, number of recombinant FSH

ampoules used, number of oocytes retrieved, serum levels for estradiol and progesterone, thickness of endometrium, and the zygote- and blastocyst-development rate. It is seemly that high quality embryo rate was higher in the antagonist protocol, but the data did not reach a statistical significance which is compatible with the present study results too.

However, several randomized controlled trails have been designed to compare the efficacy of GnRH-ant with that of GnRH-a long protocol ,but these studies often show conflicting results. It has been shown that no significant difference in the clinical pregnancy rate and the live birth rates between the two different regimens, although the implantation rate and clinical and ongoing pregnancy tented to be lower in GnRH-ant group in a study for.[20]

Another study found that clinical pregnancy rate (odds ratio (OR): 0.87, 95% CI: 0.75−1.0), and ovarian hyperstimulation syndrome (OHSS) incidence (OR: 0.59, 95% CI: 0.42~0.82) were significantly lower in GnRH-ant protocol than GnRH-a protocol.[21] However, the endometrial thickness on the day of HCG (MD: −0.04, 95% CI: −0.23~0.14), the ongoing pregnancy rate (OR: 0.87, 95% CI: 0.74~1.03), live birth rate (OR: 0.89, 95% CI: 0.64~1.24), miscarriage rate (OR: 1.17, 95% CI: 0.85~1.61), and cycle cancellation rate (OR: 1.11, 95% CI: 0.90~1.37) did not significantly differ between the two groups which were similar to this research. Nevertheless, implantation rate and clinical pregnancy rate were significantly higher in the antagonist protocol (10.64% and 30.26%, respectively) than that of the agonist protocol (5.26% and 15.82%, respectively). These data also suggest that the GnRH antagonist protocol is likely to have the advantage for improving the outcome of pregnancy in those patients with a history of multiple failures for the IVF-ET treatment.[21]

The total pregnancy rate for all the patients in the study was 21.5%.In Janpalpijn IVF center PR was 40% while in Kamal Al-Samarei IVF center it was 19.4%. This difference between the two centers may be related to the environmental condition as the percentage of air pollution in Iraq is much higher than Belgium,the type of ovulation stimulation drugs (in Belgium they prefer the long acting FSH injection) or due to the low number of male factor in Janpalpijn center.

The result of present study clearly indicated that total oocytes number was higher in pregnant than non-pregnant women for both the long and short protocols. However it was not reached the statistical significant difference.While the total number of metaphase II oocytes and good MII oocytes were higher in pregnant than in non-pregnant women in both the short and long

protocols which reached statistical significant with PR (p=0.045). A study by [19] revealed the same findings. Another study showed that normal MII oocytes and abnormal MII oocytes morphology does not unfavorably alter the fertilization, cleavage ,and high quality embryo rate but it revealed a highly significant difference in implantation rate which was higher in the group of normal oocytes morphology than abnormal oocyte morphology, oocyte with cytoplasmic, extracytoplasmic and combined abnormality.[22]

While a study of [23] showed a strong relationship between the number of egg and the live birth rate in fresh ICSI cycle which rose with increasing number of eggs up to ~15,plateaued between 15-20,and steadily declined beyond 20 eggs.

The data of this work found that low in oocytes number coupled with high gonadotrophin dose in conventional ovarian stimulation method can employ lower oocyte quality. Furthermore, endometrial quality can also be hampered in high–dose stimulation protocols as the GC in antral follicle stimulated by FSH increasingly express inhibin ,a physiological antagonist of activin.[24] This might have a deleterious effect on the maturation of the oocyte. Thus milder form of stimulation resulted in a better quality oocytes , and embryos with lower incidence of chromosomal aneuploidies.[22] On the other hand ,higher number of oocytes simply allows for better selection of quality embryos from a larger cohort of available embryos.[25]

The use of GnRH-Ag in the COS of women involved in this study may affect the process of follicular development ,luteinization, steroidogenesis, and apoptosis in the ovary. Pharmacological and high dose of GnRH-A showed a dose dependant increase in the expression of caspase-3 expression and increase cleaved poly ADP-ribose polymerase (PARP) expression   in the ovary ,also it result in increased expression of LH receptor, steroidogenic acute regulatory protein (StAR) and 3ß-hydroxysteriod dehydrogenase (3ß-HSD) proteins. Thus it affect the apoptosis and steroidogenesis process which in turn affect the number and quality of oocytes.[26] It has been emphasized that environmental factor may influence the outcome of IVF programs ,smoking and obesity have a deleterious effect on oocyte development due to the increased oxidative stress in the oocyte microenvironment.[27]

The relationship of embryo quality and pregnancy rate

This study showed a significant difference between the G1 embryos of pregnant and non-pregnant women. But the total embryo numbers and the G2 embryo numbers were not

statistically different. A studies of [25 ,28] showed a strong association between embryo quality from one side and implantation and live birth rates from the other side ,both with cleavage and blastocyst embryos; they showed a two folds proportion of live births from the implantation of good quality embryos compared with that from poor quality embryos. However; once a clinical pregnancy is achieved it has a similar chance of reaching a live birth as a good quality embryo. [25]

The other factors that may influence the outcomes of ICSI treatment in this study may be the embryo morphology which is the most common and useful tool in selecting the best embryo for transfer. Because of the single or double embryo transfer procedures have been recently recommended, choosing good quality embryos to enhance the rates of implantation ,pregnancy ,and live birth is very important. The decision of embryo selection depends on useful tools such as pronuclear morphology, early cleavage, blastomere morphology, and blastocyst grading. It has been shown that the embryo characteristic was an independent predictor of live birth in ICSI cycles.[29]

This study was accomplished all ET at Day 2-3 after ovum pick up apart from one patient had transferred a blastocyst and she got pregnancy.

The other factor that may interfere with PR was the program of ovarian stimulation that done to improve the ICSI outcome which resulted in retrieving multiple oocytes, but at the same time it affect luteal phase endocrinology, endometrial receptivity ,and oocyte and embryo quality. [28]In addition to the mentioned factors , abnormal sperm DNA is a possible cause of poor quality embryos obtained after ICSI besides the laboratory incubation environment and techniques.[30]

Fibrillin-3 hormone in relation to pregnancy through ICSI

This study revealed no statistical significant difference in the fibrillin-3 levels of any combination of stimulation protocol and cycle phase, indicating that this hormone has little predictive use for the pregnancy prediction. In early follicular and late luteal phase,fibrillin-3 level was higher in pregnant women than in non-pregnant in both protocols although it does not reach statistical significance .It has been thought that FBN-3 is lower in PCO ovaries, by measuring hormone level as a gene expression in ovarian tissues.[31] It is proposed that FBN-3 forms a specialized stroma around a transition follicle during its development from primordial to primary follicles and subsequently influence the growth and maturation of

follicle and the competence of oocytes.[7] It is possible that FBN-3 may serve as a marker of a specific transforming follicles and its level might correlate with the severity of PCOS.

In general during this study FBN-3 reduced in the mid-cycle and then increased again in the late-luteal phase for both pregnant and non-pregnant in both protocols which is on the contrary of the findings of [32] study which founded an increase of this hormone level at preovulatory stage of the non IVF cycle then the level was decreased following 14 days post ovulation ,but it also confirmed that FBN-3 in day 2 and day 13 had no statistical significant difference in relation to pregnancy rate.This may indicate that circumstances that raising the E2 level may decrease the level of FBN-3 production .Therefore the values of this hormone was decreased at mid cycle in this study.These findings was on the contrary of [32] which postulated that mean FBN-3 hormone may interfere with positive feedback of E2 to elevated FSH and LH levels .At the same time ,the peak of E2 was associated with the peak of FBN-3 level at CD 13 of the non-IVF cycle .This controversy may be due to pituitary suppression and high levels of FSH in the IVF cycle or may be due to an unkown autocrine-paracrine mechanisim.It was postulated that FSH may be responsible for FBN-3 biosynthesis in the ovaries.Thus further studies are needed to confirm these findings.

The relationship between serum and follicular fluid hormones level and its impact on oocyte, embryo, and PR following ICSI cycle

In follicular fluid, firillin-3 is found in levels far exceeding those in serum .This can be explained by the physiological production of these hormones in the ovarian tissue first and then can be measured through the blood serum**.**

Fibrilin-3 has a high or relatively high correlation within the pregnant subject and low till very low correlation in the non-pregnant subjects, independent of the stimulation. This may indicate a role of this hormone on the endometrium receptivity and implantation rate as the follicular fluid fibrillin was positively correlated with the G2 embryos numbers, and very little correlated with the total embryo numbers .But may have a role on advanced stage in oocyte maturation and associated with higher chance of achieving pregnancy. It has been recorded that fibrillins perform regulatory functions by binding and sequestering growth factors.[4] Because many members of the TGF- β superfamily are involved in the development and function of the ovary.[10] Thus these hormones may facilitate the mechanism of implantation through preparing the factors involved in uterine receptivity**.**

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