Role of Fibrillin-3 on Fertilization Capacity in Pregnant and Non Pregnant Women

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Abstract:

Background

Fibrillin- 3 is a recently discovered hormone .However, there are no studies till now regarding the role of fibrillin-3 hormone on fertilization capacity in infertile pregnant and non-pregnant women.

Objectives

The goals of this study are concentrated on the fertilization capacity parameters by examining the effect of in vivo concentration of fibrillin-3 hormone on the ovarians status at different menstrual cycle phases and to elucidate the correlation between the levels of this hormone with pregnant and non -pregnant women following Intra-uterine insemination (IUI) outcome or through natural pregnancy .

Methods

Sixty infertile couples were involved in this prospective study. The couples were divided according to the type of insemination and ovulation induction program into two groups Group(1): Thirty spouses were subjected to induced ovulation stimulation by using clomiphene citrate(CC) and recommended for natural coitus. Group(2): Thirty couples, the females were induced ovulation stimulation by CC, Gonal F-75IU /ml and Fostimon 50IU/ml with IUI. Measurements of Fibrillin-3 with other reproductive hormones were done at different phases of menstrual cycle and for pregnant and non-pregnant women following natural coitus and IUI .

Results

The mean of fibrillin-3 hormone level at day 2 of menstrual cycle was 3.7ng/ml (ranged from 3.50 to3.91) in the two groups. The mean of fibrillin-3 hormone at cycle day 13 was 6.6ng/ml(ranged from 5.83to 7.0). Whereas, The basal level of Fibrillin-3 at 14 days post insemination was 3.3ng/ml. The range is from 2.76,395 to 3.29 ng/ml. There was no significant difference in the mean of Fibrillin -3hormone between pregnant and non-pregnant women through the phases of menstrual cycle or between the two groups, but there is a significant value between fibrillin-3 post insemination and E2 day 2 and day 13 $P<0.05$.

Conclusion

The present study concluded that Fibrillin -3 hormone has no role in pregnancy, but it may have a main relationship with estrogens reaction in folliculogenesis , oocyte maturation and follicular contents. Therefore, this hormone may be one of the new hormones that interfere with fertilization capacity

Key word:Fibrillim-3,Women,Pregnancy,Fertilization

Introduction

A couple may be considered infertile if, after two years of regular sexual intercourse, without contraception, woman has not become pregnant (and there is no other reason, such as breast feeding or postpartum amenorrhea)([1]).A twelve months is the lower reference limit for time to pregnancy (TTP) for a woman under 35 years and for a woman over35 years is 6 month.([2])

Both women and men can have problems that cause infertility. About one- third of infertility cases are

caused by women 's problems. Another one third of fertility problems are due to the man. The other cases are caused by a mixture of male and female problems or by unknown factors (unexplained infertility)([3]). Most cases of female infertility (an ovulatory cycle)(4) related to problems with ovulation. Without ovulation there are no eggs to be fertilized. The other factors of female infertility are irregular or absent menstrual periods, blocked fallopian tubes due to pelvic inflammatory disease, endometriosis, or surgery for an ectopic pregnancy, physical problems with the uterus, uterine fibroids and others(5).

It is well known that there are many reproductive hormones which have a big role in folliculogenesis, oocyte maturation, corpus luteum formation and endometrial preparation for implantation of fertilized ovum and they can affect fertility if any disturbance occurred in their level. Gonadotropin- releasing hormone(GnRH) is the first hormone in a complex reproductive cascade. GnRH is released by the hypothalamus and stimulates the secretion of luteinizing hormone (LH) and follicle stimulating hormone(FSH) from the pituitary;these gonadotropins then stimulate the gonads to produce sex steroids and follicles/sperm. (6)

The mechanisms regulating follicle growth and development are under the control of changing concentrations of ligands(i.e. hormones and growth factors).(7) Although GnRH, FSH, and LH action are critically important in regulating folliculogenesis, hormones and growth factors, which are themselves products of the follicle, can act locally to modulate (amplify or attenuate) FSH and LH action. This is the autocrine/ paracrine system of developing follicles which play an important role in the timing of folliculogenesis and whether a follicle becomes dominants or atretic.(8))

The most important hormones and factors that regulates the follicle and ovum develops are various growth factors, cytokines, peptide, hormones, and steroids such as epidermal growth factor, transforming growth factor(TGF), platelet-derived growth factor, fibroblast growth factors, activin, inhibin, AntiMüllerian hormone, insulin - like growth factors.(9-10) Fibrillin- 3 is a new discovered hormones and is thought to be primarily present during development of follicles.([11])

Although there are many studies revealing the association of fibrillin-3 gene with growth factors in women with polycystic ovary syndrome (PCOS) but at our knowledge, there are no studies till now regarding the relationship of fibrillin-3 hormone and the pregnancy in infertile women. Therefore the objectives of this study are :

1-To examine the effect in vivo concentration of fibrillin-3 hormone on the ovarian status at follicular phase ,at preovulatory phase and luteal phase.

2- To elucidate the correlation between the levels of this hormone with pregnancy rate following IUI outcome or through natural pregnancy.

Patients, Materials and Method

This study was conducted at the High Institute for Infertility Diagnosis and Assisted Reproductive Techniques, Al-Nahrain University, through the period from November 2012 to May 2013.The spouses ages ranged between 18-40 years old.

All women involved had regular menstrual cycles, normal serum prolactin level and normal thyroid function. Patients with PCOS or patients with elevated FSH levels (>16ml/mL) were excluded from the study. All the spouses involved in this study were physically examined by a specialist gynecologist in charge at the Institute. The men of each couple was examined physically by the urologist include in the Institute and the semen analysis was done in Institute laboratory. The couples were divided according to their infertility status into two groups:-

Group (1): Include thirty couples, the wife's were superovulated by using clomiphene citrate (CC) and recommended for natural coitus.

Group(2): Thirty couples, the female were superovulated by CC, Gonal F-75IU /ml and fostimon 50IU/ml with IUI.

Inall groups hormonalanalysis (FSH,LH,E2,Fibrillin-3) were done for all patients at a day2-3 and the day of hCG injection and Fibrillin-3 at 14 days post insemination.

The ultrasonography study was performed at day 3 of the menstrual cycle to detect antral follicle number and diameter in the two groups, and any uterine abnormalities. Diameter of the follicle(s) was monitored by serial examinations by vaginal ultrasound. At least one or two mature (dominant) follicles were recognized about 17-20mm, Hormone Pregnyl 10000IU was used to induce ovulation, then insemination either by natural coitus or performed by (IUI) catheter after 34-36hr.Simple layer (migration - sedimentation) procedure was performed for the semen samples.

Statistical Analysis

Data analysis using SPSS (Statistical Package for Social Science) version 16 and Microsoft office excel 2007. Numeric variables were expressed as mean±SE. Different statistical tests were performed depending on the nature of the data such as student's t-test, Analysis of variance (Anova), Receiver Operating Characteristic (ROC) curve analysis was done to calculate the cutoff values of numeric variables. Comparison of frequency was done using Chi-square test. Logistic regression analysis was used to study the correlation among the different variables in relation to induction/ IUI outcome. The level of significance was p value < 0.05. ([12]).

Results

1- Results of semen analysis of natural coitus group:

the mean of certain sperm function parameters for group 1of the men and their spouses induced ovulation with clomiphene citrate and prepared for natural coitus was 59.23±3.57 (million /ml) for sperm concentration. Regarding the progressive sperm

motility all the grades of progressive sperm motility(%) grade A,B and A+B (30.80±1.7225,13±1.34 and 55.27±2.63, respectively). The percentage of MNS(%) was 63.50±2.42.(table-1)

2 -Results of in vitro sperm activation:

the mean of certain sperm function parameters following in vitro activation by migration sedimentation technique for group 2 of the men their spouses induced ovulation with Clomid® and gonadotropins and prepared for IUI The mean of sperm concentration(million/ml) was shown (37.13±4.19) after activation a highly significant (P<0.001) reduction compared to before activation (41.10±4.32).The mean values of progressive motility (%) grade A after activation (34.53±2.86) was a highly significant(p<0.001) improved compared to before activation (24.67±2.30). The percentage of progressive motility grade B after activation (25.50±1.80) was significantly (p<0.012) increased compared to before activation (19.50±1.63). The mean of progressive motility grade A+B after activation (58.47±4.09) was highly significant (p<0.001) increment compared to before activation (44.17±3.56). The mean of MNS (%) showed a highly significant (p<0.001) difference between after (67.17±3.08) and before (58.83±3.56) activation(table 2).

3-The results of hormonal levels in two studied groups.

The basal level of E2 at day 2 in group 1 was 447.73±120.67 and at day 13 was 1124.40±318.36 while basal level of E2 at day 2 in group 2 was247.55±48.28 and at day 13 was 926.28±291.63. There was no statistically significant difference P>0.05 among two groups.

The basal level of Fibrillin-3 at day 2 of menstrual cycle was (3.67±0.79) in group 1, in group 2 the level of fibrillin-3 was (3.50±0.64), The basal level of Fibrillin-3 at day 13 of menstrual cycle in group 1 was (6.89±1.19) ,and in group2 was (5.83±o.89) and The basal level of Fibrillin-3 at 14 days post insemination was (2.76±0.59)in group 1and in group 2 was (3.29±0.57) . There was no significant P>0.05 difference among the two groups (table 3). But there is significant difference between Fibrillin -3 and E2 day 2 and day 13 P<0.05 in 14 days post insemination. Table(4)

4-The results of pregnancy rates .

The total pregnancy rate in two groups was (27.77%). The pregnancy rate was 23.33% in group 1 , 33.33% in group2, There was statistically no significant P>0.05 difference in pregnancy rate among the two groups.(Table5).In table (6) there was no significant difference P>0.05 in pregnant and non-pregnant women for fibrillin-3 in three phases of menstrual cycle.

5-relationship of pregnancy rate and FBN-3 day 2 and day 13.

There was no significant negative correlation between FBN-3 D2 and pregnancy rate(r=-0.137, P>0.05) according to spearman's rank correlation line. As shown in figure 1.

There was no significant negative correlation between FBN-3 D13 and pregnancy rate (r=-0.023, p>0.05) according to superman's rank correlation line as shown in figure 2.

Table 1: Certain semen parameter of mean their spouses prepared for natural coitus.(group 1).

Certain sperm function Parameter		Natural on clomid=30	
		Mean	SE
Sperm Concentration ($X10^6$/ml)		59.23	3.57
Active sperm motility (%)	Grade A	30.80	1.72
	Grade B	25.13	1.34
	Grade A+B	55.27	2.63
Normal sperm morphology (%)		63.50	2.42
volume		2.79	0.77
pH		7.26	0.26
Agglutination		1.87	1.27
Round cell		2.35	1.05

The number of patients= 30 values are expressed as mean ±SE

Table2: Certain semen parameters of men their spouses prepared for IUI by ovulation induction using Clomid® and gonadotropin (group 2)

Certain semen Parameter		before activation		after activation		
		Mean	SE	Mean	SE	P
Sperm Concentration ($X10^6$/ml)		41.10	4.32	37.13	4.19	<0.001**
Active sperm motility (%)	Grade A	24.67	2.30	34.53	2.86	<0.001**
	Grade B	19.50	1.63	25.50	1.80	0.012*
	Grade A+B	44.17	3.53	58.47	4.09	<0.001**
Morphologically normal sperm%		58.83	3.56	67.17	3.08	<0.001**

The number of patients = 30 values are expressed as mean± SE

Table 3: Comparison of mean hormonal levels at day 2 and 13 of menstrual cycle among the two groups before the insemination

Parameter	Natural on clomid		IUI clomid +Gn before activation		P
	Mean	SE	Mean	SE	
FSHD2	7.56	0.64	7.01	0.37	0.253
FSHD13	7.13	0.96	7.33	0.87	0.978
LHD2	5.00	0.39	5.93	0.72	0.595
LHD13	11.07	1.36	14.26	2.11	0.447
E2D2	447.73	120.67	247.55	48.28	0.309
E2D13	1124.40	318.36	926.28	291.63	0.732
FIBRILIND2	3.67	0.79	3.50	0.64	0.930
FIBRILIND13	6.89	1.19	5.83	0.89	0.668
FIBRILIN14 post insemination	2.76	0.59	3.29	0.57	0.418

Value are expressed as mean± SE

Table 4: Comparison of pregnancy rate among the three groups

Pregnancy	Natural on clomid		IUI clomid +Gn		Total	
	No.	%	No.	%	No.	%
Positive	7	23.33	10	33.33	25	27.77
Negative	23	76.67	20	66.67	65	72.23
Total	30	100	30	100	90	100
P=0.67						

Table5:correlation of fibrillin-3 hormone with NF., ET.,FSH, LH and E2

Hormone	P, r	FSHD2	FSHD13	LHD2	LHD13	E2D2	E2D13
FIBRILIND CD2	r	0.050	0.035	0.035	-0.154-	-0.088-	0.050
	P	0.793	0.855	0.856	0.417	0.643	0.791
FIBRILIND CD13	r	0.137	0.094	-0.251-	-0.295-	-0.233-	-0.158-
	P	0.472	0.622	0.181	0.114	0.216	0.405
FIBRILIN Post-insemination	r	-0.013-	-0.181-	-0.006-	-0.245-	0.449*	0.426*
	P	0.947	0.337	0.975	0.193	0.013	0.019

CD=cycle day of Menstruation

*= significant correlation with CD 3 and 13.

Table 6:Mean ,SE for fibrillin-3 hormone in pregnant and non-pregnant women.

Hormone	Pregnant		Non-Pregnant		P
	Mean	SE	Mean	SE	
FIBRILIND 2	3.50	0.89	3.77	0.49	0.784
FIBRILIND 13	6.42	1.09	6.63	0.70	0.875
FIBRILIN14postinsem	2.70	0.56	3.58	0.46	0.291

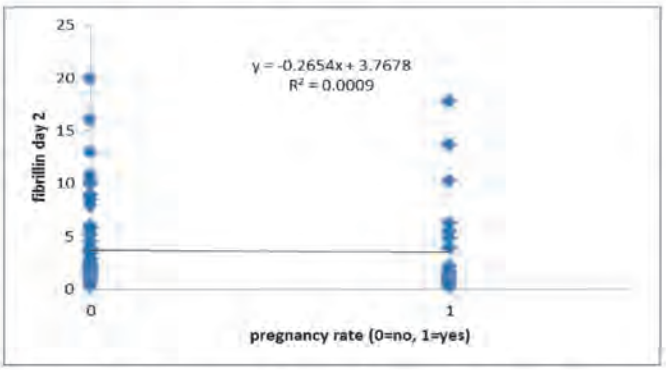

Figure 1: Spearman's rank correlation between (pregnancy rate) and fibrillin day 2 in the two groups.

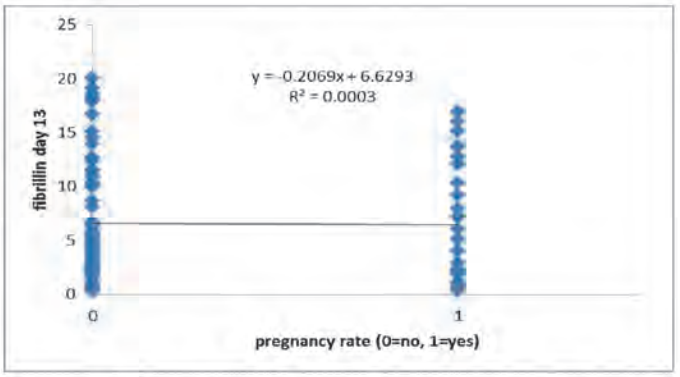

Figure 2:Sperman's rank correlation between (pregnancy rate) and fibrillin day 13 in the two groups.

Discussion

1-Semen analysis of natural coitus group

In the present study the seminal fluid analysis for the males whose females were prepared for natural coitus seemed to be normal according to WHO. This finding may increase the chance of pregnancy when the spouse has normal criteria for conception .It has been found that 30% of men with semen analysis actually have abnormal sperm function([13]).

2-In vitro sperm activation.

In this study males whose spouses were prepared for ovulation induction program and IUI, were submitted to in vitro sperm activation by doing migration sedimentation technique. All cases showed highly significant benefit from in vitro sperm activation. the mean was significantly declined after activation in group(2), this decline was improved in other study done by Al- Dujaily, et al when they found that the numeric yield of spermatozoa may decrease due to inability of dead and abnormal sperm with poor motility to swim up and migrate to the upper layer of culture medium(14). Regarding progressive sperm motility shows increase progressive sperm motility Grade A, Grade B and Grade A+B in terms of percentage, the same result was found by Al-Dujaily, et al(15) in which the activation techniques gained significantly higher percentage of motility due to separation of spermatozoa based on their motility and morphology. Schlegel and Girardi, found that the improvement of sperm motility and grade activity was obtained as a result of special basic components of culture media(16). The culture media used for ARTs may contain protein source and buffers to promote sperm capacitation and hyperactivity(17-18).

3-The relation of hormone with pregnancy rate in three groups.

The level of E2 did not reveal a difference between the two women groups. The serum E2 level on day 13 was showed negative correlation to pregnancy following natural and IUI (figure16).Our finding is in agreement with Scott et al that women for whom ovulation induction prior to IUI is likely to be effective for those women with functioning ovaries therefore they should be selected for this procedure(19).

4-Fibrillin-3 hormone and relation to pregnancy outcome.

The result of Fibrillin-3 in this study found an increase of this hormone level at preovulatory stage of the cycle then the level decreased following 14 days post insemination whether the insemination was naturally or through IUI. At the same time the kind of ovulation induction program did not influence the level of this hormone in cycle day 13 and 14 days post insemination. Consequently, it seems that the correlation between the hormone and E2 through folliculogenesis and ovulation events when Fibrillin-3 starts to elevate through follicular and oocyte development. The Fibrillin-3 hormone may interfere with positive feedback of E2 to increase the level, of FSH and LH and the peak of E2 is associated with the peak of FBN-3 level at cycle day 13. It postulated that the FSH may be responsible of FBN-3 production. The circumstances that raise the E2 level may interact with FBN-3 production leading to increase the follicular fluid , maturation of the ovum and to facilitate LH action for ovulation .

The other action of FBN-3 may be postulate the action of E2,by increasing the aromatase action by the FBN-3 causing the production of E2.(20)

This explanation may be in parallel with two cells-two concentration theory when the granulosa cells and theca cells cooperate to produce steroidal hormone inside the ovaries by the action of FSH and LH?[21]?,with the FBN-3 hormone to produce the steroidal hormone at the ovaries .More studies are recommended to elucidate our study results.

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