Relationship between antiMüllerian ovarian hormone, activin-A, and follistatin hormones levels with pregnancy rate following intrauterine insemination

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Abstract:

Background:
Recently the reproductive hormones, Anti-Mullerian hormone (AMH), activin A, and follistatin (FS) have been found to play an important role in folliculogenesis, oocyte maturation and corpus luteum function by changing the pattern of granulosa cell expression which in turn affects the success of fertilization potential.

Objectives:
To examine the relation of in vivo concentrations of AMH, activin A, and FS hormones on the ovaries status in the phases of menstrual cycle and to elucidate the relationship of these hormones with pregnancy rate following intrauterine insemination (IUI).

Materials and methods:
Seventy seven infertile couples were recruited from infertility clinic population at the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies through the period from September 2011 to May 2012. Those infertile couples were divided to 3 groups according to the infertility cause (male factors causes, anovulatory causes and unexplained causes of infertility). Measurements of AMH, activin A, and FS hormones levels were done at cycle day (CD -25). Then measurements of activin A, and FS hormones levels were done only at preovulatory cycle (CD12 -14) when the size of the Graffian follicle range between 18 -24mm and at least one follicle was present and before hCG injection and at day 28 of the cycle (CD28). IUI was performed 36 -40 hours after hCG injection then measurement of the three hormones was performed after successful IUI. The result of successful pregnancy was recorded and statistical relation with AMH, activin A and FS hormones was assessed.

Results:
There was no significant relation (P>0.05) between AMH level and pregnancy rate. A significant correlation was found between follistatin levels in preovulatory (CD 12 -14) with a cutoff value 0.20ng/ml to predict pregnancy. There was a significant relation between activin-A level and pregnancy rate with a predictive cut off value 397.5 ng/ml on CD 28 only.

Conclusions:
Activin-A can be regarded as a biomarker candidate for diagnosing very early pregnancy at luteal phase following IUI. Follistatin preovulatory (average CD13) has the most predictive value for pregnancy following IUI.

Key Words: AntiMüllerian ovarian hormone, activin-A hormone, follistatin hormone, IUI

Introduction

Female infertility can be resulted from numerous causes and the most common causes are issues with ovulation such as polycystic ovarian syndrome (PCOS) and early menopause(1). Other causes include endometriosis, tubal obstruction due to damage to the fallopian tubes due to pelvic inflammatory disease such as sexually transmitted diseases or chlamydia infection, or surgery. Pelvic adhesions, thyroid problems, cancer treatment, medication and certain health issues such as drugs, drinking alcohol, smoking cigarettes, eating poorly and being overweight or underweight and even excessive athletic training can also contribute to female infertility. Age is also considered a common factor for female infertility(2). Women over 40 have a smaller number of eggs that tend to be less healthy(3). Combined infertility arises from the combination of male and female causes and it may be that each partner is independently fertile but the couple cannot conceive together without assistance(4). Unexplained infertility is infertility that is idiopathic in the sense that its cause remains unknown(5).

It is well known that there are many reproductive hormones (hypothalamic, pituitary and ovarian) 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(6). The growth of follicles and the function of the corpus luteum, while under the primary direction of the pituitary, are highly influenced by intra-ovarian factors that modulate the action of gonadotropins. These intra-ovarian factors includes a list of potential paracrine factors that can influence steroid production by theca and granulosa cells and also includes various growth factors like AMH, activins, follistatin and inhibins and hence they involved in infertility and understanding their functions and how they interact help better to understand fertility and conception(7). Therefore, the objective of this study is to find out the relationship between AMH, activin -A, and follistatin hormones with pregnancy rate following intrauterine insemination. This study will include the estimation of in vivo concentrations of AMH, activin A, and follistatin hormones on different phases of menstrual cycle and to elucidate the correlation between the levels of these hormones with pregnancy rate following IUI.

Materials and Methods

Seventy seven infertile couples were enrolled in the current study following complete history with physical examination (systemic and local) of both the husband and spouse to role out the factors that could be the cause of impairing fertility. Accordingly, the infertile couples divided into two groups:

1. The first group includes 12 infertile couples due to male causes diagnosed by doing seminal fluid analysis (SFA) which showed either the sperm concentration was $<20x10^6$/ml (oligozoospermia) or total progressive motility (grade A and B) < 50% (asthenoozoospermia) or sperm morphology showing <50% normal forms (teratozoospermia) or all of them are present at the same time (OAT). The samples were analyzed as the following depending on guidelines of WHO(8).
2. The second group consist of 65 infertile couples due to female factors and divided into 2 groups according to the cause of infertility.
   A. Group A includes 50 infertile couples due to anovulatory causes except sever PCOS and endometriosis.
   B. Group B includes 15 infertile couples due to unexplained causes

Female investigations

A complete hormonal study was done for all females included in this study to exclude anovulation. These hormones include FSH, LH, TSH and prolactine on day 2-3 of the cycle (CD2) for assessment of the hypothalamus-pituitary function and to exclude premature menopause. Serum E2 (on CD2) and progesterone (on CD21) were measured for assessment of ovarian function. The levels of AMH, activin A, and follistatin hormones were measured on early follicular phase of the cycle (CD-25).

Pelvic ultrasonography was done in early follicular phase (CD-25) to exclude any pelvic pathology, i. e. ovarian cyst or uterine myomas. Hysterosalpingography (HSG) was performed to exclude tubal blockage or congenital malformations of the uterus like bicournuate uterus.

Postcoital test was done to exclude cervical hostility. All females of the three groups started ovulation induction. **Ovulation induction medicine and protocols:** In this study the protocols used for ovulation induction were Clomiphene Citrate (Clomid®) 50 mg (Aventis Company_ France) twice daily from day two or three of menstrual cycle for 5 days(9). Recombinant FSH injections (r-FSH) which is either Gonal-F injections 75 IU of r-FSH/(Merck Serono Company, Geneva, Switzerland.) vial alone in a dose of 75mg once daily for 2 to 10days starting from day 2 or 3 of the cycle or in combination with clomiphene citrate at the same dose(10). (The dose was adjusted depending on the ovarian response till

maturity of the follicle) or Pureagon injections 50IU of r-FSH/vial (Organon Company, The Netherlands). Vaginal ultrasonography was performed after induction of ovulation on day (12 -14) of the cycle (average CD13) to ensure at least one mature Graffian follicle was present with a size between (18- 24) mm. Measurement of the levels of activin A and follistatin hormones only was performed. Then Oviterlle injections 6500IU/vial of human chorionic gonadotrophin (hCG) were given subcutaneously. IUI performed 36-40hours after hCG injection after the semen of the male partner was prepared by layering swim-up activation technique as described by Al-Dujaily(11). Measurement of the activin A and follistatin hormones levels only was done again on day 28 of the cycle (late luteal phase) and 14 days later, biochemical pregnancy test was done to find out the pregnancy result(12). If pregnancy occurred, the levels of AMH, activin A and follistatin hormones were measured again during the first 8 weeks of pregnancy.

Statistical Analysis: Data analyzed using SPSS (Statistical Package for Social Science) version 16 and Microsoft office excel 2007. Numeric variables were expressed as mean ±SD where as nominal variables were expressed as numbers and percentage. Comparison of mean values of the three studied groups was done using one way analysis of variance (ANOVA). Comparison of frequency was done using Chi-square test. Receiver Operating Characteristic (ROC) curve analysis was done to calculate the cut off values of numeric variables. 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(13).

Results

Results of *in vitro* sperm activation using layering swim-up technique

Table1 showed that the mean value of sperm concentration (10^6/ml) in male factor infertility after activation (15 ±4.29) was significantly (p<0.05) decreased compared to before activation (20± 3.23). The percentage of progressive motility grade A and grade B was significantly (P<0.05) increased compared to before activation. The percentage of morphologically normal sperm (MNS) after activation was significant (P<0.05) improved compared to before activation (Table-1).

Table 2 illustrated the data of sperm activation anovulatory infertility women and unexplained infertility couples. The mean of sperm concentration after activation was significantly (P<0.05) decreased compared to before activation. There was a significant (P<0.05) improvement in the percentage of active sperm motility grade A and grade B in addition to MNS after activation compared to before activation as shown in table-2.

Table -1: Certain sperm function parameters following *in vitro* activation by layering swim-up technique of male factor infertility group.

Certain Sperm Function Parameters		*In vitro* activation	
		Before activation	After activation
Sperm concentration (x106/ml)		20 ±3.23	15± 4.29
Active Sperm Motility (%)	grade A	5 ±2.47	20± 3.25
	grade B	28 ±1.54	60± 4.41
	grade c	40 ±3.22	10± 2.17
Morphologically Normal Sperm (%)		35 ±2.66	50 ±1.89

Table -2: Certain sperm function parameters following *in vitro* activation by layering swim-up technique of normal male for anovulatory infertility group and unexplained infertility group.

Certain Sperm Function Parameters		*In vitro* activation	
		Before activation	After activation
Sperm concentration (x106/ml)		40± 2.21	20± 3.28
Active Sperm Motility (%)	grade A	50± 2.92	55± 1.26
	grade B	33± 2.84	40 ±1.41
	grade C	10 ±4.24	5± 3.17
Morphologically Normal Sperm (%)		55 ±2.86	65 ±1.27

Anti-Mullerian hormone level in pregnant and non pregnant infertile women

In table 2 the mean level of AMH was 0.90 ± 0.14 ng/ml in the pregnant females of the male factor infertility in the early follicular phase of the cycle (Cycle day 2=CD 2) with a range of (0.80–1.00). This value was increased to 1.45 ± 0.64 ng/ml and a range of (1.00–1.90) in the pregnant women (2 out of 10, Pregnancy rate was 20%) but the increment in AMH level did not reach the significant level (P= 0.361). The mean of AMH was 1.01± 0.29 ng/ml (range 0.70- 1.60) in pregnant females of anovulatory causes at early follicular phase (CD2). This value was increased to 1.42 ±0.33 ng/ml in the same females when ten of them became pregnant (25%) with the range (0.89 -2.00). The different between the two intervals was statistically significant (P< 0.001).

In the group of infertility with unexplained causes, the value of AMH level was increased in the females who became pregnant from 0.90 ± 0.10 ng/ml with the range (0.80 -1.00) on the CD2 to 1.12 ± 0.24 ng/ml with the range (0.85–1.30) ng/ml at pregnant of three women (pregnancy rate was 25%). However no significant (P>0.05) difference was found between them.

Table-3: Anti-Mullerian hormone level at early follicular phase and pregnancy of the studied groups

Type of infertility	pregnancy status	regnancy Rate No.(%)	AMH Level (ng/ml)		P value
			Early follicular phase	At pregnancy	
Male factors	Pregnant	2(20%)	0.90 ±0.14 (0.80- 1.00)	1.45± 0.64 (1.00- 1.90)	0.122
	Non pregnant	10	1.01± 0.21 (0.70 -1.30)	-	
	P value		0.509	-	
Anovulatory causes	Pregnant	10(25%)	1.01± 0.29 (0.70- 1.60)	1.42± 0.33 (0.89 -2.00)	0.001#
	Non pregnant	40	1.02 ±0.38 (0.60 -2.30)	-	
	P value		0.915	-	
nexplained infertility	Pregnant	3(25%)	0.90 ±0.10 (0.80- 1.00)	1.12 ±0.24 (0.85- 1.30)	0.361
	Non pregnant	12	0.98± 0.22 (0.70 -1.40)	-	
	P value		0.586	-	

Values are expressed as mean± SEM.

Levels of activin hormone in pregnant and non pregnant women.

In the three infertility groups, activin A hormone level was increased from early follicular phase to mid cycle and at the luteal phase of pregnant females compared the non pregnant women. There was a statistical significant (P<0.05) elevation in activin A level between pregnant and non pregnant women at luteal phase as shown in table-4.

Table 4: The activin hormone level at different phases of menstruation cycle of the studied groups according to pregnancy status

Type of infertility	Pregnancy status	Pregnancy Rate No.(%)	AMH Level (ng/ml)		P value Late-Luteal phase	At pregnancy
			Early follicular phase	Mid-Cycle phase		
Male factors	Pregnant	2(20%)	305.00± 63.64 (260- 350)	377.50± 16.26 (366 -389)	408.00 ±11.31 (400 -416)	423.1± 10.08 (423- 430)
	Non pregnant	10	323.90± 96.17 (179 -520)	379.50 ±62.13 (200- 500)	335.00± 13.68 (285- 360)	-
	P value		0.899	0.987	0.0271	-
Anovulatory causes	Pregnant	10(25%)	365.30 ±139.40 (150- 650)	436.90 ±143.89 (240- 680)	572.20± 155.16 (310 -790)	583.3± 126.19 (323- 811)
	Non pregnant	40	323.97± 116.27 (120 -650)	424.75± 131.32 (220- 720)	340.85± 115.01 (130 -670)	-
	P value		0.339	0.798	0.0001*	-
Unexplained infertility	Pregnant	3(25%)	490.00± 103.92 (370 -550)	570.00± 134.54 (420 -680)	646.67± 140.12 (490 -760)	660.9± 122.73 (504- 774)
	Non pregnant	12	393.58± 113.79 (261- 650)	493.83± 140.89 (301- 720)	404.25 ±114.60 (265- 660)	-
	P value		0.206	0.414	0.008*	-

Values are expressed as mean+ SEM

Effect of follistatin hormone on IUI outcome

Table-5 showed the result of follistatin hormone levels at different cycle phases and at pregnancy of the studied groups of infertile females according to IUI outcome. No statistical significant difference was recorded between the values of follistatin hormone through the menstrual cycle and after pregnancy between the pregnant and non pregnant women of the three infertile groups as shown in table -5.

Table 5: The follistatin hormone level at different phases of menstruation cycle of the studied groups according to pregnancy status.

Type of infertility	pregnancy status	Follistatin (ng/ml)				
		regnancy Rate No.(%	Early follicular phase	Mid-Cycle phase	Late luteal phase	At pregnancy
Male factors	Pregnant	2(20%)	0.17 ±0.07 (0.12- 0.22)	0.15± 0.07 (0.10- 0.20)	0.14	0.10± 0.01 (0.09 -0.10)
	Non pregnant	10	0.22 ±0.15 (0.04- 0.47)	0.17 ±0.12 (0.02 -0.34)	0.21	-
	P value		0.668	0.871		-
Anovulatory causes	Pregnant	10(25%)	0.30 ±0.12 (0.12- 0.48)	0.26 ±0.12 (0.10- 0.46)	0.24	0.23± 0.11 (0.09 -0.40)
	Non pregnant	40	0.21± 0.19 (0- 0.75)	0.17± 0.15 (0.01 -0.60)	0.20	-
	P value		0.141	0.068		-
nexplained infertility	Pregnant	3(25%)	0.33 ±0.12 (0.20- 0.44)	0.29± 0.13 (0.16- 0.41)	0.27	0.24 ±0.12 (0.12 -0.35)
	Non pregnant	12	0.24± 0.13 (0.06- 0.50)	0.21± 0.12 (0.04- 0.46)	0.19	-
	P value		0.306	0.304		-

Values are expressed as mean+SEM

The correlation between AMH, Activin-A and follistatin hormones and IUI outcome in combined setting:

Figure 1shows the Receiver operating characteristic (ROC) curve for various hormones of early follicular and midcycle versus pregnancy rate. It was shown that follistatine midcycle data was significantly (P<0.05) correlated with pregnancy rate.

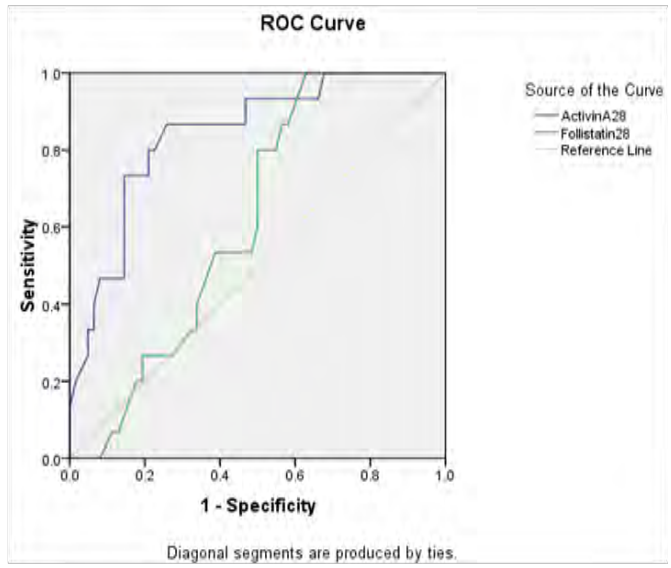

Figure1: Receiver operating characteristic (ROC) curve for various hormones of early follicular and midcycle versus pregnancy rate showing Cutoff values of the hormones related with successful pregnancy.

Figure -2 shows the ROC curve for Activin-A and follistatin hormones in late luteal phase(day 28 of the cycle) versus pregnancy rate.It was shown that Activin-A data was significantly correlated with pregnancy rate (P<0.05).

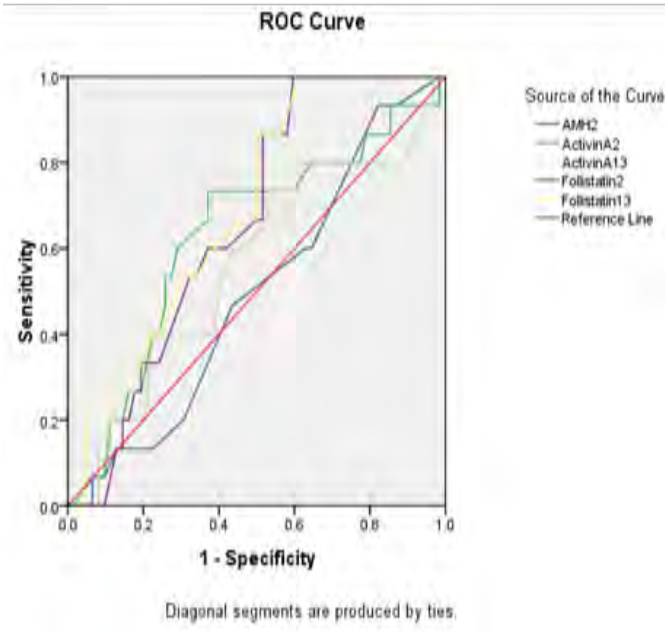

Figure 2: ROC curve for activin A and follistatin hormones of late luteal phase versus pregnancy rate showing Cut off values of the hormones related with successful pregnancy

Table-6 shows the best hormonal prediction according to ROC curve in pregnant women at cycle day 2 and 13 (early follicular and midcycle) and at day 28 (late luteal phase). The follistatin hormone level at day 13 has the most predictive value for pregnancy (p =0.024) and the best cut off predictive value is ≥ 0.20ng/ml. Activin A hormone has also a predictive value for pregnancy (p< 0.001) and the best cut off predictive value is ≥ 397.5ng/ml

Table 6: Best hormonal prediction according to ROC curve

Hormone	Best cut-off value	AUC	P-value	Sensitivity	Specificity
AMH Day 2	≥ 1.000	0.494	0.944	20%	69.4%
Activin A Day 2	≥ 355.00	0.630	0.121	53.3%	72.6%
Activin A Day 13	≥ 536.00	0.561	0.467	40%	77.4%
Activin day 28	≥ 397.5	0.840	<0.001	80%	79%
Follistatin Day 2	≥ 0.24	0.659	0.058	60%	62.9%
Follistatin Day 13	≥ 0.20	0.689	0.024	60%	61.7%
Follistatin day 28	≥ 0.225	0.619	0.155	53.7%	61.3%

Discussion

The present study showed a significant increase in sperm motility of grades A and B in the semen of men involved in IUI program. The significant improvement in active sperm motility may resulted from the effect of swim-up technique and culture medium on intact sperms to generate their capability to migrate to the upper layer of the medium. While the dead, immotile and sluggish sperms remained at the down layer of the culture medium. This finding is in consistent with other studies(14)(15).

It has been found that there was no significant relation between AMH level and pregnancy rate and there is no significant changes in its level between early follicular phase and early pregnancy. A low AMH suggests a poor ovarian reserve(16). The study found no effect of AMH on the fertilization capacity.

The data of the present work showed significant relationship between activin A level and pregnancy with the best cutoff value is ≥ 397.5ng/ml at late luteal phase. These findings may help in detecting early pregnancy (EP). In the maternal circulation serum activin A concentrations are higher in pregnant than in non pregnant women and increase throughout pregnancy until delivery(17). It is suggested that an impaired secretion of activin A occurs in the presence of problems related to trophoblast invasion and implantation(18). Positive predictive value for ectopic pregnancy (approximately 97%) is possible when low serum activin levels are observed (19,20). These procedures may select pregnancies at higher risk of ectopic pregnancy earlier and possibly to prevent unnecessary interventions(21).

This study found no significant differences between FS level of early follicular phase, midcycle and late luteal phase of the menstrual cycle and even in early pregnancy and midcycle or preovulatory stage (day12- 14 of the cycle). Follistatin value has a cut-off value of ≥ 0.20ng/ml that's mean the spouse has a very poor chance of getting pregnant if she has a follistatin value less than 0.20ng/ml in prcovulatory stage.

It was concluded that Activin-A can be regarded as a biomarker indicator for diagnosing very early pregnancy at luteal phase (at CD 28). Follistatin level has the most cut off value to predict pregnancy before IUI.

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