Journal of Molecular Pathophysiology
www.scopemed.org
DOI: 10.5455/jmp.20160226124043

Original Research

ScopeMed

Clinical Significance of Follistatin in Obese and Non-obese Egyptian Polycystic Ovarian Patients

Mostafa El-Shafey[1], Maghawry Hegazy[2], Mohammed El-Zahabi[1], Mohammed Farahat[3]

[1]Biochemistry Department, Faculty Of Pharmacy (Boys) Cairo, Al-Azhar University, Egypt.
[2]Demonstrator at Biochemistry Department, Faculty Of Pharmacy (Boys) Cairo, Al-Azhar University, Egypt.
[3]Obstetrics and Gynecology Department, Faculty Of Medicine (Boys) Cairo, Al-Azhar University, Egypt.

Address for correspondence:
Maghawry Hegazy,
Demonstrator at Biochemistry department, Faculty of Pharmacy (Boys) Cairo,
Al-Azhar University, Egypt.
drmegawry@yahoo.com

Received: January 13, 2016
Accepted: February 26, 2016
Published: March 20, 2016

ABSTRACT

Objective: Polycystic ovarian syndrome (PCOS) is one of the most common endocrine disorders in women, characterized by hyperandrogenism, infertility, chronic oligo or an ovulation and obesity. In this present study we investigate the level of follistatin in obese and non-obese women with PCOS and define any correlation between follistatin and hormonal parameters. **Methods:** The study group included 23 obese and 17 non-obese PCOS patients. The control group included 18 obese and 22 non-obese subjects. Blood samples were obtained from the patients on day 2–5 of menstrual cycle and were assayed for Leutinizing hormone (LH), follicle stimulating hormone (FSH), total testosterone, follistatin, prolactin, sex hormone binding globulin (SHBG) and fasting insulin using enzyme-linked immunosorbent assays (ELISA), fasting glucose was measured spectrophotometry. Hemostasis model assessment - insulin resistance (HOMA-IR), free androgen index (FAI), LH/FSH ratio were calculated. **Results:** Follistatin concentrations were significantly higher in obese and non-obese PCOS patients (mean ± SE; 1207 ± 37.99 and 1106 ± 30.8 pg /ml respectively) than their respective controls (721.9 ± 23.44 and 653.3± 25.88pg./ml, $P < 0.0001$ respectively) and there was weak significant difference between obese and non-obese control $P < 0.042$ and weak significant difference between obese and non-obese PCOS $P < 0.022$. Stepwise regression analyses for relationships between follistatin and all other variables in obese PCOS group indicated that follistatin was negatively affected by FSH ($P < 0.09$), and positively affected by insulin and HOMA-IR ($P < 0.009$) in non-obese PCOS group. **Conclusions:** Serum follistatin is increased in PCOS patients compared to control subjects and there is weak significant increase in obese subjects. PCOS is the most significant variable relates to high follistatin serum concentration. Follistatin correlated negatively with FSH and a high follistatin levels may contribute to the pathophysiology of PCOS.

KEY WORDS: Follistatin; hyperandrogenism; infertility; obesity; Polycystic ovarian syndrome.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is an endocrine-metabolic disorder characterized by multiple hormonal imbalances, reflecting on a clinical presentation dominated by manifestations of hyperandrogenism, which generate short and long term consequences on female health characterized by typical ovarian morphology, follicular arrest, oligo or anovulation, hyperandrogenism, obesity and insulin resistance, which are not always fully associated. Among these, infertility is one of the most alarming associated morbidities, as it currently affects approximately 48.5 million women aged 20–44 years, with PCOS accounting for 6–15% of these cases, although up to 70% of women with PCOS may be undiagnosed [1]. Indeed, its optimal diagnosis is often hindered due to its apparent similarities with several other pathologies remarkably, obesity as well as Cushing's syndrome, ovarian and adrenal neoplasms, and congenital adrenal hyperplasia [1]. The aetiology of PCOS has not yet been fully elucidated, although the observation of familial aggregation [2, 3] is consistent with a genetic basis for this disorder. Growth factors are heavily involved in the pathophysiology, either contributing to or as a consequence of the arrested development of follicles, abnormal steroidogenesis, and hyperinsulinaemia [4].

Follistatin is a monomeric glycosylated polypeptide chain, which was initially identified in and isolated from follicular fluid on the basis of its inhibition of pituitary FSH secretion [5, 6]. Later it was well documented that follistatin exerts this and other activities by neutralizing activin bioactivity, being an activin-binding protein [7]. Both follistatin and activin are expressed in numerous tissues, including the gonads, pituitary, adrenal cortex, liver and pancreas. Activin, a member of the transforming growth factor-β superfamily, promotes ovarian follicular development, inhibits theca cell androgen production, increases pituitary follicle stimulating hormone (FSH) secretion and modulates pancreatic β-cell insulin secretion [7, 8, 9]. A decrease in concentration or functional activity of activin, as well as an increase in follistatin, might therefore encourage characteristic features of PCOS. Some studies support the assumption that high follistatin gene expression might be linked to PCOS. In transgenic mice, overexpression of follistatin resulted in arrested ovarian folliculogenesis, with or without suppression of serum concentrations of FSH. [10] tested 37 candidate genes for linkage and association with PCOS or hyperandrogenism in data from 150 families [3]. The strongest evidence for linkage was with the follistatin gene. Although later studies failed to identify any mutation in the follistatin gene in PCOS [10], these observations led us to

investigate whether women with PCOS showed disordered follistatin serum concentrations. Recently, [11] published their findings that circulating follistatin concentrations are higher and activin concentrations are lower in PCOS [11]. However, the question of a correlation of these findings with body mass index (BMI) has been left open. Therefore, we compared follistatin serum concentrations in obese and non-obese patients with PCOS or normal ovarian function.

SUBJECTS

Eighty subjects aged 20–38 years were chosen from outpatient gyna clinic from Al-Hussein University Hospital, and Sayed Galal Hospital from Jan 2015 till July 2015were included in the study. The study group (with PCOS) diagnosed according to Rotterdam criteria included 40 patients with typical ultrasonic ovarian morphology (the presence of 12 or more follicles in either ovary measuring 2-9 mm in diameter and/ or increased ovarian volume > l0 ml) [12] and chronic menstrual irregularity (oligo- or amenorrhea). The control group included 40 subjects with normal ovulatory cycles with a mean length of 27–32 days and no endocrine abnormalities, such as hyperprolactinaemia or abnormal thyroid function. Each group was further divided into obese and non-obese (BMI ≥ and <30 kg/m^2 respectively). Patients being treated with steroids, oral hypoglycemic agents or insulin were not included. All the subjects are acknowledged about this study by the researchers. All patients were in good physical and mental health. Al-Hussein University Hospital, and Sayed Galal Hospital and Ethics Committee, approved the study protocol.

METHODS

Blood samples were obtained from PCOS patients on day 2–5 of the cycle after 35–90 days of amenorrhea with no hormonal treatment. After ruling out the possibility of pregnancy (β-human chorionic gonadotrophin <10 IU/l) or spontaneous ovulation (progesterone <15 nmol/l). Blood samples were obtained from the control group subjects on day 2–5 (early follicular) of the cycle. Blood sampling and anthropometric parameters for normal healthy control and PCOS groups were done at the Departments of Obstetrics and Gynecology, Al-Hussein University Hospital, and Sayed Galal Hospital, Cairo, Egypt. 10 - 14 hours fasting venous blood samples were withdrawn after 10 minutes rest in the sitting position. Sample were divided into 2 aliquots, the first aliquot was collected in tube containing fluoride for blood glucose estimation immediately by colorimetric method, the second aliquots was collected in gel separating tube, allowed to clot for 30 minutes and centrifuged at 4000 r.p.m for 15 minutes at room temperature to estimate the other biochemical parameters. The yielded sera were stored at -80 ^{0}C until time of analysis for determination of biochemical parameters including : Serum follistatin ,FSH , leutinizing hormone (LH) , prolactin , total testosterone (TT) , insulin and sex hormone binding globulin (SHBG) which were determined by enzyme-linked immunosorbent assays (ELISA) technique [13].Two ml of blood were withdrawn into NaF-potassium oxalate BD vacutainer tube and centrifuged for 4000 r.p.m for 5 minutes and the separated plasma were used immediately for determination of Fasting plasma glucose that was determined by colorimetric method [14]. Homeostasis model assessment of insulin resistance ($HOMA_{IR}$) was calculated according to $HOMA_{IR}$ = Fasting insulin (μIU/ml) X fasting blood glucose (FPG) (mg/dl) / 405 [15].Free androgen index (FAI) was calculated according to FAI =100 X (Total testosterone / SHBG) [16].

STATISTICS

All analysis and graphics were performed using Graphpad prism 6 (windows version 7; Graphpad software 2010). All results were presented as mean values ± standard error unless indicated otherwise. Differences between groups were evaluated by the calculation of unpaired Student's t-test and one-way ANOVA. Correlations between biochemical markers and other continuous variables were tested using the Spearman or the Pearson's correlation coefficients. *P* value of <0.05 was considered to be statistically significant. All reported *p*-values are based on two-sided tests and compared to a significance level of 5%.D'Agostino-Pearson omnibus test was used to identify whether the variables were normally distributed. Multivariate linear regression models were performed using follistatin as the dependent variable, and BMI or other hormonal parameters as the independent variables for women with PCOS and controls.

RESULTS

Serum follistatin concentrations on cycle day 2–5 in PCOS were comparable between obese and non-obese PCOS patients (mean ± SE; 1207 ± 37.99 and 1106 ± 30.8 pg /ml P < 0.0001 respectively) and significantly higher than their respective controls (721.9 ± 23.44and 653.3± 25.88pg./ml, *P* < 0.0001 respectively) and there was weak significant difference between obese and non-obese control (721.9 ± 23.44and 653.3± 25.88pg./ml, P < 0.042 respectively) and weak significant difference between obese and non-obese PCOS (1207 ± 37.99 and 1106 ± 30.8 pg /ml P < 0.022 respectively).

Serum follistatin on cycle day 2–5 in obese and non-obese PCOS and control groups are shown in Figure 1. Clinical data and cycle day 3–5 serum hormone concentrations (mean ± SE) in obese and non-obese PCOS and control patients are shown in Table I. LH concentrations were 1.5-fold higher (*P* < 0.001) in both obese and non-obese PCOS patients compared with their respective controls, in the face of comparable FSH concentrations. LH concentrations were 50–60% higher (*P* < 0.05) in obese than non- obese groups. Testosterone concentrations were 1.3 and 1.5 fold higher in non-obese (*P* < 0.05) and obese (*P* = 0.05) PCOS patients than in their respective controls. LH/FSH ratio was double in obese PCOS patients compared with their respective controls (*P* < 0.05) and 2 fold higher in non-

obese patients compared with their respective controls ($P < 0.05$). FAI levels was 3 fold higher in obese PCOS patients compared with their respective controls ($P < 0.05$) and 1.5 fold higher in non-obese patients compared with their respective controls ($P < 0.05$). Concentrations of fasting insulin and HOMA-IR levels were 1.6 times higher in obese ($P < 0.05$) than in their respective controls. To determine which independent variables affect serum follistatin concentrations, stepwise linear regression analyses with follistatin as the dependent variables and BMI and other hormonal parameters were performed as independent factors. Follistatin concentrations were negatively affected by FSH (r =-0.355, P < 0.09) in obese pcos group (figure 2). Follistatin concentrations were significantly and independently positively affected by HOMA –IR (r=0.6943, p=0.002) and insulin(r =0.7341, p=0.0008) in non-obese patients with PCOS. LH correlated with follistatin concentrations in both obese PCOS and non-obese pcos groups ($r = 0.22$ and $r = 0.06$ respectively,). However, stepwise regression analysis showed that LH was not a statistically significant independent factor affecting follistatin concentrations; it also positively correlated with BMI ($r = 0.11$), and negatively correlated with FAI ($r = –0.008$) and SHBG (r = - 0.2) in the non-obese PCOS patients and it also positively correlated with BMI ($r = 0.14$),FAI($r = 0.22$) and LH/FSH ratio($r = 0.35$) and negatively correlated with SHBG ($r = –0.35$),total testosterone ($r = - 0.22$)and HOMA-IR($r = -0.22$) in the obese PCOS patients

Table 1. Clinical data and day 3–5 serum hormone concentrations in obese and non-obese polycystic ovary syndrome (PCOS) and control patients (values are mean ± SE).

	Obese PCOS	Non-obese PCOS	Obese Control	Non-obese Control
Number	**23**	**17**	**18**	**22**
Age (years)	25.04± 0.65b	24±0.79	30.83±0.91a	26.41± 1.25
BMI (kg/m^2)	34.13±0.56a,c	25.64+0.89	34.05±0.69a	24.54±0.64
Follistatin(pg/ml)	1207±37.99a,b	1106±30.80a,b	721.9±23.44	653.3±25.88
LH (mIU/ml)	9.063 ± 1.079a,b	8.155 ± 1.076a	6.182 ± 0.389	5.422 ± 0.317
FSH (mIU/ml)	6.517 ± 0.3703	6.142±0.4344b	7.517 ± 0.379	7.050 ± 0.2517
LH/FSH	1.539 ± 0.223a,b	1.404±0.172a,b	0.8333 ±0.047	0.77 ±0.041
PRL (ng/ml)	15.41 ±1.239	16.02 ±1.453b	12.39 ±0.8698	13.89 ±1.078
SHBG(nmol/ml)	25.19 ± 2.77a,b	29.08 ± 1.51b	35.68± 1.58	34.85 ±2.85
Total testosterone (ng/dl)	0.527±0.023a,b,c	0.410±0.027a,b	0.3347±0.0176	0.3213±0.0087
FAI	8.604 ±0.67a,b,c	5.249±0.562a,b	3.313 ±0.1863	3.755 ±0.3625
Fasting glucose (mg/dl)	103.4 ±3.012a	94.71 ±2.995	99 ±1.913	94.86 ±1.979
Fasting insulin (mIU/ml)	18.33 ±1.87a,b,c	9.52 ±0.86	11.79 ±0.996a	9.38 ± 0.52
HOMA-IR	4.78±0.59a,b,c	2.286 ±0.2712	2.932 ±0.2851a	2.202 ±0.1352

a: Significant from non-obese control group at p < 0.05.
b: Significant from obese control group at p < 0.05.
c: Significant from non-obese PCOS group at p < 0.05.

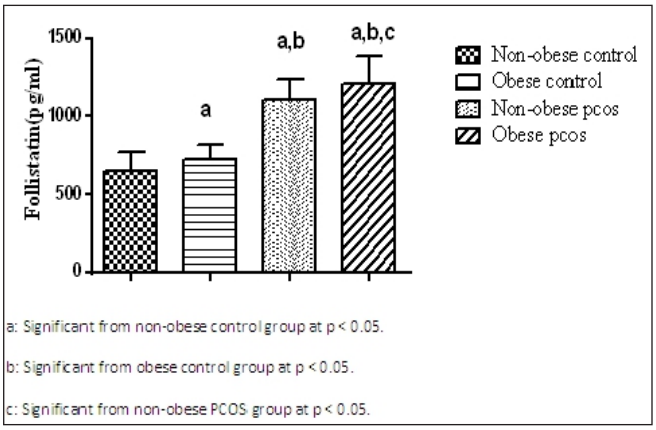

Figure 1. Mean ± SEM of Follistatin in non-obese control, obese control, non-obese PCOS and obese PCOS groups.

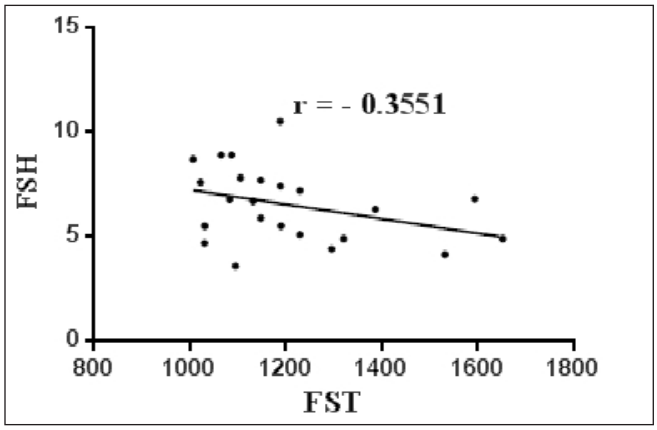

Figure 2. Correation betwen Follistatin and FSH in obese PCOS patients

DISCUSSION

Our study was instigated by recent reports that follistatin can encourage many features of PCOS, such as increased ovarian androgen synthesis, decreased pituitary FSH, disturbed pancreatic insulin secretion and inhibition of follicular development. [11].Our data indicated that the serum follistatin was 1.7 fold higher in PCOS patients compared with controls, and 1.1 fold higher in obese subjects compared to non-obese ones which means slight increase. A negative correlation between PCOS and FSH levels was found by stepwise regression analysis but still non-significant ($P < 0.09$). Moreover, PCOS was the most significant variable that independently increased follistatin and decreased FSH serum levels when weighed against BMI and hormonal status. Studies on the pathogenesis of PCOS could not identify a single causative gene involved. It has been identified that there is a relation between PCOS and disordered insulin metabolism, and this indicates that the syndrome may be the presentation of a complex genetic trait disorder [17].

The features of obesity, hyperinsulinaemia and hyperandrogenaemia, which are commonly seen in PCOS, are also known to be factors, which confer an increased risk of cardiovascular disease and non-insulin dependent diabetes mellitus (NIDDM), significantly towards the severity of many problems, such as the risk of miscarriage [18]. Evidence for linkage between the follistatin gene and PCOS was identified. This sheds some light on the genetic basis of PCOS implicating the role of follistatin gene in the disease process. While the exact contribution of the ovary to circulating concentrations of this protein is not known, these data raise the possibility that alterations in secretion of follistatin from the ovary or other organs may explain the change in circulating concentration of the protein. As reported earlier, PCOS is closely linked to areas near the follistatin gene in genetic studies in women with PCOS [3].

Following the completion of our study, [14] published their findings and quite independently discovered follistatin concentrations in PCOS, very similar to our findings [11]. We have, in addition, answered the question which left open [11] i.e. a correlation of these findings with BMI. We have demonstrated that weight is one of the explanatory factors.

A study by [19] reported that follistatin was increased by 80% - 90% in PCOS patients, independent of obesity. Moreover [11] reported PCOS as the most significant variable that independently increased follistatin and it has been shown that virtually all-circulating follistatin in women is activin bound. In contrast, a substantial amount of free follistatin has been detected in the follicular fluid and pituitary. These observations are compatible with the hypothesis that the main biological role of circulating follistatin is to restrict activin bioavailability. Currently, there is no reliable method to measure free activin levels due to disruption of activin/follistatin complexes during the assay procedures. Follistatin is found to be increased in PCOS subjects selected for the present study in comparison with the normal controls and there is weak significant increase due to obesity [19].

However, follistatin concentrations remain stable with no consistent trend across the menstrual cycle, the source of serum follistatin in women is unascertained [15, 20]. This peptide is secreted from ovarian granulosa cells in response to FSH stimulation. The concentrations of follistatin in follicular fluid exceed serum concentrations by as much as 100–200 folds [15, 21]. Furthermore, serum follistatin concentrations have been found to be similar in eugonadal women, women with hypothalamic amenorrhea and women after oophorectomy [22], and did not change during puberty or after ovarian suppression with gonadotrophin-releasing hormone analogue administration. However, follistatin concentrations in late puberty were less than those in early puberty Post-menopausal women had higher or similar follistatin levels than young cycling women [23, 24].

This data is supported by the report of [25] that over expression of follistatin will be expected to lead to increased ovarian androgen production and reduction in circulating FSH levels, which are the features of PCOS supporting the present investigation, however, LH level and follistatin concentration was not related both in PCOS and control subjects and did not show any significant correlation [25].

CONCLUSION

In summary, we found that the serum follistatin was increased in PCOS patients compared with controls and slight increase in obese subjects when compared to non-obese subjects that making obesity one of the possible explaining factors of PCOS. Using stepwise regression, we showed that PCOS was the most significant variable that increased follistatin serum levels when weighed against BMI and hormonal status. We postulate that by neutralizing the bioactivity of activin, high serum follistatin levels may contribute to the pathophysiology of PCOS and the measurement of follistatin may be relevant for the diagnosis of PCOS. Further investigations are recommended.

ACKNOWLEDGMENTS

The authors are grateful to all staff members the Departments of Bio-chemistry, faculty of pharmacy, Al-Azhar University, the nursing and all staff members the Departments of Obstetrics and Gynecology, Al-Hussein University Hospital, and Sayed Galal Hospital.

REFERENCES

1. Teede H, Deeks A, Moran L. Polycystic ovary syndrome: a complex conditions with psychological, reproductive and metabolic manifestations that impact on health across the lifespan. BMC Med 2010; 8:41.
2. Legro RS, Spielman R, Urbanek M, Driscoll D, Strauss JFIII, Dunaif A. Phenotype and genotype in polycystic ovary syndrome. Recent. Prog. Horm. Res 1998; 53: 217–256.
3. Urbanek M, Legro RS, Driscoll DA, Azziz R, Ehrmann DA, Norman RJ, Strauss JF 3rd, Spielman RS, Dunaif A. Thirty-seven candidate genes for polycystic ovary syndrome: strongest evidence for linkage is with follistatin. Proc. Natl Acad. Sci. USA 1999; 96: 8573–8578.
4. Homburg R. Involvement of growth factors in the pathophysiology of polycystic ovary syndrome. Gynecol. Endocrinol 1998; 12: 391–397.
5. Robertson DM, Klein R, de Vos FL, McLachlan RI, Wettenhall RE, Hearn MT, Burger HG, de Kretser DM. The isolation of polypeptides with FSH suppressing activity from bovine follicular fluid which are structurally different to inhibin. Biochem. Biophys. Res Commun 1987 ; 149: 744–749.
6. Ueno N, Ling N, Ying SY, Esch F, Shimasaki S, Guillemin R. Isolation and partial characterization of follistatin: a single-chain Mr. 35 000 monomeric protein that inhibits the release of follicle-stimulating hormone. Proc. Natl Acad. Sci. USA 1987; 84: 8282–8286.
7. Mather JP, Moore A, Li RH. Activins, inhibins, and follistatins: further thoughts on a growing family of regulators. Proc. Soc. Exp. Biol. Med 1997; 215: 209–222.
8. Shibata H, Kanzaki M, Takeuchi T, Miyazaki JI, Kojima I. Two distinct signaling pathways activated by activin A in glucose-responsive pancreatic beta-cell lines. J. Mol. Endocrinol 1996; 16: 249–258.
9. Florio P, Luisi S, Marchetti P, Lupi R, Cobellis L, Falaschi C, Sugino H, Navalesi R, Genazzani AR, Petraglia F. Activin A stimulates insulin secretion in cultured human pancreatic islets. J. Endocrinol. Invest 2000; 23:231–234.
10. Urbanek M, Wu X, Vickery KR, Kao LC, Christenson LK, Schneyer A, Legro RS, Driscoll DA, Strauss JF 3rd, Dunaif A, Spielman RS. Allelic variants of the follistatin gene in polycystic ovary syndrome. J. Clin. Endocrinol. Metab 2000; 85: 4455–4461.
11. Teede H1, Ng S, Hedger M, Moran L. Follistatin and activins in polycystic ovary syndrome: relationship to metabolic and hormonal markers. Metabolism 2013; 62(10):1394-400.
12. Balen AH, Laven JS, Tan SL, Dewailly D. Ultrasound assessment of the polycystic ovary: international consensus definitions. Hum Reprod Update 2003;9:505–514.
13. Evans LW, Muttukrishna S, Groome NP. Development, validation and application of an ultra-sensitive two-site enzyme immunoassay for human follistatin. J. Endocrinol 1998; 156: 275–282.
14. Bergmeyer HU, Bernt E. Methods of Enzymatic Analysis, H.U. Bergmeyer, Ed., New York, Academic Press, 1974 (2); 1205-1212.
15. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985; 28(7):412-9.
16. Fraser Marianne, Farrokh Sohrabi. "Free Androgen Index". Online Medical Encyclopedia. University of Rochester Medical Center. Via https://en.wikipedia.org/wiki/Free_androgen_index .Retrieved 11 July 2014.
17. Rajkowha M, Glass MR, Rutherford AJ, Michelmore K, Balen AH. Polycystic Ovary Syndrome: a risk factor for cardiovascular disease? Br J Obs. Gynaecol 2000; 107: 11–18.
18. Wang JX, Davies MJ, Norman RJ. Obesity increases the risk of spontaneous abortion during infertility treatment. Obs. Res 2002; 10: 551–554.
19. Ramaswamy Suganthi, Subbian Manonayaki, Jahangir Ali, Fathima Benazir. Follistatin concentrations in women from Kerala with polycystic ovary syndrome. Iranian Journal of Reproductive Medicine 2010; 8(3): 131-134.
20. Vale W, Hsueh AJ, Rivier C, Yu J. The inhibin/activin family of hormones and growth factors. In Sporn, M.B. and Roberts, A.B. (Eds) Peptide Growth Factors and Their Receptors II. Springer-Vertag, New York 1990; 211–248.
21. Foster CM, Phillips DJ, Wyman T, Evans LW, Groome NP, Padmanabhan V. Changes in serum inhibin, activin and follistatin concentrations during puberty in girls. Hum. Reprod 2000; 15: 1052–1057.
22. Erickson GF, Chung DG, Sit A, Louis DePaolo, Shunichi Shimasaki , Nicholas Ling. Follistatin concentrations in follicular fluid of normal and polycystic ovaries. Hum. Reprod 1995; 10: 2120–2124.
23. Khoury RH, Wang QF, Crowley WF JR, Hall JE, Schneyer AL, Toth T, Midgley AR Jr, Sluss PM. Serum follistatin levels in women: evidence against an endocrine function of ovarian follistatin. J. Clin. Endocrinol. Metab 1995; 80: 1361–1368.
24. Kettel LM, DePaolo LV, Morales AJ, Apter D, Ling N, Yen SS. Circulating levels of follistatin from puberty to menopause. Fertile. Steril 1996; 65: 472–476.
25. Mei-Jou Chen1, Der-Sheng Han, Jehn-Hsiahn Yang, Yu-Shih Yang, Hong-Nerng Ho, Wei-Shiung Yang. Myostatin and its association with abdominal obesity, androgen and follistatin levels in women with polycystic ovary syndrome. Human Reproduction 2012; 27(8): 2476–2483.

Source of Support: Nil, Conflict of Interest: None declared