

ORIGINAL ARTICLE

Longitudinal analysis of maternal serum Follistatin concentration in normal pregnancy and preeclampsia

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Summary

Objective Follistatin (FST) is a regulator of the biological activity of activin A (Act A), binding and blocking it, which could contribute to the modulation of its pro-inflammatory activity during pregnancy. We sought to investigate, in this nested case-control study, FST serum levels during normal pregnancy and correlate it with the FST profile in preeclamptic pregnant women, normal pregnant women followed 3 months postpartum and eumenorrheic nonpregnant women throughout the menstrual cycle.

Subjects and Methods Follistatin serum levels determined by ELISA, biochemical and anthropometric variables were measured in normal pregnant ($n = 28$) and preeclamptic ($n = 20$) women during three periods of gestation. In addition, FST serum levels were measured in a subset of normal pregnant women ($n = 13$) followed 3 months postpartum and in eumenorrheic nonpregnant women ($n = 20$) during the follicular and luteal phases of the menstrual cycle.

Results Follistatin serum levels in the eumenorrheic nonpregnant and postpartum group were significantly lower when compared to levels throughout gestation ($P < 0.01$). Serum FST levels increased in each period of pregnancy analysed, being significantly higher towards the end of gestation ($P < 0.01$). FST levels were lower in late pregnancy in preeclamptic women

compared to normal pregnant women ($P < 0.05$). Finally, FST levels were higher in the luteal phase when compared with the follicular phase of the menstrual cycle ($P < 0.05$).

Conclusions These analyses would permit the consideration that changes in FST levels during pregnancy contribute to the control of the Act A system.

(Received 13 November 2014; returned for revision 30 November 2014; finally revised 30 December 2014; accepted 31 December 2014)

Introduction

The transforming growth factor (TGF- β) superfamily consists of over 40 members, which have related structural and functional characteristics, grouped into the subfamilies of TGF- β , activins, inhibins, anti-Müllerian hormone (AMH), bone morphogenetic proteins (BMPs), nodal and growth-differentiation factors (GDFs).¹ Additionally, members of the TGF- β superfamily are involved in regulating a wide range of biological processes such as extracellular matrix production, cell growth, apoptosis, cellular differentiation and immunity.¹ The regulation of gene expression mediated by the members of this superfamily is conducted through the canonical signalling pathway, cell-surface serine/threonine kinase receptors to the intracellular Smad family proteins.²

Furthermore, activin (Act) was initially described as a positive regulatory factor of follicle-stimulating hormone (FSH) secretion, but recently new roles have been identified in processes such as angiogenesis, inflammation, immunity, fibrosis and cancer.³ There are three Act isoforms formed by disulphide linked dimers of $\beta\beta$ and $\beta\alpha$ inhibin subunits: Act A ($\beta\alpha$ - $\beta\alpha$), Act B ($\beta\beta$ - $\beta\beta$) and Act AB ($\beta\alpha$ - $\beta\beta$).³ The binding of Act to type II receptor (ActRII) leads to the binding and phosphorylation of

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the type I receptor (ActRI) and subsequent activation of Smad-2, 3 and 4, which forms complexes that translocate to the nucleus and act as the intracellular mediators of gene transcription.³ It has been shown that Act inhibits cell division in different types of cancer, but on the other hand, participates in the activation of cell division in the ovary, testis and adrenal and pituitary glands.³ Act also plays an important role in programmed cell death by caspase activation and cell cycle arrest.^{4,5} Furthermore, it has been shown that Act modulates dendritic spine morphogenesis by modulating actin dynamics in spines.⁶

Follistatin (FST) is a single-chain glycosylated protein that binds with high affinity to Act A, blocking its biological activity, by shielding the type II and I receptor binding sites.⁷ Besides, FST is able to inhibit the activity of various ligands from the TGF- β superfamily containing signalling pathways similar to those of Act A heteromeric receptors.⁸ Act A is stimulated in conditions of early inflammation, via the Toll-like receptor (TLR) 4 signalling pathway, and treatment with FST reduces inflammation and mortality in different models of disease.⁹ Act A and FST serum levels have been determined in cross-sectional studies throughout gestation, finding that circulating levels of these two factors rise towards the end of the gestational period.¹⁰ Additionally, previous studies have shown that Act A serum levels were significantly elevated while FST did not change at the end of pregnancy in women with preeclampsia.¹¹

Recently, FST expression has been studied in different tissues of C57BL/6 mice, showing an elevated gene expression in BAT, skeletal muscle, inguinal WAT and liver.¹² Additionally, Act, inhibins and FST are produced principally by granulosa cells of the ovaries.¹³ Previous studies have observed elevated FST levels in ovarian follicular fluid and blood during the late luteal phase, suggesting that follistatin plays a key role in the development of the new follicle cohort and participates in luteolysis.¹⁴ Studies in human placenta have demonstrated both the presence of inhibin, activin and follistatin messengers and their respective bioactive proteins in the amnion, chorion and maternal decidua.^{15,16} Finally, studies in sheep and humans have shown that circulating levels of FST do not change throughout the menstrual cycle.^{14,17} Thus, both human and animal model studies suggest that changes in the ovary during the menstrual cycle have no impact on circulating levels of FST.

As there is a lack of longitudinal studies assessing the profile of FST, the objective of this nested case-control study was to analyse the levels of FST during early, middle and late pregnancy, and 3 months postpartum. Furthermore, there are no cohort studies of pregnant women to adequately assess the relationship between FST and the development of preeclampsia. Thus, FST levels were also analysed during the three periods of gestation in a longitudinal cohort of pregnant women who developed mild preeclampsia. Finally, FST levels were determined in eumenorrheic women during the follicular and luteal phase of the menstrual cycle. Thus, this study can contribute to the understanding of the physiological conditions and pathological role of FST throughout gestation.

Subjects and methods

Ethics statement

Ethics approval for this study was obtained from the Ethics Committee Board of The School of Medicine – Universidad Nacional de Colombia, and all participants signed a written informed consent. The research was conducted in the clinic: Hospital de Engativá (Bogotá, Colombia), and all women were enrolled in this study by the gynaecology and obstetrics services attending prenatal care between 2012 and 2014.

Subjects and study design

We conducted a nested case-control study. The cohort consists of 450 pregnant women recruited early in pregnancy and followed until 3 months postpartum. All patients who developed preeclampsia were included (cases); controls were pregnant women with normal obstetric and perinatal outcomes selected randomly from the cohort. The randomly selected subjects were representative of the larger cohort in terms of demographic data. This study included: 28 normal pregnant women (control), 20 pregnant women who developed mild preeclampsia (cases), 13 normal pregnant women followed three months postpartum and 20 age-matched nonpregnant eumenorrheic women.

Follow-up of the pregnant women was performed during three periods of gestation: early [mean gestational age 12.15 weeks (range: 11.48–12.5)], middle [mean gestational age 24.4 weeks (range: 24.2–24.6)] and late [mean gestational age 34.6 weeks (range: 34.2–35.4)], continued until 3 months postpartum. Gestational age was calculated by an ultrasound performed between the 11th and 13th week of pregnancy in all of the women. Additionally, all women were subjected to general examination and blood pressure measurement.

Diagnosis of mild preeclampsia in this study was based on the criteria by the International Society for the Study of Hypertension in Pregnancy (ISSHP) guidelines, as previously described.¹⁸ Mild preeclampsia was defined as the presence of hypertension (pressure $\geq 140/90$ mmHg or greater on at least two occasions, at least 4 h apart), detected for the first time after 20 weeks of gestation up to 24 h after delivery, combined with proteinuria (≥ 300 mg in a 24-h urine collection).¹⁸ The patients were diagnosed with mild preeclampsia on average in the 34.9th week of gestation (range: 34.2–35.52). None of the preeclamptic women or the controls received any medication before blood sampling.

Additionally, serum FST levels were analysed during the follicular (days 3–5) and luteal phases (days 21–23) of menstrual cycles in eumenorrheic nonpregnant women ($n = 20$). The nonpregnant women who participated in the study were recruited during the same period.

Briefly, the exclusion criteria consisted of chronic hypertension, vascular disease, renal disease, history of diabetes mellitus, gestational diabetes mellitus, polycystic ovary syndrome, or use of corticosteroids, beta-blockers, beta-adrenergic receptor agonists and other drugs that may have direct effects on metabolism.

Laboratory assays

Maternal blood samples were collected with BD Vacutainer® Serum Tubes, after overnight fasting. Eumenorrheic blood samples were drawn after an overnight fast during the follicular and luteal phase of the menstrual cycle. Serum samples were separated after centrifugation at $1000\times g$ for 5 min and divided into aliquots and frozen at -80°C until assays were performed. Serum glucose, triacylglycerol, total cholesterol and HDL cholesterol levels were measured by enzymatic methods (LIAISON® Analyzer, 129 Saluggia, Italy). Additionally, serum insulin levels ($\mu\text{U/ml}$) were analysed by chemiluminescence assay (Elecsys 2010, Roche Diagnostics, Mannheim, Germany). The homeostasis model assessment of insulin resistance score (HOMA-IR) was calculated using the formula proposed by Matthews (fasting blood insulin concentration in $\mu\text{U/ml} \times$ fasting blood glucose in $\text{mmol/l}/22.5$).¹⁹ Serum levels of progesterone were measured in eumenorrheic women as described elsewhere (Roche Elecsys 1010 Immunoanalyzer, Boulder, CO, USA).

Serum concentrations of FST were quantified with an enzyme-linked immunosorbent assay kit, according to manufacturer's protocols (Reference No. ab113319, Abcam®, Cambridge, MA, USA). Average intra- and interassay coefficients of variation for FST immunoassays were $<10\%$ and $<12\%$, respectively. Sensitivity was <0.5 ng/ml. Circulating concentrations of FST (ng/ml) were measured in duplicate for each participant. According to information provided by the manufacturer, this FST ELISA kit shows no cross-reactivity with a broad list of cytokines tested.

Statistical analysis

Statistical tests were conducted using R software (version 3.1.1). Data with normal distribution were reported as mean \pm standard deviation (SD), while data with non-normal distribution were reported as median and interquartile range (IQR). Statistical differences between groups were tested on the anthropometric and metabolic variables using the Friedman's test followed by the Wilcoxon signed rank test for repeated measurements. The Wilcoxon–Mann–Whitney test was used to compare unpaired groups. The correlation between the FST serum levels and the variables through pregnancy was studied as well. Univariate correlations were assessed on the groups by partial Spearman's correlation coefficient with adjustment of gestational age. *P*-values were adjusted using the Bonferroni correction. Statistical values are presented as $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$. Significance was assumed at $P < 0.05$.

Results

Demographic and clinical characteristics

Table 1 and Table S1 show the demographic and clinical characteristics of healthy eumenorrheic nonpregnant women, normal pregnant women, preeclamptic women and healthy postpartum

women. In Table 1, it was observed that serum progesterone levels are significantly elevated in the luteal phase of the menstrual cycle when compared to circulating levels in the follicular phase ($P < 0.001$). Additionally, no correlation was found between the levels of FST and progesterone during the follicular and luteal phase of the menstrual cycle. The Spearman's correlations between these variables were 0.05 ($P = 0.84$) and 0.43 ($P = 0.06$), respectively (Figures S1 A,B).

Serum Follistatin levels in normal and preeclamptic pregnant women

In eumenorrheic women, FST serum levels are significantly higher in the luteal phase compared to the follicular phase of the menstrual cycle ($P < 0.05$) (Fig. 1) (Table 1). In addition, FST serum levels in the eumenorrheic group (in both phases) are significantly lower when compared to the levels of FST in each of the three gestational periods studied in normal women ($P < 0.001$) (Fig. 1). Furthermore, in normotensive pregnant women, serum FST levels are significantly elevated throughout gestation, with a peak at the end of this period ($P < 0.001$) (Fig. 1 and Table 1). Serum FST levels determined 3 months postpartum were significantly decreased when compared to the third trimester of gestation and reached similar levels to those observed in eumenorrheic nonpregnant women ($P > 0.05$) (Fig. 1 and Table 1).

Serum FST levels in preeclamptic pregnant women were similar to the ones observed in normal pregnant women in early and middle pregnancy (Fig. 2). However, in late pregnancy, serum FST levels were notably lower in preeclamptic women compared to normotensive pregnant women ($P < 0.05$) (Fig. 2 and Table 1 and Table S2).

Correlations between Follistatin and anthropometric, clinical and biochemical features

In the partial univariate correlation analysis between FST serum levels and anthropometric and metabolic parameters, it is worth noting that a significant correlation was present in early pregnancy between serum levels of FST and cholesterol levels for normal pregnant women (Table S3). In preeclamptic women, a direct correlation between circulating levels of FST and glycaemia is observed during late pregnancy (Table S3).

Discussion

The present study shows, for the first time, that serum levels of FST are significantly elevated in the three gestational periods studied when compared to FST serum levels in eumenorrheic women and 3 months postpartum. Additionally, we show that serum FST levels significantly increased in each of the gestational periods analysed, being higher at the end of gestation. Moreover, we demonstrate that the serum levels of FST in preeclamptic women are significantly lower when compared to the levels of the normotensive pregnant women, only towards the end of pregnancy. In addition, circulating levels of FST varied during

Table 1. Anthropometric and metabolic characteristics of healthy eumenorrheic women, normal pregnant, preeclamptic women and healthy postpartum women

Variable	Healthy eumenorrheic women (<i>n</i> = 20)	Normal pregnant (<i>n</i> = 28)			Preeclamptic women (<i>n</i> = 20)			Healthy postpartum women (<i>n</i> = 13)
		EP	MP	LP	EP	MP	LP	
Age, years (median (IQR))	20 (19–22)	23.5 (19–30)	23.5 (19–30)	23.5 (19–30)	19.5 (18–26)	19.5 (18–26)	19.5 (18–26)	24 (19–25)
Weight, kg (mean \pm SD)	55.5 (± 6.3)	57.1 (± 7.4)	61.8 (± 8.4)	66 (± 9.2)	59.5 (± 7.4)	65.4 (± 7.3)	72.9 (± 8.1)	59.6 (± 7.0)
Body mass index, kg/m ² (mean \pm SD)	21.6 (± 2.2)	22.9 (± 2.3)	24.8 (± 2.5)	26.4 (± 2.6)	24 (± 2.8)	26.4 (± 2.8)	29.4 (± 2.7)	23.9 (± 2.2)
Gestational age, weeks [median (IQR)]	–	12.1 (11.4–12.5)	24.4 (24.2–24.6)	34.6 (34.2–35.42)	12.2 (11.5–12.6)	24.2 (24.0–24.5)	34.9 (34.2–35.5)	–
Systolic blood pressure, mm Hg [median (IQR)]	110 (104–115)	93 (90–100)	90 (86–100)	97 (90–103)	105 (100–110)	100 (100–110)	107 (100–113)	100 (90–108)
Diastolic blood pressure, mm Hg [median (IQR)]	71 (65–76)	60 (58–62)	60 (58–62)	61 (58–67)	68 (60–70)	63 (60–68.5)	60 (60–70)	62 (60–64)
Mean arterial pressure, mm Hg [median (IQR)]	85.3 (78.7–87.7)	72 (67.8–74.1)	70 (67.8–74)	72.33 (69.8–78.5)	80.3 (73.1–82.3)	76.6 (74.3–81.6)	80 (74.8–81)	73.33 (70–76.6)
Glucose, mg/dl [median (IQR)]	84.2 (80–90.2)	78.5 (73–82.2)	75 (69.7–78.2)	74.5 (68.7–76.5)	80.5 (75.9–84)	78.5 (70–82)	73 (69.7–78)	79 (77–83)
Insulin, μ U/mL [median (IQR)]	9.1 (4.2–12.8)	11.3 (7.9–15.4)	11.55 (9.7–14.6)	12.3 (8.1–16.7)	11.5 (9.7–13.5)	15.05 (11.3–17.9)	14.9 (11.4–18.2)	5.8 (4.4–6.8)
HOMA IR [median (IQR)]	1.80 (0.95–2.72)	2.12 (1.51–2.87)	2.14 (1.74–2.73)	2.41 (1.55–3.13)	2.40 (1.84–2.71)	2.94 (2.24–3.56)	2.57 (2.14–3.48)	1.10 (0.81–1.26)
Total cholesterol, mg/dl [median (IQR)]	157 (142–174)	165 (146–192)	208 (189–230)	237 (209–269)	173 (160–189)	219 (196–243)	234 (209–255)	167 (144–181)
HDL chol, mg/dl [median (IQR)]	49.6 (46.1–59.1)	56.9 (51.4–65.4)	67.6 (59.8–76.1)	64.8 (59.8–71.9)	51.7 (44.3–58.5)	62.78 (51.6–73.9)	54.8 (51.6–62.5)	48 (39.1–53)
Triglycerides, mg/dl [median (IQR)]	70 (62–91)	109 (89–137)	188 (139–231)	263 (215–316)	105 (77–133)	165 (133–203)	243 (191–316)	81 (51–117)
Follistatin ng/ml (mean \pm SD)	Fo: 9.6 (± 5.6) Lu: 14.6 (± 8.8)	63.2 (± 25.6)	121.7 (± 32.1)	151.2 (± 36.2)	70.2 (± 31.2)	131.5 (± 50.9)	116.2 (± 50.1)	19.4 (± 9.6)
Progesterone, ng/ml [median (IQR)]	Fo: 0.49 (0.33–0.66) Lu: 6.43 (3.79–13.45)	–	–	–	–	–	–	–

Variables with normal distribution are shown as mean \pm standard deviation (SD). Variables with non-normal distribution are shown as median and interquartile range (IQR). Phases in eumenorrheic women: follicular (Fo) and luteal (Lu). Periods of pregnancy: early (EP), middle (MP) and late (LP). HOMA stands for homeostasis model assessment. A $P < 0.05$ is statistically significant.

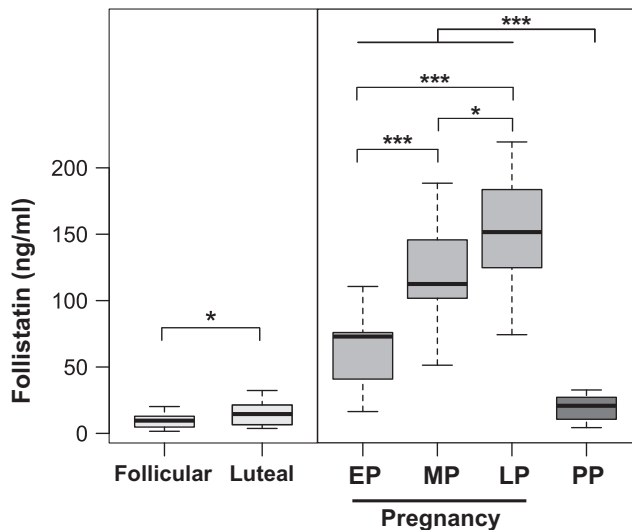


Fig. 1 Box and whisker plot with follistatin serum levels in a group of pregnant women during the three periods of pregnancy (EP, early pregnant; MD, middle pregnant and LP, late pregnant), 3 months postpartum and a control group of eumenorrheic women during both the follicular and luteal phase. A significant progressive increase in serum follistatin was observed throughout pregnancy when compared with eumenorrheic and 3 months postpartum group. * $P < 0.05$, *** $P < 0.001$.

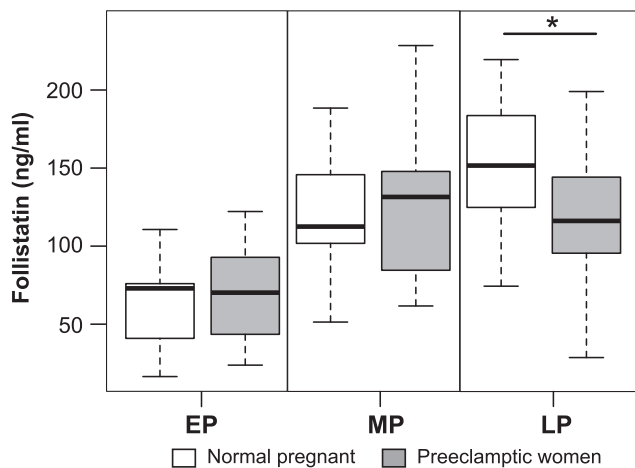


Fig. 2 Box and whisker plot with follistatin serum levels in a cohort of healthy pregnant women and preeclamptic women throughout gestation (EP, early pregnant; MD, middle pregnant and LP, late pregnant). A significant decrease of serum follistatin levels was only observed in preeclamptic pregnant women compared with normal pregnant women at the late gestational period * $P < 0.05$.

the menstrual cycle, being significantly higher at the luteal phase compared to the follicular phase.

Previous studies demonstrated that the expression of Act A is significantly increased during the secretory phase of the menstrual cycle in human endometrium, while FST levels are not altered.²⁰ The present study shows that serum levels of FST substantially vary during the menstrual cycle in eumenorrheic

women and that these levels are clearly lower than the serum FST levels measured in pregnant women in each of the three periods analysed. It is possible that progesterone levels could contribute to the regulation of serum levels of FST along the menstrual cycle, similar to the possible regulatory role that it exerts on the levels of Act A.

In a cross-sectional study, Schneider-Kolsky *et al.*¹⁰ demonstrated that Act A levels markedly rose towards the end of gestation and FST levels also increased, despite not reaching significance. In the present study, it is worth noting that FST levels are significantly elevated in each of the studied periods, when these are compared to the serum of eumenorrheic women. In addition, circulating FST levels are progressively and significantly elevated during each period of gestation. Thus, our results suggest that it is possible that FST plays a relevant immunomodulatory role and could participate in the control of placental cell death by modulating Act A activity during pregnancy, whereas in diseases such as preeclampsia, this anti-apoptotic regulatory role could be significantly reduced by the decrease in circulating levels of this factor at the end of gestation.

It has been shown that FST binds with high affinity to Act A, thereby inhibiting its biological activity.²¹ FST antagonizes the biological activity of Act A, inhibiting the interaction of this ligand with the serine/threonine kinase receptors type II (ActRII) and type I (ActRI), which signal through Smad 2 and 3 molecules.²¹ Act A is part of the superfamily of TGF- β cytokines, and its serum levels are elevated in different pro-inflammatory conditions.²² Also, Act A plays a role in the regulation of the levels of different inflammatory mediators such as tumour necrosis factor- α (TNF- α), interleukin-1 (IL-1) and the production of nitric oxide.²³ Studies in the mouse model of endotoxemia showed that blocking of the biological activity of Act A by administering FST caused a marked reduction of inflammation and mortality in this animal-based study.²⁴ Furthermore, it has been shown that plasma levels of Act A rise as pregnancy progresses, being these circulating levels significantly higher at the end of the gestational period.²⁴ Additionally, it has been shown that high doses of Act A increase apoptosis in human trophoblast cells.^{24–26} Moreover, various studies have shown an elevation of placental expression and plasma levels of Act A in preeclamptic women as compared to those of normotensive women.^{24,27,28} The elevation of placental expression and plasma concentrations of Act A correlates with increased apoptosis in placental cells of preeclamptic women.^{24–26}

It has been reported that expression of Act A is mediated by different pro-inflammatory signals, such as TNF- α , several TLR ligands and IL-1 among other signals.²⁹ Furthermore, *in vitro* studies on monocytes/macrophages have shown that Act A, at low concentrations, induces the degradation of inhibitor of kappa B (I κ B) and therefore the translocation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) to the nucleus, thereby stimulating the activation of pro-inflammatory mediators such as nitric oxide, prostanoids, IL-6, IL1 β and TNF- α .²⁹ By contrast, in activated macrophages, Act A plays an inhibitory role in the pro-inflammatory mediators.²⁹ FST is an activin-binding protein and hence a regulator of the biological

activity of this molecule. Thus, elevation of serum FST observed in the present study in each of the periods analysed, could contribute to the modulation of pro-inflammatory activity of Act A during pregnancy.

During placental development and ageing, programmed trophoblast cell death is a normal process. However, in conditions such as preeclampsia and intrauterine growth retardation, trophoblast apoptosis is significantly elevated.^{25,26,30} Moreover, in these conditions, the levels of pro-apoptotic factors, such as TNF- α , are elevated significantly in the maternal-foetal interface.^{31,32}

Furthermore, in a cross-sectional cohort study, Keelan *et al.*³³ showed that serum levels of FST are increased in preeclamptic women compared to normotensive pregnant women. In contrast, Casagrandi *et al.*³⁴ demonstrated that placental FST expression does not differ between preeclamptic women and normotensive pregnant women. The present longitudinal study demonstrates, for the first time, that serum FST significantly declines in preeclamptic women compared with normotensive pregnant women. It has been demonstrated that cell death is significantly elevated in different placental cell populations of preeclamptic women when compared to normal placentas of pregnant women.³⁵ Thus, it is possible that the decrease in circulating levels of FST during late pregnancy in preeclamptic women, allows apoptosis to be greater in the different cell populations of placenta, mediated by increased levels of Act A under this pathological condition.

Recent studies have shown that the Act A–FST system plays an important role in the control of lipid and glucose metabolism during pregnancy, with consequences on foetal growth.³⁶ In a study from Näf *et al.*³⁶, serum Act A and FST levels were determined in normal pregnant women and in pregnant women with diabetes mellitus (GDM) during the initial phase of the third gestational period. It was found that serum FST levels in patients with GDM are significantly lower than those of normal pregnant women, while circulating levels of Act A do not differ between groups. Additionally, this study also showed that FST serum levels are negatively correlated with the HOMA index. In the present study, a correlation was observed between FST levels and cholesterol levels in the first period of gestation (Table S3). Thus, our results contribute to the determination of the mechanisms of modulation of the Act A–FST system, which could participate in the control of the mother and foetus metabolism.

Previous studies have shown that human placental trophoblasts are a source of Act A and FST.³² The immunoreactivity of these proteins was mainly detected on the cytotrophoblast and syncytiotrophoblast cells.¹⁵ Furthermore, in the same Act A and FST expression studies in human placenta, it was shown that these factors rise towards the end of gestation. In keeping with the results of this study, where serum FST levels rise during the three sequential periods of gestation, it could be proposed that expression of FST in human placenta could be contributing, at least in part, to the serum profile of this factor, and therefore, to control the pro-apoptotic activity and metabolism of Act A.

Conclusion

In conclusion, the present study demonstrates for the first time in a longitudinal cohort study that (i) FST serum levels vary according to the menstrual cycle, being significantly higher in the luteal phase as compared to the follicular phase; (ii) circulating levels of FST are significantly elevated during different periods of gestation when compared with eumenorrheic women; (iii) serum FST levels are significantly lower towards the end of gestation in preeclamptic patients when compared with circulating levels of FST normotensive pregnant women; (iv) it is possible that varying levels of FST during pregnancy is partly due to placental contribution. Finally, it is possible that elevated FST during pregnancy contributes to the control of the Act A–FST system.

Acknowledgements

This work has been supported by grants from the Dirección Nacional de Investigaciones (DIB), the Vicerrectoría de Investigaciones of the Universidad Nacional de Colombia, COLCIENCIAS (Cod. 110154531660), Ministerio de Economía y Competitividad (CD: BFU2011–29102; RN: BFU2012–35255), Xunta de Galicia (RN: EM 2012/039 and 2012-CP069) and Centro de Investigación Biomédica en Red (CIBER) de Fisiopatología de la Obesidad y Nutrición (CIBERObn) – Spain. BIO-0139, CTS-5051 BFU2013-43282-R CIBERObn. The research leading to these results has also received funding from the European Community's Seventh Framework Programme under the following grants: CD: n° 245009: NeuroFAST and RN: ERC-2011-StG-OBESITY53–281408.

Disclosure

The authors declare that there is no conflict of interest associated with this manuscript.

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