

Research Article

Activin A and Follistatin as Biomarkers for Ectopic Pregnancy and Missed Abortion

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Activin A as a predictor of pregnancy failure has been the focus of heated debate, but the value of a combined activin A and follistatin (FS) measurement in serum to predict pregnancy failure has not been reported yet. We assessed whether a single serum measurement of the two physiological antagonists at 6–8 weeks gestation could differentiate ectopic pregnancies (EP) or missed abortions (MA) from healthy intrauterine pregnancies (IUP). activin A concentrations were significantly lower in women with EP ($n = 30$, median value of 264 pg/mL) and women with MA ($n = 30$, median value of 350 pg/mL) compared to IUP ($n = 33$, median value of 788 pg/mL); $P < 0.001$. At a threshold value of 505 pg/mL, activin A had 87.9% sensitivity and 100% specificity and negative predictive value of 0.974 for discriminating an ectopic pregnancy from viable pregnancies. FS was able to discriminate IUP from EP (ROC curve $P < 0.001$) as was their ratio (ROC curve $P = 0.008$), but was unable to discriminate a MA from an EP. In EP, activin A did not correlate with beta HCG levels. The present findings support the thesis that activin A or FS could be considered promising biomarkers for the discrimination between an IUP and a failed pregnancy (MA or EP).

1. Introduction

The timely recognition of the outcome in pregnant women presenting with vaginal bleeding is of paramount importance for their clinical management but is presently based on costly, lengthy followup which includes serial beta HCG measurements, ultrasound scanning, and at times diagnostic laparoscopy [1]. In search of markers which can predict the outcome of a pregnancy, several serum factors have been extensively studied [2–4]. None of these investigations have systematically analyzed the role of follistatin (FS) as a credible biomarker of ectopic pregnancy (EP).

FS is a regulatory protein which exerts its pleiotropic effects via neutralization of activins. The coordinated synthesis of FS with activin is the main regulator of the local

bioactivity of activin, as binding of activin to FS is almost irreversible [5, 6]. Amongst activins, the role of serum activin A as a predictor of pregnancy failure has been the focus of heated debate amongst researchers suggesting that measurements of activin A can identify pregnant women at risk of developing missed abortion (MA) or EP, while others have failed to report such an association [2, 7–14].

We considered that the study of serum FS and activin A and their relation with beta HCG levels in women with EP and MA is of interest, due to findings in support of activin A as a prognostic indicator of failed pregnancy, and provides indirect evidence that FS may also have a similar role by the virtue of their close interlink [15, 16]. Both factors are involved in the complex mechanisms allowing the establishment and the maintenance of pregnancy. Their serum concentrations

rise throughout viable pregnancy [5, 6] and decline in a state of nonviable trophoblasts [17–20]. Furthermore, their serum concentrations are significantly lower in serial measurements in women who subsequently miscarried when compared with live births [11, 19–21].

At the tissue level, activin A and FS are expressed in the human oviduct during the different phases of the menstrual cycle, during early pregnancy, and in fallopian tubes bearing an EP [22, 23]. The reported upregulation of the proteins in EP fallopian tubes has been accompanied by a downregulation of the mRNA of these molecules [22, 23], but the serum levels of FS have not yet been studied. These data support the notion that increasing maternal serum activin A and FS levels are associated with healthy pregnancies, and they could be altered in a status of failed pregnancy (MA or EP).

At 6–8 weeks of pregnancy, the clinical differential diagnosis with ultrasound is notoriously difficult due to uncertain dates of last menstrual period or irregular cycles. Within the same period, the magnitude of the stimulatory effect of activin A is greater [24], further indicating that a valuable sampling for related biomarker measurement would be within this interval. In the same study, placental chorionic villous explants were cultured *in vitro*, and activin A stimulated the outgrowth of cytotrophoblasts into the surrounding matrix, but FS reversed that effect. These investigators have also found that when beta HCG secretion decreased activin A, FS secretion was not significantly affected [24].

This has prompted us to measure at 6–8 weeks of gestation activin A, FS, and their ratio and to compare them with serum beta HCG, in order to assess whether they can differentiate EP or MA from healthy intrauterine pregnancies (IUP).

2. Materials and Methods

2.1. Subjects. We performed a case control study consisting of 60 patients with failed early pregnancy presenting with mild abdominal pain or vaginal bleeding between 6 and 8 weeks of gestation, who were admitted to our tertiary centre between January 2009 and December 2010. Among the 60 cases included, 30 women had a ruptured EP, while 30 had MA. Serum samples were collected at the initial visit before treatment. If the clinician was unable to make a diagnosis on this first visit even after a vaginal ultrasound, the patient was admitted and followed up until a diagnosis of a viable intrauterine pregnancy or MA or EP was confirmed. All failed pregnancy diagnoses were histologically confirmed. Serum beta HCG, activin A, and FS were measured in all 60 patients and in a group of 33 women with IUP between 6 and 8 weeks of gestation that served as a control group. EP, MA, and IUP women did not differ in terms of ethnicity (all Caucasian), maternal age (IUP: median of 27 years (range of 18–39); MA: median of 35 years (range of 21–45); EP median of 32 years (range of 26–44)), BMI (IUP: median of 24 (range of 19.9–31.2); MA: median of 25.6 (range of 20.7–35); EP: median of 26.4 (range of 21–34.5)), and smoking history.

The experimental testing complied with the principles laid down in the Declaration of Helsinki. All participating individuals gave informed consent to the work. The project

was approved by the Larissa University Hospital Research Ethics Committee.

2.2. Beta HCG Measurement. Serum concentrations of beta HCG (HCG+ β) were measured by an electrochemiluminescence immunoassay (ECLIA) intended for use on the automated analyzer Modular Analytics E170 (Roche Diagnostics GmbH, Mannheim, Germany). The results were expressed as mIU/mL, and the lower limit of detection was <0.1 mIU/mL.

2.3. ELISA Measurements. Serum samples were collected at the initial visit before treatment. All samples were processed by centrifuge (1,000 g for 15 minutes), and the supernatants were stored at -80°C until assayed. Serum concentrations of human activin A and FS were determined by quantitative sandwich ELISA (R&D Systems, Minneapolis, MN) according to the instructions of the manufacturer, as follows.

2.3.1. Activin A. 200 μL /well of a monoclonal antibody against human activin A conjugated to biotin was added to 96-well polystyrene microplates precoated with streptavidin. After 15 min of incubation at 20°C on a horizontal orbital microplate shaker, the plates were washed twice, and 100 μL /well assay diluents topped up with 100 μL /well individual serum samples or activin A standards were pipetted into the wells in duplicate. The 7 standards corresponded to 1000, 500, 250, 125, 62.5, 31.2 or 15.6 pg/mL activin A and were prepared from a stock solution of 1 mL of 10,000 pg/mL activin A standard reconstituted in deionized water. A 3-hour incubation was carried out at room temperature on a horizontal orbital microplate shaker (Dynex Technologies, West Sussex, UK) set at 500 rpm. After 6 washes, 200 wells of monoclonal antibody against activin A conjugated to horseradish peroxidase with preservatives were added to each well and incubated for 1 h at 20°C . Following washing as before to remove any unbound conjugate, a reaction with hydrogen peroxide/tetramethylbenzidine as substrate was allowed for 30 min in room temperature in the dark. The colour development reaction was stopped using 2 N sulfuric acid, and absorbance values (optical density) were determined in a microplate reader (Dynex Technologies) at 450 nm, with the correction wavelength set at 570 nm. The concentration of human activin A was calculated with the Magellan reader control and data analysis software. All tests were done in duplicate, and the average of the duplicate readings was used for the analysis.

2.3.2. Follistatin. Serum concentrations of human FS were measured using a 96-well polystyrene microplate precoated with a mouse monoclonal antibody against FS (R&D Systems). Eight standards corresponding to 16,000, 8,000, 4,000, 2,000, 1000, 500, 250, and 125 pg/mL were prepared from a stock solution equivalent to 160,000 pg/mL FS A. The assay procedure was similar to that of human activin A with some modifications, as the first incubation (3 h) of serum samples, standard, and control, as well as the second incubation (2 h) with the HRP-conjugated anti-human FS specific antibody, had to be carried out at $2-8^{\circ}\text{C}$. In between incubation,

TABLE 1: Activin A and follistatin serum levels (pg/mL) in healthy intrauterine pregnancies (IUP), missed abortions (MA), and ectopic pregnancies (EP) presented as median, range, and interquartile values.

(pg/mL)	Activin A			Follistatin		
	IUP (<i>n</i> = 33)	MA (<i>n</i> = 30)	EP (<i>n</i> = 30)	IUP (<i>n</i> = 33)	MA (<i>n</i> = 30)	EP (<i>n</i> = 30)
Mean (\pm SD)	843 \pm 338	442 \pm 248	277 \pm 94	5011 \pm 1786	3510 \pm 2742	3189 \pm 3130
Median (IQR)	788 (616–1001)	350 (264–562)	265 (207–309)	4794 (3586–6159)	3241 (2207–4190)	2606 (1626–3264)

TABLE 2: Activin A and follistatin (FS) levels and activin A/FS ratio in normal (*n* = 33) versus failed pregnancies (*n* = 60).

	Pregnancy outcome			<i>P</i> value
	Normal (<i>n</i> = 33)	Failed pregnancies (<i>n</i> = 60)		
	Mean (SD)	Mean (SD)	Mean difference (95% CI)	
Activin A	843 (338)	359 (204)	484 (375–592)	<0.001
Follistatin	5011 (1786)	3350 (2922)	1661 (546–2777)	0.004
Activin A/follistatin ratio	0.2 (0.17)	0.149 (0.1)	0.054 (0.000–0.11)	0.050
	Abortion (MA) (<i>n</i> = 30)		Ectopic (EP) (<i>n</i> = 30)	<i>P</i> value
	Mean (SD)	Mean (SD)	Mean difference (95% CI)	
Activin A	442 (248)	277 (94)	164.61 (35–294)	0.013
Follistatin	3510 (2742)	3189 (3130)	321 (–1008, 1650)	0.632
Activin A/follistatin ratio	0.167 (0.114)	0.131 (0.080)	0.036 (–0.029, 0.101)	0.276

We evaluated whether activin A and follistatin serum markers levels (pg/mL) and their ratio differ between healthy pregnancies (IUP) and failed pregnancies (MA + EP) and MA and EP. Analysis of variance was conducted in order to perform orthogonal contrasts comparing IUP to MA and EP, as well as MA to EP by using Helmert contrasts. *P* values indicate the clinically important comparison of MA to IUP. Comparison between EP and MA produced statistically significant differences in the case of activin A (*P* = 0.013).

microplates were washed for a total of 4 washes in accordance to the manufacturer's instructions. The subsequent steps of colour development, stoppage of the reaction, and measurements and analysis of the data were carried out as in the case for the human activin A by ELISA.

The intra-assay coefficient of variation was 2.1% for a sample with 125 pg/mL and 3.5% for a sample with 1,123 pg/mL of human activin A and FS, respectively. The interassay coefficient of variation was 3.8% for a sample with 189 pg/mL of human activin A and 3.1% for a sample with 1,211 pg/mL human Follistatin.

2.4. Statistical Analysis. Normally distributed variables are presented as mean \pm standard deviation, and skewed distributed variables are presented as median and interquartile range (IQR).

Analysis of variance was conducted in order to perform orthogonal contrasts (Helmert contrasts) comparing IUP to MA and EP, as well as MA to EP regarding activin A, FS, and their ratio. The optimal cut-off points for sensitivity and specificity were calculated by Receiver Operating Characteristics (ROC) curve analyses. According to the design of this study, the area under the curve (AUC) depicts the probability that the single value of activin A, FS, or their ratio of a randomly selected patient with a normal pregnancy illustrated in Figure 2 (see the following) will exceed that of a single value of a randomly selected patient with an abnormal pregnancy (EP or MA).

Sensitivity and Specificity, with their corresponding 95% CI, and Positive predictive values (PPV) and negative predictive values (NPV) were calculated and are shown in Table 3.

The nonparametric Kruskal-Wallis test was used to identify differences on beta HCG among EP, MA, and viable IUP.

To perform pairwise comparisons between groups, Mann-Whitney test was conducted determining as critical value for significance *P* = 0.0167 after using Bonferroni correction.

Basic demographic characteristics such as age and BMI were compared using Kruskal-Wallis.

Spearman's rank correlation coefficient (ρ) was used to explore the relationship between beta HCG and the other measures. All statistical analyses were performed in SPSS 15 statistical software (Chicago, IL, USA). A *P* value less than 0.05 was considered statistically significant.

3. Results

A summary of the results of serum activin A and FS is given in Tables 1–3 and Figures 1 and 2.

3.1. Activin A. Activin A concentrations were significantly lower in women with EP (*n* = 30, mean 277 \pm 94, median 265 pg/mL) and women with MA (*n* = 30, mean of 442 \pm 248, and median of 350 pg/mL) compared to patients with IUP (*n* = 33, mean of 843 \pm 338, and median of 788 pg/mL), *P* < 0.001 in both cases (Table 1). In accordance, activin A levels were significantly higher in viable IUP compared to combined EP and MA pregnancy failures (*P* < 0.001). In contrast to FS, activin A had the ability to discriminate an EP from MA (*P* = 0.013) (Table 2). The corresponding ROC analyses were calculated and plotted for the diagnostic accuracy of serum activin A concentration to discriminate between the groups (AUCs in Table 3).

3.2. Follistatin. The concentration of FS was significantly lower in EP (mean of 3189 \pm 3130, median of 2606 pg/mL) and MA (mean of 3510 \pm 2742, median of 3241 pg/mL)

TABLE 3: Sensitivity and specificity of activin A, follistatin (FS), and their activin A/FS ratio as serum markers of missed abortions (MA) and ectopic pregnancies (EP) versus those of intrauterine pregnancies (IUP).

		Sens. (95% CI)	Spec. (95% CI)	Cut-off point	AUC	P value	PPV	NPV
IUP versus MA + EP	Activin A	0.879 (0.718–0.966)	0.850 (0.734–0.929)	504.66	0.912	<0.001	0.527	0.974
	Follistat.	0.697 (0.513–0.844)	0.850 (0.734–0.929)	4254.00	0.808	<0.001	0.470	0.936
	Activin A/FS	0.727 (0.545–0.867)	0.617 (0.482–0.739)	0.136	0.642	0.024	0.266	0.922
IUP versus MA	Activin A	0.939 (0.798–0.993)	0.700 (0.506–0.853)	402.87	0.845	<0.001	0.356	0.985
	Follistat.	0.636 (0.451–0.796)	0.867 (0.693–0.962)	4412.7	0.785	<0.001	0.458	0.931
	Activin A/FS	0.818 (0.645–0.930)	0.467 (0.283–0.657)	0.107	0.588	0.231	0.213	0.936
IUP versus EP	Activin A	0.879 (0.718–0.966)	1.000 (0.884–1.000)	504.66	0.979	<0.001	1.000	0.999
	Follistat.	0.727 (0.545–0.867)	0.900 (0.735–0.979)	3747.2	0.830	<0.001	0.068	0.997
	Activin A/FS	0.727 (0.545–0.867)	0.733 (0.541–0.877)	0.136	0.696	0.008	0.027	0.996
MA versus EP	Activin A	0.633 (0.439–0.801)	0.833 (0.653–0.944)	325.21	0.734	0.002	0.037	0.996
	Follistat.	0.600 (0.406–0.773)	0.700 (0.506–0.853)	2848.5	0.621	0.107	0.020	0.994
	Activin A/FS	0.500 (0.313–0.687)	0.733 (0.541–0.877)	0.142	0.570	0.352	0.019	0.993

The optimal cut-off points were calculated by Receiver Operating Characteristics (ROC) curve analyses for IUP, MA, and EP. Details of sensitivity, specificity, area under the curve (AUC), negative predictive value (NPV), and positive predictive value (PPV) of serum markers. Values are PPV and NPV for EP for serum markers and their ratio among IUP and failed pregnancies including missed abortions MA and EP.

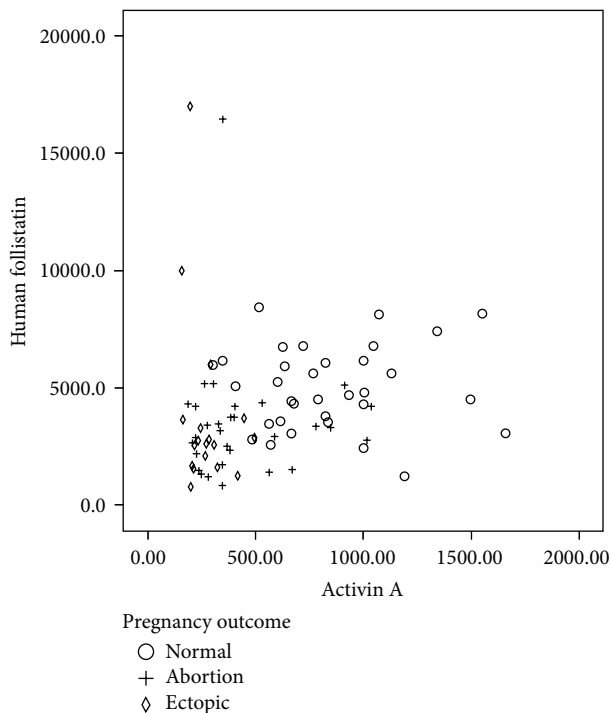


FIGURE 1: Scatter plot diagram for the distribution of (a) activin A, (b) follistatin, and (c) activin A/follistatin ratio values according to the pregnancy outcome (viable intrauterine pregnancy (IUP), missed abortion (MA), and ectopic pregnancy (EP)).

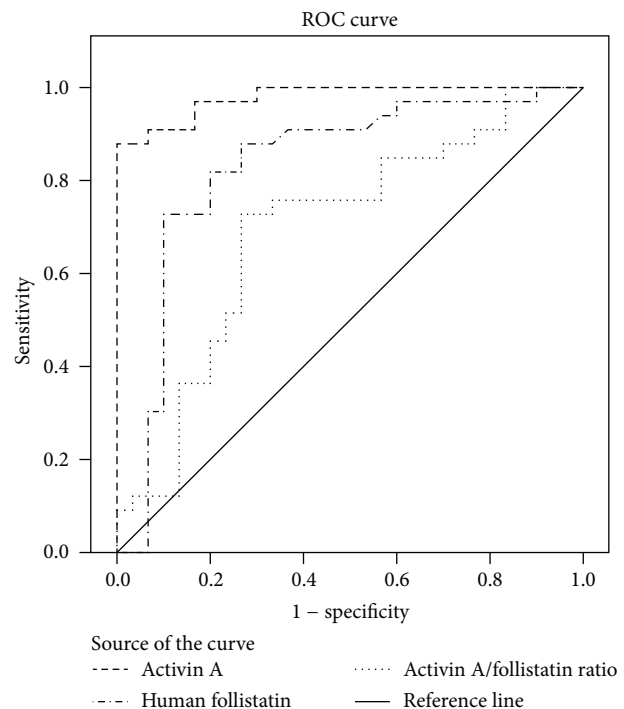


FIGURE 2: Receiver Operating Characteristics (ROC) curve analyses for the diagnostic accuracy of activin A, follistatin, and activin A/follistatin ratio values to discriminate a viable intrauterine pregnancy from an ectopic pregnancy. *Normal versus ectopic.

compared to women with a viable IUP (mean of 5011 ± 1786 , median of 4794 pg/mL) ($P < 0.001$) (Tables 1 and 2). Likewise, it was significantly higher in viable IUP compared to pregnancy failures (EP and MA) ($P < 0.001$). FS was able to discriminate IUP from EP ($P < 0.001$), but not MA from EP ($P = 0.696$) (Table 2). ROC analyses were calculated and plotted for the diagnostic accuracy of serum FS concentration to discriminate between the groups (AUCs in Table 3).

3.3. *Activin A/Follistatin Ratio.* Activin A/FS ratio was significantly lower in pregnancy failures (mean of 0.149 ± 0.099) compared to women with a viable IUP (mean of 0.203 ± 0.166) ($P = 0.05$) (Tables 1 and 2). Activin A/FS ratio was able to discriminate IUP from EP ($P < 0.001$). However, it could not discriminate ($P = 0.352$) a MA from an EP (Table 3). ROC analyses were calculated and plotted for the diagnostic

accuracy of serum FS concentration to discriminate between the groups (AUCs in Table 3).

3.4. Diagnostic Accuracy of Activin A, Follistatin, and Activin A/Follistatin Ratio. Both serum markers and their ratio were plotted in ROC curves in order to further evaluate their diagnostic accuracy for the diagnosis of healthy IUP and discriminating an ectopic pregnancy from a missed abortion. All AUCs are shown in Table 2. Activin A showed higher diagnostic accuracy compared to FS or activin A/FS ratio for the discrimination of a viable IUP from pregnancy failure (MA and EP) with areas under the curve (AUCs) of 0.912, 0.808, and 0.642, respectively. (Table 2 and Figure 1).

At the threshold of 505 pg/mL, activin A had a sensitivity of 87.9% and a specificity of 85%, PPV of 0.527 and NPV of 0.974 for discriminating a normal from an abnormal pregnancy. Similarly, FS at the threshold value of 4254 pg/mL could discriminate a normal from an abnormal pregnancy with a sensitivity of 69.7% and a specificity of 85% and a PPV of 0.470 and a NPV of 0.936.

Activin A and FS had a high diagnostic accuracy for discriminating not only a normal pregnancy from a missed abortion but also a normal pregnancy from an ectopic pregnancy as well, with AUCs of 0.845, 0.979, 0.785, and 0.830, respectively (Table 3).

For the clinically important discrimination between MA and EP, both activin A and FS showed decreasing levels, but activin A levels significantly differed statistically ($P = 0.013$). Thus, activin A showed a sensitivity of 63.3% and a specificity of 83.3% for diagnosing EP pregnancy from a missed abortion at the threshold value of 325 pg/mL (Table 3).

3.5. Relationship to Beta HCG. IUPs had a median concentration of 59,668 mIU/mL (40,156–87,906 mIU/mL), while MAs had a median of 3000 mIU/mL (1447–5500 mIU/mL) and EPs had a median of 1828 mIU/mL with an IQR of 1147–2790 mIU/mL (Kruskal-Wallis test, $P < 0.001$).

In order to further explore pairwise comparisons between groups, we conducted Mann-Whitney test using the Bonferroni correction. It was identified that IUPs have significant higher values of beta HCG compared to MAs and then to EPs, ($P < 0.001$). Between MAs and EPs, there was no statistically significant difference ($P = 0.115$).

Spearman's rank correlation coefficient (ρ) between beta HCG and activin A and FS in IUPs was 0.214 ($P = 0.284$) and 0.032 ($P = 0.873$), respectively, demonstrating that there is a weak correlation. In MAs, the coefficients were 0.454 for activin A ($P = 0.023$) and 0.411 for FS ($P = 0.041$), indicating a moderate correlation which is also statistically significant. For EPs, there was weak correlation at 0.162 ($P = 0.483$) and -0.181 ($P = 0.431$), respectively.

4. Discussion

Currently, there is no stand-alone diagnostic biomarker for tubal ectopic pregnancy that has been adequately tested and yields satisfactory results. The clinical endpoints of this study were the identification of EP cases by a single serum measurement of two physiological antagonists: activin A and

FS or their ratio. The present findings support the thesis that a single measurement of activin A or FS at 6–8 weeks of gestation enables the discrimination between an IUP and a failed pregnancy (MA or EP). More importantly, our study reveals the ability of serum activin A to differentiate a MA from an EP. Original findings were obtained showing an association between EP and decreased serum FS levels, not correlated with the corresponding low beta HCG concentrations. Although this is the first time that FS has been assessed as a serum biomarker for ectopic pregnancy, there have been a number of conflicting studies investigating the use of serum activin A [2, 7–14].

In normal pregnancy, the expression of activin A is dynamic, as it is up- and downregulated during the process of decidualization [11, 25], and studies in women with nonfunctional ovaries have suggested a fetoplacental origin for activin A [26–29]. Serum levels of activin A are higher in pregnant than in nonpregnant women and increase throughout pregnancy until about 28 weeks' gestation [18, 30–32]. However, in early pregnancy, the expression of activins by the cytotrophoblast is low, which suggests that trophoblast invasion is induced by the maternally derived activins [9]. The source of maternally derived activin A in pregnancy is primarily from newly decidualized cells, and this promotes the decidualization of neighboring cells and thus facilitates the spread of decidualization throughout the endometrium [9, 25]. Normal concentrations of serum activin A in pregnancy were reported to rise 69-fold (wide spectrum of values) throughout pregnancy from 700 ± 200 pg/mL at weeks 6–7 to a peak of $45,900 \pm 54,000$ pg/mL at weeks 38–39 [31]. In vitro, human endometrial stromal cells produce activin A subunits and drive decidualization [25, 33].

This process is expected to be compromised in failed pregnancies and possibly even more in ectopic pregnancies. Activin A levels are reduced in the presence of nonviable trophoblast [17–19], and single and serial measurements have been used to predict miscarriage [8]. Furthermore, it has been suggested that lower activin A in EP compared with those other failed pregnancies may be due to the difficulty of the ectopic trophoblast to correctly implant, compromising the decidualization process, and that some EPs could have more active trophoblasts and behave more like IUPs, whilst others will be failing and behave like failing MAs [12]. If this is true, it would explain why women with EPs in recent independent studies had variable serum activin A levels, a finding which arguably led to different conclusions as far as its discriminatory value in the differential diagnosis [2, 10, 12, 13, 34–37].

Thus, it is not surprising that there are conflicting data on the use of a serum cut-off level of activin A in discriminating EP from IUP with either poor AUC of 0.60 [12] or excellent AUCs [10, 13] in the ROC; of relevance, our study displays an AUC of 0.979.

The median EP values of 264 (range of 150–490) pg/mL found in the present study is indistinguishable to that recently reported by Warrick et al. [37] and comparable to that of 370 pg/mL (mean of 270 ± 60 ng/mL) for EP in the original study by Florio et al. [10] and not significantly different from the median of 313 pg/mL in the study by Rausch et al. [2]. In

our cohorts, as far as diagnosing a viable IUP is concerned, at a threshold value of 505 pg/mL, activin A had 87.9% sensitivity and 100% specificity for discriminating a viable pregnancy from an ectopic pregnancy. Florio also noted a 100% specificity at 430 pg/mL [13].

This reported variability of serum activin A levels supports the notion of measuring another parameter to improve diagnostic accuracy. Based on previous reports that circulating activin A is commonly detected bound with FS [35, 36] and that this fact might introduce a bias in our activin A and FS measurements, we measured FS and their ratio in all the samples. The soluble FS like 3 (FSTL3) was reported to show a progressive increase from early pregnancy through the second and third trimesters to term [24, 31]. FS concentration levels are reduced in the presence of nonviable trophoblast, as happens in complete miscarriage, and furthermore were all significantly lower in serial measurements in women who subsequently miscarried when compared with live births [11, 19, 21]. This is in accordance with our results clearly showing decreased serum FS levels in failed pregnancies.

Our analysis shows that FS is able to discriminate IUP from EP (ROC curve $P < 0.001$) as was their ratio (ROC curve $P = 0.008$).

It should be emphasized that our data do not provide information as to whether lower activin A or FS are consequence of or implicated in the events leading to EP (or failed pregnancies, in general). Hence, the aim of the study was not to dissect the mechanisms by which reduction of the above markers directly relate EP and MA. To this end, our findings need to be treated with caution.

If our findings can be replicated by larger studies, routine measurements of these markers may be of importance in patient management and counseling, especially in the case of women with uncertain gestational age presenting with possible pregnancy failure and mild abdominal pain with/without vaginal bleeding and in cases where vaginal ultrasound cannot offer a definitive diagnosis. In these patients, since they are considered to have possible pregnancy failures, a serum beta HCG is measured, and if they are stable, a follow-up visit in 48 hours is scheduled, where a new serum beta HCG takes place, and if still inconclusive, reexamination with another vaginal ultrasound and new serum beta HCG (the third measurement) is repeated in a week's time. A possible clinical application could be that if at first visit the serum measurements of the above biomarkers exceed certain cutoffs (and therefore the pregnancy is considered a healthy pregnancy, instead of being managed as possible pregnancy failures) a follow-up antenatal visit in two weeks with obvious cost benefits could be scheduled.

5. Conclusion

The present findings support the thesis that activin A or FS could be considered promising biomarkers for the discrimination between an IUP and a failed pregnancy (MA or EP). To this end, our findings need to be treated with caution, due to the small sample size. It appears that a combination of markers, including activin A, are needed in order to achieve optimal sensitivity and specificity for

the various outcomes [2]. It remains to be seen whether FS has to be included in diagnostic algorithms for early pregnancy failure. We hopefully anticipate that dissemination and external validation of our findings will generate vigorous discussion and continuous investigation in search of proper prognostic markers.

Conflict of Interests

There is no conflict of interests that could be perceived as prejudicing the impartiality of the research reported.

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