ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 12, Issue 12, 2019

Online - 2455-3891
Print - 0974-2441

Research Article

THE ROLE OF ACTIVIN A AND FOLLISTATIN IN THE DIFFERENTIATION BETWEEN VIABLE INTRAUTERINE PREGNANCY FROM MISSED MISCARRIAGE AND ECTOPIC PREGNANCY

ESRAA HAMEED HUMADI AL-MAINI[1], ISRAA TAILB ABD AL-KADIR[2]*, RAWNAQ ALI AL-SAADI[2]

[1]Department of Obstetrics and Gynecology, College of Medicine, Al-Mustansiriya University, Al-Yarmouk Teaching Hospital, Baghdad, Iraq. [2]Department of Obstetrics and Gynecology, Al-Yarmouk Teaching Hospital, Baghdad, Iraq. Email: israa_talib79@yahoo.com

Received: 03 October 2019, Revised and Accepted: 25 November 2019

ABSTRACT

Objective: The objective of the study was to investigate the role of activin A and follistatin (FS) in the differentiation between viable intrauterine pregnancy from missed miscarriage and ectopic pregnancy (EP).

Study Design: This was a case–control study.

Setting: This study was conducted at the Department of Obstetrics and Gynecology at Al-Yarmouk Teaching Hospital, from February 2017 to October 2017.

Patients and Methods: The study included 90 pregnant women, aged from 21 to 40 years old in their the first trimester attending the outpatient and inpatient clinic with single fetal pregnancy with a gestational age range between 6th and 8th weeks, they were divided into three groups; Group A: Includes 30 cases with uncomplicated pregnancies in their first trimester, Group B: Includes 30 cases diagnosed as missed miscarriages, and Group C: Includes 30 cases diagnosed as EPs.

Results: There was no significant difference in the maternal age and body mass index between the three groups, β-human chorionic gonadotropin, activin A, FS, and their ratio were significantly higher in the healthy intrauterine pregnancy compared to missed miscarriage and EP. Activin A and activin A/FS ratio had an excellent ability to discriminate EP from healthy intrauterine pregnancy, while FS alone had good ability to discriminate between EP and intrauterine pregnancy. Activin A had fair ability to discriminate missed miscarriage from intra healthy uterine pregnancy, while both FS and activin A/FS ratio had poor ability to differentiate missed miscarriage and intrauterine pregnancy.

Conclusion: Activin A can be used with high accuracy as a biomarker for EP and missed miscarriage, FS alone, and activin A/FS ratio is a possible biomarker, but it offers lower accuracy compared to activin A alone.

Keywords: Activin A, Follistatin, Viable intrauterine pregnancy, Missed miscarriage, Ectopic pregnancy.

 DOI: http://dx.doi.org/10.22159/ajpcr.2019.v12i12.36259

INTRODUCTION

Implantation is a critical step in a successful pregnancy which is supported by a healthy placenta and meticulous synchronization between the developing embryo and the cycling endometrium [1]. Molecular mechanisms that regulate early implantation events in humans are not well understood. There are several growth factors, adhesion molecules, and cytokines within the uterus, and pre-implantation blastocyst may play important roles [2]. Activin A, a dimeric growth factor belonging to the transforming growth factor-beta (TGF-β) superfamily, has key role in endometrial differentiation, trophoblastic invasion, and embryo implantation [3]. Follistatin (FS) is a cysteine-rich monomeric glycoprotein that isolated in 1987 from the porcine follicular fluid. It inhibits follicle-stimulating hormone-releasing from the pituitary gland. FS is produced by the ovary, pituitary gland, placenta, decidua, and amnion, and other tissues. It is exert local and systemic effects on folliculogenesis by blocking activins or other members of the TGF-β family [4]. There is increasing evidence that serum and tissue activin levels increased in acute and chronic inflammatory conditions, such as septicemia, preeclampsia, asthma, inflammatory bowel disease, burns injuries, and rheumatoid arthritis [5]. Miscarriage is defined as the spontaneous end of a pregnancy before fetal viability. Missed miscarriage refers to in utero death of the embryo or fetus before the 24th weeks of gestation. Vaginal bleeding may occur, and the cervix is usually closed. It is considered the most common complication with approximately 15–20% of all pregnancy suffer this condition [6,7]. Depending on the time of gestational age missed miscarriage can be classified into: Biochemical loss (<6 weeks, with no fetal heart activity), early pregnancy loss (6–10 weeks, with no fetal heart activity and empty sac), and late pregnancy loss (>10 weeks, loss of fetal heart activity and crown-rump length and fetal heart activity previously identified) [6]. Ectopic pregnancy (EP) is an extrauterine pregnancy; 98% of all EPs occur in the fallopian tube [8]. With a suspected EP transvaginal ultrasonography (TVUS) should be performed at the time of presentation and may need to be repeated, depending on the human chorionic gonadotropin (HCG) level or suspicion of rupture [9]. The discriminatory zone is the serum HCG level above which a gestational sac should be visualized by TVUS if an intrauterine pregnancy (IUP) is present. It is ranging between 2000 and 3510 international units/L [10].

Aim of the study

The aim of the study was to investigate the role of activin A and FS in the differentiation between viable intrauterine pregnancy from missed miscarriage and EP.

PATIENTS AND METHODS

Study design

A case–control study conducted at the Department of Obstetrics and Gynecology of Al-Yarmouk Teaching Hospital in correlation with the laboratories department of the hospital through a period from February 2018 to October 2018. The study protocol was approved by

the scientific council of obstetrics and gynecology specialization of the Iraqi Board of Medical Specializations.

Patient collection

The study included 90 pregnant women aged from 21 to 40-years-old within the first trimester attending the outpatient and inpatient clinic with single fetal pregnancy with a gestational age range between 6th and 8th weeks depending on accurate last menstrual period and early ultrasonography. These women were informed about the nature of the study and verbal consent taken from all of them.

They divided into three groups as follows:

- Group A: Includes 30 cases with uncomplicated pregnancies in their first trimester, which considered as the control group
- Group B: Includes 30 cases diagnosed as missed miscarriages
- Group C: Includes 30 cases diagnosed as EPs.

Exclusion criteria: History of chronic medical diseases including renal, respiratory (asthma), pancreatic diseases or previous/current history of malignancies, inflammatory bowel diseases, rheumatoid arthritis, history of the current use of chemotherapy or immunotherapy for any cause, and any patient with clinical sign of septic miscarriage.

Clinical assessment

- A detailed history obtained from all patients including name, age, history of present illness, date of last menstrual period, obstetrical history, gynecological history, and medical and surgical history
- Body mass index (BMI), systemic examination, obstetrical examination
- General examination and vital sign, measurement of weight, height, and ultrasonography for aiding in the diagnosis
- The studied groups were investigated for the following:
  a. Maternal blood sample collected at the time of presentation for the whole three groups (A, B, and C) and was sent to the laboratories for the blood group and rhesus and cross-matching if needed, full blood count, random blood sugar, serum fibrinogens, and coagulation profile if indicated
  b. Another 5 ml of venous blood were collected from each woman placed in sterile tubes which was labeled with the patient's name and centrifuged for 15 min and then freezed at −4°C.

The level of activin A was measured using enzyme-linked immunosorbent assay (ELIZA) (human activin A [ACV-A] ELIZA Kit. cat: No: YHB0086Hu), this method based on biotin double antibody sandwich technology to assay human activin A, the detection limit of this assay was 50 pg/ml. The level of FS was measured using (human

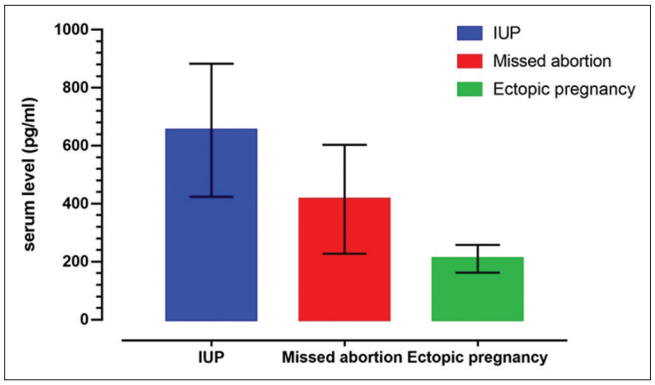

Fig. 1: Serum level of activin A in different groups (error bars are illustrated)

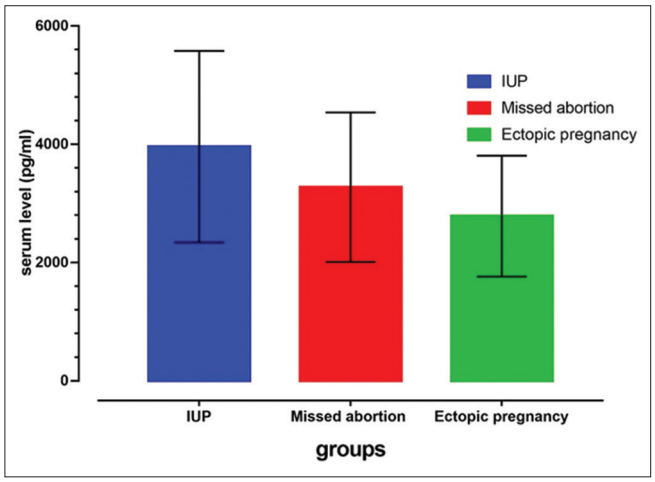

Fig. 2: Serum level of follistatin in different groups (error bars are illustrated)

Table 1: Demographic data of the subject in the study

Variables number	IUP (30)	Missed miscarriage (30)	Ectopic pregnancy (30)	p-value
Maternal age (years)	30.4±7.1	29.2±6.3	26.6±6.2	0.075
BMI (kg/m^2)	29.3±4.7	26.9±5.5	27.6±5.4	0.208

Data presented as mean±standard deviation, IUP: Intrauterine pregnancy, BMI: Body mass index

Table 2: Comparison of investigated biomarker between normal pregnancy and failed pregnancy

Variables number	Normal (30)	Failed pregnancy (60)	p-value
Activin A pg/ml	653.3±229.9	312.8±170.9	<0.001
Follistatin pg/ml	3,959.0±1,620.5	3,029.3±1,166.7	0.008
Activin A/follistatin ratio	0.199±0.119	0.125±0.100	0.003
β–HCG iu/ml	64,101.9±23,122.4	2,660.6±1,440.4	<0.001

Data presented as mean±standard deviation, HCG: Human chorionic gonadotropin

Table 3: Comparison of investigated biomarkers between missed miscarriage and ectopic pregnancy

Variables number	Missed miscarriage 30	Ectopic pregnancy 30	p-value
Activin A pg/ml	415.4±188.0	210.1±47.8	<0.001
Follistatin pg/ml	3274.2±1265.1	2784.4±1022.2	0.104
Activin A/follistatin ratio	0.158±0.122	0.091±0.058	0.010
β-HCG iu/ml	3407.8±1558.7	1913.4±797.8	<0.001

Data presented as mean±standard deviation, HCG: Human chorionic gonadotropin

FS ELIZA Kit Cat.No: YHB1232Hu) the detection limit of this assay was 20 pg/ml. HCG measured by an immune-radiometric assay; the detection limit of this assay was 0.03 mIU/ml.

Statistical analysis

All continuous data presented as mean and standard deviation since they follow a normal distribution, while categorical variables presented as number and percentage. t-test to compare between two independent continuous groups, Chi-square test to compare between categorical groups, while linear regression analysis used to find the relationship between two continuous variables.

Receiver operator curve used to see the validity of different parameters in separating cases with torsion from none torsion and area under the curve (AUC), i.e., AUC and its p-value prescribe this validity (if AUC ≥0.9 mean excellent test, 0.8–0.89 means good test, 0.7–0.79 fair test otherwise unacceptable). The trapezoidal method used to calculate the curve. SPSS 20.0.0, Minitab 17.1.0, MedCalc 14.8.1, GraphPad Prism 7.0 software package used to make the statistical analysis, p-value considered when appropriate to be significant if <0.05.

RESULTS

Mean maternal age for IUP women was 30.4±7.1 years; for a missed miscarriage, it was 29.2±6.3 years, and for EP, it was 26.6±6.2 years, and there were no significant differences among the groups. Mean BMI 29.3±4.7 kg/m^2 for IUP group and for missed miscarriage, it was 26.9±5.5 kg/m^2 for a missed miscarriage and for EP it was 27.6±5.4 kg/m^2 and no significant difference was found among the groups, as illustrated in Table 1.

Serum level of activin A, FS, and activin A/FS was significantly higher in normal pregnancy compared to failed pregnancy, as illustrated in Table 2.

Activin A and activin A/FS ratio were significantly higher in missed miscarriage compared to EP, while in FS levels were not significant

Table 4: Receiver operating characteristics analysis of investigated markers for missed miscarriage (MA) and EP

Variables	AUC	Validity	p-value
IUP versus (MA+EP)			
Activin A	0.886	Good	<0.001
Follistatin	0.686	Poor	0.007
Activin A/FS ratio	0.762	Fair	<0.001
IUP versus MA			
Activin A	0.771	Fair	<0.001
Follistatin	0.643	Poor	0.053
Activin A/FS ratio	0.663	Poor	0.025
IUP versus EP			
Activin A	0.999	Excellent	<0.001
Follistatin	0.853	Good	<0.001
Activin A/FS ratio	0.937	Excellent	<0.001
MA versus EP			
Activin A	0.847	Good	<0.001
Follistatin	0.596	Poor	0.200
Activin A/FS ratio	0.697	Poor	0.005

EP: Ectopic pregnancy, IUP: Intrauterine pregnancy, MA: Missed abortion

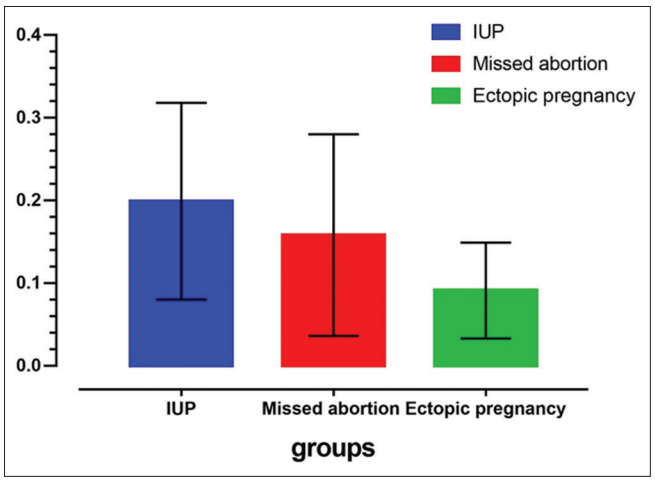

Fig. 3: Activin A/follistatin ratio in different groups (error bars are illustrated)

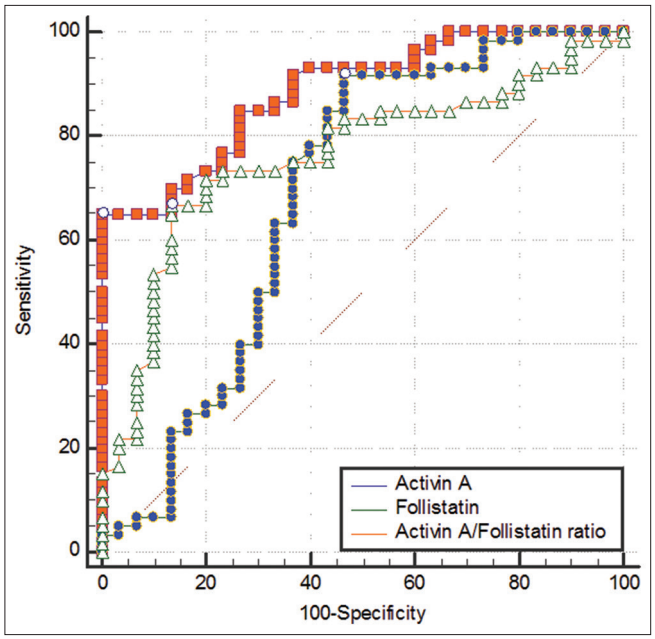

Fig. 4: Receiver operating characteristics curve analyses for the diagnostic accuracy of activin A, follistatin, and activin A/follistatin ratio values to discriminate a viable intrauterine pregnancy from failed pregnancy (ectopic pregnancy+missed abortion)

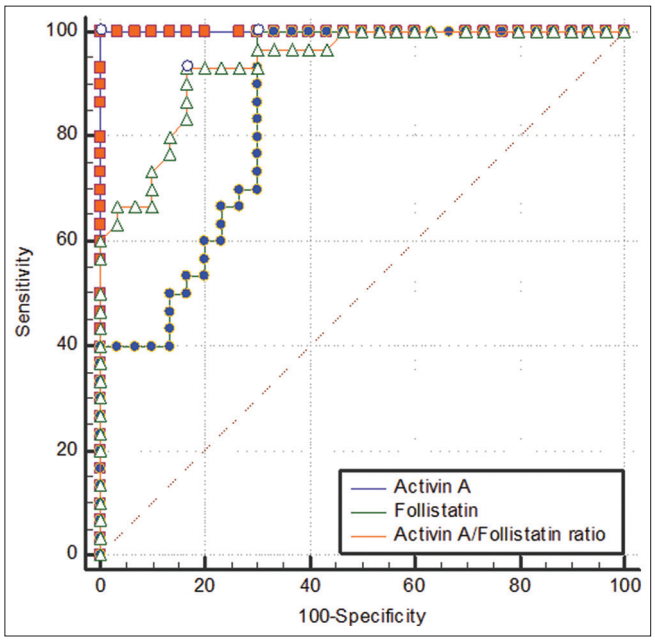

Fig. 5: Receiver operating characteristics curve analyses for the diagnostic accuracy of activin A, follistatin, and activin A/follistatin ratio values to discriminate a viable intrauterine pregnancy from ectopic pregnancy

difference between a missed miscarriage and EP, as illustrated in Table 3 and Figs. 1-3.

Activin A had good ability to discriminate normal pregnancy from failed pregnancy and to discriminate missed miscarriage from an EP, while it has excellent ability to discriminate normal pregnancy from an EP and fair ability to discriminate normal pregnancy from the missed miscarriage, as illustrated in Tables 4 and 5 and Figs. 4-7.

FS had a good ability to discriminate normal pregnancy from an EP, but it had poor ability to discriminate normal pregnancy from failed pregnancy and normal pregnancy from missed miscarriage and to discriminate missed miscarriage from EP, as illustrated in Tables 4 and 5 and Figs. 4-7.

Activin A/FS ratio had excellent ability to discriminate normal pregnancy from an EP; it had fair ability to discriminate normal pregnancy from failed pregnancy, it had poor ability to discriminate normal pregnancy from missed miscarriage and to discriminate missed miscarriage from EP, as illustrated in Tables 4 and 5 and Figs. 4-7.

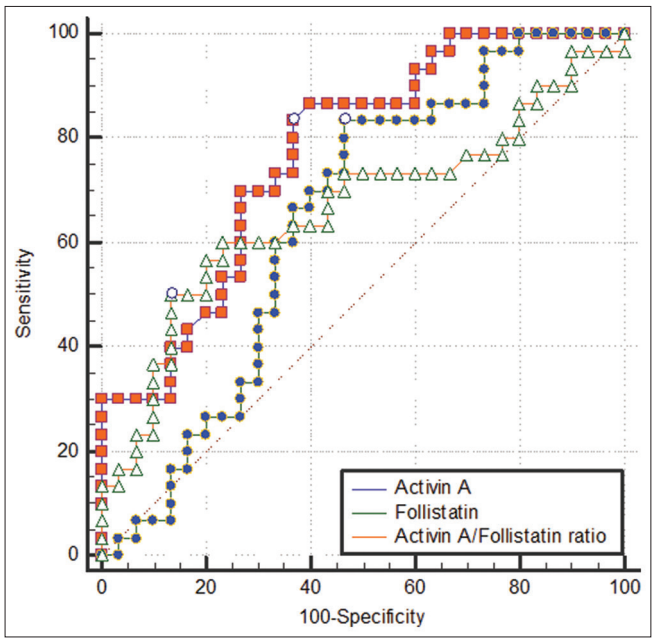

Fig. 6: Receiver operating characteristics curve analyses for the diagnostic accuracy of activin A, follistatin, and activin A/follistatin ratio values to discriminate a viable intrauterine pregnancy from a missed miscarriage

DISCUSSION

In the current study, the clinical usefulness of single serum activin A, FS, their ratio, and β-HCG for the discrimination of EP and missed miscarriage were assessed. Activin A and FS were found to be secreted by cytotrophoblasts *in vitro* [11]. While *in vivo,* they are secreted from the fetoplacental unit, from the gestational sac during the first trimester in large quantities suggesting that they act locally in paracrine/autocrine fashion [12]. At the time being there is no single best biomarker for identifying tubal EP; however, some can differentiate between EP and IUP better than others [13]. In our study beta-hCG levels were significantly higher in IUP group compared to failed pregnancy group ($p<0.001$), and also significantly lower in EP compared to MA ($p<0.001$), these results was in agreement with Warrick *et al*, and in agreement with Florio *et al*, in which they found that beta-hCG is significantly low in EP compared to spontaneous miscarriage [14,15]. The explanation of these findings is related to fact β-HCG production dependent on viable fetoplacental unite, while in normal viable IUP the fetus and uterus continue to grow; in EP and MA this grows arrested leading to placental dysfunction and lower production of β-HCG as a result of primary trophoblast dysfunction. In our study serum level of activin A, FS and activin A/FS ratio were significantly higher in normal pregnancy compared to failed pregnancy; these findings were in agreement with the results of several studies Rausch *et al*., Florio *et al*., and Johns *et al*. [13,15,16]. Activin A had good ability to differentiate a normal IUP from failed pregnancy with a sensitivity of 65.0% and 100% specificity with a cutoff value ≤284 pg/ml, while Daponte *et al*. reported excellent ability with 87.9% sensitivity, 85.0% specificity, and ≤504.66 pg/ml as a cutoff value [17]. Activin A had fair ability to differentiate a normal IUP from missed miscarriage, while Muttukrishna *et al*. [18]. who stated that activin A did not show a statistical difference between the two groups. (Pregnancy failure after 6–8 weeks can mainly be linked to placental dysfunction either secondary to fetal abnormality or as a result of primary trophoblast dysfunction). In this study, gestational age of pregnancy was above 8 weeks, but this was in contrast to Daponte *et al*. [17]. who reported good ability to differentiate between them. In the current study, activin A had excellent ability to differentiate a normal IUP from an EP, and there are some studies correlate with this finding, but with varying accuracy and cutoff values which are Rausch *et al*., Florio *et al*., and Daponte *et al*. [13,17]. While other studies found that activin A was not useful in diagnosing EP which are Warrick *et al*. and Kirk *et al*. [14,19]. Another study found that if the adnexal mass was found by transvaginal scan; then activin A levels could not differentiate between an IUP and EP [20]. These findings suggest that

Table 5: Validity and predictive values of activin A, follistatin, and their ratio for differentiating failed pregnancy, from IUP missed miscarriage and EP from normal pregnancy and MA from EP

Variables	Cutoff	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
IUP versus (MA+EP)					
Activin A pg/ml	≤284	65.0	100	100	58.8
Follistatin pg/ml	≤4213	91.7	53.3	79.7	76.2
Activin A/FS ratio	≤0.117	66.7	86.7	90.0	56.5
IUP versus MA					
Activin A pg/ml	≤553	83.3	63.3	69.4	79.2
Follistatin pg/ml	≤4144	83.3	53.3	64.1	76.2
Activin A/FS ratio	≤0.117	50.0	86.7	78.9	63.4
IUP versus EP					
Activin A pg/ml	≤298	99	99	99	99
Follistatin pg/ml	≤4467	100	10	76.9	100
Activin A/FS ratio	≤0.117	93.3	83.3	84.8	92.6
MA versus EP					
Activin A pg/ml	≤277	100	73.3	78.6	100
Follistatin pg/ml	≤3904	93.3	30.0	57.1	81.8
Activin A/FS ratio	≤0.092	66.7	70.0	69.0	67.7

PPV: Positive predictive value, NPV: Negative predictive value, EP: Ectopic pregnancy, IUP: Intrauterine pregnancy, MA: Missed abortion

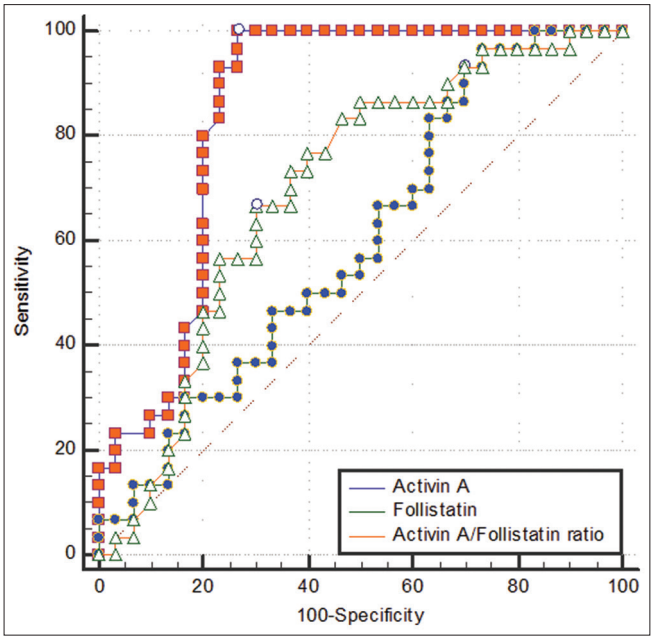

Fig. 7: Receiver operating characteristics curve analyses for the diagnostic accuracy of activin A, follistatin, and activin A/follistatin ratio values to discriminate a missed miscarriage from ectopic pregnancy

activin A can predict EP more precisely and quickly as both single tests and complementary tools in the differential diagnosis of EP.

As for discrimination between EP and MA, activin A had good ability with a cutoff value of ≤277 pg/ml, 100% sensitivity, and 73.3% specificity, which meant that it could detect all EP cases, and with a 100.0% negative predictive value (NPV) compared to MA, Daponte *et al.* reported less accuracy (SN 63.3% and SP 83.3%), with a higher cutoff value 325.21 pg/ml [17]. Serum FS showed weak ability to differentiate IUP from failed pregnancy and MA and from differentiating MA from EP, and this was consistent with the results of Muttukrishna *et al.* [18], and Daponte *et al.* [17] showed that serum FS lacked the ability to discriminate miscarriage from EP and could discriminate IUP from EP similar to this study results, as it showed good ability to discriminate IUP from EP, with 100% sensitivity, 70% specificity, at ≤4467 pg/ml cutoff value, with a 100% NPV for detecting all cases with EP compared to IUP. FS is regulatory proteins that have neutralizing properties of activins, so their synthesis is coordinated for optimal regulation of activin homeostasis, as the binding of FS to activins is irreversible. Since activin A is directly predicted pregnancy abnormalities (as function of its synthesis from fetomaternal unit), FS suggested to be indirectly correlated to pregnancy abnormality through its action on activin A, however as the results of the current confirmed; the association of FS with pregnancy abnormalities is modest at most (i.e., weaker than that of activin A) which may suggest FS is not the only regulator of activin A and hence its alone is not enough as predictor of EP and MA.

Similar to serum FS; the ratio of activin A/FS showed that it was better at discrimination IUP from EP, from others, with sensitivity of 93.3%, 83.3% of specificity, at cutoff value ≤0.117, similar to Daponte *et al.* found that this ratio was able to discriminate IUP from EP but not MA from EP [17].

Activin A levels are reduced in the presence of dead trophoblast as in Florio *et al.*, Muttukrishna *et al.*, and Luisi *et al.* [15,18,21]. With both single and serial measurements used to anticipate miscarriage [22]. There are conflicting data on setting a serum cutoff level of activin A in discriminating EP from IUP with either poor AUC of 0.60 or excellent AUCs [15,23], while this study showed an excellent AUC of 0.999. Activin A is excreted by cytotrophoblasts, and in tubal pregnancy, there is usually abnormal decidualization with poorly implanted trophoblast [15]. However, on the other hand, elevated serum activin A may be found in EPs if it was heterogeneous (one fetus inside the uterus) [24]. This could partly explain that the previously mentioned studies showed varying results. Furthermore, the type of kit (regarding method and brand) could lead to different results among studies; for example, the study was done by Rausch *et al.* [13]. Which used assays by Quantikine, while all other studies used activin A immunoassays manufactured by Serotec, and this study measured it using a two-site ELISA manufactured by Cusabioi [15,19].

CONCLUSION

- A single measurement of activin A can be used with high accuracy as a biomarker for EP and missed miscarriage
- FS alone and activin A/FS ratio are a possible biomarker, but it offers lower accuracy compared to activin A alone.

ACKNOWLEDGMENTS

Big thanks to the Department of Obstetrics and Gynecology and the Department of Laboratories of Al-Yarmouk Teaching Hospital for their help in the execution of the study.

AUTHORS' CONTRIBUTIONS

All authors have contributed equally to this work.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

REFERENCES

1. Sasson IE, Norwitz ER. Implantation events. In: Farquharson RG, Stephenson MD, editors. Early Pregnancy. New York, Cambridge, USA: Cambridge University Press; 2010. p. 199-212.
2. Al-hakeim HA, Jebur IM, Alkhafaji RS. Serum anti-zona pellucida antibodies is correlaterd with estradiol level in women with *in vitro* fertilization failure. Int J Pharm Pharm Sci 2016;8:41-4.
3. Reis FM, Luisi S, Abrão MS, Rocha AL, Viganò P, Rezende CP, *et al*. Diagnostic value of serum activin a and follistatin levels in women with peritoneal, ovarian and deep infiltrating endometriosis. Hum Reprod 2012;27:1445-50.
4. Koninger A, Schmidt B, Damaske D, Birdir C, Enekwe A, Kimmig R, *et al*. Follistatin during pregnancy and its potential role as an ovarian suppressing agent. Eur J Obstet Gynecol Reprod Biol 2017;212:150-400.
5. Hedger MP, Winnall WR, Phillips DJ, de Kretser DM. The regulation and functions of activin and follistatin in inflammation and immunity. Vitam Horm 2011;85:255-97.
6. Ekechi CI. Spontaneous miscarriage. In: Edmonds KD, editor. Dewhurst's Textbook of Obstetrics and Gynaecology. 9th ed. USA: John Wiley and Sons Ltd.; 2018. p. 559-67.
7. Alomar MJ. Evaluation of caffeine consumption and effect during pregnancy among women in UAE. Int J Pharm Pharm Sci 2016;8:101-3.
8. Kirk E, Bottomley C, Bourne T. Diagnosing ectopic pregnancy and current concepts in the management of pregnancy of unknown location. Hum Reprod Update 2013;20:250-61.
9. Ekechi CI, Stalder CM. Spontaneous miscarriage. In: Edmonds DK, editor. Dewhurst's Textbook of Obstetrics and Gynaecology. 19th ed. Chichester, UK: Wiley-Blackwell; 2019. p. 559-67.
10. NICE Clinical Guidelines 126. Ectopic Pregnancy and Miscarriage, Diagnosis and Initial Management. NICE Clinical Guidelines; 2019.
11. Bearfield C, Jauniaux E, Groome N, Sargent I, Muttukrishna S. The secretion and effect of inhibin A, activin A and follistatin on first-trimester trophoblasts *in vitro*. Eur J Endocrinol 2005;152:909-16.
12. Muttukrishna S, Jauniaux E, Pahal G, Groome N, Rodeck C, editors. *In vivo* Distribution of Inhibin A, Activin A and Follistatin in Early Pregnancy. European: Congress in Obstetrics and Gynaecology Ceska Gynekol; 2002.
13. Rausch ME, Sammel MD, Takacs P, Chung K, Shaunik A, Barnhart KT. Development of a multiple marker test for ectopic pregnancy. Obstet Gynecol 2011;117:573-82.
14. Warrick J, Gronowski A, Moffett C, Zhao Q, Bishop E, Woodworth A.

Serum activin A does not predict ectopic pregnancy as a single measurement test, alone or as part of a multi-marker panel including progesterone and hCG. Clin Chim Acta 2012;413:707-11.
15. Florio P, Severi FM, Bocchi C, Luisi S, Mazzini M, Danero S, *et al*. Single serum activin a testing to predict ectopic pregnancy. J Clin Endocrinol Metab 2007;92:1748-53.
16. Johns J, Muttukrishna S, Lygnos M, Groome N, Jauniaux E. Maternal serum hormone concentrations for prediction of adverse outcome in threatened miscarriage. Reprod Biomed Online 2007;15:413-21.
17. Daponte A, Deligeoroglou E, Garas A, Pournaras S, Hadjichristodoulou C, Messinis IE. Activin A and follistatin as biomarkers for ectopic pregnancy and missed abortion. Dis Markers 2013;35:497-503.
18. Muttukrishna S, Jauniaux E, Greenwold N, McGarrigle H, Jivraj S, Carter S, *et al*. Circulating levels of inhibin A, activin A and follistatin in missed and recurrent miscarriages. Hum Reprod 2002;17:3072-8.
19. Kirk E, Papageorghiou AT, Van Calster B, Condous G, Cowans N, Van Huffel S, *et al*. The use of serum inhibin A and activin A levels in predicting the outcome of "pregnancies of unknown location". Hum Reprod 2009;24:2451-6.
20. Únior JE, Oliveira LG, Silva MO, Júnior EA, Camano L. Serum activin A levels and tubal ectopic pregnancy. Iran J Reprod Med 2014;12:227.
21. Luisi S, Florio P, D'Antona D, Severi F, Sanseverino F, Danero S, *et al*. Maternal serum inhibin A levels are a marker of a viable trophoblast in incomplete and complete miscarriage. Eur J Endocrinol 2003;148:233-6.
22. Prakash A, Laird S, Tuckerman E, Li TC, Ledger WL. Inhibin A and activin A may be used to predict pregnancy outcome in women with recurrent miscarriage. Fertil Steril 2005;83:1758-63.
23. Florio P, Reis FM, Battista R, Luisi S, Moncini I, Bocchi C, *et al*. Serum activin A levels are lower in tubal than intrauterine spontaneously conceived pregnancies. Gynecol Endocrinol 2011;27:391-5.
24. Kirk E, Condous G, Haider Z, Lu C, Van Huffel S, Timmerman D, *et al*. The practical application of a mathematical model to predict the outcome of pregnancies of unknown location. Ultrasound Obstet Gynecol 2006;27:311-5.