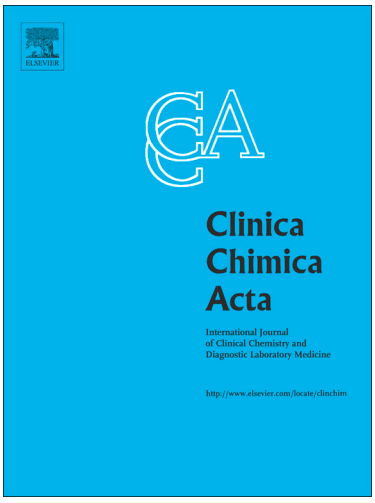

The performances of serum activins and follistatin in the diagnosis of ectopic pregnancy: A prospective case-control study

Bassem Refaat, Adil O. Bahathiq

PII: S0009-8981(19)32063-7
DOI: https://doi.org/10.1016/j.cca.2019.09.019
Reference: CCA 15856

To appear in: *Clinica Chimica Acta*

Received Date: 22 August 2019
Revised Date: 29 September 2019
Accepted Date: 30 September 2019

Please cite this article as: B. Refaat, A.O. Bahathiq, The performances of serum activins and follistatin in the diagnosis of ectopic pregnancy: A prospective case-control study, *Clinica Chimica Acta* (2019), doi: https://doi.org/10.1016/j.cca.2019.09.019

The performances of serum activins and follistatin in the diagnosis of ectopic pregnancy: A prospective case-control study

Bassem Refaat[a] and Adil O. Bahathiq[b]

[a]Laboratory Medicine Department, Faculty of Applied Medical Sciences, Umm Al-Qura University, Al Abdeyah, PO Box 7607, Makkah, Saudi Arabia.

[b]Physiology Department, Faculty of Medicine, Umm Al-Qura University, Al Abdeyah, Makkah, Saudi Arabia.

Corresponding author

Dr Bassem Refaat

Laboratory Medicine Department

Faculty of Applied Medical Sciences

Umm Al-Qura University

Holy Makkah, PO Box 7607, KSA

Telephone: +966 541162707

Fax: +966 12 5270000 Ext: 4242

Email: bassem.refaat@yahoo.co.uk

Abstract

Background

The diagnosis of ectopic pregnancy (EP) is challenging and 40-50% of patients are initially misdiagnosed.

Methods

This prospective case-control study measured activin-A, activin-B, activin-AB and follistatin for the diagnosis of EP, spontaneous abortion (SAB) and normal intrauterine pregnancy (IUP). Sera were collected from 120 women with symptoms suggesting early pregnancy failure and who were clinically diagnosed as IUP, SAB or EP (n = 40/group). The markers were measured by ELISA and their area under the curve (AUC), cut-off value, sensitivity and specificity were determined by receiver-operating characteristic curve. The results were compared with serum β-human chorionic gonadotropin and progesterone.

Results

Activin-A and activin-B decreased significantly, while activin-AB and follistatin increased, in the EP and SAB groups than the IUP group. Activin-AB (AUC = 0.961) and progesterone (AUC = 0.973) were the best markers for EP and SAB, respectively. Activin-AB (≥ 61.5 pg/mL) showed 92.5% sensitivity, 85% specificity, 75.5% positive predictive value (PPV) and 95.8% negative predictive value (NPV) for EP. Progesterone (≤ 6.3 ng/mL) had 100% sensitivity, 86.2% specificity, 78.4% PPV and 100% NPV for SAB.

Conclusions

Serum activins and follistatin were significantly altered with EP and activin-AB could be a promising marker for the diagnosis of EP.

Keywords: Activin-A; Activin-B; Activin-AB; Early pregnancy failure; Human chorionic gonadotropin; Progesterone.

1. Introduction

Ectopic pregnancy (EP) is a form of abnormal pregnancy and is the leading cause of maternal morbidity and mortality during the first trimester worldwide [1-3]. The Fallopian tube is the commonest site for ectopic implantation and tubal diseases (e.g. hydrosalpinx) are believed to increase the risk of EP by deterring ciliary motility and/or creating an abnormal milieu that favours the implantation of embryo [1, 2, 4]. Moreover, the incidence of EP ranges between 1% and 2% of all naturally occurring pregnancies and the rates increase dramatically with assisted reproductive treatments [5, 6].

The currently used diagnostic algorithm for EP consists of measuring serum β-human chorionic gonadotrophin (hCG) in combination with transvaginal ultrasound (TVS) to visualise the pregnancy and its location [7, 8]. Overall, TVS can reliably identify a gestational sac when serum β-hCG concentrations are ≥ 2000 IU/L, which is known as the discriminatory zone [9, 10]. Nevertheless, the currently applied biomarkers (β-hCG and progesterone) have limited performance and it is believed that 40 to 50% of cases are initially misdiagnosed [11]. Another diagnostic challenge arises when serum β-hCG is below the discriminatory zone level and the finding of an empty intrauterine cavity is common and could be linked with either a small early pregnancy, EP or miscarriage [6, 12]. Furthermore, serial β-hCG measurements every 48-hours with a repeat ultrasound when the hormone reaches the discriminatory level are mandatory for the accurate diagnosis of EP [11]. Therefore, there is a compelling need to develop new markers and algorithms that provide a more sensitive and specific tool for the diagnosis of EP [13-15].

The fate of EP is dependent on the activity of the trophoblast tissue and the developing placenta secrets several proteins, including activins and follistatin, that could potentially be used as biomarkers to monitor placental development and activity [1, 16]. Activins are disulphide-linked dimers of two β-subunits, and the different dimerization could produce three distinct mature proteins. The homodimers of the βA-subunit is activin-A (βAβA), βB-subunit is activin-B (βBβB) and the heterodimer of βA- with βB-subunit produces activin-AB (βAβB) [17]. The biological activities of activins are tightly regulated by their binding protein, follistatin [1, 16]. Activins and follistatin have been shown to play an important role in embryo implantation and they are expressed by the trophoblast [1, 16]. The placenta is a major source of serum activin-A during pregnancy and the protein levels increase as pregnancy progresses [15]. Therefore, activin-A and follistatin have been proposed as serum markers for differentiating between viable intrauterine pregnancy (IUP) and EP [14, 18-21]. However, the currently available results about the specificity and sensitivity of activins and follistatin are controversial as well as none of the earlier studies measured activin-AB or simultaneously investigated the different activins with follistatin.

This study, therefore, measured the serum concentrations of activin-A, -B, -AB and follistatin in serum samples collected from women diagnosed with EP and the results were compared with those obtained from gestational age-matched women either diagnosed with spontaneous abortion (SAB) or had normal early pregnancies. We also determined the sensitivity and specificity of the candidate molecules in the diagnosis of EP in comparison with serum β-hCG and progesterone at the time of presentation. The development of sensitive and specific markers for the early diagnosis of EP would offer a better chance to use more conservative and cost-effective therapeutic approaches that could provide a better prospective fertility outcome and avoidance of the morbidity and mortality associated with EP.

2. Patients and methods

2.1. Ethical approval

Approval for the clinical study was obtained from the institutional review board and ethics committee of the Faculty of Applied Medical Sciences in Umm Al-Qura University (AMSEC 10-15-9-2016). All serum samples were collected following obtaining informed written consent from all the participants.

2.2. Study design

This was a prospective case-control study and all the patients were recruited from the Emergency Department of the Maternity and Children Hospital in Jeddah city and a total of 120 women were recruited between March 2016 and February 2019. The women were presented to the Emergency Department with complaints of abdominal pain and/or vaginal bleeding, had positive pregnancy test following natural conception, a calculated gestational age ranging between 6-12 weeks from the last menstrual period and they were haemodynamically stable. A serum sample was obtained from each woman at the time of presentation prior to any medical intervention and the samples were aliquoted and stored at -80 °C till processed for measuring the different markers.

The patients were followed-up by their treating clinicians according to the hospital policies till a diagnosis was reached. The case group consisted of those women who were diagnosed with EP (n = 40) by TVS/laparoscopy or SAB (n = 40) by histopathology. Patients with a history of assisted conception treatment, gestational trophoblastic diseases, non-tubal EP, ruptured EP, recurrent abortion, recurrent EP, evidence of multiple gestations or a history of chronic diseases (e.g. diabetes mellitus, hypertension, etc.) were excluded from the study. The control group included another 40 gestational age matched women with normal singleton IUP following natural conception as determined by the ultrasound findings (e.g. intrauterine gestational sac, foetal pole, foetal cardiac activity, etc.). The β-hCG and progesterone serum concentrations

were also measured during the routine clinical management and follow-up of all the participants.

2.3. Enzyme linked immunosorbant assay (ELISA)

Sandwich ELISA was used for the quantitative measurement of human activin-A, activin-B, activin-AB and follistatin. All the samples were processed in duplicate according to the manufacturer's instructions and the used kits for each marker were from the same batch. The detection range for each of the activin kits (Cloud-Clone Corp.; TX, USA) was 16-1000 pg/mL and the cross-reactivity between the different activin proteins were negligible. As reported by the manufacturer, the intra- and inter-assay precisions for each of the activin kits were < 10% and < 12%, respectively. The detection range of the follistatin kit (R&D Systems Inc.; MN, USA) was 250-16,000 pg/mL and the intra- and inter-assay precisions were < 3.5% and < 8.5%, respectively. The minimal detection limits were 5.7 pg/mL for activin-A, 5.6 pg/mL for AB, 5.4 pg/mL for activin-B and 83 pg/mL for follistatin.

2.4. Statistical analysis

Statistical analysis of the results was performed using SPSS version 25. Normality and homogeneity of data were assessed with by the Kolmogorov and Smirnov's test and Levene test, respectively. One-way ANOVA followed by either Tukey's HSD or Games-Howell post-hoc tests were used to compare between the study groups based on variance equality. Correlations were determined by Pearson's test. Receiver-operating characteristic curve (ROC) analysis was used to determine the area under the curve (AUC) and the cut-off value for each protein to measure their sensitivity and specificity and the results were compared against β-hCG and progesterone for the diagnosis of EP and SAB. Additionally, the diagnostic performances of all the markers were also analysed in nested groups that included the women with β-hCG < 2000 IU/L at the time of presentation. P value < 0.05 was considered significant.

3. Results

3.1. Serum concentrations of the biomarkers in the overall study populations

The maternal and gestational ages were comparable between the controls, EP and SAB groups (Table 1). The β-hCG and progesterone concentrations were significantly lower in the EP and SAB groups compared with the IUP group. While there was no significant difference in the serum levels of β-hCG between the EP and SAB groups, progesterone was significantly lesser in the latter group (Table 1). Both serum activin-A and activin-B were also markedly diminished in the EP and SAB groups compared with the IUP group and they were significantly lowest in women with EP. On the other hand, serum activin-AB and follistatin were substantially higher in the EP group compared with the IUP and SAB groups. Meanwhile

activin-AB was markedly lower in the SAB group compared with the IUP group, there was no significant difference between both groups in the levels of follistatin (Table 1). Additionally, the gestational age in the 40 women diagnosed with normal IUP correlated directly and significantly with serum activin-A ($r = 0.882$; $P < 0.0001$), activin-B ($r = 0.918$; $P < 0.001$) and follistatin ($r = 0.366$; $P = 0.02$) as well as negatively with serum activin-AB ($r = -0.899$; $P < 0.001$).

3.2. Serum concentrations of the biomarkers in the women with serum β-hCG < 2000 IU/L in the different study groups

There were 36 women with serum β-hCG < 2000 IU/L at the time of presentation among whom six, 16 and 14 were clinically diagnosed with IUP, EP and SAB groups, respectively. No statistical differences were detected between the three subgroups in relation to maternal and gestational ages (Table 2). Furthermore, serum β-hCG was only significantly lower in the SAB group compared with the IUP group. However, progesterone and serum activin-A were substantially lower in the EP and SAB subgroups compared with the IUP subgroup and the lowest levels of both markers were observed with SAB compared with all subgroups (Table 2). Contrariwise, serum activin-AB and follistatin were markedly elevated in the SAB and EP subgroups and the highest significant levels were detected in the latter subgroup compared with IUP and SAB subgroups (Table 2).

3.3. Diagnostic performances of the different biomarkers for the discrimination of EP and SAB in the overall study populations

All serum markers were plotted in ROC curves to evaluate their diagnostic accuracy in discriminating an EP or SAB from a healthy IUP. While serum activin-AB (AUC 0.961; $P < 0.0001$) and activin-A (AUC 0.946; $P < 0.0001$) showed the highest scores for the diagnosis of EP, progesterone (AUC 0.973; $P < 0.0001$) and activin-AB (AUC 0.875; $P < 0.0001$) were associated with the best performance for discriminating SAB compared with the other biomarkers. The AUC values for all the markers are listed in Table 3.

Serum activin-A at the cut-off value ≤ 417.2 pg/mL demonstrated a sensitivity of 92.5%, specificity of 87.5%, positive predictive value (PPV) of 78.2% and negative predictive value (NPV) of 95.9% for discriminating an EP from SAB and IUP. Equally, the sensitivity and specificity of activin-AB for the diagnosis of EP at a cut-off value ≥ 61.5 pg/mL were 92.5% and 85%, respectively. Additionally, the PPV and NPV of Activin-AB for the diagnosis of EP were 75.5% and 95.8%, respectively. The lowest biomarkers for the diagnosis of EP were β-hCG followed by progesterone (Table 4). On the other hand, progesterone at a cut-off concentration ≤ 6.3 ng/mL had 100% sensitivity, 86.2% specificity, 78.4% PPV and 100% NPV

for the diagnosis of a SAB. Serum activin-AB showed lower performances than P and was associated with 95% sensitivity, 58.7% specificity, 53.5% PPV and 96.6% NPV for the diagnosis of SAB at a cut-off value < 61.5 pg/mL. Furthermore, the weakest markers for the discrimination of SAB were activin-A and activin-B. The diagnostic performances of all markers in the overall study populations are summarised in Table 4.

3.4. Diagnostic performances of the different biomarkers for the discrimination of EP and SAB in women with β-hCG < 2000 IU/L

Similar to the overall study population, serum activin-AB (AUC 0.956; P < 0.0001) and activin-A (AUC 0.891; P < 0.0001) showed the best performances for the diagnosis of an EP in the nested group of women (n = 36) with β-hCG < 2000 IU/L at the time of presentation. Additionally, progesterone (AUC 0.984; P < 0.0001) and activin-AB (AUC 0.932; P < 0.0001) also disclosed the utmost scores for discriminating SAB in the nested population. The AUC scores for the remaining markers are presented in Table 5.

Serum activin-AB showed the peak performances in the nested group for the diagnosis of EP at a cut-off value ≥ 61.5 pg/mL with 93.8% sensitivity, 90% specificity, 88.2% PPV and 94.7% NPV. Although serum activin-A at the cut-off value ≤ 427.4 pg/mL showed similar sensitivity (92.5%) to activin-AB, the specificity (80%), PPV (78.9%) and NPV (94.1%) for EP were lesser. Serum β-hCG was the weakest EP biomarker (Table 4). Yet again, serum progesterone at a cut-off value ≤ 4.9 ng/mL showed 92.9% sensitivity, 95.5% specificity, 92.8% PPV and 95.5% NPV for SAB. Serum activin-AB was weaker than P for the diagnosis of SAB with 92.9% sensitivity, 72.7% specificity, 81.2% PPV and 94.1% NPV at a cut-off value < 61.5 pg/mL. Furthermore, the poorest performances were observed with serum activin-B for the diagnosis of SAB (Table 6).

4. Discussion

The current study measured the diagnostic value of the different activin isoforms and follistatin to discriminate between normal IUP, EP and SAB in pregnant women presented to the emergency department with symptoms suggestive of early pregnancy failure. Activins are disulphide-linked proteins and the homo and heterodimerization of the activin βA- and/or βB-subunit produce three distinctive mature proteins that are tightly and equally neutralised by their binding protein, follistatin [16, 17]. Both activin β-subunits and follistatin are expressed by normal placenta and the three activin proteins promoted trophoblast invasion *in vitro* [22]. However, the majority of the available reports mainly focused on investigating the dynamics of serum activin-A and follistatin during normal pregnancy [23, 24]. Early studies on surrogate mothers with non-functioning ovaries have suggested a feto-placental origin for activin-A

during pregnancy [25-27] and the serum levels of activin-A and follistatin increased as pregnancy progressed [28-31]. Contrariwise, serum activin-A decreased markedly in the presence of nonviable trophoblast [4, 28, 32]. The currently available studies on activin-B and activin-AB during pregnancy are scarce and activin-B was investigated by a single study that reported positive correlations between the serum protein levels and gestational age during normal pregnancy [33]. Although mature activin-AB was recovered from human placental tissue homogenates [34], the protein was not detected in the sera of pregnant women [29].

In harmony, our data showed that the gestational age correlated significantly and strongly with serum activin-A and to a lesser extent with follistatin in the 40 women with healthy IUP, supporting the notion that the placenta could be the major source of serum activin-A during pregnancy [28-31]. However, activin-AB and activin-B were also detected in the collected serum samples and the systemic levels of both proteins were notably lower than those of activin-A. Additionally, serum activin-AB demonstrated strong positive association, whereas activin-B correlated inversely, with the gestational age within the IUP group. Our observations suggest that these activin isoforms could also be dynamically produced by the developing placenta, but to a lesser extent than activin-A. Additionally, the serum levels of all the activins and follistatin were significantly altered in the EP and SAB group compared with the IUP group, advocating that these proteins could represent plausible markers for the diagnosis of EP [16].

EP represents a universal health concern since it is the primary cause of maternal morbidity and mortality during the first trimester, and the incidence increases drastically with the use of assisted conception technology [5, 6]. Currently, the diagnosis of EP is based on the combination of TVS with the serum levels of β-hCG [6-8, 12]. However, if the hormone concentrations are below the discriminatory zone, at which a normal IUP should be visualised by ultrasound, serial quantitative measurements are performed every 48 hours till the hormone attains the predetermined cut-off concentrations to make an accurate diagnosis [9-11]. Additionally, it is believed that 40-50% of EP cases are misdiagnosed at the initial visit, thus posing a significant risk for tubal rupture with subsequent life threatening consequences or might possibly increase the odds of disturbing a desired viable pregnancy [8, 35]. Hence, accurate early diagnosis would allow the use of more conservative approaches in the management of EP as well as would decrease the potential hazard of interrupting a misdiagnosed small healthy IUP [8, 35].

The quest for reliable sensitive and specific markers of EP has long been the focus of numerous studies that have suggested several proteins, including activin-A. The original study by Florio et al. (2007) has reported that serum activin-A successfully discriminated between viable normal IUP, miscarriage and EP in women with pregnancy of unknown location with 100%

sensitivity, 99.6% specificity and 97.4% PPV at a cut-off value of 370 pg/mL [18]. These results have been confirmed by other researcher groups in the following years but with a different cut-off value of 504 pg/mL that showed a sensitivity ranging between 87.9-97% and specificity between 93.5-100% [14, 19-21]. In contrast, another two studies reported that a single measurement of serum activin-A was neither sensitive nor specific compared to serum β-hCG for the diagnosis of EP [36, 37]. More recently, another study has demonstrated that follistatin only differentiated between EP and IUP with 72.7% sensitivity and 90% specificity [14]. Similarly, serum activin-B was significantly decreased in EP compared with IUP and at the cut-off value of 23.3 pg/mL it showed 82% sensitivity and 62% specificity for the diagnosis of EP [38]. However, the serum levels of follistatin and activin-B were comparable between EP and SAB and both proteins showed poor performances in differentiating between the groups of early pregnancy failure [14, 38]. Our results agree with the prior studies as well as emphasise the earlier observations related to the superiority of serum activin-A than follistatin and activin-B in the diagnosis of EP [14, 19-21, 38]. However, the performances of activin-A, activin-B and follistatin in diagnosing SAB were weak in our study, advocating that these proteins could only be sensitive for predicting the pregnancy location but not viability [15].

Interestingly, serum activin-AB was detected in all the serum samples in the present study and the protein exhibited inverse strong correlations with the gestational age in the IUP group. Our data also exposed superior performances for serum activin-AB in differentiating between pregnancy location (EP vs. IUP and SAB) and pregnancy viability (SAB vs. IUP and EP) in the overall study population as well as in the cohort of women with serum β-hCG < 2000 IU/L. The serum levels of activin-AB were previously explored by only a single study and the researchers reported that the protein was undetectable throughout pregnancy [29]. The controversy between our observations and the earlier study could be related to the sensitivity of the used ELISA kits since the lowest detection limit in ours was 5.6 pg/mL, whereas the prior report used an inhouse kit with a lowest detection limit of 190 pg/mL [29]. Our data suggests that serum activin-AB could be a promising single marker for the diagnosis of early pregnancy failure (EP and SAB). However, progesterone was more powerful in predicting SAB than serum activin-AB. In concordance, a recent meta-analysis report that involved 26 studies have similarly shown that progesterone was sensitive and specific for predicting non-viable pregnancy [39]. Hence, we propose that measuring serum activin-AB and progesterone could be an efficient diagnostic algorithm for differentiating between early pregnancy failure and normal IUP. However, additional cross-sectional studies are mandatory to validate our suggestion in clinical settings and to measure the performances of the suggested markers in women diagnosed with pregnancy of unknown location.

In conclusion, the present study demonstrated that serum activins and follistatin are dynamic during normal early pregnancy and their concentrations were pathologically altered with early pregnancy failure. Moreover, serum activin-AB showed the superlative diagnostic performances for EP, whereas it was weaker than progesterone for predicting SAB. Therefore, the combination of serum activin-AB and progesterone could represent an efficient diagnostic tool for the differentiation between IUP, EP and SAB in women with symptoms suggesting early pregnancy failure. Nevertheless, prospective large multicentre and cross-sectional studies are still needed to measure the reference values and precision of the suggested markers in the diagnosis of EP in women with pregnancy of unknown location.

Competing interests

The authors declare no competing interests.

Funding

This project was funded by the National Science, Technology and Innovation Plan (MARRIFAH) - King Abdul Aziz City for Science and Technology (KACST), the Kingdom of Saudi Arabia, Award Number (11-MED2068-10).

Acknowledgment

The authors would also like to thank Dr. Sarah Batwa, Obstetrics & Gynaecology Department, Maternity and Children Hospital, Ministry of health for her support in patient recruitment and sample collection. The authors also acknowledge Mr. Jawwad Ahmad and Mr. Shakir Idris from the Laboratory Medicine Department, Faculty of Applied Medical Sciences, Umm Al-Qura University for processing the samples.

References

[1] B. Refaat, H. Simpson, E. Britton, J. Biswas, M. Wells, J.D. Aplin,W. Ledger, Why does the fallopian tube fail in ectopic pregnancy? The role of activins, inducible nitric oxide synthase, and MUC1 in ectopic implantation. Fertil Steril, 97: (2012) 1115-23 DOI: 10.1016/j.fertnstert.2012.02.035

S0015-0282(12)00269-5 [pii].

[2] B. Refaat, E. Dalton,W.L. Ledger, Ectopic pregnancy secondary to in vitro fertilisation-embryo transfer: pathogenic mechanisms and management strategies. Reprod Biol Endocrinol, 13: (2015) 30 DOI: 10.1186/s12958-015-0025-0.

[3] B. Refaat, J. Ahmad, S. Idris, F.F. Kamfar, A.M. Ashshi, S.A. Batwa,F.A. Malibary, Characterisation of vitamin D-related molecules and calcium-sensing receptor in human Fallopian tube during the menstrual cycle and in ectopic pregnancy. Cell Tissue Res, 368: (2017) 201-213 DOI: 10.1007/s00441-016-2519-2.

[4] S. Muttukrishna, E. Jauniaux, N. Greenwold, H. McGarrigle, S. Jivraj, S. Carter, S. Elgaddal, N. Groome,L. Regan, Circulating levels of inhibin A, activin A and follistatin in missed and recurrent miscarriages. Hum Reprod, 17: (2002) 3072-8.

[5] S. Lin, R. Yang, H. Chi, Y. Lian, J. Wang, S. Huang, C. Lu, P. Liu,J. Qiao, Increased incidence of ectopic pregnancy after in vitro fertilization in women with decreased ovarian reserve. Oncotarget, 8: (2017) 14570-14575 DOI: 10.18632/oncotarget.14679.

[6] D. Carusi, Pregnancy of unknown location: Evaluation and management. Semin Perinatol, 43: (2019) 95-100 DOI: 10.1053/j.semperi.2018.12.006.

[7] N.M. van Mello, F. Mol, H.R. Verhoeve, M. van Wely, A.H. Adriaanse, E.A. Boss, A.B. Dijkman, N. Bayram, M.H. Emanuel, J. Friederich, L. van der Leeuw-Harmsen, J.P. Lips, M.A. Van Kessel, W.M. Ankum, F. van der Veen, B.W. Mol,P.J. Hajenius, Methotrexate or expectant management in women with an ectopic pregnancy or pregnancy of unknown location and low serum hCG concentrations? A randomized comparison. Hum Reprod, 28: (2013) 60-7 DOI: 10.1093/humrep/des373.

[8] P.C. Brady, New Evidence to Guide Ectopic Pregnancy Diagnosis and Management. Obstet Gynecol Surv, 72: (2017) 618-625 DOI: 10.1097/ogx.0000000000000492.

[9] Z.Y. Chen, J.H. Liu, K. Liang, W.X. Liang, S.H. Ma, G.J. Zeng, S.Y. Xiao,J.G. He, The diagnostic value of a multivariate logistic regression analysis model with transvaginal power Doppler ultrasonography for the prediction of ectopic pregnancy. J Int Med Res, 40: (2012) 184-93.

[10] D. Desai, J. Lu, S.P. Wyness, D.N. Greene, K.N. Olson, C.L. Wiley,D.G. Grenache, Human chorionic gonadotropin discriminatory zone in ectopic pregnancy: does assay harmonization matter? Fertil Steril, 101: (2014) 1671-4 DOI: 10.1016/j.fertnstert.2014.02.023

S0015-0282(14)00176-9 [pii].

[11] C.o.P. Bulletins—Gynecology, ACOG Practice Bulletin No. 191: Tubal Ectopic Pregnancy. Obstet Gynecol, 131: (2018) e65-e77 DOI: 10.1097/aog.0000000000002464.

[12] L. Sabbioni, E. Carossino, F.M. Severi,S. Luisi, From beta-hCG values to counseling in tubal pregnancy: what do women want? Gynecol Endocrinol. 10.1080/09513590.2019.1640201(2019) 1-6 DOI: 10.1080/09513590.2019.1640201.

[13] R.N. Pillai, J.C. Konje, D.G. Tincello,N. Potdar, Role of serum biomarkers in the prediction of outcome in women with threatened miscarriage: a systematic review and diagnostic accuracy meta-analysis. Hum Reprod Update, 22: (2016) 228-39 DOI: 10.1093/humupd/dmv054.

[14] A. Daponte, E. Deligeoroglou, A. Garas, S. Pournaras, C. Hadjichristodoulou,I.E. Messinis, Activin A and follistatin as biomarkers for ectopic pregnancy and missed abortion. Dis Markers, 35: (2013) 497-503 DOI: 10.1155/2013/969473.

[15] S. Senapati, M.D. Sammel, S.F. Butts, P. Takacs, K. Chung,K.T. Barnhart, Predicting first trimester pregnancy outcome: derivation of a multiple marker test. Fertil Steril, 106: (2016) 1725-1732.e3 DOI: 10.1016/j.fertnstert.2016.08.044.

[16] B. Refaat, Role of activins in embryo implantation and diagnosis of ectopic pregnancy: a review. Reprod Biol Endocrinol, 12: (2014) 116 DOI: 10.1186/1477-7827-12-116.

[17] B. Refaat,W. Ledger, The expression of activins, their type II receptors and follistatin in human Fallopian tube during the menstrual cycle and in pseudo-pregnancy. Hum Reprod, 26: (2011) 3346-54 DOI: 10.1093/humrep/der331

der331 [pii].

[18] P. Florio, F.M. Severi, C. Bocchi, S. Luisi, M. Mazzini, S. Danero, M. Torricelli,F. Petraglia, Single serum activin a testing to predict ectopic pregnancy. J Clin Endocrinol Metab, 92: (2007) 1748-53 DOI: jc.2006-2188 [pii]

10.1210/jc.2006-2188.

[19] M.E. Rausch,K.T. Barnhart, Serum biomarkers for detecting ectopic pregnancy. Clin Obstet Gynecol, 55: (2012) 418-23 DOI: 10.1097/GRF.0b013e31825109f6

00003081-201206000-00008 [pii].

[20] M.E. Rausch, M.D. Sammel, P. Takacs, K. Chung, A. Shaunik,K.T. Barnhart, Development of a multiple marker test for ectopic pregnancy. Obstet Gynecol, 117: (2011) 573-82 DOI: 10.1097/AOG.0b013e31820b3c61

00006250-201103000-00009 [pii].

[21] M.A. Roghaei, F. Sabet,K. Mohamadi, Diagnostic accuracy of serum activin A in detection of ectopic pregnancy. J Res Med Sci, 17: (2012) 378-81.

[22] Y. Li, C. Klausen, J.C. Cheng, H. Zhu,P.C. Leung, Activin A, B, and AB increase human trophoblast cell invasion by up-regulating N-cadherin. J Clin Endocrinol Metab, 99: (2014) E2216-25 DOI: 10.1210/jc.2014-2118.

[23] R.L. Jones, J.K. Findlay,L.A. Salamonsen, The role of activins during decidualisation of human endometrium. Aust N Z J Obstet Gynaecol, 46: (2006) 245-9 DOI: AJO [pii]

10.1111/j.1479-828X.2006.00581.x.

[24] R.L. Jones, T.J. Kaitu'u-Lino, G. Nie, L.G. Sanchez-Partida, J.K. Findlay,L.A. Salamonsen, Complex expression patterns support potential roles for maternally derived activins in the establishment of pregnancy in mouse. Reproduction, 132: (2006) 799-810 DOI: 132/5/799 [pii]

10.1530/REP-06-0034.

[25] M. Birdsall, W. Ledger, N. Groome, H. Abdalla,S. Muttukrishna, Inhibin A and activin A in the first trimester of human pregnancy. J Clin Endocrinol Metab, 82: (1997) 1557-60.

[26] G.M. Lockwood, W.L. Ledger, D.H. Barlow, N.P. Groome,S. Muttukrishna, Measurement of inhibin and activin in early human pregnancy: demonstration of fetoplacental origin and role in prediction of early-pregnancy outcome. Biol Reprod, 57: (1997) 1490-4.

[27] S. Muttukrishna, T.J. Child, N.P. Groome,W.L. Ledger, Source of circulating levels of inhibin A, pro alpha C-containing inhibins and activin A in early pregnancy. Hum Reprod, 12: (1997) 1089-93.

[28] P. Florio, S. Luisi, P. Ciarmela, F.M. Severi, C. Bocchi,F. Petraglia, Inhibins and activins in pregnancy. Mol Cell Endocrinol, 225: (2004) 93-100 DOI: 10.1016/j.mce.2004.02.018

S0303720704000802 [pii].

[29] P.A. Fowler, L.W. Evans, N.P. Groome, A. Templeton,P.G. Knight, A longitudinal study of maternal serum inhibin-A, inhibin-B, activin-A, activin-AB, pro-alphaC and follistatin during pregnancy. Hum Reprod, 13: (1998) 3530-6.

[30] A.E. O'Connor, J.R. McFarlane, S. Hayward, T. Yohkaichiya, N.P. Groome,D.M. de Kretser, Serum activin A and follistatin concentrations during human pregnancy: a cross-sectional and longitudinal study. Hum Reprod, 14: (1999) 827-32.

[31] M. Schneider-Kolsky, D. D'Antona, L.W. Evans, N. Taylor, A. O'Connor, N.P. Groome, D. de Kretser,E.M. Wallace, Maternal serum total activin A and follistatin in pregnancy and parturition. BJOG, 107: (2000) 995-1000.

[32] S. Luisi, P. Florio, D. D'Antona, F.M. Severi, F. Sanseverino, S. Danero,F. Petraglia, Maternal serum inhibin A levels are a marker of a viable trophoblast in incomplete and complete miscarriage. Eur J Endocrinol, 148: (2003) 233-6.

[33] K.K. Vihko, M. Blauer, E. Kujansuu, S. Vilska, T. Alback, R. Tuimala, P. Tuohimaa,R. Punnonen, Activin B: detection by an immunoenzymometric assay in human serum during ovarian stimulation and late pregnancy. Hum Reprod, 13: (1998) 841-6 DOI: 10.1093/humrep/13.4.841.

[34] L.W. Evans, S. Muttukrishna, P.G. Knight,N.P. Groome, Development, validation and application of a two-site enzyme-linked immunosorbent assay for activin-AB. J Endocrinol, 153: (1997) 221-30 DOI: 10.1677/joe.0.1530221.

[35] J.Y. Hsu, L. Chen, A.R. Gumer, A.I. Tergas, J.Y. Hou, W.M. Burke, C.V. Ananth, D.L. Hershman,J.D. Wright, Disparities in the management of ectopic pregnancy. Am J Obstet Gynecol, 217: (2017) 49.e1-49.e10 DOI: 10.1016/j.ajog.2017.03.001.

[36] E. Kirk, A.T. Papageorghiou, B. Van Calster, G. Condous, N. Cowans, S. Van Huffel, D. Timmerman, K. Spencer,T. Bourne, The use of serum inhibin A and activin A levels in predicting the outcome of 'pregnancies of unknown location'. Hum Reprod, 24: (2009) 2451-6 DOI: 10.1093/humrep/dep066

dep066 [pii].

[37] J. Warrick, A. Gronowski, C. Moffett, Q. Zhao, E. Bishop,A. Woodworth, 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, 413: (2012) 707-11 DOI: 10.1016/j.cca.2011.12.018

S0009-8981(11)00698-X [pii].

[38] P. Dhiman, G.P. Senthilkumar, S. Rajendiran, K. Sivaraman, S. Soundararaghavan,M. Kulandhasamy, Serum activin B concentration as predictive biomarker for ectopic pregnancy. Clin Biochem, 49: (2016) 609-12 DOI: 10.1016/j.clinbiochem.2015.11.024.

[39] J. Verhaegen, I.D. Gallos, N.M. van Mello, M. Abdel-Aziz, Y. Takwoingi, H. Harb, J.J. Deeks, B.W. Mol,A. Coomarasamy, Accuracy of single progesterone test to predict early pregnancy outcome in women with pain or bleeding: meta-analysis of cohort studies. Bmj, 345: (2012) e6077 DOI: 10.1136/bmj.e6077.

The performances of serum activins and follistatin in the diagnosis of ectopic pregnancy: A prospective case-control study

Highlights

- Activins and follistatin are pathologically altered during early pregnancy failure.
- Activin-AB & activin-A showed higher performances than β-hCG & progesterone for EP.
- Progesterone followed by activin-AB were sensitive and specific for abortion.
- Combining activin-AB & progesterone could provide a better diagnostic tool for EP.

Table 1: The distribution of maternal and gestational ages in addition to each serum markers in the different study groups.

	IUP group	EP group	SAB group
Maternal age (years)*	28 (23.2-32.7)	28 (24-31)	28 (22.2-31.7)
Gestational age (weeks)*	9 (8-11)	9 (8-10)	9 (7.2-10)
β-hCG (IU/L)**	4200 (2300-7200)	2300 (1500-3300)[a]	2300 (1300-3600)[a]
Progesterone (ng/mL)**	21.5 (18.6-26.6)	7.1 (5.9-10.1)[a]	4.2 (2.9-5.3)[a,b]
Activin-A (pg/mL)**	1100 (846-1600)	281 (189-348)[a]	498 (368-600)[a,b]
Activin-B (pg/mL)**	40.8 (33.1-53.1)	22.7 (20-34.1)[a]	29.8 (24.4-34.1)[a,b]
Activin-AB (pg/mL)**	54.1 (46.6-61.7)	85.8 (71-101.2)[a]	45.2 (29.3-51.4)[a,b]
Follistatin (pg/mL)**	623 (561-718)	792 (731-878)[a]	614 (548-657)[b]

Data are shown as median (interquartile range)

* = One-way ANOVA followed by Tukey's post-hoc test

** = One-way ANOVA followed by Games-Howell post-hoc

a = $P < 0.01$ compared with IUP group

b = $P < 0.01$ compared with EP group

Table 2: The distribution of maternal and gestational ages in addition to each serum markers in the cohort of women with β-hCG < 2000 IU/L at the time of presentation from the different study groups.

	IUP group (n = 6)	EP group (n = 16)	SAB group (n = 14)
Maternal age (years)*	27.5 (22.2-33.5)	28 (22.5-31.7)	28.5 (24.5-33.2)
Gestational age (weeks)*	8.5 (7.7-9.5)	9 (8-10.7)	8.5 (7-11.2)
β-hCG (IU/L)*	1800 (1200-1900)	1500 (1200-1600)	1000 (773-1600)[a]
Progesterone (ng/mL)*	16.6 (15.4-18)	5.9 (5.1-6.7)[b]	3.0 (2.5-3.9)[b,d]
Activin-A (pg/mL)**	957 (779-1350)	274 (216-335)[a]	488 (327-613)[a,d]
Activin-B (pg/mL)**	37.3 (32.8-52.9)	22.5 (21.5-27.6)	29.4 (25.2-38.3)[c]
Activin-AB (pg/mL)**	58.8 (52.9-62.1)	80.9 (67.5-96.6)[b]	43.5 (28.1-51.5)[b,d]
Follistatin (pg/mL)**	572 (539-667)	758 (588-857)[a]	635 (562-717)[c]

Data are shown as median (interquartile range)

* = One-way ANOVA followed by Tukey's post-hoc test

** = One-way ANOVA followed by Games-Howell post-hoc

a = P < 0.05 compared with IUP group

b = P < 0.01 compared with IUP group

c = P < 0.05 compared with EP group

d = P < 0.01 compared with EP group

Table 3: The areas under the receiver-operating characteristic curve for each biomarker for the diagnosis of ectopic pregnancy (EP) and spontaneous abortion (SAB) in the overall study population (n = 120 women).

	EP AUC (95% CI)	SAB AUC (95% CI)
β-hCG (IU/L)	0.622 (0.520-0.723)*	0.624 (0.520-0.728)*
Progesterone (ng/mL)	0.527 (0.421-0.633)	0.973 (0.950-0.996)**
Activin-A (pg/mL)	0.946 (0.909-0.982)**	0.551 (0.448-0.654)
Activin-B (pg/mL)	0.860 (0.795-0.925)**	0.526 (0.422-0.625)
Activin-AB (pg/mL)	0.961 (0.926-0.996)**	0.875 (0.814-0.935)**
Follistatin (pg/mL)	0.859 (0.779-0.940)**	0.717 (0.626-0.807)**

AUC = Area under the curve

CI = Confidence interval

* = $P < 0.05$

** = $P < 0.001$

Table 4: The diagnostic performances for each biomarker for the diagnosis of ectopic pregnancy (EP) and spontaneous abortion (SAB) in the overall study population (n = 120 women).

Maker cut-off value	Diagnosis of EP				Diagnosis of SAB			
	Sensitivity	***Specificity***	***PPV***	***NPV***	***Sensitivity***	***Specificity***	***PPV***	***NPV***
β-hCG (2949.4 IU/L)	67.5%	51.2%	40.9%	75.9%	65%	50%	39.4%	74.1%
Progesterone (6.3 ng/mL)	27.5%	50%	21.5%	58%	100%	86.2%	78.4%	100%
Activin-A (417.2 pg/mL)	92.5%	87.5%	78.2%	95.9%	25%	53.7%	21.3%	58.9%
Activin-B (26.5 pg/mL)	80%	78.7%	65.3%	88.7%	35%	56.2%	28.6%	67.9%
Activin-AB (61.5 pg/mL)	92.5%	85%	75.5%	95.8%	95%	58.7%	53.5%	96.6%
Follistatin (720.6 pg/mL)	82.5%	82.2%	70.2%	90.4%	87.5%	52.5%	47.9%	89.3%

PPV = Positive predictive value

NPV = Negative predictive value

Table 5: The areas under the receiver-operating characteristic curve for each biomarker for the diagnosis of ectopic pregnancy (EP) and spontaneous abortion (SAB) in the cohort of women with β-hCG < 2000 IU/L (n = 36).

	EP AUC (95% CI)	**SAB AUC (95% CI)**
β-hCG (IU/L)	0.553 (0.360-0.746)	0.727 (0.550-0.905)*
Progesterone (ng/mL)	0.684 (0.486-0.833)	0.984 (0.952-1.000)**
Activin-A (pg/mL)	0.891 (0.782-1.000)**	0.617 (0.427-0.807)
Activin-B (pg/mL)	0.813 (0.666-0.959)*	0.591 (0.394-0.788)
Activin-AB (pg/mL)	0.956 (0.886-1.000)**	0.932 (0.851-1.000)**
Follistatin (pg/mL)	0.756 (0.581-0.931)*	0.641 (0.460-0.822)

AUC = Area under the curve

CI = Confidence interval

* = P < 0.05

** = P < 0.001

Table 6: The diagnostic performances for each biomarker for the diagnosis of ectopic pregnancy (EP) and spontaneous abortion (SAB) in the cohort of women with β-hCG < 2000 IU/L (n = 36).

Maker cut-off value	Diagnosis of EP				Diagnosis of SAB			
	Sensitivity	***Specificity***	***PPV***	***NPV***	***Sensitivity***	***Specificity***	***PPV***	***NPV***
β-hCG (1201 IU/L)	68.8%	50%	52.4%	66.7%	71.4%	77.3%	66.7%	80.9%
Progesterone (4.9 ng/mL)	93.8%	65%	68.2%	92.8%	92.9%	95.5%	92.8%	95.5%
Activin-A (427.4 pg/mL)	93.8%	80%	78.9%	94.1%	71.4%	68.2%	58.8%	78.9%
Activin-B (27.7 pg/mL)	87.5%	70%	70%	87.5%	57.1%	63.6%	50%	70%
Activin-AB (61.5 pg/mL)	93.8%	90%	88.2%	94.7%	92.9%	72.7%	81.2%	94.1%
Follistatin (672.7 pg/mL)	68.8%	70%	64.7%	73.7%	64.3%	54.5%	47.3%	70.6%

PPV = Positive predictive value

NPV = Negative predictive value