Combined Maternal Serum Inhibin A and Embryonic/ Fetal Heart Rate for the Prediction of Pregnancy Outcome in a First-Trimester Threatened Abortion†

Vorapong Phupong MD*,
Tharangrut Hanprasertpong MD*,**

† *This abstract was presented at the XIX FIGO World Congress of Gynecology & Obstetrics, Cape Town, South Africa*
** Department of Obstetrics and Gynecology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand*
*** Department of Obstetrics and Gynecology, Faculty of Medicine, Prince of Songkla University, Songkhla, Thailand*

Objective: *To examine the value of combined maternal serum inhibin A and embryonic/ fetal heart rate to predict the pregnancy outcome in a first-trimester threatened abortion.*
Materials and Method: *This was a prospective observational study. The authors measured maternal serum inhibin A and the embryonic/ fetal heart rate in women with a clinical diagnosis of a threatened abortion and in normal pregnant women. The main outcome measured was ongoing normal pregnancies.*
Results: *Thirty women with threatened abortions and 30 normal pregnant women were followed. Three women with threatened abortions ended in failed pregnancies. The mean embryonic/fetal heart rate and the median of serum inhibin A in the threatened abortion group were not different from the control group. In women with threatened abortions and failing pregnancies, the embryonic/fetal heart rate (101.7 $\pm$ 20.1 beats/min) was significantly lower than in women with threatened abortions but ongoing pregnancies (163.3 $\pm$ 19.7 beats/min, p = 0.024). Serum inhibin A in women with threatened abortions and failing pregnancies was not different from women with threatened abortions but ongoing pregnancies (median) 274.0 vs. 559.9 pg/mL, p = 0.388). When using serum inhibin A combined with embryonic/fetal heart rate, or only embryonic/fetal heart rate, the sensitivity and specificity for predicting an ongoing pregnancy were 100% and 50% or 100% and 100%, respectively.*
Conclusion: *Combined maternal serum inhibin A and embryonic/fetal heart rate is not better than embryonic/fetal heart rate for predicting the pregnancy outcome in a first-trimester threatened abortion.*

Keywords: *Serum inhibin A, First trimester, Fetal heart rate, Threatened abortion, Prediction, Outcome*

J Med Assoc Thai 2011; 94 (5): 529-34
Full text. e-Journal: *http://www.mat.or.th/journal*

A threatened abortion is a common condition in obstetrics. The clinical presentation is vaginal bleeding during early pregnancy. Patients and their physicians are anxious about the viability of a pregnancy, but there is no effective marker to predict in advance the outcome[1]. In fact, the only available tool is the measurement of serum hCG, but it does not give any predictive value[2].

Inhibins are glycoproteins produced by the granulosa and theca cells of the ovary and by the sertoli cells of the testis. Inhibin is a dimeric disulfide-linked glycoprotein molecule consisting of α and β subunits[3]. All inhibins share a common α subunit (18 to 20 kd). Depending on the type of β subunit (βA [13 kd] and βB [15 kd]), inhibins are classified as inhibin A or B. Apart from inhibin A or B, these subunits are also present in circulation in large dimeric or monomeric forms[4]. Serum inhibin A can be measured in maternal serum in significant concentration during pregnancy. The level increases as the gestational age advances because inhibin A is produced from human placenta and fetal membranes[5,6]. The precise spectrum of the function of these hormones in pregnancy has not been fully delineated. Abnormal levels have been noted in association with maternal complications of pregnancy, such as miscarriage, preeclampsia, and preterm labor. There are studies demonstrating that maternal serum inhibin A levels in women presenting with signs and symptoms of miscarriage (abdominal pain and/or vaginal bleeding) were significantly lower in women

Correspondence to:
Phupong V, Department of Obstetrics and Gynecology, Faculty of Medicine, Chulalongkorn University, Rama IV Rd, Pathumwan, Bangkok 10330, Thailand.
Phone: 0-2256-4241, Fax: 0-2254-9292
E-mail: vorapong.p@chula.ac.th

who had a miscarriage confirmed, whether it was complete or incomplete, when compared to women who had an ongoing viable pregnancy[7-9].

Cardiac function is the first activity that can be explored with non-invasive techniques such as Doppler ultrasound[10]. Previous studies demonstrated that the embryonic heart rate is about 100-200 beats per minute (bpm) when it is first visible on a sonography at approximately 6 weeks' gestation[11]. The rate increases over the subsequent 2-3 weeks[11]. Recent studies suggested that when a slow embryonic/fetal heart rate is detected, the likelihood of a subsequent first-trimester abortion is increased[11,12]. In addition, there is a report of the association between a slow embryonic/ fetal heart rate and an adverse pregnancy outcome in first trimester threatened abortions[13].

However, there has been no report on the combined measurement of serum inhibin A and embryonic/fetal heart rate in women with threatened abortions. In this present study, the authors investigated whether the measurement of serum inhibin A combined with the embryonic/fetal heart rate provided a useful tool to predict a pregnancy outcome in patients with threatened abortions.

Material and Method

Subjects

The present study was a prospective observational study and included 30 normal pregnant women (controls) and 30 pregnant women with threatened abortions between 6^{+0} and 14^{+6} weeks according to their last menstrual period (LMP). They underwent antenatal care at Department of Obstetrics and Gynecology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand. Informed consent was obtained from each participant and the Ethics Committee of the Faculty had approved the present study. Inclusion criterions were (1) singleton gestation, and (2) accurate dating based on LMP and that the calculated gestational age (GA) by crown-rump length (CRL) measurement agreed. The exclusion criteria were multiple pregnancies, diabetes, hypertension, fetal anomaly, and chromosomal abnormalities.

Study procedures

In each subject, the gestational age was confirmed by the measurement of the fetal crown- rump length (CRL) with an Aloka model Prosound 5000 (Aloka Co., Ltd., Tokyo, Japan) ultrasound machine with a 7.5 MHz vaginal- or a 3.75 MHz abdominal transducer. CRL was measured from the cranial to the caudal end of the body with the fetus in a neutral position. The measurements of embryonic/ fetal heart rates were measured by M-mode of at least 10 regular cardiac cycles. The calculation of the heart rate was made by measuring the time interval of two cardiac cycles. An average of the values from two measurements was utilized for statistical analysis. After performing ultrasonography, five ml of blood sample was collected from each subject via vacuum tubes and the blood was allowed to clot. The samples were centrifuged at 3,500 rounds per minute (rpm) for 10 min. The sera were separated and stored at -80°C until assayed. Inhibin A was measured in duplicate using a commercial sandwich enzyme-linked immunosorbent assay (ELISA) kit (Diagnostic System Laboratories, Inc, TX, USA) according to the manufacturers' protocol. The limit of sensitivity for inhibin A was < 1 pg/ml. The inter- and intra-assay coefficients of variations were < 10%.

Embryonic/fetal heart rate and serum inhibin A were compared to previously published reference values for embryonic/fetal heart rate[14] and serum inhibin A[15]. The main outcome measured was an ongoing normal pregnancy.

Statistical analysis

Statistical analysis was performed with SPSS software package version 12.0 (SPSS Inc., Chicago, IL, USA). Clinical data were presented as mean $\pm$ standard deviation (SD) or median with interquartile range depending on their distribution in the study population. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) and relative risk (RR) with a 95% confidence interval were calculated. Continuous variables were compared with student t-test or Mann-Whitney U test while χ^2 test (or Fisher exact tests when appropriate) was used to compare categorical variables. $p < 0.05$ was considered statistically significant.

Results

The mean embryonic/fetal heart rate in the threatened abortion group was not different from the control group (Table 1). The median of serum inhibin A in the threatened abortion group was also not different from the control group (Table 1).

In women with threatened abortions and failing pregnancies, embryonic/fetal heart rate (101.7 ± 20.1 beats/min) was significantly lower than in women with threatened abortions but ongoing pregnancies (163.3 ± 19.7 beats/min, $p = 0.024$). But

serum inhibin A in women with threatened abortions and failing pregnancies was not different from in women with threatened abortions and ongoing pregnancies (median (interquartile range) 274.0 (162-608.3) vs. 559.9 (215.5-718.1) pg/mL, p = 0.388).

Embryonic/fetal heart rate and serum inhibin A were compared to previously published reference values of embryonic/fetal heart rate and serum inhibin A as shown in Table 2. When using serum inhibin A combined with the embryonic/fetal heart rate, the

Table 1. Clinical data of embryonic/fetal heart rate and maternal serum inhibin A in the study population

Parameter	Healthy controls (n = 30)	Threatened abortion (n = 30)	p-value
Maternal age (years)	27.4 ± 5.2	28.0 ± 5.1	0.845
Gravida	1.7 ± 0.8	1.8 ± 0.5	0.782
Gestational age (weeks)	9.8 ± 2.8	8.5 ± 1.9	0.297
Embryonic/fetal heart rate (beats/min)	162.8 ± 17.0	156.9 ± 27.2	0.330
median (interquartile range) of inhibin A (pg/mL)	359.5 (231.8-513.2)	522.1 (209.1-687.7)	0.220

Table 2. Data and outcome of 30 patients with threatened abortion

Case No.	Gestational age (weeks^{+days})	Embryonic/fetal heart rate (beats/min)	Serum inhibin A (pg/mL)	Outcome
1	7^{+2}	133	83.36^{+}	Normal pregnancy
2	10^{+1}	160	559.98	Normal pregnancy
3	10^{+2}	180	625.70	Normal pregnancy
4	6	119	302.89	Normal pregnancy
5	13^{+2}	155	135.36	Normal pregnancy
6	8	168	463.93	Normal pregnancy
7	13	164	484.15	Normal pregnancy
8	10^{+2}	191	574.42	Normal pregnancy
9	14^{+4}	153	190.24	Normal pregnancy
10	10^{+2}	177	1,025.77	Normal pregnancy
11	6	134	3.20^{+}	Normal pregnancy
12	12^{+5}	155	296.40	Normal pregnancy
13	6	83*	608.36	Failing pregnancy
14	8^{+1}	168	627.86	Normal pregnancy
15	14^{+1}	161	736.91	Normal pregnancy
16	7^{+3}	123*	162.08^{+}	Failing pregnancy
17	10	160	215.51	Normal pregnancy
18	10^{+1}	176	933.33	Normal pregnancy
19	5^{+1}	120	47.98	Normal pregnancy
20	13^{+2}	148	295.67	Normal pregnancy
21	10^{+5}	180	718.13	Normal pregnancy
22	11^{+1}	167	853.17	Normal pregnancy
23	10^{+1}	174	641.58	Normal pregnancy
24	9^{+1}	192	593.20	Normal pregnancy
25	10^{+1}	99*	274.01	Failing pregnancy
26	9	176	763.63	Normal pregnancy
27	8^{+2}	177	880.10	Normal pregnancy
28	10	184	677.60	Normal pregnancy
29	10^{+1}	174	480.50	Normal pregnancy
30	6^{+1}	120	45.70	Normal pregnancy

* Indicated value less than mean-2SD
$^{+}$ Indicated value less than 10 centile

Table 3. The prediciton of embryonic/fetal heart rate and serum inhibin A for ongoing pregnancies

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Embryonic/fetal heart rate	100	100	100	100
Serum inhibin A	92.6	50	92.6	50
Combined	100	50	93.1	93.3

sensitivity and specificity for the prediction of an ongoing pregnancy were 100% and 50%, respectively (Table 3). Whereas, using only the embryonic/fetal heart rate, the sensitivity, and specificity for the prediction of an ongoing pregnancy were 100% and 100%, respectively (Table 3).

Discussion

The present study demonstrates that combined embryonic/ fetal heart rate and maternal serum inhibin A is not better than embryonic/ fetal heart rate alone for the prediction of a pregnancy outcome in a first-trimester threatened abortion.

The present result was similar to previous studies that showed the likelihood ratio for a subsequent first- trimester abortion remaining elevated if a slow fetal heart was detected[12,13,16]. In contrast, pregnancies with rapid embryonic heart rates in the early first trimester have a good prognosis, with a high likelihood of a normal outcome[17].

Inhibin A has been suggested as a marker for fetal or placental abnormalities in pregnancy. In the first trimester, inhibin A has a role as a marker for early pregnancy viability. Its measurement might be able to predict the pregnancy outcome in cases of recurrent miscarriage and threatened abortion[4,7,18,19]. However, the present study found no difference in inhibin A between threatened abortions with failing pregnancies and ongoing pregnancies. This result was in contrast to Florio et al's study[18]. This difference may be due to the small number of threatened abortion patients with failing pregnancies in the present study. The authors' result was similar to Illingworth et al's study[9] that inhibin A levels were not different between threatened abortions with ongoing pregnancies and failing pregnancies. The present result was also similar to Segal et al's study[20] that inhibin A levels were not different between threatened abortion and normal pregnancy.

This present study demonstrated that using serum inhibin A combined with measuring embryonic/ fetal heart rate, the sensitivity and specificity for the prediction of an ongoing pregnancy were not better than when using only the embryonic/fetal heart rate. This result showed that the measurement of serum inhibin A combined with embryonic/fetal heart rate did not provide more benefit than using the embryonic/ fetal heart rate alone in predicting the pregnancy outcome in patients with threatened abortions.

In conclusion, combined embryonic/ fetal heart rate and maternal serum inhibin A is not better than embryonic/ fetal heart rate for the prediction of a pregnancy outcome in a first-trimester threatened abortion. Further study to include more women with threatened abortions and failing pregnancies should be conducted.

Acknowledgements

The authors would like to thank Dr. Sittisak Honsawek for technical assistance.

Potential conflicts of interest

This work is supported by grant number 196/2548 and RA2/2549 of the Ratchadapiseksompotch Fund, Faculty of Medicine, Chulalongkorn University.

References

1. Reis FM, D'Antona D, Petraglia F. Predictive value of hormone measurements in maternal and fetal complications of pregnancy. Endocr Rev 2002; 23: 230-57.
2. Lipscomb GH, Stovall TG, Ling FW. Nonsurgical treatment of ectopic pregnancy. N Engl J Med 2000; 343: 1325-9.
3. Burger HG. Inhibin, a member of a new peptide family. Reprod Fertil Dev 1989; 1: 1-13.
4. 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.
5. Muttukrishna S, George L, Fowler PA, Groome NP, Knight PG. Measurement of serum concentrations of inhibin-A (alpha-beta A dimer) during human pregnancy. Clin Endocrinol (Oxf) 1995; 42: 391-7.
6. Fowler PA, Evans LW, Groome NP, Templeton A,

Knight PG. A longitudinal study of maternal serum inhibin-A, inhibin-B, activin-A, activin-AB, pro-alphaC and follistatin during pregnancy. Hum Reprod 1998; 13: 3530-6.
7. 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.
8. Phipps MG, Hogan JW, Peipert JF, Lambert-Messerlian GM, Canick JA, Seifer DB. Progesterone, inhibin, and hCG multiple marker strategy to differentiate viable from nonviable pregnancies. Obstet Gynecol 2000; 95: 227-31.
9. Illingworth PJ, Groome NP, Duncan WC, Grant V, Tovanabutra S, Baird DT, et al. Measurement of circulating inhibin forms during the establishment of pregnancy. J Clin Endocrinol Metab 1996; 81: 1471-5.
10. Leiva MC, Tolosa JE, Binotto CN, Weiner S, Huppert L, Denis AL, et al. Fetal cardiac development and hemodynamics in the first trimester. Ultrasound Obstet Gynecol 1999; 14: 169-74.
11. Doubilet PM, Benson CB. Embryonic heart rate in the early first trimester: what rate is normal? J Ultrasound Med 1995; 14: 431-4.
12. Stefos TI, Lolis DE, Sotiriadis AJ, Ziakas GV. Embryonic heart rate in early pregnancy. J Clin Ultrasound 1998; 26: 33-6.
13. Chittacharoen A, Herabutya Y. Slow fetal heart rate may predict pregnancy outcome in first-trimester threatened abortion. Fertil Steril 2004; 82: 227-9.
14. Hanprasertpong T, Phupong V. First trimester embryonic/fetal heart rate in normal pregnant women. Arch Gynecol Obstet 2006; 274: 257-60.
15. Phupong V, Hanprasertpong T, Honsawek S. First trimester serum inhibin A in normal pregnant women. Arch Gynecol Obstet 2008; 277: 307-10.
16. Doubilet PM, Benson CB. Outcome of first-trimester pregnancies with slow embryonic heart rate at 6-7 weeks gestation and normal heart rate by 8 weeks at US. Radiology 2005; 236: 643-6.
17. Doubilet PM, Benson CB, Chow JS. Outcome of pregnancies with rapid embryonic heart rates in the early first trimester. AJR Am J Roentgenol 2000; 175: 67-9.
18. Florio P, Luisi S, D'Antona D, Severi FM, Rago G, Petraglia F. Maternal serum inhibin A levels may predict pregnancy outcome in women with threatened abortion. Fertil Steril 2004; 81: 468-70.
19. Al Azemi M, Ledger WL, Diejomaoh M, Mousa M, Makhseed M, Omu A. Measurement of inhibin A and inhibin pro-alphaC in early human pregnancy and their role in the prediction of pregnancy outcome in patients with recurrent pregnancy loss. Fertil Steril 2003; 80: 1473-9.
20. Segal S, Gor H, Correa N, Mercado R, Veenstra K, Rivnay B. Inhibin A: marker for diagnosis of ectopic and early abnormal pregnancies. Reprod Biomed Online 2008; 17: 789-94.

การใช้ระดับซีรัมอินฮิบินเอของมารดาร่วมกับอัตราการเต้นของหัวใจของตัวอ่อนในการทำนายผลลัพธ์ของการตั้งครรภ์ในภาวะแท้งคุกคามในไตรมาสแรก

วรพงศ์ ภู่พงศ์, ธารารัตน์ หาญประเสริฐพงษ์

วัตถุประสงค์: *เพื่อศึกษาถึงการใช้ระดับซีรัมอินฮิบินเอของมารดาร่วมกับอัตราการเต้นของหัวใจของตัวอ่อนในการทำนายผลลัพธ์ของการตั้งครรภ์ในภาวะแท้งคุกคามในไตรมาสแรก*

วัสดุและวิธีการ: *การศึกษานี้เป็นการศึกษาแบบไปข้างหน้า โดยการวัดระดับซีรัมอินฮิบินเอของมารดาร่วมกับวัดอัตราการเต้นของหัวใจของตัวอ่อนในหญิงตั้งครรภ์ที่ให้การวินิจฉัยว่าเป็นภาวะแท้งคุกคามและหญิงตั้งครรภ์ปกติ ผลลัพธ์หลักของการศึกษาคือการตั้งครรภ์ต่อไปที่ปกติ*

ผลการศึกษา: *พบมีหญิงตั้งครรภ์ที่ให้การวินิจฉัยว่าเป็นภาวะแท้งคุกคามจำนวน 30 รายและหญิงตั้งครรภ์ปกติจำนวน 30 ราย โดยที่หญิงตั้งครรภ์ที่ให้การวินิจฉัยว่าเป็นภาวะแท้งคุกคามจำนวน 3 รายสิ้นสุดลงด้วยการตั้งครรภ์ที่ล้มเหลว ค่าเฉลี่ยของอัตราการเต้นของหัวใจของตัวอ่อนและค่ามัธยฐานของระดับซีรัมอินฮิบินเอในหญิงตั้งครรภ์ที่ให้การวินิจฉัยว่าเป็นภาวะแท้งคุกคามไม่แตกต่างจากหญิงตั้งครรภ์ปกติ หญิงตั้งครรภ์ที่ให้การวินิจฉัยว่าเป็นภาวะแท้งคุกคามที่สิ้นสุดลงด้วยการตั้งครรภ์ที่ล้มเหลวมีค่าเฉลี่ยของอัตราการเต้นของหัวใจของตัวอ่อน (101.7 $\pm$ 20.1 ครั้งต่อนาที) ต่ำกว่าหญิงตั้งครรภ์ที่ให้การวินิจฉัยว่าเป็นภาวะแท้งคุกคามที่สิ้นสุดลงด้วยการตั้งครรภ์ที่ปกติ (163.3 $\pm$ 19.7 ครั้งต่อนาที, $p = 0.024$) ส่วนระดับซีรัมอินฮิบินเอในหญิงตั้งครรภ์ ที่ให้การวินิจฉัยว่าเป็นภาวะแท้งคุกคามที่สิ้นสุดลงด้วยการตั้งครรภ์ที่ล้มเหลวไม่แตกต่างจากในหญิงตั้งครรภ์ที่ให้การวินิจฉัยว่าเป็นภาวะแท้งคุกคามที่สิ้นสุดลงด้วยการตั้งครรภ์ที่ปกติ (ค่ามัธยฐาน 274.0 vs. 559.9 พิโคกรัมต่อมิลลิลิตร, $p = 0.388$) เมื่อใช้ระดับซีรัมอินฮิบินเอของมารดาร่วมกับอัตราการเต้นของหัวใจของตัวอ่อน หรือใช้อัตราการเต้นของหัวใจของตัวอ่อนอย่างเดียว พบว่าความไวและความจำเพาะต่อการทำนายผลลัพธ์ของการตั้งครรภ์เท่ากับร้อยละ 100 และ 50, หรือ 100 และ 100, ตามลำดับ*

สรุป: *การใช้ระดับซีรัมอินฮิบินเอของมารดาร่วมกับอัตราการเต้นของหัวใจของตัวอ่อนไม่ได้มีผลดีกว่าการใช้อัตราการเต้นของหัวใจของตัวอ่อนในการทำนายผลลัพธ์ของการตั้งครรภ์ในภาวะแท้งคุกคามในไตรมาสแรก*