Original Article

Diagnosis of foetal membrane ruptures: Placental alpha-microglobulin-1 to the rescue

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ABSTRACT

Context: Pre-labour rupture of membranes (PROM) is a common obstetric complication which presents a diagnostic challenge, especially in equivocal cases. Standard methods of diagnosis are limited by high false positives and negatives. This study compared the accuracy of a biomarker placental alpha microglobulin-1 (PAMG-1) with the traditional methods of diagnosis.

Objective: To compare the accuracy of PAMG-1 in cervicovaginal secretions with other standard methods in the diagnosis of PROM.

Materials and Methods: A longitudinal prospective study was conducted among women with symptoms and signs of PROM at the Lagos State University Teaching Hospital. Standard tests and PAMG-1 assay were compared with the reported final diagnosis at delivery. Descriptive analysis was done using the Statistical Package for Social Sciences (SPSS) version 19.

Results: A total of 140 consenting pregnant women were recruited in the study. An initial diagnosis of PROM was made in 67 patients (47.9%) using the standard methods of diagnosis whereas PAMG-1 immunoassay using Amnisure ROM test diagnosed PROM in 86 patients (61.4%). Upon review of patients' clinical records, 88 women (62.8%) had a final diagnosis of PROM. In the final analysis, PAMG-1 assay had a sensitivity of 97.7%, specificity 100%, PPV 100% and NPV 96.7%. The standard methods had a sensitivity 76.1%, specificity 92.2%, PPV 90.1% and NPV 70.4%. PAMG-1 had a highest accuracy of 98.6%, followed by nitrazine test 89.3%, pooling of liquor 83.5% and fern test 51.4%.

Conclusion: The immunoassay of PAMG-1 had a higher diagnostic accuracy and is recommended for the diagnosis of PROM.

Key words: Prelabor rupture of fetal membranes; placental alpha microglobulin-1; traditional diagnostic tests.

Introduction

Prelabour rupture of membrane (PROM) is one of the most common complications of pregnancy with a major impact on perinatal outcome.[1] PROM refers to the spontaneous rupture of foetal membranes before the onset of labour.[1] It can occur at any gestational age. Rupture of membrane occurring before 37 weeks of gestation is referred as pre-term PROM (PPROM) and that occurring after 37 weeks of gestation as term PROM.[1,2]

PROM is reported in 5–10% of all pregnancies. It occurs in 8–19% of term pregnancies and 1–5% of pre-term pregnancies.[3] Management of pre-term PROM is often problematic with the need to balance the risk of prematurity with that of prolonged latency, increased risk of infection and significant risk of foetal demise.[4-6] Early and accurate diagnosis of PROM allows gestational age-specific obstetric interventions aimed at optimising perinatal outcome and reducing the risk of serious complications.[6,7] However, a

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false positive diagnosis of PROM may lead to unnecessary interventions including hospitalisation, antibiotics and corticosteroid use, stimulation of labour and the problem of prematurity.[8]

The diagnosis of PROM has been mainly clinical.[8-10] Although, the theoretical gold standard for the diagnosis of PROM is the amnio-infusion of dye, it is invasive and impracticable in clinical settings.[9,10] Other traditional tests used in diagnosis include a demonstration of an alkaline pH of cervicovaginal discharge or fluid using nitrazine paper and/or microscopic ferning of cervicovaginal discharge. These tests, however, are associated with a significant number of false positive and false negative results.[8,10,11]

The diagnosis of PROM based on obvious egress of fluid from the cervical os, though considered a definitive diagnosis, is associated with 12–30% false negative results.[10] The accuracy or reliability of these tests reduces progressively with increasing passage of time since rupture.

The fern test has a sensitivity of 51.4% and specificity of 70.8% in non-labouring women.[11] It has a false positive rate of 5–30%, which may be due to secondary contamination with finger prints on the slide or with semen or cervical mucus.[11] On the other hand, it has a false negative rate of 5–12% which may be due to dry swabs, contamination with blood or heavy vaginal discharge.

The nitrazine test has a sensitivity and specificity of 90–97% and 16–70%, respectively.[10,11] It may cause a false negative result in 9.4% of rupture cases after 48 hours.[9] It is also associated with high false positive rate which may be secondary to cervicitis, vaginitis, alkaline urine and contamination with semen or antiseptic agents.

Thus, the above tests are limited by diagnostic accuracy, cost and technical ease. Because of this diagnostic dilemma, an array of rapid, minimally invasive tests based on biochemical markers in amniotic fluid has evolved over years. These markers include lactate, alpha fetoprotein (AFP), vaginal prolactin, foetal fibronectin, beta-subunit of human chorionic gonadotropin, insulin-like growth factor binding protein-1 and more recently placental-alpha-microglobulin-1 (PAMG-1).[12-14]

The assay for PAMG-1, a 34 kD glycoprotein, was recently introduced into clinical practise to improve on traditional methods available for the diagnosis of PROM. It is synthesised by decidual cells and found in a concentration of 2000–25000 ng/ml in amniotic fluid and only 0.05–2.0 ng/ml in maternal serum and cervicovaginal secretions.

Several studies have been performed for assessing the diagnostic ability and accuracy of these biochemical markers in the diagnosis of PROM, especially in equivocal cases compared with the traditional methods adopted over the years.[12-14] These studies, however, have been largely conducted among Caucasian parturient and are uncommon in our environment. Therefore, this study compared the immunoassay of PAMG-1 and standard methods in the diagnosis of PROM with the final diagnosis of PROM at delivery.

Materials and Methods

Study setting

This study was conducted at the Department of Obstetrics and Gynaecology, Lagos State University Teaching Hospital (LASUTH) between March and November 2014. It was a longitudinal, prospective study comparing standard methods of diagnosing PROM (speculum examination for egress or pooling of liquor, nitrazine paper and fern test) with PAMG-1 immunoassay in pregnant women between 28 and 42 weeks gestational ages who presented to the obstetric emergency and antenatal clinics with symptoms and signs of PROM. Ethical clearance was obtained from the Health Research and Ethics Committee of LASUTH. Exclusion criteria included bleeding per vagina, presence of chorioamnionitis, onset of labour, foetal distress, sexual intercourse and vaginal douching within 24 hours of presentation.

Study procedure

Patients who met the study criteria and gave informed written consent were recruited in the study. Initial evaluation included both the standard clinical assessment (visualisation of pooling in the posterior fornix, nitrazine and fern test) for rupture of membranes and test for PAMG-1 using AmniSure® ROM 2012 (International LLC, Boston, USA) a rapid bedside immunoassay kit which has been used to detect foetal glycoprotein, PAMG-1, in cervicovaginal secretions. History and physical examinations were conducted. Clinical examination included a sterile speculum examination to expose the posterior fornix and cervix.

With the patient in a dorsal position, an appropriate sized, sterile Cusco speculum was passed aided by a good light source. Visual assessment of the posterior fornix for pooling of fluid and/or obvious leakage from the cervical os was done. A strip of nitrazine paper was introduced into the secretions and observed for colour change from yellow to blue. A sterile cotton swab was used to collect same secretions which was smeared thinly on a glass slide and allowed to dry for 10 minutes, and ferning was confirmed under a microscope with lens magnification of 10 × by a second assessor who had no access to patient's clinical details.

The Dacron tipped swab, a component of the AmniSure® ROM test kit, was inserted into the vagina, according to the manufacturer's specification for 1 minute and then transferred into the vial containing the eluent for 1 minute. A test strip (also a component of the kit) was then placed in the solution, and the sample in the vial was allowed to migrate through the membrane by capillary action for not more than 5 minutes. The test was positive when there was presence of 2 lines – control and test lines. It was negative if only one line (control) was seen or invalid when no line was observed.

After delivery, each patient's clinical record was reviewed for their clinical course from initial diagnosis of prelabour rupture of foetal membranes. The true positives and negatives were determined definitively upon review of the medical records after delivery.

Evaluation of findings

The standard diagnostic method of diagnosing membrane rupture was defined as positive for two of the following three examination findings: visualization of pooling of fluid in the posterior fornix, positive nitrazine test or positive fern test. For this study, the final diagnosis was taken to be the demonstration of a positive pad test, scanty or no amniotic fluid and the absence of foetal membranes on vaginal examination at delivery.[15-17]

Data processing and analysis

Data collected was entered and analysed using the Statistical Package for Social Sciences (SPSS) (version 19 Chicago Illinois, USA). Percentages, means, median, interquartile range and standard deviation of numeric variables were determined. Percentages of categorical variables were also determined. Numeric variables were compared using the Student's *t*-test and Mann–Whitney U test depending on whether they were normally distributed. Chi-square, Fisher's exact and McNemar tests were used to compare categorical variables where appropriate. Sensitivity, specificity, positive and negative predictive values and kappa were determined for each diagnostic test for PROM and compared with the final diagnosis of PROM at delivery. Confidence interval was set at 95% for all statistical tests. A *P* value less than 0.05 was considered statistically significant. Microsoft excel was used to draw the charts.

Results

A total of 140 pregnant women who presented at the antenatal clinics, emergency room and labour ward within gestational ages of 28–42 weeks and complained of passage of watery substance per vaginam and who met the inclusion criteria and gave their consent were recruited in this study from March 2014 to November 2014. The demographic data and clinical findings of the participants are shown in Tables 1 and 2.

Pooling of liquor was seen in 52.1% (73/140) patients, 24.3% (34/140) had positive fern test, 59.3% (83/140) positive nitrazine test and 61.4% (86/140) positive for PAMG-1 immunoassay. An initial diagnosis of PROM was made in 67 (47.9%) women using the standard diagnostic methods compared with 86 (61.4%) using PAMG-1 immunoassay with the Amnisure ROM kit. Upon review of patients' case notes, 88 (62.8%) women had a final diagnosis of PROM (absence of foetal membranes on vaginal examination and/or positive pad test) whereas 52 (37.2%) had their membranes intact.

Table 1: Socio-demographic data of participants

Variable	Total (%) (*N*=140)
Age group	
Less than 24 years	16 (11.4)
25-34 years	110 (78.6)
35 years and above	14 (10.0)
Mean±SD	29.5±4.2
Booking status	
Booked	121 (86.4)
Unbooked	19 (13.6)
Parity	
Nulliparous	80 (57.1)
Multiparous	60 (42.9)
Stage in pregnancy at initial diagnosis	
Preterm	59 (42.1)
Term	81 (57.9)
Occupation	
Professional	36 (25.7)
Artisan	11 (7.9)
Trader/business	49 (35.0)
Civil servant	10 (7.1)
Student	11 (7.9)
Unemployed	23 (16.4)

Table 2: Summary of maternal variables

Variable	PROM *n*=88 (%)		NO PROM *n*=52 (%)	*t*	*P*
Age	29.8±4.5		29.1±3.7	0.840	0.403
Gestational age at Diagnosis (weeks)	36.7±3.3		36.9±3.1	0.364	0.717
Duration of hospital stay (days)	4.43±3.9		3.9±2.6	0.854	0.395
Duration of Membrane rupture (h)	***n***	**Median**	**IQR**	***U***	***P***
PROM	88	8.0	3-13.5	2219.00	0.765
No PROM	52	6.0	4-24.0		

IQR - Inter-quartile range; U, Mann–Whitney U-test; *n* - Number; *t* - *t*-test

Using the final diagnosis at delivery as the confirmation of PROM, PAMG-1 immunoassay had the highest sensitivity (97.7%), specificity (100.0%), positive predictive value (100%), negative predictive value (97.6%) and accuracy (98.6%) compared with nitrazine test (86.6%, 90.4%, 94.4, 82.5 and 89.3%, respectively), pooling of liquor (78.4%, 92.3%, 94.4%, 71.6% and 83.5%, respectively) and fern test (30.7%, 67.3%, 79.4%, 42.5% and 51.4%, respectively). Of the 88 PROM cases diagnosed at delivery, the proportion of PROM diagnosed using fern test 27 (30.7%) and pooling of liquor 69 (78.4%) differed significantly (McNemar's $P < 0.05$). However, the proportion of PROM cases diagnosed using nitrazine 78 (86.6%) and PAMG-1 86 (97.7%) were not statistically significant (McNemar's $P > 0.05$). The extent to which the diagnosis of PROM at delivery and the various tests improved on chance agreement was excellent for PAMG-1 (kappa = 96.9%), good for pooling of liquor (kappa = 66.7%) and nitrazine test (kappa = 77.5%) and negligible for fern test (kappa = 14.1%), as shown in Tables 3 and 4.

Discrepancies in results between PAMG-1 and the conventional diagnostic methods were seen in 21 patients. PAMG-1 initially diagnosed PROM in 19 women, however, the conventional methods did not confirm PROM. Comparison with final diagnosis based on the absence of foetal membranes in labour and/or pad test confirmed the diagnosis of PROM in all 19 patients. In 2 patients even though PAMG-1 made an initial assessment of absence of PROM, the conventional methods diagnosed PROM. The discrepancies were sorted out using findings at final diagnosis and were confirmed to have their membranes intact at labour.

Table 3: Comparison of diagnosis of PROM using pooling of liquor, nitrazine test, fern test and PAMG-1 with the final diagnosis at delivery

Test used to diagnose PROM	Final diagnosis at delivery			Kappa	*P*
	PROM *n*=88(%)	NO PROM *n*=52(%)	Total *n*=140(%)		
Pooling of liquor					
PROM	69 (78.4)	4 (7.7)	73 (52.1)	0.667	0.003
No PROM	19 (21.6)	48 (92.3)	67 (47.9)		
Nitrazine test					
PROM	78 (86.6)	5 (9.6)	83 (59.3)	0.775	0.302
No PROM	10 (11.4)	47 (90.4)	57 (40.7)		
Fern test					
PROM	27 (30.7)	7 (13.5)	34 (24.3)	0.141	<0.001
No PROM	61 (12.5)	45 (67.3)	106 (75.7)		
PAMG – 1					
PROM	86 (97.7)	0 (0.0)	86 (61.4)	0.969	0.500
No PROM	2 (2.3)	52 (100.0)	54 (38.6)		

N/B *P*=*P* value for McNemar's test

Discussion

The main finding of this study was the demonstration that PAMG-1 immunoassay is more accurate than the conventional diagnostic methods (pooling of liquor, nitrazine and ferning tests) whether when used in combination or individually. It had a sensitivity, specificity, positive predictive value and negative predictive value of 97.7%, 100%, 100% and 97.6%, respectively for the diagnosis of rupture of foetal membranes.

This result agrees with the findings of the studies done in the USA, Malaysia and South-eastern Nigeria. Cousins *et al*. documented that PAMG-1 had sensitivity, specificity, PPV and NPV of 98.8%, 100%, 100% and 99.1%, respectively, in the diagnosis of PROM compared with standard tests. This finding was similar to 97.6%, 100%, 100% and 88.2%, respectively, documented by Eleje *et al*.[15-18] The assay of PAMG-1 was more accurate than the standard diagnostic methods because of its higher concentration (2000–25000 ng/ml) in the amniotic fluid compared with other physiologic fluids including maternal blood and vaginal secretions (0.05–0.22 ng/ml), and its absence in urine and semen.[3] Its concentration is increased in vaginal secretions with rupture of membranes. The Amnisure ROM immunoassay for PAMG-1 has a lower limit of detection of 5 ng/ml in cervicovaginal secretions, and may therefore, be more sensitive to detect subclinical PROM from small perforations (which may account for equivocal cases), which subsequently reduces the chances of obtaining false positive or negative results. Moreover, because PAMG-1 is absent in urine and semen and is not affected by blood (up to 50%)[19] in cervicovaginal secretions (as is often seen in some cases of PROM especially before the onset of labour), this further enhances its diagnostic accuracy.

Table 4: Summary of the performance matrix of diagnostic methods studied

Test	Y/N	SEN (%)	SPEC (%)	PPV (%)	NPV (%)	ACC (%)	KAPPA (%)
Standard methods	67/50	76.1	96.2	97.1	70.4	83.6	67.2
Pooling of liquor	69/48	78.4	92.3	94.5	71.6	83.5	66.7
Fern test	27/45	30.7	67.3	79.4	42.5	51.4	14.1
Nitrazine test	78/47	86.6	90.4	94.0	82.5	89.3	77.5
PAMG-1	86/2	97.7	100	100	97.6	98.6	96.9

SEN - Sensitivity; SPEC - Specificity; PPV - Positive predictive value; NPV - Negative predictive value; ACC - Accuracy; PAMG-1 - Placental alpha microglobulin-1

Poor sensitivity (high proportion of false negatives) with fern test may result from dry swabs at testing as may be seen in cases of small rupture or longer duration of rupture prior to presentation with very scanty or absent vaginal fluid on examination. In this study, the median duration of a history of membrane rupture among women with final diagnosis of PROM was 8 hours prior to presentation and diagnosis, and this may account for the lower performance index documented. Nitrazine test had a higher sensitivity than pooling of liquor and fern test in this study. However, it had a higher proportion of false positives compared to pooling of liquor. The difference may be adduced to the fact that the test is based on the detection of an alkaline pH in vaginal secretions which can be affected by the presence of urine, semen, cervical or vaginal infection with alkaline pH giving a false positive result. However, the chance of this occurring was reduced because of the exclusion selection criteria adopted in this study.

PAMG-1 had the highest diagnostic accuracy compared with the traditional tests in the diagnosis of PROM in this study. This result was comparable with the findings by Eleje *et al*. They documented a diagnostic accuracy of 98.0%, 91.8%, 82.8% and 70.7% for PAMG-1, nitrazine, pooling of liquor and fern test, respectively.[16]

Comparing the outcome of standard methods (combination of speculum, nitrazine and fern tests) with PAMG-1, the latter had a better performance. It is known that the diagnosis of PROM based on history and clinical examination can be adequate in cases with obvious drainage of liquor.[20,21] The challenge of diagnosis arises in cases where drainage of liquor is suspected or equivocal. A significantly higher proportion of PROM cases were correctly diagnosed using PAMG-1. The difference may arise from the ability of the Amnisure test to detect very small quantities of PAMG-1 in vaginal secretions which may not be detectable by the standard method in the absence of a visible leakage or accumulation of amniotic fluid in the posterior fornix.

The detection of PAMG-1 using Amnisure in the diagnosis of PROM in this study yielded 1.43% false negative results when compared with the final diagnosis. This finding was slightly higher than 0.49% false negative results documented by Cousins *et al*.,[15] however, this finding was better than 3.79% observed by Ng *et al*.[17] The reason for the difference observed may be the retesting of false negatives by Cousins *et al*. which could have minimised the degree of error.

The kappa ratio which determines the extent to which the agreement between two methods improved on chance was the highest for PAMG-1 in this study. The significance of this is that the higher the accuracy of PAMG-1 over the traditional diagnostic approach in the diagnosis of PROM was beyond a chance finding because the strength of agreement was excellent at 0.969.

The PAMG-1 immunoassay provides a quality diagnostic tool that is rapid, accurate, with higher sensitivity and specificity compared to the other methods used currently.

The immunoassay of PAMG-1 using AmniSure has the advantage of not requiring the insertion of a speculum for testing compared to conventional methods, and thus, would be more acceptable to women as it is less intrusive. It also serves as a single test that can help in determining and establishing the correct diagnosis, especially when the diagnosis of PROM is inconclusive.

The lack of a non-invasive gold standard test and reliance on clinical demonstration of absence of foetal membranes on vaginal examination, and/or positive pad test or scant or no liquor at delivery as a final diagnosis of PROM was a limitation in this study.

The diagnosis of PROM based on the detection of PAMG-1 in cervicovaginal secretions using Amnisure (a rapid, non-instrumental, qualitative immuno-chromatographic test) is highly accurate. Its performance is better than that of the conventional methods (pooling of liquor, nitrazine test and fern test) both individually and in combination. Its use as a diagnostic tool will thus improve the clinician's confidence in the correct diagnosis and institution of appropriate treatment options of prelabor rupture of membranes.

PAMG-1 assay should be recommended for the diagnosis of PROM at all levels of care as it has demonstrated a higher level of accuracy. PAMG-1 assay using Amnisure should be used as a first-line diagnostic method for PROM in women who can afford it and as a second-line for women with inconclusive diagnosis with standard methods who cannot readily afford it. There is a need for a larger sample-sized study to reinforce the findings of this study.

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Conflicts of interest
There are no conflicts of interest.

References

1. Cunningham FG, Leveno KJ, Bloom SL, Hauth JC, Rouse DJ, Spong CT. Management of Preterm labor. Williams Obstetrics. 23rd ed. New York:

McGraw-Hill; 2010. p 232-47.
2. ACOG. Committee on Practice Bulletins- Obstetrics a. Clinical management for Obstetricians and Gynaecologists. (ACOG Practice Bulletin No. 80: Premature rupture of membranes). Obstet Gynaecol 2007;109:1007-19.
3. Gian CR, LLius CR, Fabio F, Aris A, Gregor B, Eduard G, *et al*. Guidelines for the management of spontaneous preterm labour: Identification of spontaneous preterm labour, diagnosis of preterm premature rupture of membrane and preventive tools for preterm births. J Matern Fetal Neonatal Med 2011;24:659-67.
4. Liu J, Feng ZC, Wu J. The incidence rate of PROM and its influence on fetal–neonatal Health. A report from Mainland China. J Trop Paediatr 2010;56:36-42.
5. Shen TT, Defranco EA, Stamillo DM, Chang JJ, Muglich LL. A population based study of race specific risk for preterm premature rupture of membrane. Am J Obstet Gynecol 2008;199:373e1-e7.
6. Medina TM, Hill DA. Preterm premature rupture of membranes. Diagnosis and management. Am Fam Physician 2006;73:659-64.
7. Wu J, Feng ZC, Huang J, Wu G. Influence of premature rupture of membranes on neonatal health. Zhonghua Er Ke Za Zhi 2009;47:452-6.
8. Ananth CV, Oyelese Y, Srinivas N, Yeo L, Vintzileos AM. Preterm premature rupture of membrane, intrauterine infections and oligohydramnios: Risk factor for placental abruption. Obstet Gynecol 2004;104:71-7.
9. Bahasadri S, Kashaman M, Khalili S. Evaluation of vaginal fluid beta-human chorionic gonadotropin for the diagnosis of preterm premature rupture of membrane. J Obstet Gynecol Res 2013;39:777-82
10. Bornstein J, Geva A, Solt I, Fait V, Schoenfeld A, Shoham HK. Non-intrusive diagnosis of premature rupture of amniotic membranes using a novel polymer. Am J Perinatol 2006;23:351-4.
11. Erdmorglu E, MuganT. Significance of detecting Insulin-like growth factor binding protein-1 in cervicovaginal secretions. Comparison with nitrazine test and amniotic fluid volume assessment. Acta Obstet Gynaecol Scand 2005;83:622-6.
12. De Haan HH, Offerman PM, Smits F, Schouten HJ, pee teers LL. Value of the fern test to confirm or reject the diagnosis of ruptured membranes made in non-laboring women presenting with non-specific vaginal loss. Am J Perinatol 1994;11:46-50.
13. Larry MC, Dorothy PS, Stuart ML, Deborah MP. Amnisure Placental Alpha Microglobulin-1 rapid immunoassay versus standard diagnostic methods for detection of rupture of membrane. Am J Perinatol 2005;22:1-4.
14. Mulhair L, CarterJ, Postin L, Seed P, Briley A. Prospective cohort study investigating the reliability of Amnisure method of detection of spontaneous rupture of membrane. BJOG 2009;116:313-8.
15. Cousin LM, Smok DP, Lovett SM, Poetler DM. Amnisure placental alpha microglobulin-1 rapid immunoassay versus standard diagnostic methods for the detection of rupture of membrane. Am J Perinatol 2005;22:317-20.
16. Eleje GU, Ezugwu EC, Ogunyemi D, Okonkwo JE, Ikpeze OC, Igwegbe AO. The accuracy and Cost effectiveness of Amnisure ROM Test in the diagnosis of premature rupture of fetal membrane: A preliminary Report from Nigeria 2012. Unpublished.
17. Beng KN, Pei SN, Mohamad NS, Nur Azura AG, Nor Azlin MI, Mohd HO, *et al*. Comparison between Amnisure Placental Alpha Microglobulin-1 Immunoassay and Standard diagnostic methods for detection of rupture of membranes. BJOG 2012;119:2-25.
18. Birkenmaier A, Ries JJ, Kuhle J, Burki N, Lapaire O, Hosli I. Placental alpha-microglobulin-1 to detect uncertain rupture of membrane in European cohort of pregnancies. Arch Gynecol Obstet 2012;285:21-5.
19. Wilfong L. Effects of maternal blood on the readability and reliability of amnisure rapid immunoassay. Med Educ Foundation An Coll Osteopathic Obstet Gynaecol Winter Newslett 2009;30:11-2.
20. Lockwood CJ, Oner C, Uz YH, Kayasli UA, Huang SJ, Buchwalder LF, *et al*. Matrix metalloproteinase 9 (MMP-9) expression in preeclamptic decidual and MMP-9 induction by tumor necrosis factor and interleukin-1 beta in human first trimester decidual cells. Biol Reprod 2008;78:1064-72.
21. Mercer BM, Abdelrahim A, Moore RM, Novak JJ, Kumar D, Mansoor JM, *et al*. The impact of vitamin C supplementation in pregnancy and in-vitro upon fetal membranes strength and remodelling. Reprod Sci 2010;17:685-95.