

# Detection of Prom using Strip Immuno Assay Test to Detect Insulin Like Growth Factor Binding Protein-1(IGFBP-1) in Amniotic Fluid in Comparison with Fern Test

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## ABSTRACT

**Introduction:** PROM is an emergency obstetric event necessitating urgent intervention with variable outcome. Leaking of amniotic fluid has manifold obstetric implications depending upon when it occurred? at what gestation? duration of leak and colour of liquor. Study objective included evaluation of methods of detection of PROM and comparing effectiveness of two methods.

**Material and methods:** This study was a Prospective and comparative study conducted on 100 Antenatal women reported with the history of PROM to Government Maternity Hospital, Sulthan Bazaar, Osmania Medical College, Hyderabad from January 2014 to August 2015.

**Results:** The present study of 100 women with PROM, 66 cases had liquor draining clinically and 34 cases were with no obvious draining of liquor clinically. Among these 34 cases, 26 had intact membranes and 8 were without intact membranes. So total cases with Ruptured membranes are 74 and with Unruptured membranes were 26. Among the 74 cases with ruptured membranes APT is Positive in 67 cases and negative in 7 cases and Fern test is Positive in 33 cases, negative in 41 cases. P value is <0.000001 which is highly significant.

**Conclusion:** Our study shows that APT is more efficacious than FERN test in diagnosing PROM.

**Keywords:** PROM - Premature Rupture of Membranes, APT- Actimprom Test, PPV - Positive Predictive Value, NPV - Negative Predictive Value, DA - Diagnostic Accuracy

## INTRODUCTION

Spontaneous rupture of foetal membranes prior to the onset of labour, commonly known as premature rupture of membranes (PROM). Also known as prelabour rupture of membranes.

Preterm prom (PPROM) is defined as rupture of fetal membranes prior to 37 wks of gestation.

Approximately 8% to 10% term pregnancies will experience spontaneous rupture of membranes prior to the onset of uterine activity.<sup>1</sup>

PPROM complicates 2-4% of all singleton and 7-20% twin pregnancies.<sup>1</sup> PROM is responsible for approximately a third of all premature births and accounts for 18-20% of prenatal deaths and is associated with increased risk of ascending infection.

Histologic studies of the site of membrane rupture at term have demonstrated a zone of altered morphology characterized by thickening of the connective tissue components of the membranes, thinning of the cytotrophoblast layer and decidua, and disruption of the connections between amnion and chorion. These normal physiologic changes accompany cervical ripening in preparation for labour at term, and result in focal weakening of the fetal membranes in the region of the internal cervical os

that predisposes to rupture at that site. At a cellular level, these changes result from the release of phospholipases, eicosanoids (especially prostaglandin E<sub>2</sub>), cytokines, elastases, matrix metalloproteinases, and/or other proteases in response to a physiologic or pathologic stimulus.<sup>2</sup> Although the downstream cellular changes may be similar, the inciting etiologies in preterm PROM are likely different from term PROM. A number of risk factors for spontaneous PROM have been identified.<sup>1,3</sup>

## Risk factors for premature rupture of membranes

### Maternal factors:

1. PROM in prior pregnancy (16-32% as compared with 4% in women a prior uncomplicated term delivery)
2. Antepartum vaginal bleeding
3. Chronic steroid therapy
4. Collagen vascular disorders eg:Ehlerdanlos syndrome, systemic lupus erythematosus
5. Preterm labor
6. Cigarette smoking
7. Abdominal trauma
8. Illicit drugs
9. Anaemia
10. Low body mass index
11. Nutritional deficiencies
12. Low socioeconomic status

### Uteroplacental factors

1. Uterine anomalies
2. Placental abruption
3. Advanced cervical dilatation
4. Prior cervical conisation
5. Cervical shortening
6. Uterine overdistension
7. Intraamniotic infection
8. Multiple bimanual vaginal examinations

### Fetal Factors

Multiple pregnancy

### Differential Diagnosis

The differential diagnosis includes leakage of urine (urinary

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**How to cite this article:** Ushalatha Chalurkar, Ratnam Andallu. Detection of Prom using strip immuno assay test to detect insulin like growth factor binding protein -1(IGFBP-1) in amniotic fluid in comparison with fern test. International Journal of Contemporary Medical Research 2017;4(5):1118-1121.

incontinence); excessive vaginal discharge, such as physiologic discharge or bacterial vaginosis; and cervical mucus (show) as a sign of impending labour.

### Diagnosis

PROM is largely a clinical diagnosis. It is typically suggested by a history of watery vaginal discharge and confirmed on sterile speculum examination. The traditional minimally invasive gold standard for the diagnosis of ROM relies on clinician ability to document 3 clinical signs on sterile speculum examination:

1. Visual pooling of clear fluid in the posterior fornix of the vagina or leakage of fluid from the cervical os;
2. An alkaline pH of the cervicovaginal discharge, which is typically demonstrated by seeing whether the discharge turns yellow nitrazine paper to blue (nitrazine test); and/or
3. Microscopic ferning of the cervicovaginal discharge on drying.

Reliance on clinical assessment alone leads to both false-positive and false-negative results. For example, the nitrazine test is designed only to confirm an alkaline pH in the cervicovaginal secretions (the pH of the vaginal secretions is generally 4.5–6.0, whereas amniotic fluid usually has a pH of 7.1–7.3), and yet it is the most common test used to diagnose PROM. It is associated with high false-positive rates related to cervicitis, vaginitis (bacterial vaginosis), and contamination with blood, urine, semen, or antiseptic agents.<sup>4,5</sup> As such, the sensitivity and specificity of this test in diagnosing PROM ranges from 90% to 97% and 16% to 70%, respectively.<sup>6,7</sup>

**Fern test:** Amniotic fluid crystallisation, created primarily by the sodium chloride and protein content, began to dominant cytologic stains, with reported accuracies in clinically ruptured cases ranging from 73% to 98.5%. Fluid for examination should be aspirated from the vagina, spread on a slide and allowed to dry and then examined under lowpower microscope for ferning pattern. False positive results are due to fingerprints, semen and cervical mucus. False-negative results are due to technical error and contamination with blood.<sup>8,9</sup> Reported sensitivity and specificity for the fern test are 51% and 70%, respectively, in patients without labour and 98% and 88%, respectively, in patients in labor.<sup>10</sup> The results of Fern test are viewed as supportive rather than conclusive for non labouring women with non specific fluid loss.

Combination of a positive history, a positive nitrazine test, fluid crystallization, or Nile blue sulphate staining produced a diagnostic accuracy of 93.1%.

Other methods of detection of PROM include Ultrasound assessment of amniotic fluid volume.<sup>11</sup>

### Tests for the Diagnosis of PROM by detecting marker Proteins

Most commonly used are Actim prom test designed to detect insulin like growth factor binding protein -1 (IGFBP-1) and Amnisure which detects the presence of placental alpha macroglobulin -1 (PAMG-1). Other tests are ROM check test for detection of fFn and test for detection of Alphafeto protein, vaginal prolactin.

#### 1. ACTIM PROM Test:

Also known as AMNI test. It detects IGFBP-1 as a marker of amniotic fluid. Insulin like growth factor binding protein

1(IGFBP-1) is synthesized by the decidua and fetal liver and is a major protein in amniotic fluid and is detected throughout pregnancy. Although the serum concentration of IGFBP-1 increases with gestational age its concentration in amniotic fluid is 100-1000 fold higher than that in maternal serum, and the concentration in vaginal secretion is very low unless fetal membranes have ruptured.<sup>1</sup> IGFBP-1 is useful for the diagnosis of rupture of membranes. It is performed with a dipstick diagnostic device on extracted vaginal specimen. Specimen collected with or without speculum. The test principle is based on immunochromatography. Two monoclonal antibodies to human IGFBP-1 are used. In the dipstick one antibody (clone#6350) is immobilized on blue Latex particles (the detecting label), and the other (clone#6303) is bound to the membranes to act as a catching line. The cut off value of the test is 25mcg/l<sup>3</sup>. It can be performed in all gestational ages. Positive test is shown by two blue lines on dipstick, if one blue line is seen or no blue line seen then the test is negative. Vaginal discharge, urine or seminal fluid have no effect on the performance of Actimprom test. On the other hand presence of vaginal bleeding may give a positive result due to presence of IGFBP-1 in the blood. Cost of the test kit is 500 rupees.

2. AmniSure ROM test:<sup>12</sup>

3. ROM check test<sup>13</sup>

4. Detection of alpha-feto protein

5. Vaginal prolactin test

6. Microscopic fetal cell identification<sup>14</sup>

Study aimed, to know the efficacy of detection of PROM by strip immunoassay test [Actim PROM test ] by detecting insulin like growth factor binding protein1(IGFBP-1) in Amniotic fluid in comparison with fern test; with the objectives of evaluation of methods of detection of PROM and comparing effectiveness of two methods.

## MATERIAL AND METHODS

This study was a Prospective and comparative study conducted on 100 Antenatal women reported with the history of PROM to Government Maternity Hospital, Sulthan Bazaar, Osmania Medical College, Hyderabad from January 2014 to august 2015 after taking the ethical clearance and informed consent from the patients.

A sterile speculum examination was performed but digital examination was not done. In all cases Actimprom test and fern test were performed.

**Actimprom test:** Sample of leaking fluid was taken by speculum examination by keeping a poly ester swab in posterior vaginal fornix for about 10 seconds, and the swab is then rinsed in a buffered solution of Actimprom test, after rinsing the swab in buffered solution for about 5 seconds, yellow area of the dipstick provided in the kit is placed in the tube for 20 seconds then removed and placed on a flat surface. The stick contains monoclonal antibodies to IGFBP-1, and absorbs the extracted specimen. If the leaking fluid contains IGFBP-1 in the extracted sample, two blue lines will appear on the stick. It means test is positive, if no blue line is seen or one blue line is seen upto 5 min then the test is negative.

**Fern test:** Sample of leaking fluid was taken by speculum examination with a swab placed in posterior vaginal fornix and

spread on a slide. Then the slide dried and after drying examined under low power microscope for crystallisation of amniotic fluid to form fern like pattern which is considered as positive test.

The diagnostic value of the tests were determined by calculating sensitivity, specificity, PPV, NPV and Diagnostic accuracy.

Labour was actively managed in term pregnancies and induced if rupture of membranes had occurred, regardless of test results.

In these cases labour was induced after 12 hrs of observation for spontaneous onset.

## STATISTICS ANALYSIS

Sensitivity, specificity, PPV, NPV, DA were used to compare the efficacy of two tests. Chi square test was used with  $p < 0.05$  considered statistically significant.

## RESULTS

Table 1 shows that among the 74 cases with ruptured membranes APT is Positive in 67 cases and negative in 7 cases and Fern test is Positive in 33 cases, negative in 41 cases. P value is  $<0.000001$  which highly significant.

Table 2 shows that among the 26 cases with Unruptured membranes, APT is positive in 9 cases, negative in 17 cases and Fern test is positive in 6 cases and negative in 20 cases.

Table 3 shows that 'p' value for APT test for different colour of liquor was 0.2 and for Fern test chi square value is 29.96 and p value is 0.001 for clear liquor cases. It means APT diagnoses PROM irrespective of colour of liquor and diagnosis by Fern test depends on colour of liquor, better in clear liquor

Test	Positive	Negative	P value
APT	67	7	$<0.000001$ Chisquare value 35.65
Fern test	33	41	

**Table-1:** Comparison of APT and FERN test among cases with Ruptured membranes

Test	Positive	Negative	P Value
APT	09	17	P Value 0.35 Chi square value 0.8432
Fern test	06	20	

**Table-2:** Comparison of APT and Fern test among cases with Unruptured membranes

Colour of liquor	APT		FT	
	Positive	Negative	Positive	Negative
Clear (n=40)	37	3	29	11
Msl (n=23)	20	3	1	22
Blood stained (n=3)	2	1	0	3
P value	0.2		0.001	

**Table-3:** Comparison of APT and FERN test in different colour of liquor

	APT		Fern test	
	Positive (%)	Negative (%)	Positive(%)	Negative (%)
<5hrs(n=64)	55	9	23	41
>/=5hrs(n=36)	25	11	8	28
P value	0.02		0.07	
Chi square value	3.917		2.026	

**Table-4:** Duration of PROM while reporting

cases

Table 4 shows that APT is positive irrespective of duration of prom while reporting but fern test is not.

## DISCUSSION

The study was conducted at Government Maternity Hospital, Sultan Bazar, with the aim to detect efficacy of Actimprom test in comparison with Fern test in detection of PROM.

PROM is a frequent diagnostic and therapeutic dilemma in obstetrics. If the patient presents with a history of gush of fluid leakage, the diagnosis is made by physical examination. In uncertain cases, the major contaminants of amniotic fluid which have to be considered in the diagnosis or exclusion of ruptured membranes are blood, urine, cervical mucus, vaginal discharge and seminal fluid. The method reliable for demonstrating the presence of amniotic fluid in the vagina should therefore accurately discriminate between amniotic fluid and the non specific interferences. The tests measuring IGFBP-1 in cervical/vaginal secretions are useful in this respect, since IGFBP-1 levels in amniotic fluid far exceed those in interfering body fluids.

In our study among the 100 PROM cases, clinically liquor draining cases were 66, cases without draining liquor were 34; among the 34 cases, 26 cases had intact membranes and 8 were without intact membranes. So the cases with ruptured membranes are 74 and with Unruptured membranes were 26.

In our study 100 PROM cases were divided into 2 groups depending upon the presence or absence of intact membranes. In our study among the 100 cases, APT as well as FERN test were positive in most of the cases with PROM of  $<5$ hrs duration. Among the cases with PROM of  $\geq 5$  hrs duration APT was positive in more no of cases compared to FERN test. Chi square value for APT is 3.917, 'p' value is 0.02 which is significant and for FERN test, chi square value is 2.026, 'p' value is 0.07 ( $>0.05$ ), so it's not significant.

IGFBP-1 has been shown to be degraded by proteases in the vagina and it has been reported as being unreliable if  $> 12$  hours have elapsed from the time of membrane rupture.<sup>1</sup> It is of interest however that in our study a positive result obtained in two case even though the time elapse from membrane rupture to test was  $>12$ hrs.

To demonstrate the presence of amniotic fluid in the vagina, test results should not be influenced by the contamination of blood, urine, seminal fluid or cervical mucus and meconium. Rutanen et al<sup>15</sup> have reported that the IGFBP-1 concentration in urine and seminal fluid are significantly lower than that in serum. And even if amniotic fluid is contaminated by blood, the contamination is negligible, because the serum:amniotic fluid ratio of IGFBP-1 concentration is substantially high.

Comparison of our study with other studies regarding APT test in diagnosing PROM.			
Variables	Our study	Ibrahim A. Abdelzim study <sup>16</sup>	Takeyoshi Kubota study <sup>17</sup>
Sensitivity	90.54%	91.8%	94.3%
Specificity	65.38%	85.5%	93.1%
PPV	83.75%	86.3%	
NPV	65%	91.3%	
DA	80%	88.6%	

Comparison of our study with other studies regarding FERN test in diagnosis of PROM			
Variables	Our study	Ibrahim A. Abdelzim Study <sup>16</sup>	Takeyoshi Kubota study <sup>17</sup>
Sensitivity	44.7%	84.5%	42.1%
Specificity	76.92%	78.2%	78.9%
PPV	84.6%	79.5%	
NPV	32.7%	83.5%	
DA	53%	81.4%	

## CONCLUSION

Our data shows that IGFBP-1 is a ideal marker of Amniotic fluid and that rapid, simple test for the measurement of this protein in vaginal secretion by a dipstick method has diagnostic potential in the diagnosis or exclusion of rupture of fetal membranes. The high sensitivity, specificity, PPV, NPV of Actimprom test over Fern test makes it a useful test when in doubt of PROM. It is easily performed in clinical setting and no extra staff is required. Test results are unaffected by contamination with non amniotic body fluids.

Our study shows that APT is more efficacious than FERN test in diagnosing PROM.

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**Source of Support:** Nil; **Conflict of Interest:** None

**Submitted:** 16-04-2017; **Accepted:** 28-05-2017; **Published:** 07-06-2017