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Comparison Among Fern, Amnisure, Creatinine and Urea Tests in Vaginal Wash for Detection of Rupture of Membrane in Iraqi Women

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Abstract

Prelabour rupture of membrane (PROM) is a cause for 85% of the neonatal period morbidities and fatalities. PROM is the main cause of preterm deliveries and accounts for 30-40% of these cases, indeed it complicates three percent of all pregnancies. The fetal and maternal mortality and morbidity risks of PROM, increases when the rupture occurs early in the course of pregnancy. In this study we sought to compare the detection efficiency of the standard diagnostic test of PROM with other new methods such as urea and Creatinine of vaginal fluid and Amnisure tests. The study sample is composed of 90 women divided into three groups; each woman had informed consent, questionnaire with full history, clinical examination (general and abdominal and sterile speculum examination to detect cervical dilatation, amniotic fluid leakage for sample collection to do Fern, Amnisure, Creatinine and urea tests. General investigations and ultrasound were done for each patient. Mean vaginal fluid Creatinine level among the group (1) were 0.44±0.14, versus 0.38±0.12, and 0.24±0.08 among group (2) and (3) respectively. When the results of the tests compared with the standard method of diagnosis, the specificity was 100% for Amnisure, vaginal fluid Creatinine and urea, while it was 80% for fern test. The sensitivity was 97% for Amnisure, 95% for vaginal fluid urea, 91% for vaginal fluid Creatinine and 60% for fern test. Amnisure is superior for other tests followed by vaginal fluid urea level, Creatinine and lastly Fern test.

Keywords: Fern; Amnisure; Creatinine; Urea; Rupture of Membrane

1. Introduction

Premature rupture of membrane is a common daily obstetric encounter, complicating approximately 5-10% of the term pregnancy and 30% of preterm deliveries (before 37 weeks' gestation) and its management represents a challenge for the managing doctor if good prognosis for the mother and the baby is pursuit (Taylor, M [1]) PROM impairs the mechanisms that provide relative isolation and protection provided by amniotic cavity to the fetus (Medina and Hill [2]). Although the etiology of PROM is not clinically evident a degree of consensus has arisen regarding management options (Royal College of Obstetrician and Gynecologist [3]). The common major causes of neonatal death associated with PPROM are prematurity, pulmonary hypoplasia, and sepsis (Caughey, et al. [4]). Clinically it is vital to distinguish between term PROM and preterm PROM, as these two categories vary in their pathogenesis, risk factors and their management planes (Cunningham, et al. [5]). At term, three-quarters of women will deliver within 24 after membrane rupture. It is accepted that there is an inverse relationship between gestational age and latency period, at (26-27) weeks; 50% of them are in labor within a week, and the remaining will labor within 24-48hr (Ferron and Bilodeau [6]). The amniotic cavity is enclosed by two membranes; amnion and the Chorion, these are firmly attached and made of many types of cells mesenchymal cells, epithelial, and trophoblastic cells, embedded in a collagenous matrix. They hold the amniotic fluid and produce substances into the fetus against infection ascending the reproductive tract. The human amnion consists of five distinct layers that have no nerve nor blood supply; it gets its nourishment from the amniotic fluid. The innermost layer, closest to the fetus, is the made of amniotic epithelial cells that secrete collagen type III and IV and non-collagenous glycoproteins (laminin, nidogen, and fibronectin) that construct the basement membrane, that is the second inner layer of the amnion

(AmniSure International LLC [7]). The compact layer of the connective tissue adjacent to the basement membrane forms, the main fibrous skeleton of amnion. The thickest layer of the amniotic layers is the fibroblast layer, it consists of mesenchymal cells and macrophages within an extracellular matrix. The collagens in these layers form a loose network with islands of non-collagenous glycoproteins (Mohamed and Mostafa [8]). The intermediate layer (spongy layer, or zona spongiosa) lies between the amnion and the chorion. The intermediate layer absorbs physical stress by permitting the amnion to slide under the chorion, which is strongly adjacent to the maternal decidua. The chorion is thicker than the amnion, while the amnion is stronger. the chorionic membrane has polarity toward the maternal side of the decidua. As pregnancy progress Trophoblastic villi within the chorionic layer of the reflected membranes (free of the placenta) regress (Parry and Strauss [9]). Below the Cytotrophoblastic layer (closer to the fetus) are the basement membrane and the chorionic connective tissue rich in collagen fibrils (Mohamed and Mostafa [8]). There are regional variations of fetal membrane that distinguishes the membranes overlying in the placenta from the reflected membranes and there is no evidence of there are weak points where the membranes break, care must be taken to avoid overlying localized changes in the structure of the membrane and its composition in the studies of PROM (Kumar, et al.[10]). Previous PROM is the main factor risk for PROM, the recurrence rate is 16% -32% in comparison to incidence of 4% in women with no history of PROM and previous uncomplicated term delivery (ACOG Committee on Practice Bulletins-Obstetrics [11], Harger, et al. [12]). The recurrence rate is higher when there is a short cervix or uterine contractions in the second trimester. PROM resembles and should be distinguished from other conditions such as; urinary incontinence, excessive vaginal discharge (due to bacterial vaginosis and

other pathologies) and cervical mucus (show) as a sign of labor (Caughey, et al. [4]).

2. Materials and Methods

This case-control study was conducted at Tikrit teaching hospital, a tertiary center that serves a population of around one million. The study sample was recruited from patients who were admitted to or consulted at the obstetrics and gynecology department from September-2013 to April-2014. Our sample was stratified into three groups:

1- Group (1): Patients with heavy leaking liquor (35 patients).

2- Group (2): patients with mild leaking liquor and they have fern or an Amnisure positive (30 patients.).

3- Group (3): Control group or pregnant women with no leaking liquor and all tests are negative (25 patients). Our Exclusion criteria included women who met the following; vaginal bleeding, placenta previa and those with intrauterine death. All patients were informed about the study and investigated with: 1- A special questionnaire designed by the researcher (Appendix I), containing detailed information about history. 2- Clinical general, abdominal examinations, speculum examination should be sterile to detect amniotic fluid leakage, cervical dilatation and for sample collection for Fern, Amnisure, Creatinine, and urea tests were done, 3- Abdominal ultrasound.

3. Technical details of the performed laboratory test

a. Fern test

A sterile swab was used to collect vaginal secretion from the posterior vaginal pool, the sterile speculum was utilized to avoid touching the cervical mucus. Rubbing of the swab against the glass slide, creating a very thin smear immediately after collection. We waited ten minutes for the slides to dry spontaneously. Finally, the slide was examined under a microscope for arborization. Due to the sensitive nature of the test we tried our best to keep the slide free of fingerprints or letting them dry. These cautions are essential to avoid

false-positive and false-negative effects in the fern test. A positive fern test is the visualization of arborization or crystallization of amniotic fluid under the microscope (Ferron and Bilodeau [6]).

b. Amnisure test procedure

The principle of AmniSure ROM test (AmniSure® International LLC, Cambridge, MA) is based on the identification of placental alpha microglobulin 1 (PAMG-1), that is unique to amniotic fluid but almost absent in other cervicovaginal fluids such as urine and vaginal secretions in the absence of amniotic membrane rupture (AmniSure International LLC [7]). The kit manufactures instructions were strictly followed. Briefly, the patients were placed in the lithotomy position, the sterile vaginal swab provided by the kit was used to collect vaginal secretion, the swab was withdrawn after one minute to allow sucking enough amounts of secretion as shown in Figure 1. The results were interpreted according to below finding

One line: there is NO membrane rupture

Two lines: there IS a rupture

No lines: the test is invalid, retake.

c. Urea and Creatinine

The vaginal sample was obtained while the patient in a lithotomy position with good illumination. Following the confirmation of the absence of vaginal bleeding, the sterile syringe was used to wash the posterior fornix with 3 milliliters of normal saline, which was re-aspirated (Normal saline) with the same syringe. The collected fluid was sent immediately for estimation of Creatinine and urea using (Ektachem Clinical Chemistry slides, Johnson and Johnson) and generic kit respectively (Gonen, et al. [13], Ananth, et al. [14]).

4. Statistical analysis

The Statistical Package for Social Sciences (SPSS, version 18) was used for data entry and analysis. Tables, Pie chart and bar charts used to represent data. Chi ($\chi 2$) square test of association to compare

proportions of many different factors among cases with the same proportions among controls. one –way ANOVA was used to compare means of numerical variables among groups. The P-value of ≤ 0.05 was regarded as statistically significant. ROC curve used to identify the cut-off points, sensitivity, and specificity.

5. Results

The general characteristics of the sample show that the mean age was 27.5±4.5 among the group (1), 31.1±5 for the group (2), and 28.2±5.6 for the group (3), this variation was statistically not significant, as shown in table (1). About 25 (71.4%) of the group (1) from rural areas versus 18 (60%), 15 (60%) for the group (2) and (3) respectively, this variation was statistically not significant. Most of patients were Housewives, 29 (82.9%), 28 (93.3%), 18 (72%) among group (1,2) and (3) respectively. Most of the group (1) have positive medical history 5(14.3%) versus 4(13.3%), 3(12%) of the group (2) and (3) respectively, these variations were statistically not significant. Most of the group (1) have positive surgical history 13(37.1%) versus 15(50%), 8(32%) of the group (1) and (2) respectively, this variation was statistically not significant. Ectopic pregnancy was more among the group (3) 1(4%), versus group (1) 1(2.9%). The mean gestational age was 35.6±0.8, 3.9±1.9 and 3.5±2.1 for the group (1), (2) and (3) respectively, this variation was statistically not significant. The mean Parity was 1.57±1.2, 2±1.4 and 2±1.8 for the group (1), (2) and (3) respectively, this variation was statistically not significant. The mean abortion was 0.6±0.8, 0.9±0.8 and 0.5±0.9 for the group (1), (2) and (3) respectively, this variation was statistically not significant. Study groups distribution according to gestational age show that most of the gestational age was >34 weeks among 33 (94.3%) of group (1), 26 (86.7%) of group (2), and 24 (96%) among group (3), this variation was statistically not significant, as shown in Figure (2). Amnisure test was positive

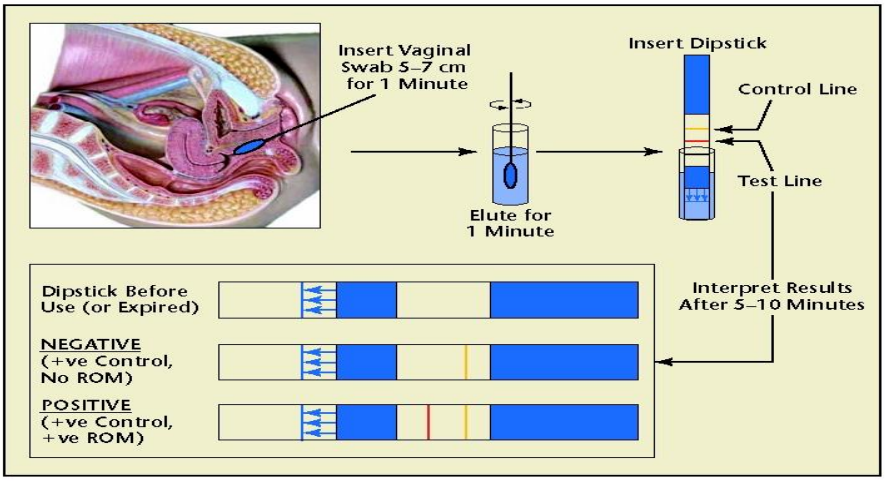

Figure (1): The steps of using AmniSure® ROM test for the diagnosis of ROM and how the results can be interpreted. Results of three scenarios can be seen in the figure; invalid, negative and positive are displayed (Mohamed and Mostafa [8]).

Table (1): General characteristics of sample

Characteristics		Study groups			P value
		Group1	Group 2	Group 3	
Age		27.5±4.5	31.1±5	28.2±5.6	<0.05
Residence	Rural	25	18	15	
		71.4%	60%	60%	
	Urban	10	12	10	
		28.6%	40%	40%	> 0.05
Occupation	Student	2	0	3	
		5.7%	%0	12%	
	Housewife	29	28	18	
		82.9%	93.3%	72%	
	Employer	4	2	4	
		11.4%	6.7%	16%	>0.05
Positive Medical History		5	4	3	
		14.3%	13.3%	12%	> 0.05
Positive surgical history		13	15	8	
		37.1%	50%	32%	> 0.05
Ectopic pregnancy		1		1	
		2.9%		4%	> 0.05
Gestational age		35.6±0.8	34.4±0.8	35.6±0.6	> 0.05
Gravida		3.11±1.6	3.9±1.9	3.5±2.1	> 0.05
Parity		1.57±1.2	2±1.4	2±1.8	> 0.05
Abortion		0.6±0.8	0.9±0.8	0.5±0.9	> 0.05

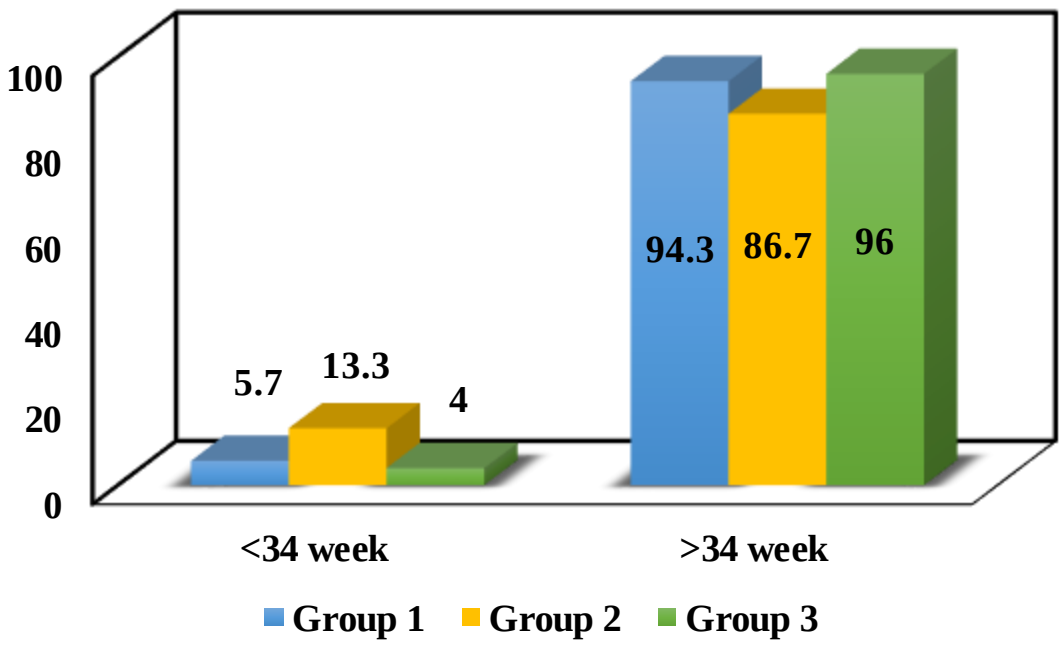

Figure (2): Gestational age distribution among study groups (df=2, x^2 =1.996 P value >0.05 NS

Table (2): The Amnisure test result among study groups

Amnisure test	Study groups			Total
	Group (1)	Group (2)	Group (3)	
Positive	35	28	0	63
	100%	93.3%	0%	70%
Negative	0	2	25	27
	0%	6.7%	100%	30%
Total	35	30	25	90
	100%	100%	100%	100%

Table (3): The Fern test result among study groups

Fern test	Study groups			Total
	Group (1)	Group (2)	Group (3)	
Positive	35	0	5	40
	100%	0%	20%	44.4%
Negative	0	30	20	50
	0%	100.0%	80.0%	55.6%
Total	35	30	25	90
	100%	100%	100%	100%

x^2 =63.7, df=2, P value < 0.05 Significant

Table (4): Mean vaginal fluid urea and Creatinine level among different study groups.

Test	Group 1		Group 2		Group 3		ANOVA test P value
	Mean	SD	Mean	SD	Mean	SD	
Urea	10.8	1.01	10.1	2.8	4.3	2.8	<0.05
Creatinine	0.44	0.14	0.38	0.12	0.24	0.08	<0.05

ANOVA = analysis of variance

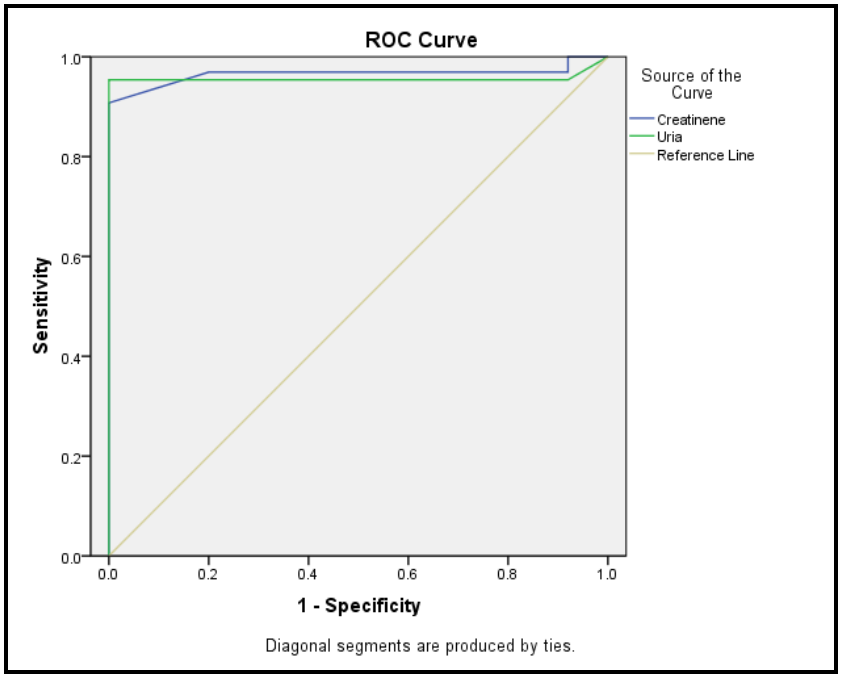

Figure (3): The ROC curve for the vaginal fluid urea and Creatinine tests as compared to Fern test

among 35(100%) in the group (1), 28(93.3%) in the group (2), and zero in the group (3), as shown in the table. Fern test was positive among 35 (100%) of the group (1), zero of the group (2), and 5 (20%) of the group (3), this variation was statistically significant (P-value <0.05), as shown in table (3). Mean vaginal fluid urea among the group (1) was 10.8±1.01, versus 10.1±2.8, and 4.3±2.8 among the group (2) and (3) respectively, this variation was statistically significant, as shown in Table (4). Mean vaginal fluid Creatinine among the group (1) was 0.44±0.14, versus 0.38±0.12, and 0.24±0.08 among the group (2) and (3) respectively, this variation was statistically significant, as shown in Table (4). When the receiver operating curve (ROC) analysis used to determine the cut-off point with higher specificity for vaginal fluid urea was > 7.5 and for vaginal fluid, Creatinine was > 0.30, as shown in figure (3). About 35(100%) of the group (1) had vaginal fluid urea level above 7.5, versus 27 (90%), and zero of the group (2) and (3) respectively, this variation was statistically significant (P<0.05) as shown in table (5). About 35(100%) of the group (1) had vaginal fluid Creatinine level above 0.30, versus 24(80%), and zero of the group (2) and (3) respectively, this variation was statistically significant (P<0.05) as shown in table (6). When the results of the tests compared with the standard method of diagnosis, the specificity was 100% for Amnisure, vaginal fluid urea, and vaginal fluid creatinine while it was 80% for the fern test. The sensitivity was 97% for Amnisure, 95% for vaginal fluid urea, 91% for vaginal fluid Creatinine and 60% for fern test, as shown in table (7).

4. Discussion

Unfortunately, PROM is a common obstetric problem that is responsible for almost one-third of premature births., PROM leads to the delivery of a fetus within hours regardless of the gestational age (Ananth, et al. [14]). Preterm prelabour rupture of membranes can be defined as

Table (5): Vaginal fluid urea results among study groups

Vaginal fluid urea	Study groups			Total
	Group (1)	Group (2)	Group (3)	
>7.5	35	27	0	62
	100%	90%	0%	68.9%
<7.5	0	3	25	28
	0%	10%	100%	31.1%
Total	35	30	25	90
	100%	100%	100%	100%

x^2 =77.4, df =2, P value < 0.05 Significant

Table (6): Vaginal fluid Creatinine result among study groups

Vaginal fluid Creatinine	Study groups			Total
	Group (1)	Group (2)	Group (3)	
>0.30	35	24	0	59
	100%	80%	0%	65.6%
<0.30	0	6	25	31
	.0%	20.0%	100%	34.4%
Total	35	30	25	90
	100%	100%	100%	100%

x^2 =68.7, df=2, P value < 0.05 Significant

Table (7): Sensitivity, specificity, positive predictive value, negative predictive value and accuracy of the tests used in the study

Test	Cutoff	Sensitivity	Specificity	PPV*	NPV**	Accuracy
Amnisure		97	100	100	93	98
Fern		60	80	89	43	66
Vaginal fluid urea	>7.5	95	100	100	89	97
Vaginal fluid Creatinine	>0.30	91	100	100	81	93

*Positive predictive value, **Negative predictive value

any rupture of amniotic membrane before term (before 37 weeks' gestation). It is not uncommon and can be seen in 3 percent of pregnancies, and it is blamed for a large significant proportion of preterm deliveries. The list of complications of PROM is long and includes substantial perinatal morbidity, due to respiratory distress syndrome, neonatal sepsis, umbilical cord prolapses, placental abruption, and fetal death. Thus it is important to appropriately identify the condition, as high stake decisions will be made depending on the diagnosis made (Lee, et al. [15]). Demographic parameters of our study sample showed that the mean age was27.5±4.5 among group 1, 31.1±5 for group 2, this was near to that found by (Al Riyami, et al. [16]) in Oman, and most of the patients were Housewives among those with PROM which higher than among the control group, this supported by what found in Iran by (Kariman, et al. [17]) among PROM group. As we know that depending on clinical diagnosis have no high specificity and sensitivity and lead to false-positive and negative results (Harger, et al. [12]), therefore we are in need of rapid noncomplex noninvasive test to diagnose PROM. This study shows that Amnisure was significantly positives in the group (1) and identify about (93.3%) of group (2) while the Fern test significantly diagnosed all group (1) but couldn't diagnose any of group (2), which means it has low sensitivity with those with mild leaking liquors. When the results of the tests compared with the standard method of diagnosis, the specificity was 100% for Amnisure, while it was 80% for fern test, this also found by (Medina and Hill [10]) except for Fern was it was 100% this may be related to technical difference or the quality control measures difference between the Iraq and Malaysia (Royal College of Obstetrician and Gynecologist [2]). The mean Vaginal fluid urea level among the group (1) and (2) was significantly higher than among the control group this was supported by previous studies (Kariman, et al. [17], Mohamed and Mostafa [8], Kafali and

Öksüzler [18]). The mean vaginal fluid Creatinine levels of the group (I) was higher than group 2 and group (3). This supported by previous studies (Kariman, et al. [17], Mohamed and Mostafa [8], Kafali and Öksüzler [18], Gurbuz, et al. [19]). The ROC curve was used to determine the cut-off point at which the vaginal fluid urea and Creatinine level are more sensitive. It was found that the cut-off point with higher specificity for urea was > 7.5 and for Creatinine was > 0.30 depending on these values, the sensitivity and specificity were calculated for the vaginal urea and Creatinine level test. When the results of the tests compared with the standard method of diagnosis, the specificity was 100% for Amnisure, Creatinine, and urea, while it was 80% for the fern test. So Amnisure, vaginal fluid urea, and Creatinine level had high specificity in diagnosis PROM than Fern test. The sensitivity was 97% for Amnisure, 95% for vaginal fluid urea, 91% for vaginal fluid Creatinine and 60% for fern test. The Aminsure was more specific and lead to less false negative test than the others. From the analysis of the results of sensitivity and specificity, therefore, we conclude that Amnisure was the best, followed by vaginal fluid urea and Creatinine levels, while the Fern test had a lower sensitivity and specificity than the other tests. These results go with the previous studies that showed Amnisure was a good diagnostic test and it's superior to other tests as found by (Cousins, et al. [20], Mi Lee, et al. [21]). (Gurbuz, et al. [19]) showed that vaginal fluid Creatinine is an extremely useful marker in doubtful cases of PROM. Other authors were also agreed that urea or Creatinine determination in vaginal fluid for the diagnosis of PROM is a reliable, simple and rapid test, sensitivity and specificity of 100% (Guinn, et al. [22], Dale, et al. [23]). (Kariman, et al. [17]) found that vaginal urea level sensitivity was 90%, the specificity of 79%, were all 100% in detecting PROM by evaluating vaginal fluid Creatinine, preferring Creatinine level on urea level.

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Author Contribution

Sarab KH performed the study, examined and reviewed results and manuscript writing.

Conflict of Interest

The author declares no conflict of interest.

Ethical Clearance

The study was approved by the Ethical Approval Committee.

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Biography

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She received the M.B.CH.B. from the College of medicine, University of Baghdad in 2002. She worked in Mesan and Kirkuk until she occupied a position of senior house officer during her permanency in obstetrics and gynecology 2008-2010

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