

# Trichomonas vaginalis infection and pregnancy outcome

Marwa M. Hamouda (✉ [dmarwahamouda@gmail.com](mailto:dmarwahamouda@gmail.com))

Mansoura University

Sara A. Mohamed

Mansoura University

Nairmen Nabih

Mansoura University

Abeer A. El-Henawy

Mansoura University

Nora Esam Eldeen

Mansoura University

Wafaa M. EL-zayady

Mansoura University

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## Research Article

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# Abstract

Trichomoniasis is the most common curable sexually transmitted disease worldwide; *T. vaginalis* increases the risk for adverse pregnancy outcomes. In this study, we aimed to detect the prevalence of *T. vaginalis* infection in pregnant women and to determine the influence of trichomoniasis on pregnancy outcome. Vaginal swab specimens were obtained from 200 symptomatic and asymptomatic pregnant females, for whom clinical examination, vaginal discharge wet mount, Giemsa stain, and culture in modified Diamond's media were performed. Metronidazole treatment was given for all positive cases. Sociodemographic information was collected, and cases were divided into two groups, the study group (infected) and the control group (not infected). Twenty four cases were positive for *T. vaginalis* infection. Cases were mostly detected by culture (12%) followed by wet mount microscopy and Giemsa staining (8.5%). Twelve of the 24 positive cases (50%) had preterm delivery. Low birth weight infants were observed in 17/24 cases (70%). The high rate of infection was observed in age group of 26–35 years ( $P = 0.017$ ). In addition, there was a significant *T. vaginalis* infection in pregnant women with vaginal discharge and a history of previous infection ( $P < 0.05$ ). Preterm delivery was statistically significant in cases with bad compliance to metronidazole treatment ( $p = 0.004$ ). Our results highlight the presence of preterm delivery and delivery of Low birth weight infants in pregnant women infected with *trichomonas vaginalis* with vaginal discharge and a history of previous infection. So married women should be screened for trichomoniasis to reduce the incidence of preterm delivery and low birth weight.

## 1. Introduction

Trichomoniasis is a communicable disease caused by the anaerobic protozoan, *Trichomonas vaginalis*. With a global incidence of 276.4 million infections each year, the disease is the most prevalent curable sexually transmitted infection ((Salakos et al. 2018). Previous studies in Egypt reported a prevalence of trichomoniasis ranging from 5–11.7% (Abdel-Magied et al. 2017; Elwakil et al. 2017; Mahmoud et al. 2015).

In 25 to 50% of women, vaginal infection is asymptomatic (Naidoo and Wand 2013; Bouchemal, Bories, and Loiseau 2017). On the other hand, symptomatized females present with vaginal discharge, pruritus and dysuria, vaginitis, and cervicitis (Kusdian and Gould 2014; Naidoo and Wand 2013).

Many health problems have been associated with trichomoniasis, including pelvic inflammatory disease, cervical cancer, prostatitis, and infertility (Swygard et al. 2004; Fichorova 2009). Early rupture of membranes, premature delivery, and low birth weight (Evidence level III) are major pregnancy risks (Oyeyemi, Fadipe, and Oyeyemi 2016; Sherrard et al. 2014).

The epidemiology of *Trichomonas vaginalis* infection can be influenced by several risk factors such as age, residence, socioeconomic level, education, marital state and the type of contraception method used, presence and type of vaginal discharge, the used drug, and history of other sexually transmitted infections (Ton Nu et al. 2015).

Diagnosis of infection is difficult since its symptoms resemble those of other sexually transmitted infections, and it needs a test with high sensitivity to confirm the infection. The diagnosis is made by conventional direct microscopic wet mount, staining, or culture technique. Other diagnostic methods include immunological detection or nucleic acid-based assays that showed higher sensitivity and specificity were also used (Divakaruni et al. 2018).

*T. vaginalis* has gotten relatively little public health attention, despite its high prevalence and substantial accompanying morbidity and associated HIV acquisition (Torok et al. 2009).

We aimed to establish the prevalence of *T. vaginalis* infection in pregnant women and determine the influence of trichomoniasis on pregnancy outcome; this study used conventional direct wet mount microscopic examination, Giemsa stain, and Diamond's medium culture.

## 2. Subjects And Methods

### 2.1. Patients selection and setting

During the period from May 2020 and April 2021, this observational cohort prospective study enrolled pregnant women who attended the Obstetrics and Gynecology Outpatient Clinic at Mansoura University Hospital. Examination of collected samples was done in Medical Parasitology Department, Faculty of Medicine, Mansoura University.

Participants of the study were randomly chosen from non-complicated pregnant women of the second and third trimesters, with or without vaginal discharge, who attended for a routine antenatal appointment at the hospital. The last menstrual cycle date was reported to calculate gestational age, which was validated by ultrasonography. A standardized questionnaire was used to collect sociodemographic data and obstetric history from participants.

Complicated pregnancies with placenta previa, multiple pregnancies, hydramnios, congenital malformations, intrauterine growth retardation, medical issues such as insulin-dependent diabetic mellitus, chronic hypertension, maternal heart illness, and preeclampsia were ruled out of the study as exclusion criteria.

200 pregnant women were enrolled in the study aging between 18 to 45 years old and had a gestational age of 13 to 40 weeks, with or without complaints of vaginal discharge. Based on the results of culture examination for *Trichomonas vaginalis* infection, pregnant women were divided between two groups: the study group (pregnant women with trichomoniasis) and the control group (pregnant women without trichomoniasis).

### 2.2. Clinical data and samples collected

All study participants were subjected to the following :

- 1• A full medical sheet fulfilling the demographic data, menstruation history, genitourinary complaints and obstetric history
- 2• Clinical examination: Each patient will be examined physically for the routine medical parameters
- 3• Two vaginal specimens (cotton swabs) were collected from the posterior fornix of the vagina regardless the complains. One sample was used for microscopic slide examination either as wet mount and/or giemsa stained, and the other one used for cultivation in Modified Diamond medium.

### 2.3. Parasitological detection of *Trichomonas vaginalis*

**Wet mount smear preparation:** the swab was immediately inoculated in a tube containing 3 ml sterile PBS (pH: 7.2). Prior to removal, the swab was placed against the tube's side, and the tube vortexed for 30 seconds to express as much fluid as feasible (Mahmoud et al. 2015).

Using a sterile pastier pipette, the vortexed fluid was put on a clean glass slide, covered with a clear coverslide and examined microscopically for motile *T. vaginalis* trophozoite in 100x or 400x magnified field.

Each slide was thoroughly examined for about 2-3 minutes. The protozon was identified by its oval or pyriform shape (10-20 $\mu$  in size) with a characteristic twitching motility (Kamal et al. 2018).

**Giemsa-stained slide preparation:** a drop from the prepared vortexed fluid was applied on a slide and allowed to dry with air. The slide was then fixed in methanol for 1 minute before being stained for 20 minutes with 20 % Giemsa stain (diluted 1:20 with distilled water). To remove any residual stain, the slide was cleaned with normal tap water then allowed to dry. Later, *T. vaginalis* trophozoites, with its characteristic smiling face and extended flagellae, were detected on the stained preparation through microscopic examination of 1000x maginified field using an oil immersion objective lens (Radonjic et al. 2006)

#### **Cultivation on Modified Diamond medium:**

The second swab was promptly put into a culture tube containing 10 ml Modified Diamond medium, which had been prepared according to the manufacturer's instructions (Gelbart et al. 1989). The prepared medium was sterilized by autoclaving at 121 °C for 15 minutes after the PH was corrected to 6.0 with HCL or NaOH. To suppress bacterial and fungal growth, 1 ml of sterile inactivated bovine serum and 0.5 ml of the antibiotics mixture (20.000 I.U./ml Penicillin G, 20 mg/ml Streptomycin sulfate, 40g/ml Fluconazole) were added after cooling. Culture tubes were preheated to 37 °C for 15 minutes before vaginal swabs were inserted into the media. Following inoculation of obtained swabs, the cultures were examined microscopically daily for the presence of *T. vaginalis* trophozoites for about seven days. .

All Positive instances according to microscopic and/or culture results were instructed for treatment. According to the 2010 Sexually Transmitted Diseases Treatment Guidelines from the US Centers for

Disease Control, the treatment regimens were provided with Metronidazole 500mg twice a day for seven days (Workowski et al. 2010).

Positive cases were also followed up on to see their pregnancy outcome.

## **2.4 Ethical approval.**

The Research Ethics Committee of the Faculty of Medicine, Mansoura University, Egypt, approved the study protocol (IRB code: R.19.01.391).

Written consents were obtained from all participants after describing the aim of the study and the examination procedures. Trichomoniasis-positive cases were instructed for the treatment after describing the nature of infection and after approval of her treating obstetrician.

## **2.5. Statistical analyses**

The data will be examined using SPSS 15 for Windows on a personal computer. The frequency distribution process will be used to calculate the number of cases and percentages for qualitative variables, while the mean and standard deviation (SD) or the median and range will be used for quantitative data. The Chi-Square Test will determine whether category variables are related. If the Chi-square assumptions are broken, Fisher's exact test will be employed. To compare the means of two groups, the independent-samples t-test will be employed. A one-way analysis of variance (ANOVA) was employed to compare the means of more than two groups. Statistical significance is defined as a p-value of less than 0.05.

# **3. Results**

## **3.1. Prevalence of *T. vaginalis* infection by different diagnostic methods:**

Out of 200 pregnant women who participated in our study, infection with *T. vaginalis* was detected in about 17/200 (8.5%) through microscopic vaginal swabs examination through wet mount smear or permanently-stained samples. Meanwhile, cultivation of samples on modified Diamond's media detected the organism in 24/200 (12%) cases. **(Table 1)**.

## **3.2. Accuracy of wet mount and Giemsa staining test in relation to Modified Diamond's culture:**

Comparing the parasitological detection of trichomoniasis by microscopic examination and culture results:

All detected positive samples (17 samples) through microscopic examination also revealed positive results during cultivation, however, some negative microscopically detected samples (7 samples) revealed positive results on cultivation. Thus on considering the modified Diamond's culture as the gold standard test for detecting trichomoniasis infection, microscopic examination of vaginal swabs (wet mount smears and/or giemsa-stained slides) revealed a sensitivity of about 70.8% and specificity of

100%. So, there was a statistically significant lower sensitivity for microscopic examination in relation to culture results ( $P = 0.001$ ). **(Table 2)**

### **3.3. Sociodemographic data among studied cases:**

Based on the results of the gold standard modified Diamond's culture, the participants were divided into cases group (24/200 trichomoniasis positive women) and control group (176/200 trichomoniasis positive women). Concerning age groups distribution, the cases group showed higher prevalence of infection in participants aged between (26-35 years) than other ages groups (53.8%), indicating a statistically significant difference ( $P < 0.05$ ) from those women of the same age in the same age group.

The residence of the participated women in rural or urban area was nearly equally distributed without any significant difference between cases and control groups. Also the occupation and educational level showed non-significant difference between the positive and negative trichomoniasis females. **(Table 3)**

On the other hand, there was a significant difference between the cases and control groups as regard the presence of vaginal discharge ( $P < 0.05$ ), as about 15/24 (62.5%) of the cases showed vaginal discharge while only 63/176 (35.8%) showed that complain. Besides, the history of previous genitourinary infection was higher in the cases group 15/24 (62.5%) than the control group 49/176 (27.8%), with a significant difference between them ( $P = 0.001$ ). **(Table 3)**

### **3.4. Descriptive data of positive trichomonas cases:**

Good compliance to metronidazole treatment was observed in 13/24 (54.2%) of the trichomoniasis-positive cases group. Partner treatment was adapted in 16/24 (66.7%) of them. More than two antenatal care visits were performed by 79.2% of those cases, About 79.2% of visits were occurred in 2nd trimester, more than one test positive were found in 8 (33.3%) cases with more than single course of metronidazole treatment. **(Table 4)**

### **3.5. Pregnancy outcome among studied groups:**

There was significant difference as regard the detected adverse pregnancy outcomes between the cases (trichomoniasis-positive) and the control (trichomoniasis-negative) groups. Premature rupture of membranes occurred in 17/24 (70.8%) of cases and in 29/176 (16.5%) of the control with  $P = 0.0001$ . Preterm delivery occurred in 12/24 (50%) of the cases group and in 29/176 (16.5%) of the control indicating significant difference of  $P = 0.0001$ . Also, low birth weight infant occurs in 17/24 (70.8%) of cases and in 28/176 (15.9%) of controls with  $P = 0.0001$ . **(Table 5)**.

### **3.6. Metronidazole treatment among positive cases:**

On correlation between metronidazole treatment compliance and the adverse pregnancy outcome among the cases group participants, premature rupture of membrane and preterm delivery were statistically significantly higher in patients with bad metronidazole compliance than patients treated well with

metronidazole (72.7% vs 30.8%; p: 0.044 for the premature rupture of membranes, 81.8% vs 23.1%; p: 0.004 for preterm delivery) respectively. (Table 6)

## 4. Discussion

Trichomoniasis is the most common non-viral sexually transmitted infection (STI) globally, affecting people of all ages, races, and socioeconomic classes (Apalata et al. 2014). *T. vaginalis* prevalence percentages vary widely worldwide, ranging from 0.9 percent to 80 percent (Valadkhani et al. 2011).

*T. vaginalis* infection was found to be prevalent in about 12% of women attending ordinary prenatal care filling the inclusion criteria. The occurrence of atypical vaginal discharge and a previous history of infection were both risk factors for infection in this population, as stated elsewhere. A recent study on pregnant women in Egypt found a prevalence of 11.7 %, which is similar to our findings (Kamal et al. 2018). In addition, pregnant women from Durban had a comparable prevalence rate of 10% for *T. vaginalis* (Dessai et al. 2020).

Despite being mostly asymptomatic sexually transmitted infection, but several sequelae and complications can occur during pregnancy like, early rupture of membranes, premature delivery, and low birth weight therefore exploring the prevalence and the sequelae of this infection with pregnancy was the aim of this study

*T. vaginalis* infection was shown to be prevalent in 11.5 % of women aged 15 to 49 years old in a previous WHO research conducted in the African region (Newman et al. 2015). Another study conducted in Egypt found a higher frequency of 37.7% (El-Gayar, Mokhtar, and Hassan 2016).

In the present study, the high rate of trichomoniasis was observed in the age group of [26- 35], followed by the age group of [18-25]. This finding was consistent with other previous studies (Mabaso et al. 2020; Kamal et al. 2018). Trichomoniasis is one of the sexually transmitted infections commonly associated with patients at child-bearing ages since these ages are more sexually active (Mabaso and Abbai 2021).

Sociodemographic characteristics such as residency, education level, and occupation did not differ between this study cases and control groups. These results contradicted Kamal et al. (2018) findings that were living in a rural location, having a poor socioeconomic standing, and having only a primary education level were associated with *T. vaginalis* infection. This discrepancy in findings could be attributed to the difference in the number of participants between the two studies.

Ibrahim et al. (2021) study indicated that *T. vaginalis* infection was more common among women living in rural regions than in urban areas, but there was no statistically significant difference (P=0.28).

A risk factor for *Trichomonas vaginalis* infection was the presence of abnormal vaginal discharge along with a previous history of genitourinary infection. The bivariate analysis of those who reported vaginal discharge revealed a significant correlation with *T. vaginalis* infection (P=0.012). Other investigations had reached the same conclusion (Chetty, Mabaso, and Abbai 2020; Ibrahim et al. 2021).

According to many researchers, the Diamond modified broth culture is the standard gold test (Domeika et al. 2010; Kamal et al. 2018). In this investigation, all *T. vaginalis* infected cases were Diamond diagnosed 24/24 (100 %), the standard gold test so that regarding the result of the wet mount and Giemsa staining tests compared to culture, the sensitivity and specificity of both were 70.8% and 100 %, respectively.

HAMDY and HAMDY (2018) declared that the wet mount and Giemsa stained smear had sensitivities of 16.7% and 50%, respectively, and specificities of 100% and 100%. When compared to the Diamond reference index, Hegazy et al. (2020) showed that Giemsa stained smear and wet mount detected 30/200 (15%) and 26/200 (13%) positive cases, respectively, with sensitivities of 67 %, 58 %, and specificities of 100 %, & 93.5 % respectively.

Also, Ibrahim et al. (2021) reported that direct wet mount smear and Giemsa stained smear detected six cases, compared to nine by Modified Diamond culture with sensitivity and specificity of both were 66.67% & 100% respectively to the Diamond reference.

The present study detected premature rupture of membrane, preterm birth and low birth weight infant in 70.8%, 50% and 70.8% of infected women, respectively.

Several studies have found a link between vaginal trichomoniasis, premature birth, and low birth weight babies (Rasti et al. 2011; Cotch et al. 1997; Kamal et al. 2018). Preterm birth and low birth weight infants were 30 % more probable in pregnant women infected with *T. vaginalis* than in uninfected women (Cotch et al. 1997). This link was also validated in meta-analysis research (Silver et al. 2014), which aligned with our findings. The increased rate of preterm birth and unfavourable post-delivery outcomes among infected women could be explained by the host's inflammatory response to infection, which may weaken the chorioamnionitis membrane and raise the risk of adverse birth outcomes (Schoonmaker et al. 1989).

Following up on the treatment of positive cases with metronidazole, only 13/24 ( 54.2%) patients received the medicine with good compliance, and 11/24 (45.8%) cases showed non-compliance in treatment with metronidazole. Some of them received repeated courses; nine out of 11 non-complained cases gave birth to a preterm newborn, indicating a statistically significant difference compared to treated instances (81.8% vs. 23.1%;  $p= 0.004$ ). The short course duration of treatment may explain this, and the repeated exposure to metronidazole in non-complained patients.

This outcome contradicted Kamal et al. findings, which found that only six (17.1%) cases of treated one gave birth to a normal baby after being treated with metronidazole.

Also, in the present study, there were 3/13 (23.1%) cases in the complied group of the patient who gave preterm birth so that the condition may be related to different factors, not only to treatment as the strain virulence may be a cause.

The effect of metronidazole treatment on pregnant women with trichomoniasis was conflicting; several studies were conducted to evaluate drug use. Most of these studies were analysed by Ajiji et al. (2021), who performed a metanalysis to test the effect of metronidazole.

Ajiji et al. (2021) stated no relationship between metronidazole treatment during pregnancy and preterm birth, stillbirth, low birth weight, and caesarian delivery. There are conflicting views on the use of metronidazole in averting or increasing the risk of preterm birth and associated outcomes (Morency and Bujold 2007; Leitich et al. 2003; Okun, Gronau, and Hannah 2005; Sheehy et al. 2015).

Sheehy et al. (2015) stated that, during pregnancy, treating bacterial vaginosis and trichomoniasis with metronidazole is effective and offers no teratogen risk. Benefit of metronidazole in the reduction of preterm birth was demonstrated for the combination of this medication with other antibiotics.

Also, some authors found that pregnant women who were administered metronidazole for less than three days may have an increased risk of preterm birth. The duration of treatment is related to two doses of 2 g administered 48 h apart (Klebanoff et al. 2001) and 400 mg for two days (Odendaal et al. 2002). This finding may support our result.

Klebanoff et al. (2001) hypothesized that this regimen might have suppressed competing flora and allowed a particular pathogen to flourish. Such a pathogen would have to be associated specifically with *T. vaginalis*.

So further studies are required to assess the hypothesis that a high dose for a short duration may increase the risk of preterm birth among women with a previous preterm birth compared to a lower dose for a longer duration (Ajiji et al. 2021).

It is essential to investigate and treat patients with trichomoniasis before pregnancy to prevent the bad sequel of trichomoniasis on the outcome of pregnancy and to reduce the probability of metronidazole treatment during pregnancy due to its doubtful safety during pregnancy.

## 5. Conclusion

The asymptomatic sexually transmitted disease of trichomoniasis is a silent danger that may affect pregnant women causing unfavourable sequels. Thus repeated examination using the favourable culture on Modified diamonds Media is preferred specially if vaginal discharge or previous infections were reported as risk factors. The association with premature rupture of membranes and premature deliveries are reported higher in infected than noninfected cases. The treatment with metronidazole during pregnancy is a point of conflict that necessitates further studies evaluating the benefit and the hazards of its administration on the pregnancy outcome and validating the optimum dose and its duration of administration.

## Declarations

**Funding:** No funding was received for conducting this study.

## Author's contributions

**Marwa M. Hamouda** : Conceptualization, writing the methods section, data collection and interpretation, literature search, drafting the work and approved the final form.

**Sara A. Mohamed**: clinical examination, data collection, revised the work critically for important intellectual content and approved the final version to be published.

**Nairmen Nabih**: visualization, data curation, helped in drafting the work, edited and revised it critically for important intellectual content.

**Abeer A El-Henawy**: Helped in laboratory investigations and statistical analysis, revised and approved the final version to be published.

**Nora E. Saleh**: helped in laboratory investigations and statistical analysis, revised and approved the final version to be published.

**Wafaa M. El-Zayady**: Helped in literature search and data analysis, reviewed and approved the final version.

#### **Conflict of interest:**

The authors have no competing interests to declare that are relevant to the content of this article.

#### **Compliance with ethical standards**

##### **Ethical approval**

This study was approved by the Research Ethics Committee of the Faculty of Medicine, Mansoura University, Egypt, approved the study protocol (IRB code: R.19.01.391).

##### **Statement of consent to participate and consent for publication**

Prior to enrollment, informed consent was gained from each patient for participation and publication  
Women positive for trichomoniasis were treated and followed up

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## Tables

**Table 1: Prevalence of *T. vaginalis* infection by different diagnostic methods:**

All examined cases (200)	No of positive results	%
Modified Diamond Culture	24	12%
Wet Mount	17	8.5%
Giemsa Stain	17	8.5%

**Table 2: Accuracy of wet mount and Giemsa staining test in relation to Modified Diamond's culture:**

Diagnosis of <i>T. vaginalis</i>		Culture (Gold standard)			Accuracy measure	
		positive	negative	total	variable	%
Wet mount& Giemsa staining	positive	17	0	17	sensitivity	70.8%
	negative	7	176	183	specificity	100%
	total	24	176	200	PPV#	100%
					NPV#	96.175%
<b>*AUC 0.82; &amp; 95%CI (0.72-0.89);p= 0.001</b>						

\*AUC (area under curve), 95%CI (confidence interval 95%);p\*

#Positive predictive value(PPV), Negative predictive value (NPV).

**Table 3: Sociodemographic data among studied groups:**

		Control		Cases		p*
		No	%	No	%	
Age	<b>18-25</b>	58	32.9%	6	25%	0.017
	<b>26-35</b>	49	27.8%	14	58.3%	
	36-45	59	33.5%	4	16.7%	
Residence	Urban	92	52.3%	12	50.0%	0.8
	Rural	84	47.7%	12	50.0%	
Education	Illiterate	23	13.1%	1	4.2%	0.2
	Primary	36	20.5%	7	29.2%	
	Vocational	72	40.9%	13	54.2%	
	Higher	45	25.6%	3	12.5%	
Occupation	Housewife	81	46.0%	13	54.2%	0.4
	Working	95	54.0%	11	45.8%	
Vaginal discharge	Absent	113	64.2%	9	37.5%	0.012
	Present	63	35.8%	15	62.5%	
Previous infection	Negative	127	72.2%	9	37.5%	0.001
	Positive	49	27.8%	15	62.5%	

\* Chi-square test

Table 4: Descriptive data of positive trichomonas cases:

Metronidazole good compliance	+ve	<b>13</b>	<b>54.2%</b>
	-ve	11	45.8%
Partener treatment	+ve	16	66.7%
	-ve	8	33.3%
Numbers of visits	1-2 visits	5	20.8%
	>2 visits	19	79.2%
Numbers of test positive	One test	16	66.7%
	>1 test	8	33.3%
Numbers of treatment coarse	non	3	12.5%
	one	13	54.2%
	>1	8	33.3%
Time of 1 <sup>st</sup> test	2 <sup>nd</sup> trimester	19	79.2%
	3 <sup>rd</sup> trimester	5	20.8%

**Table 5: Pregnancy outcome among studied groups:**

		group					p*
		Control		Case			
		No	%	No	%		
<b>Premature rupture of membrane</b>	Negative	147	83.5%	7	29.2%	0.0001	
	Positive	29	16.5%	17	70.8%		
<b>Preterm Labor</b>	Negative	147	83.5%	12	50.0%	0.0001	
	Positive	29	16.5%	12	50.0%		
<b>Full-term delivery</b>	Negative	36**	20.5%	12	50.0%	0.001	
	Positive	140	79.5%	12	50.0%		
<b>Low birth Weight</b>	Negative	148	84.1%	7	29.2%	0.0001	
	Positive	28	15.9%	17	70.8%		

\* Chi-square test

\*\*7 cases showed post-term delivery among the control group

**Table 6: Metronidazole treatment compliance in relation to pregnancy outcome:**

		Metronidazole compliance				p*
		Negative		Positive		
		No	%	No	%	
<b>Premature rupture of membrane</b>	Negative	3	27.3%	9	69.2%	0.044
	Positive	8	72.7%	4	30.8%	
<b>Preterm Labor</b>	Negative	2	18.2%	10	76.9%	0.004
	Positive	9	81.8%	3	23.1%	
<b>Full-term delivery</b>	Negative	9	81.8%	3	23.1%	0.004
	Positive	2	18.2%	10	76.9%	
<b>Low birth Weight</b>	Negative	4	36.4%	3	23.1%	0.476
	Positive	7	63.6%	10	76.9%	

\* Chi-square test