Available online at **www.ijmrhs.com**

Special Issue 9S: MedicalScience and Healthcare: Current Scenario and Future Development

ISSN No: 2319-5886

International Journal of Medical Research & Health Sciences, 2016, 5, 9S:247-253

A study on the incidence of preterm rupture of membranes in pregnant women caused by *Mycoplasmahominis*through comparison ofculture and PCR techniques in theAlavi Hospital of Ardabil Province

Arezu Ardforoosh[1],BehboodJafari[2]and RoghiyehDargahi[3]

[1]*Graduate student of Microbiology, Islamic Azad University,AharBranch*
[2]*Assistant Professor, Department of Microbiology, Islamic Azad University,AharBranch(Corresponding author)*
[3]*Assistant Professor of Obstetrics and Gynecology, Faculty of Medicine, Ardabil Branch*

ABSTRACT

Mycoplasma hominis one of the smallest free-living prokaryotes that lack peptidoglycan in the cell wall. It is also a well-known pathogenic Mycoplasmain the human reproductive system that causes abortion, bacterial vaginosis, infertility, and a low birth weight. This research investigated the incidence of preterm rupture of membrane (PROM)inpregnant women due to M.hominis in the Alavi Hospital of Ardabil Province using twocomparative PCR techniques.This study evaluated diagnostic tests on 100 pregnant womenwith rupturedmembraneand 20 healthy women withno symptoms (control) referred to the Alavi Hospital in Ardabil Province. After sampling, the samples were transferredto the laboratoryin tubes containing PBS and PPLO.Using culture ofsamples from 100 pregnant women with PROM, 41 samples (41%) were positive for the presence of M.hominiswhereas PCR revealedpositive results in62 samples (62%). PCR method displayed positive casesin 2 (10%) out of 20patients in the control group. PCR results were not significantly correlatedwith education level, parity, and gestational age. A direct correlationwas observed between the agesof pregnant women with vaginal infections before pregnancy, history of abortion, and in women with PROM.The incidence of PROMin pregnant women caused by infection of the bacterium M.hominis is high. PCR technique is more appropriatecompared with the culture method to detectM.hominis positive cases in pregnant women with PROM.

Key words: *Mycoplasma hominis*, pregnancy, premature rupture of amniotic sac

INTRODUCTION

Genital mycoplasmas are the causes of urinary-genital tract infections.*Mycoplasmahominis* is associated with bacterial vaginosis, pelvic inflammatory disease, pyelonephritis, endometritis, prostatitis, metritis, post-miscarriage fever, recurrent spontaneous abortion, low birth weight, and neonatal meningitis (1 and 2).

*M.hominis*has been implicated in the vaginasoftwo third of women with bacterial vaginosis (3). These microorganisms are free-living and often considered as normal flora of the mouth, respiratory and urinary-genitaltracts (1). These bacteria are unique amongprokaryotes because of lacking cell walls, and account for many biological properties such asnot being affectedbyGram stainingand lack of sensitivity to many common antibiotics such as beta-lactams, andare known as the pleura pneumonia-like organisms (PPLO).

Due to the lack of some features such as cell wall, very small size,andalso problems that exist in the field of their culture and separation, they have rarely beenstudied (17,18,19).These organisms stickmucosal genital surfaces by their superficial organs renderingdangerous consequences for both mother and fetus due to the high colonization capacity in the endocervical. Mycoplasmaspreventthe embryonic blastocyst stage and can lead to ovum toxicity. In addition, they bring aboutreduced sperm count and capability as well as infertility by changing the acidity of the vagina (12). The prevalence of *M. hominis*has been reported to be higher in women than in men (7). If there are no diagnoses, preventions, and proper treatments, mycoplasma infections remain to be established leading to serious consequences such as preterm rupture of membrane in pregnant women, pelvic inflammatory disease, and infertility (11 and 12).

Preterm rupture of membrane (PROM) is one of the most common problems in clinical medicine causing neonatal complications (5). Usually, PROM occurs either spontaneouslyor byurogenital infections (12). PROMtakes place in pregnant women of37 weeksand lowerchildbearing, which is identified clearly or as a leakage diagnosed by two methods: (1) nitrazinetest and (2) the use of speculum and Valsalva maneuver (12).*M.hominis*plays a very important role in creating PROM (22 and 23). Because of difficult growth and sensitivity of *M.hominis*, bacterial cultures can present false negative results. In addition, because culture is very long and time-consuming, it is not possible to access proper results in a short time. In contrast, PCR is a sensitive and fast methodwith features of high specificity that can report a precise response about the presence of these bacteria in a short time (1). This study was to evaluate the prevalence of PROM in pregnant women caused by *M.hominis*through comparison of culture and PCR techniques in the Alavi Hospital of Ardabil Province.

MATERIALS AND METHODS

This is astudy of diagnostic tests conducted on 100 pregnant women with PROM and 20 healthy women (control) referred to the Alavi Hospital in Ardabil Province within two months from 21.08.2016 to 22.10.2016. Written informed consents were collectedfrom the cases to participate in the study. The variables including pregnant maternal age, gestational age, education level, parity, abortion, record of vaginal infection, history of using antibiotics before pregnancy, duration of rupture, and rupture quality of themembrane were collected using questionnaires.

Two swabs were sampled from each patient,collected into the tubescontaining PBS and PPLO in ice boxes, and transferred to the laboratory.The samples transported to the laboratory in PPLO were filtered,cultured in liquid PPLO medium together with phenol red indicator, and evaluated for daily color change through observation.Once a color change from yellow to purple red was observed, the sample was considered positive. Afterward, DNA was extracted from all the samples transferred to the laboratory in PBS. DNA was extracted manually. Then, the resulting DNA was electrophoresed on 1% agarose gel. The sequences of primers used (32) are shown in Table 1.

Table 1: Profile ofprimer sequences used in PCR

Band length	Annealing temperature	Sequence	Primer
244bp	$56^{\cdot c}$	5′-TGAAAGGCTGTAAGGCGC-3′	Forward
244bp	$56^{\cdot c}$	5′-GTCTGCAATCATTTCCTATTGCAAA-3′	Reverse

PCR reactionwas performed in a final volume of 25 ml.

The reaction mixture contained 5.2 ml of buffer PBS 10x, 0.6 mM $MgCl_2$, 0.5 mM Dntp , 1.0 pM primer (R), 1 pMprimer (F), 2 ng of DNA, $0.3_{Macrolitre}/^{unit}$, *Taq*DNA polymerase, and17.5 mlofdeionized distilled water. After mixing all the reaction components, the microtubeswere placedin a thermocyclerunit and PCR carried out. ThePCR cycling conditions included35 cycles with an initial temperature of 94 °C, a temperature of 56 °C for binding primers and of 72 °C for reaction progresseach for one minute. To confirm the results of PCR, 50 samples of PCR products were sequenced after purification on the gel using safe-V_2 stain (Cinnagen Co.).

Data were analyzed using SPSS statistical software. For the relationship between the variables and a risk of *M.hominis* infection, odds ratio (OR) and confidence interval(CI) were calculated. A significance level of less than 0.05 was considered as a positive relationship with the risk of *M. hominis* contamination.

RESULTS

Using the culture of 100 samples, 41 samples (41%) were positive for the presence of *M.hominis* (Table 2).

Using PCR on 100 pregnant women with PROM in this study, 62 patients (62%) and 2 (10%) out of20 patients in the control group were positive for infection with *M.hominis*. Thus, greater positive cases (62%) were evaluated by PCR compared with those determined by culture method. In 41 (41%) ofcases,thepositive cultureswere detectedpositive by PCR technique as well and the presence of *M.hominis* was confirmed. An amount of 21 samples (21%) were culture-negative and PCR-positive, 30 samples (30%) showed culture-negative and PCR-negative, and 8 samples (8%) exhibitedculture-positive and PCR-negative (Table 3).

Table 2: Culture and PCR results of *Mycoplasma hominis* frequency inpregnant womenwith PROM and healthy women (controls)

	Culture		PCR	
	Positive	Negative	Positive	Negative
Patient	41%	59%	62%	38%
Control	6(30%)	140(70%)	2(10%)	18(90%)

Table 3: Comparison of the frequency of PCR and culture results *Mycoplasma hominis* in pregnant women with PROM and healthy women (control)

	Culture & PCR positive (%)	Culture negative &PCR positive (%)	Culture positive & PCR negative (%)	Culture & PCR negative (%)
Patient	41(41%)	21(21%)	8(8%)	30(30%)
Control	2(100%)	0	4(22.2%)	14(70%)

Table 4: Distribution of alleles in pregnant women with PROM and healthy women (control)

Variables		No. of patients (%)	No. of control (%)	P-value*
Age (year)	15-30	57(57%)	14 (70%)	0.01
	31-39	30(30%)	6 (30%)	
	40-45	13(13%)		
Abortion record		62 (62%)	2 (2%)	0.03
Natural rupture of amniotic sac		84 (84%)	-	0.01
Vaginal infection		60 (60%)	25 (25%)	0.02

**P < 0.05*

The results of direct sequencing was exactly similar to the genome references of *M.hominis* in the GeneBank confirming the accuracy of the samples studied, that is,all the samples belonged to*M.hominis*. The pregnant women with PROM and healthy individuals (controls) were in the age range of 15-30 years and an average age of 8^{+}_{-} 26.4 years. A significant relationshipwas observed among those aged 15-30 years and increased risk of infection with *M.hominis* followed by PROM in the pregnant women (p <0.01).

Significant correlationswere detected between abortion record (p <0.03), vaginal infections before pregnancy (p <0.02), and spontaneous PROM in the pregnant women (p <0.01)withsubsequent risk of *M.hominis*contamination followed by PROM in the pregnant women (Table 4). No significant correlations were found between theother variables studied with the risk of *M. hominis* contamination. After PCR reaction,a PCR product of 244 bp was observed on agarose gel. Samplesinfected with *M.hominis* are visible in Figure1.

DISCUSSION

According to the results of this study, the incidence of PROMin pregnant women is mostly caused by *M.hominis*. Moreover, PCR techniqueis more preferred compared with culture method to identify positive cases of*M. Hominis*inpregnant women withPROM.

Genital mycoplasmas, in particular, *M. hominis*are natural inhabitants of the urogenitaltract of men and women who are sexually active (5).Isolation of this bacterium in women has been higher than men, and is transmitted through

sexual contact or from mother to thenewborn at birth. According to the findings of the present study, a relatively high number of pregnant women with PROMwere infected with *M.hominis*. The presence of *M. hominis* in the urogenital tract is often asymptomatic and considering the serious consequences of these infections including pelvic inflammatory infection and infertility, microbial screening for women and their husbands, especially at the young ages, is inevitable (27 and 28).

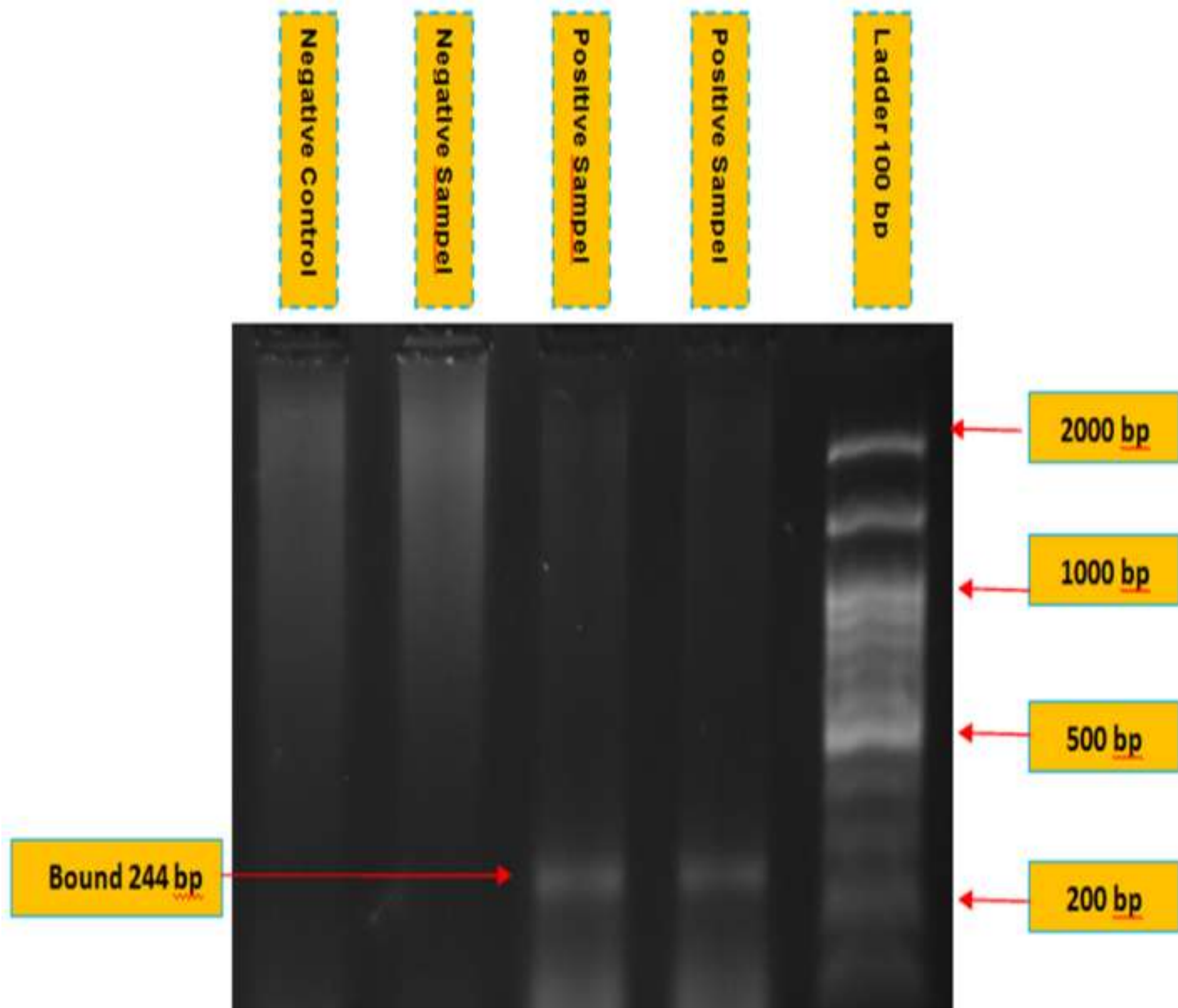

Figure 1: PCR results on an agarose gel

Because culture method is very long and time-consuming, access to desirable results is not plausible in a short time. Comparatively, PCR is a sensitive and fast methodwith unique and specific features that canidentify DNAeven from deadbacteria contrary to culture method. Mycoplasmas lack cell walls, hence, they are sensitive to environmental conditions and culture results can be falsely negative while PCR method can alsorecognize DNA from dead bacteria. In the present study, those cases of samples thatwerepositiveby PCR but became negative by culture results (18%) were considered as false positive. This is because culture is not as a gold standard method and has never been as sensitive and specific as 100 percent. In a study by Growender et al.(2009), positive results from PCR that were negativein cultures were not considered as false positive (26).

Although *M.hominis*is easier to grow *in vitro* compared to other human mycoplasmas, it is very sensitive to environmental conditions such as pH, temperature, and composition of the medium because of lacking cell wall compared to other bacteria,thus,the bacteriummay be weakened or destroyed during sampling or transferring to the

laboratoryso that it cannot beverified in the media. However, since PCRdoes notneed live bacteria, falseresults cannot be obtained under the influence ofsamplingand transporting to the laboratory. Furthermore, *M.hominis* culturetakes from over 2 to 5 daysin favorable conditions and requires very specific culture media with nutritive supplements and experienced laboratory experts, all of whichmakethe cultureoperation costly and time-consuming. Using PCR,on the other hand, several samples can be tested simultaneously andthe resultsreportedwithin hours. In this study, the prevalence of *M.hominis* bycultureand PCR methods, respectively, were 41% and 62%. A number of studieshave been conductedworldwideconcerning the prevalence of genital mycoplasmas. The prevalence of*M.hominis*detected by culture method has been variablefrom 5 percent in Mexico (25) to 26 percent in Turkey (29). Mozaffariet al.(2008)examined 210 female patients with genitalcontaminations of mycoplasmas in women with mycoplasma infections, among which39% of thebacterial genus*Mycoplasma*was identified byculture method whereas 57.1% of the samples were positive for mycoplasma infection byPCR method (10). Ramezani et al. (2004) investigatedthe prevalence of *M.hominis*and *Ureaplasmaurealyticum*in patients with abortionand concluded that 60.8 percent of the samples were positive for *M.hominis* and the aborted fetuses often caused by *M.hominis* infections (3).

In this study, the most percentage ofthe participantswerewithin the age rangeof 15-30yearswhoshowed significant associationswith increased risk of infection with *M.hominis* and subsequent PROM in pregnant women. Mousavianet al. showed that this bacterium was more frequent in peopleduring the ages of sexual activity (11). AmirMozaffariet al. reportedthe most positive cultures to be in the age group of 29-39 years (10). The current study recorded a significant relationship between the presence of *M.hominis* and the history of vaginal infection. Also,there was a significant relationship between the history of abortion with spontaneous PROM in the pregnant women and increasing risk of infection by this bacterium followed by PROM.

It is recommended that more attention be paid to the role of *M.hominis* in genital infection, diagnosis, and timely treatment of patientsas well asconsidering mycoplasma infections as a health priority. Accordingly, it is necessary toestablish identificationtestson this organism in medical diagnosticlaboratories so thatthe gynecologists and obstetricians benefit fromthese teststo confirm their observations.

CONCLUSION

The results of this study indicate that the incidence of PROM in pregnant women aremostly caused by the bacterium*M.hominis*. In addition,the findings signify that PCR technique is preferred in comparison with culture method for the identification of positive cases of *M.hominis* in pregnant women withPROM.

Acknowledgments
This article was extracted fromathesis of Master'sin Microbiology by ArezooArdforoosh atthe Faculty of Science, Islamic Azad University,AharBranch. Hereby, the respected authorities in Ardabil University of Medical Sciences andthe Alavi Hospital in Ardabil Provinceare extremely appreciated for any kind of assistance in collecting samples and providing all equipment and facilities to carry out culture and PCR techniques.

REFERENCES

[1] Javtz, Melnik, Adelberg, Brooks, Bottles, Morse, Carol. Translated by Mohammad Karim Rahimi, Amid Azhari. Javtz medical microbiology. Tehran: Aeezh Publishers, 24th edition, 2008.
[2] Jamalizadeh, S.Kheirkhah, B., Farsinejad, AliReza, et al. "Isolation and molecular identity of *Mycoplasma hominis* in the genitalia of infertile males and femalesinKerman". Kerman Medical Journal, 2014, (7) (3), 233-240.
[3] Ramezani, Amitis. Mohraz, Mino. Nazgooie, Fereshteh. Mafakheri, Maryam. The prevalence of *Mycoplasma hominis*and*Ureaplasmaurealyticum* in patients with abortion admitted to a clinic of infectious diseases in 2001. Iranian Journal of Infectious Diseases and Tropical Medicine, 2004. (24) (9), 27-32.
[4] Karmowstji, Afsaneh. Prevalence of genital mycoplasmas in women with abortion in Tehran, Hormozgan Medical Journal, 2002, (3) (6), 10-16.
[5] Karimzadeh, MohammadAli and Taheripanah, Robabeh. Infection causes in recurrent miscarriage. Quarterly Journal of Reproduction and Infertility, 137: 24-34.
[6] Hassani, Ahmad et al. Detection of the bacteria *Mycoplasma hominisUreaplasmaurealyticum* in patients with genital tract infections using PCR. Journal of Tropical and Infectious Diseases, Infectious Disease Specialists Association, 2012. 17(58), 45-50.

[7] Marashi, SayedMahmoud. The prevalence of *Mycoplasma hominis*and*Ureaplasmaurealyticum*ininfertile women.Yafteh,theIranian Journal,2002 (2), 29-32.
[8] Mousavian, SayedMojtaba. Mo'tamedi, Hossein. Maleki, Susan. Shahbazian, Nahid. Comparison of the prevalence of *M. hominis*and *U. urealyticum* in women with urogenital infections using Multiplex PCR technique and bacterial culture methods. Medical Journal of Tabriz University of Medical Sciences, 2011,33(5), 91-97.
[9] Mash'oufi, Mehrnaz. Nazari, Robab. Comchoreh, Roghiyeh. Sharghi, Afshan. Mardeh, Afrooz. GharehzadAzari, Maryam. The effectiveness of screening plan and treatment for bacterial vaginosis infections in routine cares during pregnancy in reducing preterm delivery. Journal of the University of Medical Sciences, 2012. 12(3), 312-320.
[10] Mozaffari, Nooramir. Jeddi, Farhad. MasjedianJezee, Faramarz. HaghighiLadan. Prevalence of *Mycoplasma hominis*and*Ureaplasma urealyticum* in the genital tractinfections. Journal of the University of Medical Sciences, 2008. (15) (60), 19-25.
[11] Mousavian, Mojtaba. andPordeli, HamidReza. Evaluation of mycoplasma infections in the respiratory and reproductive-urinarytracts in patients referred to the Imam Khomeini Hospital in Ahwaz. Journal of Kerman University of Medical Sciences, 2003. (10), 185-192.
[12] Williams. Translated byDr.BahramGhazi Jahan. Williams pregnancyanddelivery. Tehran: GolbanMedical Sciences Publishers, 24th edition. 2013.
[13] Vatani, Shideh.Saeedi, Kiomarth.Mohammadi, Maryam. Contaminations with genital mycoplasmas in women with bacterial vaginosis of negative culture using PCRmethod. Research Journal of Laboratory Sciences, Tehran, 2006, (8)(15), 230-235.
[14] Nosrat, Sepideh. et al. Frequency of genital mycoplasmas in vaginal infections in Gorgan. Infectious Diseases Research Center, GolestanUniversity of Medical Sciences, 2010. 20-22.
[15] Niakan, Mohammad. Ghaffari, Ma'refat. andAbedi, Fahim. The frequency of *Mycoplasma hominis*and*Ureaplasmaurealyticum* ininfertilewomencompared to infections in their husbands. Journal of Kermanshah University of Medical Sciences, 2009. 202 (13), 197-3.
English references
[16] Arreola M.GonzalezM.Tenorio A.PCR Detection of CHlamydia trachomatis, Neisseria gonorrhoae, Mycoplasma hominis And Ureaplasmaurealyticum. 2014, V(69)(5), 330-339.
[17] Blanchard A. Yanez A. Dybvig K. Watson HL Evaluation of intrespecies genetic variation within the 16s rRNA gene of *Mycoplasma hominis* and detection by polymerase chain reaction. J ClinMicrobiol, 1993, 1358-1375.
[18] BellenG.ReybrockR.Donders G. Predicitive value for preterm birth of abnormal vaginal flora, bacterial vaginosis and aerobic vaginitis during the first trimmest of pregnancy. 2009, 115(12), 240-1315.
[19] Byrne WJ. MC Cormack R. Ball HJ. Brice N SE. Ayling RD .Nicholas RAJ. Application of an indirect Elisa to milk sample to identify cow with *Mycoplasma bovis* mastitis. The veterinary record. 2000. 146, 368 -9.
[20] Bashiruddin J B. Santini F G. Santis P. Vissagio M C. Francesco G. D'AngeloA.Nicholas R A J..Detection of *Mycoplasma mycoidesmycoides* small colony in tissues from an outbreak of contagious bovine pleuropneumonia by culture, IHC and PCR. Vet Rec., 1999. 145: 10,271-274.
[21] Beverly E. Zariffard MR. Wang QJ. Chen HY. Bremer J. Cohen M, et al." Femal gen ital- tract HIV Load. Correlates *Mycoplasua hominies*. J in fect Dis2004, 191: 25-32
[22] Broan, Cloning gene.2009,90-130.
[23] EriceA.WeerayuthK.Pularj K.et.al. Comparative Genomic Analysis of Seven *Mycoplasma hyosynoviae* Strains.2014, V(4)(2),243-259..
[24] FayuraL.BoretskyYu.PynyahaYu,et.ai. Deveiopment of Cultivation Technology For the *Eschrichia coli* Recombinant Strain Producing DEIMINASE of *Mycoplasmahominis*,2014, V(10)(4),29-36.
[25] Georg F., Medical microbiology. New York: 2007.
[26] GovenderS.Theron‹GB.Chalkley‹LJ.et.al. Prevalence of Genital *Mycoplasma*,*Ureaplasma*and*Chlamydia* in Pregnancy.2009,V(29)(8),698-701.
[27] JosianM.KopardeV.BrooksJ.et.al. Identification of a gene in *Mycoplasma hominis*associatedwith Preterm Birth and Microbial Burden In Intra-aminiotic Infection.2014,V(10)(4),32-42.
[28] Jamalizade S.Naeimem.Kheirkhahb.et al.Isolation and molecular identification of *Mycoplasmahominis* in infertile female and male reproductive system,2014,6(6),1-10.
[29] Josephine Lusk.Pamela K. Peter A. White, William D,Rawlinson. Multiplex PCR Testing Detection of Higher-than-Expected Rates of Cervical *Mycoplasma*, *Ureaplasma*, and *Trichomonas* and Viral Agent Infections in Sexually Women. Journal of Clinical Microbiology, 2009;47:1358 – 1363.
[30] kundsinRB.*Mycoplasma* isolation from the urine and metabolic inhibition to t-strain in nuns. Bacteriol. Proc.1971.77-78.

[31] KwakDw.HwangHs. Kwon j.et al. Co-infection With Vaginal *Ureaplasmaurealyticum* and *Mycoplasma hominis*Inscreases Adverse Pregnancy Outcomesin Patients With Preterm PremutureRubture of Membrans, 2014,V(27)(4),333-337.
[32] Kuppeeveld,J.Loget, A. Angulo, M.et al. Genus-and species-specific identification of Mycoplasmas by 16S r RNA Amplification.1992,58(8),2606-2615.
[33] Larsen B, Hwang J. *Mycoplasma, Ureaplasma*, and Adverse pregnancy outcomes:A fresh look. Infect Dis ObstetGynecol2010, ; 8:17.
[34] Lukin.Lebel.P,et al. Comparison of polymerase chain reaction assay with culture for detection of genital *Mycoplasma* in prenatal infection. EuropJClinMicrobialInfect Dis.1998.17(4),255-263.
[35] Lanzafame M, Delma A, Lattuada E, Faggain F, Padovani G, Concia E, Vento S. Prevalence and clinical significance of *Ureaplasmaurealyticum* and *Mycoplasmahominis* in the lower genital tract of HIV-1-infected women. Le Infezioni in Medicina, 2006. 4, 213-215.
[36] Moosavian SM. PordeliHR.Survey of *Mycoplasma* infections in respiratory and urogenital tracts of patients in Ahvaz Emam Khomeini Hospital .J JKerUniv Med Sci, 2003; 10 (4): 185-195 (Persian)
[37] Pasturalm. Audardv. BraletMp. Remy Ph. salomon L. Tankonic J. et al. *Mycoplasmahominis* infection in renal transplantation. Nephrol Dial Transplant; 2002,17: 495-496
[38] ScholossbergD.,*Mycoplasma* infection. Cecil Text Book of Medicine, 2004.
[39] VasilikiM.PantelisC.KostantinosM,etal. MolecularInvestigation of Menstrual TissueFor The Presence of *Chlamydia trachomatis,Ureaplasmaurealyticum,*and*Mycoplasmahominis* Collected By Women With a History of Infertility. 2013,V(40)(1), 237-242.
[40] WaitesKB. *Ureaplasma* infection. E Med J, 2001; 2: 1-17
[41] World Organization of Animal Health (OIE). In manual of diagnostic tests and vaccines for terrestrial: Contagious Caprine Pleuropneumonia., 2004, 2, 4-6.