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Chapter 4

# Molecular Study for Diagnosis of *Ureaplasma parvum* in Women with Recurrent Miscarriage

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**Abstract**

The objects of study is concerted to investigate the occurrence of *Ureaplasma parvum* in women with recurrent abortion and to determine the distribution of *U. parvum* serovars (1, 3, 6, 14) in women with recurrent abortion by conventional polymerase chain reaction (PCR) technique. In total, 130 samples included vaginal bleeding, vaginal swab, and urine, were collected from women with recurrent abortion and 40 samples included vaginal swab and urine from control women without recurrent abortion. Through the study, two types of media were used, *Ureaplasma* broth (IH Broth) and *Ureaplasma* agar (IH Agar). The positive isolates for *Ureaplasma* spp. were investigated by conventional PCR technique for identification of *U. parvum* and subtyping to their serovars (1, 3, 6, 14). The results revealed the *U. parvum* was identified in 29.6% from patient group and 11% from the control group. *U. parvum* isolates were further subtyped by using PCR, the results showed the serovar 3 was the most frequent isolate in proportion (42.8%), whereas serovar 1 (28.5%), serovar 6 (14.2%), and serovar 14 (14.2%) in patient group but in the control group only serovar 1 was isolated in rate (11%). These results evidently indicate that *U. parvum* may be an important etiologic agent for recurrent abortion.

**Keywords:** IH medium, PCR, recurrent miscarriage, serovars, *Ureaplasma parvum*

## 1. Introduction

Recurrent miscarriage is the loss of three or more consecutive pregnancies ending of pregnancy by removing a fetus or embryo before it can survive outside the uterus [1, 2]. A miscarriage which occurs spontaneously is also known as a miscarriage and World Health Organization (WHO) explained the around 56 million recurrent miscarriage before the 24th week of gestation occur each year in the world unexplained [3].

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Any sever infection that leads to bacteraemia or viraemia can cause sporadic miscarriage. The role of infection in recurrent miscarriage is unclear; the presence of bacterial vaginosis in the first trimester of pregnancy has been reported as a risk factor for second-trimester miscarriage and preterm delivery [4].

*Ureaplasma parvum* could be important pathogen that may affect pregnancy outcomes and the health of neonates was first given serious consideration when reported of postpartum endometritis with septicemia, chorioamnionitis [5]. Since those days, numerous clinical studies have been performed in an attempt to clarify what roles, if any, these organisms play as agents responsible for invasive infections in neonates, premature labor, spontaneous miscarriage, stillbirth, and chronic lung disease of prematurity [6].

Although more than 30 years of study inside and outside of Iraq, many clinical importance of genital *Ureaplasma parvum* are still incompletely understood for a variety of reasons. These include (1) the high prevalence of these organisms in healthy persons; (2) poor design of many of the earlier research studies, which attempted to relate the more presence of *Ureaplasma parvum* in the lower urogenital tract to pathology in the upper tract or in offspring; (3) unfamiliarity of clinicians and microbiologists with the complex and fastidious nutritional requirements for *Ureaplasma parvum* and the methods detection [7].

*Ureaplasma parvum* found in the placenta and endometrium is associated with infection, the birth of a dead fetus, spontaneous miscarriage, premature delivery and lowers than normal weight of infant. *Ureaplasma parvum* penetrate into amnion in the second trimester it may cause chorioamnionitis [3]. *Ureaplasma parvum* was found in the blood of mothers who have had problems with high fever after childbirth this infection can be transmitted to about 40% of babies who were born to a mother with this infection if the mother has it, *Ureaplasma parvum* can infect the lungs of the newborn during childbirth [8].

*Ureaplasma parvum* are the most prevalent, possibly pathogenic bacteria isolated from the urogenital tract of both men and women, they are also frequently associated with preterm birth and other adverse pregnancy outcomes [9]. Genital *U. parvum* (biovar 2, serotypes 1, 3, 6 and 14) are considered natural inhabitants of the lower urogenital tract of humans as they are often isolated from healthy individuals and involved in a variety of infections in humans the isolation of *U. parvum* from patients with genitourinary tract infections [10]. Waites [5] found *U. parvum* to be dominant in patients with pelvic inflammatory disease as well as in women who had miscarriages, and it seemed to have more adverse effects on pregnancy outcome regarding birth weight, gestational age, and preterm delivery than *U. urealyticum* and shown that *U. parvum* can be isolated more frequently from patients with a history of recurrent miscarriages than from normal pregnant women.

*Ureaplasma parvum* are the microorganisms most frequently isolated from amniotic fluid (AF) or placentae in women who deliver preterm between 23 and 32 weeks pregnancy and *U. parvum* has been linked with adverse pregnancy outcomes such as late miscarriage and early preterm birth Also identified *U. parvum* in 57% of healthy non pregnant women and the organism was far more prevalent than any of the other genital mycoplasma, *Chlamydia* spp. or viruses [11]. *Ureaplasma parvum* infections require the therapeutic use of antimicrobials. Tetracyclines, macrolides and quinolones are the major antibiotics used in the treatment of

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genital *Ureaplasma*. However, their therapeutic efficacy may be unpredictable due to increasing resistance [9]. *Ureaplasma* spp. is the most prevalent, possibly pathogenic bacteria isolated from the urogenital tract of both men and women [9]. *Ureaplasma* spp. are also frequently associated with preterm birth and other adverse pregnancy outcomes and *Ureaplasma* spp. are colonies isolated in female genitourinary tract sometimes these microorganisms do not evaluate as infectious agents [12]. Detection of *Ureaplasma* is possible by the characteristic growth on appropriate media and urease activity, but species identification of *U. urealyticum* and *U. parvum* must be demonstrated by molecular methods [10]. Differentiation between *U. parvum* and *U. urealyticum* is very important, especially for correct interpretation of laboratory results and evaluation of pathogenicity [13]. *Ureaplasma* spp. do not have cell wall, are fastidious and mostly referred to as no cultivable organisms [12]. Genital tract infections with *Ureaplasma* caused approximately 50% of preterm labor and recurrent abortion [9]. Most of *Ureaplasma* infected pregnancies produced infant with low weight at birth with increased risk of recurrent abortion (at or before 14 weeks). Also, 60% of mortality among infants with no anatomic or chromosomal defects is low birth weight [14]. *U. parvum* has been linked with adverse pregnancy outcomes such as late abortion and early preterm birth. *Ureaplasma* spp. are the microorganisms most frequently isolated from amniotic fluid or placentae in women who deliver preterm between 23 and 32-weeks pregnant [13, 15]. *U. parvum* are involved in a variety of infections in genitourinary tract infections of humans [10, 16]. Identified *U. parvum* in 57% of healthy non pregnant women and the organism was far more prevalent than any of the other genital mycoplasmas, *Chlamydia* spp., or viruses [11, 17]. The proposed mechanisms for infectious causes of recurrent abortion include: direct infection of the uterus, fetus, or placenta; placental insufficiency; chronic endometritis or endocervicitis; amnionitis; infected intrauterine device [18, 19]. *Ureaplasma* can be detected in the cervix or vagina of 40–80% of sexually mature asymptomatic women [20]. *U. parvum* may play important role in pregnancy and eliciting conditions associated with prematurity [21]. The main aim of this study is to investigate the occurrence of *U. parvum* in women with recurrent abortion and to determine the distribution of *U. parvum* serovars (1, 3, 6, 14) in women with recurrent abortion by conventional polymerase chain reaction (PCR) technique.

## 2. Materials and methods

### 2.1. The bacterial isolates

In total, 130 samples included vaginal bleeding, vaginal swab, and urine, were collected from women with recurrent abortion and 40 samples included vaginal swab and urine from control women without recurrent abortion. All specimens were cultured in IH broth, which consists of PPLO broth, tryptone soya broth, yeast extract powder, distilled water, and supplements [22]. Then make a subculture to IH agar, which consists of PPLO agar, tryptone soya broth, $MgSO_4 \cdot H_2O$, yeast extract powder, agar-agar, distilled water, and supplements [22]. The *Ureaplasma* spp. isolates were identified by examination of colonial morphology on IH agar media as dark golden-brown or rich, deep brown, and granular appearance because of accumulation of magnesium oxide inside and outside the colony [17].

### 2.2. Molecular experiments

Molecular experiments included the extraction of *Ureaplasma* DNA by using the Reagent Genomic DNA Kit (Geneaid, New Taipei, Taiwan). PCR identification of *U. parvum* was done according to Kong et al. [23, 24] and master mix kit (BioNeer, Irvine, California). PCR was performed with primers specific for highly conserved regions in the 50 end of multiple band antigen gene of *U. parvum*. Primer for diagnosis of *U. parvum*, UMS-57/UMA222, is shown in **Table 1** [23, 24]. The primers for detection of serovars UMS3S/UMA26, UMS14S/UMA314A, UMS-83/UMA1A, and UMS-54/UMA269 (BioNeer, Irvine, California) are shown in **Table 2** [23, 24] and were used for subtyping of *U. parvum* to amplify the repetitive of the multiple band antigen genes of *U. parvum* serovars.

| Organism | Primer (F)/(R) | Sequence (5′-3′) | Size of amplified product (bp) | Target gene |
|---|---|---|---|---|
| *U. parvum* | UMS-57 | F (TAA ATC TTA GTG TTC ATA TTT TTT AC -57) | 326 | 5′ Ends of MBA genes and upstream regions |
| | UMA222 | R (GTA AGTGGA TTA AAT TCA ATG 222) | | |

MBA, multiple band antigen. Adapted with permission from [23–25].

**Table 1.** PCR primer employed in the detection of *Ureaplasma parvum*.

| Organism | Primer (F)/(R) | Sequence (5′-3′) | Size of amplified product (bp) | Target gene |
|---|---|---|---|---|
| *U. parvum* Serovar 1 | UMS-83/UMA1A | F (TTACT GTA GAA ATT ATG TAA GAT TGC)<br>R (TTT CTT TTG GTT CTT CAG TTT TTG AAG) | 578 | MBA |
| *U. parvum* Serovar 3 | UMS3S/UMA269 | F (TTA CTG TAG AAA TTA TGT AAG ATT ACC)<br>R (AA CTA AAT GAC CTT TTT CAA GTG TAC) | 400 | MBA |
| *U. parvum* Serovar 6 | UMS-54/UMA269 | F (AAT CTT AGT GTT CAT ATT TTT TAC TAG)<br>R (ACCA AAT GAC CTT TTG TAA CTA GAT) | 370 | MBA |
| *U. parvum* Serovar 14 | UMS14S/UMA314A | F (AAT TAC TGT AGA AAT TAT GTA AGA TTA AT)<br>R (GTT GTT CTT TAC CTG GTT GTG TAG) | 572 | MBA |

MBA, multiple band antigen; *U. parvum*, *Ureaplasma parvum*. Adapted with permission from [23–25].

**Table 2.** PCR primers employed for subtyping of *Ureaplasma parvum* in to serovars.

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### 2.3. PCR technique

The 20 ul amplification reaction mixtures contained 10 pmol of each primer, 5 ul of DNA template, and PCR water added to 20 ul for identification *U. parvum*. The PCR conditions were used as follows: initial denaturation at 95°C for 5 min, denaturation at 95°C for 30 s, annealing at 58°C for 30 s, extension at 72°C for 1 min for 40 cycles, and final extension at 72°C for 5 min in a thermo cycler. The PCR positive isolates for *U. parvum* were further subtyped into serovars as described in **Table 2**. Briefly, the PCR conditions were used as follows: initial denaturation at 95°C for 5 min, denaturation at 95°C for 30 s, annealing at 55–62°C for 30 s, extension at 72°C for 1 min for 40 cycles. Amplified PCR products (12.5 ul) were visualized under UV light after electrophoresis in 2% agarose gel which was stained with 0.5 mg/ml of ethidium bromide. A visible band of the appropriate size on UV transillumination was considered a positive result.

## 3. Statistical analysis

The data were analyzed using SPSS statistic software version 20 (IBM, Armonk, USA) for comparison of qualitative variables using $P < 0.05$ and odd ratio. Association between *U. parvum* infection and recurrent abortion was statistically significant.

## 4. Results and discussion

### 4.1. Laboratory identification of *Ureaplasma* spp. (colonial morphology)

In this study *Ureaplasma* spp. isolates was identified by examination of colonial morphology on IH agar media used in this study as dark golden-brown or rich deep brown and granular appearance. All isolates revealed positive urea analysis. These results were in accordance with [17, 26].

### 4.2. Isolation of *Ureaplasma* spp. on culture media

Detection of *Ureaplasma* spp. is possible by characteristic growth on appropriate culture media but species identification of *U. parvum* along with serovar identification by molecular method is important especially for correct interpretation of laboratory results and evaluation of pathogenicity. In present study, *Ureaplasma* broth media (IH broth) & *Ureaplasma* agar media (IH agar) were used to isolate *Ureaplasma* spp. the rate of isolate are (52.3%) from vaginal bleeding, (30.7%) from vaginal swab and (7.6%) from urine. The reason for the high isolation of *Ureaplasma* spp. on IH medium can be attributed to supplementation with DNA, Putrescine – dihydrochloride, cysteine which enhances the microorganisms with lipid materials essential for growing of these bacteria. Also a mixture of antibiotics was used (Ceftriaxone, Amoxicillin, Augmentin, Nystatine, & Fluconazole) to prevent contamination may occur in the conventional broth media. The amount of horse serum reduced from 20 to 10 ml to reduce the cost

and it is sufficient for growth of these microorganisms. *Ureaplasma* agar media (IH medium) is supplemented with essential requirements for growing of these microorganisms. These are horse serum, yeast extract, urea, cysteine, antibacterial and antifungal that inhibited the growth of other bacteria and fungi and the optimal PH (6.0) [22].

The medium contains urea and sensitive indicators of ammonia, Manganous sulfate which is firstly used by [27]. Sulfate salt of manganese was described to support the growth. Manganous sulfate was added in a final concentration of 0.03% was therefore, selected as the ammonia – detecting reagent of choice. *Ureaplasma* spp. colonies appeared within 2 days, identified as dark golden-brown owing to accumulation of manganese oxide in the colony.

Moreover, putrescine – dihydrochloride was added to enhance the *Ureaplasma* spp. growth and development of the precipitate in the colonies (Phillips et al., 1986). In addition the size of *Ureaplasma* spp. were seen to be larger when add putrescine – dihydrochloride. The originality of this media can be attributed to horse serum (10%) instead of (20%), yeast extract as a powder, BBL tryptone soya broth together with PPLO, also used DNA and putrescine in same medium. Moreover the mixture of antibiotics prevents contamination of medium by bacteria and fungi that easily contaminate conventional media. Moreover, agar – agar was added to the medium was more than 3% which believed it inhibited the bacterial growth [22]. Therefore, IH media considered as enrichment, differential and selective for *Ureaplasma* spp. because it resolved the important problem of culturing these bacteria.

Other study used IH medium for isolate *Mycoplasma* spp. and *Ureaplasma* spp. [28] isolated *Mycoplasma hominis*, *Ureaplasma urealyticum* and *Ureaplasma parvum* on IH medium from infertile male in rate (5.8%), (5.8%) and (3.5%) respectively. Also [29] isolated *Mycoplasma pneumoniae* in rate (18%) on IH media from tonsillitis patients. Another study was isolated *Ureaplasma urealyticum* in rate (16.8%) from infertile female, (20%) from infertile male and isolated *Mycoplasma hominis* in rate (27.7%), (1.6%) from infertile female and male respectively on MAU-medium similar to IH medium [30]. In the present study the results on IH medium *Ureaplasma* spp. was identified as dark golden-brown or rich deep brown colonies **Figure 1**. Moreover, *Ureaplasma* spp. was identified by its granular appearance **Figure 2**.

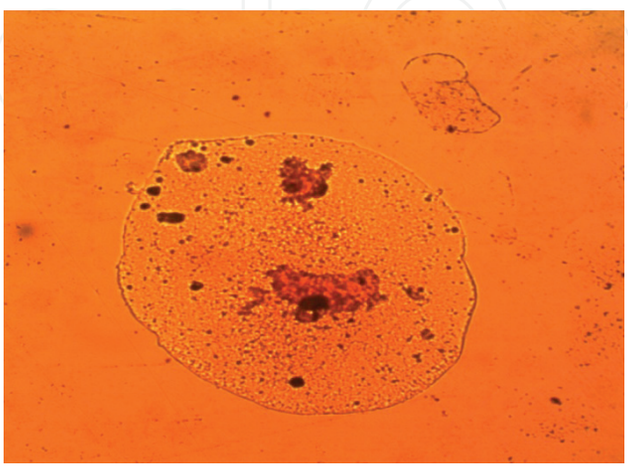


**Figure 1.** *Ureaplasma* spp. as dark golden-brown colonies in IH medium under light microscope 10X.

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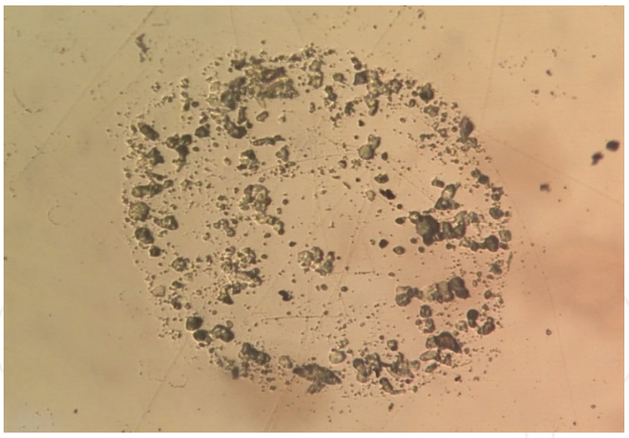


**Figure 2.** *Ureaplasma* spp. as granular appearance in IH medium under light microscope 10X.

### 4.3. Comparison between age groups of miscarried women and percentage of isolation of *Ureaplasma* spp.

The results of this study showed that the age patients range 17–26 & 27–36 years old represented a high rate (46%), (40.7%) respectively compared with group (37–46) represented (13.3%) as shown in **Figure 3** included in this study. The incidence of *Ureaplasma* spp. infection was also reported by some other studies as higher in age 26–30 years [31]. The high isolation detection in miscarried women of age group 17–26 & 27–36 years. This may be attributed to the sexual activity of females together with hormonal change, so the genital tract is more susceptible for infection [32]. The high rate of *Ureaplasma* spp. infection detected in this study suggests that this agent is widespread among miscarried women.

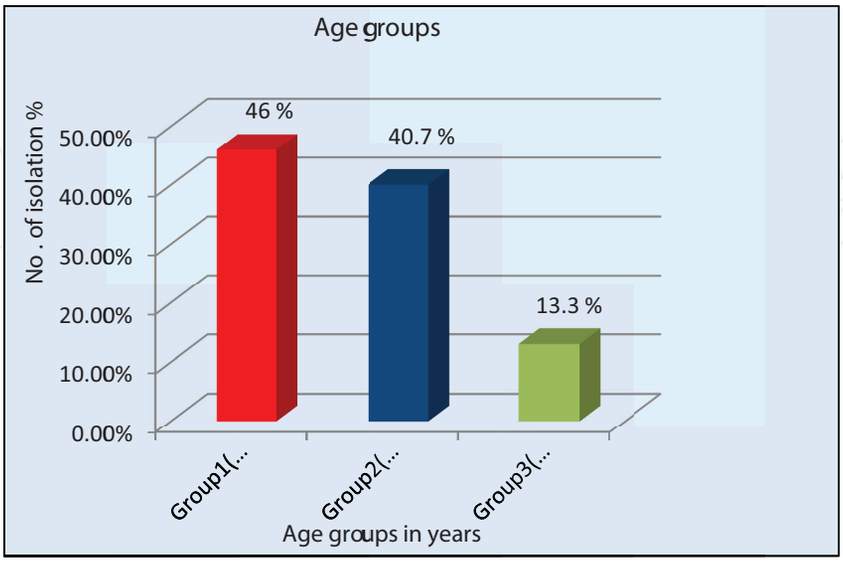



**Figure 3.** Histogram showing the distribution of *Ureaplasma* spp. according to 10 years age intervals of patients with recurrent miscarriage.

This can be attributed to the sexual activity among this groups since there is an increased in estrogen hormone produced from female genital tract leading to change the vaginal environment which is regarded as a factor for infection [33]. because the estrogen is important hormone during pregnancy, it makes to adjust the level of the hormone progesterone is essential in the formation and development of the fetus, so pregnancy is one of the reason leading to the rise in the hormone estrogen [34]. For this reason, when the increased of estrogen, it affects the hormone progesterone is necessary in pregnancy, which affects the thickness of the lining of the uterus and the difficulty of adapt fetus with her. However it is generally difficult to determine whether these agents cause colonization or infection. Since the incidence of infection is affected by some factors, such as: menstrual cycle, bacterial and protozoan infection (co-infections), and socio-economic conditions like poverty. Also the age group 17–26 & 27–36 years are the most widely accepted for marriage and reproduction in our society for this reason the proportion of isolation the *Ureaplasma* spp. be high. It has been shown in this study that the percent of isolation of *Ureaplasma* spp. was directly associated with age. The isolation rate decrease in 37–46 years this may be due to the changes that associated with a decrease in the incidence of genital *Ureaplasma*, also due to vagina multilayer lining are atrophic in ages 37–46 years old. All of these reasons associated with decrease in the incidence of genital *Ureaplasma* spp. From the results of this study, can conclude that the genital *Ureaplasma* were significant correlation with age. Statistical analysis: ($P$-value = 0.001) appeared highly significant between patients and controls according to age interval with ($P < 0.05$).

### 4.4. Relationship between the isolation of genital *Ureaplasma* spp. and type of specimen

**Figure 4** shows the distribution of bacterial isolate of genital *Ureaplasma* according to the clinical specimen. The results exhibited that vaginal bleeding from miscarried women given high percentage of isolation (52.3%) followed by vaginal swab (30.7%) and urine (7.6%). According to the

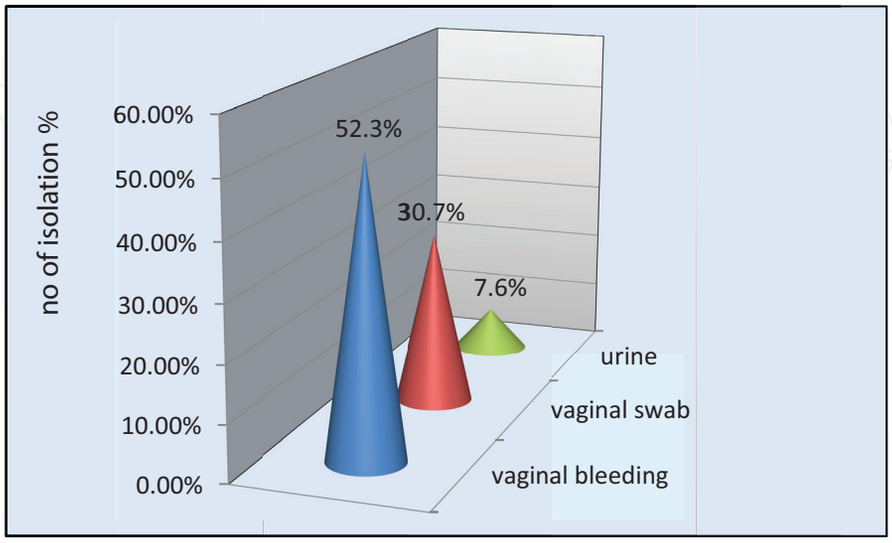



**Figure 4.** Distribution of genital *Ureaplasma* spp. isolate among clinical specimen.

available knowledge genital *Ureaplasma* spp. are not screened by routine examination of vaginal bleeding, urine, vaginal swab from miscarried woman in health laboratories in Iraq. Little studies that working culture examine for detection of these organisms in specimens taken from miscarried woman. The results obtained by this study explain isolated genital *Ureaplasma* in percentage more than 38% from vaginal bleeding samples, this may be due to dysfunction of placenta and the vaginal bleeding is a marker for placental dysfunction, vaginal bleeding is most likely to be seen around the time of the luteal-placental shift [35]. There has been little investigation of first trimester bleeding. It is interesting that the peak in bleeding episodes coincides with the development of a hormonally functional placenta. In very early pregnancy, the corpus luteum produces progesterone. The shift from luteal production to placental production of progesterone occurs by the seventh week of pregnancy and can result in a temporary reduction in progesterone levels if the placenta is not producing sufficiently [36]. Decreasing levels of progesterone are associated with the onset of menses outside of pregnancy; similarly, during pregnancy, decreasing levels may trigger an episode of vaginal bleeding and limit successful maintenance of the pregnancy. Thus, bleeding at this time in pregnancy may signal that the early placenta has not developed optimally [37]. One of the routes of intrauterine infection with *Ureaplasma* spp. by hematogenous dissemination through the placenta this mechanism occur by which microorganisms are able to pass through the cervix, infect the maternal and fetal (chorioamnion) layers of the placenta and often access the amniotic fluid and outcome common intramniotic infection lead to abortion [19].

An explanation for these variations may be related to the type of specimens investigated for isolation, the methods used for transport and storage, and media used for primary isolation of *Ureaplasma* spp. In light of the observation and experience during this study, centrifuged urine sediments yield more positive cultures than the urine specimen without centrifuged.

### 4.5. Molecular detection for diagnostic of *Ureaplasma parvum* by polymerase chain reaction (PCR)

The results showed the *Ureaplasma parvum* isolated in rate (29.6%) from women with recurrent abortion and (11%) from control as shown in **Figure 5** (*P*-value <0.05 appeared highly significant). The results revealed positive isolates for *Ureaplasma parvum* by using UMS-57/UMA222 primer as shown in **Figure 6**. The negative isolates for *U. parvum* may be because of the fact that *Ureaplasma* are divided into two species *U. parvum* and *U. urealyticum*, these two species cannot be identified by characteristic growth on appropriate media and only identified by molecular methods [23, 25]. So the negative results may be *Ureaplasma urealyticum* rather than *Ureaplasma parvum* and the results appeared to be attributable to a higher proportion of women with recurrent abortion. It may be hormonal effects which could increase *Ureaplasma parvum* counts and thus the likelihood of detection during pregnancy. A previous study showed that there is *Ureaplasma parvum* in rate (20%) from women with recurrent abortion in China by using PCR technique [38].

Although *Ureaplasma parvum* was isolated in rate (25%) from women with symptoms of urethral, cervical discharge, genital pruritis, dysuria in India [24, 39]. However, some other studies detected this organism in high rate (approximately 79%) from pregnant women and women with sexually transmitted disease in Australia [23]. *Ureaplasma parvum* positive isolates were

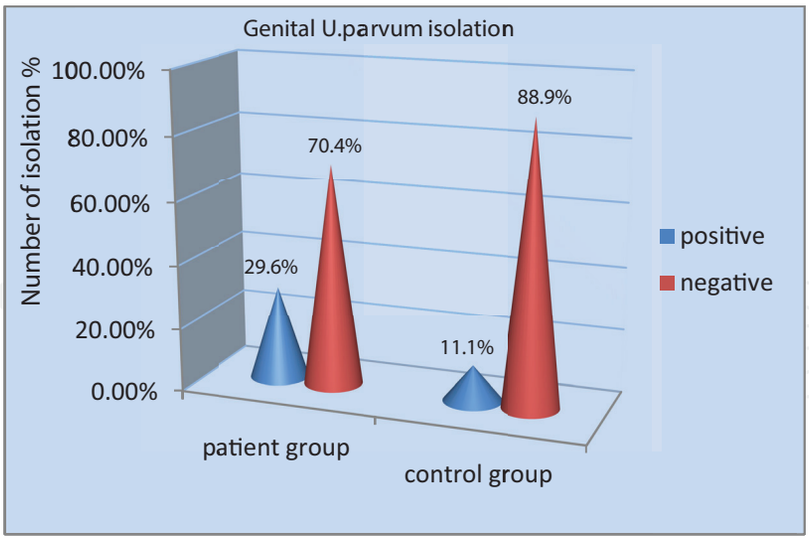



**Figure 5.** Prevalence of *Ureaplasma parvum* among patient group and control group.

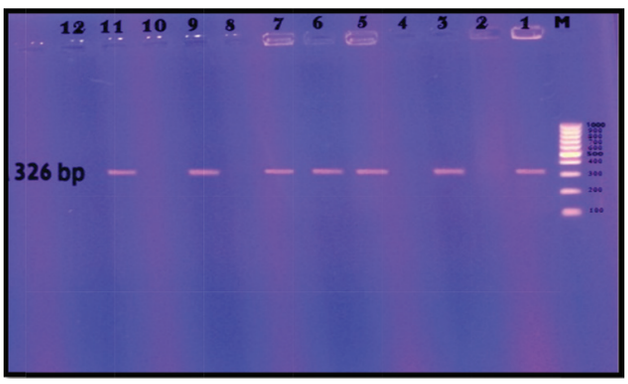



**Figure 6.** Ethidium bromide-stained agarose gel showing PCR amplification product with (326 bp) primers for *Ureaplasma parvum*. M 100 bp standard size reference marker. Lanes (1, 3, 5, 6, 7, 9, and 11): *Ureaplasma parvum* positive results. Lanes (2, 4, 8, 10, and 12): *Ureaplasma parvum* negative samples.

further subtyped into serovars 1, 3, 6, 14; the results revealed *Ureaplasma parvum* (biovar 2) serovar 3 was predominant among woman with recurrent abortion. As shown in **Figures 7–9**, *Ureaplasma parvum* serovar 3 was isolated in proportion 42.8%, the most frequent isolate in women with recurrent abortion followed by serovar 1 in proportion 28.5%, whereas serovar 6 and 14 showed the same proportion (14.2%) detected it in patient group; however, in control group, only *Ureaplasma parvum* serovar 1 was isolated in proportion of 11%. Among the different serovars of *Ureaplasma parvum*, serovar 3 was the most frequent serovar detected in the patient group. Therefore, *Ureaplasma parvum* (biovar 2) serovar 3 was predominant among woman with recurrent abortion. We suggested the *Ureaplasma parvum* serovar 3 may be playing a role in recurrent abortion and prematurity. Also may be related to intra- amniotic inflammatory response to *Ureaplasma parvum* and that this is related not only to recurrent abortion but also to early onset sepsis in the baby. Although the difference in detection rates of the different serovars of *Ureaplasma parvum* was statistically significant, the predominance of serovar 3 was consistent with previous reports [24]. Another study detected *Ureaplasma parvum* serovar

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3 is the most prevalent serovar detected in reproductive humans [40]. Another study isolated the complete genome sequence of *Ureaplasma parvum* serovar 3, clinical strain SV3F4, isolated from a Japanese patient who had an infectious abortion during the 13th gestational week in her previous pregnancy [41, 42]. Also Urszula et al. [10] isolated *Ureaplasma parvum* serovar 3/14 in 86% of women with symptomatic genital tract infections. It is possible that the combination of variable serovar-specific genes of *Ureaplasma* with generally known virulence factors determines the development of pathological processes on the mucosal surface of the human genital tract and respiratory tract in infant [43, 44].

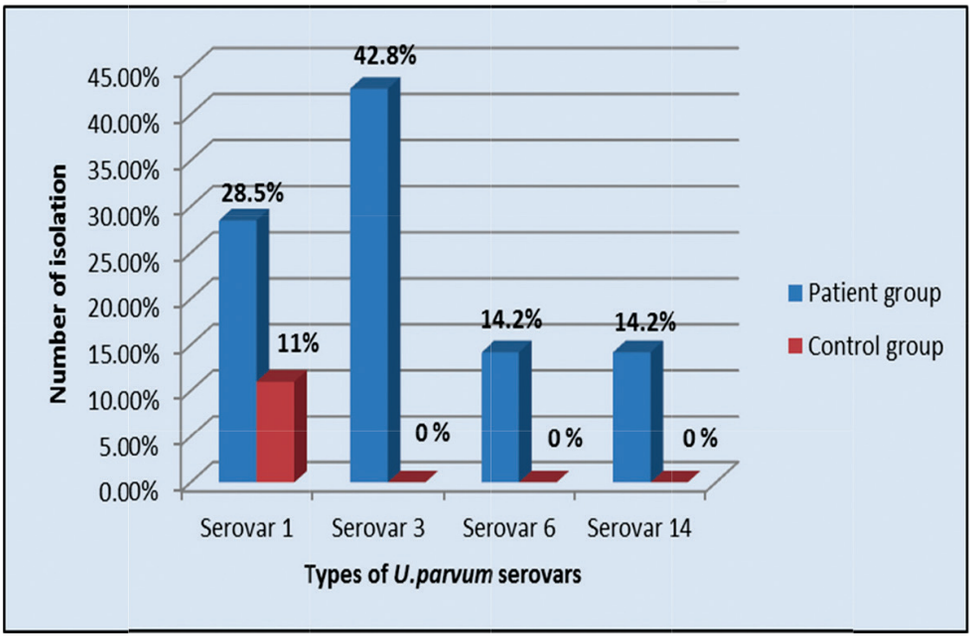



**Figure 7.** Distribution of *Ureaplasma parvum* serovars among patient group and control group.

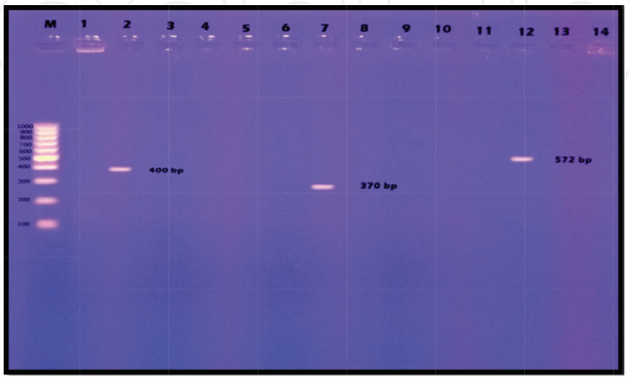



**Figure 8.** Results of PCR amplification for identification of serovar 1 (578 bp) and serovar 3 (400 bp). M 100 bp standard size reference marker. Lane (1, 5): serovar 1 positive results. Lane (2, 3, 4, 6, 7, 8, 9): negative samples. Lane (10, 14): serovar 3 positive results. Lane (11, 12, 13, 15, 16, 17): negative samples.

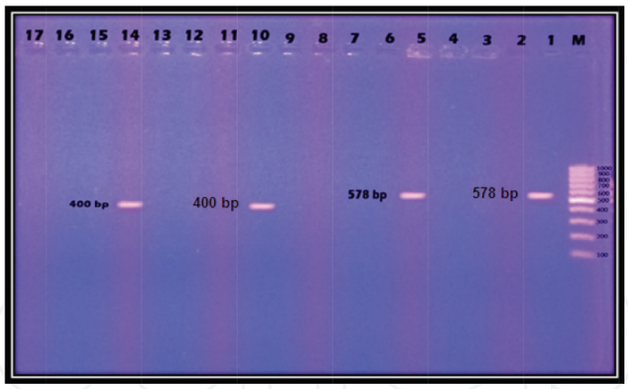



**Figure 9.** Results of PCR amplification for identification of serovar 3 (400 bp), serovar 6 (370 bp) and serovar 14 (572 bp). M 100 bp standard size reference marker. Lane (1, 3, 4, 5, 6, 8, 9, 10, 11, 13, 14): negative samples. Lane 2: serovar 3 positive results. Lane7: serovar 6 positive results. Lane12: serovar 14 positive results (Agarose Con. 2% & Voltages 100). *__Statistical analysis__ includes the *P*-value <0.05 showed highly significant between patient group and control group according to isolation of *Ureaplasma parvum* serovars.

## 5. Conclusion

The results evidently indicate that *U. parvum* may be an important etiologic agent for recurrent abortion. And *U. parvum* serovar 3 was the most frequent serovar isolated in this study. It may play a role in recurrent abortion.

## Conflicts of interest

There are no conflicts of interest.

## Author details

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