**Chlamydia Trachomatis and Waddlia Chondrophila among Women with Unexplained Early Miscarriage: Case-Control Study**

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**Abstract: Objective:** To determine the prevalence of *Chlamydia trachomatis* and other infections of the genital tract and its relation to the incidence of early unexplained spontaneous miscarriages. **Design:** a case-control study. **Setting:** Fayoum University hospital. **Patients:** The study group included 300 women presenting with unexplained spontaneous miscarriage or missed abortion of gestational age 8-14 weeks. The control group included 300 women with normal pregnancy of the same gestational age. **Intervention:** All participants of both groups were subjected to 3 cervicovaginal swabs and placental sampling was added from the study group. One swab was used for a smear test of vaginal fluid pH evaluation and direct microscopy. The second swabs, cultured for aerobic bacteria and yeasts. The third swab of both groups and placental samples from the study group were cultured for aerobic and anaerobic bacteria and used for DNA extraction. The extracted DNA was subjected to a pan-*Chlamydiales* qPCR and positive samples were subjected to qPCR specific for *Chlamydia trachomatis, Waddlia chondrophila and Parachlamydia acanthamoebae*. **Results:** *Candida* infection and *Trichomonas vaginalis* represented the main infections in both groups without a statistically significant difference between both groups. Placental samples were positive in 20%, 21 cases were positive by culture and 39 cases by PCR. Pan- *Chlamydiales* were detected in 92 cervicovaginal samples of both groups and 39 placental samples of study group with a statistically significant difference between both groups (p <0.041). *Chlamydia trachomatis* qPCR was detected in cervicovaginal samples in 54 patients of the study group and 36 patients of control group, that was a statistically significant difference (p <0.04), and 31 placental samples of study group, representing 92.3% of all chlamydial infections. *Waddlia chondrophila* was detected in 2 cervicovaginal samples of both groups and 8 placental samples of the study group, representing 7.6% of all chlamydial infection, that was statistically insignificant difference between both groups (p=1). No Parachlamydia *acanthamoebae* detected in both groups. **Conclusion:** *Chlamydia trachomatis* and *Waddlia chondrophila* infections are possible factors for miscarriage as their prevalence was higher in cervicovaginal and placental samples of women with unexplained early miscarriage.

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**Key Words:** Miscarriage, *Chlamydia trachomatis, Waddlia chondrophila, Parachlamydia acanthamoebae,* placental samples.

**1. Introduction**

Miscarriage, one of the commonly seen unfavorable pregnancy outcomes, is defined as the spontaneous pregnancy loss before viability and throughout the first 24 weeks of pregnancy (1). Early miscarriage, defined as loss of pregnancy of less than 12 weeks, occurs in up to 20% of pregnancies. Late miscarriage occurring in 1–2% of pregnancies, is defined as pregnancy loss during the second trimester (12–24 weeks). (2)

The prevalence of spontaneous abortion is not precisely known and worldwide figures are variable as it is altered by the identification method. (1) In 1988, Wilcox and colleagues declared an incidence of pregnancy loss of 31 % after nidation of which over 2/3 were clinically silent (3).

Bacterial, viral, and other lower genital tract infections that can reach the fetoplacental units either locally or through blood-born spread was suggested as a cause of spontaneous miscarriage (4). However, there

are conflicting data regarding the link between infections and abortion rate. Some reported a 2-4 fold increase in miscarriage with polymicrobial infection (5), while others declined first-trimester association and reported a relationship between only second-trimester pregnancy loss and Bacterial vaginosis. (6) These infections were reported in 15 and 66% of early and late miscarriages respectively. (7,8)

*Chlamydia trachomatis* (CT), a gram-negative non-motile bacterium, is an obligate intracellular parasite for humans and animals that tend to proliferate in epithelial cells (9). It is responsible for many disastrous pregnancy complications as ectopic pregnancy, premature delivery, premature rupture of membranes and low birth weight and many gynecological conditions as pelvic inflammatory disease and infertility as well. (8)

Recently, the incidence of *Chlamydia trachomatis* infection was dramatically increased and this increase may be due to lack of education or prevention, variations in sexual conduct or better and improved diagnostic techniques. (10)

Although the role of *Chlamydia trachomatis* in miscarriage has been investigated widely over the last three decades, the available data are conflicting and its relation to early pregnancy loss remains unrecognized (1,9). While Feist et al in their study didn’t find an indicator of placental contagion in patients with serological grounds of *Chlamydia trachomatis* and showed absence of direct involvement of *Chlamydia* in the miscarriage cases examined (11), Baud and Greub detected *Chlamydia trachomatis* in less than 1 percent of controls compared to 4% of miscarriage cases (12). Moreover, Baud et al in their earlier study reported a higher prevalence of immunoglobulin G against *Chlamydia trachomatis* in the miscarriage group than in the control group (15.2% vs. 7.3%; *p* = 0.018) that remained significant after adjustment for age, origin, education, and number of sex partners. (8)

*Waddlia chondrophila* and *Parachlamydia acanthamoebae* are newly identified family-level [progenies](https://www.synonyms.com/synonym/progenies) that have 80–90%  [indistinguishable](https://www.synonyms.com/synonym/indistinguishable) 16S rRNA-encoding gene sequence with members of the *Chlamydiaceae*, and display a *Chlamydia-*likereplicative cycle within human macrophages. (13) Baud et al in 2007 reported a strong association between miscarriage and the presence of anti-*Waddlia* antibodies. (14) Later in 2017, in *Chlamydiales*-associated miscarriage, Reid et al demonstrated the direct presence of *Waddlia* in the placenta and cervicovaginal secretions and by immunohistochemistry and polymerase chain reaction (PCR). (15) However, the pathogenesis of adverse pregnancy events caused by *Parachlamydia* and *Waddlia* needs to be clarified.

Detection of *Chlamydia trachomatis* and *Chlamydia*-like organism infections involve direct and indirect procedures. Although direct detection of the organism by culture was long considered the gold standard test for *Chlamydia trachomatis* detection, it is rarely used nowadays in diagnostic laboratories because of difficult standardization, storage and transfer, and insufficient collection of specimens. On the other hand, indirect detection of infection by measuring antibodies against *Chlamydia trachomatis* is not suitable for diagnosing acute infection, as the antibody response is only discovered after weeks of infection and often less sharp.

Moreover, serology can diagnose only chronic or invasive infections as the organism is no longer detectable in swabs. (10)

The high sensitivity (>97%) and speciﬁcity (>99%) provided by nucleic acid ampliﬁcation largely replaced cell culture and serological methods and is mostly used for the detection of *Chlamydia trachomatis*nowadays. (9,16) Moreover, the recent [availableness](https://www.synonyms.com/synonym/availableness) of the *Waddlia* and *Parachlamydia* genomes opened new research perspectives. (17)

The present study is designed to determine the prevalence of *Chlamydia trachomatis* and other infections of the genital tract and its relation to the occurrence of early miscarriage in women with early unexplained spontaneous miscarriages.

**2. Patient and methods**

**Design:**

A case-control study.

**Setting:**

Conducted at Fayoum university hospital after approval of the local Fayoum University ethics committee between September 2017 and September 2019. Informed written consent was signed by all women after explanation of the aim and benefits of the study.

**Patients:**

The study group included 300 women presenting with unexplained spontaneous miscarriage or missed abortion of gestational age 8-14 weeks. The control group included 300 women with normal pregnancy of the same gestational age 8-14 weeks, recruited from women attending the antenatal clinic. Women included aged between 18 to 45 years old.

The exclusion criteria included women with a miscarriage at a gestational age of more than 14 weeks, those with documented causes of miscarriage as antiphospholipid syndrome, endocrine factors (diabetes, thyroid dysfunction. etc) or fetal or uterine anomalies, hypertension, morbid obesity, and women who received a recent antibiotic, antifungal or antiprotozoal treatment.

All participants were subjected to full history, careful general and abdominal examination, cervicovaginal swabs in addition to placental sampling from the study group. The specimens were immediately transported to the local university lab and processed within 2 hours. Culture and qPCR of different samples were performed for both groups as described in Figure 1.

From each patient, three cervicovaginal swabs were obtained; one was used for a smear test of vaginal fluid pH evaluation and direct microscopy. The second swabs, cultured for aerobic bacteria and yeasts, were inoculated on Sabouraud Dextrose Agar (SDA) (Oxoid, UK) for detection of candida species, Blood agar ( Sheep blood) (Oxiod), Chocolate agar ( Sheep blood) (Oxiod) on CO2 and MacConkey agars (Oxiod, UK) at 35-37°C for 24-48 hrs for detection of residual microflora, *Gardnerella* selective supplement (Oxiod, UK) for *Gardnerella vaginalis* and Thayer–Martin agar plates (Oxoid Ltd., Hampshire, United Kingdom) for the detection of *Neisseria gonorrhea*. The cultured plates for the detection of *Gardnerella vaginalis* and *Neisseria gonorrhea* were incubated at 35-37°C in a moist atmosphere enriched with carbon dioxide (CO2 3-7%) for a maximum of 48 hrs.

The third swab was eluted in 2-sucrose-phosphate (2SP) based transport medium. Placental samples from the study group were obtained by aspiration in 132 cases (44 %) among the study group while the 168 remaining cases, an endometrial curettage was done. The samples were placed into a sterile Petri dish and a portion approximately 1 cm square of each tissue specimen was removed and put in sterile vials containing sterile phosphate buffered saline (PBS), or homogenized mechanically by grinding using autoclavable Mortar and pestle and then cultured for aerobic and anaerobic bacteria on blood agar, blood-chocolate agar incubated in aerobic and anaerobic conditions at 37°C for 48 hours. The rest of placental samples and the eluted third swab were stored at the temperature of −80°C until conducting DNA testing (18,19).

Identification of the isolates was performed by examination of colony morphology, microscopic examination of Gram-stained preparations and biochemical characterization using BioMerieux API kits (Marcy l’Etoile, France). Gram smears were also morphologically evaluated under oil immersion (1000x magnification) for the presence of *lactobacillus, Gardinella vaginalis, Bacteroides species*, and *Mobiluncus* species. A Nugent score system and Amsel’s composite criteria for the diagnosis of Bacterial vaginosis were used. A Nugent score system (NS) of 7-10 was classified as Bacterial vaginosis,4-6 as intermediate vaginal flora and 0-3 as normal. (20) Amsel's composite criteria including vagnial PH >4.5, homogenous thin discharge, positive whiff test or the positive amine odor after the addition of 10 percent KOH, absence of lactobacilli and the presence of clue cell in wet mount vaginal discharge under microscopic evaluation. (21) In the presence of three of four Amsel criteria, Bacterial vaginosis diagnosis was confirmed with a test characteristic (sensitivity/ specificity) 93-99%.

For DNA extraction, placenta samples were left to thaw and about 25 g of the placenta samples were cut in sterilized conditions. DNA extraction from placental samples and 2SP medium was carried by QIAamp DNA Mini KIT (QIAGEN Inc., Valencia, CA) according to the manufacturer's recommendations (22). To determine the concentration, yield and purity of DNA samples we used Thermo Scientific NanoDrop instrument (Thermo Fisher Scientific, USA).

The extracted DNA was subjected to several specific qPCR assays to detect the DNA of Chlamydial DNA using a pan-*Chlamydiales* qPCR and positive samples were subjected to qPCR specific for *Chlamydia trachomatis, Waddlia chondrophila,* and *Parachlamydia acanthamoebeae*. Primers and probes used for qPCR are listed in table 1. The primers and probe were prepared by Applied Biosystems (Foster City, California, USA). The Ct cut-off values were fixed at 35 for all qPCRs. Amplification and PCR product detection were performed with the ABI prism 7500 real time-PCR System (Applied Biosystems, USA).

The primary outcome parameter was the association of lower genital tract infection with spontaneous unexplained miscarriage.

Sample size calculation was done using the comparison of the prevalence of chlamydial infection between cases with spontaneous unexplained miscarriage and matched women with no miscarriage. The calculation was done based on comparing 2 proportions from independent samples in a case-control study using Chi test, the α-error level was fixed at 0.05, the power was set at 80% and the case: control ratio was set at 1. As previously published by Baud et al 2014, the prevalence of chlamydial infection among the miscarriage group was 30.4% while it was 13.8% in control mothers (23). Accordingly, the minimum optimum sample size should be 296 participants in each group to detect a 10% difference in chlamydial prevalence between the groups. Sample size calculation was done using PS Power and Sample Size Calculations software, version 3.0.11 for MS Windows (William D. Dupont and Walton D., Vanderbilt University, Nashville, Tennessee, USA).

Data were statistically described in terms of mean ± standard deviation (±SD), or frequencies (number of cases) and percentages when appropriate. Comparison of numerical variables between the study groups was done using Student t test for independent samples. For comparing categorical data, Chi-square (χ2) test was performed. Exact test was used instead when the expected frequency is less than 5. p values less than 0.05 were considered statistically significant. (24) All statistical calculations were done using computer program IBM SPSS (Statistical Package for the Social Science; IBM Corp, Armonk, NY, USA) release 22 for Microsoft Windows.

**3. Results**

Flow chart of participants is shown in Figure 1.

As regards basic characteristics between both groups there was no statistical difference in age, parity, BMI and gestational age at recruitment (Table 2). As regard mode of termination a statistically significant difference of vaginal infection was noted with spontaneous miscarriage compared to surgical or medical procedures in the study group (*p* 0.001). (Table 2)

Cultures of cervicovaginal swabs were positive in 128 cases (42.7%) of the study group (n=300) and 117 cases (39%) of the control group (n= 300) with a prevalence of 40.8% but with no statistically significant difference between both groups (*p* = 0.361). (Table 3)



Figure 1 flow chart

Table 1: Primers and probes used for qPCR

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Bacteria | Primer /probe | Oligonucleotide sequence (5’ 3’) | Target gene | Product size (bp) | Reference |
| *Chlamydia trachomatis* | Forward | 5′-CATGAAAACTCGTTCCGAAATAGAA-3′ | Crypticplasmid | 149 | 34 |
| Reverse | 5′-TCAGAGCTTTAC CTAACAACGCATA-3′ |
| Probe | 5’- **FAM** TCGCATG- CAAGATATCGA-3’ |
| Pan*-Clamydiales* | Forward | 5′-CCGCCAACACTGGGACT-3′ | 16S | 207to215 | 35 |
| Reverse | 5′-GGAGTTAGCCGGTGCTTCTTTAC-3′ | rRNA |
| Probe | 5′- **FAM** CTACGGGAGGCTGCAGTCGAGAATC**BHQ**-3′ | gene |
| *Waddlia chondrophila* | Forward | 5′-GGCCCTTGGGTCGTAAAGTTCT-3′ | 16S | 101 | 36 |
| Reverse | 5′CGGAGTTAGCCGGTGCTTCT-3′ | rRNA |
| Probe | 5′-**FAM**-CATGGGAACAAGAGAAGGATG-**BHQ**-3′ | gene |
| *Parachlamydia acanthamoebae* | Forward | 5′-GGT CCG GGT AAT CTT TGC AAA-3′ | 18S rRNA- | 126 | 37 |
| Reverse | 5′-GTA CAA AGG GCA GGG ACG TAA TCA AC-3′ | encoding |
| Probe | **5′-VIC-**TAAGCGCGAGTCATC**-MGB-**3′ | gene |

**Table (2): Basic characteristics of both groups**

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Study group (n = 300)** | **Control group (n = 300)** | **p value** |
| **Age (years)\*1** | 27.8±6 | 28.3±5.8 | 0.337 |
| **Parity\*1** | 1.3±1.1 | 1.4±1.2 | 0.181 |
| **BMI (kg/m2)\*2** | 28.8±4.9 | 29.1±4.7 | 0.466 |
| **Gestational age (weeks)1** | 12.1±1.8 | 12.1±1.4 | 0.721 |
| **Method of termination**†**:*** **Medical evacuation**
* **Surgical evacuation**
* **Complete spontaneous miscarriage**
 | 98 (32.7%)118 (39.3%)84 (28%) |  |  |

\*Data were described as mean ± SD; data compared using student t test for independent sample

†data was described in number (percent); compared using chi square test, BMI: body mass index

All microorganisms isolated are listed in Table 3.

When compared to women of the control group, women of the study group had less frequently normal flora and more frequently an intermediate vaginal flora with a significant statistical difference (*p*<0.001). However, bacterial vaginosis showed no significant difference between both groups (*p* = 0.661) (Table 3).

**Table (3) distribution of different infections in studied population**

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Study group (n=300)** | **Control group (n=300)** | **p value** |
| (+) ve Cervicovaginal swabs | 128 (42.7%) | 117 (39%) | 0.361 |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Study gp (n=300)** | **Control gp (n=300)** | **p value** | **Total** |
| **Flora** |  |
| Normal flora (score < 4) | 137 (45.7%) | 205 (68.3%) | < 0.001\* | 342 |
| Intermediate flora (score ≥ 4) | 115 (38.3%) | 43 (14.3%) | < 0.001\* | 158 |
| Bacterial vaginosis (score ≥ 7) | 48 (16%) | 52 (17.3%) | 0.661 | 100 |
| **Cervico vaginal Culture** |  |
| *Streptococcus agalactiae (group B)* | 5 (1.7%) | 4 (1.3%) | 0.737 | 9 |
| *Streptococcus pyogenes (group A)* | 3 (1%) | 2 (0.7%) | 0.653 | 5 |
| *Enterococcus faecalis* | 3 (1%) | 3 (1%) | 1.000 | 6 |
| *Escherichia coli* | 6 (2%) | 5 (1.7%) | 0.761 | 11 |
| *Klebsiella pneumoniae* | 3 (1%) | 3 (1%) | 1.000 | 6 |
| *Proteus mirabilis* | 4 (1.3%) | 3 (1%) | 0.704 | 7 |
| *Providencia stuartii* | 5 (1.7%) | 6 (2%) | 0.761 | 11 |
| *Pseudomonas aeruginosa* | 4 (1.3%) | 3 (1%) | 0.704 | 7 |
| *Acinetobacter baumannii* | 3 (1%) | 4 (1.3%) | 0.704 | 7 |
| *Staphylococcus aureus* | 3 (1%) | 2 (0.7%) | 0.653 | 5 |
| *Candida spp.* | 58 (19.3%) | 55 (18.3%) | 0.754 | 113 |
| *Trichomonas vaginalis* | 31 (10.3%) | 27 (9%) | 0.581 | 58 |
| **Placental culture (n=300)** |  |
| *Streptococcus agalactiae* (group B) | 3 (1%) |  |  |  |
| *Streptococcus pyogenes* (group A) | 2 (0.7%) |  |  |  |
| *Enterococcus faecalis* | 1 (0.3%) |  |  |  |
| *Escherichia coli* | 3 (1%) |  |  |  |
| *Klebsiella pneumoniae* | 3 (1%) |  |  |  |
| *Proteus mirabilis* | 1 (0.3%) |  |  |  |
| *Pseudomonas aeruginosa* | 2(0.7%) |  |  |  |
| *Staphylococcus aureus* | 2 (0.7%) |  |  |  |
| *Candida albicans* | 4 (1.3%) |  |  |  |
| **qPCR** |  |  |  |  |
| *Pan-chlamtydiales**Cervicovaginal**placental* | 94 (31.3%)5539 | 37 (12.3%)]37- | <0.001\*0.041\* | 1319239 |
| *Chlamydia trachomatis****Cervicovaginal******Placental*** | 85 (28.3%)5431 | 36 (12%)36- | < 0.001\*0.04\* | 1219031 |
| *Waddlia chondrophila**Cervicovagina**placentall* | 9 (3%)18 | 1 (0.3%)1- | <0.001\*1 | 1028 |
| *Parachlamydia acanthamoebae* | 0 | 0 |  | 0 |

\* Statistically significant difference; † data were described in number of cases (%) compared using chi square test

Subgroup analysis of type of lower genital tract infections detected in cervicovaginal swabs showed that *candida* infection *and Trichomonas vaginalis* represented the main infections in both groups with a prevalence of 18.8% and 9.7% respectively and together they represented 28.5% of all lower genital tract infections without a statistically significant difference between both groups (*p* 0.754 and 0.581 respectively). Other infections detected in both groups were *Streptococccus agalactiae, Streptococccus pyogenes, Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Providencia stuartii, Pseudomonas aeruginosa* and *Acinetobacter baumannii,* all did not show any statistical difference between both groups. No *Neisseria gonorrhoeae* or *Treponema pallidum* infections were detected in both groups (Table 3).

Placental samples were positive in 60 cases of study group (representing a total of 20%), 21 cases were positive by culture and 39 cases by PCR. When comparing to cervicovaginal swabs, placental cultures were positive to the same microorganism of cervicovaginal swabs in 3 cases for *Streptococccus agalactiae*, 2 cases of *Streptococccus pyogenes*, 1 case of *Enterococcus faecalis*, 3 cases of *Escherichia coli*, 3 cases of *Klebsiella pneumoniae* and 1 case of *Proteus mirabilis*, 2 cases of *Pseudomonas aeruginosa,* 2 cases of *Staphylococcus aureus*, 4 cases for *Candida albicans*. (Table 3).

Using qPCR for cervicovaginal and placental samples, pan- *Chlamydiales*was detected in 92 cervicovaginal samples of both groups and 39 placental samples of study group (total 131 cases of both groups) that represented a prevalence of chlamydial infection of 21.8%. pan- *Chlamydiales* qPCR of cervicovaginal samples showed a statistically significant difference between both groups of (55 cases of study group and 37 cases of control group, p <0.041). The positive samples for Pan- *Chlamydiales* qPCR showed positive *Chlamydia trachomatis* qPCR in 90 cervicovaginal samples of both groups (54 patients of the study group and 36 patients of control group), that was a statistically significant difference (p <0.04) and 31 placental samples of study group (of which 23 were also with positive cervicovaginal samples) representing 92.3% of all chlamydial infections). *Waddlia chondrophila* was detected in 2 cervicovaginal samples of both groups and 8 placental samples of the study group in patients with positive pan- *Chlamydiales* (representing 7.6% of all chlamydial infection) that was statistically insignificant difference between both groups (*p=*1). No *Parachlamydia acanthamoebeae* was detected in both groups. (Table 3)

**4. Discussion**

Maternal genital tract infection as a significant risk factor for unfavorable pregnancy outcomes has gained solid grounds. The possible mechanisms include production of toxic metabolites or cytokines (i.e. tumor necrosis factor-α) which induce uterine contractions or damage to the feto-placental unit; fetal or placental infection resulting in placental insufficiency, fetal death or life-threatening malformations; or probably excessive maternal immune response towards the trophoblastic invasion induced by inflammatory activation of endometrial immunocytes, interfering with embryo implantation, and chorio-amnionitis (25,26). With the increased accessibility to modern and strictly accurate screening diagnostics, a higher proportion of infections may now be detected during pregnancy. (10)

According to our findings, the prevalence of lower genital tract infection was 40.8% and was similar among the study group and control group. There was no statistical difference between women with miscarriage and control regarding *Candida, Trichomonas* or bacterial vaginosis. However, there was a significant difference of intermediate flora among patients with miscarriage compared to control. This is in agreement with the study of Guerra et al, that demonstrated an association between abnormal flora and adverse pregnancy outcomes. (27)

Furthermore, our study confirmed a higher prevalence of chlamydial infection in women with unexplained miscarriage when compared to those without similar history. This was in accordance with the studies that isolated *Chlamydia trachomatis* in cervical smears and urine (6,28) and conception products. (11,29)

Moreover, our results revealed a higher detection rate of *Chlamydia trachomatis*by PCR amplification of chlamydial DNA of vaginal swabs and placental samples of miscarriage group reflecting a positive association with miscarriage. This is consistent with Baud et al 2011 who documented a possible role of *Chlamydia trachomatis*in early miscarriage (12) and the meta-analysis of Check; 2010 who suggested a possible reactivation of dormant *Chlamydia* infections during pregnancy and proposed a potential value of antibiotics if given intermittently during the first trimester even with negative cultures or in unexplained miscarriages. (30) Although we don’t agree with Check’s recommendations, the value of intermittent use of antibiotics during the first trimester to prevent miscarriage should be weighed against the antibiotic resistance that would develop with its widespread use, and individualization of patient care should be our practiced behavior. We are in need of a well-constructed treatment studies to determine whetherearly intervention makes a difference.

Contrary to our findings, some studies reported no association between *Chlamydia* infection and spontaneous miscarriage (11,31,32) but most of them were done before the increased prevalence of chlamydia infection and before improvement in the accuracy of the diagnostic tools so that the higher prevalence reported can result from the low specificity of the diagnostic tests used. (12)

In our study *Waddlia chondrophila* was detected in 9 patients with spontaneous unexplained miscarriage of which 8 was detected in placental samples and only one case of the control group, that was statistically insignificant. However, the higher detection of *Waddlia chondrophila* in placental samples suggest a higher endometrial and decidual preference and a strong association between *Waddlia chondrophila* infection and miscarriage. This association had been ﬁrstly reported by Baud et al. in 2011. (33) Furthermore, in 2014, Baud et al. in their study strongly suggested the possible role of *Waddlia chondrophila* in early miscarriage. (23) On the other hand, our results disagree with the study of Reid et al, that does not show an association of *Waddlia chondrophila* with miscarriage. (15) This could be explained by the smaller sample size of Reid et al and the different selection of samples of the study that was based on PCR analysis of urine samples, which might have had lower rates of more related infections than cervicovaginal and placental samples as in our study.

To the best of our knowledge, our study is the first one to detect *Waddlia chondrophila* in cervicovaginal and placental samples and detect its association with the occurrence of unexplained miscarriage in Egypt.

The main limitation of our study is the small number of positive cases that may not reflect the actual prevalence of chlamydial infections, and mandates further studies of bigger sample size. Another limitation was its dependence on the history of abortion that could be inaccurate in determining the infection state and its severity, and without follow up of the control group to report the possible occurrence of late abortion and assessment of placental samples of the control group.

**Conclusion:**

*Chlamydia trachomatis* and *Waddlia chondrophila* infections are possible factors for miscarriage as their prevalence was higher in cervicovaginal and placental samples of women with unexplained early miscarriage.

**Declarations**

# **Compliance with Ethical Standards**

The study was performed in accordance with the Declaration of Helsinki ethical standards. The study was approved by Fayoum University ethical committee number R102. Informed written consent was taken from the study participants.

# **Disclosure**

# All authors report no conflict of interests in this work.

**Disclaimers:**

The views expressed in the submitted article are our own and not an official position of the institution or funder.

**Synopsis:**

*Chlamydia trachomatis* and *Waddlia chondrophila* infection were significantly higher in women with unexplained early miscarriage compared to the control group.

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