

The relationship between antisperm antibodies prevalence and genital Chlamydia trachomatis infection in women with unexplained infertility

Emad M. Siam¹, Enas M. Hefzy²

Abstract

Chlamydia trachomatis infection is one of the most common sexually transmitted diseases. Sperm-associated antibody could impair fertility through various mechanisms; both factors could be correlated to affect the fertility status of women. **Design:** A retrospective case-control study was performed enrolling ninety (n=90) patients with primary or secondary infertility as the case group, in addition to another eighty (n=80) healthy women attending the family planning clinic to investigate the correlation between C. trachomatis past and current infections and antisperm antibodies (ASA) in women with unexplained infertility. **Results:** The PCR prevalence of C. trachomatis didn't differ significantly among both groups (2.4 versus 1.6%, P value=0.66). In contrast, significantly higher prevalence of anti- C. trachomatis specific IgG (39% versus 19%, P value=0.87) antibodies were found among infertile women. ASA prevalence was significantly higher in infertile group (20 % versus 5%, P=0.04). The final study results have failed to find a positive correlation between current or past C. trachomatis infection and the level of antisperm antibodies level in women suffering of unexplained infertility. **Conclusion:** Anti-sperm antibodies were significantly higher in infertile women, but without a significant difference between the incidences of ASA in infertile women with past or current C. trachomatis current infection. (Afr J Reprod Health 2011; 15[3]: 101-112).

Résumé

L'infection de chlamydia trachomatis est une des maladies sexuellement transmissibles les plus communes et l'anticorps lié au sperme peut abimer la fertilité à travers divers mécanismes. Les deux facteurs peuvent être corrélés pour affecter l'état de fertilité des femmes. Une étude rétrospective basée sur l'étude de cas a été menée auprès des quatre-vingt –dix (n=90) patientes atteintes de la stérilité primaire et secondaire comme constituant le groupe de cas, y compris quatre-vingts (n=80) d'autres femmes en bonne santé qui fréquentaient la clinique de planification familiale afin d'étudier la corrélation entre l'ancien C. trachomatis et les infections actuelles et les anticorps antisperme (AAS) chez les femmes atteintes de la stérilité inexplicable. La prévalence de PCR par rapport à C. trachomatis n'a pas indiqué des différences remarquables parmi les deux groupes (2,4 contre 1,6%, Valeur de P= 0,06). Par contre, on a découvert des différences très remarquables par rapport aux anti-C. trachomatis de la spécificité IgG (39% par opposition à 19%, valeur de P= 0,87) ont été trouvés chez les femmes stériles (20% par opposition à 5% P=0,04). Les résultats définitifs de l'étude n'a pas réussi à montrer une corrélation positive entre l'infection de C. trachomatis et le niveau d'anticorps d'antisperme chez les femmes stériles, mais sans une différence remarquable entre les incidences d'AAS chez les femmes stériles de C. trachomatis passé ou actuel.

Key words: Antisperm-antibodies (ASA); Anti-C. trachomatis antibodies (ACTA); un-explained infertility; pelvic inflammatory diseases (PID).

Corresponding authors: ¹Department of Obstetrics and Gynecology, College of Medicine, El-Minya University, Egypt. Tel.: +20862346586 Fax: +20862334456 Cellular: +20103480999
E-mail: eemsalah@yahoo.com. ²Department of Microbiology & Immunology, College of Medicine, Al-fayoum University, Egypt. Tel.: +20862346586 Fax: +20862334456 Cellular: +20103480999
E-mail: emhefzy@yahoo.com

The research work had been done In Al-Fayoum University Hospital, a tertiary referral center in middle Egypt.

Introduction

Chlamydia trachomatis infection has recently become the most common genital infectious disease in the world. C. trachomatis is a common cause of cervicitis and urethritis, and sequelae include pelvic inflammatory disease (PID), ectopic pregnancy and tubal factor infertility, epididymitis, proctitis and reactive arthritis. Chlamydial PID is the most important preventable cause of infertility and adverse pregnancy outcome. Based on the available evidence, approximately 20% of women with chlamydial genital infection will develop PID, 3% develop infertility, 2% develop adverse pregnancy outcome¹⁻³. Unexplained infertility (UI) is a diagnosis of exclusion. Up to 25-30 % of patients who present for investigation in a reproductive medicine clinic are diagnosed with UI. The diagnosis is usually made after investigations show normal semen parameters, ovulatory concentrations of serum progesterone in the mid-luteal phase, tubal patency, and a normal uterine cavity¹⁰.

In the male and female, antisperm antibodies (ASA) may be found systemically (in the blood and lymph) and in local secretions (in seminal or cervico-vaginal fluids). Antibodies in the blood and lymph belong predominantly to the immunoglobulin G (IgG) isotype, while those found in external secretions are predominantly of the IgA isotype^{4,5}.

Many hypotheses assumed that C. trachomatis impaired fertility by generation of ASA. ASA are generated during the chlamydial infection process by genital tract infection leading to release of pro-inflammatory cytokines from activated T cells, which in turn activate macrophages into phagocytizing C. trachomatis microorganisms and spermatozoa. The result from this inflammatory response is the production of antibodies to spermatozoal and microbial antigens by activated B-lymphocytes⁶⁻⁹.

The alternative hypothesis for the ASA production assumes that possible cross-reactivity between antigens of spermatozoa and C. trachomatis exists. It has been suggested that antibodies against conserved epitopes on cHSP60 may cross-react with those on human HSP60 and initiate an autoimmune response. Antisperm antibodies might reduce fertility either through impairment of the migration of spermatozoa

through cervical mucus and/or through binding to the receptor by which spermatozoa attach to the ovum, thereby blocking sperm-ovum interaction^{10,11}.

Women don't generally produce antibodies against sperm; however, some infertile women have been found to possess antisperm antibodies, which may contribute to their infertility¹². Therefore, the following questions arise: who produces antisperm antibodies? and what makes women produce these antibodies?. Among the risk factors for antisperm antibody production, inflammatory diseases of the genital tract are believed to play an important role as a consequence of local inflammation.

The aim of the current work was to evaluate the relationship between either past or current C. trachomatis infection, and the level of antisperm antibodies in asymptomatic Egyptian females with unexplained infertility.

Materials and methods

Ninety married women (primary infertility=58 and secondary infertility=32) have been recruited for the current study from the Gynecology and Infertility Clinics in a University Hospital, as a case group. And eighty females attending the family planning clinic (n=80) with matching ages as a control group. All subjects were asymptomatic in terms of genital tract infection. None of the women received antibiotics, or corticosteroids at least for 15 days before samples were taken. Only cases with UI for one year or more were included in the study.

Basic infertility investigation included full detailed medical history and thorough clinical examinations. During the standard infertility investigations, all female patients were checked for: tubal patency and normal uterine cavity by hysterosalpingography (HSG) and/or laparoscopy and for the hormonal factor, including tests for ovarian, pituitary, thyroid and adrenal gland function. Sperm analysis was performed for their male partners according to the World Health Organization criteria for normal sperm picture profile.

I: Chlamydia trachomatis genital infection

Detection of C. trachomatis Specific IgG by ELISA
Circulating anti- C. trachomatis IgG antibodies were

detected in serum of both study and control groups by ELISA (enzyme-linked immunosorbent assay) using DRG Chlamydia Trachomatis IgG (DRG International Inc., U.S.A.) which provides materials for the qualitative determination of IgG-class antibodies to *C. trachomatis* in serum. The DRG *C. trachomatis* IgG ELISA kit is a solid phase ELISA. Specimen collection and preparation, assay procedure, calculation and interpretation of results were done according to the manufacturer's instructions. Blood was collected by venipuncture, allowed to clot and serum was separated by centrifugation at room temperature.

Specimens were stored -20°C. Prior to assaying each patient sample was diluted 1:100 with sample diluent. e.g. 10 µL of specimen: 1mL of sample diluent, mixed well, let stand for 15 min and mixed well before use. Prior to commencing the assay, wash solution was diluted 1+19 (e.g. 10 mL+190 mL) with fresh and germ free redistilled water. All reagents and required number of strips were allowed to reach room temperature prior to use. The distribution and identification plan supplied in the kit was established carefully for all specimens and controls.

Interpretation of the results were a: Positive, when Patient absorbance values more than 10 % above CO [OD (optical density) patient > 1.1 x CO], b: Grey zone: when Patient absorbance values from ± 10 % (CO), test was repeated 2 - 4 weeks later - with new patient samples, [(0.9 x CO OD patient 1.1 x CO)]. Results in the second test again in the grey zone negative, while c: Negative: Patient absorbance values more than 10 % below CO (OD patient < 0.9 x CO).

II. Detection of *C. trachomatis* DNA by PCR in urine specimens (i.e. diagnosis of current infection)

Since infection of the urethra occurs commonly during *C. trachomatis* infections, copies of target DNA remain present in urine and are detectable by nucleic acid amplification tests (NAATs). Urine samples for the DNA amplification test, PCR, are collected following certain precautions.

Urine sample collection and DNA extraction:

Women in both study and control groups were instructed not to wash or otherwise clean their genitalia before voiding or to spread the labia during urine collection (as should be recommended when collecting samples for bacterial urine cultures). The first void urine (FVU) specimen was collected. Women were asked not to urinate for at least 1h. About 15 to 30 ml of urine were collected (in a 50-ml crew-cap plastic sterile cup)¹³⁻¹⁵.

The FVU specimens were transported at room temperature and processed after overnight incubation at 2 to 8 °C to reduce effect of PCR inhibitors in urine. Ten ml of FVU were centrifuged at 3000 rpm for 30 min at room temperature. The precipitate was centrifuged at 14000 rpm for 30 min¹⁶. The supernatant was discarded and pellets were stored at -20°C for at most 2 months till DNA extraction. Urine specimens can be stored at 4°C for up to 96h before the urine was processed¹⁷⁻¹⁹. DNA extraction was performed using QIA amp Viral RNA Mini kit as the AVL buffer used in this kit, inactivates the numerous unidentified PCR inhibitors found in urine. Frozen specimens were thawed and tested on the same day^{15,16}.

III. Antisperm Antibody (IgG class) testing:

Blood for determination of circulating ASA was drawn at the middle of the woman's cycle. ASA, (IgG) type, in serum was assayed by ELISA test (DRG International, Inc., USA) which was done according to the manufacturer's instructions. The DRG Sperm Antibody ELISA is a solid-phase sandwich enzyme-immunoassay for the determination of anti-spermatozoa antibodies in human serum. Specimen collection and preparation, assay procedure and calculation of results were done according to the manufacturer instructions. Cut-off value was 60 U/ml²²⁻²⁵.

Statistical Analysis

A relationship between *C. trachomatis* infection and ASA was evaluated and their impact on unexplained infertility was assessed. The data were analyzed using SPSS version 15.0 (SPSS, Inc, Chicago, IL, USA) for windows. Chi square test was used for significance analysis. Significant level was established at P<0.05. Results were represented in tables and charts.

Table 1: Prevalence of anti-chlamydial antibodies (IgG) in serum of cases and control

		Anti- <i>C. trachomatis</i> IgG (ACTA)		Total
		+ve	-ve	
Groups	Case	28 (=31.1%)	62(=68.9%)	90
	Control	14 (=17.5%)	66(=82.5%)	80
Total		41	128	170

The overall prevalence of anti- *C. trachomatis* antibodies (ACTA) (IgG) in infertile women was 28/90 (=31.1%) and 14/80 (=17.5%) in control subjects, difference was not statistically significant (P=0.87).

Table 2: Prevalence of anti- *C. trachomatis* IgG in primary and secondary infertility patients

		Type of infertility		
		Primary	Secondary	
anti- <i>C. trachomatis</i> IgG	+ve	14 (= 24.1%)	14 (= 43.8%)	28
	-ve	44 (= 75.9%)	18 (= 56.2%)	62
Total		58	32	90

Higher prevalence of anti- *C. trachomatis* IgG was found in serum of secondary infertile women. 14/32 (= 43.8 %) of secondary infertile women were positive for anti- *C. trachomatis* IgG and 14/58 (= 24.1%) were positive in primary infertility patients. However, the difference between both groups was not significant (P=0.19).

Table 3: Prevalence of current genitourinary *C. trachomatis* by PCR in cases and control

		Groups		Total
		Case	Control	
Ct PCR	+ve	4 (= 4.4%)	6 (= 7.5%)	10
	-ve	86 (= 95.6%)	74 (= 92.5%)	160
Total		90	80	170

The overall prevalence of current genitourinary *C. trachomatis* by PCR was 4/90 (=4.4%) in infertile women and 6/80 (= 7.5%) in control subjects, there is no significant difference between both groups (P=0.66).

Results

Serum samples were collected from ninety (n=90) women with UI (primary infertility=58 and secondary infertility=32), and eighty (n=80) women from family planning clinic attendants, with matching ages, as control subjects. Within infertility cases, age ranged from 20-42 years, with a mean (30.18±5.3) years. In control subjects, age ranged from 22-40 years with a mean (30.05 ± 4.9) yrs. Difference between both groups wasn't statistically different (p>0.05). According to age, cases with UI either 1ry or 2ry were divided into 5 groups Figure (1); first group ranged from 20-24 yrs old [n=14 (16%)], from 25-29 yrs old [n=26 (29%)], 30-34 yrs old [n=28 (31%)], 35-39 yrs old [n=18 (20%)] and 40 yrs old or more [n=4 (4%)].

The overall prevalence of anti- *C. trachomatis* antibodies (ACTA) (IgG) in infertile women were 28/90 (=31.1%) and 14/80 (=17.5%) in control subjects (table 1). The difference was not statistically significant (P=0.87). Higher prevalence of anti- *C. trachomatis* IgG was found in serum of 2ry infertile women. 14/32 (= 43.8 %) of 2ry infertile women were positive for anti- *C. trachomatis* IgG, and 14/52 (= 24.1%) were positive in 1ry infertility patients (table 2). However, the difference between both groups was not significant (P=0.19).

The overall prevalence of current genitourinary *C. trachomatis* by PCR was 4/90 (=4.4%) in infertile women and 6/80 (= 7.5%) in control subjects (table 3), without a significant difference between both groups (P=0.66). The prevalence of current genitourinary *C. trachomatis* by PCR in 1ry infertility was 4/58 (=6.9%) and in 2ry infertility was (0%) (table 4), and this difference was not significant (p=0.53). For both tests no significant differences was noted between both groups. Difference between 1ry and 2ry infertility patients in the prevalence of *C. trachomatis* current infection or anti- *C. trachomatis* IgG was not statistically significant.

The prevalence of ASA in serum assayed by ELISA test was 18/90 (=20%) in infertile women and 4/80 (=5%) in control women, there was a significant difference between both groups (P=0.04). Prevalence of ASA in UI patients was not significantly different between 1ry and 2ry UI patients (P=0.45) (table 5 & figure II). In 1ry infertility 14/58 were positive

(=24.1%) and in 2ry infertility patients 4/32 were positive (=12.5%). There was a significant difference between both groups, with higher prevalence in 1ry than 2ry infertility patients. However, this difference was not significantly different (p=0.45).

Prevalence of ASA was 6/28 (=21.4%) in infertile women with past *C. trachomatis* infection and 12/62 (=19.4%) in infertile women without past infection (table 6). No significant difference between both groups was found (p=0.7). Prevalence of ASA was 0% in control subjects with past *C. trachomatis* infection and was 4/66 (=6.1%) in control subjects without *C. trachomatis* infection. Difference between both groups was not statistically significant (p=0.5).

Prevalence of ASA was (0%) in cases with current genitourinary *C. trachomatis* infection and 18/86 (=20.9%) in patients without current infection (table 7), difference between both groups was not statistically significant (p= 0.47). Prevalence of ASA was (0%) in control subjects with current genitourinary *C. trachomatis* infection and 4/80 (=5%) in subjects without current infection. Difference between both groups was not significant (p=0.85).

No relationship was found between the presence of ASA and current *C. trachomatis* infection (p= 0.7) or ASA and past *C. trachomatis* infection (p= 0.47), in Egyptian asymptomatic infertile females. The prevalence of ASA was (21.4%) in cases with past *C. trachomatis* infection and (19.4%) in Egyptian asymptomatic infertile females without past infection. No significant difference between both groups (p= 0.7). The prevalence was (0%) in cases with current genitourinary *C. trachomatis* infection and (20.9%) in patients without current infection, difference between both groups was not significant (p= 0.47).

Discussion

C. trachomatis is the most common bacterial cause of sexually transmitted infections. Genetic predisposition and host immune response play important roles in the pathogenesis of long-term complications after *C. trachomatis* infections^{16,17,21}. It is very important to understand why and how some women produce ASA, which react with human sperm,

Table 4: Prevalence of current *C. trachomatis* infection by PCR in UI patients

		Type of Infertility		Total
		1ry	2ry	
Ct PCR	+ve	4 (=6.9%)	0	4
	-ve	54 (=93.1%)	32	86
Total		58	32	90

Prevalence of current genitourinary *C. trachomatis* by PCR in primary infertility was 4/58=6.9%) and in secondary infertility was (0%). This difference was not significant (p=0.53).

Table 5: Prevalence of ASA in primary and secondary infertility

		Type of Infertility		Total
		Primary	Secondary	
ASA	+ve	14(=24.1%)	4(=12.5%)	18
	-ve	44=75.9%)	28=87.5%)	72
Total		85	32	18

Prevalence of current genitourinary *C. trachomatis* by PCR in primary infertility was 4/58=6.9%) and in secondary infertility was (0%). This difference was not significant (p=0.53).

Table 5: Prevalence of ASA in primary and secondary infertility

		Type of Infertility		Total
		Primary	Secondary	
ASA	+ve	14(=24.1%)	4(=12.5%)	18
	-ve	44=75.9%)	28=87.5%)	72
Total		85	32	18

Prevalence of ASA in UI patients was not significantly different between primary and secondary UI patients (P=0.45). In primary infertility 14/58 were positive (=24.1%) and in secondary infertility patients 4/32 were positive (=12.5%).

Table 7: Relationship between ASA and current *C. trachomatis* infection in UI patients

		Ct PCR		Total
		+ve	-ve	
ASA	+ve	0	18 (=20.9%)	18
	-ve	4	68 (=79.1%)	72
Total		4	86	90

Prevalence of ASA was (0%) in cases with current genitourinary *C. trachomatis* infection and 18/86 (=20.9%) in patients without current infection, difference between both groups was not statistically significant ($p=0.47$).

and how the immune reaction interferes with reproductive success. However, the factors that affect the production of ASA in some women are not fully understood. Moreover, the reason why most women don't develop an immune response upon exposure to sperm is not yet clear²³⁻²⁶.

This case-control study was performed to investigate the relationship between past *C. trachomatis* (examined by anti- *C. trachomatis* antibodies assay detected by ELISA) and current *C. trachomatis* infection (detected by PCR in FVU samples) and ASA in asymptomatic Egyptian women with primary or secondary UI. The present study aimed to determine whether *C. trachomatis* infection induces the production of ASA in women, because the association between them has not yet been clearly understood. Also impact of each variable; ASA, anti- *C. trachomatis* IgG antibodies and current *C. trachomatis* infection, on UI was tested.

In the present study prevalence of anti- *C. trachomatis* IgG in asymptomatic infertile and control women was (31.1% and 17.5 % respectively). Prevalence of current infection was (4.4% and 7.5%) in infertile and fertile groups respectively (P value=0.87), no significant differences was noted between both groups. Also no relationship was found between current or past *C. trachomatis* infection and unexplained infertility (P value=0.66). Among Egyptian infertile women, the prevalence of *C. trachomatis* infection ranged from 15-45% depending on the method of diagnosis. Authors of previous studies concluded that *C. trachomatis* plays a role in

infertility, and should be considered in the investigations of these patients²⁷⁻²⁹.

Among Egyptian asymptomatic women the reported rate of *C. trachomatis* infection was 13.3%. Studies about the prevalence of *C. trachomatis* infection in the Arab world are quite limited. The seroprevalence of *C. trachomatis*, and other pathogens, was determined in Palestinian women attended IVF center in Gaza complaining from infertility and abortion. Anti-Chlamydia IgM antibodies were assayed using ELISA. Positive results were found in 12.8% for *C. trachomatis* antibodies. These results are lower than those of the current study; one reason for this might be the submission of both infertility and abortion patients besides they assayed anti- Chlamydia IgM antibodies while the current study assayed anti- Chlamydia IgG antibodies³⁰⁻³².

IgG antibodies to *C. trachomatis* were detected in (31.1%) of the infertile women in this study. Specific anti- *C. trachomatis* IgG, in infertile women, determined by immunoenzymatic assay, was 39.1% in Poland and 39% in Ghana which was similar to the present study³³.

In another study, higher prevalence rates for ACTA than the present study was present; (55%) in women with secondary infertility. Antichlamydial IgG antibodies were present in 68% of women with infertility in another study, where Chlamydia IgG antibodies have been found in 30–60% of subfertile women, and are considered as markers for past pelvic infections. No explanation for this striking high

prevalence was found except that might be misinterpretation of results^{34,35}.

Prevalence of anti- *C. trachomatis* antibodies or positive urine samples for current infection with *C. trachomatis* didn't differ significantly between primary and secondary infertility groups in our study. In contrast to these results, prevalence of past and current chlamydial infection was found to be strongly statistically significant in women with secondary infertility.

In another study, a significant high seropositivity to chlamydial anti-cHSP60 antibodies were detected in patients with secondary infertility. According to this study authors, this increased susceptibility could be due to their longer period of active sexual life in 2ry infertility patients thus enhancing their exposure to chlamydial infection^{35,36}. However, this is not accepted as it's not accepted as it's well known that risk factors for chlamydial infections are multiple partners and not duration of active sexual life with a single partner. Besides, some women with 1ry infertility have long duration of marriage and sexual activity¹⁰⁻¹⁵.

Prevalence of *C. trachomatis* varies with the population under study and the sensitivity of the laboratory method used. The low isolation rate of *C. trachomatis* in this study is in accordance with other studies in asymptomatic infertile women which ranged from 3-8.7%. Also, from the combined data of many studies using DNA amplification tests, it can be concluded that endocervical *Chlamydia* infections are infrequent in subfertility patients³⁰⁻³⁷.

In contrast to the present study higher isolation rates ranged from 26.9% to 36.2% was detected in etiologically unexplained asymptomatic infertile women in a WHO study reported the current chlamydial infection in infertile women to be 18-20 per cent. An explanation for these high rates of infection was; difference in the study population, method of assay, male partner infection or inclusion of other causes of infertility^{22,31,36}.

No association between current or past chlamydial infection and unexplained infertility was found in the present study. In contrast, positive association

between past *C. trachomatis* infections and subfertility was confirmed. In Romania, correlation of the *C. trachomatis* past and current infection with the infertility was clearly shown¹⁵⁻²⁰. One explanation for this, is that exposure to the chlamydial heat shock proteins could significantly affect mucosal immune function by modifying the release of cytokines (IFN- α , IL-10 and TNF- α) and leading to severe immunopathological conditions related to infertility. These results were observed in infected women with infertility but not in infected fertile women²⁵⁻³⁰.

Another explanation is that cross-reactions may occur between Chsp10 and human extracellular early pregnancy factor (EPF), a factor essential for the growth and survival of the embryo during the pre- and post-implantation periods, such cross-reactions might interfere with EPF function, thereby playing a role in *C. trachomatis*-associated female infertility due to embryo implantation failure. The majority of EPF cross-reactive sera corresponded to the DR7 DQ2, DR17 DQ2, DR13 DQ6, and DR15 DQ6 haplotypes. This may explain why not all *C. trachomatis* infected women become infertile^{20,26,32}.

A third explanation is the suggestion, that antibodies against conserved epitopes on cHSP60 may cross-react with those on human HSP60 and initiate an autoimmune response. In women with pre-existing immunity to Chsp60, exposure to human hsp60 in the early stages of pregnancy can lead to reactivation of hsp60-sensitized lymphocytes. The subsequent pro-inflammatory immune response can foster immune rejection of the early developing embryo³⁵.

As a final conclusion, no relationship was found between past or current *C. trachomatis* infection and ASA in females with unexplained infertility indicates that chlamydial infections have a low probability of inducing circulating antisperm antibodies in women. ASA were significantly more prevalent in females with unexplained infertility. No difference was found in the prevalence of current or past *C. trachomatis* infection between unexplained infertility cases and fertile controls. No difference in the prevalence of current, past *C. trachomatis* infection and ASA between 1ry and 2ry infertile patients.

So we could recommend the screening of infertile

Figure 1 : Distribution of unexplained infertility cases according to age

According to age, cases with UI either 1ry or 2ry were divided into 5 groups; first group ranged from 20-24 yrs old [n=14 (16%)], from 25-29 yrs old [n=26 (29%)], 30-34 yrs old [n=28 (31%)], 35-39 yrs old [n=18 (20%)] and 40 yrs old or more [n=4 (4%)].

Distribution of unexplained infertility cases according to age

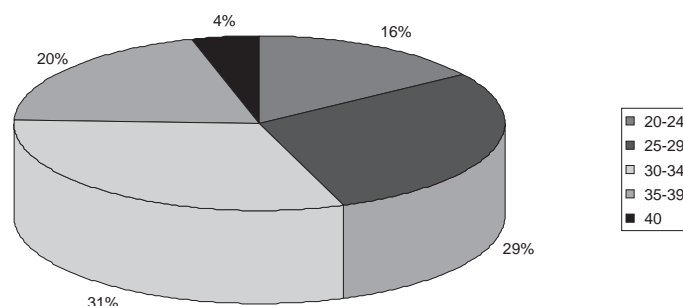
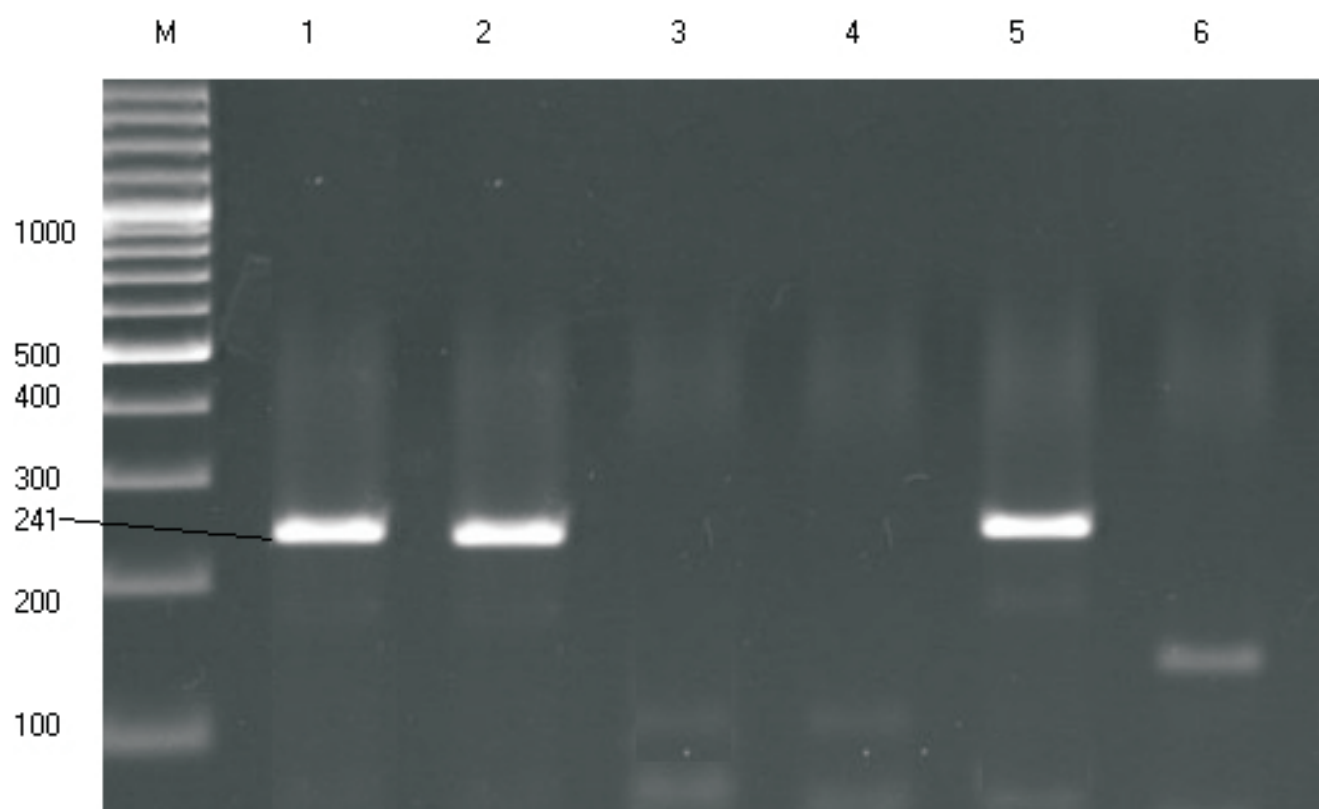


Figure 11: Detection of *C. trachomatis* plasmid amplification by agarose gel electrophoresis in 2% agarose gel. KL1-KL2 primers were used, which amplifies 241 bp segment within *C. trachomatis* cryptic plasmid.



Lane M: Molecular size markers; sizes are on the left, in base pairs.

Lanes: 1, 2: positive reaction in which the 241bp amplified segments of cryptic plasmid were obtained.

Lane 3, 4: negative reaction in which no *C. trachomatis* plasmid DNA was detected

Lane 5: positive control of *C. trachomatis*

Lane 6: negative control in which PCR reaction was performed without a template DNA.

females for ASA, anti-*C. trachomatis* IgG and current chlamydial infection, which is most frequently asymptomatic. This test still remains to be performed whether a localized mucosal female genital tract immune response to spermatozoa may be induced by *C. trachomatis* in the apparent absence of a systemic immune response to this organism. Investigating the antichlamydial and antisperm antibody status of the male partners of those previously studied women is recommended to assess their possible influence on ASA formation in women. More researches are needed to investigate antigens shared between *C. trachomatis* and sperms. Researches for the development of new contraceptives using ASA or chlamydial antigens will be helpful in family planning plans.

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