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# Role of Oxidative Stress and *Chlamydia trachomatis* in Infertile Women with Anti-Zona Pellucida Autoantibodies

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## Abstract

Female infertility could be attributed to various factors including; sperm isoantobody, zona pellucid antibody, ovarian antibody, *Chlamydia trachomatis* infection and imbalance between oxygen radical and antioxygen radical. These factors might impair infertility by different mechanisms.

A retrospective case-control study was performed on 105 infertile female including 75 (71.4%) with primary infertility and 30 (28.6%) with secondary infertility. Thirty healthy women were used as a control. Antisperm antibody (ASA), antizona pelucida antibody (AZP-Ab) and antiovarian antibody (AOA) were detected in the serum and cervical secretion using Enzyme labeled and Biotin labeled antibody. PCR technique was applied to detect the presence of *Chlamydia trachomatis* in the cervical secretion using the following primers: KL1-F and KL2-R, whereas IgG specific antibody was examined in the serum samples.

According to infertility etiology, 29 (21.5%) were classified with unexplained infertility, 32 (23.7%) with polycystic ovary, 6 (4.4%) with endometriosis, 22 (16.3%) with tubal damage, 9 (6.7%) with hormonal imbalance, while other cases showed multiple causes of infertility. ASA were detected in the serum of infertile females (13.3%) and in (30%) of fertile one. Autoantibodies to zona pelucida were detected in the serum and the cervical secretion of infertile females (9.5% and 13.3%, respectively). No detectable levels of AZP-Ab were observed in fertile females. Statistically, the prevalence of ASA was significantly correlated to the AZP-Ab detected in the serum and cervical secretion of infertile females ($P>0.05$).

Aautoantibodies to different ovarian antigens were detected in the cervical secretion of 47 (44.8%) of the cases, in the absence of any detectable levels among fertile females. A high percentage of ASA, AZP-Ab and AOA were detected in primary infertility-related cases. However, with respect to the etiology of infertility, it seems that tubal damage, polycystic ovary and unexplained cases showed the highest percentages of both iso and autoantibodies.

Serum samples obtained from fertile and infertile females were analyzed for the presence of *C. trachomatis* specific IgG. Five (4.76%) of etiologically tubal damage infertile females showed the presence of Chlamydial antibody with no detectable level in the serum of fertile females. To confirm the presence of *C. trachomatis*, PCR was used to identify Chlamydia DNA in the cervical secretion. Only positive serum samples revealed clear, sharp bands of amplified Chlamydial DNA with 241 bp. *C. trachomatis* was detected in infertile female with ASA (60%), serum and cervical secretion positive for AZP-Ab (60%, 80%, respectively) and in the cervical secretion positive for AOA (60%). A significant correlation was appeared between *C. trachomatis* infection and the presence of ASA and AZP-Ab ($p>0.05$). Infertile females with Chlamydia infection has a high probability of inducing circulating and local ASA and AZP-Ab. Diagnosis of Chlamydia infection in the endocervix is recommended to assess their possible influence on ASA, AZP-Ab formation in infertile females.

**Keywords:** Antisperm-antibodies (ASA); Antizona pellucida antibodies (AZP); *Chlamydia trachomatis*; Un-explained infertility; Polycystic ovary; Endometriosis; Tubal damage; Hormonal imbalance

## Introduction

The term fertility is known as the capacity to produce offspring and a couple is considered to be infertile if they cannot conceive after 12 months of unprotected intercourse. A more strict definition of infertility is failure to reach a pregnancy in a 12 month period for patients under 35 years of age and failure to conceive in a 6 month period for the over 35 years [1].

These couples comprise the infertile and the sterile members of the population, for who is no possibility of natural pregnancy and the remainder who are sub fertile [2]. Infertility affects 10% of all couples. About a third of infertility problems are due to female infertility and another third are due to male infertility. Comparative reports issued by WHO have estimated that 8% to 12% of couples worldwide are affected [3].

Diseases or dysfunctions of reproductive tract, neuroendocrine system and immune system in female can affect fertility. Failed of autoimmune may be due to overall activity or reaction of immune system specifically directed against ovarian antigens [4]. Although the presence of antiovarian antibodies immunoglobulin G (IgG) have been documented in different groups of infertile patients.

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The zona pellucida (ZP) is a layer surrounds the egg at ovulation and remains in place until the time of implantation. It contains receptors (ZP1, ZP2 and ZP3) for sperms which are, with some exceptions, species-specific [5]. The initial contact between the sperm and the oocyte is a receptor-mediated process. These receptors are glycoproteins and any alteration of these glycoproteins leads to a loss of receptor activity [6]. Specific antibodies directed against these glycoproteins are able to inhibit sperm attachment and penetration into oocyte and may be the cause of immunological infertility [7].

There is a convince evidence that the establishment of a chronic inflammatory response, together with the presence of local oxidative environment, could play an important role in the etiology and the progression of several human diseases. In the reproductive system, the presence of inflammatory cytokines (TNF-α, IFN-γ, IL-1) and the high levels of free radicals may damage biological molecules by oxidation and chemically modifying cell proteins which consequently can change their antigenicity and therefore become implicated in immunological deleterious reactions associated with inflammatory and/or autoimmune injury [8]. An altered protein function and the presence of circulating autoantibodies to new epitopes could block some membrane surface antigens with a receptor function in the reproductive system.

Genital infectious disease in the world, it is one of the factors affecting fertility. *Chlamydia trachomatis* is an important human pathogen associated with sexually transmitted diseases.

C. trachomatis is a common cause of cervicitis; urethritis and a condition that is the consequence of a previous disease or injury include pelvic inflammatory disease.

Repeated episodes of infection clearly play a role in stimulating the host immune response and producing some pathological changes. Redgrove and McLaughlin [9] found that the *chlamydia* infection is a local, whereby immune cells such as leukocytes are recruited to the site of infections and subsequently secrete pro-inflammatory cytokines and chemokines such as interferon gamma.

The objective of this study was to evaluate the incidence of anti-zona pellucid antibodies in cervical secretion and serum of infertile women and study the prevalence of *Chlamydia trachomatis* among infertile women.

## Materials and Methods

### Subjects

A total of 135 women (105 infertile and 30 fertile) with ages ranged between 21-44 years old which were investigated. Infertile cases were divided into four age groups consisted of: group 1 (20-25), group 2 (26-30), group 3 (31-35), group 4 (36-42) years. The blood samples were collected from Jordan University Hospital, Department of *in vitro* fertilization (IVF) (Supplementary Table).

### Sample collection

Serum (5 ml) and Cervical mucus samples (0.5 ml) were collected from 105 infertile females who visiting the Specializing Medical Center in Amman with at least (2 or 3) years duration of infertility. The control group was consisted of thirty apparently healthy fertile women.

### Cervical secretion collection

The clinical samples will be collected aseptically and transported directly to the laboratory. Samples of cervical secretions were collected from spontaneously ovulating infertile and fertile women. A sterile speculum will inserted into the vagina and a 5–10 mL syringe used to collect cervical secretions from the endocervical canal. About 0.5 ml of cervical secretion was taken by syringe from high cervix using Cusco speculum, placed in sterile tubes and stored at –21°C until analyzed. The mucus must be liquefied by mucolytic agent of N-acetyl L cysteine at concentration 0.2 mg/ml which prepared by weight 0.2 mg of N-acetyl L-cysteine and complete to one milliliter with distilled water [10].

### Diagnosis

Detection of Antizona pellucid antibodies (AZPA), in cervical secretion by ELISA kit (MyBiosource, USA). Detection procedure for Sperm Antibody in Serum by ELISA (DRG International Inc®, USA). Detection procedure for Human Anti-Ovarian Antibody (AOA) in cervical secretion by Eliza Kit obtained from (Sinnow® Medical, China).

At the detection of Chlamydia Chlamydia trachomatis of IgG was measured by ELISA from NovaTec Immundiagnostica GmbH Technologie and Waldpark (Germany).

## Results

A total of one hundred and thirty five Jordanian females were enrolled in this study, including 105 (77.8%) infertile females and 30 (22.2%) fertile control. All the females were attended the Infertility Department at Jordan University Hospital during the period Feb-Sept 2016. The mean age of infertile females was 31.27 ± 0.558 years old and the mean duration of infertility was (4.53 ± 2.875). According to the age, infertile females were divided into four groups including; group I (20-25 years), group II (26-30 years), group III (31-35 years), group IV (36-42 years) (Figure 1). No statistically significant differences in the mean age of the infertile and fertile females used as a control (25.1 ± 5.90) was reported ($p$>0.05).

According to infertility, cases were divided into primary infertility which include 75 (71.4%) of the cases and secondary which include 30 (28.6%) of the infertility cases. Furthermore, females were grouped with respect to the causes of infertility to nine groups (Table 1).

| Group identity | Years | Number |
|---|---|---|
| Group 1 | 20-25 | 21 |
| Group 2 | 26-30 | 32 |
| Group 3 | 31-35 | 24 |
| Group 4 | 36-42 | 28 |

**Supplementary Table**

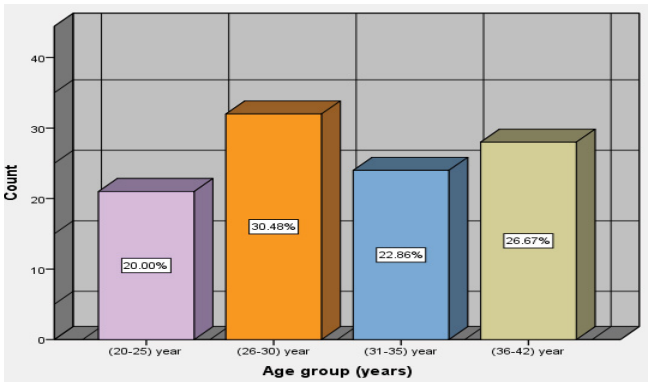


**Figure 1:** Distribution and percentages of infertile females, according to age groups.

| Causes of infertility | | | Infertility | | |
|---|---|---|---|---|---|
| | | | Primary infertility | Secondary infertility | Total |
| I | Unexplained | Count | 23 | 6 | 29 |
| | | % within infertility group | 79.3% | 20.7% | 100.0% |
| | | % within status | 30.7% | 20.0% | 21.5% |
| II | Polycystic ovary | Count | 20 | 12 | 32 |
| | | % within infertility group | 62.5% | 37.5% | 100.0% |
| | | % within status | 26.7% | 40.0% | 23.7% |
| III | Endometriosis | Count | 6 | 0 | 6 |
| | | % Within infertility group | 100.0% | 0.0% | 100.0% |
| | | % within status | 8.0% | 0.0% | 4.4% |
| IV | Tubal damage | Count | 15 | 7 | 22 |
| | | % within infertility group | 68.2% | 31.8% | 100.0% |
| | | % within status | 20.0% | 23.3% | 16.3% |
| V | Hormonal imbalance | Count | 5 | 4 | 9 |
| | | % within infertility group | 55.6% | 44.4% | 100.0% |
| | | % within status | 6.7% | 13.3% | 6.7% |
| VI | Polycystic ovary + Endometriosis | Count | 1 | 0 | 1 |
| | | % within infertility group | 100.0% | 0.0% | 100.0% |
| | | % within status | 1.3% | 0.0% | 0.7% |
| VII | Polycystic ovary + Tubal damage | Count | 2 | 0 | 2 |
| | | % within infertility group | 100.0% | 0.0% | 100.0% |
| | | % within status | 2.7% | 0.0% | 1.5% |
| VIII | Polycystic + Hormonal tubal | Count | 2 | 1 | 3 |
| | | % within infertility group | 66.7% | 33.3% | 100.0% |
| | | % within status | 2.7% | 3.3% | 2.2% |
| IX | Multiple causes | Count | 1 | 0 | 1 |
| | | % within infertility group | 100.0% | 0.0% | 100.0% |
| | | % within status | 1.3% | 0.0% | 0.7% |
| Total | | Count | 75 | 30 | 105 |
| | | % within infertility group | 55.6% | 22.2% | 100.0% |
| | | % within status | 100.0% | 100.0% | 100.0% |

**Table 1:** Distribution of females with primary and secondary infertility, according to the causes of infertility.

## Detection of ASA, AZP and AOA in the serum and cervical secretion

Antisperm antibodies were detected in the serum of 14 (13.3%) of the infertile females. Of these cases, 12 (16%) associated with primary infertility and 2 (6.7%) with secondary infertility cases (Table 2). However, fertile females revealed the presence of ASA in 9 (30%) of serum samples.

Concerning the causes of infertility, a high level of ASA was detected in infertile females with polycystic ovary (42.9%), followed by cases with tubal damage (35.7%) and unexplained infertility (21.4%) (Table 3).

From the other hand, autoantibodies to zona pelucida were detected in the serum of 10 (9.5%) of the infertile females. However, 7(9.3%) of cases are with primary infertility and 3(2.9%) with secondary infertility (Table 4). Fertile females showed no detectable levels of AZP antibodies in their serum. Infertility cases related to polycystic ovaries showed the highest level of AZP in the serum (Table 5).

We attempted to evaluate the level of AZP autoantibody in the cervical secretion and compared its levels to that observed in the serum. Fourteen (13.3%) of infertile females showed positive AZP antibody in their secretion, including 11(10.5%) with primary infertility and 3 (2.9%) with secondary infertility (Table 6). Infertile females with polysystic ovary appeared to have a high percentage of AZP autoantibody (42.9%) in their cervical secretion in comparison to other infertility causes (Table 7).

| | | | Status | | Total |
|---|---|---|---|---|---|
| | | | Primary infertility | Secondary infertility | |
| ASA | Negative | Count | 63 | 28 | 91 |
| | | % within status | 84.0% | 2.7% | 86.7% |
| | | % of Total | 60.0% | 26.7% | 86.7% |
| | Positive | Count | 12 | 2 | 14 |
| | | % within status | 16.0% | 6.7% | 13.3% |
| | | % of Total | 11.4% | 1.9% | 13.3% |
| Total | | Count | 75 | 30 | 105 |
| | | % within status | 100.0% | 100.0% | 100.0% |
| | | % of Total | 71.4% | 28.6% | 100.0% |

**Table 2:** The incidence of antisperm antibodies detected by ELISA in the serum of infertile females.

Fertile females showed no detectable levels of AZP antibodies in their cervical secretion samples. Statistically, the prevalence of ASA was significantly correlated to the AZP antibody detected in the serum and cervical secretion in infertile females (P>0.05).

Antiovarian autoantibodies were detected in the cervical secretion of 47 (44.8%) of cases with infertility. Of these, 33 (31.4%) classified with primary infertility and 14 (13.3%) with secondary infertility (Table 8). Fertile females showed no detectable levels of AOA antibodies in their cervical secretion.

| Count | Infertility causes groups | | | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|
| | Group I* | Group II | Group III | Group IV | Group V | Group VI | Group VII | Group VIII | Group IX | |
| % within ASA | 3 | 5 | 0 | 6 | 0 | 0 | 0 | 0 | 0 | 14 |
| % within infertility group | 21.4% | 35.7% | 0.0% | 42.9% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 100% |

**Table 3**: Numbers and percentages of females with serum ASA in relation to the infertility causes.

| | | | Status | | |
|---|---|---|---|---|---|
| | | | Primary infertility | Secondary infertility | Total |
| AZP | Negative | Count | 68 | 27 | 95 |
| | | % within status | 90.7% | 90% | 90.5% |
| | | % of Total | 64.8% | 25.7% | 90.5% |
| | Positive | Count | 7 | 3 | 10 |
| | | % within status | 9.3% | 10% | 9.5% |
| | | % of Total | 6.7% | 2.9% | 9.5% |
| | | Count | 75 | 30 | 105 |
| | | % within status | 100% | 100% | 100% |
| | | % of Total | 71.4% | 28.6% | 100% |

**Table 4:** The incidence of antizona pelucida antibodies detected by ELISA in the serum of infertile females.

| | Infertility causes groups | | | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|
| | Group I* | Group II | Group III | Group IV | Group V | Group VI | Group VII | Group VIII | Group IX | |
| Count | 1 | 3 | 1 | 5 | 0 | 0 | 0 | 0 | 0 | 14 |
| % within AZP serum | 10% | 30% | 10% | 50% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 100% |

**Table 5:** Numbers and percentages of females with AZP antibody in the serum of infertile females with different infertility cause (*See Table 1).

| | | | Status | | Total |
|---|---|---|---|---|---|
| | | | Primary infertility | Secondary infertility | |
| AZP | Negative | Count | 64 | 27 | 91 |
| | | % within AZP | 70.3% | 29.7% | 100.0% |
| | | % of Total | 61.0% | 25.7% | 86.7% |
| | Positive | Count | 11 | 3 | 14 |
| | | % within AZP | 78.6% | 21.4% | 100.0% |
| | | % of Total | 10.5% | 2.9% | 13.3% |
| Total | | Count | 75 | 30 | 105 |
| | | % within AZP | 71.4% | 28.6% | 100.0% |
| | | % of Total | 71.4% | 28.6% | 100.0% |

**Table 6:** The incidence of autoantibody to zona pelluicda was detected by ELISA in the cervical secretion of the infertile females.

| | | Infertility group | | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Group I* | Group II | Group III | Group IV | Group V | Group VI | Group VII | Group VIII | Group IX | |
| AZP | Count | 2 | 6 | 0 | 6 | 0 | 0 | 0 | 0 | 0 | 14 |
| | % within AZP Secretion | 14.3% | 42.9% | 0.0% | 42.9% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 100% |
| | % within infertility group | 6.9% | 18.8% | 0.0% | 27.3% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 13.3% |

**Table 7:** Distribution of infertile female with AZP antibody in the cervical secretion according to the causes of infertility (*See Table 1).

*C. trachomatis* was detected in 4 (80.0%) of infertility cases with sperm antibody; 3 (60.0%) of serum samples positive for zona antibody; 4 (80.0%) of cervical secretion positive for zona antibody; and 3 (60.0%) of cervical secretion positive for ovarian antibody (Table 9).

Serum samples obtained from fertile and infertile females were analyzed for the presence of *C. trachomatis* specific IgG using ELISA method. Five Out of 105 serum samples obtained from infertile females screened, 5 (4.76%) showed the presence of anti *C. trachomatis* IgG antibodies. No detectable level of IgG specific for *C. trachomatis* was recorded in the serum samples obtained from fertile females.

## Discussion

Reproductive failure is a major medical issue adversely affecting human health in the 21st century. It is widely accepted that during the last twenty years, the average age of having children has increased and this is a key factor for infertility. As the age of giving birth is increased, the reproductive capacity is decreased, the ovary becomes less efficient, the frequency of sexual intercourse is decreased and the possibility of chromosomal abnormalities and miscarriage is increased [9].

The present study was carried out on infertile females with different

| | | | Status | | Total |
|---|---|---|---|---|---|
| | | | Primary infertility | Secondary infertility | |
| AOA | Negative | Count | 42 | 16 | 58 |
| | | % within AOA | 72.4% | 27.6% | 100.0% |
| | | % of Total | 40.0% | 15.2% | 55.2% |
| | Positive | Count | 33 | 14 | 47 |
| | | % within AOA | 70.2% | 29.8% | 100.0% |
| | | % of Total | 31.4% | 13.3% | 44.8% |
| Total | | Count | 75 | 30 | 105 |
| | | % within AOA | 71.4% | 28.6% | 100.0% |
| | | % of Total | 71.4% | 28.6% | 100.0% |

**Table 8:** The incidence of antiovarian antibody detected by ELISA in the cervical secretion of infertile females.

| | | | *C. trachomatis* | | Total |
|---|---|---|---|---|---|
| | | | Negative | Positive | |
| Status | Primary infertility | Count | 70 | 5 | 75 |
| | | % within status | 93.3% | 6.7% | 100.0% |
| | | % of Total | 66.7% | 4.8% | 71.4% |
| | Secondary infertility | Count | 30 | 0 | 30 |
| | | % within status | 100.0% | 0.0% | 100.0% |
| | | % of Total | 28.6% | 0.0% | 28.6% |
| Total | | Count | 100 | 5 | 105 |
| | | % within status | 95.2% | 4.8% | 100.0% |
| | | % of Total | 95.2% | 4.8% | 100.0% |

**Table 9:** Incidence of *C. trachomatis* in the primary and secondary infertility cases as detected by ELISA and PCR.

infertility etiology. Although there are no statistically significant differences in the mean age of the infertile and fertile females, however, the presence of young age females might express the desire of women to have an early pregnancy immediately after marriage. No significant difference in the geographic distribution of infertile females among Jordanian cities.

Most of the infertile females enrolled in this study were classified with primary infertility (71.4%). Cystic ovary cases appeared to be the major cause of female infertility in Jordan (23.7%) followed by unexplained infertility and infertility-related to tubal damage (21.2% and 16.3%, respectively). According to American Society for Reproductive Medicine, female infertility is often caused by a woman's inability to ovulate, or release an egg [10].

Immunological infertility is one of the major causes of female infertility which entails production of isoantibodies against sperms [11]. However, the relevance of ASA in the diagnosis of infertility is still controversial [12]. The current study tried to give an answer to this controversy. Our results are slightly higher (13.3%) than the results conducted at USA [13] and reported an ASA prevalence of 5.7% among infertile females. However, the percentages of ASA reached 42.9%, 35.7% and 21.4% among females with polycystic ovary, tubal damage and unexplained infertility, respectively. Other researchers reported the frequency of circulating ASA from 2.0% to 8.0% in the serum of infertile women [14,15]. This variability may be related to the tool used to detect ASA and sample size. The disruption of mucosal layer in the female genital tract either by mechanical or chemical causes will lead to exposure of the sperm antigens to the immune system which will result in the production of ASA [16].

It is presumed that harsh sexual intercourse with heavy bleeding or coitus during times of uterine mucosal disruption for example during menses and puerperium, will bring huge amount of sperms in direct contact with circulation and so definite immune response will take place. Antibodies against limited sperm epitopes cannot cause infertility especially if the epitops involved in the immune reaction is not participated directly in the fertilization process [17].

In women, the failure of natural tolerance may lead to sensitivity resulting in sperm elimination. ASA affect fertility potential through various pre/post-fertilization processes, such as sperm agglutination and motility, cervix mucus penetration, capacitation, acrosome reaction, zona pellucida (ZP) binding and penetration, spermoocyte fusion and embryo implantation [18]. The active local immuno-regulatory mechanism is based on vaginal and cervical tissues having an active and sensitive mucosal immune system, by which the fertility potential is affected. This explains the rather high percentage of infertile women with the local reactions leading to inflammation as well as with high levels of serum anti-sperm antibodies. Furthermore, ASA-coated sperm may be more vulnerable to phagocytosis in the female reproductive tract [19]. Serum ASA is related to the long-term exposure of female to sperm antigens and then to seminal deficiency in immuno-suppressive factors [20].

Antibodies can also block the sperm's ability to bind to the development (zona pellucida) of the egg, a prerequisite for fertilization (i.e. blocking antibodies). Some studies suggest the possible immuneopathological mechanism for reproduction failure in patients with organ-specific auto antibodies. Many reports have been published indicating the incidence of AZP-Ab in females with immunological infertility [21].

In this study, we assessed the level of AZP-Ab among infertile women. A significant serum level of AZP-Ab (20.8%) was reported among infertility cases without any detectable level in fertile control. Our results are quite different from those reported the presence of anti-zona pellucida activities in the sera of fertile female [22,23]. The presence of anti-zona activity in infertile women may be an autoimmune response, possibly due to absorption and degradation of the ova into the peritoneal cavity or in the reproductive tract and the subsequent exposure of the degradation products to the immune system [24]. Our study supports the results of previous studies and showed comparable high incidence of AZP in women with primary infertility which might explain the involvement of auto-antibodies related to reproduction system in development of primary infertility. The finding that auto-immunological factors are participated in the development of infertility is supported by others [25].

One of the most common sexually transmitted diseases is *Chlamydia trachomatis* infection that could impair fertility through different mechanisms [26]. Chlamydia infection, often simply known as Chlamydia, is a common sexually transmitted disease associated with the bacterium *C. trachomatis*, which can damage a woman's reproductive tissues, thus, affecting fertility. The overall prevalence of IgG specific to Chlamydia in infertile women was 4.76% which seems to be lower than in other studies [27-29]. All cases positive to *Chlamydia trachomatis* IgG were associated with primary infertility. IgG antibodies in serum were the best predictor of a current or potential past Chlamydia infection. Host immune response plays important roles in the pathogenesis of long-term complications after Chlamydia infections [22,24]. Positive serum samples for Chlamydia specific IgG antibodies revealed bands of amplified Chlamydial DNA by PCR.

Infertile females with *C. trachomatis* infection showed a

statistically significantly incidence of ASA and AZP-Ab in the serum and cervical secretion. An early investigations for immunological and bacteriological factors associated with female infertility provides an important diagnostic and prognostic information, decrease the probability of tubal damage and might prevent further complication of infertility.

## Conclusion

In the present study, the possible contribution of female reproductive biomarkers of oxidative stress to the development of Chlamydia induced infertility was examined in Chlamydia positive women. The Mechanisms of damage is related to DNA oxidation and reduced antioxidant capacity which might induce infertility related to tubal damage. The mean level of antioxidant also has been shown to be significantly related to iso-antisperm antibodies among the infertile females in comparison to fertile one.

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