Saber, Universidad de Oriente, Venezuela.Vol. 27 Nº 2: 269-275. (2015)
ISSN: 2343-6468 Digital / ISSN: 1315-0162 Impreso / Depósito Legal pp 198702SU187

ANTI-*Chlamydia trachomatis* SECRETORY IgA DETECTION IN A VENEZUELAN INFERTILE WOMEN POPULATION

DETECCIÓN DE IgA SECRETORA ANTI-*Chlamydia trachomatis* EN UNA POBLACIÓN DE MUJERES INFÉRTILES VENEZOLANAS

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ABSTRACT

Importance of microorganisms that cause sexually transmitted diseases associated to infertility in many couples have been remarked in several epidemiological and clinical studies. *Chlamydia trachomatis* is a frequent agent of sexual transmission of the female genital tract that can induce a persistent local immunological reaction causing tissue damage, provoking obstruction of Fallopian tubes altering the normal transport and nurture of gametes and therefore producing infertility. In sexually active Venezuelan women, either asymptomatic or symptomatic for genital infection, prevalence of *C. trachomatis*, estimated by several methods, ranges between 9% and 35%. But prevalence of anti *C trachomatis* secretory IgA in cervical mucus. from infertile women has not been studied. The aim of this study was to detect secretory IgA anti- *C. trachomatis* in cervical mucus samples from Venezuelan women and its possible relationship with female infertility. Secretory IgA was assessed using an indirect immune enzymatic assay of solid phase. Eleven out thirty-eight studied patients (29%) had antecedents of Pelvic Inflammatory Disease (PID), 21% of spontaneous abortion, 5.2% of ectopic pregnancy and 7.8% showed signs and symptoms of blennorrhagia. IgA Antibodies anti-*C. trachomatis* were not identified in cervical mucus in studied patients. However, to discard the presence of these antibodies in the cervical mucus is convenient after genital infection, as a cause of female factor for infertility.

Key-words: *Chlamydia trachomatis*, secretory IgA, cervical mucus.

RESUMEN

Numerosos estudios clínicos y epidemiológicos han revelado la importancia que tienen los microorganismos causantes de las enfermedades de transmisión sexual en el origen de la infertilidad de muchas parejas. *Chlamydia trachomatis* es un agente de trasmisión sexual frecuente del aparato genital femenino, que puede inducir una reacción inmunológica local persistente que provoca daño tisular, que resulta en obstrucción de las trompas de Falopio, alterando finalmente el transporte normal de los gametos y su nutrición, causando infertilidad. Se ha estimado que la prevalencia de infección genital por *C. trachomatis*, detectada por varios métodos, en mujeres venezolanas sexualmente activas, con o sin síntomas, varía entre 9% y 35%. Sin embargo, no existen estudios sobre la prevalencia de anticuerpos anti-*C. trachomatis* en moco cervical de mujeres infértiles. El objetivo de esta investigación fue detectar IgA secretora anti-*C. trachomatis* en muestras de moco cervical de mujeres venezolanas infértiles. Para la determinación de IgA secretora se utilizó un ensayo inmunoenzimático indirecto de fase sólida. El 29% de las pacientes presentaron antecedentes de Enfermedad Inflamatoria Pélvica (EIP), 21% tenían como antecedente abortos espontáneos, 5,2% embarazo ectópico y 7,8% presentaron signos y síntomas de blenorragia. A pesar de que no se identificaron anticuerpos IgA anti-*C. trachomatis,* es conveniente descartar su presencia luego del tratamiento de infecciones genitales, por su asociación con infertilidad.

Palabras clave: *Chlamydia trachomatis*, IgA secretoria, moco cervical.

INTRODUCTION

According to the level of country development, between 10% and 30% of couples in reproductive age are unable to have children as a consequence of alterations in the structure and function of male and/or female reproductive system (Dabekausen *et al*. 1994, Brito *et al*. 1998).

Several clinical and epidemiological studies have shown the importance of micro organisms as a cause of sexually transmited infectios (STI) on infertility. *Neisseria gonorrhea*, *Chlamydia trachomatis*, *Ureaplasma urealyticum*, *Mycoplasma hominis* and *Trichomonas vaginalis* produce sequela in women that alter the cervico-vaginal and/or endometrial enviroment which becomes unfavourable for the movement of the oocyte through the fallopian tubes and for the arrival of spermatozoa that can alter motility and provoque agglutination (Brito *et al*. 1998).

Recibido: enero 2015. Aprobado: marzo 2015.
Versión final: abril 2015.

C. trachomatis is a frequent agent of sexual transmission in male and female genital tract. In sexually active men younger than 40 years-old it is the main cause of epididymitis, and it has also been involved in cases of prostatitis, urethritis and proctitis. It has been isolated from endocervix in 30-60% of women with gonococcal cervicitis, in 10-20% of women that consult to a venereologic clinic and up to 10% of young asymptomatic women (Thomas *et al*. 2000).

C. trachomatis is also a frequent cause of endometritis, salpingitis and ectopic pregnancy as well as low-weight newborns, prematurity and spontaneous abortions. It is associated at least to 50% of cases of Pelvic Inflammatory Disease (PID) in developed countries and it is considered one of the main causes of alterations of fallopian tubes leading to infertility, and between 50-80% of patients are asymptomatic. Percentage of isolation of this micro organism increases with sexual promiscuity. Importance of these infections is due not only to their morbility but also to infertility than they may produce (Thomas *et al*. 2000).

According to serological studies, infertility associated to cicatrization of oviducts was related to a previous *C. trachomatis* infection. However, many unfertile women with cicatricial tubal obstruction and with antibodies anti-*Clamydia* did not have any antecedent of previous PID and it was suggested that subclinical tubal infection was enough to produce cicatrization (Stamm 1999).

Frequency of chlamydial infection is variable among sexually active females. In North America between 2007 and 2012, estimated prevalences were 1.6% (2007-2008); 1.7% (2009-2010) and 1.9% (2011-2012) (Torrone *et al*. 2014). On the other hand, in latin-american countries like Brazil, prevalence of chlamydial infection in infertile women is 10.9% (Fernandes *et al*. 2014). Eventhough general prevalence could be higher in general female population, low incidence as 1% could be found in previosly treated infertile women with STI (Pantoja *et al*. 2012).

In serum of *C. trachomatis* infected individuals, a conventional immunological response, (i.e. IgM high titres) has been detected the first week after infection, and IgG response a week later, persisting then through years. Sometimes, variations in IgG titres are not correlated with evolution of the infection, therefore these titres have a diagnostic but not a pronostic value. A protective value of serum antibodies against a reinfection is uncertain, although *in vitro* they can neutralize *C. trachomatis* infectivity (Thomas *et al*. 2000).

In women, *C. trachomatis* genital infection tends to persist for a long time and it is suggested that is transfered cell to cell without a productive infection, for that reason, local concentrations of secretory IgA (sIgA) remain constant in cervical mucus. This sIgA is locally synthesized in the lamina propria of subepithelial cells of cervix glands and it is expressed in their basolateral surface, thus explaining its intercellular transference. Secretory IgA levels are very low in plasma in comparison with those found in secretory glands (lacrimal, salivary, intestinal, mammary, lung and cervix). IgA Secretion in cervical mucus depends on the secretory activity and response of the secretory cells to circulating hormones. In women with natural menstrual cycles peak concentrations of sIgA are high previously to ovulation and decrease after it, when the levels of progesterone are high (Franklin and Kutteh 1999, WHO 1999).

Pathogeny of complications induced by *Chlamydia* in the female genital tract is not well known, but infertility due to tubal factor, ectopic pregnancy and Fitz-Hugh-Curtis syndrome (Perihepatitis) have been related to antibodies sIgA against to heat shock protein 60 (CHSP60) (Stamm 1999).

Tests for anti-*Chlamydia* antibodies detection are suitable and sensitive methods for diagnosing this kind of infections. Nevertheless, in some cases, cross-reactions between species alter significatively the interpretation of results (Meikle *et al*. 1994).

Species specific IgA anti-*C. trachomatis* antibodies originated by active infections and directed against lipopolysaccharides (LPS) are the best indicators of acute, chronic or recurrent infections by *Chlamydia*. A high degree of correlation between specific IgA antibodies and the presence of antigens like LPS of *Chlamydia* has been reported in many studies (Bjercke and Purvis 1993).

Clad *et al*. (1994) have shown in fertile and sterile women, that ELISA detection with elemental bodies and without LPS of IgA anti-*C. trachomatis* in secretions of the reproductive tract has a higher sensitivity (65%) and specificity (95%) than microimmunofluorescence (MIF), therefore it has a great diagnostic value in routine tests for *C. trachomatis* in the genital tract. Furthermore, this is a screening test easy to perform that serves as an indicador of high risk for tubal factor and infertility in many couples positive to this immunoglobulin.

Persson *et al*. (1999) studied the relationship between antibodies against *C. trachomatis* and factors of infertility and they reported that IgA anti-*C. trachomatis* levels may suggest an either active or silent infection but the difference of the values of prevalence between control group and infected cases was not significative.

In sexually active asymptomatic venezuelan women incidence of *C. trachomatis* infection ranges between 9% and 35%. Nevertheless sIgA anti-*C. trachomatis* has not been detected in cervical mucus from infertile patients (Brito *et al*. 1998, Alfieri *et al*. 2005, Arraiz *et al*. 2006).

The aim of this study was to detect secretory IgA anti-*C. trachomatis* antibodies in cervical mucus from a population of venezuelan infertile women.

PATIENTS AND METHODS

Patients

Thirty-eight women who voluntarily attended two private clinics for infertility in two venezuelan cities (Centro Médico "Orinoco" - Ciudad Bolívar, and to Centro Médico "Uno" - Caracas) were evaluate. Patients signed their consent to participate in this study and all of them fullfilled the following criteria of inclusion: more than 2 years infertility, absence of anatomical defects of the reproductive tract, normal cytology and hormonal profile and not use of contraceptive methods.

The age group was between 22 and 35 years-old (Median: 27). Epidemiological data like cause of consultation, antecedents of blennorrhagia, salpingitis, ectopic pregnancy and PID, previous or present symptoms of infections of the genital tract were collected.

This study was approved by the Thesis Committee of the School of Health Sciences (Universidad de Oriente-Núcleo de Bolívar), that reviewed the ethical and methodological aspects of the research.

Cevical mucus samples

Samples of cervical mucus were obtained at the 12 to 16 day of menstrual cycle. It was pointed out to the patients that they should abstain of: *a*) Having sexual intercourse during 7 days previous to the test and *b*) Have vaginal wash 24 hours before the exam. A speculum without lubricant in vagina and uterine cervix was placed. A swab with plastic handle was introduced 1-2 cm into the endocervical channel in order to withdraw external contaminants from vagina (the swab was rotated strongly but without injuring the mucose) and removed carefully. Mucus was aspirated with a 20 mL syringe without the needle. Suction was performed when the tip of the syringe was 1 cm inside the cervical channel and was maintained up to the moment just before the instrument get out of the external cervical orifice. Cervical mucus was maintained in polyethilene tubes sealed with a stopper in order to avoid dehydration. Samples were stored up to for 7 days at 2-8ºC before processing or at -20ºC when storage time was longer than 7 days.

Secretory IgA anti-*Chlamydia trachomatis* detection

IgA anti-*C. trachomatis* antibodies were determined using an immunoenzymatic assay of solid phase (ImmunoComb II ® *Chlamydia trachomatis* monovalent IgA Orgenics) following the kit instructions. Briefly, the solid phase is a plastic comb with 12 teeth, sensitized at different spots with reactive materials and an internal control. Each tooth contains constant aliquots of *C. trachomatis* L2 elementary bodies which reacts with the cervical mucus sample diluted 1:4 with the supplied diluent solution. After incubation, the comb reacts with alkaline phospatase conjugated to anti-human goat serum. Staining is done at room temperature with nitro-blue-tetrazolium and 5-bromo-4-chloro-3-indolyl-phosphate. The test result is semi-quantitatively read by an optical reading instrument (Comb Scale) supplied by the manufacturer.

RESULTS

Mean time of infertility was 4.37 years (range 1-10 years). Only 11/38 (29%) patients showed clinical criteria of PID during the physical exam, 5/38 (54.5%) had antecedents of salpingitis and 45.5% of endometritis (Table 1). Two out of 38 patients (5.2%) had had ectopic pregnancy and 8/3 (21%) had suffered spontaneous abortion (Table 2).

Three out 38 patients (7.8%) have signs and symptoms of blennorrhagia and 21% (n = 8) had had this antecedent. In the latter group, dyspareunia (13%; n = 5), pruritus, hemorrhage and pain (5.2%; n = 2) were the predominant signs and symptoms. Only one patient (2.6%) showed adenopathies and colposcopic changes related to papillomavirus infection.

Eigth out 38 patients (21%) had pathological features in hysterosalpingography and 3 of them had clinical

criteria of PID.

Secretory IgA anti-*C. trachomatis* was not detected in cervical mucus samples of these patients.

Table 1. Antecedents of inflammatory pelvic disease in infertile women.

Antecedent	Actual	Past	Absent	Total
Endometritis	5 (13.2)*	4 (10.6)	29 (76.4)	38 (100)
Salpingitis	6 (15.8)	10 (26.4)	22 (57.8)	38 (100)

*Values in parentheses are percentages

Table 2. Obstetric antecedents in infertile women.

Antecedent	Yes	No	Total
Abortion	8 (21.0)*	30 (79.0)	38 (100)
Ectopic pregnancy	2 (5.2)	36 (94.7)	38 (100)

*Values in parentheses are percentages

DISCUSSION

Infections and inflammatory processes of female genital tract are common. Effect of these infections on women's infertility is a controversial topic and further research may clarify their real importance. Relationship between chlamydial infection and infertility is complex and multifactorial, Bahamondes *et al*. (1994), studying the relationship between tubal factor infertility (TFI) produced by *C. trachomatis* and other posible risk factors to which patients with this infection found that STI, multiple sexual partners, sexual intercourse at an early age, abortion, tobacco, alcohol, educational and socio-economical level, use of contraceptives, previous either abdominal or pelvic surgery have in some extent a relationship with obstruction and fibrosis of fallopian tubes (Bahamondes *et al*. 1994, Rhoton-Vlasak 2000).

C. trachomatis is the sexually transmitted bacteria with higher prevalence all over the world. It is 3-4 times more common that *N. gonorrhoeae*. It is considered the agent that causes the higher frequency of asymptomatic infections in the female reproductive system and it can produce obstruction and irreversible tubal damage due to local inflammatory processes that destroy ciliated epithelium, leading to infertility (Rhoton-Vlasak 2000). Eventhough seminal IgA anti-*C. trachomatis* antibodies are not associated with alterations in sperm parameters, its prevalence is high in venezuelan asymptomatic infertile men with STI antecedent, indicating thus an infection risk factor for their wives, who could become infertile (Penna-Videau *et al*. 2001).

This study showed the abscence of IgA anti-*C. trachomatis* antibodies in samples of cervical mucus from infertile patients. Similar results were found by MacMillan and Templeton (1999) in the United Kingdom. They evaluated 400 samples of cervical mucus of unfertile women using the ELISA method and Ligase Chain Reaction (LCR) and they found a prevalence of 0% and 1.9%, respectively, for IgA antibodies anti-*C. trachomatis.* Indeed, Torrone *et al*. (2014) estimated recently that prevalence of *C. trachomatis* infection in women with ages between 14 and 39 years-old is 1.8/million in USA. This suggests that prevalence of this infection in the fertile female population of developed countries is low. On the contrary, Brito *et al*. (1998) in Venezuela, studied 174 infertile couples and found a prevalence of 14.4% in women. In Brasil, estimated prevalence in infertile women is 10.9% (Fernandes *et al*. 2014). These results indicate that prevalence of *C. trachomatis* infection is statisticaly higher in underdeveloped countries than in developed countries.

PID has an annual estimate of 1% in women in USA and it causes a higher morbility that the other infections together. In USA, about 1 million women had at least 1 episode of PID yearly, about 200 cases/1000 hospitalizations and more than 100/1000 major surgical procedures. The disease has a wide spectrum of infections, it may begin as a cervicitis, that if it is left untreated it may progress to endometritis and salpingitis. The infection is often initiated by *N. gonorrhoeae*, *C. trachomatis* or both. Polymicrobial infections with elements of the normal vaginal flora can be found in association with these two microorganisms. Bacteria can ascend from the lower part of the genital tract to the endocervix and then to the endometrium. The result is a mixed microbial infection frequently associated to intraluminal damage and destruction of epithelium that is necessary for the normal transport and nurture of gametes. Hidrosalpinx is a consequence and can interfere with reproduction since the distal end of tube becomes impermeable (Rhoton-Vlasak 2000).

Eventhough, 20% of the women with genital chlamydial infection develop PID (Price *et al*. 2013) and it is the most important preventable cause of infertility, the majority of those infections are asymptomatic (Malhotra *et al*. 2013).

Diagnose of PID can be difficult because there are many variations in the presentation of signs and symptoms particularly in the group of patients with atypical or silent PID. Considering these factors and that

only a fraction of women with TFI have a clear history of PID makes clear that this is a puzzling problem. This remarks the importance of the development of permanent tubal damage in patients with silent PID (Rhoton-Vlasak 2000).

In this study we observed that 29% (n = 11) of patients had clinical criteria of PID. Association between PID and the presence of antibodies anti-*C. trachomatis* has been found. Studies in USA showed that 10% of women between 15 and 25 years-old suffered from PID. Also, in this study 21% of patients presented antecedents of blennorrhagia at least in one opportunity. Evidences suggest that women with chlamydial PID have a risk 4 to 6 times higher to be sterile than those with gonnococcal PID or without PID (Bahamondes *et al*. 1994, Kinnunen *et al*. 2000, Rhoton-Vlasak 2000).

Vigil *et al*. (2002), studied 66 samples of cervical mucus from chilean women with antecedents of spontaneous abortion using a method of direct immuno fluorescence and demonstrated a prevalence of 21% (n = 14) for *C. trachomatis* thus establishing a relationship between *C. trachomatis* infection and this complication. Other similar investigations have estimated a prevalence of 5.4% and 8.2%. In this work 21% and 5.2% of infertile patients also showed spontaneous abortion and ectopic pregnancy as antecedents, respectively (Aliaga *et al*. 1985, Martínez *et al*. 1985, Romero *et al*. 1997). Since patients of our study had been evaluated for infertility, it is possible that previous therapy for STI have reduced the humoral response against *C. trachomatis* antigens, as described elsewhere (Cunningham 1995), and reducing the incidence of infection (Pantoja *et al*. 2012).

In spite of the method that demonstrate infection by *C trachomatis* is the culture using cellular lines, this is not commonly performed by clinical laboratories. Furthermore, PCR and LCR can detect active infection (Marrazzo *et al*. 2005). Serum detection of IgM, IgG, and even IgA, can give contradictory results due to immune systemic response not always reflects the local mucosal response in the genital tract (Mittal *et al*. 1996). Indeed, local secretory antibodies levels have been associated to common epitopes of HSP60, also present in tubal epithelium cells, even though the bacteria and/or the antigens are not present (Tiitinen *et al*. 2006).

Immunological response of prolonged defense against chlamydial HSP60 has been pointed out as responsable for tubal obstruction. However, others try to explain a possible damage of fetal tissues due to chlamydial contamination of uterus (Witkin 1999, Beagley and Timms 2000).

Secretory IgA antibodies against *C. trachomatis* have been correlated with *in vitro* fertilization failure and associated and continued presence of the microorganism in female reproductive tract. Its detection have been recommended previous to antibiotic therapy prescription to avoid unnecessary treatment and antibiotic resistance (Witkin and Linhares 2002).

To our knowledge, this is the first study on detecting local immune response to *C trachomatis* in Venezuelan infertile women with antecedents of infection, demonstrating it absence in the lower reproductive tract, probably due to previous infection therapy.

ACKNOWLEDGMENTS

To Dr. Carlos Aguilar and MSc. Maria Luisa Saatdjian for their review of the manuscript and to Dr. José R. Orta and Dr. Héctor L. Hernández for their collaboration in collecting samples.

REFERENCES

Alfieri A, Ramírez LG, Arcila N, Guevara Y. 2005. Determinación de anticuerpos contra *Chlamydia trachomatis* en pacientes del servicio de infertilidad del Centro Médico "Dr. Rafael Guerra Méndez", Valencia, Venezuela. Rev. Soc. Ven. Microbiol. 25(1):47-49.

Aliaga P, Bernal J, Martínez M, Dabancens A, Fernández P, Nazer J. 1985. Incidence of *Chlamydia trachomatis* in pregnancy. Rev. Chil. Obstet. Ginecol. 50(2):140-149.

Arraiz N, Ginestre M, Castellano M, Perozo A, Urdaneta V. 2006. Detección de *Chlamydia trachomatis* en muestras de hisopado endocervical por inmunofluorescencia directa y reacción en cadena de la polimerasa. Rev. Soc. Ven. Microbiol. 26(1):14-18.

Bahamondes L, Bueno J, Hardy E, Vera S, Pimentel E, Ramos M. 1994. Identification of main risk factors for tubal infertility. Fertil. Steril. 61(3):478-482.

Beagley KW, Timms P. 2000. *Chlamydia trachomatis* infection: incidence, health costs and prospects for vaccine development. J. Reprod. Immunol.

48(1):47-68.

Bjercke S, Purvis K. 1993. Characteristics of women under fertility investigations with IgA/IgG seropositivity for *Chlamydia trachomatis.* Eur. J. Obstet. Gynecol. Reprod. Biol. 51(2):157-161.

Brito A, Bellorín E, González I, Graffe L, Carmona O. 1998. Microorganismos identificados en parejas infértiles. Bol. Soc. Ven. Microbiol. 18(1):7-15.

Clad A, Freidanck H, Plünnecke J, Jung B, Peterson E. 1994. *Chlamydia trachomatis* species Specific Serology: ImmunoComb *Chlamydia* bivalent versus Microinmunofluorescence (MIF). Infection. 22 (3):165-173.

Cunningham DS. 1995. Immune response characteristics in women with chlamydial genital tract infection. Gynecol. Obstet. Invest. 39(1):54-59.

Dabekausen Y, Evers J, Land J, Stals F. 1994. *Chlamydia trachomatis* antibody testing is more accurate than hysterosalpingography in predicting tubal factor infertility. Fertil. Steril. 61(5):833-837.

Fernandes L, Arruda J, Approbato MS, Garcia-Zapata M. 2014. Infecção por *Chlamydia trachomatis* e *Neisseria gonorrhoeae*: fatores associados à infer. tilidade em mulheres atendidas em um serviço público de reprodução humana. Rev. Bras. Ginecol. Obstet. 36(8):353-358.

Franklin RD, Kutteh WH. 1999. Characterization of inmunoglobulings and cytokines in human cervical mucus: influence of exogenous and endogenous hormones. J. Reprod. Immunol. 42(2):93-106.

Kinnunen A, Molander P, Laurila A, Rantala I, Morrison R, Lehtinen M, Karttunen R, Tiitinen A, Paavonen J, Surcel M. 2000. *Chlamydia trachomatis* reactive T lymphocytes from upper genital tract tissue specimens. Hum. Reprod. 15(7):1484-1489.

MacMillan S, Templeton A. 1999. Screening for *Chlamydia trachomatis* in subfertile women. Hum. Reprod. 14(12):3009-3012.

Malhotra M, Sood S, Mukherjee A, Muralidhar S, Bala M. 2013. Genital *Chlamydia trachomatis*: An update. Indian J. Med. Res. 138(3):303-316.

Marrazzo JM, Johnson RE, Green TA, Stamm WE, Schachter J, Bolan G, Hook EW, Jones RB, Martin DH, Louis ME, Black CM. 2005. Impact of patient characteristics on performance of nucleic acid amplification test and DNA probe for detection of *Chlamydia trachomatis* in women with genital infections. J. Clin. Microbiol. 43(2):577-584.

Martínez M, Gubelin W, Pinto M, Ojeda J. 1985. Diagnosis of *Chlamydia trachomatis* by direct specimen test with monoclonal antibodies. Rev. Med. Chile 113(8):769-771.

Meikle S, Zhang X, Marine W, Calonge B, Hamman R, Betz G. 1994. *Chlamydia trachomatis* antibody titers and hysterosalpingography in predicting tubal disease in infertility patients. Fertil. Steril. 62(2):305-312.

Mittal A, Kapur S, Gupta S. 1996. Host immune response in chlamydial cervicitis. Br. J. Biomed. Sci. 53(3):214-220.

Pantoja M, Campos EA, Pitta DR, Gabiatti JE, Bahamondes MV, Fernandes AM. 2012. Prevalence of *Chlamydia trachomatis* infection among women candidates for in vitro fertilization at a public institution of the State of São Paulo, Brazil. Rev. Bras. Ginecol. Obstet. 34(9):425-431

Penna-Videau S, Cermeño-Vivas J, Salazar N. 2001. IgA antibodies to *Chlamydia trachomatis* and seminal parameters in asymptomatic infertile males. Arch. Androl. 46(3):189-195.

Persson K, Osser S, Birkelund S, Christiansen G, Brade H. 1999. Antibodies to *Chlamydia trachomatis* heat shock proteins in women with tubal factor infertility are associated with prior infection by *C. trachomatis* but not by *C. pneumoniae.* Hum. Reprod. 14(8):1969-1973.

Price MJ, Ades AE, De Angelis D, Welton NJ, Macleod J, Soldan K, Simms I, Turner K, Horner PJ. 2013. Risk of pelvic inflammatory disease following *Chlamydia trachomatis* infection: analysis of prospective studies with a multistate model. Am. J. Epidemiol. 178(3):484-492.

Rhoton-Vlasak A. 2000. Infections and infertility. Prim. Care Update Ob/GYNS 1:200-206.

Romero J, Prado J, Gaete V, Martínez J, Pizarro E. 1997. *Chlamydia trachomatis* infection in asymptomatic men with urethritis. Usefulness of first catch urine samples. Rev. Med. Chile. 125(10):1165-1171.

Stamm WE. 1999. *Chlamydia trachomatis* infections: progress and problems. J. Infect. Dis. 179(S2):S380-383.

Thomas K, Coughlin L, Mannion PT, Haddad NG. 2000. The value of *Chlamydia trachomatis* antibody testing as part of routine infertility investigations. Hum. Reprod. 15(5):1079-1082.

Tiitinen A, Surcel HM, Halttunen M, Birkelund S, Bloigu A, Christiansen G, Koskela P, Morrison SG, Morrison RP, Paavonen J. 2006. *Chlamydia trachomatis* and chlamydial heat shock protein 60-specific antibody and cell-mediated responses predict tubal factor infertility. Hum. Reprod. 21(6):1533-1538.

Torrone E, Papp J, Weinstock H. 2014. Prevalence of *Chlamydia trachomatis* genital infection among persons aged 14-39 years-United States, 2007-2012. MMWR Morb Mortal Wkly Rep. 63(38):834-838.

Vigil P, Tapia S, Zacharias S, Riquelme R, Salgado A, Varleta J. 2002. First-trimester pregnancy loss and active *Chlamydia trachomatis* infection: correlation and ultraestructural evidence. Andrologia. 34(6):373-378.

Witkin S. 1999. Immunity to heat shock proteins and pregnancy outcome. Infect. Dis. Obstet. Gynecol. 7(1-2):35-38.

Witkin S, Linhares I. 2002. *Chlamydia trachomatis* in subfertile women undergoing uterine instrumentation, an alternative to direct microbial testing or prophylactic antibiotic treatment. Hum. Reprod. 88(8):1938-1941.

WHO (World Health Organization). 1999. WHO laboratory Manual for the examination of human semen and sperm-cervical mucus interaction. Cambridge University Press. 4th ed. Cambridge, England, p. 59.