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► To cite this version:

Clara Gadenne, Laura Miquel, Cindy Faust, Julie Berbis, Jeanne Perrin, et al.. Impact of a positive *Chlamydia trachomatis* serology on cumulative IVF live birth rate. *Reproductive BioMedicine Online*, 2024, 48 (2), pp.103586. 10.1016/j.rbmo.2023.103586 . hal-04412334

HAL Id: hal-04412334

<https://amu.hal.science/hal-04412334v1>

Submitted on 9 Apr 2024

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Impact of a positive *Chlamydia trachomatis* serology on cumulative IVF live birth rate

Clara Gadenne^{a,*}, Laura Miquel^a, Cindy Faust^b, Julie Berbis^b, Jeanne Perrin^{a,c}, Blandine Courbiere^{a,c}

KEY MESSAGE

There are few data on the impact of *Chlamydia trachomatis* serology on IVF outcomes, although serology is often performed in reproductive medicine centres. In this matched cohort study, there was no impact on IVF results, which raises the question of the value of systematic screening for *Chlamydia* before IVF.

ABSTRACT

Research question: Does positive *Chlamydia trachomatis* serology have an impact on the cumulative live birth rate from IVF?

Design: A retrospective matched cohort study compared women with positive *Chlamydia trachomatis* serology (group A) who underwent IVF treatment between January 2016 and December 2021 with a control group of women with negative *Chlamydia trachomatis* serology (group B). The main outcome measures were the cumulative live birth rate per IVF cycle and the live birth rate per embryo transfer. Secondary outcomes were the cumulative rates of clinical pregnancy, ectopic pregnancy and pregnancy loss calculated per IVF cycle and per embryo transfer.

Results: A total of 151 women in group A were matched 1:2 to 302 women in group B, representing 220 and 440 IVF cycles, respectively. Women with a history of *Chlamydia trachomatis* infection had a significantly higher rate of tubal obstruction ($P < 0.001$), excluded or operated hydrosalpinx ($P = 0.002$) and/or history of chronic endometritis ($P < 0.001$). There were no statistically significant differences between the two groups in the mean number of mature oocytes retrieved, fertilization rate or implantation rate. The IVF cumulative live birth rate per cycle was similar in the two groups (36.7% in group A versus 34.9% in group B, $P = 0.692$). The cumulative rates of clinical pregnancy, pregnancy loss, biochemical pregnancy and ectopic pregnancy were comparable between the two groups.

Conclusion: Positive *Chlamydia trachomatis* serology has no impact on IVF pregnancy outcomes.

INTRODUCTION

Chlamydia trachomatis is an intracellular bacterium and represents the most widespread bacterial sexually

transmitted infection. *Chlamydia trachomatis* infections especially affect young women, with an estimated 129 million new *Chlamydia* infections worldwide in 2020 ([Sexually Transmitted Infections \(STIs\)](#), *n.d.*).

At least 70% of *C. trachomatis* infections of the female genital tract are asymptomatic ([Facts about Chlamydia](#), *n.d.*), and are therefore often undiagnosed or untreated, leading to transmission of the infection to sexual partners and increasing

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KEYWORDS

Chlamydia trachomatis
Cumulative live birth rate
IVF
Pregnancy outcome

the risk of chronic infection. *Chlamydia trachomatis* can ascend into the upper genital tract and lead to a pelvic inflammatory disease (PID) (Haggerty et al., 2010). After antibiotic treatment, upper genital infection may have no clinical consequence. Otherwise, long-term sequelae from PID include irreversible tubal damage, which can lead to tubal factor infertility (Land et al., 2010) with an increased risk of ectopic pregnancy (Egger et al., 1998). Previous studies have reported that positive *C. trachomatis* serology was associated with a significantly lower rate of naturally conceived pregnancy (Coppus et al., 2011; Ness et al., 2008) and an increased risk of miscarriage (Baud et al., 2011).

The impact of *C. trachomatis* infection can be explained by the pathogenesis of the disease. Under certain conditions, *C. trachomatis* can enter into a persistent state, representing the chronic phase of the infection in which production of the stress protein Chlamydia heat shock protein 60kDa (CHSP60) becomes up-regulated (Stephens et al., 2011; Witkin et al., 2017). When released from infected cells, CHSP60 can induce a local pro-inflammatory immune response, especially in the Fallopian tube epithelium, that leads to tissue damage, including fibrosis and luminal occlusion (Hafner, 2015). In cases of tubal obstruction after *C. trachomatis* infection, the strategy for managing infertility is based on IVF.

Studies investigating the impact of *C. trachomatis* infection on IVF outcomes have shown conflicting results. Some studies have reported no association between positive *C. trachomatis* serology and IVF pregnancy rates (Claman et al., 1996; Osser et al., 1990; Spandorfer et al., 1999). Others, however, have observed that the presence of anti-*C. trachomatis* antibodies, through a chronic inflammatory process, could lead to endometrial damage resulting in an impaired implantation rate (Jakus et al., 2008) and lower IVF pregnancy rate (Lunenfeld et al., 1989; Witkin et al., 1994). Licciardi and colleagues reported an increased miscarriage rate after IVF in women with positive *C. trachomatis* serology (Licciardi et al., 1992). Keay and co-workers reported a significantly higher prevalence of *C. trachomatis* antibodies in the serum of women with a poor ovarian response (Keay et al., 1998). In addition, Pacchiaroti and colleagues observed a significantly reduced number of mature

oocytes retrieved from women in whom antichlamydial antibodies were detected in the serum and follicular fluid (Pacchiarotti et al., 2009).

With regard to the current literature, the impact of positive *C. trachomatis* serology on IVF pregnancy rates is still unclear. The objective of the study was therefore to evaluate the IVF cumulative live birth rate in women with positive *C. trachomatis* serology, compared with a control population of women with negative *C. trachomatis* serology.

MATERIALS AND METHODS

Study population

A single-centre retrospective observational matched cohort study was conducted between January 2016 and December 2021 at the Department of Gynecology, Obstetric and Reproductive Medicine of a university teaching hospital. January 2016 marked the start of routine screening for *C. trachomatis* serology for all the infertile women in the hospital's assisted reproductive technology (ART) unit.

Patients were divided into two groups: group A included women with positive *C. trachomatis* serology, and group B included women with negative *C. trachomatis* serology. The inclusion criteria were as follows: women aged 18–43 years who had undergone at least one IVF or intracytoplasmic sperm injection (ICSI) cycle during the study period in order to subsequently transfer a fresh or frozen-thawed embryo, and for whom *C. trachomatis* serology was documented. To limit bias and factors that could impact the live birth rate, the exclusion criteria were as follows: a diagnosis of endometriosis stage III or IV according to the revised American Fertility Society classification ("Revised American Society for Reproductive Medicine Classification of Endometriosis," 1997), involvement in an oocyte donation programme, embryo adoption and missing data for matching criteria.

Each *C. trachomatis*-positive woman was matched 1:2 to two women with negative *C. trachomatis* serology with regard to the following matching criteria: age, anti-Müllerian hormone (AMH) concentration, body mass index (BMI), tobacco consumption, the rank of the IVF cycle and the ART technique (IVF or ICSI). Regarding age matching, women were classified into

four categories: less than 30 years old, 30–35 years old, 36–40 years old and over 40 years old. For the AMH concentration, women were matched according to the following categories: less than 2 ng/ml, 2–5 ng/ml and more than 5 ng/ml. Two categories of BMI were defined: BMI below 30 kg/m² or obesity, with a BMI \geq 30 kg/m². For women who consumed tobacco, those who smoked and those who did not were distinguished. Each woman in group B had to have had the same number of ovarian stimulation cycles resulting in oocyte retrieval as the woman in group A to whom they were matched.

Chlamydia trachomatis detection

In this study, *C. trachomatis* detection was decided in accordance with the results of *Chlamydia* immunoglobulin G (IgG) antibody testing of the serum. Among the different techniques available (micro-immunofluorescence, enzyme-linked immunosorbent assay [ELISA] and indirect immunofluorescence), the most commonly used in the laboratories was the ELISA immunoassay technique. For *C. trachomatis*-positive women, a vaginal swab for direct detection of the bacteria using the polymerase chain reaction was systematically performed, and appropriate antibiotic treatment was administered to infected women.

Chronic endometritis

Endometrial biopsy for microbiological assay was performed in three situations: before hydrosalpinx surgery, after recurrent pregnancy loss (RPL) or after repeated implantation failure (RIF). RIF was defined according to Coughlan and collaborators by implantation failure after the transfer of at least four good-quality embryos in women younger than 40 years of age (Coughlan et al., 2014). As described by Bouet and colleagues, chronic endometritis was diagnosed if five or more plasma cells were observed on 10 non-overlapping high-power fields (\times 400 magnification) in the endometrial tissue, with histological confirmation using immunohistochemistry stains for syndecan-1 (CD138) (Bouet et al., 2016).

If chronic endometritis was diagnosed, empirical bi-antibiotic therapy was systematically administered before IVF using doxycycline (100 mg twice daily) and metronidazole (500 mg twice daily) for 14 days. In our center, a second biopsy is systematically performed in the next cycle after treatment to confirm resolution of

the chronic endometritis. If the second sample was still positive for chronic endometritis, a second line of antibiotics was used, with ofloxacin (400 mg twice daily) and metronidazole (500 mg twice daily) for 14 days, according to French recommendations on PID (Verdon, 2019).

IVF protocol

Women underwent ovarian stimulation with exogenous recombinant FSH or human menopausal gonadotrophin. Daily doses were individualized in accordance with the woman's age, BMI, AMH values and ovarian response to previous stimulation. The usual protocols were performed, using analogues of gonadotrophin-releasing hormone (GnRH): the fixed antagonist protocol with daily GnRH antagonist injection from the fifth day of stimulation, the long protocol with GnRH agonist administration in the luteal phase of the previous cycle, and the short protocol with daily GnRH agonist injection from the first day of the IVF cycle.

Women routinely underwent serial transvaginal ultrasonography, starting on day 8 of ovarian stimulation, and serum measurements of oestradiol, LH and progesterone were made. When at least three follicles reached a mean diameter of 17 mm, ovulation was triggered by injection of a pre-filled ampoule of 250 µg of recombinant human chorionic gonadotrophin (HCG; choriogonadotropin alfa) or 0.3 mg of subcutaneous triptorelin, or both for dual triggering. Oocyte retrieval was scheduled for 36 h after triggering of ovulation under local or general anaesthesia, at the woman's discretion, considering the authors' previous study (Rolland et al., 2017).

The retrieved oocytes were fertilized by conventional IVF or ICSI. ICSI was performed in cases of male infertility, according to the sperm characteristics. After fertilization, one or two embryos were transferred under ultrasound guidance at 48 h (day 2 embryo), at 72 h (day 3 embryo) or at the blastocyst stage (day 5 embryo), depending on the biological characteristics and quality of the embryos, the woman's age, the gynaecological surgical and obstetric history and the rank of the attempt. Supernumerary embryos were vitrified for subsequent embryo transfer.

For luteal phase support after fresh embryo transfer, oral dydrogesterone

(30 mg per day) was given from the day of oocyte retrieval to the pregnancy test. For the frozen-thawed embryo transfer (FET) regimens, FET was performed after a modified natural cycle or after HCG-triggered ovulation in cases of dysovulation or anovulation. Luteal phase support after FET was performed with intravaginal natural progesterone (400 mg twice a day). No changes were made to the luteal phase support protocol over the duration of the study.

Study end-points

The primary end-points were the cumulative live birth rate per IVF/ICSI cycle (including the first live birth per woman) and the live birth rate per embryo transfer. Live birth was defined as the birth of a live infant after 22 completed weeks of gestational age, according to the International Glossary on Infertility and Fertility Care (Zegers-Hochschild et al., 2017). A twin birth was equivalent to two live births. Secondary outcomes were the cumulative rates of clinical pregnancy, ectopic pregnancy and pregnancy loss per cycle as well as per embryo transfer, the implantation rate (corresponding to the number of gestational sacs observed divided by the number of embryos transferred), the number of mature oocytes retrieved per cycle, the fertilization rate (corresponding to the number of diploid embryos obtained divided by the number of mature oocytes retrieved) and the rate of chronic endometritis.

A clinical pregnancy was defined by the visualization of an embryo with a positive fetal heartbeat on pelvic ultrasound performed from 6 weeks of gestation. Pregnancy losses included biochemical pregnancies (corresponding to a pregnancy diagnosed only by the detection of HCG in the serum (Zegers-Hochschild et al., 2017), early spontaneous miscarriages before 14 weeks, late miscarriages between 14 and 22 weeks and ectopic pregnancies.

Statistical analysis

All analyses were performed using IBM SPSS Statistics 20 software (SPSS Inc., USA). Categorical variables were presented as numbers and percentages. Quantitative variables were presented as means ± standard deviations for continuous variables. To compare categorical variables, a Pearson's chi-squared test or Fisher's exact test was used as appropriate, and quantitative variables

were compared using a Student's t-test or Mann–Whitney U-test. All tests were bilateral, and for all analyses a P-value <0.05 was considered statistically significant.

Ethics statement

In this retrospective study, informed consent to the use of their data was obtained from all the individual participants included. This study was approved by the local Ethical Committee of the university on 11 July 2022 (reference 2022-39).

RESULTS

During the study period, 1512 women underwent at least one IVF or ICSI cycle at the Reproductive Medicine Center and 1141 women were eligible for the study after the inclusion and exclusion criteria had been applied. Of these, 151 women with positive *C. trachomatis* serology were included in group A and matched 1:2 to 302 women with negative *C. trachomatis* serology in group B, representing 220 and 440 stimulation cycles, respectively (FIGURE 1).

The baseline and clinical characteristics of the women and their partners are presented in TABLE 1. No significant differences were found between the two groups of women in terms of age, smoking status, BMI, AMH concentrations and basal FSH and oestradiol concentrations. The baseline characteristics of the male partners did not differ between the two groups. The rate of secondary infertility of the women in group A was higher than those in group B (53.6% versus 40.7%, $P = 0.009$) and the duration of infertility was longer in group A. Women with positive *C. trachomatis* serology had significantly higher rates of tubal obstruction ($P < 0.001$) and excluded or operated hydrosalpinx ($P = 0.002$). The rate of chronic endometritis was significantly higher in group A, with 18 positive biopsies out of 28 performed (64.3%), while only 11 biopsies were positive out of 47 in group B (23.4%) ($P < 0.001$).

The characteristics of the IVF/ICSI cycles are presented in TABLE 2, with no significant differences concerning the total progressively motile sperm count in the ejaculate, the total dose of gonadotrophins, the mean duration of stimulation, the oestradiol concentration

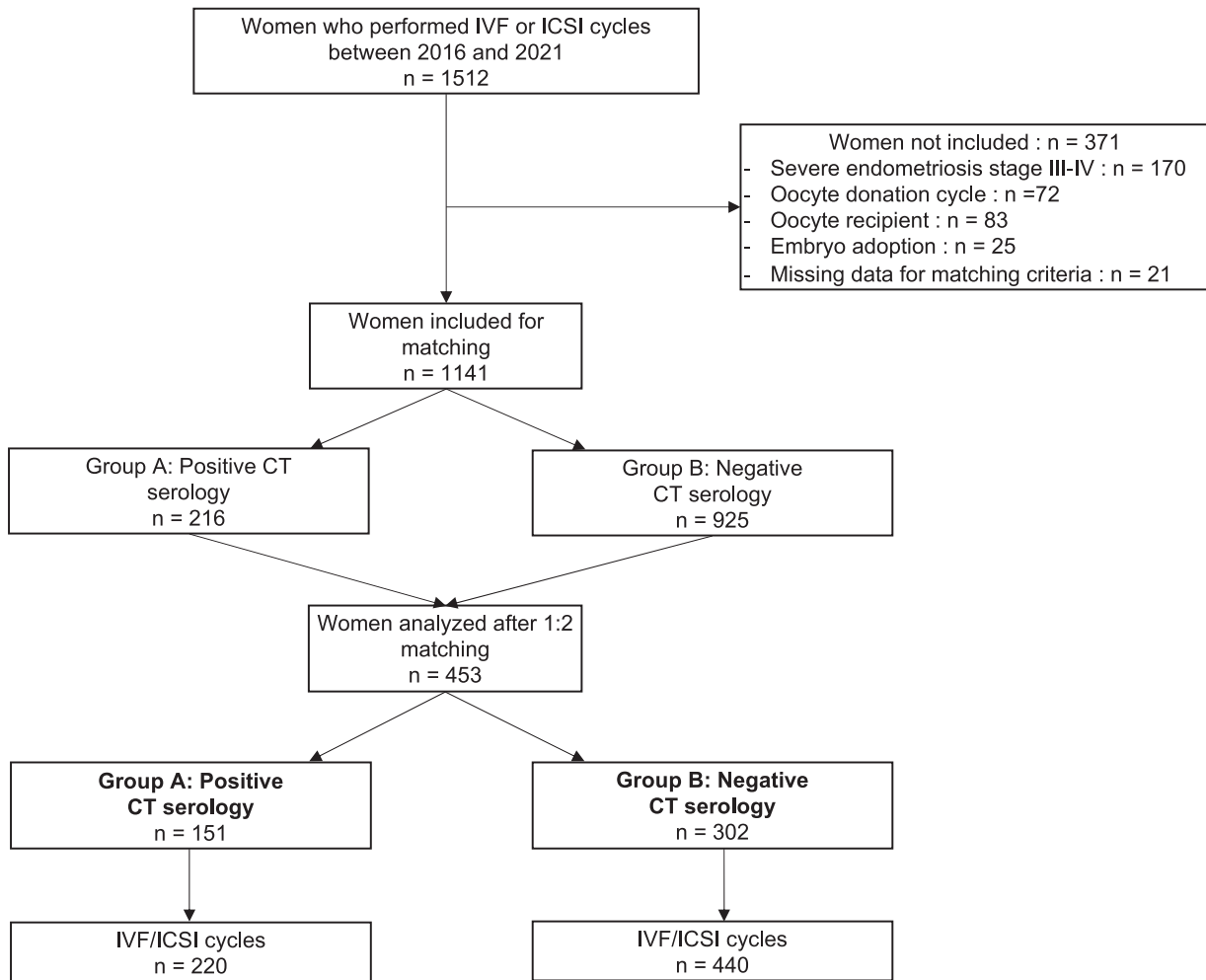


FIGURE 1 Flow chart showing the selection of the study cohort. CT, Chlamydia trachomatis; ICSI, intracytoplasmic sperm injection.

on the day of triggering, the type of stimulation protocol and the triggering mode. There were no statistically significant differences between the two groups in the mean number of mature oocytes retrieved, the fertilization rate, the rate of ovarian hyperstimulation syndrome and the transfer cancellation rate. There were several reasons for cancelling a transfer: no oocyte retrieved, no good quality embryo, inadequate progesterone, premature ovulation, an insufficient or excessive ovarian response to stimulation, inadequate endometrium and ovarian hyperstimulation syndrome, as well as treatment error, intercurrent illness or personal problems.

The mean number of diploid embryos and transferred embryos per cycle was not statistically different. Taking into account all the transferred embryos, significantly more day 5 embryos were transferred per cycle in group A (22.8 %, $n = 41$) than in

group B (10.5 %, $n = 39$) ($P < 0.001$). A subgroup analysis was performed for the mean implantation rate per cycle of the day 2–3 embryos (17.5% in group A versus 22.1% in group B, $P = 0.469$) and of the day 5 embryos (44.6% in group A versus 60.4% in group B, $P = 0.567$): no significant difference was found between the two groups.

The IVF outcomes are presented in [TABLE 3](#). No significant difference was found between the two groups with regard to the cumulative live birth rate per IVF cycle: 36.7% in group A versus 34.9% in group B ($P = 0.692$) and the live birth rate per embryo transfer. The outcomes of the two groups were similar in terms of the cumulative clinical pregnancy rates per IVF cycle and clinical pregnancy rates per transfer. An analysis was performed in women with a positive HCG result 14 days after embryo transfer in order to calculate the ectopic pregnancy, pregnancy loss and

biochemical pregnancy rates, with no significant difference between the two groups. The groups were comparable in terms of twin pregnancy rates: 3.3% ($n = 6/180$) in group A and 6.2% ($n = 23/372$) in group B ($P = 0.160$).

DISCUSSION

These results suggest that the *C. trachomatis* serology status has no impact on IVF pregnancy outcomes. In other words, in this study, a history of *C. trachomatis* infection had no adverse IVF outcomes once hydrosalpinx had been excluded and chronic endometritis had been treated. The results are consistent with the study of Claman and colleagues in which 118 women with positive *C. trachomatis* serology were compared with 77 women with negative *C. trachomatis* serology. No significant difference was observed in the

TABLE 1 BASELINE AND CLINICAL CHARACTERISTICS OF THE STUDY POPULATION ACCORDING TO *CHLAMYDIA TRACHOMATIS* SEROLOGY

Characteristic	Group A Positive CT serology (n = 151)	Group B Negative CT serology (n = 302)	P-value
Characteristics of the female partner			
Age (years)	33.7 ± 4.6	33.8 ± 4.4	0.716
Tobacco smoker	28 (18.5)	56 (18.5)	1.000
BMI (kg/m ²)	24.0 ± 4.2	23.8 ± 4.5	0.463
FSH (IU/ml)	7.3 ± 2.5	7.1 ± 2.2	0.260
Oestradiol (pg/ml)	49.9 ± 56.6	51 ± 37.2	0.763
AMH (ng/ml)	2.9 ± 3.8	2.8 ± 2.8	0.891
Characteristics of the male partner			
Age (years)	36.3 ± 5.8	37.2 ± 6.8	0.072
Tobacco smoker	56 (37.1)	108 (35.8)	0.782
BMI (kg/m ²)	26.0 ± 3.9	25.2 ± 6.0	0.117
Type of infertility			
Couples with secondary infertility	43 (28.5)	86 (28.5)	1.000
Women with secondary infertility	81 (53.6)	123 (40.7)	0.009
Duration of infertility (years)	4.5 ± 2.8	3.9 ± 2.4	0.024
Aetiology of infertility			
Diminished ovarian reserve	46 (30.5)	89 (29.5)	0.828
Ovulatory	29 (19.2)	46 (15.2)	0.283
Tubal	73 (48.3)	66 (21.9)	<0.001
Male factor	83 (55.0)	164 (54.3)	0.894
Idiopathic	8 (5.3)	47 (15.6)	0.002

Data are expressed as mean ± standard deviation or n (%).

AMH, anti-Müllerian hormone; BMI, body mass index; CT, *Chlamydia trachomatis*.

pregnancy rates per cycle: 23% in the serology-positive group versus 13% in the serology-negative group ($P = 0.125$) (Claman et al., 1996).

Zhang and co-workers' study showed similar results, although they used a cervical swab rather than serology to detect *C. trachomatis*. Zhang and co-workers performed a comparative study between women with a positive *C. trachomatis* cervical swab (treated with appropriate antibiotic therapy) and a control group of women with a negative cervical swab. One of the exclusion criteria was the presence of hydrosalpinx. No significant difference was found between the two groups ('Chlamydia trachomatis infection group' versus 'no infection group') in terms of the clinical pregnancy rate (39.5% versus 47.0%, $P = 0.152$), live birth rate (30.2% versus 40.1%, $P = 0.053$), ectopic pregnancy rate (2.0% versus 1.3%, $P = 0.709$) and spontaneous abortion rate (21.6% versus 12.7%, $P = 0.127$) (Zhang et al., 2022).

Regarding the number of mature oocytes retrieved and the fertilization rate, the results of the current study are consistent with those of Jakus and collaborators who conducted a double-blind case-control study of 253 women who underwent IVF, with assay of IgG antibodies to CHSP60 in follicular fluid. CHSP60 antibody detection, which represents the serological scar indicative of a persistent *C. trachomatis* infection, was not related to the number of oocytes collected or the fertilization rate (Jakus et al., 2008).

The current study showed a higher rate of chronic endometritis in women with a history of *C. trachomatis* infection. *Chlamydia trachomatis* infection can lead to local inflammation throughout the genital tract, resulting in a pro-inflammatory immune response and thus an alteration in the distribution of lymphocytes involved in embryo implantation (Park et al., 2016). Numerous studies have found a high prevalence of chronic endometritis in women with RIF

and RPL (Bouet et al., 2016; Kimura et al., 2019; Kitaya et al., 2018). In the current study, despite a higher rate of chronic endometritis in the group with positive *C. trachomatis* serology, the implantation and live birth rates were not decreased. This may be related to the systematic treatment of chronic endometritis at our center with appropriate antibiotic therapy, with a histological confirmation of cure.

The subject of chronic endometritis is controversial as there is no standardized definition and the value of antibiotic treatment remains debated, with no randomized controlled trial on this currently available. Some studies have demonstrated that women with cured chronic endometritis show higher live birth and pregnancy rates than women with persistent disease (Cicinelli et al., 2015; Vitagliano et al., 2018). On the other hand, in the study of Herlihy and colleagues, no difference was observed between women with and without chronic endometritis in terms of the rates of implantation, clinical pregnancy, pregnancy loss or live birth after the transfer of a single euploid embryo (Herlihy et al., 2022). Given the divergent outcomes between studies and the potential benefit of antibiotics, it seemed unethical in the current study to withhold treatment once chronic endometritis had been diagnosed. As chronic endometritis was not left untreated the results of this study are valid in relation to situations in which the treatment of chronic endometritis is undertaken.

The women who were followed up at our reproductive medicine centre had *C. trachomatis* serology performed at the laboratory of their choosing. However, the *C. trachomatis* detection technique can be different depending on the laboratory involved. The ELISA technique is the one most commonly employed, usually using IgG antibodies directed against the major outer membrane protein present on the *C. trachomatis* cell surface, which contains species- and serovar-specific epitopes. Therefore, there is interlaboratory variability, in addition to the fact that the choice of the threshold of positivity differs from one laboratory to another.

The interpretation of *C. trachomatis* serology is also difficult: as most infections are asymptomatic, the discovery of positive *C. trachomatis* serology may occur several months or years after the infection. Several

TABLE 2 CHARACTERISTICS OF THE OVARIAN STIMULATION CYCLES IN RELATION TO CHLAMYDIA TRACHOMATIS SEROLOGY

Characteristic	Group A Positive CT serology (n = 220 cycles)	Group B Negative CT serology (n = 440 cycles)	P-value
ART technique			
IVF	128 (58.2)	257 (58.4)	
ICSI	92 (41.8)	183 (41.6)	0.955
Semen parameter: total progressively motile sperm count in ejaculate (million)	43.5 ± 48.9	43.8 ± 51.9	0.944
Characteristics of the cycle			
Total gonadotrophin dose (IU)	2893.3 ± 1290.6	2802.6 ± 1339.5	0.407
Duration of stimulation (days)	10.8 ± 1.9	10.7 ± 1.9	0.465
Oestradiol levels on the day HCG triggering (pg/ml)	2208.8 ± 1435.7	2298.9 ± 1552	0.471
Freeze-all cycle	67 (30.5)	128 (29.1)	0.717
Ovarian stimulation protocol			
Fixed antagonist protocol	155 (70.5)	331 (75.2)	
Long protocol	60 (27.3)	95 (21.6)	
Short protocol	5 (2.3)	14 (3.2)	0.405
Mode of triggering ovulation			
Chorionadotropin alfa	143 (65)	295 (67)	
Triptorelin	15 (6.8)	31 (7)	
Dual trigger	62 (28.2)	114 (25.9)	0.824
Characteristics of oocyte retrieval			
Number of mature oocytes	7.3 ± 4.8	7.0 ± 4.8	0.449
Retrieval with no oocyte	7 (3.2)	13 (3.0)	0.872
Ovarian hyperstimulation syndrome rate	22 (10)	45 (10.2)	0.927
Characteristics of the embryos per cycle			
Number of diploid embryos	2.46 ± 2.1	2.27 ± 1.7	0.228
Number of transferred embryos	1.76 ± 1.3	1.75 ± 1.2	0.858
Number of vitrified embryos	1.37 ± 2.2	1.09 ± 1.9	0.107
Characteristics of embryo transfers per participant			
Number of embryos transferred	1.76 ± 1.3	1.75 ± 1.2	0.858
Number of transfers	1.20 ± 0.9	1.13 ± 0.8	0.349
Transfer cancellation rate	41 (18.6)	62 (14.1)	0.129

Data are expressed as mean ± standard deviation or n (%).

CT, *Chlamydia trachomatis*; HCG, human chorionic gonadotrophin; ICSI, intracytoplasmic sperm injection, .

factors affect the magnitude of the IgG response and the ability of serological assays to detect a previous *C. trachomatis* infection; these include the technique used, the target antibody, the time since infection, the number of previous *C. trachomatis* infections and patient characteristics such as age, sex and ethnicity (Woodhall et al., 2018). The concentration of IgG antibodies against *C. trachomatis* does not define the age of the infection as *Chlamydia trachomatis* serology can remain positive for years

(Horner et al., 2016). In the current study, if the *C. trachomatis* polymerase chain reaction result based on the vaginal swab came back negative, it was not possible to consider the duration of *C. trachomatis* infection and its possible impact on local inflammation and endometrial receptivity.

Chlamydia trachomatis serology does not on its own have a sufficient positive predictive value or negative predictive value to confirm or rule out tubal pathology (Muvunyi et al., 2012; Raoufi

et al., 2013). In order to increase the specificity of the test, studies have investigated antibodies directed against proteins involved in inflammation. Clinical and experimental data have shown the role of antibodies against CHSP60, secreted during the persistent state, in the chronic inflammatory process, which is involved in the immunopathogenesis of tubal factor infertility (Land and Evers, 2002; Tiitinen et al., 2006). CHSP60 antibody testing appears to be more specific than *C. trachomatis* antibody testing for predicting tubal infertility but does not currently have sufficient diagnostic sensitivity (Berestoviy et al., 2021; Rantsi et al., 2018).

The first and most important limitation of this study is its retrospective and monocentric design, but a randomized controlled trial is not possible in this case. Another weakness of the study is related to the lack of data on the treatment received by women with a history of *C. trachomatis* infection. The study is the first to have included a large number of women over a long study period in a reproductive medicine centre with a systematic *C. trachomatis* screening protocol for all women.

The greatest strength of the study is its matching of the two groups on factors that may have an impact on the implantation rate. Multiple matching criteria were employed – the woman's age, BMI, smoking status and AMH concentration and the ART technique used – as these factors have an impact on the study end-points. However, even though a homogeneous study sample was obtained, the number of these matching criteria had the inconvenience of significantly reducing the number of women included in the study. Compared with previous studies, the current investigation tightly controlled the possible confounders between the two groups studied. Women with stage III or IV endometriosis were not included as endometriosis could have an impact on the number of oocytes retrieved, the fertilization rate and embryo implantation (Barnhart et al., 2002; Horton et al., 2019). In order to analyse oocyte quality and fertilization rate, women who had undergone oocyte donation or embryo adoption were not included. To compare the two groups it was also necessary to match the rank of the IVF cycles performed.

The study results suggest that positive *C. trachomatis* serology has no impact on

TABLE 3 OUTCOMES PER IVF CYCLE IN RELATION TO CHLAMYDIA TRACHOMATIS SEROLOGY

Outcome	Group A ^a Positive CT serology (n = 180)	Group B ^b Negative CT serology (n = 372)	P-value
Cumulative live birth rate	66 (36.7)	130 (34.9)	0.692
Cumulative clinical pregnancy rate	76 (42.2) ^c	166 (44.6) ^d	0.594
Cumulative pregnancy loss rate	21/86 (24.4) ^e	56/188 (29.8) ^e	0.388
Cumulative biochemical pregnancy rate	8/86 (9.3)	17/188 (9)	1.000
Cumulative ectopic pregnancy rate	2/86 (2.3)	2/188 (1.1)	0.592
Cumulative early spontaneous miscarriage rate	9/86 (10.5)	34/188 (18.1)	0.151
Cumulative late miscarriage rate	2/86 (2.3)	3/188 (1.6)	0.651
Vanishing twin syndrome rate	4/86 (4.7)	5/188 (2.7)	0.468

Data are expressed as n (%). Only IVF cycles resulting in at least one embryo transfer were included.

^a n = 151 women were included in group A.

^b n = 302 women were included in group B.

^c n = 6 pairs of twins, including 8 individual live births.

^d n = 23 pairs of twins, including 32 individual live births.

^e The number represents women with a positive human chorionic gonadotrophin level 14 days after embryo transfer. CT, *Chlamydia trachomatis*.

pregnancy outcomes after IVF. It can be supposed that there would be no impact of any history of upper genital *C. trachomatis* infection on the IVF pregnancy outcome as hydrosalpinx and chronic endometritis were treated before IVF. In addition to the fact that a positive *C. trachomatis* serology result cannot determine the age of the infection, or confirm tubal pathology on its own, the findings of this study raise the question of the value of routine *C. trachomatis* serology testing. The results must, however, be analysed with caution, given the retrospective nature of the study and the fact that not all the women were systematically tested for chronic endometritis. These data could help to reassure women with a history of *C. trachomatis* infection, but a complementary prospective study with a homogeneous microbiological

assessment of *C. trachomatis* antibodies would be useful to validate the results.

DATA AVAILABILITY

Data will be made available on request.

AUTHOR CONTRIBUTIONS

This study was conceived and conducted by physicians specialized in assisted reproduction techniques at a university teaching hospital. B.C. and C.G. designed the study and drafted the manuscript. J.P. and L.M. participated in the development and improvement of the study. J.B. and C.F. performed the matching between the cases and controls, and the statistical analysis. The manuscript has been read and approved by all the named authors.

REFERENCES

- Barnhart, K., Dunsmoor-Su, R., Coutifaris, C., 2002. Effect of endometriosis on in vitro fertilization. *Fertil. Steril.* 77 (6), 1148–1155. [https://doi.org/10.1016/s0015-0282\(02\)03112-6](https://doi.org/10.1016/s0015-0282(02)03112-6).
- Baud, D., Goy, G., Jaton, K., Osterheld, M.-C., Blumer, S., Borel, N., Vial, Y., Hohlfeld, P., Pospischil, A., Greub, G., 2011. Role of *Chlamydia trachomatis* in miscarriage. *Emerg. Infect. Dis.* 17 (9), 1630–1635. <https://doi.org/10.3201/eid1709.100865>.
- Berestoviy, V.O., Sokol, I.V., Mahmood, A.A., Ginzburg, V.G., Govsieiev, D.O., 2021. A prospective study to analyze the specificity of *Chlamydial Heat Shock Protein (CHSP60) antibodies to diagnose tubal infertility*. *Wiadomosci Lekarskie (Warsaw, Poland: 1960)* 74 (2), 184–189.
- Bouet, P.-E., El Hachem, H., Monceau, E., Gariépy, G., Kadoch, I.-J., Sylvestre, C., 2016. Chronic endometritis in women with recurrent pregnancy loss and recurrent implantation failure: Prevalence and role of office hysteroscopy and immunohistochemistry in diagnosis. *Fertil. Steril.* 105 (1), 106–110. <https://doi.org/10.1016/j.fertnstert.2015.09.025>.
- Cicinelli, E., Matteo, M., Tinelli, R., Lepera, A., Alfonso, R., Indraccolo, U., Marrocchella, S., Greco, P., Resta, L., 2015. Prevalence of chronic endometritis in repeated unexplained implantation failure and the IVF success rate after antibiotic therapy. *Hum. Reprod. (Oxford, England)* 30 (2), 323–330. <https://doi.org/10.1093/humrep/deu292>.
- Claman, P., Amimi, M.N., Peeling, R.W., Toye, B., Jessamine, P., 1996. Does serologic evidence of remote *Chlamydia trachomatis* infection and its heat shock protein (CHSP 60) affect in vitro fertilization-embryo transfer outcome? *Fertil. Steril.* 65 (1), 146–149. [https://doi.org/10.1016/s0015-0282\(16\)58042-x](https://doi.org/10.1016/s0015-0282(16)58042-x).
- Coppus, S.F.P.J., Land, J.A., Opmeer, B.C., Steures, P., Eijkemans, M.J.C., Hompes, P.G.A., Bossuyt, P.M.M., van der Veen, F., Mol, B.W.J., van der Steeg, J.W., 2011. *Chlamydia trachomatis* IgG seropositivity is associated with lower natural conception rates in ovulatory subfertile women without visible tubal pathology. *Hum. Reprod. (Oxford, England)* 26 (11), 3061–3067. <https://doi.org/10.1093/humrep/der307>.
- Coughlan, C., Ledger, W., Wang, Q., Liu, F., Demiroglu, A., Gurgan, T., Cutting, R., Ong, K., Sallam, H., Li, T.C., 2014. Recurrent implantation failure: Definition and management. *Reprod. Biomed. Online* 28 (1), 14–38. <https://doi.org/10.1016/j.rbmo.2013.08.011>.
- Egger, M., Low, N., Smith, G.D., Lindblom, B., Herrmann, B., 1998. Screening for chlamydial infections and the risk of ectopic pregnancy in a county in Sweden: Ecolog. anal. *BMJ (Clinical Research Ed.)* 316 (7147), 1776–1780. <https://doi.org/10.1136/bmj.316.7147.1776>.
- Facts about *chlamydia*. (n.d.). European Centre for Disease Prevention and Control. Retrieved August 6, 2022, from <https://www.ecdc.europa.eu/en/chlamydia/facts>
- Hafner, L.M., 2015. Pathogenesis of fallopian tube damage caused by *Chlamydia trachomatis* infections. *Contraception* 92 (2), 108–115. <https://doi.org/10.1016/j.contraception.2015.01.004>.
- Haggerty, C.L., Gottlieb, S.L., Taylor, B.D., Low, N., Xu, F., Ness, R.B., 2010. Risk of sequelae after *Chlamydia trachomatis* genital infection in

- women. *J. Infect. Dis.* 201 (Suppl 2), S134–S155. <https://doi.org/10.1086/652395>.
- Herlihy, N.S., Klimczak, A.M., Titus, S., Scott, C., Hanson, B.M., Kim, J.K., Seli, E., Scott, R.T., 2022. The role of endometrial staining for CD138 as a marker of chronic endometritis in predicting live birth. *J. Assist. Reprod. Genet.* 39 (2), 473–479. <https://doi.org/10.1007/s10815-021-02374-z>.
- Horner, P.J., Wills, G.S., Righarts, A., Vieira, S., Kounali, D., Samuel, D., Winston, A., Muir, D., Dickson, N.P., McClure, M.O., 2016. Chlamydia trachomatis Pgp3 Antibody Persists and Correlates with Self-Reported Infection and Behavioural Risks in a Blinded Cohort Study. *PloS One* 11 (3), e0151497. <https://doi.org/10.1371/journal.pone.0151497>.
- Horton, J., Sterrenburg, M., Lane, S., Maheshwari, A., Li, T.C., Cheong, Y., 2019. Reproductive, obstetric, and perinatal outcomes of women with adenomyosis and endometriosis: A systematic review and meta-analysis. *Hum. Reprod. Update* 25 (5), 592–632. <https://doi.org/10.1093/humupd/dmz012>.
- Jakus, S., Neuer, A., Dieterle, S., Bongiovanni, A.M., Witkin, S.S., 2008. Antibody to the Chlamydia trachomatis 60 kDa heat shock protein in follicular fluid and in vitro fertilization outcome. *Am. J. Reprod. Immunol. (New York, N.Y.: 1989)* 59 (2), 85–89. <https://doi.org/10.1111/j.1600-0897.2007.00539.x>.
- Keay, S.D., Barlow, R., Eley, A., Masson, G.M., Anthony, F.W., Jenkins, J.M., 1998. The relation between immunoglobulin G antibodies to Chlamydia trachomatis and poor ovarian response to gonadotropin stimulation before in vitro fertilization. *Fertil. Steril.* 70 (2), 214–218. [https://doi.org/10.1016/s0015-0282\(98\)00145-9](https://doi.org/10.1016/s0015-0282(98)00145-9).
- Kimura, F., Takebayashi, A., Ishida, M., Nakamura, A., Kitazawa, J., Morimune, A., Hirata, K., Takahashi, A., Tsuji, S., Takashima, A., Amano, T., Tsuji, S., Ono, T., Kaku, S., Kasahara, K., Moritani, S., Kushima, R., Murakami, T., 2019. Review: Chronic endometritis and its effect on reproduction. *J. Obstet. Gynaecol. Res.* 45 (5), 951–960. <https://doi.org/10.1111/jog.13937>.
- Kitaya, K., Takeuchi, T., Mizuta, S., Matsubayashi, H., Ishikawa, T., 2018. Endometritis: New time, new concepts. *Fertil. Steril.* 110 (3), 344–350. <https://doi.org/10.1016/j.fertnstert.2018.04.012>.
- Land, J.A., Evers, J.L.H., 2002. Chlamydia infection and subfertility. *Best Pract. Res. Clin. Obstet. Gynaecol.* 16 (6), 901–912. <https://doi.org/10.1053/beog.2002.0319>.
- Land, J.A., Van Bergen, J.E.a.M., Morr e, S.A., Postma, M.J., 2010. Epidemiology of Chlamydia trachomatis infection in women and the cost-effectiveness of screening. *Hum. Reprod. Update* 16 (2), 189–204. <https://doi.org/10.1093/humupd/dmp035>.
- Licciardi, F., Grifo, J.A., Rosenwaks, Z., Witkin, S.S., 1992. Relation between antibodies to Chlamydia trachomatis and spontaneous abortion following in vitro fertilization. *J. Assist. Reprod. Genet.* 9 (3), 207–210. <https://doi.org/10.1007/BF01203814>.
- Lunenfeld, E., Shapiro, B.S., Sarov, B., Sarov, I., Insler, V., Decherney, A.H., 1989. The association between chlamydial-specific IgG and IgA antibodies and pregnancy outcome in an in vitro fertilization program. *J. In Vitro Fertil. Embryo Trans.: IVF* 6 (4), 222–227. <https://doi.org/10.1007/BF01132869>.
- Muvunyi, C.M., Claeys, L., De Sutter, T., De Sutter, P., Temmerman, M., Van Renterghem, L., Claeys, G., Padalko, E., 2012. Comparison of four serological assays for the diagnosis of Chlamydia trachomatis in subfertile women. *J. Infect. Dev. Ctries.* 6 (5), 396–402. <https://doi.org/10.3855/jidc.1740>.
- Ness, R.B., Soper, D.E., Richter, H.E., Randall, H., Peipert, J.F., Nelson, D.B., Schuback, D., McNeeley, S.G., Trout, W., Bass, D.C., Hutchison, K., Kip, K., Brunham, R.C., 2008. Chlamydia antibodies, chlamydia heat shock protein, and adverse sequelae after pelvic inflammatory disease: The PID Evaluation and Clinical Health (PEACH) Study. *Sex. Transm. Dis.* 35 (2), 129–135. <https://doi.org/10.1097/olq.0b013e3181557c25>.
- Osser, S., Persson, K., Wrambsy, H., Liedholm, P., 1990. Dose previous Chlamydia trachomatis infection influence the pregnancy rate of in vitro fertilization and embryo replacement? *Am. J. Obstet. Gynecol.* 162 (1), 40–44. [https://doi.org/10.1016/0002-9378\(90\)90816-p](https://doi.org/10.1016/0002-9378(90)90816-p).
- Pacchiarotti, A., Sbracia, M., Mohamed, M.A., Frega, A., Pacchiarotti, A., Espinola, S.M.B., Aragona, C., 2009. Autoimmune response to Chlamydia trachomatis infection and in vitro fertilization outcome. *Fertil. Steril.* 91 (3), 946–948. <https://doi.org/10.1016/j.fertnstert.2007.12.009>.
- Park, H.J., Kim, Y.S., Yoon, T.K., Lee, W.S., 2016. Chronic endometritis and infertility. *Clin. Exp. Reprod. Med.* 43 (4), 185–192. <https://doi.org/10.5653/ceerm.2016.43.4.185>.
- Rantsi, T.,  hman, H., Puolakainen, M., Bloigu, A., Paavonen, J., Surcel, H.-M., Tiitinen, A., Joki-Korpela, P., 2018. Predicting tubal factor infertility by using markers of humoral and cell-mediated immune response against Chlamydia trachomatis. *Am. J. Reprod. Immunol. (New York, N.Y.: 1989)* 80 (5), e13051. <https://doi.org/10.1111/aji.13051>.
- Raofi, Z., Barchinegad, M., Haghighi, L., 2013. The value of negative Chlamydia trachomatis antibody in prediction of normal tubes in infertile women. *Clin. Exp. Obstet. Gynecol.* 40 (1), 95–97.
- Revised American Society for Reproductive Medicine classification of endometriosis: 1996. *Fertil. Steril.* 67 (5), 817–821. [https://doi.org/10.1016/s0015-0282\(97\)81391-x](https://doi.org/10.1016/s0015-0282(97)81391-x).
- Rolland, L., Perrin, J., Villes, V., Pellegrin, V., Boubli, L., Courbiere, B., 2017. IVF oocyte retrieval: Prospective evaluation of the type of anesthesia on live birth rate, pain, and patient satisfaction. *J. Assist. Reprod. Genet.* 34 (11), 1523–1528. <https://doi.org/10.1007/s10815-017-1002-7>.
- Sexually transmitted infections (STIs)*. (n.d.). Retrieved August 6, 2022, from [https://www.who.int/news-room/fact-sheets/detail/sexually-transmitted-infections-\(stis\)](https://www.who.int/news-room/fact-sheets/detail/sexually-transmitted-infections-(stis))
- Spandorfer, S.D., Neuer, A., LaVerda, D., Byrne, G., Liu, H.C., Rosenwaks, Z., Witkin, S.S., 1999. Previously undetected Chlamydia trachomatis infection, immunity to heat shock proteins and tubal occlusion in women undergoing in-vitro fertilization. *Hum. Reprod. (Oxford, England)* 14 (1), 60–64. <https://doi.org/10.1093/humrep/14.1.60>.
- Stephens, A.J., Aubuchon, M., Schust, D.J., 2011. Antichlamydial Antibodies, Human Fertility, and Pregnancy Wastage. *Infect. Dis. Obstet. Gynecol.* 2011, 525182. <https://doi.org/10.1155/2011/525182>.
- Tiitinen, A., Surcel, H.-M., Halttunen, M., Birkelund, S., Bloigu, A., Christiansen, G., Koskela, P., Morrison, S.G., Morrison, R.P., Paavonen, J., 2006. Chlamydia trachomatis and chlamydial heat shock protein 60-specific antibody and cell-mediated responses predict tubal factor infertility. *Hum. Reprod. (Oxford, England)* 21 (6), 1533–1538. <https://doi.org/10.1093/humrep/del014>.
- Verdon, R., 2019. [Treatment of uncomplicated pelvic inflammatory disease: CNGOF and SPLIF Pelvic Inflammatory Diseases Guidelines]. *Gynecol. Obstet. Fertil. Senol.* 47 (5), 418–430. <https://doi.org/10.1016/j.gofs.2019.03.008>.
- Vitagliano, A., Saccardi, C., Noventa, M., Di Spiezo Sardo, A., Saccone, G., Cicinelli, E., Pizzi, S., Andrisani, A., Litta, P.S., 2018. Effects of chronic endometritis therapy on in vitro fertilization outcome in women with repeated implantation failure: A systematic review and meta-analysis. *Fertil. Steril* 110 (1), 103–112.e1. <https://doi.org/10.1016/j.fertnstert.2018.03.017>.
- Witkin, S.S., Minis, E., Athanasiou, A., Leizer, J., Linhares, I.M., 2017. Chlamydia trachomatis: The Persistent Pathogen. *Clin. Vaccine. Immunol.: CVI* 24 (10), e00203–e00217. <https://doi.org/10.1128/CVI.00203-17>.
- Witkin, S.S., Sultan, K.M., Neal, G.S., Jeremias, J., Grifo, J.A., Rosenwaks, Z., 1994. Unsuspected Chlamydia trachomatis infection and in vitro fertilization outcome. *Am. J. Obstet. Gynecol.* 171 (5), 1208–1214. [https://doi.org/10.1016/0002-9378\(94\)90134-1](https://doi.org/10.1016/0002-9378(94)90134-1).
- Woodhall, S.C., Gorwitz, R.J., Migchelsen, S.J., Gottlieb, S.L., Horner, P.J., Geisler, W.M., Winstanley, C., Hufnagel, K., Waterboer, T., Martin, D.L., Huston, W.M., Gaydos, C.A., Deal, C., Unemo, M., Dunbar, J.K., Bernstein, K., 2018. Advancing the public health applications of Chlamydia trachomatis serology. *Lancet. Infect. Dis.* 18 (12), e399–e407. [https://doi.org/10.1016/S1473-3099\(18\)30159-2](https://doi.org/10.1016/S1473-3099(18)30159-2).
- Zegers-Hochschild, F., Adamson, G.D., Dyer, S., Racowsky, C., de Mouzon, J., Sokol, R., Rienzi, L., Sunde, A., Schmidt, L., Cooke, I.D., Simpson, J.L., van der Poel, S., 2017. The International Glossary on Infertility and Fertility Care, 2017. *Hum. Reprod. (Oxford, England)* 32 (9), 1786–1801. <https://doi.org/10.1093/humrep/dex234>.
- Zhang, D., Wang, Z., Hu, X., Ma, C., Ma, Y., Xu, Y., 2022. Effect of previous Chlamydia Trachomatis infection on the outcomes of ivf/icis treatment: A retrospective study. *BMC Pregnancy Childbirth* 22 (1), 305. <https://doi.org/10.1186/s12884-022-04624-z>.