
Evaluation of the effect of bacterial colonization in cervical mucus on the outcome of ICSI cycles

Farahat M., Nouh B., Rageh K.,
Ismael M.S., Fata A., Hablas W.,
Wafa Y
International Islamic center for
population studies and research,
Al Azhar University, Cairo, Egypt.

Abstract

Objective: Evaluation the effect of chlamydial infection and microbial flora in cervical mucus at the time of embryo transfer on fertilization and implantation in women undergoing ICSI procedures.

Patients and Methods: 500 infertile women were enrolled in this study. All were recruited from the outpatient clinic of Assisted Reproduction unit in International Islamic Centre for Population Studies and Researches, Al-Azhar University. All participants underwent pituitary down-regulation using either long or short protocol followed by ovarian stimulation, oocytes retrieval, fertilization, and embryos transfer. In the study group, cervical swabs were taken with the first folliculometry and screened for Chlamydia & other bacterial growth; antibiotics were given accordingly for positive cases. During ET, cervical swabs were taken from control group and tested for Chlamydia & other bacterial infection but with no usage of any antimicrobials, pregnancy tests were done two weeks after ET, and the participants were accordingly divided into two subgroups: pregnant group and non-pregnant group.

Results: Overall incidence of pregnancy: 193/500 (38.6 %), and for those who got pregnant, the incidence of Chlamydia alone (in study group) was 8.89 % compared to 8.74% (in control group). Incidence of other bacterial growth (in study group) was 35.56% compared to 30.1% (in control group). Incidence of associated Chlamydia & other bacterial growth (in study group) was 8.89 % compared to 5.83% (in control group)

Conclusion: Chlamydial infection and microbial flora of the cervix detected during ET has no role in the implantation process, and does not affect pregnancy rates significantly in women undergoing ICSI, and antimicrobial has no significant role in improving outcome of pregnancy rate.

Key words: Cervical mucus, bacterial colonization, Chlamydia, ICSI.

Introduction

The hostility or receptivity of the cervix to spermatozoa is dependent to a great degree upon the condition of the cervical mucus, a subject that deserves more attention in the gynecological literature. It is unfortunate that so much stress has been placed on evaluation of tubal patency, determination of ovulation and analysis of semen with relative neglect of the cervical factor. In intracytoplasmic sperm injection (ICSI), embryo transfer (ET) is usually performed 72 hours after oocyte retrieval. For standard ET procedure, transfer is accomplished via transcervical cannulation and injection of embryos into the uterine cavity. [1]

Embryo implantation is the main event that limits the success of ICSI-ET. Despite the presence of satisfactory embryo morphological analysis as well as adequate histological and histochemical endometrial characteristics, overall ICSI –ET pregnancy rates remain poor. This indicates that additional factors might interfere with the normal embryo implantation process. These factors range from culture conditions, hormone stimulation, gamete immaturity or quality of chromosomal abnormality to the possi-

bility of bacterial contamination of the uterine cavity. [2] ICSI-ET procedures involve needle puncture of the vagina for egg retrieval and placement of embryos through the cervix, thus contamination is possible from vaginal-cervical microorganisms particularly because vaginal antiseptics usually are not used during egg retrieval or embryo transfer to avoid injury of the egg or embryos [3]. Prophylactic antibiotics administered to women at time of oocyte retrieval, were claimed to be associated with a reduction in positive microbiology cultures of embryo catheter tips 48 hours later in 78.4% of patients [4]. The minimal inflammation in response to microorganisms that enter the endometrium from the cervix during embryo transfer provides another mechanism that could damage the developing embryo and prevent pregnancy [4].

Regarding the mechanisms that may be responsible for damaging the developing embryo. First, it is conceivable that intense concentrations of microorganisms on the cervix may be associated with subclinical chronic endometritis and therefore poor uterine receptivity. Second, the ET procedure may inoculate cervical microorganisms into the uterine cavity, potentially altering the biochemical or ultrastructural characteristics of the endometrium required for satisfactory embryo implantation and early development. Third, the possible direct contamination of embryos during transcervical embryo transfer may cripple their ability to implant [5].

Subjects and Methods

The aim of this study was to evaluate the effect of bacterial colonization in cervical mucus on the outcome of ICSI cycles.

- Study setting: The study was carried out from February 2012 to June 2013, 500 participants with infertility were enrolled in the study in international Islamic center for population studies and research, Al Azhar University, Cairo, Egypt. All were recruited from the outpatient clinic of Assisted Reproduction unit.
- Research Design: A prospective follow-up study design was used in carrying out the study.
- Study subjects: The study included 500 consecutive patients from those attending the unit with the diagnosis of infertility who were assigned for treatment with intra-cytoplasmic sperm injection (ICSI). The inclusion criteria included women age less or equal to 36 years, whose uteri were morphologically normal as confirmed by HSG and U/S to limit additional factors that may affect the results of the study. Moreover, none of the women includ-

ed in this study had clinical evidence of vaginitis or cervicitis or has a concurrent use of antibiotics. Each woman participated only once in the study. Ethical committee approval was obtained and all subjects gave their oral consent to be included in the study.

- Study Maneuver: Taking history, abdominal and pelvic examination was also performed. Routine investigations were carried out for all participants according to the protocols applied in the unit. Down regulation and induction of ovulation were initiated for every patient according to the applied protocol. Follicular monitoring and estimation of endometrial thickness were performed using TVS every other day starting six days after initiation of gonadotropins. In study group, cervical swabs were taken with the first folliculometry and screened for Chlamydia and other bacterial growth, antibiotics were given accordingly for positive cases), then after hCG administration and oocytes retrieval, embryo transfer (ET) was done 48-72 hours after oocyte using a standardized technique, with a cook ET catheter. A maximum of four embryos were transferred at a time. No antiseptic solution was used to prepare the cervix for embryo transfer, but this was only washed with normal saline. In the control group, cervical swab was taken and tested for Chlamydia and other bacterial growth but with no any antimicrobials given even in positive cases), luteal phase support was given and patients were scheduled for B-hCG two weeks after ET, and the participants were accordingly divided into two subgroups: a pregnant group , and a non-pregnant group.
- Bacteriological investigation methods:
 - a. Polymerase chain reaction (PCR): was used for the detection of the Chlamydia trachomatis. PCR involved three steps, namely DNA extraction, amplification, and detection of specific DNA product.
 - b. Culture: Swabs were cultured on blood agar, chocolate agar and ma ckonky's agar. The plates were incubated aerobically and anaerobically in 5% CO₂ at 37 °C. Bacteria were isolated for identification by standard laboratory procedures.

Results

All data were collected, tabulated and subjected to statistical analysis. Comparison of the socio-demographic characteristics of the study and control groups, revealed no differences of statistical significance, their age was around 29 years. Their BMI around 26.5, the

mean duration of infertility was respectively 5.8 and 6.1 years, and the mean of FSH level was 6.6 and 6.3 respectively, they were mostly housewives. Concerning the cause of infertility in the two groups, the figure (1), shows no statistically significant difference between study and control in the cause of infertility, it was mainly male factor with 62% to 64%.

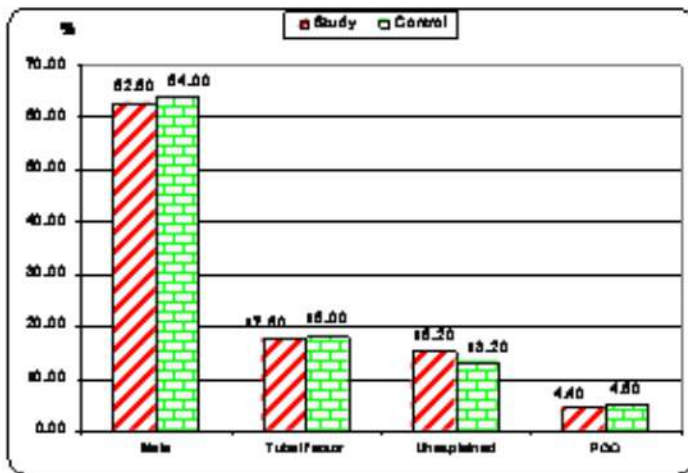


Figure 1: Relation between study and control as regard to cause of infertility.

Regarding the characters of ICSI cycles, there were no statistically significant differences between the two groups in number of administered HMG ampoules (37 to 38 ampoules), duration of stimulation is around 12.5 days, endometrial thickness at time of embryo transfer ranged from 10.5 to 10.9 mm. Nearly, 8 oocytes were retrieved, 4 of them were fertilized and almost 2 embryos were transferred, as shown in figure (2)

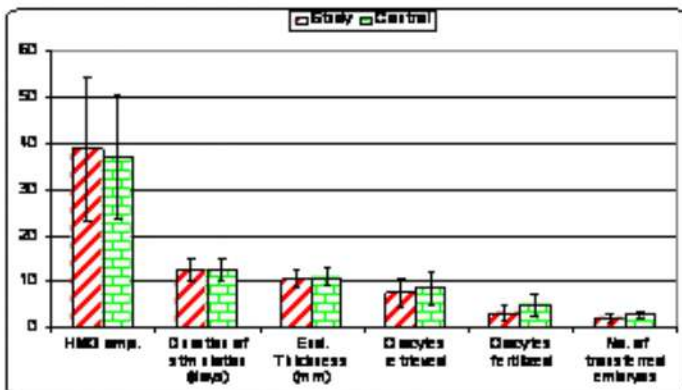


Figure 2: Comparison between study and control as regard characters of ICSI cycle.

Concerning bacterial growth, figure (3) and (4) indicate that the organisms isolated varied from staphylococci, Klebsiella, E-coli, lactobacilli and to less extent anaerobic cocci. However, no differences of statistical significance could be noticed between the study and control group

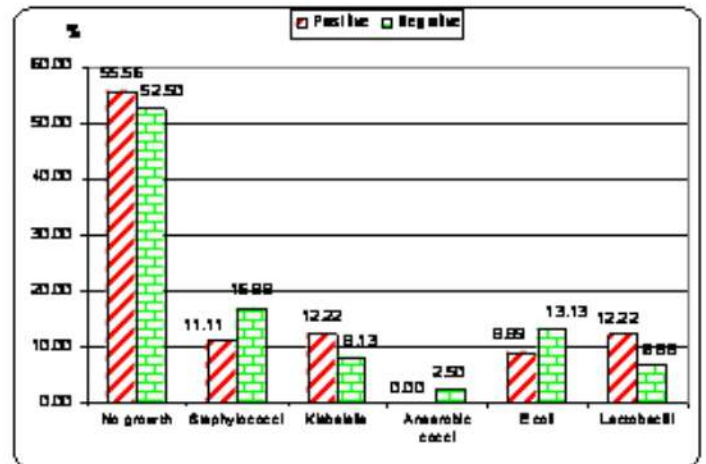


Figure 3: Relation between cultures of bacterial growth as regard to pregnancy test in study group.

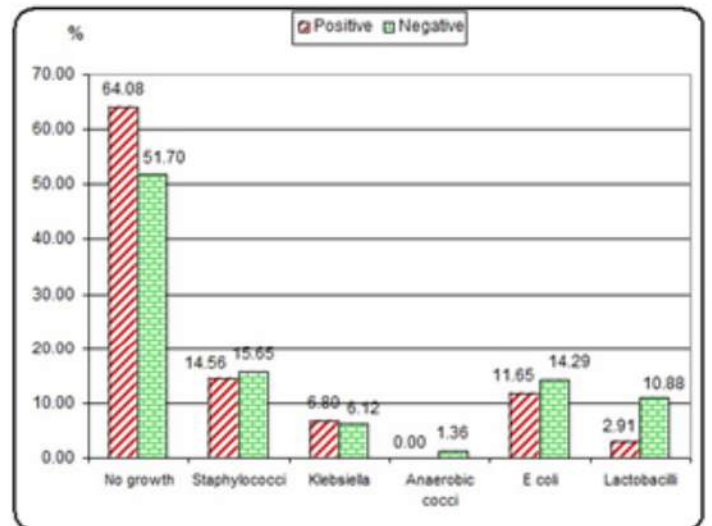


Figure 4: Relation between cultures of bacterial growth as regard to pregnancy test in control group.

The chlamydial growth data were also similar in the two groups. As illustrated in figure (5), no differences of statistical significance could be revealed between them, the positive cases was 17.7% and 14.5% in preg-

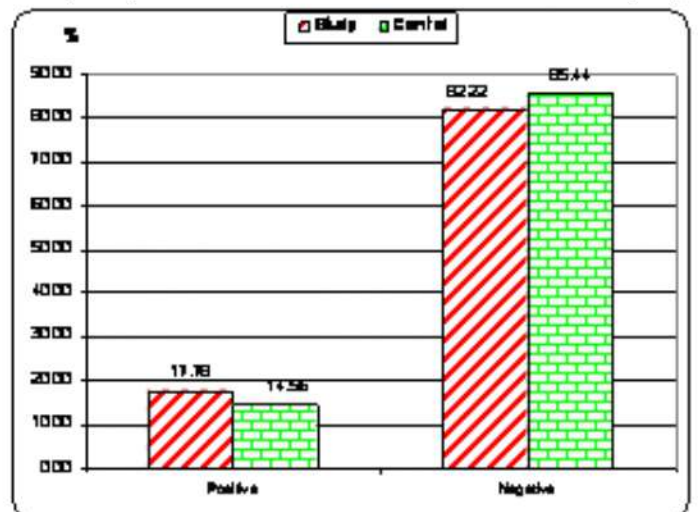


Figure 5: Relation between study and control pregnant cases as regard to Chlamydia.

Comparison of various combinations of bacterial and or chlamydial growth did not show any differences of statistical significance between the pregnant cases in the two groups. Also, figure (6) shows that overall incidence of pregnancy: 193/500 (38.6 %), and for those who got pregnant, the incidence of Chlamydia alone (in study group) is 8.89 % compared to 8.74% (in control group). Incidence of other bacterial growth (in study group) is 35.56% compared to 30.1% (in control group). Incidence of associated Chlamydia & other bacterial growth (in study group) is 8.89 % compared to 5.83% (in control group)

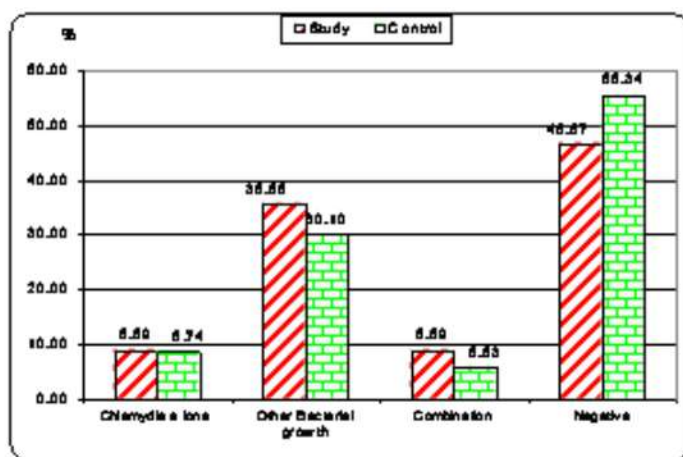


Figure 6: Relation between study and control pregnant cases as regard to combination between positive culture and Chlamydia

Discussion

Embryo implantation is the main event that limits the success of IVF-ET [5]. Genital infections, particularly those caused by sexually transmitted microorganisms, rank among the leading causes of infertility [6]. The fear that genital bacterial contamination may interfere with embryo implantation has been suggested as far back as 1978 [7]. Clinical studies have shown that bacterial contamination of the embryo transfer catheter has a significant negative effect on the clinical pregnancy rates [8,9,10]. In addition, cervical sterility at the time of ART procedures cannot be achieved with the routine use of vaginal antiseptics since there is evidence these solutions have been shown to have a negative impact on the quality of the oocytes collected and the embryos available for transfer [11]. Moreover, there is insufficient evidence about the effects of different antibiotic prophylaxis regimens on ART cycle outcomes [4,12,13]. The influence of the cervical mucus present at time of embryo transfer and its bacterial contamination on the outcome of ICSI cycles has been highly

debated due to conflicting results in the medical literature. Some authors have demonstrated diminished pregnancy rates, while others have shown not. In addition, most of studies supported the use of samples of cervical mucus from catheter tips at time of embryo transfer. *C. trachomatis* is believed to be one of the major causes of cervical factor infertility, as a result of the alterations of the epithelium and mucus composition, and by the presence of inflammatory cells; anyway, the overall impact of cervical disorders on fertility still needs to be assessed [14]. It has been suggested that impaired ovarian function and low ovarian response to ovulation induction are associated with *C. trachomatis* infection [15]. Earlier studies have shown that pathogenesis of the disease was not only induced by the infectious agent but also due to immune response to infected tissues [14]. Detection of Chlamydia species in the endocervices of women undergoing IVF-ET has been associated with decreased implantation rates [16]. The cervix is usually colonized by other potentially pathogenic microorganisms that can be introduced into the uterine cavity or contaminate embryos during ET [17]. The consequences of this septic milieu on the outcome of embryo implantation are unclear. Egbase P.E., et al, in 1996, has reported poor IVF-ET results in patients with positive microbial growth after catheters used in ETs were cultured. From a pathophysiologic standpoint, at least three mechanisms may be considered for explaining the lower pregnancy rates observed among patients with positive cultures: First, it is conceivable that intense concentrations of microorganisms on the cervix may be associated with subclinical chronic endometritis and, therefore, poor uterine receptivity. Second, the ET procedure may inoculate cervical microorganisms into the uterine cavity potentially altering the biochemical or ultrastructural characteristics of the endometrium required for satisfactory embryo implantation and early development. Third, the possible direct contamination of embryos during transcervical ET may cripple their ability to implant [18].

In light of this controversy, and the need to clearly identify the potential value of bacterial contamination of the cervical mucus and role of antibiotic on the outcome of ICSI cycles, it was decided to perform this study. The vaginal flora contains a large variety of bacterial species, including aerobic and anaerobic organisms, as revealed by modern microbiologic methods [19]. Moreover, the diversity and kinds of organisms that comprise the vaginal microbial community vary among women [20]. Since the lower genital tract is a naturally inhabited with vaginal flora and pathogenic organisms, operative procedures through or adjacent to this field leads to a moderate to high incidence of

infection. Therefore recommendations for antibiotic prophylaxis have been established in many procedures, including vaginal hysterectomy, abdominal hysterectomy, and cesarean section [21]. However, unlike most assisted reproductive techniques, these are major operative procedures that may carry a high morbidity rate from infections. With regards minor operative procedures related to ART, such as during trans-vaginal oocyte retrieval and embryo transfer, there are no clear recommendations by any society (e.g. American society of Reproductive Medicine [ASRM], European Society for Human Reproduction and Embryology [ESHRE], Middle East Fertility Society [MEFS], Mediterranean Society for Reproductive Medicine [MSRM]) or other evidence-based guidelines (e.g. NICE guidelines). However, these procedures have a high possibility of ascending infection from the lower genital tract to the upper genital tract, especially for those procedures that pass through the endocervical canal into the uterine cavity (e.g. intrauterine insemination and embryo transfer). Since these procedures have only small areas of tissue trauma, it is questionable whether or not antibiotic prophylaxis, the use of antibiotics for the prevention of infection, for these procedures protects against ascending infection. Therefore, antibiotic prophylaxis might have a role to prevent infection in these procedures, but this has yet to be officially quantified. In essence, in today's evidence based medical environment, any recommendation must be built on two main questions: (a) whether ascending infections occur as a result of the procedure and (b) whether this results in a decreased pregnancy rate in such cases. Only then can a proper set of guidelines be proposed to answer this clinical query.

The present study aimed to evaluate whether the presence of cervical microorganisms in cervical mucus at embryo transfer has an effect on the success of preg-

nancy in women undergoing ICSI procedure, and what is the benefit of antimicrobials on the outcome of ICSI cycles. The present study has shown that there were no statistically significant differences between the study group and control group women as regards to their age, duration of infertility, and serum FSH level measured on day 3 of the cycle. This was important in order to be able to compare pregnant versus non-pregnant women as regards the presence of Chlamydia and other organisms, and to investigate the relation between the presence of these microorganisms and the success or failure rates of the process of ICSI. Similarly, there were also no statistically significant differences between the two groups, study group and control group, as regards to the number of retrieved oocytes and the number of transferred embryos. This is again to ensure that both groups had equal chance of success, and of getting pregnant. The present study has demonstrated that there were no statistically significant differences between the two groups of study and control groups as regards to bacterial growth in pregnant cases, despite of antibiotics given to study group according to culture and sensitivity tests. The present study findings have also shown that subjects in the study group, who were positive for Chlamydia and received anti-chlamydial medications, show no statistically significant difference between them and other positive chlamydial cases in control group who did not receive any antichlamydial medications as regard to the pregnancy rate.

Conclusion

The present study reports that chlamydial infection and microbial flora of the cervix detected during ET has no role in the implantation process, and does not affect pregnancy rates significantly in women undergoing ICSI procedure for the treatment of infertility, and antimicrobial has no significant role in improving outcome of pregnancyrate.

The following table will summarize and demonstrate some published studies in relation to our study (arranged in a chronological manner)

Study	Year	Sample size	Maneuver	Conclusion
Lunenfeld E., et al, [22]	1989	200	Chlamydial-specific IgG and IgA antibodies in the serum of 106 patients that have conceived in an in vitro fertilization and embryo transfer (IVF-ET), and in a group of 94 patients that went through the program at the same period of time and did not conceive.	The authors have concluded that their results indicate the possible role of past or chronic active chlamydial infection on the success of an IVF-ET program.
Lessing J.B., et al, [23]	1991	86	The prevalence of specific chlamydial IgG and IgA antibodies have determined in 86 infertile women undergoing in vitro fertilization (IVF). Pregnancy was later achieved by IVF in 13 of 32 seropositive and 19 of 32 seronegative women.	The results have demonstrated that high levels of specific antichlamydial antibodies (IgG and IgA) are not correlated with the outcome of IVF-embryo transfer treatment.

Witkin S.S., et al, [24]	1995	307	Chlamydia trachomatis in the endocervices of 307 asymptomatic culture-negative women undergoing in vitro fertilization (IVF). C. trachomatis was detected by polymerase chain reaction (PCR) in 20 subjects (6.5%)	There were strong correlations between a positive finding and both failure to become pregnant (p=0.013) and spontaneous abortion after embryo transfer (p=0.004). They have concluded that an undetected C. trachomatis infection may be responsible for implantation failure or spontaneous abortion after IVF and embryo transfer.
Egbase P.E., et al, [8]	1996	110	Microbiological cultures were performed on endocervical swabs and embryo transfer catheter tips.	The clinical pregnancy was 57.1 % in the group of patients without growth and 29.6% in the group with positive microbial growth from catheter tips. Concluded that the presence of microorganisms on the cervix, as detected on the catheter used for ET, was associated with poor IVF-ET outcome.
Claman P., et al, [25]	1996	195	To examine IVF-ET outcome in patients with and without serologic evidence of Chlamydia trachomatis infection and chlamydia heat shock protein 60 (CHSP 60) antibodies, these investigators have conducted a retrospective case control study on 195 IVF-ET patients with tubal factor infertility who underwent oocyte pick-up	There are no differences in pregnancy rates or outcomes in patients with and without serologic evidence of previous C. trachomatis infections.
Sharara F.I, et al, [26]	1997	194	These authors have carried out a study to evaluate the impact of elevated serum Chlamydia IgG antibodies (Ab) on in vitro fertilization (IVF) outcome in a large infertility population. 194 women undergoing a total of 316 IVF cycles were evaluated. All couples with positive serum Chlamydia IgG Ab were pretreated with doxycycline, 100 mg twice daily, for 10 days prior to the first IVF cycle. They have found that one hundred seven women (55.2%) had elevated serum Chlamydia IgG Ab. One hundred seventy-two IVF cycles (54.4%) were in patients with elevated Ab as compared to 144 cycles (45.6%) in controls with negative Ab.	Concluded that there was no correlation between IVF outcome and quantitative IgG Ab titers in women with elevated serum Chlamydia Ab.
Fanchin R., et al, [9]	1998	279	The tips of catheters were subjected to quantitative (≥ 10 colonies = positive culture group; < 10 colonies = negative culture group) and qualitative microbial assessment.	The presence of cervical microbial flora (particularly E. coli) at the time of ET, as detected through bacteriologic assessment of ET catheter is associated with decreased IVF-ET success
Liversedge N.H., et al, [27]	1999	301	High vaginal swabs taken at the time of oocyte collection were assessed by Gram staining.	Although the prevalence of bacterial infection was much higher in infertile patients having IVF treatment, compared to others in antenatal and general gynecological populations, no significant effect upon fertilization and implantation rates was found.
Johnson K. [28]	2000	297	In the study, cultures were taken from catheter tips used during the cycles, both at the time of egg retrieval and again at embryo transfer. All patients were then routinely treated prophylactically with <u>ceftriaxone</u> (2 g IV) and <u>metronidazole</u> (1 g IV) at egg retrieval. They later underwent embryo transfer.	Implantation rates were statistically less for those who tested positive on both occasions (9.3%), compared with those who tested negative both times (21.6%) or who tested positive only at egg retrieval (19.3%). %)

Study	Year	Sample size	Maneuver	Conclusion
Moore D. [29]	2000	91	cultures were taken from catheter tips used during the cycles, both at the time of egg retrieval and again at embryo transfer, All patients were then routinely treated prophylactically with 100 mg of <u>doxycycline</u> twice a day for 5 days, starting before embryo transfer	The negative impact on pregnancy rates was only true of one particular bacteria, Streptococcus viridans, which reduced the pregnancy rates to 7%. Most other bacteria seemed to have no effect, being associated with a 37% pregnancy rate, and administration of prophylactic antibiotics, in this case, was ineffective in changing any of the vaginal microbial growth in the patients
Salim R., et al, [10]	2002	204	Bacteriological culture of cervical canal	Failure to conceive in ART is significantly associated with bacterial colonization of the uterine cervix.
Cortinas P., et al, [30]	2004	41	The relationship between presence of anti-Chlamydia trachomatis and anti-HSP60 antibodies in serum and follicular fluid of infertile women has investigated. Serum IgG and follicular fluid IgA to Chlamydia trachomatis and human heat shock protein 60 (HSP60) were determined in 41 women undergoing in vitro fertilization (IVF).	A significant association was found between the presence of bacterial antibodies in serum and IgA anti-HSP60 in follicular fluid. Although the authors have reached to the conclusion that Chlamydia trachomatis infection might be triggering an autoimmune process that could negatively affect the success of IVF, they have not reported the pregnancy rates achieved, and whether it was associated with the levels of these markers.
Meherafza M., et al, [31]	2007	260	After transferring the embryo, about 3cm of the end of catheters were cut and put in selective culture media. After incubating in special conditions by using current bacteriological methods; bacteria were isolated and characterized	The study revealed that microbial flora of the cervix didn't influence in poor ICSI-ET outcome.
Selman H., and Mariani M. [32]	2007	152	During embryo transfer, separate samples were collected for microbial examination from the following sites: the fundus of the vagina, the cervix, the embryo culture medium prior and post-embryo transfer, the tip of the catheter samples were separately cultured to identify any bacteria or yeast present.	the presence of vaginal-cervical contamination at the time of E.T. is associated with significantly decreased pregnancy rate
Aboul Fotouh I., and Al-Inany M.G. [33]	2008	25	Cervical mucus samples were taken immediately prior to embryo transfer and the tips of the post-transfer catheters were examined for bacterial contamination, and their levels were recorded.	The presence of bacterial contamination of catheter tips during embryo transfer is evidently limited and doesn't affect the cycle outcomes.
Abd- Raboh S., et al. [34]	2011	300	A study group of 150 infertile females attending infertility centers for ICSI and a control group of 150 multiparus females attending outpatient clinics for IUD introduction have been included. Endocervical swabs from cases and controls have been examined for chlamydial antigen using immunochromatography. Also sera were examined for IgG and IgM for Chlamydia trachomatis in both groups.	Results: 12 positive cases for serum Ig G in study group (8.0%) and 16 in controls (10.67%) has been revealed. While serum IgM was found in 4 study cases (2.7%), with no positives in the controls. Regarding Chlamydia antigen detection in endocervical swab, there was 6 positive study group cases (4.2%), while no cases were positive in controls. Conclusion: the study reports a very low prevalence rate of Chlamydia trachomatis infection in Egyptian females, which minimizes its role as cause of infertility in Egyptian population and subsequently its impact on success of ICSI is not much expressed. Cultural impact on sexual life style in Egyptian population could justify these findings.

Study	Year	Sample size	Maneuver	Conclusion
Van Oostrum N., et al, [35]	2013	27 studies	This study is a meta-analysis of data on the prevalence of BV in women with infertility, the association between BV and the cause of infertility, and the associations between BV and conception rates and early pregnancy loss following IVF over 27 Systematic literature searches of the electronic databases, PubMed, EMBASE, CINAHL, Cochrane Library and ISI Web of Knowledge.	None of the studies found an association between abnormal vaginal flora and conception rates following IVF treatment.
Our study	2013	500	To evaluate the effect of bacterial colonization in cervical mucus on the outcome of ICSI cycles. 500 participants who underwent ICSI were enrolled in the study. As regard study group 250 cases, cervical swabs were taken with the first folliculometry and screened for Chlamydia and other bacterial growth; antibiotics were given accordingly for positive cases. During ET, cervical swabs were taken from control group 250 cases and tested for Chlamydia and other bacterial infection, but with no usage of any antimicrobials. Pregnancy tests were done two weeks after ET, and the participants were accordingly divided into two subgroups: a pregnant group and non-pregnant group	Our study reports that chlamydial infection and microbial flora of the cervix detected during ET has no role in the implantation process, and doesn't affect pregnancy rates significantly in women undergoing ICSI procedure for the treatment of infertility, and antimicrobial has no significant role in improving outcome of pregnancy rate.

References

- Elder K. and Dale B. (2011): In vitro Fertilization, 3rd edition. U.K., New York, Australia: Cambridge University Press, P.152.
- Paulson R.J., Sauer M.V. and Lobo R.A. (1995): Factors Affecting Embryo Implantation after Human In vitro Fertilization. A Hypothesis. Am J Obstet Gynecol; 163:2020-3.
- Yaron Y., Peyser M., Samuel D., and Lessing J. (1994): Infected Endometriotic Cysts Secondary to Oocyte Aspiration for In vitro Fertilization. Hum Reprod; 9:1759-60.
- Egbase P.E., Sharhan M., and Grudzinskas J.G. (1999): Prophylactic Antibiotics and Endocervical Microbial Inoculation of the Endometrium at Embryo Transfer. Lancet; 354:651-2.
- Tabibzadeh S. and Babakina A., (1995): the Signals and Molecular Pathways Involved in Implantation. Asymbiotic Interaction between Blastocyst and Endometrium Involving Adhesion and Tissues Invasion. Hum Reprod; 10:1579-1602.
- Faro S. (1991): Chlamydia Trachomatis: Female Pelvic Infection. Am J.Obstet Gynecol; 164:1767-70.
- Czernobilsky B. (1978): Endometritis and infertility. Fertil Steril. 1978 Aug; 30(2):119-30.
- Egbase P.E., Al-Sharhan M., Al-Othman S., et al, (1996): Incidence of Microbial Growth from the Tip of the Embryo Transfer Catheter after Embryo Transfer in Relation to Clinical Pregnancy Rate Following in-Vitro Fertilization and Embryo Transfer. Human Reproduction; 11(8): 1687-1689
- Fanchin R., Harmas A., Benaoudia F., et al, (1998): Microbial Flora of the Cervix Assessed at the Time of Embryo Transfer Adversely Affects In vitro Fertilization Outcome. Fertil Steril; 70:866-70
- Salim R., Ben-Shlomo I., Olodner R., et al, (2002): Bacterial colonisation of the uterine cervix and success rate in assisted reproduction: results of a prospective study. Human Reproduction 17, 337–340.
- Van Os H.C, Roozenburg B.J, Janssen-Caspers H.A, et al, (1992): Vaginal disinfection with povidon iodine and the outcome of in-vitro fertilization. Hum Reprod. 1992 Mar; 7(3):349-50.
- Peikrishvili R, Evrard B., Pouly J.L, and Janny L., (2004): Prophylactic antibiotic therapy (amoxicillin + clavulanic acid) before embryo transfer for IVF is useless. Results of a randomized study. J Gynecol Obstet Biol Reprod (Paris). 2004 Dec; 33(8):713-9.
- ACOG (2006): American College of Obstetricians and Gynecologists Committee on Practice Bulletins, ACOG Practice Bulletin No. 74. Antibiotic prophylaxis for gynecologic procedures. Obstet Gynecol. 2006 Jul; 108(1):225-34.
- Paavonen J. and Eggert-Kruse W. (1999): Chlamydia trachomatis: impact on human reproduction. Hum Reprod Update 1999; 5:433–47.
- Malik M., Rizvi S., Hakim A. and Jain M. (2006): Chlamydia trachomatis in females and Infertility in India. J. Med. 2006; (123): 770 – 5.
- Witkin S.S., Sultan K.M., Neal G.S., et al, (1994): Unsuspected Chlamydia Trachomatis Infection and IVF Outcome. Am J Obstet Gynecol; Nov

-
- 171(5):1208-14.
17. Bartlett J.G., Moon N.E., Goldstein P.R., et al, (1978): Cervical and Vaginal Bacterial Flora: Ecologic Niches in the Female Lower Genital Tract. *Am J Obstet Gynecol*; 130:658-61.
 18. Lessey B.A., Damjanovich L., Coutifaris C., et al, (1992): Integrin Adhesion Molecules in the Human Endometrium. Correlation with the Normal and Abnormal Menstrual cycle. *J Clin Invest*; 90:188-95.
 19. Zhou X., Bent S.J, Schneider M.G., et al, (2004): Characterization of vaginal microbial communities in adult healthy women using cultivation independent methods. *Microbiology*, 2004 Aug; 150(Pt 8):2565-73.
 20. Heinemann C. and Reid G. (2005): Vaginal microbial diversity among postmenopausal women with and without hormone replacement therapy. *Can J Microbiol*. 2005 Sep; 51(9):777- 81.
 21. ACOG (2003): American College of Obstetricians and Gynecologists Committee. Prophylactic antibiotics in labour and delivery. *ACOG Practice Bulletin, clinical management guidelines for obstetrician-gynecologists 2003*; Number 47.
 22. Lunenfeld E., Shapiro B.S, Sarov B., et al, (1989): The association between chlamydial-specific IgG and IgA antibodies and pregnancy outcome in an in vitro fertilization program. *J In Vitro Fert Embryo Transf.*; 6(4): 222-7.
 23. Lessing J.B, Kletter Y, Amster R, et al, (1991): Success rates in in vitro fertilization treatment and its correlation with high titer antibodies for Chlamydia trachomatis. *Isr J Med Sci.*; 27(10):546-9.
 24. Witkin S.S, Klingman I., and Bongiovanni A.M., (1995): Relationship between an asymptomatic exposure to Chlamydia trachomatis and an autoimmune response to spermatozoa. *Hum Reprod* 1995;10: 2952-5.
 25. Claman P, Amimi M.N, Peeling R. W, et al, (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):146-9.
 26. Sharara F.I, Queenan J.T., Springer R.S., et al, (1997): Elevated serum Chlamydia trachomatis IgG antibodies. What do they mean for IVF pregnancy rates and loss? *J Reprod Med.*; 42(5): 281-6.
 27. Liversedge N.H., Jenkins J.M. and Keay S.D. (1999): Antibiotic Treatment Based on Seminal Cultures from Asymptomatic Male Partners in IVF is Unnecessary and May be Detrimental. *Hum. Reprod*; 11, 1227-1231.
 28. Johnson K., (2000): Normal Vaginal Flora May Hamper IVF Success, *OB/GYNNews*.(2000), FindArticles.com. 30Mar,2011 .http://findarticles.com/p/articles/mi_m0CYD/is_1_35/ai_59579586/
 29. Moore D.E, Soules M.R, Klein N.A, et al, (2000): Bacteria in the transfer catheter tip influence the live-birth rate after in vitro fertilization. *Fertil Steril*. 2000 Dec; 74(6):1118-24.
 30. Cortinas P, Munoz M.G, Loureiro C.L and Pujol F.H., (2004): Follicular fluid antibodies to Chlamydia trachomatis and human heat shock protein-60 kDa and infertility in women. *Arch Med Res*; 35(2):121-5.
 31. Mehrafza M., Heidarzadeh A., Jafari M., et al, (2007): survey of the effect of cervix microbial flora on outcome of intracytoplasmic sperm injection (ICSI), *Journal of Guilan University of medical sciences spring 2007*; 16 (61):1-8
 32. Selman H. and Mariani M., (2007): Examination of bacterial contamination at the time of embryo transfer and its impact on the IVF/pregnancy outcome, *J. Assist. Reprod. Genet* (2007) 24: 395-399.
 33. Aboul Fotouh I. and Al-Inany M.G. (2008): The levels of bacterial contamination of the embryo transfer catheter relate negatively to the outcome of embryo transfer. *Middle East Fertility Society Journal Vol. 13, No. 1, 2008*.
 34. Abd-Raboh S., Saleh H.A., Hanafy N.H., and Darwish H.B., (2011): The detection of Chlamydia Trachomatis Antigen in cervical secretions and serum antibodies in infertile females undergoing ICSI and its impact on pregnancy success. *Life Science Journal*. 2011; 8(2):259-263] (ISSN: 1097-8135).
 35. Van Oostrum N., Petra D.S., Joris M. and Hans V. (2013): Risks associated with bacterial vaginosis in infertility patients: a systematic review and meta-analysis, *Hum. Reprod.* (2013).
-