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Serodynamic of *Chlamydia Trachomatis* Infection in Women with in Vitro Fertilization-Embryo Transfer Failures

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Abstract

The role of *Chlamydia trachomatis* as one of the main causes of salpinigitis and infertility in women has been well acknowledged. A total of 93 sera was screened with antibodies (IgG) to *Chlamydia trachomatis* detected in 31(33.3%) suggesting past infections. All sera tested negative for IgM. Antibodies were found significantly more in patients with tubal damage 12(40.0%) while the least antibodies was found in women with Polycystic ovary syndrome 2(30.0%). Antibodies to *Chlamydia trachomatis* were detected 14(45.1%) of the 31 pregnant women and in 26(41.9%) in the 62 of the nonpregnant women. 47 women had four embryos transferred and 20 became pregnant with 7(10.0%) of these pregnant having antibodies to Chlamydia trachomatis, while 13(65.0%) were seropositive for *C. trachomatis* but not pregnant. A total of 214 oocytes was recovered with 199(92.9%) satisfactorily fertilized. There was no difference in fertilization rate of oocytes in-vitro between those with and those without antibody to *C. trachomatis* and between those who became pregnant and those who did not. Thus, past infected with *C. trachomatis* halved the success rate of in-vitro fertilization; in these patients. The implications of these findings are relevant to all aspects of infertility particularly in in-vitro fertilization treatment/procedures.

Keywords: *Chlamydia trachomatis*, Serology, IVF-ET failure.

1. Introduction

Chlamydia infections are now reported to be the most prevalent and among the most damaging of all sexually transmitted disease worldwide (1). Its manifestations are well acknowledged in women in whom it is responsible for the majority of cases of salpingitis (2, 3); and pelvic inflammatory disease and thus accounts for a large number of cases of female factor infertility (4,5). More than 70% of women with signs of tubal damage have circulating antibodies *C. trachomatis* compared to none in a group of controls with normal fallopian tubes (6) reported a 64% incidence of Chlamydial antibodies in women with residual inflammatory adnexal lesions, compared to 28% in women with normal adnexae. The implication is that the organism has predilection the female may also have relevance to its pathological significance in the male.

C. trachomatis, an obligate intracellular parasite, infects the epithelial cells lining the genital tract of the human host. *C. trachomatis* can cause ascending infection resulting in tubal infertility (7), ectopic pregnancy(8) and is associated with an increased risk of developing invasive squamous cell carcinoma of the uterine cervix (2). *C. trachomatis*, infection in women are often asymptomatic.

C. trachomatis, infection has a direct cytotoxic effect on the uterine tube mucosa, causing loss of microvilli and disruption of cell junctions due to

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rupture of epithelial cells (9), often resulting in scarring, tubal osculation and infertility.

In natural pregnancies, once *C. trachomatis* infects that female UGT, there is also the potential for the embryo to become infected/colonized after ovulation and whilst travelling through the uterine tubes to the uterus (10).

In 1984 the possible implications of *C. trachomatis* infection for in vitro fertilization and embryo replacement (IVF/ET) were first reported (18). This study was designed to attempt to define the association of *C. trachomatis* with infertility together with its management by IVF. We have looked at the prevalence of exposure to *C. trachomatis* among patients attending the University of Benin Teaching Hospital IVF Centre with IVF/ET.

2. Patients and methods

During February and ending of August, 2013, 93 infertile women attending the University of Benin Teaching Hospital in vitro fertilization center were enlisted in this study. The women were attending the initial consultation, preliminary routine laboratory/laparoscopy, and various stages of assessment for In vitro Fertilization Treatment. Their mean age, was 38 ± 2years and they had experienced an average of 8-22 years (range, 2-16) of infertility. None had evidence of acute salpingitis either clinically or at laparoscopy done by the clinician.

The techniques of IVF/ET as practiced by this center involve the patients being stimulated for ovarian follicular growth with a combination of agonist and antagonist. This growth was monitored by serial ultrasound scanning of the ovaries. Once follicular growth was judged to be adequate, by the clinician follicular maturation was induced with 10,000 IU of human chorionic gonadotropin (hCG) and oocyte recovery was timed for 34-36 hr later depending on the time of hcG administration.

Specimen collection:

4mLs of venous blood was collected each of the female patients into a sterile plain tube for cloth retraction to obtain serum for the seroanalysis of *C. trachomatis* antibodies using a modified Enzyme Immuno Assay.

Oocyte recovery in all cases involved an ultrasound guided technique and the number of ripe oocytes recovered ranged from 1 to 13. Each oocyte was offered for fertilization by placing it in a calculated containing a seminal plasma free suspension of spermatozoa at a concentration of 300,000 motile spermatozoa/ml/oocytes fertilization was usually confirmed 24hr later by observing pronuclei or by demonstrating the presence of a cleaving embryo. Embryo replacement was performed after 2 to 3 days, and up to four embryos were replaced into the uterine cavity by the trans-cervical route. The establishment of a pregnancy was confirmed by both laboratory hcG tests beyond 14 days from oocyte recovery and with ultrasound confirmation.

3. Result

Antibodies of *C. trachomatis* were detected in 31(33.3%) out of the 93 sera screened Detected antibodies were IgG classes. All sera tested for IgM were negative. The mean age of the patients screened was 38.0 ± 2years. Among the 93 patients examined, tubal factor infertile women had 12(40.0%), male factor 7(30.4%), antibodies to *C. trachomatis*. Endometrosis had 6(33.0%) *C. trachomatics* antibodies, unexplained 4(33.0%), polycystic ovary syndrome (Pcos) 2(20.0%) trachomatis all antibody presence (table II). The difference in the prevalence of trachomatis antibodies between the women with history of tubal damage and each of the others etiology was significant by the chi-square test ($p < 0.01$). Out of the 93 patients programmed for IVF-ET, 31(33.3%) became pregnant.

The transfer of four embryos was the optimum and the most common treatment outcome in this our environment (table II). Antibodies to Chlamydia trachomatis were detected in 14(45.1%) of the 31 pregnant women and in 26(49.9%) in the 62 not pregnant women. (table III). Forty-five women had four embryos transferred and 20 became pregnant and antibodies to Chlamydia trachomatis was found in 7(10.0%) of these pregnant women. (table IV). The difference in pregnancy rate following four embryo transfer is significant by the chi-square test ($P = 0.001$). There was no age bias in the course of this study.

A total of 214 oocytes was recovered through ultrasound guided oocytes retrieval techniques with an average yield of 5.18 oocytes per patient range (1 – 16). Out of these, 199 (92.9%) were satisfactorily fertilized.

Table I: Antichlamydia Trichomatis antibodies prevalence according to the aetiology of infertility.

Etiology	No. screened (%)	No. Positive (%)	No. Negative (%)
Endometrosis	18(20.4)	6(33.0)	12(66.7)
Male factor	23(24.7)	7(30.4)	16(69.6)
Idiopathic (Unexpl.)	12(12.9)	4(33.0)	8(66.7)
Polycystic Ovary Syndrome	10(10.7)	2(20.0)	8(80.0)
Tubal factor	30(32.2)	12(40.7)	18(60.0)
Total	93	31(33.3)	62(66.6)

Table II: Pregnancy rate of 93 women programmed for IVF-ET according to the number of embryo transferred.

Number transferred	No. of women	No. of pregnant	% pregnant
1	8	2	25
2	15	4	26.6
3	21	7	30.4
4	47	18	38.3
Total	93	31	33.3

Table III: Seroprevalence of Chlamydia Trichomatis among 93 women programmed for IVF-ET.

Result of treatment	No. of women	Number with antibodies	Number with no antibodies
Pregnant women	31(33.3)	14(45.1)	17(54.8)
Non-pregnant women	62	26(49.9)	36(58.1)
Total	93	40	62

Table IV: Seropositive of Chlamydia Trichomatis among 47 women receiving four embryo in IVF-ET.

Treatment outcome	No. of women	No. of seropositive *C. trachomatics* in Preg. Women	No. of seropositive *C.trachomatics* in non Preg. Women
Pregnant women	20	7 (35.0)	13(65.0)
Non pregnant women	27	19(70.3)	8(29.6)
Total	47	26	21

4. Discussion

The findings of this study have implications for many methods of treating the infertile couple and for the prevention of infertility. In vitro fertilization and embryo transfer directly provides an end point for which many other treatments strive: the presence in the uterus of a cleaving embryo. However, progress from that point is independent of the technique and any influences that lessen the chance of implementation will impede all methods of treating the infertile couple. The profound impact of previous infection with *C. trachomatis* is clearly shown in these results. There is a seroevidence rate (30.7%)

exposure to *C. trachomatis* in our patients. Control populations are difficult to define in chlamydial serology. Levels of background seropositivity are recorded in prevalence studies of blood donors (3%) and gynaecological patients (6%) (11). A majority of women (40.0%) in this study was those with tubal disease. The data clearly show the strong association between the presence of tubal damage and previous infection with *C. trachomatis* (12). The strong association between tubal disease and positive chlamydial serology has been reported by other workers (13) and is not surprising since *C. trachomatis* is associated with acute salpingitis (14). The destruction resulting from an episode of chlamydial salpingitis is often accompanied by a relatively benign clinical course(6). Many women with tubal infertility give no history of pelvic inflammatory disease. Despite high levels of chlamydial antibody (5). Often treatment has been ineffective or inappropriate for the organism (15). This draws attention to the need for early diagnosis and treatment of *C. trachomatis* infection to prevent tubal occlusion and ectopic pregnancy.

The most striking new finding from this study is the association of *C. trachomatis* infection with the failure of IVF/ET. The replacement of three embryos is regarded as the optimum and most common treatment outcome at University of Benin Teaching Hospital following IVF. The serological results are statistically significant for this group of patients only. Among 47 women receiving four embryos, 26 had antibodies to *C. trachomatis* of these 26 antibody-positive women, only (35.0%) became pregnant. This compares with (29.6%) pregnancies among the 19 patients who showed no serological evidence of previous chlamydial infection. It would appear that past infection with *C. trachomatis* halved the chance of the successful establishment of a pregnancy in these patients. The groups were similar in other respects i.e., age, duration of infertility, and method of IVF/ET. The presence of chlamydial antibody did not seem to significantly alter the rate of fertilization of oocytes recovered. This implies that *C. trachomatis* is not present within the oocyte and then the embryo transferred. The possibility cannot be excluded that by latency the organism may adversely affect the developing embryo. Should this occur, it may be manifest only after transferring. It has been observed that many “spare” embryos fail to develop satisfactorily after the time of embryo transferred.

Mechanisms for the adverse effect of *C. trachomatis* infection on IVF/ET may be postulated.

First, this disease is an ascending infection and will therefore include an endometriosis (16). *C. trachomatis* may lie dormant in the crypts of the endometrium persisting through menstruation and after the salpingitis has subsided or after inappropriate treatment. Second, the instrumentation may transfer the organism from the cervical canal to the endometrium at the time of ET (17). Third, *C. trachomatis* may be present in the region of the ovary and be aspirated with the oocyte and later be transferred to the uterus in the fluid surrounding the embryo or in the embryo itself (18). Fourth, it has been shown that the means, be transferred to the fertilized oocyte. These four possible events would result in a local transient endometritis or infection of the embryo and hence reduce the chance of implantation occurring successfully. The final possibility is that the endometrium has been permanently damaged by the original *C. trachomatis* infection and rendered less receptive to implantation. Our results have a wide significance.

C. trachomatis infection is recognized as an increasing problem, particularly among teenage girls. Our results indicate that the infection does affect a younger age group, which will lead to more tubal damage and to greater need for IVF, albeit with a lessened chance of establishing a pregnancy. The majority of women receiving tubal microsurgery will have suffered from chalmydial salpingitis. Few of these women or their partners will have received antichlamydial therapy. We postulate that the presence of *C. trachomatis* antibodies will be associated with a reduced pregnancy rate following surgery even if tubal patency is restored and an embryo enters the uterine cavity. The implication of this organism for tubal surgery should not be ignored and we suggest that serological investigations should precede operative procedures. The role of antichlamydial therapy in such patients should be evaluated. A prospective serological study of patients, and their partners, selected for tubal microsurgery is in preparation. These suggestions are supported indirectly by results using IVF/ET to treat male infertility. The results are significantly inferior if the female partner has tubal disease, compared to couples in whom no such tubal factor exists.

This study will be followed by a more detailed microbiological investigation of couples being assessed for IVF. This will include standard genital urinary screening supplemented by the analysis of endometrial biopsies. The findings from our present study support our earlier belief that investigation for *C. trachomatis* together with effective treatment is important for the management of the infertile couple at all levels.

References

1. Westrom L, Mardh P.A: (1983) Chlamydial salpinigitis. *Br Med Bull*: 39:145-150.
2. Moller BR, Mardh PA: (1980): Experimental salpingits in grivet monkeys by *Chlamydia trachomatis*: Mode of spread of infection to the Fallopian tubes. *Acta Path Microbiol Scand* (Sect B) 88:107-114
3. Cohen J, Edwards R, Fehilly C, Fishel S, Hewitt J, Purdy J. Rowland G, Steptoe P, (2005) Webster J: In vitro fertilisation: A treatment of male infertility *Fertil Steril* (in press)
4. Bjerke, S. and Purvis, K. (1992). *Chlamydia* serology in the investigation of infertility. *Human Reproduction* 7, 136-130.
5. Eggert-Kruse, W., Rohr, G. and Demirakca, T. (1997) *Chlamydia* serology in 1303 asymptomatic subfertile couples. *Human Reproduction* 12,1464-1475.
6. Moore, D.E., Foy, H.M., Daling, J. R., Grayson, J.T., Spadonl, L. R., Wang, S.P., (1992). Increased frequency of serum antibodies to *Chlamydia trachomatis* in fertility due to distal tube disease. *Lancet* 2,574-577.
7. Swenson, C.E., Donegan, E. And Schachter, J. (1983) *Chlamydia trachomatis* induced salpingitis in mice. *Journal Infection Disease*, 148:1101-1107.
8. Wolner-Hanssen, P. and Mardh, P., Kiviat, N., and Holmes, K. (1990) A typical pelvic inflammatory disease, Subacute, chronic, or subclinical upper genital tract infection in women. In Holmes, K.K. *et al*. (eds). *Sexually Transmitted Disease*. McGraw-Hill, New York.
9. Cooper M.D., Rapp, J., Jeffery-Wiseman, C., Barnes, R.C. and Stephens, D.S. (1990), *Chlamydia trachomatis* infection of human Fallopian tube organ cultures. *Journal for General Microbiology*. L136:1109-115.
10. Vigil, P., Tapia, A., Zacharias, S., Riquelme, R., Salgado, A. M. And Varleta, J. (2002b) First-trimester pregnancy loss and active *Chlamydia trachomatis* infection: Correlation and ultra-structural evidence. *Andrologia* 34:373-378.
11. Darougar S, Forsey T, Brewerton DA, Rogers L. (1980): Prevalence of antichlamydial antibody in London blood donors. *Br. J Vener Dis*. 56:484-487.
12. Ibadin K.O., Onalwu, I., Enabulele, O.I., Eghafona, N.O., and Aziken, M.E., (2010) *Sero*evidence *of Chlamydia trachomatis* antibody in infertile women in University of Benin Teaching Hospital, (UBTH) Benin City, Nigeria. *Malaysian Journal of Microbiology.* Vol. 6(1):91-93.
13. Mardh, P.A., Svensson, L. and Westrom, L.(1997). *Chlamydia trachomatis* infections with acute salpingitis. *New England Journal of Medicine* 296. 1377-1379.
14. George F. Rowland, Timothy Forsey, Timothy R. Moss, Patrick C. Steptoe, Jonathan Hewitt and Sorhab Darougar (1985) Failure of In-Vitro Fertilization and Embryo Transferred following Infection with *Chlamydia trachomatis. Journal of in Vitro Fertilization and Embryo Transfer*. Vol. 2, No. 3.
15. Conway D, Caul EO, Hull MGR, Glazener MA, Hodgson J, Clarke SKR, Stirral GM (1984): Chlamydial serology in fertile and infertile women. *Lancet* 1:191-193.
16. Cohen, C.R., Mugo, N.R., Astete, S.G., Odondo, R., Manhart, L.E., Klehlbauch, J.A., Stamm, W. E., Walyaki, P.G. and Totten, P.A. (2005). Detection of *Chlamydia trachomatis* in women with laparoscopically diagnosed acute salpingitis. *Sexually Transmitted Infection* 81,462-466.
17. Moss TR, Steptoe PC (1984): *Chlamydia trachomatis*: Importance in in vitro fertilisation. *J. Roy Soc Med* 77:70-72.