

**PREVALENCE OF *CHLAMYDIA* INFECTION ASSOCIATED WITH FEMALE INFERTILITY AT
UNIVERSITY OF PORT HARCOURT TEACHING HOSPITAL**

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ABSTRACT

This study was carried out to determine the prevalence of *Chlamydia* infection in female infertility cases at the University of Port Harcourt Teaching Hospital (UPTH). Subjects were 50 infertile females attending infertility clinic at the Teaching hospital and 30 apparently fertile females working at the University of Port Harcourt Teaching Hospital (UPTH) served as Control. All the subjects had their 21 day Progesterone and oestradiol concentrations determined by enzyme immunoassay method (EIA) while detection of *Chlamydia* antibodies was done by enzyme linked immunometric assay method (ELISA). There was a significant difference between the mean progesterone concentration of 27.29 ± 0.47 nmol/l, for Control subjects, and 9.50 ± 0.25 nmol/l for the infertile subjects. Also, there was significant difference in the mean oestradiol concentration of 0.38 ± 0.01 nmol/l for Control subjects and 0.12 ± 0.002 nmol/l for the infertile subjects. However, there was no significant difference in the *Chlamydia* infection in both the Control and infertile female subjects. This suggests that *Chlamydia* infection is prevalent worldwide and might not be a cause of infertility among women.

Keywords: Eostradiol, Infertile, *Chlamydia*, Progesterone.

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INTRODUCTION

Infertility is the inability to conceive and become pregnant after 12 months of unprotected regular intercourse. If a couple does not conceive after a year of effort, it is likely that either of or both partners are infertile. Female infertility is as a result of a complex chain of hormonal, physiological, environmental and genetic complications which prevent a woman that is exposed to regular unprotected sex for a period of one year without getting pregnant (Smith *et al.*, 2002). For a woman to conceive, certain things have to happen, which include intercourse must take place around the time when an egg is released from her ovary, the systems that produce eggs and sperm have to be working at optimum levels; and her hormones must be balanced (Smith *et al.*, 2002). Infertility affects men and women equally. Infertility in women is usually due to anovulation (absence of ovulation), amenorrhea (absence of menstruation), blocked fallopian tubes, uterine abnormalities, or immunological causes, due to consequences of infection with sexually-transmitted bacterial pathogens (Hu *et al.*, 2004). The issue of infertility is a serious problem, it has led to divorce, abandonment, maltreatment, clinical depression etc (Domar-Tomar *et al.*, 1993). Adegoke *et al.* (2007) reported increase concentrations of Luteinizing hormone (LH) and Follicle stimulating hormone (FSH) in both primary and secondary infertile subjects compared with their respective Controls while Braide *et al.* (2011) in their study has confirmed

the role of endocrinology in diagnosis and treatment of infertility.

Chlamydia are obligate intracellular parasites of eucaryotic cells. Two species, *Chlamydia trachomatis* and *Chlamydia psittaci* cause human disease and can be distinguished on the basis of antigenic composition. *C. trachomatis* is very sensitive to sulfonamides and forms inclusions containing glycogen, while *C. psittaci* is resistant to sulfonamides and produces a single inclusion that is glycogen negative (Harley and Wein, 2001). *Chlamydia* is widespread geographically and highly prevalent among the economically disadvantaged young women between 16 and 24 years old. A typical distribution of etiology in infertile couple indicated that 40% women with untreated *Chlamydia* develop pelvic inflammatory disease (PID) (Schmidt *et al.*, 2005). Undiagnosed PID caused by *Chlamydia* is common. Of those with PID, 20% will become infertile 18% will experience debilitating, chronic pelvic pain, and 9% will have a life – threatening tubal pregnancy (Schmidt *et al.*, 2005). *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infections cause substantial morbidity in the United States (Center for Disease Control and Prevention, 2002). In women, Chlamydial and gonococcal infections may cause pelvic inflammatory disease, tubal infertility, chronic pelvic pain, and ectopic pregnancy (Cates and Wasserheit, 1991). Chlamydial infection may also be linked to cervical cancer (Wallin *et al.*, 2002). In fact, studies have shown that spermatozoa with

adhering Chlamydial bodies in the peritoneal fluid of women with salpingitis at laparoscopy (Friberg *et al.*, 1987). Tubal Pregnancy is the leading cause of first-trimester related deaths in most women (Schmidt *et al.*, 2005). An infected male has a 25% chance per sexual encounter to transmitting the infection to a female partner. In Africa, couples who are unable to bear as many children as they wish may feel anguish or emotional pain. Hence several reports have focused on the causes, prevention and treatment of infertility in the continent (Brunham *et al.*, 1992). This study was done to determine the prevalence of *Chlamydia* infections among female partners of infertile couples attending infertility clinic at University of Port Harcourt Teaching Hospital, Port Harcourt, Nigeria

MATERIALS AND METHODS

Sample Size Determination

Sample size was determined using the formula of Araoye (2004):

$$n = Z^2pq/d^2$$

Where, n = sample size minimum, z = 95% confidence interval = 1.96, p = proportion of the target population, q = 1.0 - p, and d = degree of accuracy (95% interval) = 0.05%

Selection of Subjects

Eighty (80) subjects made up of 50 (fifty) infertile women and 30(thirty) apparently

healthy fertile women as Control were studied.

Sample Collection and Processing

Five milliliter (5ml) of blood was collected from each subject of the study groups to carry out estradiol, progesterone estimation, and *Chlamydia* detection. The subjects in sitting position had tourniquet tied on the upper arm while the venous blood sample were drawn from participants using needle and syringe (Venepuncture) at day 21 of cycle .

Serum was separated from cell by centrifugation at 2,500rpm for 15 minutes and samples for assay were analyzed as soon as possible or stored frozen until needed for analysis analysis

Source of Reagent

Commercially prepared EIA (Enzyme Immuno Assay) reagents for quantitative measurement of human hormones in serum were purchased from immunometrics (UK limited London). The reagents include estradiol and progesterone.

Biochemical and serological determination

Progesterone assay is a direct 2-step serum EIA with no pre-extraction of samples. It is of a "competitive" design with the first stage being the displacement of progesterone from serum binding proteins. Fifty microlitres (50ul) of standards, samples and Control were pipetted into their respective

plastic tubes in a rack. One hundred microlitres (100ul) of progesterone EIA blocking reagent, 100ul of progesterone EIA antiserum and 100ul of progesterone EIA separation reagent was added to each tube. Tubes were vortex mixed, covered with foil and incubated for 2 hours at 37°C in a water bath. Assay was washed with 500ul of dilute progesterone wash buffer 1. Two hundred microlitres (200ul) of dilute progesterone EIA enzyme label was added to the tubes. Tubes were mixed, covered and incubated at 37°C in a water bath for 15 minutes. Wash step was repeated twice by adding 500ul of progesterone wash buffer 2. Five hundred microlitres (500ul) of EIA stop buffer was added to the tubes to stop the reaction. Absorbances were read at 490nm, using a spectrophotometer and unknown extrapolated from calibration curve prepared as described by the manufacturers. The estradiol EIA is a direct assay of a limited (competitive) type A. Specific agent is used to displace estradiol from binding proteins, thus making it available for antibody binding. One hundred and fifty microlitres (150ul) of standards, samples and Control were pipetted into their respectively labelled plastic tubes. Two hundred microlitres (200ul) of estradiol antibody were added, mixed, covered and incubated at 37°C in a water bath for 20 minutes. Two hundred microlitres (200ul) of enzyme labeled estradiol solution was added to each tube and reincubated at 37°C in a water bath for 20 minutes. Two hundred microlitres (200ul) of separation reagent was added to each tube and incubated at

37°C for 5 minutes. The tube rack was placed on a magnetic base for 5 minutes before decanting the supernatant. Five hundred microlitres (500ul) of dilute estradiol wash buffer was added to the assay for washing purpose. This wash step was repeated, after which 500ul of EIA substrate buffer solution was added to each tube, and incubated at 37°C in a water bath for 60 minutes. One millilitre (1.0ml) of EIA stop buffer was added to the tubes to end the reaction and stabilize the colour formed. Absorbances were read at 490nm in a spectrophotometer and unknown values extrapolated from the calibration curve already prepared.

Chlamydia determination was done using the Immunocomb *Chlamydia* bivalent IgG (*C. trachomatis* and *C. pneumonie*) test which is an indirect solid phase enzyme Immuno Assay. The developing plate was incubated at 37°C for 20minutes in the incubator. The reagent was mixed by shaking the developing plate. A 1:32 dilution of sample was made in a microtitre well and vortex mixed. Row A of the developing plate was perforated and 20ul of the sample transferred into it. A card was inserted into the well and left for 60 minutes. At 60 minutes it was removed, dried on absorbent paper and transferred into Row B (First wash) for 2 minutes with agitation every 10 seconds. The card was removed at 2 minutes and the liquid absorbed then, transferred to Row C for 30 minutes. The card was removed at Row C at 30 minutes and transferred to Row D and E with agitation

for 2 minutes each (for washing). While, at F it was allowed to stay for 10 minutes for color reaction. The card was inserted back into Row E for 1 minute to stop the reaction, withdrawn and the liquid on it absorbed and allowed to dry in the air. *Chlamydia* antibodies, if present, will specifically bind to the respective *Chlamydia* antigens on the lower and middle spots. The result was read with positive showing red colorations at the Upper Spot (Control), the middle Spot (*C. pneumonia*) or the lower spot (*C. trachomatis*) and mixed infection at both middle and lower spots while negative showed red colorations at the Upper Spot (Control) only.

Statistical Analysis

The biochemical data were subjected to some statistical analysis. Values were reported as Mean \pm SEM while student's t-test was used to test for differences between treatment groups, using Statistical Package for Social Sciences (SPSS) version 16. A value of $P < 0.05$ was accepted as significant.

RESULTS

The mean \pm SEM of progesterone in Control was 27.79 ± 0.47 while it was 9.50 ± 0.25 in the test subjects, showing a significant difference ($P < 0.001$) in the mean. The Estradiol level in the Control was 0.38 ± 0.01 while it was 0.12 ± 0.002 in the infertile female subjects. There was significant difference in the two mean ($P < 0.001$) as shown in Table 1 below.

Table 1: Mean progesterone and E2 levels in Control and infertile women

Parameters	Control	Infertile subject	P Value
Progesterone(nmol/l)	27.79 ± 0.47	9.50 ± 0.25	$P < 0.001$
E2(nmol/l)	0.38 ± 0.01	0.12 ± 0.002	$P < 0.001$

Table 2 showed the prevalence of *Chlamydia* spp in the infertile female. The prevalence of *Chlamydia trachomatis* in Control subjects was 7 (23.3%) while it was 8 (16%) in the infertile female subjects. There was no significant difference in the means ($P < 0.05$). *Chlamydia pneumonia* had 13 (43.3%) in the Control while it was 15 (30%) in the test

subject showing no significant difference in the two means ($P < 0.05$). Mixed infection of *Chlamydia pneumonia* and *Chlamydia trachomatis* was 5 (16.7%) in Control and 7 (14%) in the test subject. There was no significant difference in the two means ($P < 0.05$).

Table 2: Prevalence of *Chlamydia* infection in infertile and fertile women attending the University of Port Harcourt Teaching Hospital, Port Harcourt

Organism	Infertile subjects	Control	P value
	No (%)	No (%)	
<i>Chlamydia trachomatis</i>	0 (0.00)	2 (6.70)	P > 0.05
<i>Chlamydia Pneumoniae</i>	5 (10.00)	8 (26.70)	P < 0.05
Mixed infection	9 (18.00)	5 (16.60)	P > 0.05
Negative	36(72.00)	15(50.00)	P< 0.05
TOTAL	50(100.00)	30(100.00)	

The progesterone and estradiol concentrations for infertile females at 18-27 years was 9.36 ± 0.78 and 0.12 ± 0.006 while the Control were 27.39 ± 0.77 and 0.39 ± 0.024 respectively showing significant difference. The infertile women at age group 28-32 years had progesterone and estradiol concentrations of 8.38 ± 0.84 and

0.12 ± 0.006 while the Control had 18.77 ± 2.83 and 0.23 ± 0.04 , respectively. At age group 33-36, infertile women had progesterone and estradiol concentrations of 9.37 ± 0.34 and 0.12 ± 0.007 while the Control had 19.15 ± 3.69 and 0.23 ± 0.05 , respectively, as shown below in table 3.

Table 3: Mean progesterone and estradiol (E₂) levels in different age group of infertile and fertile women at University of Port Harcourt Teaching Hospital, Port Harcourt

Age group (Years)	Progesterone (nmol/l)			Estradiol (nmol/l)		
	Infertile subjects	Control	P Value	Infertile subject	Control	P Value
18-27	9.36 ± 0.78	27.39 ± 0.77	0.000	0.12 ± 0.006	0.39 ± 0.024	0.000
28-32	8.38 ± 0.84	18.77 ± 2.83	0.006	0.12 ± 0.006	0.23 ± 0.04	0.018
28-32	9.37 ± 0.34	19.15 ± 3.69	0.057	0.12 ± 0.007	0.23 ± 0.05	0.067

The prevalence (%) of *Chlamydia pneumonia* in infertile females and Controls at age groups 18-27, 28-32 and 33-36 were 0(0.0), 4(14.2), 1(7.7) and 5(38.5), 3(23.1), 0(0.0) respectively. The prevalence (%) of *Chlamydia trachomatis* in infertile females for all age group was 0(0.0), while Controls at age groups 18-27, 28-32 and 33-36 had 0(0.0), 1(7.7), 1(25.0), respectively. Mixed infection of *C. pneumonia* and *C. trachomatis*

in infertile females was 0(0.0), 5(17.9), 4(30.8) at age groups 18-27, 28-32 and 33-36, respectively, while in Control they were 3(23.0), 2(15.4) and 0(0.0), respectively. The infertile females without *Chlamydia* infections include 9(100.0), 19(67.9) and 8(61.5) at age groups 18-27, 28-32 and 33-36 with the prevalence in Control at 5(38.5), 7(53.8) and 3(75.0), respectively, at the same age groups as shown below in table 4.

Table 4: Prevalence of *Chlamydia* infection in infertile females at different age group

Status of Chlamydial Infection	Subjects	18-27	28-32	33-36
<i>Chlamydia pneumonia</i>	Infertile female	0(0.00)	4(14.20)	1(7.20)
	Control	5(38.50)	3(23.10)	0(0.00)
<i>Chlamydia trachomatis</i>	Infertile female	0(0.00)	0(0.00)	0(0.00)
	Control	0(0.00)	1(7.70)	1(25.00)
Mixed infection	Infertile female	0(0.00)	4(14.20)	5(35.70)
	Control	3(23.00)	2(15.40)	0(0.00)
Negative	Infertile female	9(100.00)	19(70.40)	8(57.10)
	Control	5(38.50)	7(53.80)	3(75.00)

DISCUSSION

In this study, the prevalence of *Chlamydia trachomatis* infection in fertile women showed no significant difference from infertile women; hence *Chlamydia trachomatis* screening alone should not be the main infertility indicator among female. Individual may be screened to prevent the organisms from causing future damage to

the female reproductive organ, endocrine system which could eventually lead to infertility. Previous studies have found a correlation between genital *Chlamydial* infection and sperm quality (Custo *et al.*, 1989), although other studies have reported contradictory findings (Eggert-Kruse *et al.* 1996; Gdoura *et al.*, 2001). *C. trachomatis* can attach to spermatozoa (Hanssen and Mardh, 1984) and can be present in

cytoplasmic droplets of spermatozoa (Villegas *et al.*, 1991). It is important to resolve this issue, since *Chlamydiae* present in semen could be transmitted by adhering to spermatozoa, which may serve as vectors, spreading the pathogen to the uterus and Fallopian tubes.

The prevalence of *Chlamydia pneumoniae* in this study showed significant difference between the infertile and fertile groups suggesting that this organism is likely to be of concern than *C. trachomatis* in infertility. Hamdad-Daoudi *et al.*, (2004) suggested that the number of cases is probably underestimated, particularly for men, who are less likely to seek diagnosis than women because *C. trachomatis* often causes asymptomatic genital tract infections in both men and women, and the high number of unrecognized infected individuals provides a reservoir for spreading the infection to men and women via sexual contact. In women, *Chlamydial* and gonococcal infections have been suggested to cause pelvic inflammatory disease, tubal infertility, chronic pelvic pain, and ectopic pregnancy (Cates and Wasserheit, 1991). Chlamydial infection has also been linked to cervical cancer (Wallin *et al.*, 2002).

The significant differences observed in Mean progesterone and oestradiol (E2) concentrations showed that hormonal imbalance is a factor in fertility hence the low progesterone and estradiol in infertile subjects compared with the fertile subjects. Also in the age group there was significant

difference in Mean progesterone and oestradiol (E2) concentrations between the fertile and infertile groups. The presence of ovulation is always confirmed by measurement of serum progesterone in the mid-luteal phase on day 22 or approximately 7 days before menses (Yu and Yap, 2003). The ovarian reserve is related to the size of the primordial follicle store within the ovary and this decline with age (Faddy and Gosden 1996). The rate of depletion of the ovarian follicle store hastens at around the age of 37 years (Yu and Yap, 2003). The report of the progesterone and estradiol in this study was to confirm the incidence of infertility in the subjects.

Age group 28-32 had the highest prevalence of *C. pneumoniae* in infertile group while the *C. trachomatis* were not present as single infection in the infertile subjects. The incidence of mixed infection of *C. pneumoniae* and *C. trachomatis* was high in the infertile group compared with the Controls. The age group 33-36 had the highest mixed infection in infertile group suggesting that these groups are more prone than the other groups to infertility due to the presence of these organisms. Infected women are usually asymptomatic and because of the serious morbidity of these infections, *Chlamydia* programs have traditionally focused on screening women (Hocking and Fairley, 2003). Men are less likely to be infected than women probably due to the presence of prostatic fluid which has antibiotic property and most men with

urethral *Chlamydia* infection, like women, are free of symptoms.

The result of this study further showed that infertile women at 28-32 had the highest prevalence of *Chlamydia* infection. This may be as result of increase sexual activity in this group of women. The other groups might have been under reported because of awareness of this organism in infertility. Screening for *Chlamydia* had always been difficult in many developing countries. This might be due to factors such as high cost and poor awareness about infection. The true incidence of *Chlamydia* infection in developing countries is difficult to establish because of several factors. There is a sociocultural inhibition that prevents women from reporting sexual symptoms, non-availability of facility to detect the organism in many health units and the largely asymptomatic nature of the disease (Westrom *et al.*, 1992; Harry *et al.*, 1994; Okonofua *et al.*, 1995). In spite of these limitations, it is still reported that there is a high prevalence of the *Chlamydia* infection in most parts of Africa (Okonofua, 2003).

The prevalence of *Chlamydia* infections (Mixed and either *C. pneumonia* or *C. trachomatis*) in infertile women in this study was 28%, which indicated high prevalence but lower than the 50% in Controls. This prevalence level is higher than incidence of 18.2% reported by Oloyede *et al.* (2009) in infertile subjects. This is significantly above the range of incidences from other studies that were between 9.33 and 12.0% (Thander

et al., 2001; Sobocinsk *et al.*, 2002; Geisler and James *et al.*, 2008). The results suggests that *Chlamydia* infection may not be a factor responsible for infertility since it is higher in Control who are apparently fertile than infertile women. The incidence of *Chlamydia* in infertile subjects (28%) was less than the Controls (50%) suggesting that *Chlamydia* infection is not a peculiar problem of infertility. The commonest cause of tubal disease in this environment is infection arising from postarbotal, post-partum or sexually transmitted infections (Oloyede and Osagie 2003; Okonofua 2003). However, infection from *Chlamydia* is mainly sexually transmitted, with studies showing a relationship between *Chlamydia* infection and secondary infertility (Okonofua 2003; Okonofua *et al.*, 1995). These infections are easy to diagnose and curable with a single dose of oral antibiotics, early detection and treatment are an important component of efforts to reduce the disease burden. Bamigbowu *et al.* (2011) has shown in their study that even though *Chlamydia* infection is prevalent worldwide, the infection may not necessarily cause reduction in sperm count vis- a- vis infertility in males.

CONCLUSION

In this study, the prevalence of *Chlamydia trachomatis* infection in fertile women showed no significant difference from infertile women; hence, *Chlamydia trachomatis* screening alone should not be a major reason to determine female infertility but individual may be screened to prevent

the organisms from causing future damage to the female reproductive organ, endocrine system which could eventually lead to infertility.

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