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Raised inflammatory markers in semen from men with asymptomatic chlamydial infection

Running title: Interleukin-8 in men with chlamydia

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Abstract

The aim of this study was to determine if interleukin (IL) -6 and IL-8 concentrations, as well as numbers of seminal leukocytes in a population of infertile men, some of whom were *Chlamydia trachomatis* positive, were related to chlamydial infection.

Our patient group included 255 men attending for diagnostic semen analysis as part of infertility investigations. Significantly raised levels of IL-8, but not IL-6, were found in *C.trachomatis*-infected patients but not in uninfected patients. Raised IL-8 levels in semen were also associated with an increase in semen volume. There was a relationship between *C.trachomatis* infection and lower progressive motile sperm as well as an increase in seminal leukocytes. The overall prevalence rate for *C.trachomatis* was 6.2% and more infections were detected in semen than in first void urine.

This study supports the suggestion that IL-8 might be used as a marker for male genital tract infection, especially when due to *C.trachomatis*. In this study there was a relationship between the presence of *C.trachomatis* in semen and alterations of some semen parameters. Further investigations should be performed to understand the disparities of first void urine and semen testing for detection of *C.trachomatis* in males.

Keywords: *Chlamydia trachomatis*/interleukin-8/ interleukin-6/semen quality.

Introduction

Chlamydia trachomatis causes the most prevalent sexually transmitted bacterial infection and affects more than 90 million people annually worldwide (Norman, 2002). In general it is assumed the effect of *C.trachomatis* on female reproduction (Keck et al, 1998) is more important than chlamydial infections in men. In an attempt to link chlamydial infections in man with male reproductive problems, a number of studies have investigated the relationship between infection and semen quality. While some studies have shown that infection is associated with poorer semen quality (Custo et al, 1989; Wolff et al, 1991; Witkin et al, 1995; Cengiz et al, 1997), others have claimed that this is not the case (Gregoriou et al, 1989; Nagy et al., 1989; Eggert-Kruse et al, 1990; Soffer et al, 1990; Dieterle et al, 1995; Eggert-Kruse et al, 1996; Weidner et al, 1996; Eggert-Kruse et al, 1997; Habermann et al, 1999; Hosseinzadeh et al, 2004). However, it is difficult to make like for like comparisons between many of these studies since the methodology for *C.trachomatis* detection as well as the techniques of semen analysis used, are not always comparable (Pacey and Eley, 2004). Irrespective of the findings between chlamydial infection and semen quality, there has been a report stating that the function of human spermatozoa can be significantly affected by direct exposure to *C.trachomatis* (Eley et al., 2005a). This may contribute to sub-fertility in infected individuals by a route that is independent of any damage to the reproductive epithelium (Pacey and Eley, 2004). Other evidence has focused on epididymitis which in young men is often attributable to *C.trachomatis* (Eley et a., 1992). Epididymitis is thought to be important because fertility might be affected due to inflammation and obstruction, especially where both testes are

25 affected (Oriel and Ridgway, 1983). As well as creating a physical blockage to the
26 movement of sperm, *C.trachomatis* can also cause epithelial damage that reduces
27 spermatogenesis, induces immunological responses that destroy or hinder sperm, and
28 reduces the female partner's fertility (Gonzalex et al., 2004).
29
30 Higher numbers of leukocytes (>1×10^6 per ml) are thought to be a sign or marker of
31 microbiologically-induced inflammation (WHO, 1999). Nevertheless, there is much
32 controversy as to whether leukocytospermia is closely related to the presence of
33 pathogenic micro-organisms (Trum et al, 1998) and whether markers such as
34 leukocytospermia can be interpreted as an aid for the diagnosis of infections in
35 asymptomatic patients (Barratt et al, 1990; Eggert-Kruse et al, 1992; Tomlinson et al,
36 1992; Aitken et al, 1995; Kiessling et al, 1995; Yanushpolsky et al, 1996). A number of
37 studies have specifically excluded a correlation between leukocytospermia and semen
38 quality (Eggert-Kruse et al, 2001; Ludwig et al, 2003). However, as the commonly used
39 method for diagnosis of leukocytospermia only detects granulocytes that are intact,
40 degranulated granulocytes could be missed (Kopa et al, 2005) suggesting that additional
41 markers of inflammation such as cytokines in the seminal plasma may be of value in
42 diagnosing infections in semen.
43
44 Cytokines are regulatory proteins produced by leukocytes and other cells that control
45 inflammation. Certain pro-inflammatory cytokines such as interleukin (IL)-6 and IL-8 are
46 involved in inflammatory processes. Previous studies have investigated levels of seminal
47 IL-6 and/or IL-8 in infertile patients and controversy exists as to whether elevated

48 cytokine levels are related to semen quality (Comhaire et al, 1994; Dousset et al, 1997;
49 Eggert-Kruse et al, 2001; Kopa et al, 2005). To our knowledge, there has been no study
50 in which levels of IL-6 and IL-8 have been measured in semen from males infected with
51 *C.trachomatis,* and comparisons made with a group shown not to be infected with
52 chlamydia. This focus on *C.trachomatis* infected patients was suggested by Eggert-Kruse
53 *et al.,* (2001) who found one sub-fertile male who was positive for *C.trachomatis* and had
54 high IL-6 and IL-8 concentrations in seminal plasma. Therefore, the purpose of the
55 present study was to determine concentrations of IL-6 and IL-8 in seminal plasma as well
56 as numbers of seminal leukocytes in male partners of infertile couples, some of whom
57 were *C.trachomatis* positive, and relate the findings to semen quality.

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61

62 Materials and Methods

63

64 Patient recruitment and sample collection

65 A total of 255 consecutive men attending the Avesina Research Institute in Tehran, Iran
66 for diagnostic semen analysis were recruited to the study. Ethical approval for the use of
67 semen samples in this study was granted by the 15th session of the Ethical Committee for
68 Researches in Medical Sciences (Feb 23rd 2004) at the Avesina Research Institute,
69 Tehran. All men were from primary care and undergoing semen analysis as part of a
70 work-up for infertility investigations with their partner after failing to conceive after 1
71 year of unprotected intercourse. None of the men reported any symptoms of genitourinary
72 infections and were therefore considered asymptomatic of sexually transmitted disease.
73 There were no age restrictions for inclusion in the study, although individuals with a

74 history of chemotherapy or radiotherapy treatment, or a vasectomy, or an abnormally low
75 semen volume and with any retrograde ejaculation or hypogonadotropic hypogonadism
76 were excluded.
77
78 Prior to semen analysis, the men were provided with written information on the study and
79 requested to abstain from sexual intercourse for at least 48 h, but no longer than 5 days,
80 before attending the clinic. A record was made of the number of days of abstinence at the
81 time of sample production. All semen samples were produced on site and collected into
82 standard containers previously shown to have no cytotoxic effects on human
83 spermatozoa, according to the methods outlined in WHO (1999). In addition, all men
84 were asked to bring 20ml of first void urine (FVU) in a sterile pot to the clinic for
85 detection of *C.trachomatis*.
86
87 **Semen quality**
88 Immediately following semen production, the sample was placed in an incubator and
89 allowed to liquefy at 37°C for up to 30 min before analysis. Semen analysis was
90 performed according to WHO (1999) guidelines with all measures of semen quality being
91 completed within 1h, apart from sperm morphology which was completed later after
92 slides had been stained. Sperm morphology was observed on Papanicolaou-stained
93 smears by an experienced technician according to WHO (1999) criteria. The presence of
94 leukocytes in semen was determined within 1h by the peroxidase test as recommended by
95 WHO (1999). Peroxidase positive cells (leukocytes) which were brown and round in
96 shape were counted, using a haemocytometer. Throughout the study, the laboratory was a

97 member of an external quality assessment scheme for andrology (UK NEQAS, St.
98 Mary's Hospital, Manchester, U.K.).
99
100 **Sample transportation from Tehran to Sheffield**
101 Following semen analysis, both semen and FVU samples were lyophilized in
102 microcentrifuge tubes and transportation of the lyophilized samples to Sheffield was
103 carried out in carrier vessels (Airsea container Ltd, Birkenhead, U.K.) at room
104 temperature. Lyophilization using an Alpha 1-2 ld plus (Martin Christ, Osterode am Herz,
105 Germany) was carried out according to the manufacturer's instructions. In Sheffield, both
106 semen and FVU samples were reconstituted with sterile, endotoxin-free water based on
107 their initial volume and tested immediately as described below.
108 **Strand Displacement Amplification (SDA) for *C.trachomatis* in semen and urine**
109 Four ml of reconstituted urine or 200µl of reconstituted semen were tested by SDA
110 (Becton Dickinson, Cowley, U.K.) at the Northern General Hospital. Sheffield, U. K. The
111 laboratory is a member of the NEQAS scheme for microbiology and where this test is
112 routinely performed on clinical samples for the Sheffield Teaching Hospitals NHS
113 Foundation Trust. Positive results were confirmed by retesting the sample with the same
114 SDA test. SDA is a DNA amplification system, the BDProbeTec ™ ET, based on
115 simultaneous strand displacement amplification and real-time fluorescence detection. The
116 system uses sealed microwells to minimize release of amplicons to the environment. The
117 following nested PCR confirmatory test was performed as although commercial NAAT's
118 such as SDA have now become the method of choice for routine *C.trachomatis* detection

119 (Hamdad and Orfila, 2005; Gaydos et al., 2008), these methods have rarely been applied
120 to testing of semen.
121

122 **Nested plasmid PCR confirmatory testing for *C.trachomatis* in semen and urine**

123 DNA extraction was carried out on semen and urine samples which tested positive for
124 *C.trachomatis* by SDA, using a QIAamp DNA Mini Kit (Qiagen, Hamburg, Germany)
125 according to the manufacturer's instructions. The extracted DNA was stored at -20°C
126 until nested PCR analysis. Initially the extracted DNA was tested for β-globin according
127 to the method of Saiki *et al*., (1985) to check there were no PCR inhibitors in the
128 samples. When samples were shown to be β-globin positive, they were tested by the
129 nested PCR method using primers directed against the cryptic plasmid, as described
130 previously (Hosseinzadeh et al, 2004). Products were analysed by gel electrophoresis in
131 1.5% agarose with ethidium bromide staining. Each PCR run included *C.trachomatis*
132 DNA as a positive control. The amplicon was sequenced and compared to GenBank
133 accession number 144462.
134

135 **Human interleukin-8 (IL-8) immunoassay on semen samples**

136 Each reconstituted sample of seminal plasma was diluted 1:4 prior to testing. 100µl of
137 each diluted sample were tested using a commercial quantitative sandwich enzyme
138 immunoassay (R+D Systems, Abingdon, U.K.) according to the manufacturer's
139 instructions. A monoclonal antibody specific to IL-8 had been pre-coated onto a
140 microplate. Standards and samples were pipetted into the wells and any IL-8 present
141 bound by the immobilized antibody. After washing to remove any unbound substances,

142 an enzyme-linked polyclonal antibody specific for IL-8 was added to the wells.
143 Following a further washing step to remove unbound antibody-enzyme reagent, a
144 substrate solution was added to the wells for 30 min and colour developed in proportion
145 to the amount of IL-8 bound in the initial step. The colour development was stopped by
146 adding 50µl stop solution to each well and the intensity of colour measured. A standard
147 curve was constructed for each 96 - well plate by plotting the mean absorbance for each
148 standard on the y–axis against the concentration on the x-axis: a best fit curve was drawn
149 through the points on the graph. The minimum detectable level was 1.5pg/ml.
150
151 **Human interleukin-6 (IL-6) immunoassay on semen samples**
152 Each sample of reconstituted seminal fluid was diluted 1:2 prior to testing. 100µl of each
153 diluted sample were tested using a commercial quantitative sandwich enzyme
154 immunoassay (R+D Systems, Abingdon, U.K.) according to the manufacturer's
155 instructions. Essentially the assay method was as described above for IL-8 but a luminol,
156 rather than a colorimetric substrate was used. This necessitated the use of a microplate
157 luminometer to measure the intensity of the light emitted. The minimum detectable level
158 was 0.7pg/ml.
159
160 **Statistical analysis**
161 Data were processed using SPSS 12.0 for Windows (SPSS Inc., Chicago, USA).
162 Independent Student's t-tests or Mann-Whitney tests were employed depending on
163 whether data were normally distributed or not. A P value of <0.05 was accepted as

significantly different. Pearson or Spearman correlation coefficients were appropriately used to investigate the correlation between variables.

Results

A total of 16 subjects (6.2%) were found to be positive for *C.trachomatis* in semen and urine samples and/or in samples positive by SDA and confirmed by PCR (Table 1). Using SDA, 18 (7.1%) subjects gave positive semen samples whilst nine (3.5%) showed positive urine samples. PCR confirmed the SDA results in 16 semen samples with one sample insufficient for testing, and in six of the urine samples with two samples insufficient for testing. None of the samples were identified as positive for Nucleic Acid Amplification Test (NAAT) inhibitors.

The median seminal plasma concentration of IL-8 was 400 (range 70-12,000) pg/ml. Concentrations of >788 pg/ml, defined as high levels of IL-8 (based on 75% percentile) were found in 62 samples. When the median IL-8 concentrations in *C.trachomatis*-infected and non-infected groups were compared, a statistical significant difference (P<0.05) was found (Table 2), with IL-8 concentrations significantly greater in *C. trachomatis*-infected, as compared to uninfected, individuals. In six samples, insufficient semen was available for testing.

The median seminal plasma concentration of IL-6 was 6.1 (range 1-150) pg/ml. Concentrations of >13.5 pg/ml, defined as high levels of IL-6 (based on 75% percentile)

188 were found in 62 samples. When median IL-6 concentrations in *C.trachomatis* infected
189 and non-infected groups were compared, there was no statistical significance between
190 them (Table 2). In six samples, insufficient semen was available for testing.
191
192 A degree of correlation was seen between IL-8 and IL-6 concentrations (r=0.376,
193 P<0.001) with respect to all samples tested. The presence of *C.trachomatis* had no effect
194 on this correlation.
195
196 A bivariate analysis of the independent correlation of different semen parameters with
197 levels of seminal plasma IL-6 and IL-8 found that semen volume was correlated with IL-
198 8 (r=0.18; P<0.01) and leukocyte count was correlated with IL-6 (r=0.51; P<0.01) and
199 IL-8 (r=0.28; P<0.01) respectively. These results are presented in Table 3.
200
201 At semen analysis, it was found that the percent of progressively motile sperm was lower
202 in subjects infected with *C.trachomatis* but that there were no significant differences in
203 the percent of immotile or viable sperm (P<0.05) (Table 4). The age of the subjects, the
204 duration of infertility and days of sexual abstinence were not significantly different
205 between the infected and uninfected groups (Table 4). Interestingly, *C.trachomatis*-
206 infected men had a significantly raised pH in semen (P<0.05) (Table 4), although this
207 difference was small and probably clinically insignificant.
208

209 Table 4 shows that the mean seminal leukocyte counts were significantly ($P<0.05$)
210 greater in subjects showing evidence of infection with *C.trachomatis* ($1.0 \pm 0.6 \times 10^6$
211 leukocytes per ml) in comparison to those who did not ($0.2 \pm 0.6 \times 10^6$ leukocytes per
212 ml). Moreover, 5 of 16 (31.2%) patients with *C.trachomatis* infection, in contrast to only
213 12 of 239 (5.0%) patients without *C.trachomatis* infection, showed leukocytospermia
214 according to the WHO (18) definition.
215

216 **Discussion**
217

218 In a study by Eggert-Kruse *et al.,* (2001), it was observed that in a sole *C.trachomatis*
219 positive, subfertile male, levels of both IL-8 and IL-6 were very high. Therefore, in the
220 current study, IL-8 and IL-6 concentrations were examined in a larger number of
221 *C.trachomatis* positive patients to observe any possible association between
222 *C.trachomatis* infection and raised IL-8 and IL-6 levels in semen. The findings showed
223 significantly raised levels of IL-8, but not IL-6, in semen from patients who were
224 *C.trachomatis* positive. In a recent *in vitro* study (Al-Mously and Eley, 2007), it was
225 suggested that raised IL-6 and IL-8 levels might be useful as a marker for upper genital
226 tract infection, especially prostatitis. Therefore, increased IL-8 levels in *C.trachomatis*
227 infected patients in the current study suggest these infections are more likely derived
228 from the upper genital tract, which correlates with the observation of lower progressively
229 motile sperm observed in *C.trachomatis* infected men. However, the lack of high levels
230 of immotile sperm in the ejaculates of *C.trachomatis*-infected men, suggests that the
231 mechanism of *C.trachomatis* mediated sperm death observed *in vitro* (Hosseinzadeh et al,

232 2001; Hosseinzadeh et al, 2003; Eley et al, 2005b) may differ from that observed *in vivo*
233 in the presence of seminal plasma.
234
235 Controversy exists as to whether elevated cytokine levels are related to semen quality.
236 Previous studies suggested that elevated cytokine levels were not related to semen quality
237 (Comhaire et al, 1994; Dousset et al, 1997; Matalliotakis et al, 1998; Eggert-Kruse et al,
238 2001; Matalliotakis et al, 2002) although more recent studies have supported such a
239 relationship (Furuya et al, 2003; Kopa et al, 2005). The advantage of the present study is
240 that it is the first to specifically investigate levels of both IL-6 and IL-8 in semen from
241 *C.trachomatis* infected and non-infected patients. Of course, it is realised that since other
242 micro-organisms could be present in semen, and that these may also be responsible for
243 altered cytokine levels, this could be a complicating factor in interpretation of the results.
244 However, Eggert-Kruse et al, (2001) found no relationship between a wide range of
245 bacteria present in semen samples and interleukin concentrations and Bezold et al, (2007)
246 showed that pathogen DNA in semen was not associated with inflammatory markers.
247 Nevertheless, the fact that a relationship between raised IL-8 levels and semen volume
248 was found would lean towards our previous findings of increased semen volume levels in
249 *C.trachomatis*-infected patients (Hosseinzadeh et al, 2004), which was not observed in
250 our present study. Not surprisingly, raised IL-6 and IL-8 levels were associated with an
251 increased leukocyte count in semen as both these cytokines have neutrophil chemotactic
252 and activating factors (Eggert-Kruse et al, 2001). No other semen parameters were
253 associated with raised levels of either IL-6 or IL-8.
254

255 In our previous UK study which investigated the presence of *C.trachomatis* in semen of
256 men with asymptomatic chlamydial infection who were undergoing infertility
257 investigations, a prevalence of 4.9% using NAATs was observed (Hosseinzadeh et al,
258 2004). The prevalence rate of 6.2% in the current study involving Iranian men was
259 therefore similar. An earlier study which investigated the presence of *C.trachomatis* using
260 cell culture in male patients attending a Genito-urinary medicine clinic in Tehran showed
261 a prevalence rate of 8.8% (Darougar et al, 1982), while a more recent study conducted on
262 women attending Obstetrics and Gynaecology clinics in Tehran gave an overall
263 prevalence rate of at least 6.4% using both SDA and PCR testing (Chamani-Tabriz,
264 2007). This suggests that the level of *C.trachomatis* infection in adult males and females
265 in Iran is relatively high and comparable to the UK.
266
267 With regard to the relationship between semen parameters and chlamydial infection, the
268 current study found that men infected with *C.trachomatis* had a lower percentage
269 progressive sperm motility, a higher leukocyte count and a raised concentration of IL-8
270 compared to men without infection. In a previous study (Hosseinzadeh et al, 2004), a
271 raised leukocyte count was also observed in semen from patients with a chlamydial
272 infection but no difference was observed in percentage motile sperm. However, the
273 present study is different in that urine was also examined for *C.trachomatis* and a newer
274 molecular method was used to test for *C.trachomatis*. Therefore, direct comparisons with
275 our previous study cannot be made. In the current study a much higher percentage of
276 patients with leukoctyospermia had *C.trachomatis* infection than in those without

277 *C.trachomatis* infection (31.2% v 5.0%, respectively); and these findings are again
278 similar to Hosseinzadeh *et al*., (2004).
279
280 Rather unexpectedly in the current study, using SDA, twice as many semen samples,
281 (n=18), than FVU samples, (n=9), were found to be positive for *C.trachomatis*. This is in
282 spite of there being insufficient semen sample left for two patients to run confirmatory
283 tests (and therefore defined as negative). It is acknowledged that there can be a
284 discrepancy between detection of *C.trachomatis* in FVU and semen from the same patient
285 (Pannekoek et al, 2003; Gdoura et al., 2008). As suggested by Gdoura et al., (2008), the
286 presence of *C.trachomatis* DNA in FVU and its absence in semen may indicate a urethral
287 infection, whereas its presence only in semen may indicate an infection of the epididymis
288 or seminal vesicles. It is also generally believed that a higher number of positive results
289 are found in urine (Pannekoek et al, 2003; Hamdad-Daoudi et al, 2004). However, a
290 recent study by Gdoura *et al*., (2008) showed a good correlation between PCR detection
291 of *C.trachomatis* in FVU and in semen, with a marginally higher proportion of *C.*
292 *trachomatis* positives detected in semen (42.3%) compared to FVU (39.4%). Similarly, in
293 a study by Bornman *et al*., (1998) more semen samples (35/131) were positive for
294 *C.trachomatis* than FVU samples (33/131). These reports, along with the data presented
295 here, therefore raise an important question as to which is the best test specimen in males
296 to look for *C.trachomatis* infection in the genital tract. This is compounded by the fact
297 that unlike FVU, there is no approved method of testing for *C.trachomatis* in semen. It is
298 difficult to fully understand why in our study more patients were positive for
299 *C.trachomatis* in semen than in FVU samples. One suggestion is that despite clear

guidelines, there could have been a misunderstanding of the patients on the strict requirement to collect a urine sample immediately on waking and not at a later time in the morning. However, in the light of the above findings, it is therefore suggested that further comparative studies of *C.trachomatis* testing in semen and FVU be undertaken and efforts made to determine and recommend the best test to detect the presence of *C.trachomatis* in the male.

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498 **Summary sentence**: Interleukin-8 might be used as a marker for male genital tract
499 infection, especially when caused by *C.trachomatis*.

Table 1. SDA and PCR testing to detect genital chlamydial infection in male partners of infertile couples (n=255).

	Positive		Negative	
	Urine	Semen	Urine	Semen
SDA	9	18	246	237
PCR	6 [a]	16 [b]	249	239

[a] 2 samples insufficient for testing

[b] 1 sample insufficient for testing

Table 2. Relationship between the levels of IL8 and IL 6 with genital chlamydial infection in male partners of infertile couples [a] (n=249).

	IL-8			IL-6		
	Chlamydia **infected men (n=16)**	***Chlamydia*** **uninfected men (n=239)**	**Total (n=255)**	***Chlamydia*** **infected men (n=16)**	***Chlamydia*** **uninfected men (n=239)**	**Total (n=255)**
Mean ± SD (pg/ml)	1,457.8 ± 1,778.7	727.1 ± 1,254.4	773.9 ± 1,301.9	22.8 ± 31.2	16.3 ± 23.3	16.7 ± 23.9
Median (pg/ml)	1,000.0 [b]	350.0 [b]	400.0	12.0	6.0	6.1
Interquartiles, 25-75 percentile (pg/ml)	168.8-2,275.0	150.0-637.5	150.0-788.0	2.8-25.0	2.0-12.6	2.0-13.5
Range (pg/ml)	70.0-12,000.0	100.0-7,000.0	70.0-12,000.0	1.0-120.0	1.0-150.0	1.0-15.0

[a] **6 samples insufficient for testing**

[b] **Independent sample *t*-test ($p < 0.05$)**

Table 3. Correlation between different semen parameters with levels of IL-6 and IL-8 in semen (n=249).

Semen parameters	Median (range)	Correlation [a] with IL-6 levels	Correlation [a] with IL-8 levels
Age (years)	34.0 (22.0-57.0)	r= 0.06, p>0.05	r= 0.01, p>0.05
Duration of infertility (years)	4.0 (1.0-32.0)	r= 0.01, p>0.05	r= 0.09, p>0.05
Semen volume (ml)	3.0 (0.1-9.6)	r= -0.093, p>0.05	**r= 0.18, p<0.01**
pH	7.6 (6.8-7.9)	r= 0.054, p>0.05	r= 0.04, p>0.05
Sperm concentration (million/ml)	90.0 (0.008-750)	r= -0.001, p>0.05	r= 0.05, p>0.05
Progressive percent motile	43.0 (0-85.0)	r= 0.06, p>0.05	r= 0.72, p>0.05
Percent immotile	39.0 (7.0-98.0)	r= 0.04, p>0.05	r= 0.12, p>0.05
Percent viable	93.0 (0-99.0)	r= 0.02, p>0.05	r= -0.07, p>0.05
Percent normal morphology	28.0 (1.0-64.0)	r = 0.03 , p>0.05	r= 0.04 , p>0.05
Mean leukocyte count (million/ml)	0.4 (0-5.7)	**r=0.51, p<0.01**	**r =0.28, p<0.01**

[a] Spearman Rank Correlation Test

Table 4. The semen parameters (Mean ± SD) of men with and without *C. trachomatis* infection (n=255).

Semen parameters	*Chlamydia* infected men (n=16)	*Chlamydia* uninfected men (n=239)	Statistical significance [a]
Age (years)	35.5 ± 6.4	34.8 ± 6.0	$p>0.05$
Duration of infertility (years)	8.5 ± 7.1	5.7 ± 5.9	$p>0.05$
Semen volume (ml)	2.9 ± 1.4	3.2 ± 1.4	$p>0.05$
Abstinence (days)	5.3 ± 5.4	4.8 ± 3.3	$p>0.05$
pH	7.7 ± 0.1	7.6 ± 0.5	**$p<0.05$**
Sperm concentration (million/ml)	110.0 ± 68.8	120.0 ± 102.7	$p>0.05$
Progressive percent motile	32.7 ± 20.0	43.4 ± 18.0	**$p<0.05$**
Percent immotile	45.6 ± 20.3	40.3 ± 17.9	$p>0.05$
Percent viable	90.2 ± 8.8	90.4 ± 9.7	$p>0.05$
Percent normal morphology	32.6 ± 9.3	28.0 ± 11.9	$p>0.05$
Leukocyte count (million/ml)	1.0 ± 0.6	0.2 ± 0.6	**$p<0.05$**

[a] Independent student *t*-test