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Research Article

EFFECT OF TORCH AGENTS AND *CHLAMYDIA TRACHOMATIS* ON REPRODUCTIVE PARAMETERS AND FERTILITY HORMONES OF IRAQI INFERTILE MALES

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ABSTRACT

Objective: The aim of this study is to investigate the effect of acute TORCH infection [*Toxoplasma gondii*, *Rubella virus*; cytomegalovirus (CMV), herpes simplex virus 2 (HSV2)] and *Chlamydia trachomatis* on semen parameters and fertility hormones; follicular stimulating hormone (FSH); luteinizing hormone (LH); prolactin; progesterone; testosterone among infertile males.

Methods: Serum and semen samples collected from (155) infertile males. Enzyme-linked immunosorbent assay test used to determine TORCH and *C. trachomatis* immunoglobulin M (IgM), FSH, LH, prolactin; progesterone; testosterone among infertile males. Standard world health organization protocol used to perform semen analysis.

Results: A significant correlation between age group and CMV - IgM, HSV2 - IgM positivity (p=0.05 and 0.024). Type of infertility inversely correlated with *T. gondii* - IgM positivity (p=0.039) and positively correlated with Rubella - IgM (p=0.021). Significant correlation of *T. gondii* - CMV co-infection (p=0.048); *T. gondii* - Rubella co-infection (p=0.007); *T. gondii* - *C. trachomatis* co-infection (p=0.001). Significant correlation between semen parameters and TORCH positivity testosterone level significantly correlated with *T. gondii* - IgM; Rubella - IgM. Inverse significant correlation between *C. trachomatis* - IgM and FSH level. Significant correlations between TORCH - IgM positivity, semen parameters and hormones were following *T. gondii*, CMV, total sperm count and LH. *T. gondii*, Rubella, active motility and LH. *T. gondii*, total sperm count and prolactin. CMV, HSV2, *C. trachomatis* trachomatis, active sperm motility and progesterone. Rubella, sluggish motility and LH level. *T. gondii*; Rubella, sperm dead % and LH. *T. gondii*; sluggish motility and FSH. *T. gondii*; CMV; sluggish motility and FSH. *T. gondii*, sluggish motility and prolactin.HSV2, sluggish motility and progesterone level. *T. gondii*, CMV, HSV2, sperm dead % and progesterone. Sluggish motility, *T. gondii*, *C. trachomatis*, HSV2 and testosterone. *T. gondii*, *C. trachomatis*, sperm dead % and testosterone. *C. trachomatis*, HSV2; normal sperm morphology and LH. *T. gondii*, normal sperm morphology and prolactin level. HSV2, normal sperm morphology and testosterone. *T. gondii*, abnormal sperm morphology and prolactin. *C. trachomatis*, abnormal sperm morphology and LH.

Conclusions: A current study proved several important and outstanding results about the role of TORCH agent and *C. trachomatis* in male infertility. TORCH agent and *C. trachomatis* have direct effects on sperm parameters. *T. gondii* and Rubella have a direct effect on testosterone levels; *C. trachomatis* has direct effect on FSH among infertile males. TORCH agent plays a vital role in hormonal disturbances that lead to sperm abnormalities and subsequently to infertility problem.

Keywords: TORCH, *Chlamydia trachomatis,* Semen parameters, Fertility hormones.

INTRODUCTION

Infertility, a major problem of modern medicine, is defined as the inability of sexually active couples to achieve pregnancy after 12 months [1]. Infertility affects nearly 20% of reproductive-aged couples, in which 40-50% of these cases are male infertility [2]. The major causes of male infertility include varicocele, endocrine disturbances, immunological conditions, genital duct obstruction, gonadotoxins, medications, cryptorchidism, infection, sexual dysfunction, and ejaculatory failure [1].

About 70% of cases with male infertility are idiopathic (of unknown etiology) [1]. Several microorganisms such as *Chlamydia trachomatis*, gonococcus, mycoplasma and herpes simplex viruses (HSV), cytomegalovirus (CMV), and *Toxoplasma gondii* can colonize in the male genital tract [3-5]. Seminal tract infections may play a contributing role in male infertility. Infections impair fertility by different mechanisms including damaging spermatogenesis, impairment of sperm function, and obstruction of the seminal tract [6,7].

There is increasing evidence that viral and *C. trachomatis* infections play a role in the pathogenesis of male infertility [8]. Infections impair male fertility either by directly invading the male genital tract cells or by indirectly causing local inflammatory or immunological responses that could deteriorate reproductive functions [1,6]. In addition, pro-inflammatory cytokines and reactive oxygen species (ROS) may play an important role in infertility [9]. ROS may damage fertility by decreasing polyunsaturated fatty acid on sperm membrane including DNA damage and impairing acrosomal reaction [1].

The aim of this study is to investigate the effect of acute TORCH infection and *C. trachomatis* in semen parameters and fertility hormones; follicular stimulating hormone (FSH); luteinizing hormone (LH); prolactin; progesterone; testosterone among infertile males. As well as the correlation between semen parameters, hormonal level and TORCH infection and *C. trachomatis* immunoglobulin M (IgM) positivity.

METHODS

This cross-sectional study was performed on 155, age range 17-55 years, mean (32.98±9.16) years infertile males which visited private and public infertility clinics in Baghdad during the period from September 2014 to march 2015. This study was conducted according to the principles of Helsinki declaration. A full explanation about the purpose of this study to all patients was done. Dully-filled consent form obtained from all patients that agree to participate in the study. Approval of Ethical Review Committee of College of Medicine, Diyala University, Iraq, was taken before initiation of the work.

Blood collection

About 5 mL of blood were collected by vein puncture using syringe with needle gauge 23, transported to unheparinized tube and allowed to

clot at room temperature and sera were separated by centrifugation at 1500 g for 5 minutes, then stored and frozen at (−20°C) [10].

Serological tests
Enzyme-linked immunosorbent assay test for detection of *T. gondii* - IgM product code, BC1087, CMV - IgM product code, BC1091, Rubella - IgM product code, BC1083, HSV2 product code, BC1099 was run for all the samples using ELISA Kits (BioCheck, Inc., USA) [11] and, *C. trachomatis* trachomatis IgM product code (EIA-3463) from DRG [12]. Final results were recorded by ELISA reader (optical absorbance, OD=450).

ELISA kits from Monobilds Inc. USA [13] for hormonal assays were used to determine the levels of FSH (product code: 425-300), LH (product code: 625-300), prolactin (product code: 7225-300), progesterone (product code: 4825-300), and testosterone (product code: 3725-300) in serum samples of all patients.

According to these kit, the normal level of FSH in men serum should be 1-14 IU/ml, LH 0.7-7.4 IU/ml, prolactin 1.8-17 ng/ml, progesterone 0.13-1.22 ng/ml, and testosterone 2.5-10 ng/ml. Hence, patients less or more than this level were considered as abnormal.

Semen collection
These men samples collected after a minimum of 3 days of sexual abstinence, and then every patient was given a clean, wide mouth, sterile, dry, graduated plastic, and warm disposable container. The container was labeled with the man's name, identification number, and the date and time of collection. After masturbation, the seminal fluid samples were immediately incubated at 37°C, and waiting for complete liquefaction (during 30 minutes).

Semen analysis
After 60 minutes for liquefaction of the sample, sperms motility, morphology viability, and count of Toxoplasma seropositive infertile men were assessed according to WHO standard procedures [14].

Preparation for routine semen analysis
A fixed volume of semen (10 µl) was delivered onto a clean glass slide with a micropipette and covered with a 22 mm × 22 mm cover slip. It is important that the volume of semen and the dimensions of the cover slip are standardized so that the analysis was carried out in a preparation of fixed depth (i.e., about 20 µm). This depth allows full expression of the rotation movement of normal spermatozoa. The weight of the cover slip spreads the sample for optimum viewing. The freshly made wet preparation was left to stabilized for 1 minute, and the preparation was then examined at magnification of ×40.

Sperms motility
A standard volume of semen (10 µ) was placed onto a clean glass slide which was examined with phase (contrast optics at ×200 or ×400 magnifications). According to WHO the following types of motility reported in percentage after counting of 100 spermatozoa, rapid progressive (PR) motility, slow or sluggish PR motility, non PR motility, immobility. Men with PR motility less than 32% were suffered from abnormality in their sperms motility.

Sperms count
The concentration of spermatozoa was determined using hemocytometer method WHO, 1992). In this procedure, a 1:20 dilution was made from each well-mixed sample by diluting 50 µl of liquefied semen with 950 µl of a diluent (sodium bicarbonate 50 g, formalin 35% [V/V] 10 ml, saturated aqueous gentian violate [5 ml]).

If the preliminary examination of the semen indicates that the concentration of spermatozoa present was either excessively high or low, then the extent to which the sample is diluted should be adjusted accordingly. For samples containing less than 20×10^6 spermatozoa/ml, a 1:10 dilution was used. For samples containing more than 100×10^6 spermatozoa per ml, 1:50 dilution was appropriate.

The diluted specimen should be thoroughly mixed, and a drop (10-20 µl) was transferred to each chamber of an improved neubauer hemocytometer and covered with cover glass. The hemocytometer was allowed to stand for about 5 minutes in humid chamber to prevent drying out.

The cell sedimented during this time and was then counted only normal mature spermatozoa with tails were counted under microscope at ×40. One chamber grid by grid was examined and continued counting until at least 200 spermatozoa had been observed. The lower reference limits for sperms concentration is 15×10^6 spermatozoa per ml.

Sperms morphology
Preparing a smear of semen on a slide, then air (drying, fixing and staining with Papanicolaou stain the slide were done. The slide was examined with bright field optics at ×1000 magnification. The lower reference limit for normal forms is 4%. Sperms with abnormal shape more than 4% were considered abnormal.

Sperm viability (vitality)
Sperm vitality test performed in the case of low progressively motile sperms percentage, e.g. 30-40%. This test is important to determine if the non-motile spermatozoa are alive or dead. Live spermatozoa characterized an intact cell membrane. Dye exclusion method used for studying of sperm viability. If sperms were died, this mean the plasma membrane was damaged which facilitate the entrance of the stain inside the cells. Therefore, viable cells will not appear stained, but non-viable cells will take up the stain.

To assess viability, a drop of liquefied semen placed on a clean slide. An equal volume of a vital stain (trypan blue) was added then covered with a coverslip. Allow color to develop for 5 minutes. Count 100 cells (both motile and non-motile cells). During the count, differentiate between the non-stained cells (living) and stained cells (non-living).

Statistical analysis
Frequency of variables express as percentage, Pearson test and spearman's test for correlation were used for non-categorical and categorical data. Chi-square test used to compare the semen parameters according to TORCH and *C. trachomatis* IgM. The level of significance was 0.05 (two-tail) in all statistical testing; significant of correlations (Pearson, spearman) include also 0.01 (two-tail). Statistical analysis was performed using SPSS for windows TM version 17.0 and Microsoft Excel for windows 2010.

RESULTS

In current study, 155 infertile males fully investigated by semen analysis and hormonal assays. As shown in Table 1, the frequency of age groups as following: 7.74% positive to *T. gondii* belong to the age group of 41-48 years, followed by 33-40 years (5.80%), and finally among 49-56 years (0.64%). Significant differences in *T. gondii* - IgM positivity among age groups (p=0.000). No significant correlation between age group and *T. gondii* - IgM positivity (p=0.529). CMV - IgM positive detected among 18.06% of 25-32 and 33-40 years; 11.61% of 41-48 years. No significant differences in CMV - IgM positivity among age groups (p=0.475). Significant correlation between age group and CMV - IgM positivity (p=0.05). Rubella - IgM positive detected among 10.97% of 33-40 years, followed by 9.03% of 25-32 years; 8.39% of 17-24 years. No significant differences in Rubella - IgM positivity among age groups (p=0.144). No significant correlation between age group and Rubella - IgM positivity (p=0.201). HSV2 - IgM positive detected among 14.84% of 25-32 years, 10.32% of 17-24 and 33-40 years, and finally 0.64% of 49-56 years. No significant differences in HSV2 - IgM positivity among age groups (p=0.064). Significant correlation between age group and HSV2 - IgM positivity (p=0.024). *C. trachomatis* - IgM positive detected among 23.87% of 25-32 years, 12.26% of 33-40 years, and finally in 3.87% of 49-56 years. Significant differences in *C. trachomatis* - IgM positivity among age groups (p=0.023). No significant correlation between age group and *C. trachomatis* - IgM positivity (p=0.657).

As shown in Table 2, *T. gondii* - IgM positive cases detected among 22.58% with primary versus (0.64%) secondary infertility. Significant differences and inverse correlation between *T. gondii* - IgM positivity and type of infertility (p=0.039). CMV - IgM positive cases detected among 58.06% with primary versus (5.81%) secondary infertility. Significant differences in CMV - IgM positivity and type of infertility (p=0.053). No significant correlation between CMV - IgM positivity and type of infertility (p=0.060). Rubella - IgM positive cases detected among 25.16% with primary versus (7.1%) secondary infertility. Significant differences and correlation between Rubella - IgM positivity and type of infertility (p=0.021). HSV2 cases detected among 34.84% with primary versus (5.81%) secondary infertility. Neither significant differences nor correlation between HSV - IgM positivity and type of infertility (p=0.424 and 0.673). *C. trachomatis* - IgM positive cases detected among 52.26% with primary versus (5.81%) secondary infertility. Neither significant differences nor correlation between *C. trachomatis* - IgM positivity and type of infertility (p=0.153 and 0.207).

As shown in Table 3, a significant correlation between acute *T. gondii* - IgM positive cases and CMV - IgM positive cases. Inverse significant correlation between *T. gondii* - IgM positive cases and Rubella - IgM positive cases (p=0.007); *C. trachomatis* - IgM positive cases (p=0.001).

As shown in Table 4, *T. gondii* - CMV co-infection detected in 18.06% with significant correlation (p=0.048). *T. gondii* - Rubella co-infection detected in 3.23% with significant correlation (p=0.007). *T. gondii* - HSV 2 co-infection detected in 9.03% without significant correlation (p=0.808). *T. gondii* - *C. trachomatis* co-infection detected in 7.74% with significant correlation (p=0.001).

As shown in Table 5, significant correlation between *T. gondii* - IgM positivity; sperm dead % (p=0.022); sperm normal morphology % (p=0.000). Inverse significant correlation between *T. gondii* - IgM positivity and total sperm count (p=0.054); sperm sluggish motility (p=0.029); sperm abnormal morphology (p=0.000).

As shown in Table 5, significant correlation between CMV - IgM positivity; sperm dead % (p=0.004); sperm normal morphology (p=0.016). Inverse correlation between CMV - IgM positivity; sperm active motility (p=0.006);

Table 1: Correlation between TORCH - *C. trachomatis* and age groups of 155 infertile males

Age group (years)	*T. gondii* - IgM positive (%)	CMV - IgM positive (%)	Rubella - IgM positive (%)	HSV2 - IgM positive (%)	*C. trachomatis* - IgM positive (%)
17-24	7 (4.52)	17 (10.97)	13 (8.39)	16 (10.32)	13 (8.39)
25-32	7 (4.52)	28 (18.06)	14 (9.03)	23 (14.84)	37 (23.87)
33-40	9 (5.80)	28 (18.06)	17 (10.97)	16 (10.32)	19 (12.26)
41-48	12 (7.74)	18 (11.61)	2 (1.29)	7 (4.52)	15 (9.68)
49-56	1 (0.64)	8 (5.16)	4 (2.58)	1 (0.64)	6 (3.87)
Total	36 (23.23)	99 (63.87)	50 (32.26)	63 (40.65)	90 (5.81)
	T. gondii - IgM negative	CMV - IgM negative	Rubella - IgM negative	HSV2 - IGM negative	*C. trachomatis* - IgM negative
17-24	23 (14.84)	13 (8.39)	17 (10.97)	14 (9.03)	17 (10.97)
25-32	44 (28.39)	23 (14.84)	37 (23.87)	28 (18.06)	14 (9.03)
33-40	32 (20.64)	13 (8.39)	24 (15.48)	25 (16.13)	22 (14.19)
41-48	10 (6.45)	4 (2.58)	20 (12.90)	15 (9.68)	7 (4.52)
49-56	10 (6.45)	3 (1.94)	7 (4.52)	10 (6.45)	5 (3.23)
Total	119 (76.77)	56 (36.13)	105 (67.74)	92 (59.35)	65 (41.94)
χ^2 value	67.631	32.845	41.612	46.164	51.142
p value	0.000	0.475	0.144	0.064	0.023
Pearson correlation value	0.051	0.153 0.157*	−0.103	−0.181	0.036
p value	0.529	0.057 0.050*	0.201	0.024	0.657

*Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed). *C. trachomatis: Chlamydia trachomatis, T. gondii: Toxoplasma gondii,* IgM: Immunoglobulin M, CMV: Cytomegalovirus, HSV2: Herpes simplex virus 2

Table 2: Correlation between TORCH - *C. trachomatis* and type of infertility among 155 infertile males

Parameters	Type of infertility		χ^2	p value	r	p value
	Primary	Secondary				
T. gondii - IgM						
Negative	100 (64.52)	19 (12.26)	4.278	0.039	−0.166	0.039
Positive	35 (22.58)	1 (0.64)				
CMV - IgM						
Negative	45 (29.03)	11 (7.1)	3.455	0.053	−0.161	0.060
Positive	90 (58.06)	9 (5.81)				
Rubella - IgM						
Negative	96 (61.93)	9 (5.81)	5.435	0.021	0.187	0.02
Positive	39 (25.16)	11 (7.1)				
HSV2 - IgM						
Negative	81 (52.26)	11 (7.1)	0.181	0.424	0.034	0.673
Positive	54 (34.84)	9 (5.81)				
C. trachomatis - IgM						
Negative	54 (34.84)	11 (7.1)	1.610	0.153	0.102	0.207
Positive	81 (52.26)	9 (5.81)				

*Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed). *C. trachomatis: Chlamydia trachomatis, T. gondii: Toxoplasma gondii,* IgM: Immunoglobulin M, CMV: Cytomegalovirus, HSV2: Herpes simplex virus 2, r: Rho

sperm abnormal morphology (p=0.016). No significant correlation between Rubella - IgM positivity and semen parameters. Significant correlation between HSV - IgM positivity and sperm normal morphology % (p=0.050). Inverse significant correlation between HSV - IgM positivity and sperm abnormal morphology % (p=0.050). Significant correlation between *C. trachomatis* - IgM positivity and sperm abnormal morphology % (p=0.004). Inverse significant correlation between HSV - IgM positivity and sperm normal morphology % (p=0.004).

As shown in Table 6, normal LH level detected in 16.77% of *T. gondii* - IgM positive cases; above normal LH (1.93%). No significant correlation between *T. gondii* - IgM positive and LH levels (p=0.567). Normal LH level among CMV - IgM positive cases (42.58%) of CMV - IgM positive cases; above normal LH (7.1%). Significant correlation between CMV - IgM positive and LH levels (p=0.03). Rubella - IgM positive cases with LH normal level (18.06%), above normal level of LH (9.68%) without significant correlation between IgM positivity and LH levels (p=0.773). HSV2 - IgM positive cases with normal LH level (22.58%), above normal levels (12.26%) without significant correlation (p=0.703). *C. trachomatis* - IgM positive cases with normal LH level (29.03%), with above normal level (11.61%) without significant correlation between IgM positivity and LH levels (p=0.238).

Normal FSH level detected in 31.30% of *T. gondii* - IgM positive cases with No significant correlation between *T. gondii* - IgM positive and LH levels (p=0.134). Normal FSH level among CMV - IgM positive cases (61.94%) 1.94% was above normal FSH with no significant correlation between CMV - IgM positive and FSH levels (p=0.291). Rubella - IgM positive cases with FSH normal level was 30.97%, above normal level of FSH (1.29%) without significant correlation between IgM positivity and

Table 3: Co-infections with TORCH - *C. trachomatis* among 155 infertile males

Parameter	Spearman's correlation	CMV - IgM	Rubella - IgM	HSV2 - IgM	*C. trachomatis* - IgM
T. gondii - IgM	r	0.159*	−0.216**	−0.020	−0.276**
	p value	0.048	0.007	0.808	0.001
CMV - IgM	r		−0.027	0.021	−0.040
	p value		0.740	0.797	0.618
Rubella - IgM	r			−0.093	−0.085
	p value			0.248	0.294
HSV2 - IgM	r				−0.095
	p value				0.238

*Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed). *C. trachomatis: Chlamydia trachomatis, T. gondii: Toxoplasma gondii,* IgM: Immunoglobulin M, CMV: Cytomegalovirus, HSV2: Herpes simplex virus 2, r: Rho

Table 4: Coinfections of *T. gondii* with Rubella, HSV2 and *C. trachomatis* among 155 infertile males

Parameter	*T. gondii* - IgM		Total	χ^2 value	p value	Pearson's correlation (R) value	P value
	Negative (%)	Positive (%)					
CMV - IgM							
Negative	48 (30.97)	71 (45.81)	119 (76.77)	3.930	0.047	0.159	0.048
Positive	8 (5.16)	28 (18.06)	36 (23.23)				
Rubella - IgM							
Negative	74 (47.74)	45 (29.03)	119 (76.77)	7.241	0.007	−0.216	0.007
Positive	31 (20)	5 (3.23)	36 (23.23)				
HSV2 - IgM							
Negative	70 (45.16)	49 (31.61)	119 (76.77)	0.060	0.807	−0.020	0.808
Positive	22 (14.19)	14 (9.03)	36 (23.23)				
C. trachomatis - IgM							
Negative	41 (26.45)	78 (50.32)	119 (76.77)	11.778	0.001	−0.276	0.001
Positive	24 (15.48)	12 (7.74)	36 (23.23)				

*Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed). *C. trachomatis: Chlamydia trachomatis, T. gondii:* Toxoplasma gondii, IgM: Immunoglobulin M, CMV: Cytomegalovirus, HSV2: Herpes simplex virus 2

Table 5: Correlation between TORCH - *C. trachomatis* agents and semen parameters among 155 infertile males

Parameters	Active motility	sluggish Motility	Dead	Normal morphology	Abnormal morpholog	Total count
T. gondii - IgM						
r	−0.125	−0.176*	0.183*	0.284**	−0.284**	−0.155
p value	0.121	0.029	0.022	0.000	0.000	0.054
CMV - IgM						
r	−0.221**	−0.139	0.228**	0.192*	−0.192*	−0.072
p value	0.006	0.085	0.004	0.016	0.016	0.374
Rubella - IgM						
r	−0.002	0.116	−0.051	0.003	−0.003	−0.032
p value	0.983	0.149	0.529	0.968	0.968	0.695
HSV2 - IgM						
r	−0.074	−0.129	0.119	−0.158	0.158	−0.027
p value	0.359	0.110	0.139	0.050	0.050	0.739
C. trachomatis - IgM						
r	0.005	−0.015	−0.007	−0.228**	0.228**	−0.026
p value	0.948	0.855	0.931	0.004	0.004	0.751

*Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed). *C. trachomatis: Chlamydia trachomatis, T. gondii: Toxoplasma gondii,* IgM: Immunoglobulin M, CMV: Cytomegalovirus, HSV2: Herpes simplex virus 2, r: Rho

Table 6: Correlation between TORCH - *C. trachomatis* agents and fertility hormones among 155 infertile males

Parameters	*T. gondii* - IgM positive (%)	CMV - IgM positive (%)	Rubella - IgM positive (%)	HSV2 - IgM positive (%)	*C. trachomatis* - IgM positive (%)
LH (IU/ml)					
Under normal <0.7	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Normal 0.7-7.4	26 (16.77)	66 (42.58)	28 (18.06)	35 (22.58)	54 (29.03)
Above normal >7.4I	3 (1.93)	11 (7.1)	15 (9.68)	19 (12.26)	18 (11.61)
Not detected	7 (4.51)	22 (14.19)	7 (4.52)	9 (5.81)	18 (11.61)
r	0.046	0.101 (0.167*)	0.023	−0.031	−0.095
p value	0.567	0.211 (0.03*)	0.773	0.703	0.238
FSH (IU/ml)					
Under normal <1	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Normal 1-14	36 (31.30)	96 (61.94)	48 (30.97)	62 (40)	89 (57.42)
Above normal >14	0 (0.0)	3 (1.94)	2 (1.29)	1 (0.64)	1 (0.64)
r	0.121	−0.085	0.116	−0.058	−0.176*
p value	0.134	0.291	0.151	0.473	0.028
Prolactin (ng/ml)					
Under normal <1.8	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Normal 1.8-17	14 (9.03)	39 (25.16)	24 (15.48)	26 (16.77)	42 (27.1)
Above normal >17	21 (24.14)	59 (38.06)	25 (16.13)	37 (23.87)	47 (30.32)
Not detected	1 (0.64)	1 (0.64)	1 (0.64)	0 (0.0)	1 (0.64)
r	0.045	−0.016	−0.090	−0.045	−0.083
p value	0.579	0.847	0.267	0.580	0.306
Progesterone (ng/ml)					
Under normal <0.13	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Normal 0.13-1.22	4 (2.58)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Above normal >1.22	2 (1.29)	11 (7.1)	5 (3.23)	6 (3.87)	5 (3.23)
Not detected	30 (19.35)	87 (56.13)	45 (29.03)	57 (36.77)	85 (54.84)
r	0.001	−0.066	−0.112	0.089	0.141
p value	0.990	0.412	0.166	0.271	0.079
Testosterone (ng/ml)					
Under normal <2.5	0 (0.0)	7 (4.51)	0 (0.0)	7 (4.52)	5 (3.23)
Normal 2.5-10	28 (18.06)	79 (50.97)	39 (25.16)	50 (32.26)	66 (42.58)
Above normal >10	3 (1.93)	4 (2.58)	4 (2.58)	1 (0.64)	10 (6.45)
Not detected	1 (0.64)	8 (5.16)	7 (4.51)	5 (3.23)	5 (3.23)
r	−0.174*	−0.134	0.259**	−0.120	−0.013
p value	0.031	0.095	0.001	0.137	0.868

* Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed). *C. trachomatis: Chlamydia trachomatis, T. gondii: Toxoplasma gondii,* IgM: Immunoglobulin M, CMV: Cytomegalovirus, HSV2: Herpes simplex virus 2, FSH: Follicular stimulating hormone, LH: Luteinizing hormone, r: Rho

FSH levels (p=0.151). HSV2 - IgM positive cases with normal FSH level (40%), above normal levels (0.64%) without significant correlation (p=0.473).

C. trachomatis - IgM positive cases at normal FSH level (57.42%); above normal level (0.64%), with significant correlation between IgM positivity and FSH levels (p=0.028). Normal prolactin level detected in 9.03% of *T. gondii* - IgM positive cases, above normal levels (24.14%) with no significant correlation between *T. gondii* - IgM positive and prolactin levels (p=0.579). Normal prolactin level among CMV - IgM positive cases (25.16%) 38.06% was above normal prolactin with no significant correlation between CMV - IgM positive and prolactin level (p=0.847). Rubella - IgM positive cases with prolactin normal level was 15.48%, above normal level of prolactin (16.13%) without significant correlation between IgM positivity and prolactin level (p=0.267). HSV2 - IgM positive cases with normal prolactin level (16.77%), above normal levels (23.87%) without significant correlation (p=0.580). *C. trachomatis* - IgM positive cases with normal prolactin level (27.1%), with above normal level (30.32%) with a significant correlation between IgM positivity and prolactin levels (p=0.306).

Normal progesterone level detected in 2.58% of *T. gondii* - IgM positive cases; above normal levels (1.29%) with no significant correlation between *T. gondii* - IgM positive and progesterone level (p=0.990). Above normal progesterone level among CMV - IgM positive cases (7.1%) with no significant correlation between CMV - IgM positive and progesterone level (p=0.412).

Rubella - IgM positive cases with progesterone above normal level was 3.23%, without significant correlation between IgM positivity and progesterone levels (p=0.166). HSV2 - IgM positive cases with above normal progesterone level (3.87%), without significant correlation (p=0.271). *C. trachomatis* - IgM positive cases with above normal progesterone level (3.23%), without significant correlation between IgM positivity and prolactin level (p=0.079).

Normal testosterone level detected in 18.06% of *T. gondii* - IgM positive cases, above normal level (1.93%) with significant correlation between *T. gondii* - IgM positive and testosterone levels (p=0.031). under normal testosterone level among CMV - IgM positive cases (4.51%) normal level in 50.97%, above normal level (5.16%) with no significant correlation (p=0.095). Rubella - IgM positive cases with testosterone normal level was 25.16%, above normal (2.58%) with significant correlation (p=0.001). HSV2 - IgM positive cases with under normal testosterone level (3.23%), normal (42.58%), above normal (6.45%) without significant correlation (p=0.271). *C. trachomatis* - IgM positive cases with above normal testosterone level (3.23%), without significant correlation (p=0.868).

As shown in Table 7, significant correlations between *T. gondii*, CMV - IgM positivity and total sperm count according to LH (p=0.003, 0.024).Significant correlations between *T. gondii*, Rubella - IgM positivity and active motility count according to LH (p=0.024 and 0.042). No significant correlations between TORCH agents, total sperm count; active motility and LH level. Significant correlations between *T. gondii* - IgM positivity; total sperm count according to prolactin (p=0.038). Inverse Significant correlations between CMV, HSV2, *C. trachomatis* IgM positivity; Active sperm motility and progesterone (p=0.002, 0.019, and 0.023). No significant correlations between TORCH agents, total sperm count; active motility and testosterone level.

Significant correlations between Rubella - IgM positivity, sluggish motility and LH level (p=0.041). Inverse correlations between *T. gondii*; Rubella - IgM positivity, sperm dead % and LH (p=0.021; 0.016). Significant correlations between *T. gondii* - gM positivity, sluggish motility and FSH level (p=0.042). Significant correlations between *T. gondii* - IgM positivity, Sluggish motility and FSH (p=0.042). Significant correlations between *T. gondii* - IgM positivity, sluggish motility and prolactin (p=0.032). Inverse significant correlation between HSV2,

Table 7: Correlation between semen parameters and serum levels of fertility hormones in 155 infertile males with positive TORCH - *C. trachomatis* agents

Parameters	*T. gondii* - IgM positive	CMV - IgM positive	Rubella - IgM positive	HSV2 - IgM positive	*C. trachomatis* - IgM positive	*T. gondii* - IgM positive	CMV - IgM positive	Rubella - IgM positive	HSV2 - IgM positive	*C. trachomatis* - IgM positive
	Total count					**Active motility**				
LH										
r	0.478	0.226	0.006	0.013	0.037	0.113 0.375*	0.031	0.067 0.288*	−0.050	−0.052
p value	0.003	0.024	0.969	0.920	0.730	0.514 0.024*	0.758	0.642 0.042*	0.700	0.628
FSH										
r	0.150	0.115	−0.032	0.150	0.052	0.303	0.146	0.008	0.228	0.001
p value	0.381	0.257	0.828	0.241	0.625	0.072	0.149	0.956	0.073	0.993
Prolactin										
r	0.347	0.076	−0.115	0.111	0.085	0.214	0.023	0.026	0.135	0.025
p value	0.038	0.453	0.427	0.386	0.425	0.209	0.818	0.862	0.291	0.818
Progesterone										
r	−0.099	−0.006	0.026	−0.096	−0.092	−0.298	−0.310	−0.158	−0.294	−0.240
p value	0.567	0.953	0.858	0.454	0.386	0.077	0.002	0.274	0.019	0.023
Testosterone										
r	0.054	−0.030	0.079	−0.079	−0.089	0.066	−0.023	0.019	0.071	−0.048
p value	0.753	0.769	0.586	0.540	0.403	0.703	0.819	0.897	0.582	0.652
	Sluggish motility					**Dead%**				
LH										
r	0.216	−0.013	0.179 0.290*	−0.129	−0.083	−0.193 −0.383*	−0.010	−0.142 −0.338*	0.104	0.081
p value	0.205	0.895	0.215 0.041*	0.312	0.435	0.260 0.021*	0.921	0.325 0.016*	0.419	0.449
FSH										
r	0.341	0.173	0.112	0.095	0.040	−0.388	−0.192	−0.068	−0.193	−0.023
p value	0.042	0.088	0.437	0.459	0.707	0.019	0.057	0.639	0.129	0.832
Prolactin										
r	0.326 0.358*	0.054	0.031	0.010	−0.073	−0.320	−0.047	−0.033	−0.088	0.023
p value	0.053* 0.032	0.598	0.829	0.940	0.495	0.057	0.645	0.818	0.492	0.830
Progesterone										
r	−0.272	−0.162	−0.041	−0.262	−0.026	0.347	0.282	0.121	0.328	0.179
p value	0.108	0.110	0.778	0.038	0.805	0.038	0.005	0.403	0.009	0.092
Testosterone										
r	0.413	0.118	−0.037	0.275	0.154 0.301*	−0.268 −0.423*	−0.059	0.009	−0.199	−0.051 −0.204*
p value	0.012	0.246	0.801	0.029	0.148 0.004*	0.114 0.010 *	0.561	0.951	0.118	0.634 0.054*
	Normal morphology					**Abnormal morphology**				
LH										
r	0.196	0.220	0.026	0.320	0.236	−0.196	−0.220	−0.026	−0.320	−0.236
p value	0.251	0.029	0.855	0.011	0.025	0.251	0.029	0.855	0.011	0.025
FSH										
r	0.143	0.053	−0.004	0.204	0.168	−0.143	−0.053	0.004	−0.204	−0.168
p value	0.407	0.600	0.981	0.109	0.113	0.407	0.600	0.981	0.109	0.113
Prolactin										
r	0.358	0.107	0.067	−0.070	0.155	−0.358	−0.107	−0.067	0.070	−0.155
p value	0.032	0.294	0.643	0.584	0.144	0.032	0.294	0.643	0.584	0.144
Progesterone										
r	−0.194	−0.073	0.165	−0.129	0.179	0.194	0.073	−0.165	0.129	−0.179
p value	0.258	0.475	0.253	0.314	0.090	0.258	0.475	0.253	0.314	0.090
Testosterone										
r	−0.118	0.131	0.229	0.245	0.015	0.118	−0.131	−0.229	−0.245	−0.015
p value	0.492	0.198	0.110	0.053	0.891	0.492	0.198	0.110	0.053	0.891

*Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed). *C. trachomatis: Chlamydia trachomatis, T. gondii: Toxoplasma gondii*, IgM: Immunoglobulin M, CMV: Cytomegalovirus, HSV2: Herpes simplex virus 2, FSH: Follicular stimulating hormone, LH: Luteinizing hormone, r: Rho

sluggish motility and progesterone level (p=0.038). Significant correlations between *T. gondii*, CMV, HSV2 - IgM positivity, sperm dead % and progesterone level (p=0.038, 0.005, and 0.009). Significant correlations between sluggish motility, *T. gondii*, *C. trachomatis*, HSV2 and testosterone levels (p=0.012, 0.029, and 0.004).

Inverse significant correlation between *T. gondii*, *C. trachomatis* sperm dead % and testosterone levels (p=0.010 and 0.054). Significant correlations between *C. trachomatis* HSV2; normal sperm morphology and LH level (p=0.011 and 0.025). Significant correlations between *T. gondii* - IgM positivity, normal sperm morphology and prolactin level (p=0.0.032). Significant correlations between HSV2 - IgM positivity, normal sperm morphology and testosterone level (p=0.0.053). Inverse significant correlation between *T. gondii*, abnormal sperm morphology and prolactin (p=0.0.032). Inverse significant correlation between *C. trachomatis*, abnormal sperm morphology and LH (p=0.0.025).

DISCUSSION

In current study, 155 infertile males were fully investigated by semen analysis and hormonal assays. *T. gondii* - IgM detected in 23.23% of infertile males which come in contrary with previous report determine IgM positive - *T. gondii* (62.96%, 2.5%, and 2%) [15-17] of infertile males.

The current study revealed significant differences in *T. gondii* - IgM positivity among age groups.No significant correlation between age group and *T. gondii* - IgM positivity. These results come in concordance with local study achieved among blood donor in Baghdad [18] and includes approximately similar age groups with obvious differences in frequency of IgM positive cases among selected groups. The current study revealed increasing in the incidence of acute toxoplasmosis among age under 20 years to under 50 years, which come in line with others [19,20]. In current study, total number of *T. gondii* - IgM positive cases (23.2%). This indicates that the presence of *T. gondii* infection among infertile males was not rare, and this agree with other studies [5,21].

In current study, CMV - IgM positivity detected in 63.87% of infertile males which considered relatively high compared with previous studies in Iraq, in Babylon (32-33.1%) [17,22], Najaf (11%) [23], and in Sudan (8.7%) [24]. With respect to age groups of this study are consistent with previous studies that the infection get clearly in ages from 20 to 40 years with obvious differences in incidence according to study design [24]. In current study, CMV - IgM positivity detected mainly among 25-32, 33-40 years, 18.06%; 41-49 years, 11.61%. Significant correlation between age group and CMV - IgM positivity (p=0.05), which come in line with previous local study in Najaf province, found that the highest rate of CMV - IgM was detected in 15-20 years, 25% and the lowest rate was 8.4% among 39-44 years [23].

The prevalence of Rubella - IgM positive cases was 32.26% which come in accordance with other local studies in Babylon (32% and 38%) [17]. In current study, Rubella - IgM positive cases detected among 10.97% of 33-40 years, followed by 9.03% of 25-32 years; 8.39% of 17-24 years which agree with Areej [17] proved that positive cases concentrated between >30 to <40. current result indicates no correlation between Rubella - IgM positivity and age groups which come in line with [17,24,25]. The relatively high prevalence of Rubella - IgM reflects the fact the endemic nature of *Rubella virus* in Iraq although the vaccination policies applied for last 35 years and give an impression for the errors in general vaccination program after American invasion for Iraq in 2003.

Prevalence of HSV2 - IgM was 40.65% which considered relatively high compared with others in Iraq, Babylon province (10%) [17], and in Sudan (4.5%) [24]. HSV2 - IgM positive detected among 14.84% of 25-32 years, 10.32% of 17-24 and 33-40 years, and finally 0.64% of 49-56 years. Significant correlation between age group and HSV2 - IgM positivity (p=0.024) which may be associated with sexual activity among age 20-40 years as well as hygienic measures and endemic nature of HSV in local community.

C. trachomatis - IgM positive detected among 23.87% of 25-32 years, 12.26% of 33-40 years, and finally in 3.87% of 49-56 years, which considered higher than 5% in Romanian study [26], indicates the high prevalence of asymptomatic *C. trachomatis* infections among infected individuals as well as infertile males [7,27]. Current study determines significant differences in *C. trachomatis* - IgM positivity among age groups (p=0.023). No significant correlation between age group and *C. trachomatis* - IgM positivity (p=0.657) which come in line with other local studies [7], as asexually transmitted pathogen the pathogen can be transmitted and infects regardless of the age groups [27].

In current study, inverse correlation between *T. gondii* - IgM positivity and type of infertility, mainly primary (p=0.039). This might be associated with the fact that the patients were not selected according to the type of infertility during preliminary investigation and such difference come in contrary with other local studies, found association between secondary infertility and toxoplasmosis in men [17]. Current inverse association reflects the disruptions in hypothalamo-pituitary-gonadal axis [28] due to toxoplasmosis which cause indirect effect on gonadotropin-releasing hormone [29].

In current study, CMV - IgM positive cases detected among 58.06% with primary versus (5.81%) secondary infertility. No correlation with type of infertility reflects the fact that the majority of patients enrolled in current study claimed primary infertility and failed to conceive for more than 1 year, which come in line [17]. Significant correlation between Rubella - IgM positivity and type of infertility, mainly primary type (p=0.021) come in contrary with Areej [17] indicating no such correlation and this might be due to relatively small sample size [17] and statistical bias. No correlation between HSV - IgM positivity and type of infertility come in contrary [17]. No correlation between *C. trachomatis* - IgM positivity and type of infertility, come in line [26], which indicates the high prevalence of *C. trachomatis* characterized by asymptomatic infections among infected individuals as well as infertile males [7,30].

One of the most prominent features in current study, detection of co-infection. *T. gondii* - CMV coinfection detected in (18.06%) with significant correlation (p=0.048), *T. gondii* - Rubella co-infection detected in (3.23%) with significant correlation (p=0.007). *T. gondii* - HSV2 co-infection detected in (9.03%) without significant correlation (p=0.808). *T. gondii* - *C. trachomatis* trachomatis co-infection detected in (7.74%) with significant correlation (p=0.001). This might be due to frequent exposure to *T. gondii* via feco-oral route and multiple sources of infection through contaminated water and food or even contact with animals or contaminated environment. All these factors accompanied by the asymptomatic and latency and immunosuppressive natures of HSV, CMV, asymptomatic *C. trachomatis*, and weakness of health surveillance and the possibility of asymptomatic infections with TORCH agents among partners which give opportunity of male partners to get infection [31].

The current study proved marginal inverse correlation between *T. gondii* - IgM positivity and total sperm count (p=0.054). The effect of *T. gondii* in spermatogenesis come in line with clinical studies which proved the presence of *T. gondii* in seminal fluid and tissue of infected human [32,33] which affect directly on decreasing sperm count [5]. Experimental studies proved the isolation of *T. gondii* from reproductive system (testicles, epididymis, seminal vesicle, and prostrate) of experimentally infected rams [34] and mice [35]. Correlation between *T. gondii* and sperm counts also proved by experimental study in rat fed with *T. gondii* [36,37]. Decrease in sperm count in *T. gondii* positive patients probably due to increase in apoptosis of germ cells that is triggered by a decreased gonadotropin level [35,38]. Current study proved inverse correlation between sperm sluggish motility and *T. gondii* - IgM, and this can be explained by testicular involvement

or even, epididymis, seminal vesicle, and prostrate by this pathogen and possible attachment with sperm specific receptors. This come in accordance with local study in Baghdad proved direct correlation between toxoplasmosis and decreased sperm motility [36]. Other experimental study come in line with this result [37], which proved that decreased motility extended for up to day 70 after *T. gondii* infection. Current study proved a significant correlation between *T. gondii* - IgM positivity and sperm dead % (p=0.022); sperm normal morphology % (p=0.000). Inverse significant correlation between *T. gondii* - IgM positivity and sperm abnormal morphology (p=0.000). Current study proved significant correlation between *T. gondii* - IgM positivity and sperm dead % (p=0.022); This results supported by experimental observation [37], stated that a temporary decreasing in sperm viability after infection for up to two months. Increased number of dead sperms indicate an increased apoptotic process triggered by acute *T. gondii* infection [35,38].

In current study, inverse significant correlation between normal sperms morphology and *T. gondii* - IgM positivity; positive correlation between abnormal sperms morphology and *T. gondii* - IgM positivity. This come in line with others [36,37] stated that sperm abnormalities increased throughout 70 days after infection; These results indicate that *T. gondii* may cause direct or indirect defect in sperm quality which is the main cause of infertility in men [5].

Current study revealed significant correlation between CMV - IgM positivity and sperm dead %; sperm normal morphology. Inverse significant correlation between CMV - IgM positivity and sperm active motility; sperm abnormal morphology. which come in contrary with others found no association between abnormalities in sperm count, motility, morphology and CMV infection [39,40]. HSV2 - IgM significantly correlated with abnormalities in sperm morphology which come in line [39,40]. Viral infection has the ability to impaired male fertility by a direct effect on spermatogenesis which leads to impaired sperm functions; induction of inflammatory response and elevated levels of proinflammatory cytokines mainly IL1 which has direct effects on spermatogenic cell differentiation and testicular steroidogenesis.as well as transforming growth factor-β which implicated in testicular development. Local or systemic up-regulation of cytokine expression during contribute to the disruption of testicular function and fertility; induction of antisperm antibodies formation and subsequent immunologic infertility [41]; endocrine changes via influence on testicular Leydig cells and/or the hypothalamic-pituitary gonadal and/or-adrenal axis" [31].

Current study determine significant correlation between *C. trachomatis* IgM positivity and sperm abnormal morphology % (p=0.004), Which come in line with other studies showed that men with *C trachomatis* in semen have reduced sperm concentration, motility, velocity, viability, morphology [6,39,42,43]. These effects were attributed to lipopolysaccharide that has the ability to induce sperm cytotoxicity and leading to sperm DNA fragmentation; apoptosis and finally leads to impaired fertility [43].

In current study, testosterone level correlated inversely with *T. gondii* - IgM positivity and positively correlated with Rubella - IgM positivity. It is possible that the disturbance of testosterone is an adaptive mechanism of infected individual aimed to compensate toxoplasmosis (induced immunosuppression observed during latent Toxoplasma infection [44]. The same assumption may be acceptable in the case of Rubella infection. The inverse association between testosterone and toxoplasmosis reflect the plasticity of testosterone-dependent signal intensity response, which adjusted according to the potential costs of parasite pathogenicity versus the benefits of increased reproductive success for *T. gondii* or even *Rubella virus* afforded by signal exaggeration (4). Beside other factors like infection load, nutritional status which indirectly affected the immune response against toxoplasma or Rubella. The inverse correlation between testosterone and *T. gondii* - IgM or Rubella reflects the possibility of immunosuppressive effect of testosterone that play a role in protection of spermatozoa, which are antigenic because they are formed long after the development of the immune system. Testosterone play a role in reduce the ability of lymphocytes that pass to seminiferous tubules to produce antibodies pleiotropically and their by protect the sperms [45,46].

In current study, significant correlation between CMV and LH levels. As CMV have the ability for latency and induce a status of immune suppression, the reactivation of latent infection due to various factors is possible and determination of serum IgM is reasonable depending on viral load, immunological responsiveness, CMV infection may leads to endocrine changes via influence on testicular Leydig cells and/or the hypothalamic-pituitary gonadal and/or-adrenal axis" affecting male hormones including LH and subsequently normal spermatogenesis [31,47]. The inverse correlation between *C. trachomatis* and FSH levels may attributed to functional disturbances in the hypothalamic-pituitary gonadal axis.

Current study revealed significant correlation between TORCH - *C. trachomatis* infection, semen parameters and fertility hormones. This result comes in line with others stated that circulating levels of FSH in men are associated with semen quality parameters, total sperm count; mainly spermatogenesis and normal Sertoli cell function [28]. This study revealed that among patients with *T. gondii* positive IgM have abnormal sperm sluggish motility, which affected by testosterone; FSH, prolactin levels, come in line with other clinical [28] and experimental studies [48]. In the present study, LH levels has significant correlation with sperm count and active motility among *T. gondii* positive IgM cases which agree with finding of other studies among infertile cases without toxoplasmosis [28,49,50]. Correlation among *T. gondii* - IgM positivity; total count; sluggish motility, normal and abnormal morphology according to prolactin level, may be due to negative effects of prolactin in FSH, LH serum level as the majority of patients in current study presented with hyperprolactinemia, this explanation come in line with Aleem *et al*. [51] who stated that in hyperprolactimic rat there was a loss for sperm chromatin integrity which lead to sperm dead at the final fate which may explain the significant correlation between progesterone levels with *T. gondii*; sperm dead %.

Significant correlations between CMV - IgM positivity and total sperm count according to LH come in line with Kapranos *et al*. [4] and come in contrary with Bezold *et al*. and Eggert-Kruse *et al*. [31,39], reported that CMV-DNA detected in 6.5% and 8.7% semen samples of infertile males, respectively, without significant differences with regard to the total sperm count, motility, morphology or viability. This controversy may be due to low copy number of CMV-DNA. Although CMV have the ability to attach with and enters to intact sperm plasma membrane during spermatogenesis in germinal epithelia obviously affect sperm motility [2] and have negative impacts on semen quality beside the ability of latency and reactivation under its own immunosuppressive properties for other viruses [42]. CMV - IgM inversely correlated with teratospermia and LH, which supported by the ability of CMV to ability to attach with and enters to intact sperm plasma membrane during spermatogenesis in germinal epithelia of seminiferous tubules [2]. CMV - IgM inversely correlated with sperm active motility and progesterone. CMV - IgM inversely correlated with sperm dead % and FSH and progesterone. This appears to be associated with teratogenic effects on sperm due to CMV-DNA within spermatozoa [2] beside the fluctuation in FSH and progesterone leading to increase in sperm DNA fragmentation and apoptosis [43].

Current reported that Rubella - IgM positivity has significant correlations with sperm active and sluggish motility and inversely correlated with sperm dead % according to LH level which above normal in 9.68% of infertile males. No previous studies reported this finding. The possible explanation for such correlation belongs to Rubella cause stress induces on hypothalamus-pituitary-gonadal axis which induce elevation of LH

above normal level and subsequently leads to testosterone production which is important for normal spermatogenesis from leyding cells although above normal testosterone reported only in 0.64% of current infertile cases.

In current study HSV2 Inversely correlated with progesterone; sperm active and sluggish motility and positively correlated with sperm dead %. These results due to the fact that the balance of progesterone–testosterone is important for normal spermatogenesis as progesterone considered as a precursor for testosterone which in turn have a role in normal spermatogenesis that appears in current study, testosterone level positively correlated with normal sperm morphology; sluggish motility depending on its level [47]. HSV2 produce oxidative damage to sperms that probably affect their motility and activity [52]. HSV2 correlated with normal sperm morphology and LH level which come in line with others reported the presence of HSV2 inside spermatozoa of normal morphology [53].

In current study, *C. trachomatis* - IgM positivity correlated with sluggish motility and testosterone levels; normal sperm morphology and LH level which explained by the ability of *C. trachomatis* elementary bodies to attached in spermatozoa using specific receptors and hence sperm motility affected as well as viability affected by cytotoxic lipopolysaccharide of *C. trachomatis* elementary bodies causing sperm apoptosis [54] which appear to be enhanced in current study by the disturbance of testosterone, LH and progesterone levels.

CONCLUSION

The current study proved several important and outstanding results about the role of TORCH agents and *C. trachomatis* in male infertility. TORCH agents and *C. trachomatis* have direct effects on sperm parameters. *T. gondii* and Rubella have direct effect on testosterone levels; *C. trachomatis* has direct effect on FSH among infertile males. TORCH agents plays a vital role in hormonal disturbances that lead to sperm abnormalities and subsequently to infertility problem.

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