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Association between latent toxoplasmosis and fertility parameters of men

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Abstract

Background: About a third of people in the world are infected with *Toxoplasma gondii*. This parasite has been found in the reproductive organs and semen of males of many animal species as well as humans. The effects of toxoplasmosis on sperm count, motility and morphology were confirmed in rats. A higher prevalence of toxoplasmosis has been observed in infertile men. On the other hand, no significant effect of infection on semen parameters in men was found in one already published study.

Objectives: To compare the prevalence of toxoplasmosis in men with and without semen pathology and to examine in detail the possible impact of infection on semen volume, sperm count, motility and morphology.

Materials and methods: The pre-registered cross-sectional study included 669 men who visited the Centre for Assisted Reproduction in Prague from June 2016 until June 2018.

Results: The incidence of fertility problems was significantly higher in the 163 *Toxoplasma*-infected men (48.47%) than in the 506 *Toxoplasma*-free men (42.29%), $\tau = 0.049$, $P = 0.029$. After correction for multiple tests, we found significantly lower sperm concentration, concentration of progressively motile sperm, and concentration of non-progressively motile sperm in *Toxoplasma*-positive men than in *Toxoplasma*-negative men using partial Kendall correlation with age controlled. In addition, toxoplasmosis correlated with sperm quality in smokers but not in non-smokers.

Discussion and conclusion: Our results suggest that latent toxoplasmosis affects certain semen parameters (sperm count and motility), but does not seem to affect sperm morphology and semen volume. Impairment of semen parameters may be either a side effect of the presence of *Toxoplasma gondii* in male reproductive organs or a product of manipulation activity of the parasite aimed to increase the efficiency of the sexual route of its transmission. Tobacco smoking also appears to exacerbate the negative impact of toxoplasmosis on semen parameters.

KEYWORDS
infertility, prevalence, reproduction, semen, sexual transmission, *Toxoplasma gondii*

1 | INTRODUCTION

Infertility is defined as a couple's inability to achieve pregnancy with regular unprotected intercourse within one year.[1] Approximately one third of infertility cases in couples are attributed primarily to women, one third to men, and one third to female-male interaction.[2] In up to 50% of infertile men, the cause of infertility remains unknown.[3] Known factors affecting male fertility include age, hormonal imbalance, stress, alcohol consumption and tobacco smoking.[4-6] Other possible causes of lowered sperm quality are a decrease in seminal plasma fructose level, which is necessary for normal sperm motility,[7] and overproduction of reactive oxygen species, which may likewise negatively affect sperm quality.[8]

Toxoplasma gondii is an intracellular parasitic protozoan which infects approximately one third of world's population. It is estimated that seroprevalence rates of toxoplasmosis depend on the environmental conditions, hygienic standards and eating habits.[9,10] In the 1990s, seroprevalence in women of childbearing age ranged from 37% to 58% in Central European countries, Poland, Croatia, Slovenia, Australia and northern Africa. Lower seroprevalence has been reported in Southeast Asia, China and Korea, as well as in countries with cold climates, such as in the Scandinavian region (4%–39%). Seroprevalence was higher in West African (54%–77%) and several Latin American (51%–72%) countries.[9] The prevalence in the male population of the Czech Republic tested between 2000 and 2004 was 23%.[11] In that study, *Toxoplasma* infection was significantly associated with raw meat consumption, gardening, current life in a small village and the location of the childhood residence in a small village.

The definitive host of *Toxoplasma* is felines, while its intermediate hosts can be various warm-blooded animals, including humans. The acute phase of toxoplasmosis is accompanied by symptoms similar to viral or bacterial infection. During this phase, tachyzoites rapidly multiply in the cells of various body tissues, including blood. In immunocompetent individuals, this acute phase spontaneously evolves into a latent phase, during which bradyzoites, another form of the parasite, slowly proliferate within host cells in the form of tissue cysts.[9] Aside from that, oocysts form in the small intestine of the definitive host, usually cats, and are released with faeces into the environment.[12] Common sources of infection include food or water contaminated by cat faeces containing oocysts and consumption of tissue cysts in raw or undercooked meat of intermediate hosts.[9] There is currently no effective treatment for the latent form of toxoplasmosis. Only pregnant women are treated for acute infections, which causes an accelerated transition from the acute to the latent phase of the infection to prevent infection of the foetus.[13]

Toxoplasma persists in hosts for the rest of their lives in the form of bradyzoites in tissue cysts, which can be located in the central nervous system, eyes, skeletal and cardiac muscles, but also lungs, liver or kidneys.[9] *Toxoplasma* has also been found in the reproductive organs and in the semen. For instance, *Toxoplasma* DNA has been found in the semen of male rabbits[14] and male goats,[15] while *Toxoplasma* cysts have been observed in the epididymis and semen of male rats[16] and in the testes of male mice.[17] Tachyzoites have been found in the semen as well as testicular and epididymis samples of dogs with acute toxoplasmosis[18] and *Toxoplasma* was found in the testes, seminal vesicles and semen samples of bovines[19] as well as in the epididymis and seminal vesicles of pigs.[20]

In rats, a negative impact of toxoplasmosis on sperm count, motility and morphology was observed up to 70 days after infection. Infected rats moreover had decreased fructose levels in the seminal vesicles and lower testosterone levels.[21,22] Lower sperm counts and higher rate of abnormalities in sperm morphology were also found in infected male mice four weeks after infection, but no changes in sperm motility or the weight of testes were observed.[17] No changes in sperm parameters in connection with *Toxoplasma* infection were found in sheep, but various histopathological changes were observed in the testes, prostate and seminal vesicles of infected rams.[23]

Toxoplasma has also been found in the reproductive organs of men and several studies report that it affects male fertility. Haskell et al.[24] found tachyzoites of *Toxoplasma gondii* in the testes of an HIV-positive man and Wong et al.[25] found cysts with bradyzoites in the testes of an immunocompetent man. Moreover, it has been observed that there is a significantly higher prevalence of toxoplasmosis in infertile couples (34.83%) compared to fertile couples (12.11%) and a higher level of anti-sperm antibodies was observed in *Toxoplasma*-infected men than in men free of this parasite.[26] The presence of anti-sperm antibodies in the semen significantly reduces sperm motility and concentration.[27] Another study, conducted on Chinese men, also confirmed a higher prevalence of toxoplasmosis in infertile men, namely 36%, while its prevalence in fertile men was only 11%.[28] Several studies made links between male genital damage and testicular toxoplasmosis,[29] testicular inflammation,[24,30] and hypogonadotropic hypogonadism caused by congenital toxoplasmosis.[31]

All in all, available evidence suggests that toxoplasmosis in men could be associated with infertility. On the other hand, a pilot study conducted in 60 men (15 *Toxoplasma*-positive) found no significant effect of *Toxoplasma* infection on sperm parameters, in particular semen volume and sperm count, motility and morphology.[32] Nonetheless, further indirect evidence to the effect that toxoplasmosis has a negative impact on male fertility comes from a study where the authors found that 77 *Toxoplasma*-positive men had fewer children than 343 *Toxoplasma*-negative men.[33]

The aim of our research was to compare the prevalence of toxoplasmosis in men with and without semen pathology and to investigate the possible effect of *Toxoplasma* infection on semen volume and sperm count, motility and morphology.

2 | MATERIALS AND METHODS

2.1 | Study design and participants

Data collection was conducted in the form of a prospective cross-sectional study from June 2016 until June 2018 in collaboration with the Centre for Assisted Reproduction (CAR) at the Department

of Obstetrics and Gynaecology of the First Faculty of Medicine of Charles University and General University Hospital in Prague. This study initially involved 758 men who visited the CAR with their partner for fertility problems. Informed consent was obtained from all participants. Blood samples for serological testing for toxoplasmosis were taken during a routine examination. Semen samples were processed and examined at the CAR laboratory according to the WHO protocol.[34]

A total of 89 men were excluded from the study: One man had acute toxoplasmosis. Another 26 men had inconclusive test results for toxoplasmosis (in most cases, complement fixation test result was positive, while the ELISA was negative). In another 62 men, either blood tests or semen analysis were missing.

Participants completed a questionnaire that included the following question: "Do you smoke cigarettes? (a) I do not smoke; (b) occasionally; (c) 1–5 cigarettes a day; (d) 6–10 cigarettes a day; (e) more than 10 cigarettes a day". In the exploratory part of the study, 72 men who answered that they smoked occasionally and 44 men who did not answer the question were excluded. For statistical analysis, a group of non-smokers (who answered that they do not smoke) and a group of smokers (who selected answers 3–5) were created.

This research was approved by the Ethics Committee of General University Hospital in Prague (No. 384/16; 92/17) and by the Institutional Review Board of Faculty of Science, Charles University (No. 2015/29). The confirmatory part of the study, including stopping rules and hypotheses to be tested, was pre-registered on the OSF, https://osf.io/974wc. For the Differences between pre-registered and realized protocol, see the chapter 4.1. in the Discussion.

2.2 | Serological testing for toxoplasmosis

Testing for toxoplasmosis was performed in the National Reference Laboratory for Toxoplasmosis at the National Institute of Public Health in Prague by a complement fixation test and the ELISA (TestLine Clinical Diagnostics, Brno). Negative results of both tests indicated that a person is not infected with *Toxoplasma*, while positive results of both tests indicated the presence of anamnestic anti-*Toxoplasma* antibodies and therefore a high likelihood of latent toxoplasmosis. Subjects with discordant test results (one positive, one negative) were excluded from the study. In the case of high levels of IgG antibodies, IgM and IgA antibodies assays were also performed, because high levels of these antibodies indicate acute toxoplasmosis, meaning the person was probably infected recently.

2.3 | Semen analysis

Semen analysis is a basic examination for the assessment of male fertility. It includes an evaluation of semen volume, pH, sperm concentration (sperm count in 1 mL), concentration of progressively motile sperm (number of progressively motile sperm in 1 mL), concentration of non-progressively motile sperm (number of non-progressively motile sperm in 1 mL), concentration of immotile sperm (number of immotile sperm in 1 mL), and the percentage of normally formed sperm. In the present study, we used the terminology related to semen quality and lower reference limits that was set by the WHO[34]: Less than 15 million spermatozoa per mL or under 39 million spermatozoa per ejaculate was called oligozoospermia (low sperm count). Sperm motility was graded as (a) progressive motility: sperm moving actively, either in a linear fashion or in a large circle, (b) non-progressive motility: absence of progression, for instance by swimming in small circles or due to flagellar force hardly pushing the head, (c) immotility: no movement. Pathology characterized by less than 32% of progressive motility or less than 40% of total motility (both progressive and non-progressive) was called asthenozoospermia. In analysing sperm morphology, if less than 4% of the sperms had normal form, the pathology was named teratozoospermia. The three basic pathologies listed above, that is oligozoospermia, asthenozoospermia and teratozoospermia, can all combine depending on which of the parameters fall below WHO reference levels. A combination of all three disorders was called oligoasthenoteratozoospermia. Ejaculate may also contain either no sperm (azoospermia) or no live sperm (necrozoospermia). Absence of any pathologies with respect to semen and sperm quality was called normozoospermia. We calculated total sperm count by multiplying the semen volume and sperm concentration, while sperm quality was determined by the proportion of progressively motile sperm in all other kinds of sperm.

2.4 | Statistical analysis

The data were analysed in statistical program R, version 3.5.1.[35] Non-parametric tests were used for all analyses because many variables did not have a normal distribution and, moreover, non-parametric tests reduce the impact of outliers. The Wilcoxon test was used to compare age among groups of men. We detected a strong correlation between age and many focal variables. This is why, using the R package ppcor,[36] a partial Kendall correlation controlled for age was used to compare the prevalence of toxoplasmosis in different groups of men and to analyse the relationship between toxoplasmosis and semen parameters. A correction for multiple tests was done using the Benjamini-Hochberg procedure with false discovery rate pre-set to 0.1.[37] This method takes into account the distribution of *P* values of all tests, so that even a *P* value higher than 0.05 before the correction can prove significant after the correction if the results of several or even many tests approach this threshold. The method moreover allowed us to define the fraction of false-positive tests (eg 10%) we were prepared to tolerate. In accordance with pre-registration, we used one-sided tests because a negative association between toxoplasmosis and fertility was expected. All data are available in an online open-access repository Figshare[63] (https://doi.org/10.6084/m9.figshare.12213749.v2).

3 | RESULTS

3.1 | Confirmatory analyses

The final sample included 669 men with an average age of 35.7 years (SD = 5.5) ranging from 21 to 58 years, of whom 163 were *Toxoplasma*-positive (24.36%). The mean age of infected men (36.3; SD = 5.5) did not significantly differ from the mean age of uninfected men (35.5; SD = 5.5; $P = 0.058$), but the difference was close to statistical significance. The mean age of men with pathology in semen (36.1; SD = 5.5) was higher than the mean age of men with normozoospermia (35.4; SD = 5.5; $P = 0.019$).

The prevalence of toxoplasmosis was 26.96% in men with semen pathologies (n = 293) and 22.34% in men with normozoospermia (n = 376). A partial Kendall correlation controlled for age was used for all following analyses. Binary variables toxoplasmosis and pathology in semen significantly correlated ($\tau = 0.049$; $P = 0.029$; one-sided test). Adverse results of semen analyses were found in 48.47% of infected men (n = 163) and 42.29% of non-infected men (n = 506). Table 1 shows men divided in groups according to the type of disorder, while Table 2 shows the proportion of men according to the results of semen examination. There was a significant difference between toxoplasmosis prevalence in men with normozoospermia and toxoplasmosis prevalence in men with particular disorders, namely oligozoospermia, asthenozoospermia and teratozoospermia (Table 1).

We found correlations between toxoplasmosis and total sperm count ($\tau = -0.039$; $P = 0.068$) and sperm quality ($\tau = -0.042$; $P = 0.050$); these two correlations turned significant after a correction for multiple tests with the Benjamini-Hochberg method with a false discovery rate pre-set to 0.1.

Follow-up analyses were performed to investigate the specific effects of latent toxoplasmosis on semen parameters. We analysed associations between toxoplasmosis and particular semen parameters, namely semen volume, sperm concentration, concentration of progressively motile sperm, concentration of non-progressively motile sperm, concentration of immotile sperm and percentage of sperm with normal form. Significant correlations between toxoplasmosis and sperm concentration, concentration of progressively motile sperm and concentration of non-progressively motile sperm were found (Figure 1, Table 3).

3.2 | Exploratory analyses

The final sample included 174 smokers and 379 non-smokers. The mean age of smokers (35.3; SD = 6.1) did not significantly differ from the mean age of non-smokers (36.1; SD = 5.5; $P = 0.126$). The percentage of smokers (32.8% vs. 30.36%) did not differ between 250 men with semen pathologies and 303 men with normozoospermia ($\chi 2 = 0.377$; $P = 0.539$) using contingency tables and the Pearson chi-square test. No significant correlation between smoking and semen parameters was found by partial Kendall correlation with age controlled for.

TABLE 1 Prevalence of toxoplasmosis (shown in parenthesis) in groups of men according to the type of disorder

Diagnosis	n	*Toxoplasma*-positive	Partial Kendall τ	*P*
All				
Normozoospermia	376	84 (22.34%)		
Oligozoospermia	210	60 (28.44%)	0.064	0.010
Asthenozoospermia	104	31 (29.81%)	0.066	0.015
Teratozoospermia	226	64 (28.32%)	0.061	0.013
Smokers				
Normozoospermia	92	18 (19.57%)		
Oligozoospermia	59	18 (30.51%)	0.124	0.024
Asthenozoospermia	26	10 (38.46%)	0.184	0.003
Teratozoospermia	62	23 (37.10%)	0.191	<0.001
Non-smokers				
Normozoospermia	211	49 (23.22%)		
Oligozoospermia	126	35 (27.78%)	0.043	0.237
Asthenozoospermia	62	15 (24.19%)	−0.004	0.931
Teratozoospermia	130	32 (24.62%)	0.005	0.886

The table shows results for group of all men and also for two subgroups of smokers and non-smokers. Men who had more than one semen pathology appear in two or three groups at the same time. Associations between toxoplasmosis in men with normozoospermia and men with oligozoospermia, asthenozoospermia and teratozoospermia were analysed using partial Kendall correlation with age controlled for.

TABLE 2 Prevalence of toxoplasmosis (shown in parenthesis) in groups of men according to the results of semen examination

	n	*Toxoplasma*-positive
Normozoospermia	376	84 (22.34%)
Asthenozoospermia	15	3 (20.00%)
Oligozoospermia	36	6 (16.67%)
Teratozoospermia	35	5 (14.71%)
Asthenoteratozoospermia	22	8 (36.36%)
Oligoteratozoospermia	107	34 (31.48%)
Oligoasthenozoospermia	5	3 (60.00%)
Oligoasthenoteratozoospermia	62	17 (27.42%)
Azoospermia	10	3 (30.00%)
Necrozoospermia	1	0 (0.00%)

The prevalence of toxoplasmosis did not differ between smokers (25.29%) and non-smokers (24.01%; $\chi 2 = 0.105$; $P = 0.746$) using contingency tables and the Pearson chi-square test. A partial Kendall correlation controlled for age was used for all following analyses, separately in smokers and non-smokers. Binary variables toxoplasmosis and pathology in semen significantly correlated in smokers ($\tau = 0.138$; $P = 0.007$) and non-significantly correlated in non-smokers ($\tau = 0.013$; $P = 0.711$). Adverse results of semen analyses were found in 59.09% of *Toxoplasma*-infected smokers (n = 44), 46.15% of *Toxoplasma*-infected non-smokers (n = 91), 43.08%

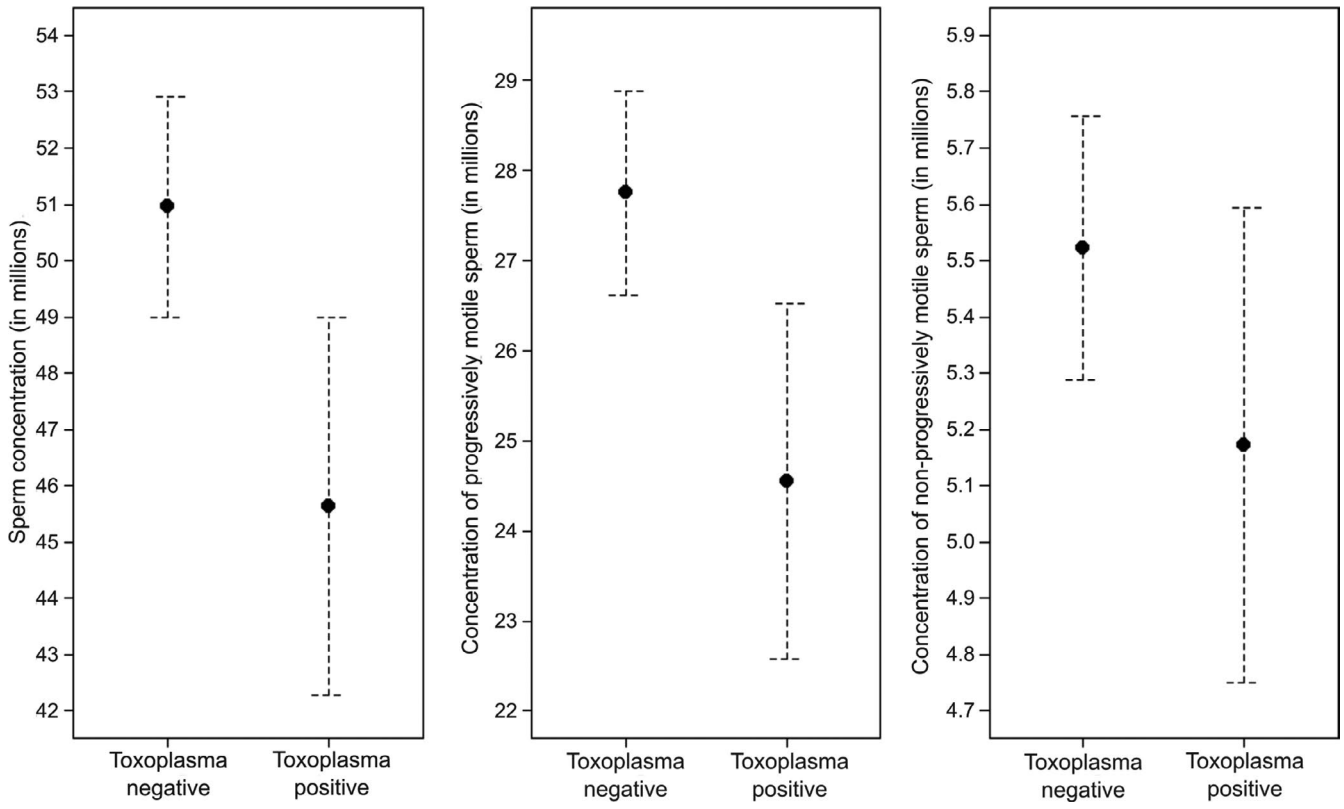

FIGURE 1 Semen parameters in 506 *Toxoplasma*-negative and 163 *Toxoplasma*-positive men. This figure shows the mean and standard error of the mean of sperm concentration, concentration of progressively motile sperm and concentration of non-progressively motile sperm. Concentration denotes sperm count in 1 mL

of non-infected smokers (n = 130) and 43.75% of non-infected non-smokers (n = 288). There was a significant difference between toxoplasmosis prevalence in men with normozoospermia and toxoplasmosis prevalence in men with particular disorders, namely oligozoospermia ($P = 0.024$), asthenozoospermia ($P = 0.003$) and teratozoospermia ($P < 0.001$) in smokers, but not in non-smokers (for more details see Table 1).

We found a significant correlation between toxoplasmosis and sperm quality in smokers ($\tau = -0.150$; $P = 0.003$), but not in non-smokers ($\tau = 0.014$; $P = 0.688$). No significant correlations existed between toxoplasmosis and total sperm count in smokers ($\tau = 0.040$; $P = 0.436$) and non-smokers ($\tau = -0.061$; $P = 0.078$). Also, no significant correlations between toxoplasmosis and particular semen parameters were found after a correction for multiple tests with the Benjamini-Hochberg method (Table 3).

4 | DISCUSSION

In our study, the prevalence of toxoplasmosis was 24.36%. The observed number of *Toxoplasma*-positive men corresponds to the prevalence (23%) in Czech men tested between 2000 and 2004.[11] The present study found a higher prevalence of toxoplasmosis in men with semen pathology or pathologies than in men without such pathologies. A more detailed analysis of specific pathologies revealed a higher prevalence of toxoplasmosis in men with low sperm count, low sperm motility and in men with a low percentage of normally formed sperm than in men without fertility problems. Our results are consistent with those of Qi et al.[28] In their study performed on 200 men, the authors found a higher prevalence of toxoplasmosis in infertile men.

In 2015, a study with a relatively small sample conducted on 60 men (15 *Toxoplasma*-positive) found no significant effect of *Toxoplasma* on semen volume, sperm count, motility or morphology, but this result may well be due to small sample size and the absence of control over confounding age variable. In the sample, 6 of the 15 *Toxoplasma*-positive men (40%) and 14 of the 45 *Toxoplasma*-negative men (31.11%) exhibited a modified spermiogram.[32] Based on the results of power analyses, the authors estimated that 994 subjects would be needed to demonstrate the effect of latent toxoplasmosis on semen parameters. On the other hand, we have shown that our sample size (669 men) is sufficient once subjects' age is controlled for.

As summarized in a recent review,[38] infections of the reproductive tract contribute to important causes of impaired fertility in men. Higher levels of pro-inflammatory cytokines in the male reproductive tract during inflammation are highly detrimental to sperm production. Moreover, inflammation is associated with oxidative stress, which impairs sperm function. *Toxoplasma* has been found in the reproductive organs and semen of several species of animals.[16-20] The authors of case reports also found tachyzoites and bradyzoites of *Toxoplasma gondii*

TABLE 3 Partial Kendall correlation between toxoplasmosis and semen parameters after age filtering in groups of all men and also in two subgroups of smokers and non-smokers

	Partial Kendall τ	*P* value
All (n = 669)		
semen volume ~toxoplasmosis	−0.007	0.395[a]
sperm concentration ~toxoplasmosis	−0.053	0.020[a,b]
concentration of progressively motile sperm ~toxoplasmosis	−0.053	0.020[a,b]
concentration of non-progressively motile sperm ~toxoplasmosis	−0.044	0.045[a,b]
concentration of immotile sperm ~toxoplasmosis	−0.038	0.146
percentage of sperms with normal form ~toxoplasmosis	−0.029	0.135[a]
Smokers (n = 174)		
semen volume ~toxoplasmosis	0.101	0.049
sperm concentration ~toxoplasmosis	−0.081	0.116
concentration of progressively motile sperm ~toxoplasmosis	−0.103	0.043
concentration of non-progressively motile sperm ~toxoplasmosis	−0.043	0.397
concentration of immotile sperm ~toxoplasmosis	−0.036	0.477
percentage of sperms with normal form ~toxoplasmosis	−0.086	0.092
Non-smokers (n = 379)		
semen volume ~toxoplasmosis	−0.069	0.047
sperm concentration ~toxoplasmosis	−0.056	0.103
concentration of progressively motile sperm ~toxoplasmosis	−0.046	0.183
concentration of non-progressively motile sperm ~toxoplasmosis	−0.075	0.030
concentration of immotile sperm ~toxoplasmosis	−0.046	0.181
percentage of sperms with normal form ~toxoplasmosis	−0.026	0.513

[a]One-sided *P* values.

[b]Results that remained significant after the correction for multiple tests.

in the testes of men.[24,25] Infection with this parasite is accompanied by a Th1 immune response characterized by overproduction of pro-inflammatory cytokines. Cytokine-activated macrophages that produce reactive oxygen species play an important role in host defence against *Toxoplasma*.[39] Excessive production of reactive oxygen species can, however, adversely affect sperm quality,[8] and indeed, up to 80% of infertile patients have elevated levels of reactive oxygen species in their semen samples.[40] It should be stressed that the abovementioned changes in the reproductive tract are present during the acute phase of *Toxoplasma* infection. It is yet to be seen whether and how long they persist during the latent stage, but it has been observed in mice with latent toxoplasmosis that local inflammatory sites are present in brain, another immune-privileged organ.[41] It is assumed that toxoplasmosis is maintained in a latent form by continuous activity of the host immune system.[42]

Excessive production of reactive oxygen species reduces the levels of male sex hormones that regulate testicular function, coordinate testosterone synthesis, and maintain normal spermatogenesis, sperm health, and density.[43] In accordance, a case-control study has shown that the serum concentration of testosterone in 365 men with latent toxoplasmosis was significantly lower than in *Toxoplasma*-negative men.[44] Similarly, Kaňková et al.[45] have shown that *Toxoplasma* infection causes a decrease in serum testosterone concentration in male mice two months after acute *Toxoplasma* infection. However, studies of testosterone levels in *Toxoplasma*-infected subjects appear to depend on the duration of infection and the type of measured testosterone. Contrary to previously, it has been reported that salivary testosterone concentration in *Toxoplasma*-positive men was significantly higher than in *Toxoplasma*-negative men.[46] Some authors observed indirect evidence of increased testosterone levels in 20 and 18 *Toxoplasma*-infected young men (undergraduate biology students) compared to 66 and 71 *Toxoplasma*-free controls, respectively.[47,48] Lim et al.[49] also found higher testicular testosterone levels in *Toxoplasma*-positive rats measured between six and eight weeks after infection in a laboratory. In summary, *Toxoplasma gondii* could affect male reproductive function through changes in testosterone levels, however, more studies are needed to describe the exact physiological pathway.

Reduced fertility in *Toxoplasma*-positive men may be related to the sexual transmission of *Toxoplasma gondii*. This transmission route has been confirmed in rats,[16] sheep,[50] goats,[51] dogs[18] and rabbits.[52] Hypotheses of the existence of *Toxoplasma* transmission during sexual intercourse[53] and oral sex[54] in humans have been proposed recently. So far, however, these two hypotheses are supported only by indirect evidence. A high prevalence of toxoplasmosis has been observed in men who have sex with men,[55] sex workers[56] and fathers of congenitally infected children.[57] In 2020, an analysis of toxoplasmosis in couples was performed, revealing that one of the risk factors for women, but not for men, is an infected sexual partner.[58] Impairment of male semen parameters may be either a side effect of *Toxoplasma* reproduction in the body of the host, especially its immune-privileged reproduction organs, or a result of parasite-specific manipulation activity aimed at increasing the probability of transmission from an intermediate to a definitive host.[59] Infected men's reduced ability to conceive a child might thus lead to a higher frequency of sexual intercourse, and therefore also to an increased rate of sexual transmission of the parasite. It is, however, unclear whether such manipulative activity is at work only in modern human society or also in *Toxoplasma's* natural intermediate hosts, rodents. On the other hand, the higher observed attractiveness[16] and phenotypic changes in male rats analogical to those observed in infected men, namely higher visual dominance[48] and stature of infected male students taller by 3 cm than that of controls,[47] could indeed effectively increase the number of sexual partners, frequency of sex, and therefore also the effectiveness of sexual transmission of *Toxoplasma* in any species.

In our study, the effect of toxoplasmosis on semen parameters was more significant than the effect of smoking. No significant association was found between tobacco smoking and individual semen parameters. These results are in contrast to studies where tobacco smoking was found to have a negative effect on sperm quality.[60,61] However, these two studies on tobacco smoking have been performed on large numbers of men (2542 and 2105). In our study, a higher prevalence of toxoplasmosis was found in men with sperm pathology compared to the prevalence in men without pathology, but only in smokers. Similarly, only in smokers, there was a higher prevalence in groups of men with individual types of disorders compared to the prevalence in men without pathology in sperm. Most men with sperm pathology were among smokers with toxoplasmosis and the least among smokers without toxoplasmosis. Toxoplasmosis correlated with sperm quality only in smokers, not in non-smokers. Probably due to the relatively small sample, no correlation was observed between toxoplasmosis and individual semen parameters in smokers and non-smokers. The results of this study suggest that the combined effects of toxoplasmosis and tobacco smoking could exacerbate the negative impact on sperm parameters.

4.1 | Differences between pre-registered and realized protocol

Initially, we wanted to average the semen parameters where a patient had multiple examinations. In the end, however, based on the analysis of 355 men who underwent more than one semen examination during their treatment, we decided to use only the results from the first semen collection during the first visit to the Centre for Assisted Reproduction. This was because examination of the data revealed statistically significant differences in sperm concentration ($P < 0.001$), concentration of progressively motile sperm ($P = 0.002$) and concentration of immotile sperm ($P < 0.001$) when comparing semen parameters from the first examination with averaged semen parameters using a paired Wilcoxon test. It suggests that the results of subsequent examinations may have been affected by medical intervention (medication and vitamins), which men with unfavourable sperm test results had undergone.

4.2 | Limitations and strengths of the study

The effect of latent toxoplasmosis on specific fertility parameters in males has so far been studied in detail only in non-human animals. Leaving aside a pilot study conducted on a small sample of men, this study is one of the first to demonstrate a negative effect of latent toxoplasmosis on sperm count and motility in men. But even here, association does not necessarily imply causality. Subfertility is not very likely to increase the risk of infection, but it is possible that some further factors, such as immune deficiency or lifestyle, could increase both the risk of infection and the risk of subfertility.

5 | CONCLUSIONS

Results of our pre-registered prospective study indicate that latent toxoplasmosis selectively affects some fertility parameters, namely sperm count and motility, but not sperm morphology and semen volume. The effects were stronger for the subpopulation of smokers. Generally speaking, the underlying causes of infertility are difficult to determine because many genetic and environmental factors and their interactions could be co-responsible. Some of these factors could also independently affect the risk of *Toxoplasma* infection. This makes it difficult, if not impossible, to unravel the complex mechanism of infertility using observational studies performed on humans. On the other hand, latent toxoplasmosis affects about one third of the world's population. Therefore, its effects on human reproduction, although sometimes small on the level of individuals, could be important on the level of the large populations.[62] The results of our observational study suggest that more attention should be paid to the study of the effects of latent toxoplasmosis on male reproductive functions in the future.

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CONFLICT OF INTEREST

All authors have nothing to declare.

AUTHORS' CONTRIBUTIONS

ŠK provided oversight of the study. JH coordinated the study and processed blood samples. KŘ, PC supervised the patient recruitment and the data collection in CAR. JH, JF and ŠK analysed the data and wrote the first manuscript draft. All authors critically revised and approved the final manuscript.

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