Asian Journal of Andrology (2023) 25, 29–37
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REVIEW

Semen Analysis

The influence of *Mycoplasma* species on human and canine semen quality: a review

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Mycoplasma **species (spp.) are bacteria that are difficult to detect. Currently, the polymerase chain reaction (PCR) is considered the most effective diagnostic tool to detect these microorganisms in both human and veterinary medicine. There are 13 known species of human *Mycoplasma* and 15 species of canine *Mycoplasma*. Owing to the difficulties in identifying the individual species of *Mycoplasma*, there is a lack of information regarding which species are saprophytic and which are pathogenic. The prevalence of the individual species is also unknown. In addition, in both humans and dogs, the results of some studies on the impact of *Mycoplasma* are conflicting. The presence of *Mycoplasma* spp. on the epithelium of reproductive tract is often associated with infertility, although they are also detected in healthy individuals. The occurrence of *Mycoplasma* spp. is more common in dogs (even 89%) than in humans (1.3%–4%). This is probably because the pH of a dog's genital is more conducive to the growth of *Mycoplasma* spp. than that of humans. Phylogenetically, human and canine *Mycoplasma* are related, and majority of them belong to the same taxonomic group. Furthermore, 40% of canine *Mycoplasma* spp. are placed in common clusters with those of human. This suggests that species from the same cluster can play a similar role in the canine and human reproductive tracts. This review summarizes the current state of knowledge about the impact of *Mycoplasma* on canine and human male fertility as well as the prospects of further development in this field.**

Asian Journal of Andrology (2023) **25**, 29–37; doi: 10.4103/aja2021124; published online: 04 March 2022

Keywords: *Mycoplasma*; sperm morphology; sperm motility

INTRODUCTION

In human medicine, infertility is defined as a failure to conceive after 12 months of regular intercourse without contraception,[1] and it affects 8%–12% of couples.[2] Infectious organisms in the reproductive tract may affect male fertility. Although some researchers suggested a correlation between *Mycoplasma* and infertility in humans and dogs, this phenomenon has not been proved in other studies.[3] It is suspected that these bacteria may be commensals, although it is difficult to estimate their role. This article summarizes the current state of knowledge about the impact of *Mycoplasma* species (spp.) on fertility in dogs and men.

Mycoplasma spp. are the smallest self-replicating organisms, belonging to the *Mycoplasmataceae* family, and are detectable in humans, animals, as well as in plants.[4] There is a theory that *Mycoplasma* spp. evolved from Gram-positive bacteria, and phylogenetically they are close to *Clostridia*.[4] Morphologically, *Mycoplasma* spp. stand out because of the total lack of a cell wall, and because they are included in the *Mollicutes* class (from Latin: *mollis* means soft, *cutis* means skin). The *Mycoplasma* cell contains only the organelles that are essential for growth and replication.[4] Taxonomically, *Mycoplasma* spp. are divided into the following groups: *anaeroplasma*, *asteroleplasma*, *hominis*, *pneumoniae,* and *spiroplasma*.[5] The majority of both canine (Ca) and human (Ho) genital *Mycoplasma* belong to the hominis group, which shows that they are relatively closely related. In the *hominis* group, among others, there are three clusters: *hominis*, *bovis,* and *synoviae*, in which both human and canine *Mycoplasma* are placed. The *hominis* cluster includes *Mycoplasma* (*M.*) *arginini* (Ca), *M. gateae* (Ca), *M. spumans* (Ca), *M. buccale* (Ho), *M. faucium* (Ho), *M. hominis* (Ho), and *M. orale* (Ho); the *bovis* cluster includes *M. bovigenitalium* (Ca), *M. maculosum* (Ca), *M. opalescens* (Ca), *M. fermentans* (Ho), *M. primatum* (Ho), and *M. spermatofilum* (Ho); and the *synoviae* cluster includes *M. cynos* (Ca), *M. edwardii* (Ca), *M. felis* (Ca), and *M. canis* (Ca).[6] Therefore, on the basis of *Mycoplasmataceae* taxonomy, it has been estimated that 40% of canine species are in the same cluster as human (not published).

All phylogenetic are shown in **Figure 1**. The 16S ribosomal DNA sequences of *Mycoplasma* species were retrieved from GenBank (NCBI), as shown in **Table 1**. Alignment of the sequences was constructed using GeneDoc using Blosum62 matrix (gap open cost: 8, gap extend cost: 4). Aligned sequences were trimmed to the longest overlapping region and sequences of *M. primatum, M. haemocanis,* and *M. arginini* were rejected due to small overlapping region, and rest of the sequences were aligned again using aforementioned parameters. An evolutionary tree was constructed with Molecular Evolutionary Genetic Analysis (MEGA) software using the maximum likelihood method and Tamura-Nei model with bootstrap consensus inferred from 10 000 replicates.

This affinity between species of human and canine *Mycoplasma* suggests that they could influence semen quality similarly. Accordingly, the dog can probably be treated as a model organism for research on

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Received: 04 October 2021; Accepted: 20 December 2021

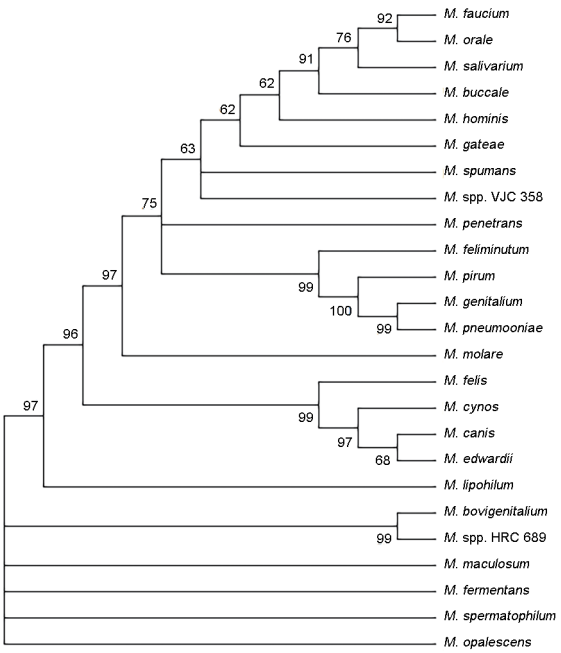

Figure 1: The evolutionary tree of 16S ribosomal DNA sequences of canine and human species of *Mycoplasma*. Numbers above the branches show the percentage of probability of the result. *M.*: *Mycoplasma*.

mycoplasmosis of the genital tract. In addition, it is possible for canine *Mycoplasma* to colonize the human body. Klein *et al.*[7] have isolated *M. canis* from human tissue after a dog bite.

The primary habitats of human and canine *Mycoplasma* are the mucous surfaces of the respiratory and urogenital tracts, eyes, digestive system, mammary glands, and joints.[4] In addition, there is a report about their occurrence in pathological canine brain tissues.[8] As well as other mollicutes, *Mycoplasma* spp. can be present intracellularly in the host's cells. In both humans and animals, *Mycoplasma* is taken up by leukocytes and macrophages, but the mechanism of entry into the cells is still unclear. However, it has been described that this invasion may affect cell function.[4] Díaz-García *et al.*[9] demonstrated that *M. hominis* can also infect spermatozoa.[9]

Mycoplasma adheres to the surface of the epithelium in the reproductive tract, and this process is strong enough to prevent their elimination in their secretions or urine.[4] It is also known that *M. genitalium* has a major surface adhesion complex known as the nucleoid-associated protein (NAP) on its surface, and because of this, it can adhere to surfaces and remains motile.[10] Furthermore, no specific toxins or virulence factors of *M. genitalium* have been described, and it is suspected that the lipoproteins exposed on their surface can stimulate local inflammatory response in the reproductive tract.[11] There is limited knowledge about the virulence factors of canine *Mycoplasma* spp. However, some species can cause hemolysis during culturing; therefore, it has been suggested that some of them can synthesize hemolytic enzymes.[12] Genital *Mycoplasma* in humans and possibly in veterinary patients are natural inhabitants of the male urethra, and therefore, they can be present in spermatozoa during ejaculation.[13] There are 13 known species of human *Mycoplasma* which occur in the genital tract including *M. buccale*, *M. faucium*, *M. fermentans*, *M. genitalium*, *M. hominis*, *M. lipophilum*, *M. orale*, *M. penetrans*, *M. pirum*, *M. pneumoniae*, *M. primatum*, *M. salivarium*, and *M. spermatophilum*,[14] but the more common are *M. genitalium* and *M. hominis*.[15]

Table 1: List of species of *Mycoplasma* and their numbers in the GenBank used to create a phylogenetic tree

Species of Mycoplasma	*GenBank number*
Mycoplasma facium	NR_024983.1
Mycoplasma orale	NR_043199.1
Mycoplasma salivarium	NR_041745.1
Mycoplasma hominis	NR_041881.1
Mycoplasma gateae	NR_029180.1
Mycoplasma spumans	NR_24980.1
Mycoplasma spp. VJC 358	AY246564.1
Mycoplasma penetrans	RCH401000003.1
Mycoplasma feliminutum	NR_029181.1
Mycoplasma pirum	NR_029165.1
Mycoplasma genitalium	NR_026155.1
Mycoplasma pneumooniae	NR_041751.1
Mycoplasma molare	NR_041931.1
Mycoplasma felis	U09787.1
Mycoplasma cynos	NR_025181.1
Mycoplasma canis	AB680678.1
Mycoplasma lipohilum	AB680693.1
Mycoplasma bovigenitalium	AB680692.1
Mycoplasma spp. HRC 689	AF527624.1
Mycoplasma maculosum	AB680679.1
Mycoplasma fermentans	NR_044666.2
Mycoplasma spermatophilum	NR_025069.1
Mycoplasma opalescens	NR_025067.1

In the canine reproductive tract, *M. arginini*, *M. bovigenitalium*, *M. canis*, *M. cynos*, *M. edwardii*, *M. feliminutum*, *M. felis*, *M. gateae*, *M. haemocanis*, *M. maculosum*, *M. molare*, *M. opalescens*, *Mycoplasma* spp. HRC 689, *Mycoplasma* spp. VJC 358, and *M. spumans* can be detected, and the more common are *M. canis*, *M. spumans*, and *M. maculosum*.[16] Both canine and human *Mycoplasma* are shown in **Figure 2**.

It has been estimated that their prevalence in the human reproductive tract in countries with high levels of development is 1.3%, while it is almost 4% in countries with lower levels of development.[17] In veterinary medicine, the occurrence of *Mycoplasma* spp. in animals is more common. It has been estimated that among dogs, up to 89% can be *Mycoplasma* positive.[18] There are possible reasons that *Mycoplasma* spp. is more common in dogs than in humans. On the one hand, dogs have more different sexual partners than humans, and in addition, people are using safeguards against contracting venereal diseases. On the other hand, *Mycoplasma* spp. may be present in the prepuce of some dogs before the first mating. The pH value of the canine reproductive tract may be potentially more suitable for the growth of this microorganism. The best pH conducive for *Mycoplasma* growth is between 7.8 and 8.[19] In canine females, the pH value in the vagina is 7.4–8.3[20] and 6.3–6.7 in prepuce of males,[21] as opposed to humans who have lower values of 5.71 in men's prepuce[22] and 3.8–4.5 in women's vagina.[23] The pH values of canine semen are as follows; first fraction: 6.37, second fraction: 6.37, and the third one is 7.2;[24] and human semen pH values are between 7.2 and 8.[25] The most important factor seems to be pH in the place of arising the *Mycoplasma*. In the tunica mucosa of the human reproductive tract, the pH is inappropriate for growth and development of these bacteria. This phenomenon can be a reason that *Mycoplasma*-positive results are more common in the dog than in the human reproductive tract. In a few publications, the presence of *Mycoplasma* was in semen, not the prepuce.[26,27] Ultimately, the

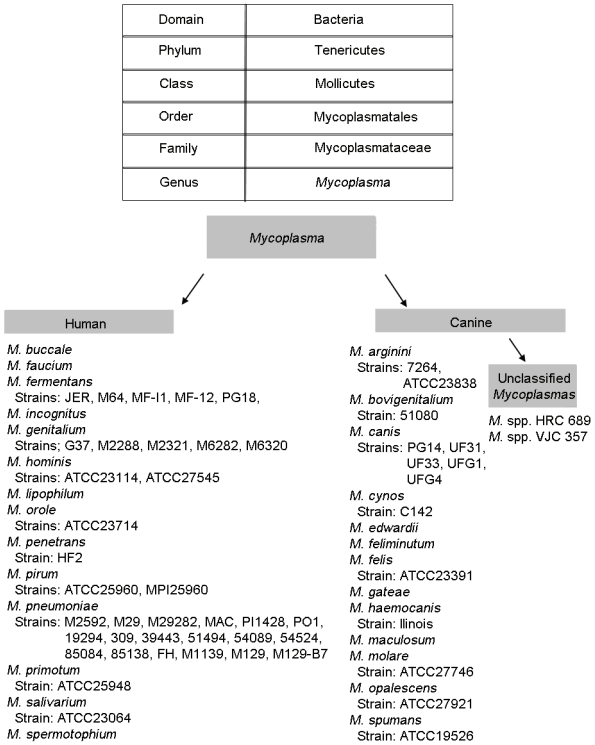

Figure 2: Scientific classification of human and canine genital *Mycoplasmas*, based on: ncbi.nlm.nih.gov (strains) and patricbrc.org/view/Taxonomy/ (taxonomy). *M.*: *Mycoplasma*.

hypothesis is that in the canine reproductive tract, the environmental conditions are better for *Mycoplasma* spp. can be given. However, more research is needed to confirm this theory. Moreover, the prevalence of *Mycoplasma* in the respiratory tract is higher in dogs than that in man; in humans, it ranges from 2% to 35%,[27] while in dogs, it ranges from 86% to 90%.[28] Nevertheless, a study performed in mice showed that those infected by *Mycoplasma* intranasally were more resistant to *Mycoplasma* infections of the reproductive tract than the noninfected.[29] Probably, a similar phenomenon can be observed in dogs and humans; however, further studies are required to confirm this suggestion.

Similar to *Mycoplasma* spp. are *Ureaplasma* (*U.*) spp. which reside in the urogenital tract. These bacteria, by evolution, have also lost their cell wall. In humans, there are two known species: *U. urealyticum* and *U. parvum*. Like *Mycoplasma* spp., *Ureaplasma* spp. are also considered to be a cause of infertility, but it has also been suggested that they could be a part of the normal genital flora.[30] Since *Mycoplasma* and *Ureaplasma* are related and very similar, some researchers have named them together as "*Mycoplasmas*", and their effect on the semen is examined together in studies.

SPECIFYING THE *MYCOPLASMA*

In the past, the main method of detecting *Mycoplasma* spp. was by culturing them, but owing to the high requirements of these bacteria, this method is not used nowadays in commercial laboratories. The polymerase chain reaction (PCR) is now the most commonly used method in both veterinary and human medicine. Peerayeh and Samimi[31] have shown that the PCR method enables a higher rate of detection of *Mycoplasma* than standard microbiologic cultures.

The ribosomal 16S gene sequence is frequently used in molecular techniques owing to its universal presence among bacteria. The 16S rRNA gene contains nine hypervariable regions (V1–V9) that show differences among bacteria. These specific sequences are useful for diagnostic assays, *e.g.*, V6 helps to distinguish among most bacterial species except *Enterobacteriaceae*. In the case of 16S rRNA analysis, identification of the bacteria is easier when the entire gene can be sequenced. Unfortunately, this technique is not rapid, so it is not common. A faster and commonly used method is based on assays that combine nucleic acid amplification with a sequence-specific probe of the amplified product. In this technique, there is a possibility to query short DNA sequences. Therefore, the identification of the regions within the target gene is important.[32]

In human medicine, there are primers which are capable of detecting *M. hominis*, *M. genitalium*, and *U. urealyticum* simultaneously.[33] In addition, highly specific primers have been developed for the detection of *M. hominis*, *U. urealyticum*, and two others reproductive tract pathogens,[34] and they are based on the ribosomal 16S gene. There are also commercial biochemical assay-based kits available for the identification of *M. hominis*, but the PCR method is faster, more reliable, and more sensitive.[35] The primers which can be used for the identification of human *Mycoplasma* are shown in **Table 2.**

The current knowledge regarding their molecular nature is very limited. Chalker and Brownlie[5] revealed that most canine *Mycoplasma* have a variable phylogenetic origin, but a great part of them lies in a variety of clusters within the hominis group of *Mycoplasma*. Owing to the similarity between the 16S rRNA genes of canine *Mycoplasma*, PCR tests have been created to identify the species-specific regions in the 16S/23S rRNA intergenic spacer region.[36] **Table 3** shows the primers that can be used in the PCR assay to detect canine *Mycoplasma*.

Recently, a novel quantitative qPCR to monitor *Mycoplasma* infection in dogs has been developed by Hemmatzadeh *et al*.[37] A single band of bacterial 16S ribosomal DNA was amplified by using universal *Mycoplasma* primers. The band was excised from the gel, and the purified DNA was submitted to the Australian Genome Research Facility Ltd. for Sanger sequencing. This sequence was used to search GenBank using BLAST for matching a sequence. Thereafter, the prepared DNA was used as a standard for qPCR reactions. The number of copies of the *Mycoplasma* plasmid was calculated on an online calculator. This method was developed because conventional PCR fails to detect less than 100–200 genomes per μl.[37]

INFLUENCE OF INFECTION ON SEMEN QUALITY

The influence of human and canine *Mycoplasma* on the quality of the semen seems to be similar. Infections of the reproductive tract in both humans and animals play an important role in infertility. It is suggested that bacterial and viral infections are two of the factors responsible for male infertility.[38] However, this correlation and the underlying pathogenesis remain unclear. It has been suggested that decreased effectiveness of spermatogenesis, obstruction of the seminal tract, and dysfunction of the spermatozoa are among the adverse effects of bacterial infections.[39] *In vitro* studies have shown that bacterial infection can affect sperm function, in addition to inducing sperm agglutination and apoptosis.[40,41]

The role of *Mycoplasma* infection in both dogs and humans remains unclear. In veterinary medicine, this issue is even more complicated than in human medicine because not all veterinary laboratories specify the species of *Mycoplasma* because of difficulty in their recognition. Previously, the identification of canine *Mycoplasma* was by serological methods which were dependent on specific antisera for each species. However, cross-reactions were also observed; consequently, antisera are not readily available in laboratories.[42] Moreover, owing to the

Table 2: Polymerase chain reaction primers for specifying human *Mycoplasmas*

Mycoplasma spp.	*Mycoplasma primer sequence (5'–3')*	*Source*
Mycoplasma buccale	Forward: ATGCATGTCGAGCGGAAGTA Reverse: AATCCGAAGACCGTCATCATGC	GenBank: AF125586.1[a]
Mycoplasma faucium	Forward: CATGTCGAGCGGAAGTAGCA Reverse: TTAGCTGCGTCAGTGGCTC	GenBank: NR_024983.1[a]
Mycoplasma fermentans	Forward: GGACTATTGTCTAAACAATTTCCC Reverse: GGTTATTCGATTTCTAAATCGCCT	Vojdani and Franco[87] 1999
Mycoplasma genitalium	Forward: TACATGCAAGTCGATCGGAAGTAGC Reverse: AAACTCCAGCCATTGCCTGCTAG	Jensen *et al*.[88] 2003
Mycoplasma hominis	Forward: GGAAGA-TATGTAACAAAAGAAGGTGCTG Reverse: TTTATCTTCTGGCGTAATGATATCTTCG	Baczynska *et al*.[89] 2004
Mycoplasma lipophilum	Forward: CAATATTTAACCGCCGCGCA Reverse: AGCACCCATTAAAGCACGGT	GenBank: DQ112177.1[a]
Mycoplasma orale	Forward: AAGCTTGATGGAGCGACACA Reverse: GCGTTAGCTGCGTCAGTAGT	GenBank: NR_043199.1[a]
Mycoplasma penetrans	Forward: CATGCAAGTCGGACGAAGCA Reverse: AGCATTTCCTCTTCTTACAA	Vojdani and Franco[87] 1999
Mycoplasma pirum	Forward: TACATGCAAGTCGATCG-GAT Reverse: CATCCTATAGCGGTC-CAAAC	Grau *et al*.[90] 1993
Mycoplasma pneumoniae	Forward: CAAGCCAAACACGAGCTCCGGCC Reverse: CAGTGTCAGCTGTTTGTCCTTCCCC	Chaudhry *et al*.[91] 2013
Mycoplasma primatium	In the GenBank, there is no sequence based on which the primer designing could be possible.	-
Mycoplasma salivarium	Forward: ATGATGCTAACCGTGCGCT Reverse: CCATCTTGTCGCCGACTCT	GenBank: EU797448.1[a]
Mycoplasma spermatophilum	Forward: TGACGCTAACCGTGCATTGA Reverse: TGTTACCGTGACGACCTGAC	GenBank: DQ219487.1[a]

[a]Primers not published previously. Parts of the data from the table are cited from the articles and other part of the data are primers not published previously. They are designed based on the sequence from GenBank (ncbi.nlm.nih.gov/genbank). -: no data

Table 3: Polymerase chain reaction primers to specifying canine *Mycoplasma*

Mycoplasma spp.	*Mycoplasma primer sequence*	*Source*
Mycoplasma arginini	Forward: CA-CCGCCCGTCACACCA Reverse: GTTGTATGACCTATTGTTGTC	Chalker[36] 2004
Mycoplasma bovigenitalium	Forward: CGTAGATGCCGCATGGCATTTACGG Reverse: CATTCAATATAGTGGCATTTCCTAC	Kobayashi *et al*.[92] 1998
Mycoplasma canis	Forward: CA-CCGCCCGTCACACCA Reverse: CTGTCGGGGTTATCTCGAC	Chalker[36] 2004
Mycoplasma cynos	Forward: CA-CCGCCCGTCACACCA Reverse: GATACATAAACACAACATTATAATATTG	Chalker[36] 2004
Mycoplasma edwardii	Forward: CA-CCGCCCGTCACACCA Reverse: CTGTCGGGTTATCATGCGAC	Chalker[36] 2004
Mycoplasma feliminutum	Forward: AAGGTCCGTTTGGATCGCTT Reverse: TTTTGGAGCGGGACATGGTT	GenBank: U16758.1[a]
Mycoplasma felis	Forward: CA-CCGCCCGTCACACCA Reverse: GGACTATTATCAAAAGCACATAAC	Chalker[36] 2004
Mycoplasma gateae	Forward: CA-CCGCCCGTCACACCA Reverse: GTTGTATGACCTATTGTTGTC	Chalker[36] 2004
Mycoplasma haemocanis	Forward: GTGCTACAATGGCGAACACA Reverse: TCCTATCCGAACTGAGACGAA	Barker *et al*.[93] 2010
Mycoplasma maculosum	Forward: CA-CCGCCCGTCACACCA Reverse: CCTATGATTGTTACAGATG	Chalker[36] 2004
Mycoplasma molare	Forward: CA-CCGCCCGTCACACCA Reverse: AGCCTATTGTTTTTGATTTG	Chalker[36] 2004
Mycoplasma opalescens	Forward: CA-CCGCCCGTCACACCA Reverse: TAAGCTTTGTAGACCATAA	Chalker[36] 2004
Mycoplasma spp. HRC 689	Forward: CA-CCGCCCGTCACACCA Reverse: CTTGCGACCTAACAAGTCC	Chalker[36] 2004
Mycoplasma spp. VJC 358	Forward: AGGGAGACTGCCCGAGTAAT Reverse: TCGGGTTATCTCGACACATGAC	GenBank: AY246564.1[a]
Mycoplasma spumans	Forward: CA-CCGCCCGTCACACCA Reverse: GTTGTATGACCTATTGTTGTC	Chalker[36] 2004

[a]Primers not published previously. Parts of the data from the table are cited from the articles and other part of the data are primers not published previously. They are designed based on the sequence from GenBank (ncbi.nlm.nih.gov/genbank)

high similarity between the 16S rRNA genes of canine *Mycoplasma*, diagnosis by PCR is also challenging.[12] This is the reason that *Mycoplasma* spp. associated with negative changes in the semen are still unknown.

In human medicine, a meta-analysis has suggested that the presence of *M. hominis*, rather than *M. genitalium*, correlates with male infertility.[43] This indicates that some *Mycoplasma* spp. may also affect male fertility in dogs and some may not. The impact of *Mycoplasma* spp. on the basic semen parameters is described below.[26,44,45]

IMPACT ON BASIC SEMEN PARAMETER VALUES

Volume of the ejaculate

Following the World Health Organization (WHO) guidelines, the volume of the ejaculate should be measured in all semen evaluations. The influence of *Mycoplasma* on the semen volume is not clear. Gdoura *et al*.[44] did not find a significant influence on the semen volume in *Mycoplasma*-positive patients. On the other hand, a study by Ahmadi *et al*.[46] showed a significant increase in the semen volume after treatment of *Mycoplasma* infection. Owing to these contradictory study results, it is not possible to evaluate the impact of *Mycoplasma* on the semen volume, and more studies on this issue are needed.

Progressive sperm motility and sperm concentration

The effect of both canine and human *Mycoplasma* infection on sperm concentration and motility remains unclear. However, a study performed by Gdoura *et al*.[44] showed a negative correlation between the sperm concentration and detection of *M. genitalium* in the semen. Furthermore, semen was analyzed in a Greek study performed to investigate the influence of *Chlamydia* spp., *Ureaplasma* spp., and *Mycoplasma* spp. on sperm concentration, total motility, and progressive motility. No correlation was found between these bacteria and sperm parameter values.[47] However, it has been demonstrated

that *M. genitalium* can influence semen quality by adhering to the sperm heads, midpieces, and tails, owing to which the spermatozoa become immotile.[48] Similarly, the research by Köhn *et al.*[49] showed that spermatozoa incubated with *M. hominis* are less motile than spermatozoa from the control group. In addition, it revealed that for men who were *M. hominis*-positive group, the sperm concentration and motility were significantly lower.[50]

In veterinary medicine, studies on the impact of *Mycoplasma* on dog semen are very limited and many of them are old. In one study from 1977, the researchers tried to infect the reproductive tracts of male dogs. In this study, the *M. canis* isolates were inoculated into the ductus deferens via vasotomy in three dogs (examined group). The control was one dog who received uninoculated broth. All dogs were clinically healthy during this experiment. An increase in the scrotal temperature as well as changes in the testes and epididymides was noticed in two animals (from the examined group) on days 23 and 29. In all dogs in the study group, a significant increase in abnormal spermatozoa and a decrease in the sperm motility were reported, although *Mycoplasma canis* were detected in only one dog.[16] It may be suggested that the abnormalities in the sperm morphology occurred because of the inflammation caused by manipulations during vasotomy, and not because of the *Mycoplasma* infection. In addition, the examined group of dogs was too small to draw final conclusions. There is also a case report of a male dog which was found to be positive for *M. spumans* and *M. maculosum*, and of which seminal sperm concentration was low (1.5×10^6 ml^{-1}) and the spermatozoa were immotile. After *Mycoplasma* treatment, semen quality improved.[51] To confirm the negative effect of those two species of *Mycoplasma* on the semen quality, more research is needed.

In a study by Schäfer-Somi *et al.*[26] andrological examination was correlated with the presence of *Mycoplasma* spp. and other bacteria in the reproductive tract and semen of dogs. *M. canis* was isolated from the semen samples of 18% of dogs whose semen was collected for cryopreservation, 40% of infertile dogs, and 45% of dogs with benign prostatic hyperplasia (BPH). This study showed that these bacteria may be present even in the high-quality semen of a young dog. The authors suggested that the number of the microorganisms is not a decisive factor, but the duration of the infection, degree of epithelial damage, or local immune response may be important. In addition, it has been suggested that the concentration of the spermatozoa may be lower after germinal epithelium damage.[26] To confirm this hypothesis and estimate the real impact of *Mycoplasma* spp. on the morphology of dog spermatozoa and sperm concentration, further studies are needed.

Effect on sperm morphology

A normal human sperm tail should be without cytoplasmic residues and should have a length of approximately 45–50 μm.[52] In veterinary medicine, the assessment of sperm morphology is more difficult owing to the lack of morphometry information.[53] Only a few of the more popular breeds of dogs have been evaluated by morphometrical examination,[54] and this is not enough to define the standard values for all dogs. In male dogs, during the evaluation of the morphology of the spermatozoa, mainly the cytoplasmic residues and tail are considered.

In humans, the lower reference limit for normal forms of spermatozoa is 4%,[55] while in dogs, it should be greater than or equal to 80%.[18] In the past, the reference limit of this parameter was different for men. It was 30% in 1992 and 14% in 1999. The reference values are based on the sperm parameters of fertile men in the fifth percentile in the percentile distribution of results of pregnancy rates. The discrepancy in the lower reference limit is probably because in humans, the sperm counts fall with every decade of life.[56]

Rose *et al.*[45] investigated the influence of *Mycoplasma* spp. on the morphology of spermatozoa. After semen incubation with *Mycoplasma*, there was a significant increase in abnormal midpieces and tails compared with the control group, which suggests that *in vivo Mycoplasma* spp. can have an influence on sperm morphology. Moreover, older reviews have suggested that ejaculates contaminated by *Mycoplasma* spp. contain coiled forms as well as swollen necks of the spermatozoa.[57] An electron microscopical study showed that the spermatozoa from *Mycoplasma*-positive ejaculates had several distinctive features. *Mycoplasma* was attached to the sperm cells by interlacing fibrils of variable diameter, and was associated with spherules. Another characteristic feature was numerous sperms with coiled tails.[58] In addition, a study investigated the real influence of *Mycoplasma* on sperm morphology. In this research, *Mycoplasma* were detected by a *Mycoplasma* IST kit (BioMerieux, Marcy-l'Étoile, France), and the changes in the sperm morphology were found to be as follows: abnormalities in the head's shape, disrupted nuclear membrane, vacuoles within the nuclear chromatin, protuberances in acrosomes, cytoplasmic residues, and vacuoles inside the chromatin.[59]

Since the effect of *Mycoplasma* on sperm morphology remains unclear, and because of limited publications, new studies are needed on this issue. Owing to the similarity between *Mycoplasma* spp. and *Ureaplasma* spp., the impact on the sperm quality of these two bacteria could also be comparable. In one study on the influence of *Ureaplasma* on sperm morphology, it was shown that the *U. urealyticum*-positive group had a higher proportion of abnormal spermatozoa than the control group.[60] This indicates that both *Ureaplasma* spp. and *Mycoplasma* spp. can influence sperm morphology. However, another study showed that *U. urealyticum* had a more significant impact on sperm morphology than *Mycoplasma* and four other pathogens.[61]

IMPACT ON CELLS OTHER THAN SPERMATOZOA AND SPERM AGGLUTINATION

The ejaculate contains cells other than spermatozoa, including epithelial cells, leukocytes, and immature germ cells. All of them can be identified by examining a stained smear.[55] There is a controversial report suggesting that epithelial cells can phagocytose the spermatozoa, which possibly acts as a removal process for abnormal spermatozoa. This phenomenon was noted in men infected by *Chlamydia trachomatis* and *Mycoplasma* spp.[62]

Leukocytes in the ejaculate

The occurrence of leukocytes in the ejaculate is due to infections of the male reproductive tract. This process can be divided into three stages. The first stage occurs shortly after infection, and is not associated with a significant number of leukocytes. During the second stage, it is assumed that the leukocytes take part in the immune response, and therefore, activated leukocytes appear in the semen. During the third stage, the bacteria are eliminated by the immune system, but the leukocytes persist in the ejaculate.[63]

A study has revealed that the presence of *Mycoplasma* in the semen is not correlated with leukocytospermia in humans.[64] In dogs, there was a similar study in which the semen cytology was investigated. Only in 15 of 41 *Mycoplasma*-positive dogs did the cytology show a higher amount of leukocytes than noninflammatory samples.[65] These two studies suggest that *Mycoplasma* spp. may not be related with infections of the male reproductive tract. However, one report has claimed that leukocytes are present in the ejaculate of *Mycoplasma*-

and *Chlamydia*-positive men, and they could phagocytose abnormal spermatozoa. The researchers described a process in which, during the early stages, the sperm head adheres to the surface of the leukocyte, and in the later stages, it is surrounded by the leukocytic pseudopodia. They also found that the leukocytes contained spermatozoa.[59] This study did not comment on the amount of leukocytes in the ejaculate.

Agglutination and aggregation of spermatozoa

Aggregation is the adherence of spermatozoa to other cells or debris,[66] it has been suggested that in *Mycoplasma*-positive men, the number of cells other than spermatozoa was not increased. The phenomenon of the motile spermatozoa sticking to each other is called agglutination.[55] It can be positively correlated not only with anti-sperm antibodies but also with other causes such as genital tract infections and ascorbic acid deficiency.[67] There are two reports on the effect of *Mycoplasma* on sperm agglutination. Both of them involved humans, and did not find a relationship between the presence of anti-sperm antibodies and *Mycoplasma* spp.[68,69] This may indicate that *Mycoplasma* have no influence on sperm agglutination.

IMPACT ON THE FUNCTIONAL PROPERTIES OF SPERMATOZOA

Sperm DNA fragmentation

Any abnormalities in the sperm chromatin or damage to the DNA can cause infertility because the sperm DNA must decondense during fertilization.[70] In a study performed on 143 infertile patients with diagnosed genitourinary infection with *Chlamydia* spp. and *Mycoplasma* spp., sperm DNA fragmentation was examined by the sperm chromatin dispersion (SCD) method. The result showed that the mean percentage of spermatozoa with fragmented DNA in the infertile patient group was 3.2 times higher than that in the control fertile group. After antibiotic and anti-inflammatory treatment, the frequency of the sperm cells with fragmented DNA decreased from 37.7% to 24.2%.[71] This suggests that *Mycoplasma* spp. can influence sperm DNA fragmentation, which is associated with infertility in men. In another study in which flow cytometry was performed after staining with acridine orange (AO), the chromatin integrity, measured by the presence of single-stranded DNA (ssDNA) and double-stranded DNA (dsDNA) breaks in the sperm chromatin in men with semen positive for *Ureaplasma* and *Mycoplasma* strains, was not disturbed.[72] However, in these studies, as the *Mycoplasma* spp. were not specified, it could not be determined which *Mycoplasma* spp. could affect DNA fragmentation.

Acrosome reaction

There are only two studies in this field. In the first study, the spermatozoa were incubated with *Mycoplasma* (*M. hominis* and *U. urealitycum*). The authors showed that spermatozoa from the experimental group were less likely to undergo an acrosome reaction in response to calcium ionophore treatment than the control cells.[45] The second study also showed that *M. hominis* can reduce the inducibility of human sperm acrosome reaction.[49] However, no similar studies have been performed on dogs.

SPERM VITALITY

In both veterinary and human medicine, the most common method to assess the sperm vitality is a test using eosin-nigrosin. This method is based on the principle that the damaged plasma membrane (in dead spermatozoa) allows the entry of membrane-impermeant stains.[73]

In the flow cytometry method, the most common stain used is SYBR-14 with propidine iodine (PI). SYBR-14 penetrates undamaged cell membranes to cause light green fluorescence. Damaged cell membranes allow PI penetration, which displaces SYBR-14, causing red fluorescence. This double staining shows three subpopulations of spermatozoa: live cells (SYBR-14$^+$, PI$^-$), dead cells (SYBR-14$^-$, PI$^+$), and moribund cells (SYBR-14$^+$, PI$^+$).[74,75]

Gallegos *et al.*[71] found no significant impact of these bacteria on sperm vitality. Andrade-Roha[64] also investigated the influence of *Mycoplasma* on this parameter. Sperm vitality was lowest in semen with more than 10^3 colony-forming units per ml of semen (cfu ml^{-1}), but it was not statistically significant. In another study in which the intracellular location of *M. hominis* was investigated, it was noted that this species of *Mycoplasma* does not affect sperm viability.[9]

Although the influence of canine *Mycoplasma* on sperm vitality is unknown, in a dog which was a carrier of *M. spumans* and *M. maculosum*, 100% of the spermatozoa were dead.[51]

EFFECT OF *MYCOPLASMA* SPP. ON PROSTATE FUNCTIONS

In men, the seminal vesicles are the main accessory gland of the male reproductive system,[76] while in dogs, the prostate is the only accessory sex gland.[18] In humans, acute bacterial prostatitis is not associated with infertility in contrast to chronic prostatitis. This phenomenon can be attributed to the impairment of the secretory capacity of the prostate, which might have a negative effect on all semen parameters.[76] It has been suggested that *M. genitalium* is associated with chronic prostatitis in humans, because it is detected more frequently in patients with prostatitis than in healthy ones.[77] However, Mändar *et al.*[78] reported that both *Mycoplasma* and *Ureaplasma* occurred more frequently in the semen of men with prostatitis than in healthy ones, and the most frequently occurring species was *U. parvum*. In another research, *M. hominis* was detected in 13% of men with prostate cancer, while these bacteria was not detected in any of the men with BPH.[79]

In dogs, the correlation between prostate diseases and infertility has not been proven. However, in a study performed on nine stud dogs who presented with infertility, five had prostatitis and one had BPH.[18] In a study by Schäfer-Somi *et al.*[26], *M. canis* was detected in 83.3% of the dogs that were diagnosed with BPH, although it remains unknown if these bacteria play a role in the pathogenesis of this disease.

IMPROVEMENT IN SEMEN QUALITY AFTER TREATMENT OF *MYCOPLASMA* INFECTION

Treatment of *Mycoplasma* infection is based on antibiotic therapy, but because of the lack of a cell wall, these bacteria are resistant to β-lactam antibiotics. Some species are also resistant to macrolides, sulfonamides with trimethoprim and rifampicin.[80] Doxycycline is widely used to treat infections by *Mycoplasma* spp.[81] Treatment with doxycycline (twice daily, for 7 days) in patients with *Mycoplasma* infection results in a significant improvement in all semen parameter values except for volume, pH, and nonprogressive sperm motility.[82,83] However, in another study, 3 months after antibiotic treatment, only 55.3% of men were free from microorganisms, and no significant improvement in any of the investigated semen parameters was noted.[72] It should be noted that doxycycline is a drug that stops bacterial protein synthesis; therefore, the duration of doxycycline therapy should be longer than bactericidal antibiotics. In dogs, the most common drug used for treatment is also doxycycline. Successful treatment has also been reported by the use of doxycycline for 15 days, followed by azithromycin for 9 days.[51] In this case, although the semen quality improved after therapy, a control PCR test was not performed.[51]

In case of low-grade infections with no changes in the semen quality parameters, it has been suggested that preputial irrigation

Table 4: Influence of human and canine *Mycoplasmas* on semen parameters and prostate diseases

Andrological finding	*Human Mycoplasmas*			*Canine Mycoplasmas*	
	Mycoplasma spp.	*Mycoplasma hominis*	*Mycoplasma genitalium*	*Mycoplasma spp.*	*Mycoplasma canis*
Prevalence	1.3%–4%[17]	No data	No data	89%[18]	No data
Infertility	No data	Negative influence[43]	No influence[43]	No data	Present in 17.8% high quality ejaculates and in 40.4% poor semen quality[16]
Volume	Conflicting results[44,82]	No influence[39]	Conflicting results[44,82]	The ejaculate volume is not so important as in human patients	
Concentration	No data	Conflicting results[44,82]	Conflicting results[44,82]	No data	No data
Motility	No data	Conflicting results[44,82]	Conflicting results[44,82]	No data	Temporary decreased spermatozoa motility[16]
Morphology	Negative influence[45]	No influence[63]	No influence[63]	No data	Temporary increased numbers of abnormal spermatozoa[16]
Number of leukocytes	No data	No influence[63]	No influence[63]	No data	In 15 of 41 dogs, the semen cytology showed a higher amount of leukocytes[64]
Sperm agglutination	No influence[67,68]	No data	No data	No data	No data
DNA fragmentation	Conflicting results[71,94]	No data	No data	No data	No data
Acrosomal reaction	No data	Negative influence[45,49]	No data	No data	No data
Viability	Negative influence[63]	No influence[9]	No data	No data	No data
Prostate diseases	Prostatitis[77]	Cancer, BPH[78]	Prostatitis[78]	No data	Prostatitis, BPH[26]

BPH: benign prostatic hyperplasia

with 2.5% marbofloxacin can be a form of therapy,[26] but there is no report about the effectiveness of this method. After treatment, it is recommended that stud dogs should have an 8-week break in mating in order to regenerate and improve the quality of the semen from new germ cells formed during spermatogenesis. Supplementation with vitamin E for 10 weeks has also been suggested to regenerate the epithelium of the seminal tubules.[26]

CONCLUSIONS

Mycoplasma spp. occur on mucosal surfaces in both humans and dogs. Previous studies have described their effect on pelvic diseases in women,[84,85] reproductive tract of female canines,[86] respiratory tract in dogs,[36] and fertility in men.[69,49] In these studies, bacteria were detected in both healthy and diseased study participants; consequently, the impact of *Mycoplasma* remains unclear. A summary of current state of the knowledge about influence of *Mycoplasma* spp. on fertility is shown in **Table 4**.

Almost 89% of the dog population has been reported to be *Mycoplasma* positive,[18] suggesting that not all species or strains are pathogenic, or their virulence is low. Some authors have identified which bacterial species can cause infertility in dogs.[51] However, the knowledge about all strains is still limited.

Further research is required to compare the mechanisms underlying mycoplasmosis in the genital tract in both humans and dogs, especially in close phylogenetic species. It is also necessary to investigate if antibodies induced by *Mycoplasma* infection of the respiratory tract can potentially protect the genital tract during contact with pathogenic species of *Mycoplasma*. Importantly, there is a need to identify which *Mycoplasma* species and strains are pathogenic and which are not.

AUTHOR CONTRIBUTIONS

KD reviewed the literature and wrote the main body of the manuscript. IK helped with designing the manuscript, and made linguistic and stylistic corrections. PJ, SK, and MS critically reviewed and substantially contributed to the final draft of the manuscript. All authors read and approved the final manuscript.

COMPETING INTERESTS

All authors declare no competing interests.

ACKNOWLEDGMENTS

All authors acknowledge Dr. Ricardo Faúndez (InviMed Fertility Clinics, Warsaw, Poland) who provided critical insights into the reviewed topic during manuscript preparation. We also thank Dr. Piotr Bąska (Warsaw University of Life Sciences, Institute of Veterinary Medicine, Department of Preclinical Sciences, Division of Pharmacology and Toxicology, Warsaw, Poland) for designing primers used in **Table 2** and **3**, and **Figure 2**.

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