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Research Article

Impact of Ureaplasma Infection on Sperm Morphometry and Fertility

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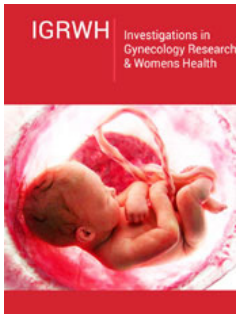
ISSN: 2577-2015



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Submission: July 30, 2024

Published: August 16, 2024

Volume 5 - Issue 1

How to cite this article: K. Aruna, A. Smitha Sree, Pranati Mahapatro, Thota Sivanarayana and G.A. Rama Raju*. Impact of Ureaplasma Infection on Sperm Morphometry and Fertility. Invest Gynecol Res Women's Health. 5(1). IGRWH. 000604. 2024.
DOI: 10.31031/IGRWH.2024.05.000604

Abstract

This study presents novel findings on the impact of *Ureaplasma urealyticum (U. urealyticum)* infection on sperm morphometry and male infertility. We conducted a retrospective study involving 594 infertile male participants diagnosed with teratospermia at a specialized infertility centre. Semen samples were analysed using Computer-Assisted Sperm Analysis (CASA) to assess sperm motility, morphology, and concentration, while PCR techniques were employed to detect *U. urealyticum* infection. Our comprehensive dataset revealed significant differences in the midpiece area between *U. urealyticum*-positive and -negative groups, with the midpiece area emerging as a potential marker for infection. Specifically, the midpiece area in U. urealyticum-positive samples was significantly reduced ($1.70\pm0.38\mu m^2$) compared to *U. urealyticum*-negative samples ($1.77\pm0.47\mu m^2$, $p = 0.0013$). Other morphometric parameters, such as head area, head length, head width, and midpiece width, did not show significant differences. Advanced statistical analyses, including Random Forest and t-tests, were utilized to identify these differences. Correlation analyses further revealed stronger associations between morphological defects in *U. urealyticum*-infected samples. These unique findings underscore the significant impact of U. urealyticum on the midpiece area of spermatozoa, potentially compromising sperm motility and fertilizing capacity. Our results highlight the importance of screening for *U. urealyticum* in the evaluation of male infertility and suggest that targeted antibiotic therapy combined with antioxidant supplementation may improve reproductive outcomes. This study deepens our understanding of the implications of *U. urealyticum* infection on male fertility, paving the way for further research in clinical settings to explore the specific mechanisms by which *U. urealyticum* affects sperm quality and to develop targeted interventions.

Keywords: Ureaplasma; Sperm morphometry; Midpiece area; Male infertility; Random forest; Statistical analysis

Introduction

Ureaplasma urealyticum is a common pathogen in the urogenital tract and has been implicated in various reproductive disorders. *Ureaplasma urealyticum* (*U. urealyticum*) is found in the male urogenital tract that has been associated with male infertility and alterations in sperm parameters [1]. While several studies have examined the effects of *U. urealyticum* on overall semen quality, its specific impact on sperm morphometry remains unclear. Sperm morphometry refers to the detailed measurements and analysis of sperm head, midpiece, and tail dimensions, which can provide valuable insights into sperm function and fertility potential [2,3].

Previous research has demonstrated that *U. urealyticum* infection can negatively affect sperm concentration, motility, and overall morphology. For example, studies have reported lower sperm concentrations, decreased progressive motility, and a higher percentage of abnormal sperm forms in semen samples positive for *U. urealyticum* compared to uninfected controls [4]. However, the precise effects on specific morphometric parameters of individual sperm cells have not been thoroughly characterized.

Understanding how *U. urealyticum* impacts sperm morphometry is important for several reasons. First, subtle changes in sperm head dimensions or midpiece length could potentially

affect sperm hydrodynamics and fertilizing capacity, even if overall morphology appears normal by standard criteria. Second, detailed morphometric analysis may reveal specific patterns of sperm abnormalities associated with *U. urealyticum* infection, which could serve as sensitive biomarkers. Finally, elucidating the effects on sperm ultrastructure could provide insights into the mechanisms by which this microorganism impairs male fertility [1,5-7].

Therefore, this study aimed to conduct a comprehensive analysis of sperm morphometric parameters in a population of infertile population diagnosed with teratospermia using Computer-Assisted Sperm Morphometry Analysis (CASA). We hypothesized that Ureaplasma urealyticum-positive samples within this teratospermia population would exhibit significant alterations in sperm head dimensions, midpiece length, and tail characteristics. The findings of this research may enhance our understanding of how *U. urealyticum* affects sperm at the cellular level and could have implications for the diagnosis and management of male infertility associated with this infection.

Materials and Methods

Study design

To evaluate the impact of *Ureaplasma urealyticum* infection on semen quality, we conducted a retrospective study involving male participants at a single centre specializing in male infertility. A total of 594 semen samples were collected from infertile males diagnosed with teratospermia. Participants provided informed consent, and the study was approved by the institutional review board. Initially, all participants underwent semen analysis using Computer-Assisted Sperm Analysis (CASA) to assess sperm motility, morphology, and concentration. This analysis was conducted by our experienced technicians, each with over 10 years of expertise in CASA as per World Health Organization WHO guidelines [8,9]. Additionally, our technicians have been active participants in the External Quality Assurance Schemes for Reproductive Medicine (EQASRM) program since 2012, ensuring high standards of accuracy and reliability in semen analysis. Following the CASA assessment, participants diagnosed with teratospermia were tested for *Ureaplasma urealyticum* infection using Polymerase Chain Reaction (PCR) techniques on both semen and urine samples. Age and Body Mass Index (BMI) were also recorded at the time of semen analysis to study their potential influence on semen quality.

By investigating the differences in sperm morphometry between Ureaplasma positive and negative samples, focusing on identifying potential markers that could elucidate the infection's impact on fertility, this study aims to provide comprehensive insights into the factors affecting male fertility. Employing both traditional statistical methods and machine learning techniques, we will analyze how *Ureaplasma urealyticum* infection alters sperm structure and function, considering age and BMI as additional variables. This multifaceted approach aims to enhance our understanding of the infection's role in male infertility and identify key markers associated with compromised semen quality.

Inclusion criteria

A. All semen samples were obtained by external ejaculation after masturbation in the semen collection room at the hospital at 25 °C. Patient information and data were complete, and follow-up requirements.

B. Men diagnosed with infertility

C. Males aged between 18 and 50 years.

D. Diagnosed with teratospermia based on standard semen analysis criteria.

E. No history of antibiotic treatment within the past three months.

F. No concurrent urogenital infections other than Ureaplasma urealyticum.

Exclusion criteria

A. Those who did not meet the inclusion criteria had used antibiotics in the two weeks before the test, had systemic diseases, had a long-term drug use history, had a history of exposure to radioactive substances or toxic substances, had chromosomal abnormalities, had Azoospermia Factor (AZF) gene deletion, had varicocele, had azoospermia, or had testicular tumors.

B. Presence of other urogenital infections.

C. Recent use of antibiotics or other medications affecting sperm parameters.

D. Chronic systemic illnesses that could influence semen quality.

Sample collection

Samples of human semen were collected by masturbation during an abstinence period, which was standardized to be 3-5 days in line with the World Health Organization (WHO) guidelines [8,9]. The exact duration of the abstinence period was recorded. All males were instructed on semen and genital specimen collection according to the national standard of reproductive laboratories to minimize microbial contamination during semen extraction. Each semen sample was poured into a sterile sample container and liquefied in specific incubators at 37 °C. When the semen sample was entirely liquefied, the volume of each semen sample was measured by weight and converted to millilitres. Each semen sample was used for three purposes:

(i) routine semen analysis,

(ii) analysing the ratio of normal sperm morphology, and

(iii) for pathogen testing.

Sperm count and motility analysis by CASA

Sperm count and motility parameters were evaluated by using a CASA system, WHO version 6 Sperm Class Analyzer (SCA), version 6.0 (Microptic) and A binocular phase contrast microscope is fitted with a high-resolution video camera. Type of capture was manual. After initial automated detection of spermatozoa, manual validation was performed to exclude debris, leucocytes and other non-sperm

cells. Spermatozoa were categorised into progressive (μm/s), non-progressive (μm/s) and immotile based on WHO guidelines [8,9].

The following parameters were assessed:

A. **Volume:** Measured in millilitres (mL).

B. **Liquefaction Time:** Time taken for the semen to become liquid at room temperature.

C. **Appearance:** Visual assessment of semen.

D. **Viscosity:** Evaluated subjectively as normal or increased.

E. **pH:** Measured using pH strips.

F. **Concentration:** Sperm concentration measured in million sperm per millilitre.

G. **Motility:** Assessed and categorized into progressive, non-progressive, and immotile.

H. **Morphology:** Percentage of normal and abnormal sperm shapes, with detailed assessment of head, midpiece, and tail abnormalities.

Morphology assessment

Morphology of spermatozoa was analyzed using Computer-Aided Sperm Analysis (CASA) systems, following the World Health Organization (WHO) guidelines [8,9]. Parameters assessed included volume, liquefaction time, appearance, viscosity, pH, concentration, motility, and detailed sperm morphometry. Key morphometry measurements included head area, head length, head width, midpiece width, length and tail length.

Preparation of semen smear and staining method for morphological analysis of spermatozoa by CASA

All semen samples were subjected to staining procedure after complete liquefaction. Depending on the concentration, the volume of the sample was adjusted (ranging from 3 to 7μl) for the smear preparation which results in 2-10 spermatozoa per field. The air-dried smears were fixed in fixative solution and stained with Diff-Quick stain [10,11].

For morphology, at least 100 stained spermatozoa were manually analysed by using 100X oil immersion objective in phase contrast microscope is fitted with a high-resolution video camera with CASA system, Sperm Class Analyzer (SCA), version 6.0 (Microptic). The percentages of normal sperm, head defects, middle and main segment defects, excessive residual cytoplasm, and round cells were observed under an oil microscope. The operation was strictly performed following the SOP. In line with the WHO guidelines [8,9], a spermatozoon with a malformed head, middle, and tail parts was recorded as teratospermia. The Sperm Deformity Index (SDI) was calculated as the ratio of deformed sperm cells to the total number of sperm cells.

Detection of pathogenic microorganisms

All Teratozoospermia semen and urine samples from the study population were collected and evaluated for *U. urealyticum*. A modified method of Lacroix JM et al. [12] the semen and urine samples were centrifuged, and the supernatant was removed. The bacterial DNA was extracted from the semen and Urine pellet (by using MN extraction kit) and *U. urealyticum* was detected using Polymerase Chain Reaction (PCR) with specific kits (Thermofisher Scientific Real time PCR Kit). A standard curve was formulated for each test based on the kit contents. Concentrations of *U.* urealyticum ≥500 copies/ml were considered positive [13]. *U. urealyticum* was analyzed under high-quality control in the professional Molecular biology laboratory, Genetics Department.

Statistical analysis

Descriptive statistics were used to summarize the semen parameters and morphometric measurements. Independent t-tests were conducted to compare the midpiece areas between the Ureaplasma positive and negative groups. A p-value of <0.05 was considered statistically significant. Effect sizes were calculated using Cohen's d and confidence intervals were provided for key parameters. Corrections for multiple comparisons were applied using the Bonferroni method. Correlation analysis was performed to identify relationships between Ureaplasma status and sperm features. Multivariate analyses including MANOVA were conducted to validate findings and control for potential confounders.

Result

Among the 594 men included in this study, 72 (12.12%) were positive for Ureaplasma urealyticum, while 522 (87.87%) were negative (Table 1). The Table 2 shows that the mean CT value for *Ureaplasma urealyticum* infection positive samples is 28.44 with a standard deviation of 3.83. CT values range from 24 to 30, indicating variability in infection load or detection sensitivity among the samples. The results in Table 3 show a significant difference in the midpiece area between the Ureaplasma positive and Ureaplasma negative groups. The midpiece area for Ureaplasma positive samples was significantly smaller (1.70±0.38 μm^2) compared to the Ureaplasma negative samples (1.77±0.47μm^2, p= 0.0013). No significant differences were found for other morphometric parameters such as head area, head length, head width, and midpiece width.

Table 1: Distribution of *Ureaplasma urealyticum* infection among participants.

Variables	Number(n)	Rate (%)
Ureaplasma urealyticum infection positive group	72	12.12
Ureaplasma urealyticum infection negative group	522	87.87

Table 2: Distribution of *Ureaplasma urealyticum* infection among participants.

Mean CT value of *Ureaplasma urealyticum* infection positive group (standard deviation)	Minimum CT value	Maximum CT value
28.44±3.83	24	30

Note: CT value: Cycle Threshold value.

Table 3: Descriptive statistics of sperm parameters.

Parameter	Ureaplasma Positive (Mean ± SD)	Ureaplasma Negative (Mean ± SD)	p-value
Head Area (μm^2)	6.39±1.08	6.53±1.62	>0.05
Head Length (μm)	4.95±0.55	4.98±0.61	>0.05
Head Width (μm)	3.45±0.30	3.48±0.32	>0.05
Midpiece Area (μm^2)	1.70±0.38	1.77±0.47	0.0013
Midpiece Width (μm)	0.67±0.12	0.69±0.14	>0.05

Note: SD: Standard Deviation.

Correlation Analysis of Sperm Morphological Parameters

Figure 1 illustrates the correlation matrices for sperm morphological parameters in both Ureaplasma infected and uninfected individuals. In the infected group (left matrix), strong positive correlations (red) are seen between multi-headed sperm and head defects, indicating these abnormalities often co-occur. Negative correlations (blue) between normal sperm morphology and abnormal forms suggest that as normal morphology decreases, abnormalities increase. The uninfected group's matrix (right) shows similar patterns but with fewer strong correlations, suggesting that Ureaplasma infection intensifies sperm abnormalities, leading to stronger associations between different morphological defects.

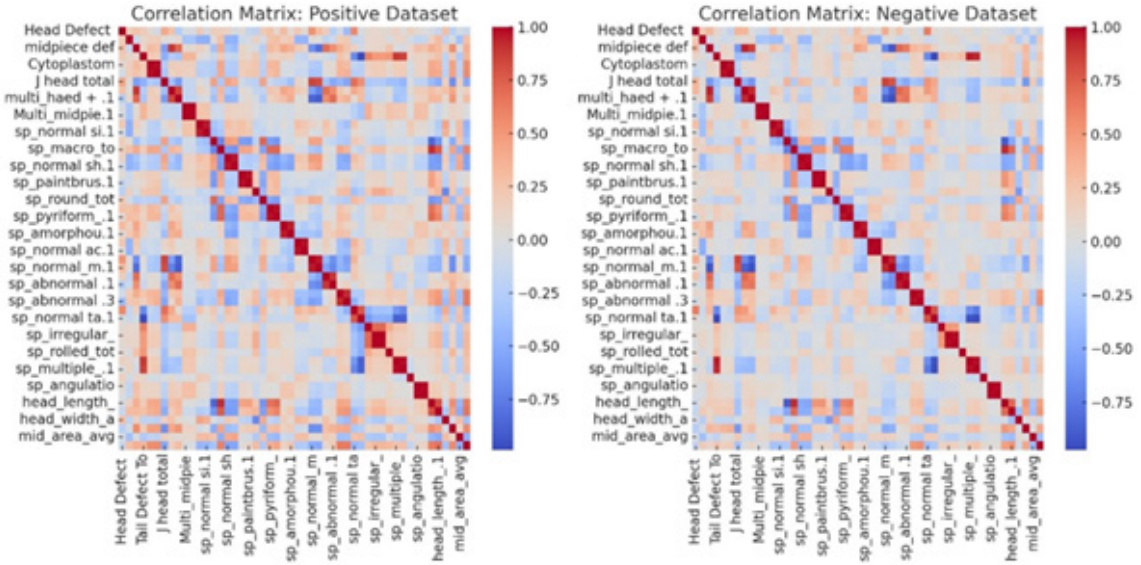

Figure 1: Correlation analysis of sperm morphological parameters.

Comparison of Sperm Head Morphology Parameters

Figure 2 compares sperm head morphology parameters head area, head length, and head width between Ureaplasma positive and Ureaplasma negative individuals. The top row histograms show higher densities and broader distributions for all three parameters in the Ureaplasma negative group, indicating more diverse sperm head morphologies. The middle row violin plots further illustrate these differences, with the Ureaplasma negative group displaying greater variability and wider interquartile ranges. The bottom row scatter plots highlight more clustered data points for the Ureaplasma negative group, suggesting that Ureaplasma infection may be associated with alterations in sperm head morphology, potentially impacting fertility.

Impact of Ureaplasma Infection on Sperm Parameters

Figure 3 shows the impact of Ureaplasma infection on sperm morphology by comparing key parameters head area, head length, head width, midpiece width, and midpiece angle between Ureaplasma positive and Ureaplasma negative individuals. The box plots reveal that Ureaplasma negative individuals generally have higher median values for head area and head length, suggesting that Ureaplasma infection may be associated with reduced head dimensions. The head width parameter shows a slight increase in the Ureaplasma positive group. Additionally, the midpiece width and midpiece angle parameters indicate higher median values in the Ureaplasma positive group, pointing to potential structural changes in the sperm midpiece due to the infection. These findings under-

score the significance of screening for Ureaplasma in the evaluation of male infertility, as the infection appears to affect both the head and midpiece morphology of sperm.

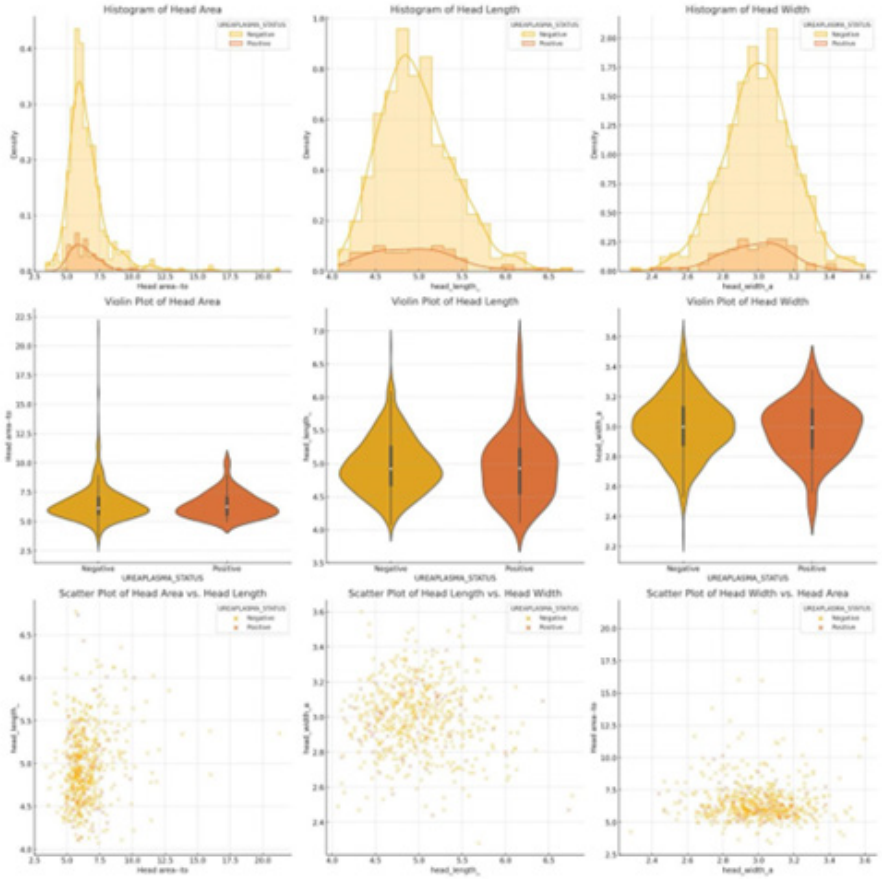

Figure 2: Comparison of sperm head morphology parameters.

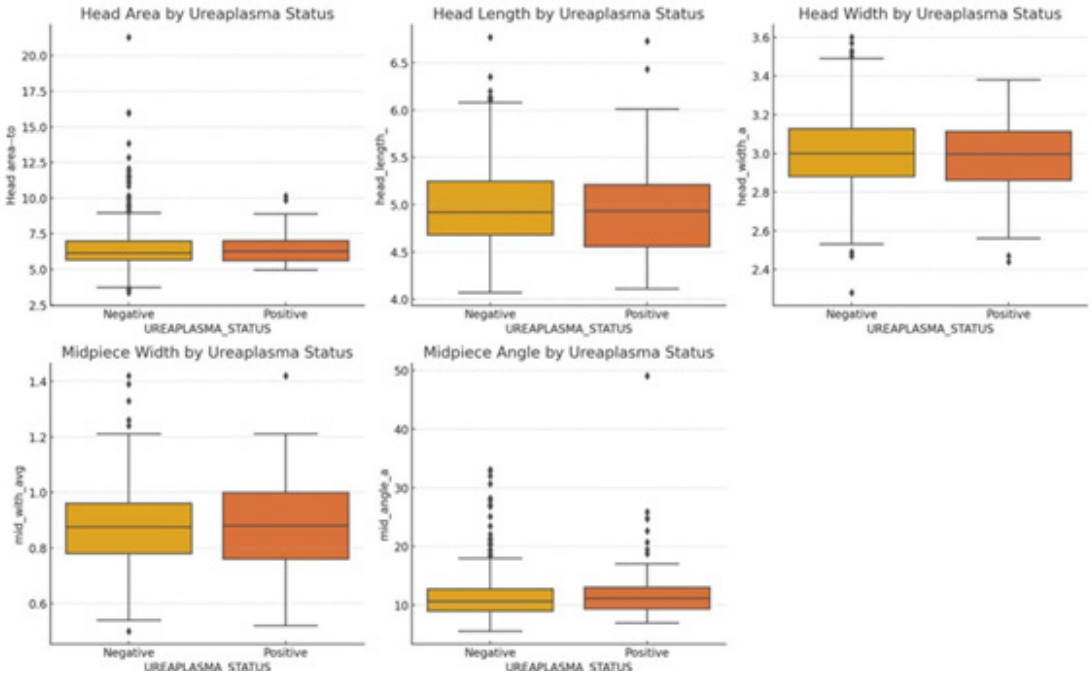

Figure 3: Impact of Ureaplasma infection on sperm parameters.

Impact of Ureaplasma Infection on Sperm Motility

Figure 4 demonstrates the distribution of two key sperm motility parameters, Curvilinear Velocity (VCL) and Straight-line Velocity (VSL), among Ureaplasma positive and Ureaplasma negative individuals. The histograms reveal that sperm from Ureaplasma negative individuals exhibit higher motility, with a pronounced peak in VCL around 40-60µm/s and in VSL around 30-40µm/s. In contrast, the Ureaplasma positive group shows lower and less defined peaks for both parameters. These findings suggest a negative impact of Ureaplasma infection on sperm motility, highlighting the importance of screening for Ureaplasma in the assessment of male infertility.

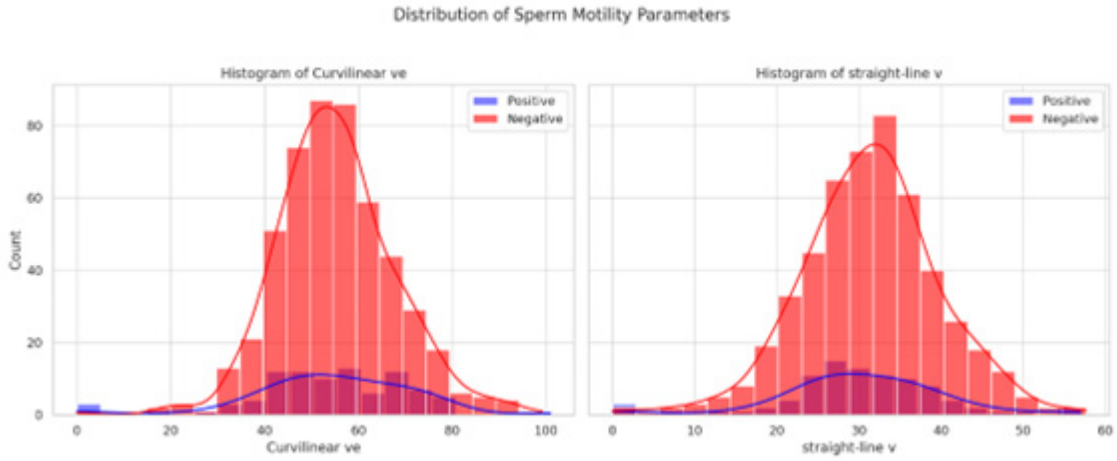

Figure 4: Impact of Ureaplasma infection on sperm motility.

Summary of Key Findings

1. *Ureaplasma urealyticum* infection was detected in 12.12% of the infertile men with teratospermia in this study.

2. The midpiece area of sperm was significantly smaller in Ureaplasma-positive samples compared to negative samples (1.70µm^2 *vs* 1.77µm^2, p=0.0013).

3. Other morphometric parameters like head area, length, width and midpiece width did not show significant differences between infected and uninfected groups.

4. Correlation analysis revealed stronger associations between sperm abnormalities in the Ureaplasma-infected group compared to the uninfected group.

5. Visualization of sperm head morphology parameters showed greater variability in the Ureaplasma-negative group.

Discussion

In this study, we investigated the impact of *Ureaplasma urealyticum* infection on sperm morphometry and its potential implications for male infertility. Our results demonstrate that *U. urealyticum* infection is associated with significant alterations in the midpiece area of spermatozoa, which may contribute to reduced fertility.

Ureaplasma urealyticum is a common pathogen in the urogenital tract and has been implicated in various reproductive disorders. The infection is known to induce oxidative stress, leading to lipid peroxidation and inflammatory responses, which can negatively affect sperm function [14]. This oxidative stress can result in a reduced midpiece area, as seen in Ureaplasma positive samples, impacting sperm motility and fertilizing capacity. Moreover, the inflammatory response associated with Ureaplasma infection can alter the structural integrity of the spermatozoa, further compromising fertility [14-16]. The midpiece of the spermatozoon is particularly susceptible to oxidative damage due to its high concentration of mitochondria, which are crucial for ATP production and sperm motility [17-19].

The significant reduction in midpiece area associated with Ureaplasma infection is a novel finding. This could be due to the inflammatory response triggered by *U. urealyticum* infection, where proinflammatory cytokines released can alter the structural integrity of spermatozoa, particularly in the midpiece where mitochondria are densely packed [19].

The specific impact on the midpiece raises questions about how Ureaplasma infection might target this structure. Possible mechanisms could include direct damage to mitochondria, alterations in protein expression crucial for midpiece formation, or disruption of lipid membranes. Furthermore, the stronger correlations between different sperm abnormalities in infected samples may indicate that Ureaplasma infection exacerbates or triggers multiple aspects of sperm dysfunction simultaneously, potentially due to systemic effects on the testicular or epididymal environment.

While previous studies have reported overall increases in abnormal morphology with Ureaplasma infection, our results suggest the effects may be more specific to certain structures like the midpiece. The lack of significant differences in head dimensions indicates the infection may not uniformly affect all aspects of sperm structure. Therefore, it is crucial to consider both the direct impact on sperm morphometry and the indirect effects mediated through alterations

in seminal plasma composition. Interestingly, other morphometric parameters such as head area, head length, head width, and midpiece width did not show significant differences between Ureaplasma positive and negative samples. This suggests that *U. urealyticum* infection specifically targets the midpiece region, which houses the mitochondria. The selective impact on the midpiece could be due to the localized accumulation of Reactive Oxygen Species (ROS) in this region, leading to targeted damage [15,20].

Zhou et al. [21] reported that infertile men have a significantly higher prevalence of Ureaplasma spp. (39.6%) compared with fertile men (19.2%). Our study revealed a significant reduction in the midpiece area in Ureaplasma positive samples compared to Ureaplasma negative samples. This finding aligns with previous research indicating that oxidative stress and lipid peroxidation can compromise mitochondrial function, leading to reduced ATP production and impaired sperm motility [15,22,23]. However, it is important to note that while many studies show a clear impact of *U. urealyticum* on sperm quality, the pathogenic impact may depend on individual susceptibility and the presence of other factors. For instance, while our study strongly suggests a negative impact of *U. urealyticum* on male fertility, the clinical relevance of the differences in morphometry needs further investigation. Future studies should examine whether these subtle changes in midpiece dimensions correlate with functional deficits or reduced fertility outcomes.

In addition to the structural changes, *U. urealyticum* infection can affect the biochemical composition of seminal plasma, further influencing sperm function. Increased levels of ROS in seminal plasma can exacerbate oxidative stress, leading to a vicious cycle of mitochondrial damage and impaired sperm function [24]. Our findings highlight the importance of screening for *U. urealyticum* infection in the evaluation of male infertility. Given the significant impact on the midpiece area and the potential downstream effects on sperm motility and fertilizing ability, early detection and treatment of *U. urealyticum* infection could improve reproductive outcomes. Antibiotic therapy targeting *U. urealyticum*, combined with antioxidant supplementation to mitigate oxidative stress, may offer a comprehensive approach to managing infection-related infertility [25].

Conclusion

In conclusion, *Ureaplasma urealyticum* infection is significantly associated with adverse effects on sperm morphology and motility, which are critical factors in male fertility. The presence of *Ureaplasma urealyticum* correlates with a reduction in sperm midpiece area, highlighting potential structural changes in the sperm caused by the infection. This study underscores the importance of screening for *Ureaplasma urealyticum* in the evaluation of male infertility, as the infection's impact on sperm morphology and motility can compromise reproductive outcomes. Further research is warranted to explore the specific mechanisms by which *Ureaplasma urealyticum* affects sperm quality and to establish targeted interventions to mitigate its negative effects on male fertility.

Acknowledgement

We would like to thank Mr. Ramana, Mrs. Sivani, and Mr. Ramesh for their help with the sperm analysis. Their expertise was essential to our study. We also thank our institution's staff and the participants for their support.

Conflict of Interest

The authors declare that the research was conducted without any commercial or financial relationships that could be seen as a potential conflict of interest.

References

1 Xianchun F, Jun F, Zhijun D, Mingyun H (2023) Effects of *Ureaplasma urealyticum* infection on semen quality and sperm morphology. Frontiers in Endocrinology 14: 1113130.

2 Jia YL, Wu YB, Yu L, Zheng Y, Yang TT, et al. (2024) Normal sperm head morphometric reference values in fertile Asian males. Asian Journal of Andrology 26(3): 315-320.

3 Vasan SS (2011) Semen analysis and sperm function tests: How much to test? Indian Journal of Urology 27(1): 41-48.

4 Gdoura R, Kchaou W, Chaari C, Znazen A, Keskes L, et al. (2007) *Ureaplasma urealyticum*, *Ureaplasma parvum*, *Mycoplasma hominis* and *Mycoplasma genitalium* infections and semen quality of infertile men. BMC infectious diseases 7: 1-9.

5 Gdoura R, Kchaou W, Ammar-Keskes L, Chakroun N, Sellemi A, et al. (2008) Assessment of *Chlamydia trachomatis*, *Ureaplasma urealyticum*, *Ureaplasma parvum*, *Mycoplasma hominis*, and *Mycoplasma genitalium* in semen and first void urine specimens of asymptomatic male partners of infertile couples. Journal of Andrology 29(2): 198-206.

6 Liu H, Yang K, He L, Zhu S, Pang T, et al. (2022) Risk prediction of *Ureaplasma urealyticum* affecting sperm quality based on mathematical model and cross-sectional study. Computational and Mathematical Methods in Medicine 2022(1): 2498306.

7 World Health Statistics (2010) World Health Organization, Geneva, Switzerland.

8 (2021) WHO laboratory manual for the examination and processing of human semen. World Health Organization, Geneva, Switzerland.

9 Kruger TF, Ackerman SB, Simmons KF, Swanson RJ, Brugo SS, et al. (1987) A quick, reliable staining technique for human sperm morphology. Archives of Andrology 18(3): 275-277.

10 Rama Raju GA, Jaya PG, Murali KK, Madan K, Siva NT, et al. (2012) Noninsulin-dependent diabetes mellitus: Effects on sperm morphological and functional characteristics, nuclear DNA integrity and outcome of assisted reproductive technique. Andrologia 44(Suppl 1): 490-498.

11 Lacroix JM, Jarvi K, Batra SD, Heritz DM, Mittelman MW (1996) PCR-based technique for the detection of bacteria in semen and urine. Journal of Microbiological Methods 26(1-2): 61-71.

12 Yoshida T, Maeda SI, Deguchi T, Ishiko H (2002) Phylogeny-based rapid identification of mycoplasmas and ureaplasmas from urethritis patients. J Clin Microbiol 40(1): 105-110.

13 Potts JM, Sharma R, Pasqualotto F, Nelson D, Hall G (2000) Association of *Ureaplasma urealyticum* with abnormal reactive oxygen species levels and absence of leukocytospermia. The Journal of Urology 163(6): 1775-1778.

14 Taylor-Robinson D (1986) Evaluation of the role of *Ureaplasma urealyticum* in infertility. Pediatr Infect Dis 5(6 Suppl): S262-5.

15 Nunez-Calonge R, Caballero P, Redondo C, Baquero F, Martinez-Ferrer M, et al. (1998) *Ureaplasma urealyticum* reduces motility and induces membrane alterations in human spermatozoa. Human Reproduction (Oxford, England) 13(10): 2756-2761.

16 Fraczek M, Kurpisz M (2007) Inflammatory mediators exert toxic effects of oxidative stress on human spermatozoa. Journal of Andrology 28(2): 325-333.

17 Aitken RJ, Smith TB, Jobling MS, Baker MA, Iuliis GND (2014) Oxidative stress and male reproductive health. Asian journal of Andrology 16(1): 31-38.

18 Aitken RJ, Jones KT, Robertson SA (2012) Reactive oxygen species and sperm function-in sickness and in health. Journal of Andrology 33(6): 1096-1106.

19 Wang X, Sharma RK, Sikka SC, Thomas AJ, Falcone T, et al. (2003) Oxidative stress is associated with increased apoptosis leading to spermatozoa DNA damage in patients with male factor infertility. Fertility and Sterility 80(3): 531-535.

20 Zhou YH, Ma HX, Shi XX, Liu Y (2018) *Ureaplasma spp.* in male infertility and its relationship with semen quality and seminal plasma components. Journal of Microbiology, Immunology and Infection 51(6): 778-783.

21 Aitken RJ, Baker MA (2004) Oxidative stress and male reproductive biology. Reproduction Fertility and Development 16(5): 581-588.

22 Armstrong JS, Rajasekaran M, Chamulitrat W, Gatti P, Hellstrom WJ, et al. (1999) Characterization of reactive oxygen species induced effects on human spermatozoa movement and energy metabolism. Free Radical Biology and Medicine 26(7-8): 869-880.

23 Agarwal A, Saleh RA, Bedaiwy MA (2003) Role of reactive oxygen species in the pathophysiology of human reproduction. Fertility and Sterility 79(4): 829-843.

24 Gallegos G, Ramos B, Santiso R, Goyanes V, Gosálvez J, et al. (2008) Sperm DNA fragmentation in infertile men with genitourinary infection by *Chlamydia trachomatis* and Mycoplasma. Fertility and Sterility 90(2): 328-334.

25 Paira DA, Olivera C, Tissera AD, Molina RI, Olmedo JJ, et al. (2023) *Ureaplasma urealyticum* and *Mycoplasma hominis* urogenital infections associate with semen inflammation and decreased sperm quality. Journal of Leukocyte Biology 113(1): 18-26.