Ultrastructural findings in semen samples of infertile men infected with *Chlamydia trachomatis* and mycoplasmas

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The aim of this study was to describe the ultrastructural features observed in semen samples of men with infertility and subfertility of unknown cause infected with *Chlamydia trachomatis* and mycoplasmas. The findings observed by ultrastructure included destruction or persistence of bacteria in leukocytes, phagocytosis of spermatozoa by leukocytes, and structural damage of spermatozoa. (Fertil Steril® 2009;91:915–9. ©2009 by American Society for Reproductive Medicine.)

Key Words: *Chlamydia trachomatis*, mycoplasmas, infertile men, semen, ultrastructure

There is conflicting evidence regarding the influence of infection with *Chlamydia trachomatis* and mycoplasmas on male infertility. Electron microscopy can be of great help in the study of this male infertility condition. This is especially obvious when the small size of *C. trachomatis* and mycoplasmas is considered (1, 2). The aim of this study was to describe the ultrastructural features observed in semen samples of men with infertility and subfertility of unknown cause infected with *C. trachomatis* and mycoplasmas.

A total of 143 male partners from couples with infertility or subfertility of unknown cause were included in this study. The median duration of infertility was 4 years (range, 1–23 years). Primary infertility was present in 111 couples (78%) and secondary infertility in 32 couples (22%). The median age of the patients was 34 years (range, 22–55 years). Ninety-five percent of their partners were infected with *C. trachomatis* and/or mycoplasmas. The control group comprised 10 healthy and fertile volunteers with a median age of 31 years (range, 23–44 years). All control subjects had normal results on seminal analysis performed according to World Health Organization criteria (3) and negative results on microbiologic tests performed as described below. None of the control individuals or their female partners had clinical signs or symptoms of a genital tract infection. Approval for the study was obtained from our institutional review board. Informed consent to use samples was obtained from all subjects. The detection of *C. trachomatis* was performed in urethral samples using a direct specimen test kit (MicroTrak; Trinity Biotech, Wicklow, Ireland). The isolation of *Mycoplasma hominis* and *Ureaplasma urealyticum* was done using the Mycoplasma IST kit (BioMerieux, Marcy L'etoile, France). Finally, ejaculates were analyzed using an electron microscopic technique.

Four groups of patients were defined: 16 patients (11%) positive for *C. trachomatis*, 62 (43%) positive for mycoplasmas, 35 (25%) positive for *C. trachomatis* and mycoplasmas, and 30 (21%) negative for the two analyzed bacteria. The findings observed by ultrastructure included destruction or persistence of bacteria in leukocytes, phagocytosis of spermatozoa by leukocytes, and structural damage of spermatozoa (Figs. 1–3). These findings were observed in the four groups of patients, whereas in the control group only abnormal forms of spermatozoa were observed but in the normal values established by the World Health Organization (3).

DISCUSSION

We found in the analyzed semen samples that *C. trachomatis* and mycoplasmas were phagocytized and killed by polymorphonuclear leukocytes and macrophages. This observation

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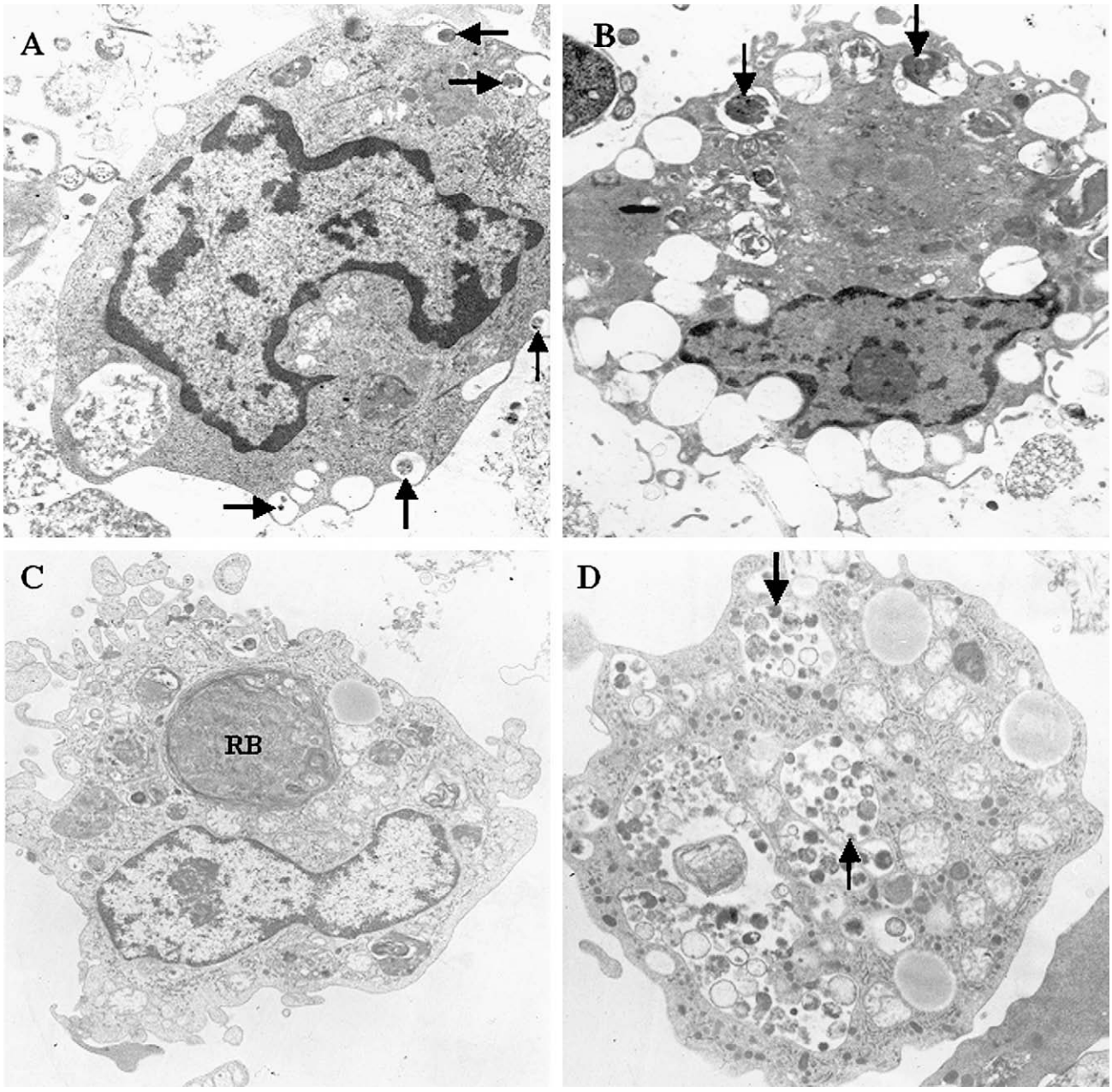
FIGURE 1

Interaction between *C. trachomatis* and mycoplasmas with leukocytes in semen samples of infertile or subfertile men. (**A**) In an early phase of the interaction, internalization of both microorganisms was observed (*arrows*). Two alternatives were possible after this first phase: (**B**) degraded bacteria were found in phagolysosomes (*arrows*) and (**C**) residual bodies (RB), or colocalization of the two kinds of bacteria was observed in inclusion bodies (**D**, *arrows*). Original magnification, ×4,400.

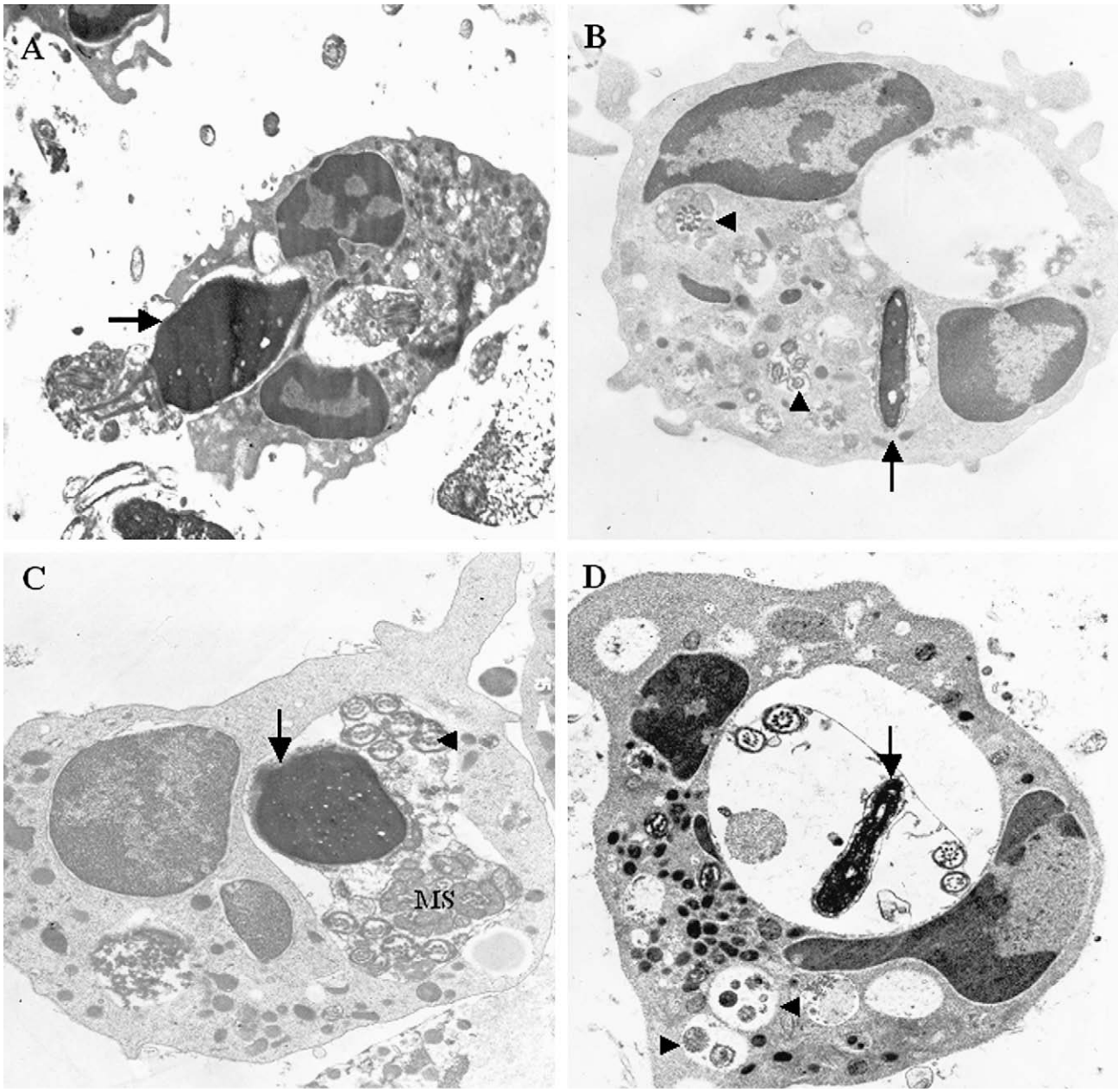


Gallegos-Avila. Semen ultrastructure in infected men. Fertil Steril 2009.

indicates that human leukocytes have the capability to phagocytize these bacteria and thus their contribution in the immune response against these microbes in genitourinary male infections. However, we also observed localization of intact bacteria in cell inclusions. This intracellular location would results in several pathogenic consequences. Intracellular residence promotes the establishment of latent or chronic infection states and circumvents bactericidal immune mechanisms and selective drug therapies (4). In addition, this physical association may act as a factor for the dissemination of the bacterial infections to the female partners.

FIGURE 2

Phagocytosis of sperm cells in semen samples of infertile or subfertile men infected with *C. trachomatis* and mycoplasmas. (**A**) In early stages the sperm head adhered to the surface of the leukocytes, and at later stages the sperm head (*arrow*) was surrounded by the leukocytic pseudopodia. (**B**) Afterward, the sperm head underwent complete phagocytosis (*arrow*) along with segments of the tail (*arrowheads*). (**C**) Leukocytes were observed phagocytizing the mid-piece (mitochondrial sheath, MS) and others segments of the tail (*arrowhead*), along with the head of sperm cells (*arrow*). (**D**) Leukocytes often contained sperm cells (*arrow*), along with bacterial inclusions (*arrowheads*). Original magnification, ×4,400 in **A**; ×7,000 in **B–D**.



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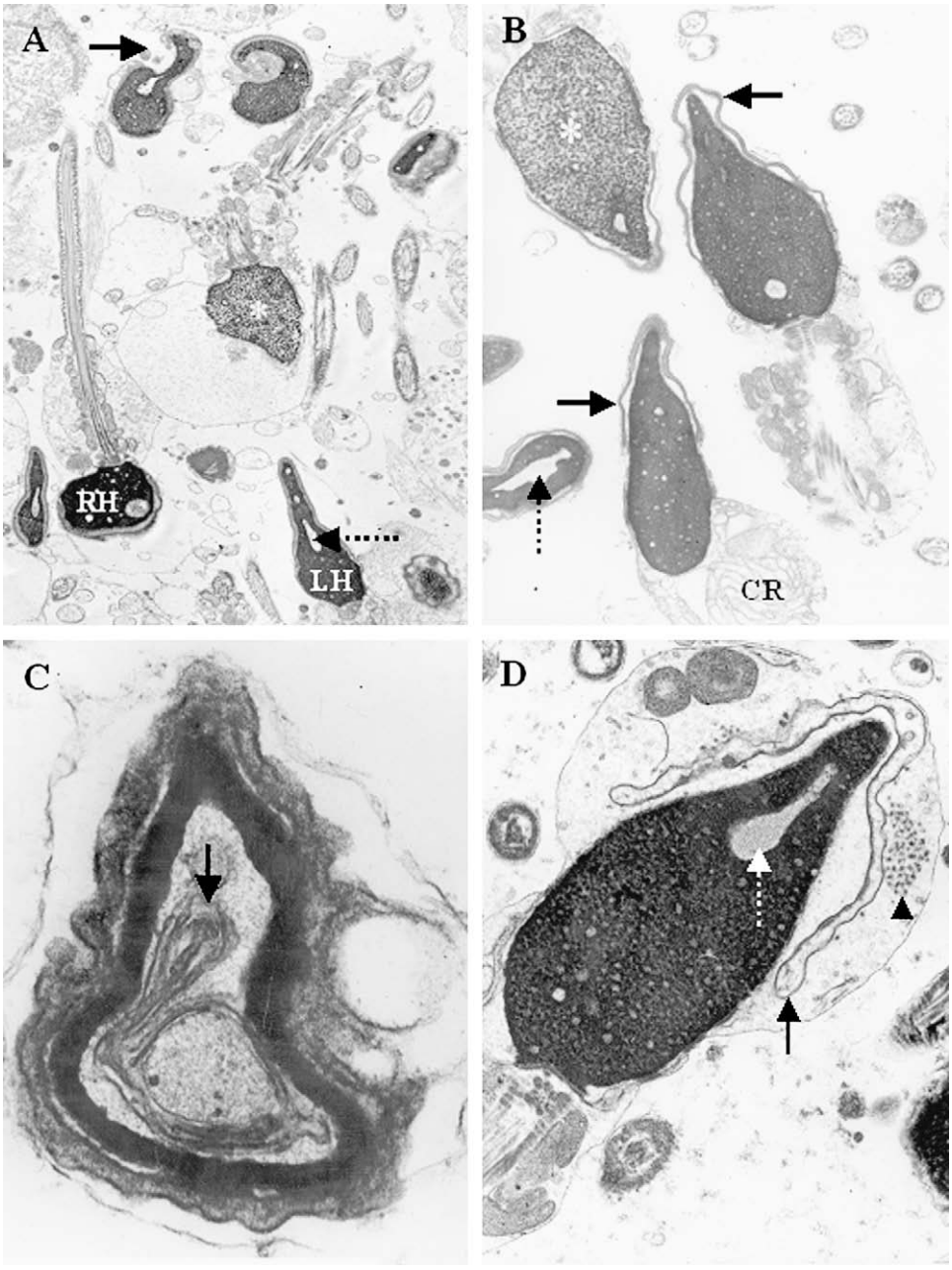
The presence of abnormal spermatozoa was evident in the analyzed semen samples of the patients. As in other infectious processes, the cell damage would be caused directly by the microorganisms or alternatively by the host immune responses. The presence of spermatophagy in the semen samples of the analyzed patients was demonstrated in this study, and their possible role includes the clearing of degenerate or abnormal spermatozoa. We observed several sperm abnormalities, and most of them were also represented in the spermatophagy encountered.

FIGURE 3

Damage of spermatozoa in semen samples of infertile or subfertile men infected with *C. trachomatis* and mycoplasmas. (**A**) Abnormalities included round heads (RH), large heads (LH), and partially disrupted nuclear membranes (*arrow*). Vacuoles within the nuclear chromatin (*dotted arrow*) and spermatozoa with a pattern of a loose fibrillar–microgranular chromatin network (*asterisk*) were also observed. (**B**) The acrosomes presented protuberances (*arrows*), and cytoplasmic residues were evident, containing vacuoles and multilayered membranous bodies (CR). Vacuoles inside the chromatin (*dotted arrow*) and spermatozoa with a pattern of a loose fibrillar–microgranular chromatin network (*asterisk*) were also observed. (**C**) Multilayered membranous bodies within a vacuole were also present in the nuclear chromatin (*arrow*). (**D**) A physical association between spermatozoa and bacteria (*arrowhead*) was present in several semen samples. The acrosomes were detached in several cells (*arrow*). The *dotted arrow* indicates a nuclear vacuole. Original magnification, ×3,000 in **A**; ×7,000 in **B**, ×20,000 in **C**; and ×12,000 in **D**.



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In conclusion, we have presented the ultrastructural features observed in semen samples of men with infertility and subfertility of unknown cause infected with *C. trachomatis* and mycoplasmas. However, these observations must be taken with caution because the features were also found in patients negative for *C. trachomatis* and mycoplasmas. It is possible that the microbiologic methods used were unable to detect these bacteria in such patients. More work must be performed to assess the real prevalence of *C. trachomatis* and mycoplasmas in our patient group. This includes the use of the polymerase chain reaction technique, which is the most reliable method for detecting these organisms (5). On the other hand, this fact demonstrates the usefulness of ultrastructural analysis of semen samples of men with infertility of unknown cause, including those with no apparent genitourinary infection.

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