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Morphological Characteristics of Spermatozoa from Men in a Barren Marriage with Identified Chlamydia

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Abstract

Using scanning electron microscopy (SEM) the spermatozoa of men in a barren marriage, having normal sperm, but identified with chlamydia are studied. Chlamydia causes the deformation of the spermatozoa tails, leading to their bifurcation and scalloped appearance. Deformation of the heads can occur producing an hour-glass like appearance, with the formation of ball-like heads with hollows and inclusions on their surface. IJBM 2012; 2(1):62-65.
Key words: *barren, spermatozoa, chlamydia, scanning electron microscopy.*

Introduction

In recent years, the investigation of an ejaculate of a male to identify the presence of the viral infection has been performed due to the rising problem of infertility and intensive development of auxiliary reproductive technologies. Cytomegalovirus, hepatitis C virus, HIV, human papillomavirus, adenovirus, and herpes simplex virus can be found in the ejaculate It has been proposed that the spermatozoa carrying the genetic information of a virus could cause an anomaly in the fetal development, leading to a miscarriage of the pregnancy [15]. If this hypothesis is proven true and if a viral infection of the spermatozoa is proven a reason for congenital reproductive problems, then diagnosis and prophylactics of these violations will become easy to discover. The presence of chlamydia is the second problem of the urogenital tract post viral infection, which leads to infertility in men and women. However, the interrelationship between the chlamydia and spermatozoa warrant further research. Although electron microscopy, as well as transmission and scanning microscopy have been used to investigate different biological objects for about half a century, they have begun to be used only recently to determine the functional morphology of the spermatozoa [2, 5-10]. This method has been introduced to practical Andrology because now there is sufficient knowledge regarding the normal functional morphology of the spermatozoa to be able to proceed. Electron microscopy has revealed that spermatozoa are not a homogenous population of cells. In the ejaculate of fertile men, not less than 30% of spermatozoa should be morphologically normal [3, 4, 9], while their considerable quantity have different pathological deviations. Morphological changes in the spermatozoa are caused by many factors, including the effect of running a temperature. [1].

Chlamydia greatly affects male fertility. Firstly, the infection has an indirect effect on the spermatozoa [11]. However, the effect of chlamydia infection on the condition of the spermatozoa of the ejaculate, particularly their morphology, has not been studied yet using scanning electron microscopy (SEM).

Methods

In order to study the spermatozoa with SEM, we used a modified method of SEM tissue processing. After receiving the semen into clean bottles by masturbation, the sperm was kept at room temperature for 30-40 min for

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liquefaction of the ejaculate. Then five times the volume (normally, the ejaculate volume did not exceed 2 mL) of 2.5% solution of glutaraldehyde with phosphate buffer was added to the ejaculate in the bottles, until the quantity of the fixative averaged 10 ml. After fixation for 1 hour and 30 min, the ejaculates were placed in centrifuge tubes and centrifuged at 5000 rpm for 5 min. After removal of the supernatant of the fixative, the sediment was rinsed with phosphate buffer, twice. Then, 1% solution of osmium tetroxide with the same buffer was added for fixation for 1 h. After carefully pouring out the fixator, the sediment was rinsed with cold 50% alcohol, and left in the same concentration alcohol for 10 minutes. After that, the samples were dehydrated with alcohol and acetone, according to conventional methods. After final dehydration with acetone, the precipitate was fragmented into pieces that were later placed into containers and dried by transition through the critical point, in the HCP-2 apparatus. Then, the dried pieces were mounted on foil plates using current-conducting adhesive and sputtered with gold in the IB-3 apparatus.

Samples were studied under the Hitachi S-405A microscope and photographs were obtained from the microscope display screen using a Canon digital camera. Photomicrographs were processed using the computer software "Computech".

The ejaculate studied was taken from 18 patients diagnosed with chlamydia infection and from 8 individuals without any pathology of the genital organs used as control.

Results

In normospermia, the majority of spermatozoa have a regular oval shape. The contours of the head, neck and tail are even. Small cytoplasmic droplets are sometimes defined in the neck. The ratio of length to width of the head by SEM samples is 1.5:1.75. Head length ranges within 4-5 microns while width is 2.5-3.5 microns. As a rule, the tail is straight or slightly curved with a normal length of 45 microns. The flatness of head is seen under SEM (Fig. 1).

Substantial changes occur in the spermatozoa of patients diagnosed with chlamydia. The surface of the tails and heads becomes uneven and indentations and inclusions are visible (Fig. 2 and 3). Quite often, spermatozoa with twin tails and deformed heads are detected (Fig. 3 and 4). Bulges alternating with constrictions are observed on the tail surfaces, showing a scalloped appearance (Fig. 3 and 7). Some also tails show curled tips (Fig. 5). Some spermatozoa appear to have tails that are thick and bifurcated at the distal part (Fig. 3) while others, have two thin extensions from one head; the bifurcation of tails is sometimes seen in case of thin tails as well (Fig. 4). Quite often, the spermatozoa appear deformed having hourglass heads (Fig. 6) or ball-shaped heads with indentations (Fig. 7).

The surfaces of the tails, and particularly the heads, contain discrete circular formations, 0.2-0.3 microns in diameter, depending on the size of the chlamydia (Fig. 8). Thus, the results of the studies conducted on normospermia, suggest that chlamydia causes significant

Figure 1.
Spermatozoa with regularly shaped heads and tails in normospermia. SEM ×2000

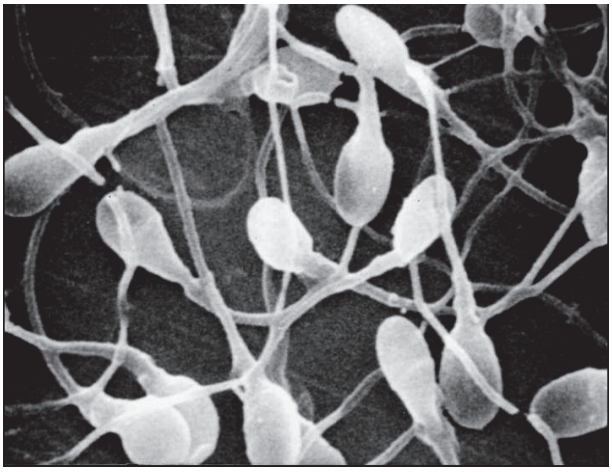


Figure 2.
Spermatozoa of patients with Chlamydia, have deformed heads and bifurcated tails. SEM ×2000.

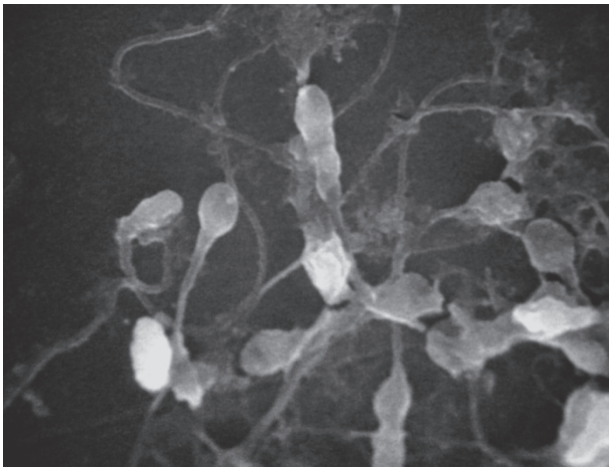


Figure 3.
Bifurcated tails and deformed heads in spermatozoa, taken from patients with chlamydia. SEM ×2000.

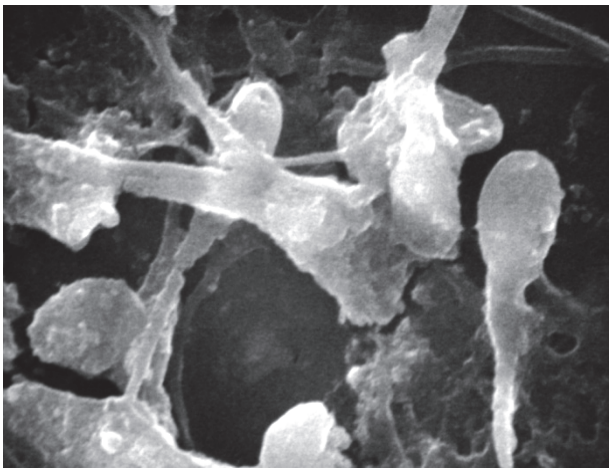


Figure 4.
Spermatozoon with bifurcated tail. SEM ×4000.

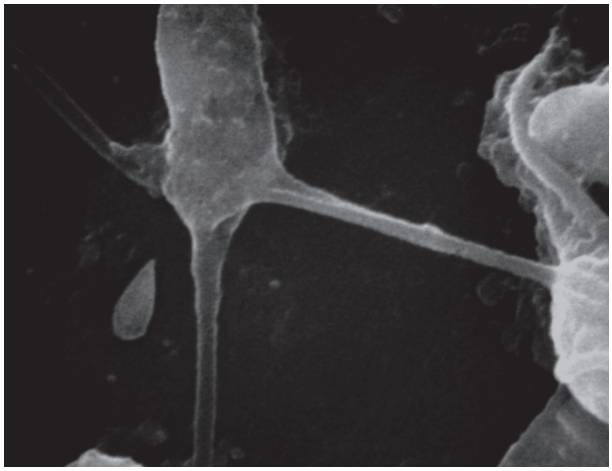


Figure 5.
Spermatozoon with curled tip of the tail. SEM ×4000.

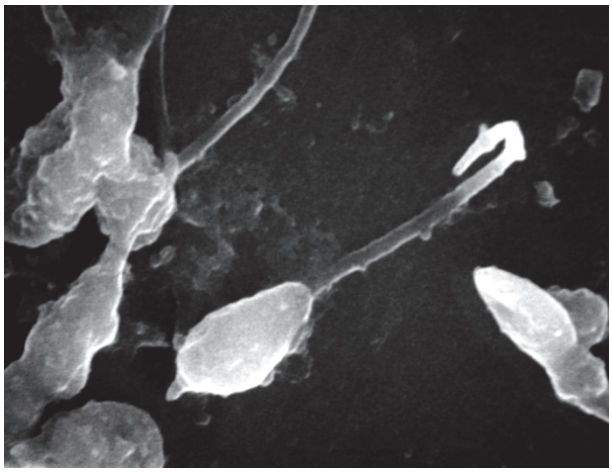


Figure 6.
Spermatozoon with the hourglass-shaped head. SEM ×4000.

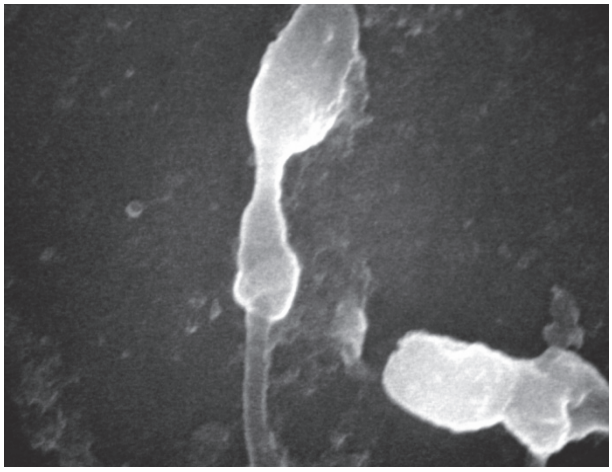
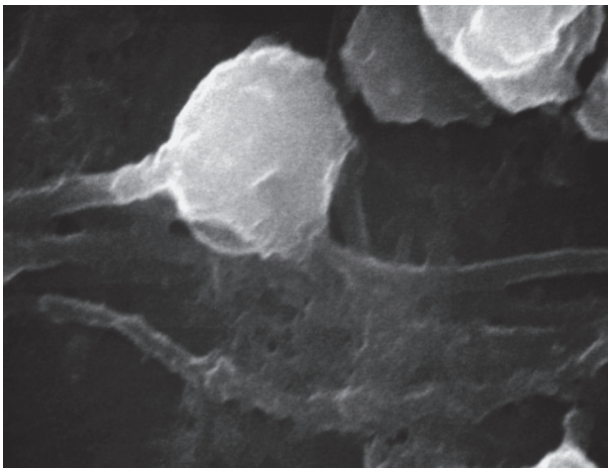
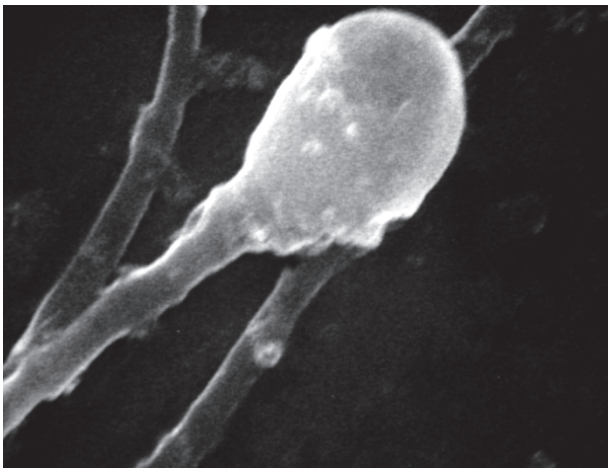


Figure 7.



Spermatozoon with spherical head. SEM ×4000.

Figure 8.
Spermatozoon with inclusions on the surface of the head



SEM ×10000.

changes in the shape of the spermatozoa, producing the appearance of deformed heads, double and deformed tails in the spermatozoon.

The discrete formations on the surface of the tails, and particularly the heads, correspond to the chlamydia in size as well as shape.

Discussion

Early experiments performed to investigate the nature of any interaction between spermatozoa and C. trachomatis relied upon electron microscopy to examine the possibility of interaction between them [11]. However, although these experiments generated useful micrographs of bacteria closely associated with the spermatozoon, the observations provided no information on the functional status of the spermatozoa, nor did they provide support for the argument that “piggy-backing” on the spermatozoon

was the mechanism by which C. trachomatis was spread through the female reproductive tract (see above).

Our studies, performed with SEM, revealed that a significant number of spermatozoon with deformed heads and tails appeared in the ejaculate of individuals with chlamydia. The spherical discrete particles, detected on the surfaces of tails, and particularly the heads, could represent chlamydia.

The question as to whether C. trachomatis infection leads to a reduction in the semen quality has been difficult to answer until recently, because the several studies conducted have often provided conflicting and confusing results.

The SEM-revealed abnormally changed forms of a significant part of the spermatozoa, supports the view that chlamydia, inducing deformation of spermatozoon, may cause disturbances in the sperm quality and contribute to infertility [10, 11, 13].

Conclusions

1. Chlamydia causes significant changes in the structure of the spermatozoon.
2. The changes occur in the heads, as well as in the tails; particularly varied changes are observed in the heads of the spermatozoa.
3. On the surface of spermatozoon, discrete spherical formations corresponding to the chlamydia in shape and size were detected.

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