# Sperm immobilized before intracytoplasmic sperm injection undergo ultrastructural damage and acrosomal disruption

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The aim of this study was to describe morphologic sperm changes observed via scanning electron microscopy and the acrosomal status resulting from immobilization and micromanipulation. The manipulations made on sperm before intracytoplasmic sperm injection were the possible cause of sperm ultrastructural damages and could trigger an acrosome reaction. (Fertil Steril® 2007;88:702–4. ©2007 by American Society for Reproductive Medicine.)

**Key Words:** Acrosome reaction, human spermatozoa, ICSI, scanning electron microscopy

The aim of this study was to increase our knowledge of the morphologic sperm changes observed via scanning electron microscopy (SEM) and the acrosomal status that result from immobilization and micromanipulation (1).

Semen samples were obtained from three consenting donors with normozoospermia. Three groups of approximately 200 sperm per group were established for all the semen samples. Sperm in the first group were immobilized by squeezing the principal piece (immobilized group). The second group were also manipulated by aspiration into an intracytoplasmic sperm injection (ICSI) pipette (micromanipulated group). The third group received no treatment and was used as a control (control group).

## EFFECTS OF EXPERIMENTAL PROCEDURES ON SPERM MORPHOLOGY

In the control group, every analyzed sperm showed normal ultrastructural morphologic characteristics (Fig. 1A, B, C). In the micromanipulated group, every cell presented a denuded terminal piece (see Fig. 1F), and the flagella underwent noticeable coiling, resulting in a figure-eight loop (see Fig. 1E). Some of sperm did show damage in the neck membrane, but this was less marked than in those of the immobilized group (see Fig. 1D).

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In the immobilized group, we observed that the terminal piece of flagellum had lost its plasma membrane and that the axoneme was disassembled, forming a kind of "tuft" made of microtubules (see Fig. 1I). We also found many sperm with solution continuity of the cell membrane at the neck level (see Fig. 1G, H).

In some of the micromanipulated and immobilized sperm we also saw two different morphologic changes on the head: [1] membrane fracture lines and patch membrane loss (Fig. 2A) and [2] grayish halos around the head of the spermatozoa (see Fig. 2B).

## EFFECTS OF EXPERIMENTAL PROCEDURES ON SPERM ACROSOME STATUS

The percentage of acrosome-reacted sperm was calculated according to the fluorescence pattern of their acrosomes using fluorescein isothiocyanate (FITC)-labeled *Pisum Sativum* lectin (FITC-PSA) (Fig. 3). The proportion of spermatozoa affected by the acrosome reaction was greater in the immobilized sperm group (95.5%) than in the micromanipulated sperm group (43.2%) or the control group (24%). The differences among the three groups, compared by chi-square analysis test, were statistically significant ($P<.001$).

## DISCUSSION

This is the first study to show the detailed morphologic features obtained via SEM of human sperm after being immobilized before ICSI.

The ultrastructural state of sperm immobilized for ICSI has been previously evaluated using transmission electron



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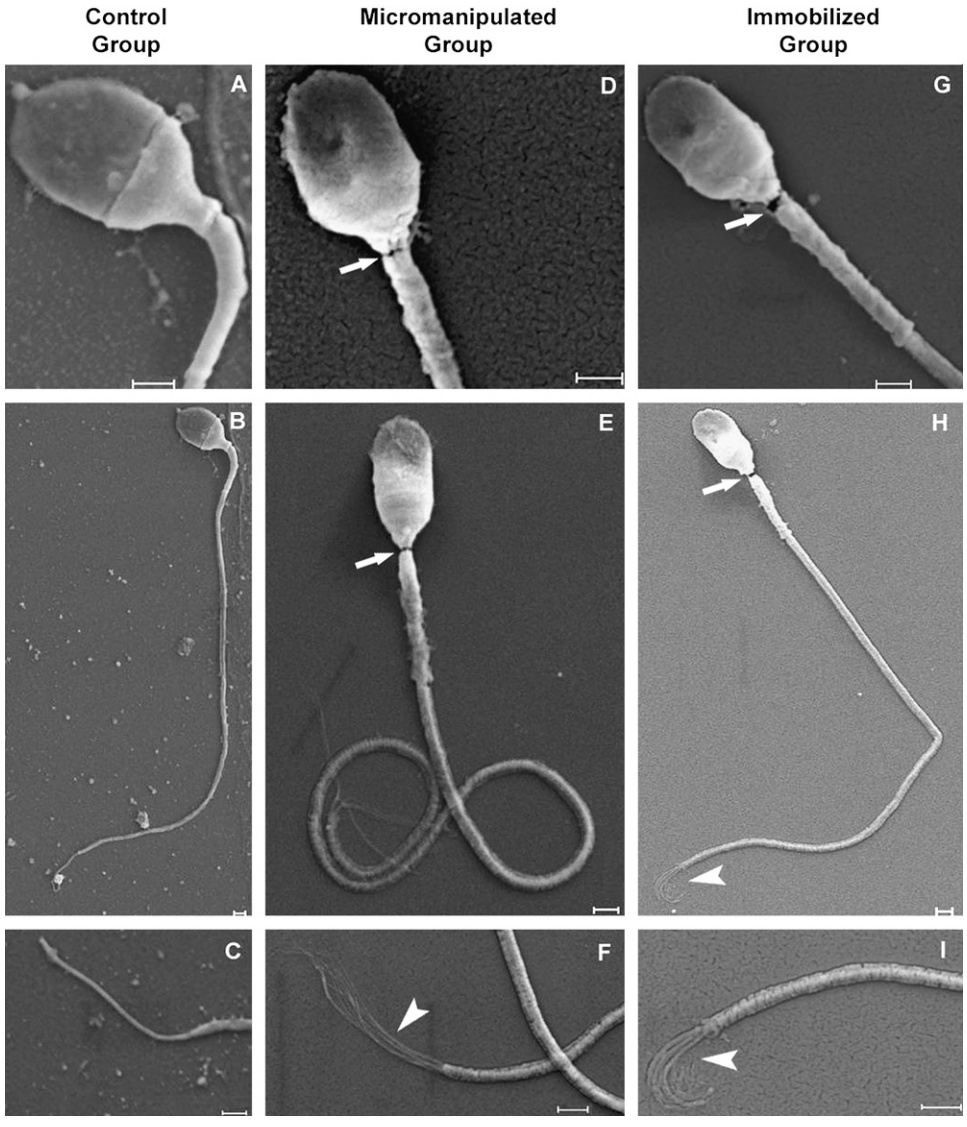
## FIGURE 1

Scanning electron microscopy images of human sperm. (**A**) Morphologic features of a normal sperm head. (**B**) Whole spermatozoa with normal morphologic features. **(C)** Detail of a normal terminal piece. (**D**) Sperm head with damage at the neck level (*arrow*). (**E**) Whole sperm showing damage on the connecting piece (*arrow*) and flagellum with coiling. (**F**) Detail of denuded terminal piece showing disassembled axoneme (*arrowhead*). (**G**) Sperm head with solution continuity in the connecting piece (*arrow*). **(H)** Whole spermatozoa with injury at the neck level (*arrow*) and denuded terminal piece (*arrowhead*). (**I**) Aspect of terminal piece with axoneme disassembled (*arrowhead*). Scale bar = 1 $\mu$m.

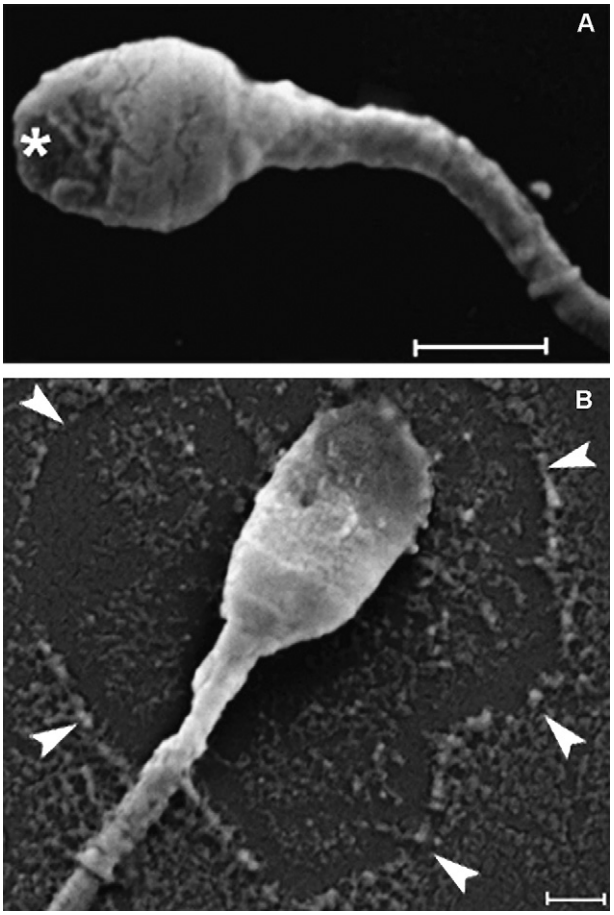



*Gómez-Torres. SEM images of damage in immobilized sperm. Fertil Steril 2007.*

microscopy (2). The investigators demonstrated that immobilization elicited changes in the plasma membrane and acrosome, which were always altered in the immobilized sperm in contrast to the control population though by varying degrees. Because acrosomal disruption can sometimes occur spontaneously (3), that study and ours found a similar percentage in the sperm in the control group. Some investigators have noted that the grayish halo around the head corresponds to more or less advanced stages of acrosome reaction (4), which coincides with our results.

## FIGURE 2

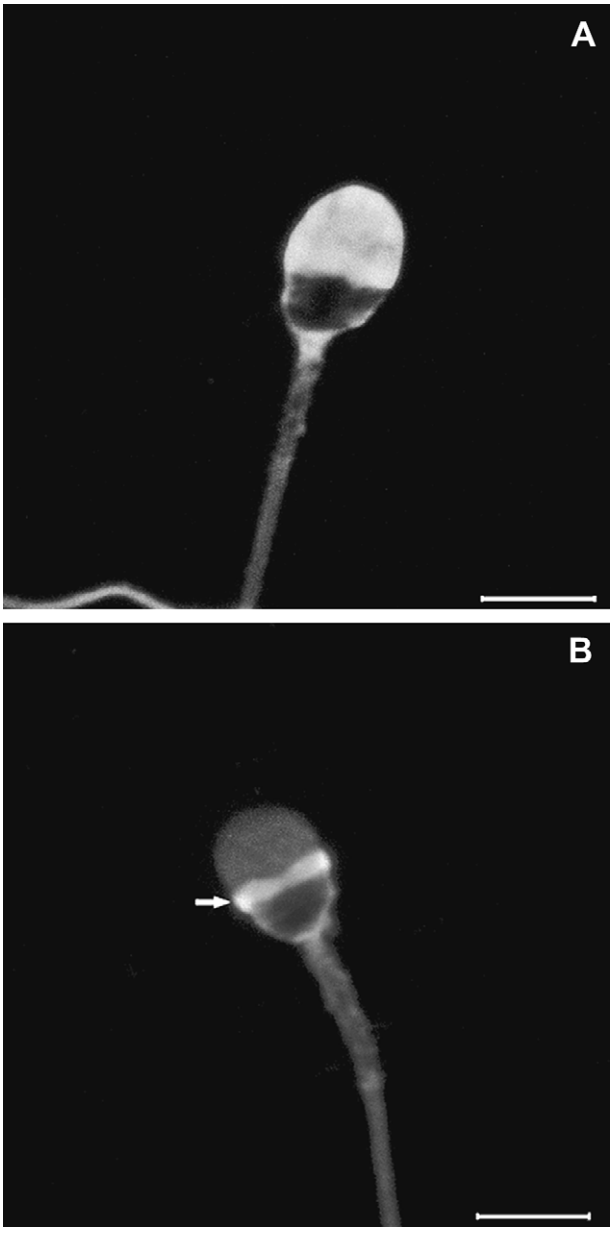
(**A**) Scanning electron microscopy image of sperm head and middle piece. Lines of fracture and membrane loss (*) can be seen on the head. Scale bar = 4 μm. (**B**) Scanning electron microscopy image of grayish halo around the sperm head (*arrowheads*). Scale bar = 1 μm.




*Gómez-Torres. SEM images of damage in immobilized sperm. Fertil Steril 2007.*

## FIGURE 3

Laser scanning confocal fluorescence images of sperm labeled with FITC-*Pisum sativum* lectin. (**A**) Acrosome-intact sperm. (**B**) Acrosome-reacted sperm. Scale bar = 4 μm.




*Gómez-Torres. SEM images of damage in immobilized sperm. Fertil Steril 2007.*

We conclude that the immobilization and micromanipulation of sperm before ICSI can be a possible cause of the sperm ultrastructural damage we observed and the acrosome reaction trigger. We also think that the rupture of the cellular membrane can favor the release of the sperm–oocyte activating factors.

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