# Acute epididymitis induces alterations in sperm protein composition

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**Objective:** To use a proteomic approach to evaluate possible postinflammatory alterations in the protein composition of motile sperm in patients 3 months after acute epididymitis.
**Design:** Prospective case-control study.
**Setting:** University medical school research laboratory.
**Patient(s):** Eight patients 3 months after acute unilateral epididymitis and 10 healthy controls.
**Intervention(s):** None.
**Main Outcome Measure(s):** Proteome analysis of sperm samples collected by swim-up from control and acute epididymitis patients analyzed by two-dimensional gel electrophoresis and subsequent protein identification by matrix-assisted laser desorption/ionization–time-of-flight (MALDI-TOF) mass spectrometry; immunofluorescence staining for mitochondrial ATP synthase subunit $\beta$ (ATP5B), $\alpha$-tubulin (TUBA1A), and tubulin-$\beta$2c (TUBB4B) for validation purposes.
**Result(s):** Proteome analysis identified 35 proteins in sperm from epididymitis patients that were down-regulated, irrespective of subcellular localization and biologic function. Furthermore, immunofluorescence microscopy confirmed ATP5B, TUBA1A, and TUBB4B were less abundantly expressed in epididymitis samples compared with controls.
**Conclusion(s):** Despite normal semen parameters observed by conventional semen analysis in patients after epididymitis, significant changes to sperm protein composition were observed. These changes may be implicated as additional factors contributing to subfertility/infertility in men after episodes of epididymitis. (Fertil Steril® 2014;101:1609–17. ©2014 by American Society for Reproductive Medicine.)
**Key Words:** Epididymitis, male fertility, proteome analysis, semen quality

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Infection and inflammation of the genital tract are considered important factors contributing to male infertility (1). Epididymitis is the only ascending urinary tract infection that can have a direct impact on the epididymis and in up to 60% of cases the testis as well (epididymo-orchitis) (2, 3). Despite epididymitis occurring frequently in patients in their reproductive years (4), its impact on fertility has not been systematically investigated (5). Previous studies have shown reduced sperm concentrations after acute infection with overall recovery within 3 months after antibiotic treatment (2, 6). However, persistent oligozoospermia and azoospermia even after successful treatment have been reported in up to 40% of patients (5). Aside from changes in sperm concentration, no information is currently available on sperm protein composition and

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function or possible alterations in viable and motile sperm cells from patients diagnosed as "cured" after epididymitis.

With recent developments in mass spectrometry, high throughput for the identification and study of sperm protein composition is now possible (7). Currently, proteomic profiling has identified several promising markers that are associated with various forms of infertility/subfertility in both animals and humans (8–19). Given that approximately 60% of men after an acute episode of infectious epididymitis appear to have normal sperm parameters by conventional semen analysis, we evaluated possible postinflammatory alterations in the protein composition of motile sperm using a proteomic approach.

## MATERIALS AND METHODS

### Study Population

Between October 2010 and October 2011, 38 men were diagnosed with acute epididymitis. Eight of these men were recruited for our study (median age: 31 years; range: 19–47 years) and provided a semen sample suitable for proteome analysis (Supplemental Figure 1, available online). The participants were part of the wider Giessen Epididymitis Study (institutional review board number: 100/7, German Clinical Trials Register number: DRKS00003325). The diagnosis was based on the medical history, palpation of a painful enlarged epididymis, blood parameters demonstrating inflammation, urine analysis showing urogenital tract infection and color-coded duplex sonography for confirmation. Following the U.S. Centers for Disease Control and Prevention (CDC) recommendations, all participants received 500 mg/day of levofloxacin for 10 days (20). Three months after antibiotic treatment, semen samples were collected after 3 days of sexual abstinence. After approval of the study from the institutional review board (institutional review board number: 32/11), 10 healthy fertile men (median age: 44 years; range: 33–62 years) requesting vasectomy were recruited from May 2011 to November 2011 as controls. Written informed consent was obtained from all participants.

### Semen Analysis and Swim-up Technique

Within 1 hour of collection, the semen analysis was performed in a blinded fashion according to World Health Organization (WHO) recommendations (21). In addition to standard parameters, the concentration of peroxidase-positive granulocytes as well as neutrophil elastase was determined in each sample to assess seminal inflammation. To select motile/viable sperm for proteome analysis, direct swim-up technique was performed (15, 22, 23). Briefly, 1 mL of fresh semen was placed in a sterile centrifuge tube, and 1 mL of human tubal fluid (HTF) medium (24) layered on top. After 1 hour of incubation at 37°C, 1 mL of the upper medium containing highly motile sperm was removed, diluted with 2 mL of HTF medium and centrifuged at 500 × *g* for 5 minutes. After resuspension in 0.5 mL of HTF medium, the sperm concentration and progressive motility were assessed. For immunofluorescence staining 10 μL of freshly prepared sperm was smeared onto clean grease-free slides (SuperFrost; R. Langenbrinck), air-dried, and fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS, pH 7.4) for 20 minutes at room temperature. The remaining sample was immediately snap frozen and stored at −80°C until the proteome analysis.

### Proteome Analysis

Protein extraction from sperm was performed by ultrasonication in 200-μL lysis buffer containing 6M urea (Roth), 2M thiourea (Sigma-Aldrich), 4% 3-[(3-cholamidopropyl) dimethyl ammonio]-1-propane sulfonate (CHAPS; Roth), 1% dithiothreitol (DTT; GE Healthcare), and 2% Pharma Lyte 3–10 (GE Healthcare). Protein quantification was performed using a two-dimensional (2D) Quant kit (GE Healthcare). Because of the low protein concentration, all 10 individual control swim-up samples and eight postepididymitis swim-up samples were pooled into respective study groups.

To examine the protein concentration and remove interfering compounds, proteins were precipitated twice with acetone. Protein pellets were resolubilized in 780-μL lysis buffer. For isoelectric focusing, IPG-strips (pH 3–10 nL; GE Healthcare) were rehydrated at 20°C with the protein extract. Technical replicates of 260 μL of protein extract were applied on three strips (11, 25–27), and isoelectric focusing was performed with 32.05 kV·h. After focusing, the IPG-strips were equilibrated for 10 minutes in 2 mL equilibration stock solution (ESS; 6M urea, 0.1 mM EDTA [Sigma-Aldrich], 0.01 % bromophenol blue [Serva], 50 mM Tris-HCl [Roth], pH 6.8, 30% glycerol) for 15 minutes in 2 mL ESS I (10 mL ESS containing 200 mg SDS [Roth]), 100 mg DTT (GE Healthcare) followed by 15 minutes in ESS II (10 mL ESS containing 200 mg SDS [Roth], 480 mg iodoacetamide [Sigma-Aldrich]). Protein separation in 2D was performed by electrophoresis on 12.5% SDS polyacrylamide gels (28). Electrophoresis was performed in a Hoefer 600 system with the following program: 15 minutes at 60 mA/gel and 5 hours at 110 mA at 25°C. Gels were fixed in 40% ethanol and 10% acetic acid, and were stained overnight with flamingo (Bio-Rad Laboratories).

Gel images were acquired with a Typhoon 9200 laser scanner (GE Healthcare) with an excitation of 532 nm using an emission 555 BP filter. Gel images were analyzed with PdQuest software (Bio-Rad Laboratories). Comparison between groups (controls vs. epididymitis) revealed 292 matched spots with variations in spot intensities by at least a factor of 2. We found that 192 spots showed statistically significant spot intensities using a Student's *t*-test (significance level >90%). The intersection of both analyses revealed 79 spots. These spots were excised with the ExQuest spot cutter, and proteins were digested with trypsin on a liquid-handling robot system (MicroStarlet; Hamilton Robotics).

For protein identification matrix-assisted laser desorption/ionization–time-of-flight (MALDI-TOF) mass spectrometry was performed on an Ultraflex I TOF/TOF mass spectrometer (Bruker Daltonics) equipped with a nitrogen