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The Amount of Zinc Detected in Washed Human Spermatozoa

JAN FRIBERG and OVE NILSSON

Department of Obstetrics and Gynecology, and Institute of Human Anatomy, University of Uppsala, Uppsala, Sweden

ABSTRACT

Samples of human semen, the supernatants from washings of these samples, and the suspensions of washed spermatozoa, were examined for zinc in a Perkin-Elmer spectrophotometer. Almost all the zinc was found in the original semen samples and in the first washings. Zinc could not be detected in the heads of fourfold-washed spermatozoa on analysis in a wavelength dispersive X-ray spectrophotometer attached to a scanning electron microscope.

INTRODUCTION

High concentrations of zinc have been demonstrated in seminal fluid (1, 8, 9, 11) and in prostatic fluid (2, 6, 7). Mawson & Fischer (8) performed zinc analysis of the ashed infranatant after centrifugation of human ejaculates and observed about 2 mg zinc/g dry weight. Hall (3) used an X-ray microprobe to examine the zinc content of individual spermatozoa and found a high concentration in the heads of the sperm cells. Since unwashed spermatozoa were used, an unintentional contamination of the samples with zinc from the seminal fluid cannot be excluded.

This possibility is strengthened by the finding that spermatozoa which were obtained from patients with infertility problems and/or suspected prostatitis and then separated by density gradient centrifugation and analysed by atomic absorption spectrophotometry showed a mean zinc concentration of 5.5 $\mu g/10^8$ spermatozoa (5). To obtain information about the content of zinc in carefully washed human spermatozoa from normal donors, analyses were performed in the present study both by atomic absorption spectrophotometry and by a wavelength dispersive X-ray spectrophotometer attached to a scanning electron microscope.

MATERIAL AND METHODS

Two masturbated ejaculates from each of 4 fertile donors were used for the present study. The fertility of the donors was previously demonstrated by artificial insemination. Two ml of each semen sample (average 40–60 million sperm cells/ml) were spun at 1 500 rpm, washed 4 times in 0.01 M phosphate-buffered saline (PBS), pH 7.4, and finally suspended in the same volume of PBS as the original sperm sample. The motility of the spermatozoa was not markedly affected by this treatment.

The semen samples, the supernatants from each washing, and one lot of the fourfold-washed sperm suspension, were subjected to zinc analysis in a Perkin-Elmer spectrophotometer using the method described by Parker (10) and Kahnke (4).

Small drops of suspended, washed spermatozoa were placed on a stage of pure aluminium adapted for a Jeol SMU-3 scanning electron microscope. The heads of single spermatozoa were then examined for their zinc content by means of a wavelength dispersive X-ray spectrophotometer. The microscope was operated at 25 kV with 0.01 μA and 10 cps for full scale on the recorder. The minimum detection level of zinc under the conditions used was calculated to be about 1×10^{-10} gram zinc.

RESULTS

Zinc analyses of the sperm samples, the supernatants from each washing, and the final sperm suspensions showed that almost all the zinc was found in the original semen samples and the supernatants from the first washings. The concentration of zinc in the final solution of fourfold-washed spermatozoa was about 9×10^{-8} mol/ml or about 12 $\mu g/10^8$ spermatozoa.

No zinc could be demonstrated in the heads of individual spermatozoa when investigated by means of the dispersive X-ray spectrophotometer attached to the scanning electron microscope. This is only to be expected since the amount of zinc in a single washed spermatozoon was about 1×10^{-13} gram.

DISCUSSION

The results in the present study indicate that only minute amounts of zinc can be present in

the sperm cells. The high zinc concentrations previously ascribed to spermatozoa were probably due to contamination with zinc from the seminal fluid. The minute amounts of zinc present in human spermatozoa, according to the results in this study, may derive from contamination by epithelial cells and cellular debris or from the carbonic anhydrase contained in the spermatozoa. The experiments do not exclude the possibility that some zinc may have leaked out from the spermatozoa during the washing procedures.

The healthy donors were found to have a zinc concentration of about 12 $\mu g/10^8$ spermatozoa. This figure lies in the upper range of values obtained from patients with infertility problems and/or suspected prostatitis (mean 5.5 $\mu g/10^8$ spermatozoa, range 0.20–12.63) (5). However, the results are sufficiently similar to permit the conclusion that the zinc concentration of human spermatozoa is low, lying around some $\mu g/10^8$ spermatozoa.

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Address for reprints:
Jan Friberg
Department of Obstetrics and Gynecology
University Hospital
S-750 14 UPPSALA 14
Sweden