Relationship between Spermatozoon Movement Velocity and Expression of Testicular Isoform of Angiotensin-Converting Enzyme on Their Surface

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The expression of testicular isoform of angiotensin-converting enzyme on the surface of human spermatozoa was evaluated by flow cytometry with monoclonal antibodies to epitopes of C-terminal domain of human angiotensin-converting enzyme and the mean path, mean curvilinear and straight-line velocities were determined by computer analysis of spermatozoon movement. A positive correlation between the expression of testicular isoform of angiotensin-converting enzyme on the cell surface and spermatozoon movement velocity was revealed.

Key Words: *angiotensin-converting enzyme; spermatozoon mobility; flow cytofluorometry; monoclonal antibodies*

Angiotensin-converting enzyme (ACE; EC 3.4.15.1, CD143) is a zinc-dependent peptidyl dipeptidase with a wide spectrum of effects. Removing His-Leu C-terminal dipeptide ACE converts angiotensin I into angiotensin II and inactivates bradykinin, thus regulating blood pressure and electrolyte homeostasis [2].

Two ACE isoforms are described: somatic (sACE) expressed on the surface of lung endotheliocytes [9], intestinal and renal epitheliocytes, cerebral neuroepithelial cells, epitheliocytes of male genital tract, and Leidig cells, and testicular (tACE) isoform detected in postmeiotic spermatogenesis cells (spermatides) and spermatozoa [12].

The molecule of sACE has two homologous domains, while tACE molecule has only one domain with the peptide chain identical to sACE C-terminal part, except the first 36 amino acids [3].

The role of ACE in the reproduction is still disputed. Knockout of ACE encoding gene in mice does not change the number of spermatozoa, their mobility and morphology, but these cells cannot move through the female genital tract and fertilize oocytes [6,8]. A positive correlation between ACE activity and mobility of human [4] and cattle spermatozoa was detected. Other authors detected a negative correlation between ACE activity and mobility of spermatozoa in humans and swine [10,11].

We quantitatively evaluated the expression of tACE on the surface of human spermatozoa using for the first time monoclonal antibodies (MAb) to tACE, which we first obtained and characterized [7], and studied mobility of these cells.

MATERIALS AND METHODS

Seventeen ejaculates from donors (mean age 30±7 years) were analyzed. The semen was collected into

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sterile containers and analyzed according to the WHO recommendations [13].

ACE on cell surface was detected using native seminal spermatozoa and fraction of active mobile spermatozoa, obtained by the flotation method (in medium M199). Cell suspensions (5×10^6 cells/ml) in medium M199 with 0.5% BSA were incubated at 4°C for 15 min with MAb to ACE (1E10, 4E3, i2H5+9B9). Murine nonimmune FITC-labeled IgG (Sigma-Aldrich) in a concentration of 10 μg/ml served as the negative control. After incubation polyclonal FITC-labeled goat antibodies specific to mouse IgG Fc fragment (Zymed) diluted 1:100 were added for 20 min at 4°C.

Cell viability was evaluated using propidium iodide (2 μg/ml). The expression was analyzed by flow cytofluorometry on a FACScan device (Becton Dickinson). The percentage of ACE-positive spermatozoa was evaluated in each sample.

Computer Assisted Semen Analysis (CASA) was carried out on a Hamilton Thorn device. Straight-line (VSL), average path (VAP), and curvilinear (VCL) velocities (μ/sec) of spermatozoon movement were measured (Fig. 1).

The results were statistically processed using MedCalc applied software. The means were presented as the median and standard deviation. The significance of differences in two groups was evaluated using Wilcoxon test. The differences were considered significant at $p<0.05$. Correlations were evaluated using nonparametric Spearman test.

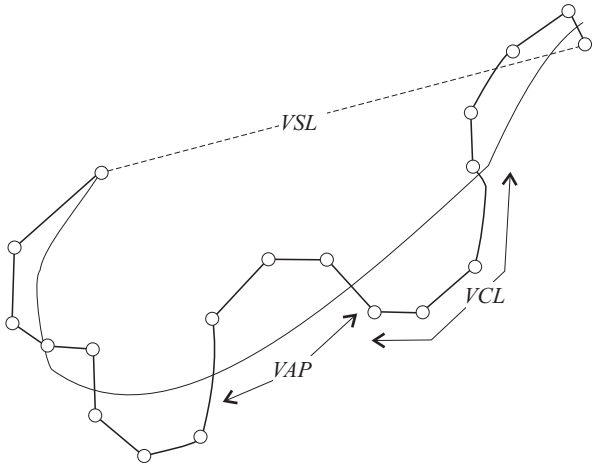

Fig. 1. Parameters of the trajectory of spermatozoon movement. VCL: curvilinear velocity (μ/sec); VAP: average path velocity (μ/sec); VSL: straight-line velocity (μ/sec).

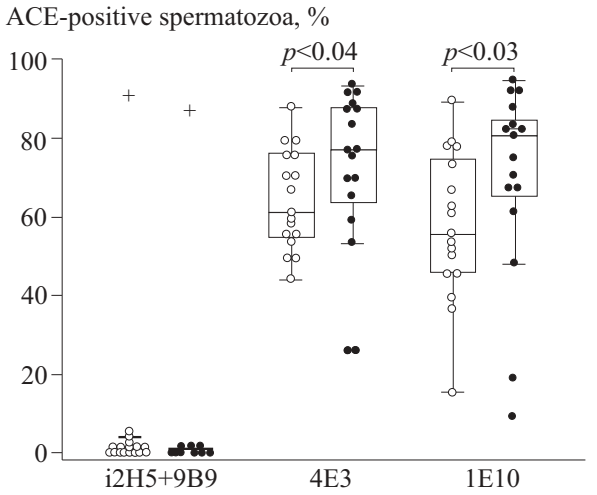

Fig. 2. Expression of angiotensin-converting enzyme (ACE) on the surface of native seminal spermatozoa (light signs) and active mobile cells isolated by flotation method (dark signs). The data are presented as a Box and Whisker diagram. The upper and lower lines of the rectangle correspond to lower and higher quarters of the range of values (25-75 percentile). Median line shows position of the median. Vertical lines show the lowest and highest significant values.

RESULTS

Monoclonal antibodies 1E10 are specific to tACE, i2H5 and 9B9 to sACE, and 4E3 bind both isoforms of the enzyme [7].

High level of tACE (64.1±22.4%) was detected in native seminal spermatozoa and on active mobile cells isolated by the flotation method (Fig. 2), while sACE was virtually absent (less than 5% sACE-positive spermatozoa). The percentage of tACE-positive cells was significantly higher among spermatozoa isolated by the flotation method than in native semen ($p<0.04$).

A positive correlation between tACE expression and velocity of spermatozoon movement was detected. Positive relationships between VAP and VSL, on the one hand, and the expression of tACE, on the other, were detected for active mobile spermatozoa isolated by flotation and for native seminal cells (Fig. 3), while VCL did not depend on the enzyme expression.

Our findings are in line with the data on positive correlation between ACE activity and mobility of spermatozoa [4], but contradict other reports [10,11]. Possible nonspecific adsorption of sACE on the surface of seminal fluid cells was neglected in previous studies. The use of MAb specific to the two enzyme isoforms helped us to control the effect of sACE removal from spermatozoon surface.

The mechanisms underlying the effects of ACE on spermatozoon mobility remain unclear. The effect can be due to angiotensin II binding to type I receptors located on spermatozoon neck and tail [1]. Angiotensin II is a potential agonist of capacitation stimulation, when the pattern of spermatozoon movement is changed significantly.

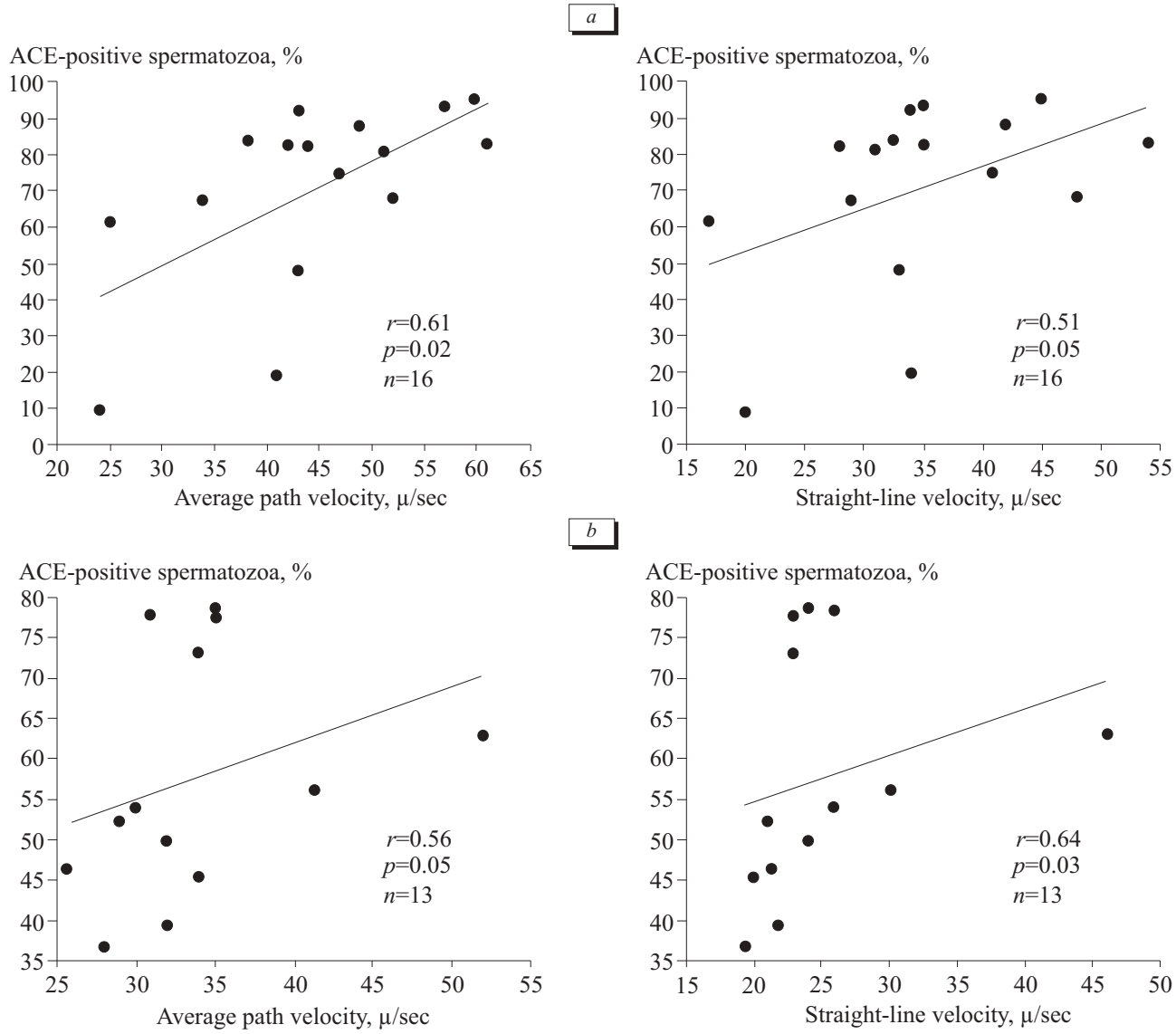

Fig. 3. Relationship between expression of testicular ACE isoform (1E10 monoclonal antibodies) on spermatozoon surface and trajectory of spermatozoon movements. *a*) active mobile fraction of spermatozoa; *b*) native seminal spermatozoa. *r*: Spearman correlation coefficient.

A new (different from peptidase) activity of ACE is described: release of glycosylphosphatidylinositol-bound (GPI-bound) peptides from the cell surface [5]. Presumably, this enzyme activity is essential for spermatozoon mobility, because removal of some proteins from the cell surface during capacitation is associated with a sharp increase in the spermatozoon mobility.

Hence, functionally active spermatozoa with high expression of tACE are characterized by pronounced forward motion, which can promote their more effective movement in the female genital tract and fertilization of the oocyte.

These data provide the basis for further studies aimed at evaluation of the clinical significance of evaluating tACE expression on human spermatozoon surface for the diagnosis of some forms of male sterility.

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