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Method Article

Zeta potential technique for analyzing semen quality

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A B S T R A C T

The presented study shows the possibility of using the zeta potential technique in sperm selection. Results suggest that the characteristics of semen may be reflected in the sperm surface charge, which can be measured by a simple Zeta technique. This is a pilot study that answers question whether a commercially available Zeta Potential analyzer can be used to determine the quality of human semen. Semen samples were obtained from young adult men donors and divided into portions to analyze the motility, viability, morphology, concentration and zeta potential. Results indicate that zeta potential of semen samples with right structural and functional parameters was significantly more negative in comparison to the other samples. Our use of a Zeta potential analyzer to investigate sperm surface charge adds a new dimension to data on semen quality. It is an additional simple method that helps in the widely-used routine methods of semen analysis.

- Characteristics of semen may be reflected in the sperm surface charge, which can be measured by Zeta potential technique.
- Only 20 µl semen being needed to analyze spermatozoa surface charge.
- The commercially available Zeta Potential analyzer can be applied for semen quality investigation.

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Specifications Table

Subject Area:	Agricultural and Biological Sciences
More specific subject area:	Cell biology of cell migration Measurements in biology and medicine Human cells functions Flow measurements
Method name:	ζ Potential assay for semen analysis
Name and reference of original method:	1. Smoluchowski M. in: L. Graetz (Ed.), Handbuch der Elektrizität und de Magnetismus, 2nd ed. Barth, Verlag, Leipzig. (1921) 366. 2. Sze A, Erickson D, Ren L, Li D, Zeta-potential measurement using the Smoluchowski equation and the slope of the current–time relationship in electroosmotic flow. Colloid. Interface Sci. 261 (2003) 402–410.
Resource availability:	https://www.sciencedirect.com/topics/pharmacology-toxicology-and-pharmaceutical-science/zeta-potential

Method details

Introduction

Diagnostic methods of semen selection for assisted reproduction programs have already been developed on the basis of research and are recognized by the reproductive clinics. The method of preparing sperm for intracytoplasmic sperm injection (ICSI) and classic in vitro fertilization (IVF) depends on the values of the parameters sperm count, motility, morphology and viability [1]. They rely on centrifugation and separation of the semen on a colloidal sperm concentration gradient. The innovative program we present relies on the introduction of new methods dividing samples of spermatozoa into two categories on the basis of the electric potential on the sperm surface. Sperm samples provided by the Gravita Lodz Laboratory were used for a method based on detection of low-voltage potential.

The World Health Organization (WHO) recently indicated the increase in the tendency of use of Assisted reproductive technology (ART) for the treatment of infertile couples [2,3]. Selection of good quality semen is one of the important steps for successful ART. The selection of mature sperm cells needed for ICSI is currently based on determination of the motility and morphology of the sperm samples available [2,4–6]. Sperm samples collected from infertile patients may have functional or pathological defects [6–8]. The routine determination of semen quality may not reflect pathological changes of the spermatozoa [9]. Analysis of sperm quality has been improved by other methods, the monitoring of the zeta potential that measures surface charge of semen cells being among them [10,11]. Previous studies [6,10] suggests that spermatozoa with a high negative membrane potential are undamaged and mature. We have correlated sperm quality analyzed by routine procedures with the examination of the zeta potential of spermatozoa by using a Nano-ZS Malvern Zeta Potential analyser. Measurements of electrophoretic mobility (zeta potential) have been used to estimate cell surface charge. Cells move under an applied electric field, and this movement can be measured and is related to the cell surface charge [12,13].

The main goal of this study was to determine the correlation between sperm cells quality parameters and their zeta potential as well as answer the question whether a Zeta technique can be applied to characterize the semen.

A total of 29 young adult men who were sperm donors at the Gravita Fertility Clinic Sperm Bank of Lodz in 2016 (January-November) were included in this retrospective cross-sectional laboratory investigation of biophysical properties of their semen samples. The sample from each of the donors was divided into five portions to be analyzed for spermatozoa motility, viability, morphology, concentration and zeta potential. The samples prepared for zeta experiments were divided into two groups depending the quality of their morphology, viability, motility and concentration (highest and lowest values). All samples were measured for sperm quality and zeta potential and were divided into optimal and inferior values. Overall experimental design showing the steps of sample analysis is present in Fig. 1.

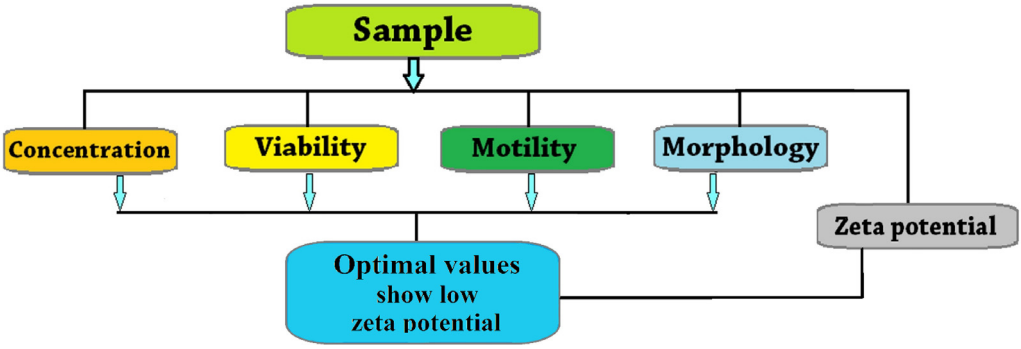

Fig. 1. Overall experimental design showing the steps of sample analysis.

The zeta potential of samples in each of the groups was measured and averaged, any difference giving $p<0.05$ being considered significant. The zeta potential of semen samples was significantly more negative in the optimal values group than in the other samples. Obtained data can suggest that an inexpensive, simple and fast Zeta potential technique offers a promising method of selecting semen of good quality both for human and veterinary artificial insemination. The results can help to improve the standard protocols for sperm selection before being used in ART procedures.

Materials and method

Ethics statement and study design

We examined the semen samples of young adult men - sperm donors at the Gravita Fertility Clinic Sperm Bank of Lodz – who had signed informed consent forms during their first visit to the human sperm bank, agreeing that their semen samples or data could be used by the bank for scientific research. The study was approved by the Ethics Committee of Lodz University (NR 3/KBBN-UŁ/I/20016). All methods were performed in accordance with the relevant guidelines and regulations.

Semen samples were obtained from 29 patients. In accordance with the WHO criteria, their semen was analyzed in the routine manner [2], i.e. motility, viability and morphology of semen before their zeta potential was measured.

Zeta potential analyser

To analyze the zeta potential of semen samples we used a commercially available Zeta Potential analyser (Zetasizer Nano-ZS) constructed and produced by Malvern Instruments limited UK, https://www.malvernpanalytical.com/en/products/productrange/zetasizer-range/zetasizer-nano-range/zetasizer-nano-zs. The charge or zeta potential of particles is determined by measuring their velocity while they are moving in an electrophoretic field. Particles and molecules that have a zeta potential will migrate towards an electrode if a field is applied. The speed they move is proportional to the field strength and their zeta potential. The technique called phase analysis light scattering (PALS) was used in the tests. The whole measurement procedure is automated to simplify the measurement process

Specificaton:

- Measurement range: 3.8 nm – 100 µm (diameter)
- Measurement principle: Electrophoretic Light Scattering
- Minimum sample volume: 150 µL - 800 µL (using Malvern capillary plastic cells DTS1061)
- Accuracy: 0.12 µm.cm/V.s for aqueous systems
- detection angle 90, and a laser wavelength 633 nm

- Capillary internal size 2 × 4 mm, length 6.5 cm.
- Capillary optical path length 4 mm

Semen analysis

Sperm morphology was examined with commercial pre-stained Testsimplets® slides, which helps detect abnormalities in their morphology [14]. Five µl well-mixed semen was placed directly on a slide under a coverslip and examined after 30 min at room temperature under oil immersion (l00x magnification). For each sample, >200 sperm cells were classified as normal or abnormal. Sperm motility was analyzed with a Makler counting chamber [15,16] which have been designed for the measurement of the concentration and motility of human sperm in semen [17]. Three types of sperm motility were distinguished. The first contained spermatozoa moving actively and linearly, regardless of speed (Progressive motility). The second group had non-progressive sperm that contained all of the other patterns of motility but with an absence of progression, for instance, swimming in small circles, or only when a flagellar beat was seen (Non-progressive motility). The third group contained non-motile spermatozoa (Immobile). Over 200 sperm cells from each sample were examined.

Sperm viability was estimated by assessing the integrity of the cell membranes. This method is based on the principle that dead cells have damaged plasma membranes that allow a dye to move into the cells, unlike live spermatozoa which have intact cell membranes and therefore remain unstained. Each sample was prepared by combining 5 µl semen with 5 µl eosin solution. They were mixed and examined at 200x magnification in negative phase-contrast microscopy. Again >200 sperm cells were examined.

Zeta potential technique

Zeta potential of semen samples was measured by using phase analysis light scattering with the Zetasizer Nano-ZS. The electrophoretic mobility of the dynamic light scattering (DLS) samples in an applied electric field was measured in Malvern capillary plastic cells with gold electrodes (DTS1061) in 0.75–0.85 mL. Samples were not specially prepared for zeta potential measurements. The sperm cells were not washed free of protein, were not filtered, the contaminating particles were not removed.

The main advantage of the method applied is the simplicity of the analysis. 20 µl of semen were taken from the fresh semen, diluted in 10 mM PBS (pH) 7.4 up to 1.5 million cells/ml, and zeta potential was measured. Measurements were taken with an automatic optimization of laser power and voltage settings. Five runs, repeated 7 times without equilibration time, were collected for each sample, and the results were averaged. The refractive index (R_i), absorption (Abs), viscosity and dielectric properties used were those of PBS 1.333; 0.001; 08984; 78.5 respectively. The zeta potential value was calculated directly from the Helmholtz–Smoluchowski equation [18,19] with Malvern software.

Statistical analysis

Statistical analysis was carried out in GraphPad Prism 5. Data are shown as mean ± standard deviation of the mean (SEM). Unpaired *t*-test with Welch's correction was used to determine statistically significant differences between two means (marked with * when $P < 0.05$, ** when $P<0.01$ and *** when $P<0.001$).

Extraction of the data

Table 1 shows concentration, motility, viability and morphological properties (% of normal forms) of the semen samples. Samples were divided into two groups with the optimal and inferior values of these parameters. The zeta potential of each group was measured and averaged. All the samples assayed were included as one of the "optimal" or "inferior" group.

Fig. 2 shows analysis of the zeta potential of semen samples and their quality. The samples with the better characteristics were invariably more negatively charged than the samples having lower

Table 1
Analysis of semen parameters of the samples investigated.

	1-st group				2-st group			
	Mean ±SEM	Min	Max	n	Mean ±SEM	Min	Max	n
Motility, [%]	71.1 ± 2.34	59	83	13	47.6 ± 1.40	38	57	16
Morphological property, [%]	14.5 ± 1.17	11	30	19	5.5 ± 0.58	4	8	10
Concentration, [mln/ml]	78.9 ± 8.74	36	144	14	17.1 ± 2.57	7	34	12
Viability, [%]	87.1 ± 1.01	83	92	10	70.8 ± 2.11	49	81	19

Mean values are presented as ± SEM.

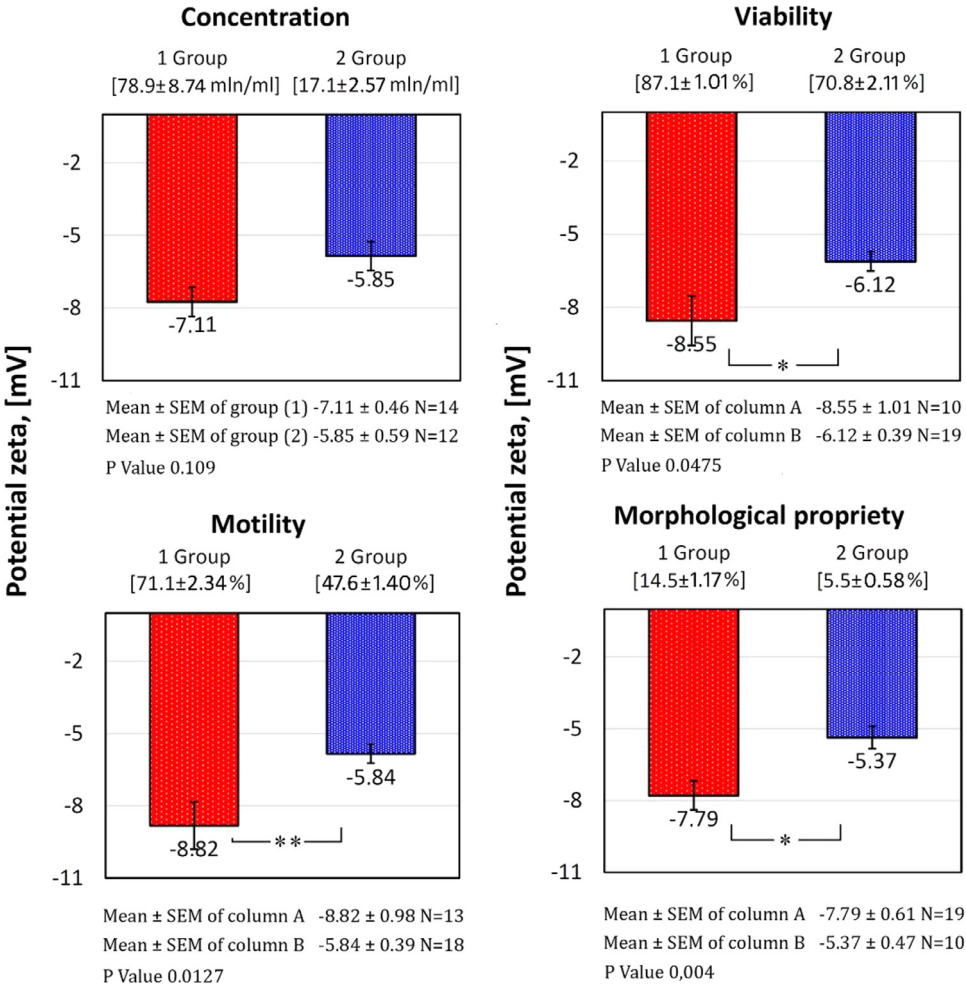

Fig. 2. Zeta potential of the semen samples versus their quality parameters (concentration, total motility, viability and morphological propriety). The Y-axis of each graph gives the averaged zeta potential values in mV of the samples. The measurements were in 10 mmol/L phosphate-buffered saline, pH 7.4 at 37 °C. The results give the mean ± standard deviation of the mean (SEM), * - $P < 0.05$ and ** - $P < 0.01$.

functional parameters. The difference in zeta potential values between the groups categorized by semen concentration (1st group, 78.9 ± 8.74 and 2nd group 17.1 ± 2.57 mln/ml) was not significant, -7.11 ± 0.46 mV and -5.85 ± 0.59 mV, respectively ($p = 0.109$). In contrast, the samples divided according to semen motility, viability and morphological property were significantly different its sperm quality and surface charge of the semen cells. Samples with normal morphology (1st group, $14.5 \pm 1.17\%$) had zeta potentials that were significantly more negative (-7.79 ± 0.61 mV) than those that had a lower percentage of morphologically normal cells (2nd group, $5.5 \pm 0.58\%$) with the zeta potential values -5.37 ± 0.47 mV ($p = 0.004$). The difference between the 1st and 2nd groups differing in their motility and viability was also significant (Fig. 2.). In both cases, the 1st group was more negatively charged, with averaged zeta potential values of -8.82 ± 0.98 mV and -8.55 ± 1.01 mV, respectively. Of all the samples, the most significant difference was between the two groups characterized by semen motility.

Discussion

For successful intra-cytoplasmic sperm injection in ART morphologically "normal" sperm should be selected. The development of new techniques permits the analysis of individual semen samples more accurately and would make it possible to treat (i.e. by intra-cytoplasmic sperm injection) infertile couples with improved efficiency [20]. Reports have shown that different effective methods which can be used for the diagnosis of semen; among them, the most commonly employed include analysis of: a) semen volume and concentration; b) semen pH, c) sperm morphology; d) sperm motility; and e) sperm viability [2,21,22]. However, these parameters do not clearly show the true fertility potential of semen samples [23,24]. To improve the routine protocols of sperm analysis, we have tested the dependence of spermatozoa quality in relation to their surface charge.

Depending on the types of anomalies, abnormal spermatozoa generally have a lower fertilizing potential. Morphological defects were also associated with increased DNA fragmentation, structural chromosomal aberrations, immature chromatin and aneuploidy. The relationship between sperm Zeta potential and nuclear DNA damage was not explored in our work. Furthermore, the low number of analyzed samples (only 29 donors) could influence the statistical significance of the data. Therefore, the main goal of this work has been to compare the structural and functional parameters of semen samples with their zeta potential. Samples selected on the base of the acceptable parameters are more negatively charged. This data suggests that the zeta potential method can be used as a further technique for a better selection of suitable semen. Consequently, the zeta method may have considerable application in testing semen, a finding that could be implemented in analysis of preparations for ICSI clinical procedures. However, further investigation in the area of semen zeta potential is required to improve our understanding of the relationship between semen quality and surface charge, and to improve this method for possible use in the ART protocols.

The equipment used in this study is completely suitable for the measurement of living cells [13]. The internal size of capillary used was 2×4 mm with the length of 6.5 cm what is near 400 times wider than human sperm cells head (4–5 μm) and 40 times wider than sperm cells length (45 μm). The results seem to indicate that the zeta potential of semen samples correlated with the sperm motility, viability, and morphology in being more negative in samples with highest proportion of spermatozoa with normal morphology than in samples with poorer characteristics from conventional (routine) tests.

It is difficult to establish that a new method is better than the current gold standard in semen analysis that is accurate and fast. The evidence that measuring the zeta potential of spermatozoa is a promising method in sperm selection for infertility treatment seems robust. The results suggest that semen cells selected on the base of zeta potential analysis can indicate per se good functional and structural parameters of sperm. The zeta potential is a fast, cheap and simple technique that can be considered as another effective method of sperm selection for the needs of the ART procedures.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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