Micro-electrophoresis: a noninvasive method of sperm selection based on membrane charge

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Objective: To develop a technique with the potential of isolating genetically fit sperm for assisted reproductive technology (ART) treatment without compromising its structural or functional competence.
Design: Observational study.
Setting: University hospital.
Patient(s): Fifty patients undergoing infertility diagnosis and 88 couples undergoing ART treatment.
Intervention(s): None.
Main Outcome Measure(s): Under an electric field, the percentage of positively charged sperm (PCS), negatively charged sperm (NCS), and neutrally charged sperm was determined for each ejaculate before and after density gradient centrifugation (DGC), and evaluated for sperm DNA damage, histone retention, and couples' ART outcomes. Subsequently, PCS, NCS, and neutrally charged sperm were selected using an intracytoplasmic sperm injection needle and directly analyzed for DNA damage.
Result(s): There was a reduction in the NCS population (95.10% ± 0.94% vs. 54.48% ± 2.39%) and an increase in the PCS population (4.28% ± 0.58% vs. 42.52% ± 2.36%) after DGC. The DNA damage was inversely proportional to %NCS ($r^2 = -0.242$) and directly proportional to the %PCS ($r^2 = 0.206$). When sperm were picked according to their charge and directly analyzed, sperm DNA damage was lower in the NCS population (3.9% ± 1.5%) compared with control (17.3% ± 3.2%) and %PCS populations (27.8% ± 6.0%). The % NCS was positively associated with fertilization rate ($r^2 = 0.469$) and blastocyst development ($r^2 = 0.308$) and inversely associated with embryo arrest ($r^2 = -0.253$). Implantation rate and clinical pregnancies were higher in patient groups with increased NCS.
Conclusion(s): Selection of NCS through micro-electrophoresis has the potential to isolate sperm relatively free of DNA damage to be used in ART. (Fertil Steril® 2015;103:361–6. ©2015 by American Society for Reproductive Medicine.)
Key Words: ART outcomes, histone retention, micro-electrophoresis, sperm selection, sperm DNA damage

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The development of IVF, and particularly intracytoplasmic sperm injection (ICSI), highlighted the benefits of being able to select genetically fit sperm to improve pregnancy outcomes. Our understanding of sperm physiology has progressively improved, and techniques have been developed to separate functional sperm from those that are immotile, have poor morphology, or are not capable of fertilizing the egg (1). Initially sperm washing, and later more sophisticated separation techniques based on principles such as migration, filtration, density gradient centrifugation, cell surface protein binding, and membrane charge, have been used to separate sperm. The majority of methods currently available are based on sperm motility and morphology (2). However, the external appearance of sperm does not indicate the maturity (3) or the genetic qualities of the sperm (4). To measure parameters such as DNA damage, the sperm must be either lysed or fixed, making it unusable for assisted reproductive

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technology (ART) treatment. Therefore, novel methods to isolate the best-quality sperm without compromising structural or functional integrity are needed (5).

It is well known that the sperm membrane undergoes multiple chemical changes during sperm maturation, and these changes are important for various cellular interactions, such as capacitation, cell-to-cell recognition, and sperm–egg interaction during fertilization (6, 7). The sperm head is covered by a negatively charged 20–60-nm-thick glycocalyx with an electric charge of −16 to −20 mV that facilitates the interaction of sperm with its extracellular environment (8). The highly negative charge-dense glycocalyx adjacent to the sperm plasma membrane also helps to prevent sperm from undergoing self-agglutination or nonspecific binding with the genital tract epithelium during its transport and storage (9, 10).

Despite the established role of electrostatic charge in sperm physiology, few research groups have applied this principle to selection. Currently two methods of separation exist based on differences in sperm membrane charge; a simpler version known as the Zeta test (11, 12) and a more sophisticated method known as electrophoretic sperm separation (13, 14). In this study we have developed a novel method for determining the charge of individual sperm within a sample based on the electrostatic properties, called "micro-electrophoresis." This technique, which is focused on isolating mature sperm with decreased DNA damage, is suitable for real-time use during ICSI.

MATERIALS AND METHODS

Study Population and Semen Analysis

Eighty-eight consecutive infertile couples undergoing ART treatment between January 2012 and November 2012 and 50 men undergoing semen analysis were included in the study. Semen samples with severely low sperm count ($<1 \times 10^6$/mL) were excluded from the study. Charge ratios and aniline blue retention were determined for all samples. Semen analysis samples were used for determination of DNA damage levels in differentially charged sperm populations. The institutional review board governed by the University of Utah approved this study. Sperm samples for research were obtained after each couple gave written consent. Semen samples, surplus to clinical requirements, were collected by masturbation from the infertile men on the day of IVF treatment after 2–5 days of recommended abstinence. After liquefaction, routine semen analyses were performed according to World Health Organization guidelines (15).

Sperm Preparation for Micro-electrophoresis

Semen was processed using a two-step discontinuous gradient, or density gradient centrifugation (DGC, 90%–35%; Irvine Scientific). This step facilitates removal of somatic cells and suspended debris and washes off the excess glycol-proteins anchored on the sperm surface. After DGC, immobilized sperm samples were adjusted to a concentration of 20×10^6/mL.

Micro-electrophoresis Instrumentation

The micro-electrophoresis sperm separation unit consists of three parts: [1] the power supply, [2] the connecting electrodes, and [3] the disposable sterile electrophoresis unit. The power supply consists of a basic power-pack unit that can control and supply 0–300 V and 0–300 mA of electricity. Reusable platinum electrodes are used to connect the electrophoresis unit to the power supply. The electrophoresis unit consists of an electrophoresis chamber, egg injection chambers, and bubble restriction chambers (Fig. 1).

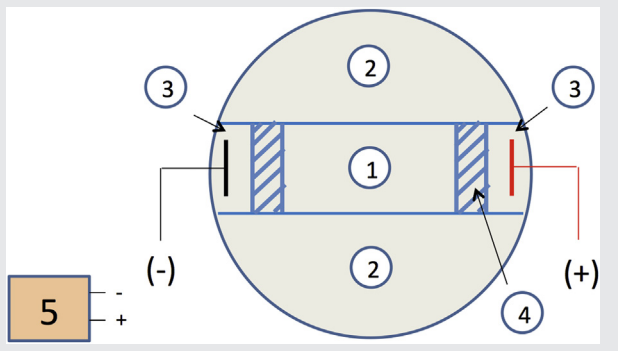
Micro-electrophoresis Sperm Separation

Two milliliters of electrophoresis buffer (10 mM Tris and 20 mM NaOH, pH 7.8) was added to the electrophoresis chamber. An approximately 10–15-μL aliquot of immobilized sperm was added to the electrophoretic buffer and allowed to settle for 2 minutes. Sperm were separated according to surface charge by applying current between 6 and 14 mA (increased from low to high) and a variable voltage of 25 to 75 V, respectively. The sperm were separated at low current first (6 mA) to ensure the isolation of sperm with the highest negative charge. The current was gradually increased to increase the movement of sperm in the electrophoretic field. During electrophoresis, sperm were assessed for their morphology. Fifty to one hundred sperm with normal morphology and a negative charge were selected using the ICSI needle. Simultaneously, the percentage of positively charged sperm (PCS), negatively charged sperm (NCS), and neutrally charged sperm was calculated by microscopic observation of sperm migrating toward the negative electrode, toward the positive electrode, or not migrating, respectively. All sperm within a microscopic field were counted, and this process was repeated until a total of 100 sperm were counted.

ART Treatment

Ovarian stimulation was performed using standard techniques. Oocytes were obtained using ultrasound-guided, transvaginal aspiration. Standard IVF (n = 31) involved

FIGURE 1



Schematic representation of micro-electrophoresis instrument. Electrophoresis chamber (1), egg injection chambers (2) and bubble restriction chambers (3), conductive bridge (4), and power pack (5).

Simon. Sperm selection by micro-electrophoresis. Fertil Steril 2015.