ABSTRACT

Objective: During the journey through the female reproductive tract to reach the oocyte, spermatozoa exhibit a variety of motility modes and swimming patterns, including linear as well as rotational motion. Currently, only those sperm that show rotational motion are chosen for intracytoplasmic sperm injection (ICSI). Whether this leads to better oocyte activation and embryo development competence after ICSI, however, is unknown. The objective of this study was to compare the activation of mouse oocytes after ICSI of human sperm with linear and rotational motion.

Design: In vitro experimental study

Materials and Methods: Fresh human semen samples (n=2) from fertile normospermic men according to 2010 WHO guidelines and B6C3F1 frozen metaphase II mouse oocytes (n=88) were used. Spermatozoa were prepared by the density gradient centrifugation and selected for ICSI based on either linear or rotational motion in deep regions of a PVP microdroplet. Spermatozoa with linear motion changed their position, but not their location by exhibiting two-dimensional movement in the bottom of a PVP droplet. Spermatozoa with rotational motion were the ones that changed position by gyration over their own axis. After thawing, surviving oocytes (n = 81) were randomly assigned into two groups (n = 39) spermatozoa with linear motion were microinjected into oocyte cytoplasm; in group 2 (n=42) spermatozoa with rotational motion were injected. In both cases, selected spermatozoa were immobilized by squeezing the tail using the injection micropipette prior to injection. After injection, the oocyte degeneration rates and oocyte activation rates (recorded as a percentage of surviving embryos at the two-cell stage 24 hours after microinjection) were assessed. Chi-square or Fisher exact tests were used to compare the outcome measures (expressed as mean ± 95% confidence interval) among the groups using an alpha level of p < 0.05.

Results: The oocyte degeneration rates and activation rates were not statistically different between the mouse oocytes microinjected with human spermatozoa with linear motion (46.1%, 95% CI: 30.0-62.8%) and those injected with spermatozoa with rotational motion (42.85%, 27.7%-59.0%). The oocyte activation rate was higher in the group of oocytes injected with spermatozoa with rotational motion (79.16%, 95% CI: 84.92-92.8%) compared to those with linear motion (52.4%; 95% CI: 29.8%-74.3%), these differences trended towards significance (p = 0.057).

Conclusions: Our results indicate that selection of human spermatozoa with rotational motion may be beneficial to oocyte activation. Further evaluation using a human oocyte model involving an expanded panel of embryo viability scores is needed.

INTRODUCTION

An integral laboratory aspect of ICSI is sperm selection, which is usually based on viability – often expressed by motility and morphology. However, during the journey through the female reproductive tract and before reaching the oocyte spermatozoa exhibit a variety of motility motion and swimming patterns, including linear as well as rotational motion. Using laser tweezers, it has recently been suggested that distinguishing normal and abnormal sperm on the basis of beat frequency may help to select better quality sperm for use in assisted reproductive techniques (ART). In order to facilitate the selection, handling and immobilization of the male gamete prior to ICSI, post-washed sperm specimens are usually loaded into a micropipette containing a viscous medium, such as polyvinylpyrrolidone (PVP) or hyaluronic acid (HA) that slows down sperm movement. Increased medium viscosity dampens the strong linear component, converting sperm motion into rotational motion that is believed to be the result of a reversible change in the molecular components of the mitochondrial apparatus. It has been hypothesized that such sperm-swimming mode is similar to the one sperm exhibit when approaching the oocyte in vivo. Nevertheless, it is at present unclear whether sperm with rotational motion prior to selection for ICSI have better oocyte activation than those selected by linear motion. This is an interesting concept that deserves further investigation. We conducted an experiment using an ICSI experimental model to assess the activation of mouse oocytes after intracytoplasmic injection of human sperm with linear or rotational motion.

MATERIALS and METHODS

Fresh human semen samples (n=2) from fertile normospermic men according to 2010 WHO guidelines and B6C3F1 frozen metaphase II mouse oocytes (n=88; Embryotek, Haverhill, MA) were used in this experiment. Sperm were prepared by the density gradient centrifugation and selected for ICSI based on either linear or rotational motion in deep regions of a PVP (Origio, Denmark) microdroplet. Sperm with linear motion were those that changed their position but not their location by exhibiting two-dimensional movement in the bottom of a PVP droplet. On the other hand, sperm with rotational motion were the ones that changed position by gyration over its own axis (Figure 1). Frozen mouse oocytes were thawed and the surviving oocytes (n = 81) were randomly assigned into two groups for microinjection. Sperm with linear motion (Group 1; n = 39) and rotational motion (Group 2; n = 42) were selected and microinjected into the oocyte cytoplasm. In both cases the selected sperm were immobilized by squeezing the tail using the injection micropipette prior to injection. After sperm injections, the oocyte degeneration rates and oocyte activation rates (recorded as a percentage of surviving embryos at the two-cell stage 24 hours after microinjection) were assessed (Figure 2). Chi-square or Fisher exact tests as appropriate were used to compare the outcome measures (expressed as mean ± 95% confidence interval) among the groups using an alpha level of p < 0.05.

RESULTS

1. The rates of oocyte degeneration and activation after intracytoplasmic injection of human sperm selected based on linear or rotational motion are presented in Table 1 and Figure 3.

2. The oocyte degeneration rates were 46.15% and 42.85% in the groups of linear and rotational motion, respectively, and results were not statistically different.

3. In contrast, higher oocyte activation rates were achieved in the group of rotational motion (79.16%) than linear motion (52.38%); these results were marginally significant (p = 0.057).

Table 1. Degeneration and activation rates of mouse oocytes after intracytoplasmic injection of human sperm with linear and rotational motion

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Linear motion (n = 39)</th>
<th>Rotational motion (n = 42)</th>
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<tbody>
<tr>
<td>Oocyte degeneration rate (%)</td>
<td>46.15; 30.09-62.82  (18/39)</td>
<td>42.86; 27.72-59.04 (18/42)</td>
</tr>
<tr>
<td>Surviving embryos at 2-cell stage (%)</td>
<td>52.38; 29.78-74.29 (11/21)</td>
<td>79.16; 57.84-92.87 (19/24)</td>
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</table>

Data are presented as mean and 95% confidence interval. *Difference was marginally significant (p = 0.057).

CONCLUSIONS

1. Our findings add to the understanding of the sperm selection step that precedes the sperm injection.

2. Selection of human sperm with rotational motion may be beneficial to oocyte activation and further ICSI outcomes.