Sperm Evaluation by 3D Laser Scanning Method Microscopy (LEXT OLS4000)


1. AnPath GmbH | AnPath Services GmbH, Switzerland | RESP BioService Scientific Laboratories GmbH, Germany
2. Vivotecia Research S.L., Spain | Olympus Switzerland AG, Switzerland

INTRODUCTION

Reproductive toxicology testing for a variety of pharmaceutical, chemical or other xenobiotics is a necessary step in the evaluation of a compound's potential to affect the reproductive system of animals. The test utilizes the same endpoints that are used in the standard reproductive toxicology test battery, with the addition of a qualitative and quantitative analysis of sperm parameters. The purpose of the study is to provide a comprehensive and detailed understanding of the effects that a compound may have on sperm quality and quantity, as well as other reproductive parameters.

METHODS

Test Sperm

C57Bl/6J rats (BEL Bioservice Laboratories GmbH, Planegg, Germany)

- sperm samples were collected from the vas deferens
- test samples were collected from the vas deferens

H98 cells (BEL Bioservice Laboratories GmbH, Planegg, Germany)

- sperm samples were collected by means of an artificial vagina (AV) system
- AVs were assembled by inserting a cone and filling collection tube
- temperature was adjusted to 37°C
- animals were euthanized and collected in the morning
- genomic monkey (Macaca fascicularis; Vivotecia Research S.L., Madrid, Spain)
- sperm samples were collected from overnight-anesthetized animals
- samples were collected and stored in liquid nitrogen

RESULTS

Table 1: Measured Values on Rat Sperm

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head Circumference</td>
<td>100</td>
<td>5</td>
</tr>
<tr>
<td>Head Diameter</td>
<td>30</td>
<td>2</td>
</tr>
<tr>
<td>Head Lateral Diameter</td>
<td>20</td>
<td>2</td>
</tr>
<tr>
<td>Head Length</td>
<td>20</td>
<td>5</td>
</tr>
<tr>
<td>Tail Circumference</td>
<td>20</td>
<td>5</td>
</tr>
<tr>
<td>Tail Length</td>
<td>10</td>
<td>3</td>
</tr>
</tbody>
</table>

Discussion

- Only 10 animals were used
- Fixed
- Samples were placed in 2% glutaraldehyde solutions prior to evaluation
- Sample Preparation
  - Fixation with Aquadest followed by 3% osmium tetroxide with 0.1% sodium nitroprusside
- Preparation of samples
  - Evaluation by LEX
  - LEXT 3D Imaging Laser Microscope OLS4000 (Olympus Deutschland AG)

CONCLUSION

LEXT 3D imaging laser microscopy is a promising technology for sperm evaluation. 3D pictures are similar to those obtained by scanning electron microscopy with high-magnification accuracy. The application provides results within minutes once a spermatozoa is evaluated. The images and 3D imaging analyses are useful tools for the interpretation of spintronic properties.

REFERENCES