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

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INTRODUCTION

Reproductive toxicology testing for a variety of pharmaceutical, chemical or agrochemical substances is necessary to ensure that there are no harmful effects on the reproductive organs. Sperm analysis is one of the endpoints in toxicity studies. Different methods for quantitative sperm analysis (total count of motile (live) and non-motile (dead) sperm, motility percent, motility grade profile, pH, white blood cell count, agglutination and if necessary, vitality and fructose count) have been described, e.g. computer-assisted sperm analysis (CASA) that requires a vast investment; the MTT method with a microplate and sperm quality analyzer (SQA) etc.[1]

For qualitative morphological sperm analysis, either the above-mentioned techniques or smears of sperm (stained e.g. by eosin or toluidine blue) may be used. The technique provides morphological results on a light microscopy level.

Laser Scanning Microscopy is a technique using a focused laser for scanning an object. The Olympus LEXT OLS4000 is using Differential Interference Contrast (DIC), a method for visualizing sub-nanometer micro asperities, which are far beyond the resolving power of a typical laser microscope. The obtained live images are comparable to those of a scanning electron microscope under relatively low power magnifications. The technique was developed to evaluate technical surfaces and materials. So far it has not been used in biology. An attempt was made to use LEXT for the morphological analysis on sperm of rats, mice, rabbits and cynomolgus monkeys.

METHODS

Test System

Crl: WI(Han) rats (BSL BIOSERVICE Laboratories GmbH, Planegg / Germany):

- sperm samples were collected from the vas deferens

BALB/c mice (BSL BIOSERVICE Laboratories GmbH, Planegg / Germany):

- sperm samples were collected from the vas deferens

NZW rabbits (BSL BIOSERVICE Laboratories GmbH, Planegg / Germany):

- sperm samples were collected by means of an artificial vagina (AV) (COLLAP system with temperature maintenance, IMV Technologies, L'Aigle / France)
- AV was assembled by inserting a cone and fitting collection tube; temperature was adjusted to 50-55°C
- to increase the animals' libido a teaser doe was presented to the bucks
- AV was held tight in the collector's hand with a preserved fur from a skinned female rabbit covering his forearm, which was laid flat onto the ground of the buck's cage to allow the buck to mount

Cynomolgus monkeys (Macaca fascicularis) (Vivotecnia Research S.L., Madrid/Spain):

- sperm samples were collected from overnight fasted animals but allowed access to water ad libitum by electro-ejaculation using an electro-stimulator (PTE Model 304) coupled to a rectal probe (0.7 cm diameter)
- animals were placed on a padded table in lateral recumbency after sedation with 5 mg/kg of ketamine/HCl i.m.
- rectal probe was dipped in lubricating oil and inserted gently in the pelvic rectum so that the brass bands laid approximately at the level of the prostate gland
- rhythmic pulse stimulations were applied at a voltage ranged 5-10V (carried out in pulses of 6s duration interspersed with rest periods of 3s between pulses; 3 series of 8 – 10 pulses; voltage level increased gradually between the series if required)

RESULTS

Table 1: Measured Values on Rat Sperm

Parameter	Mean	SD
Head: Circumference	42.4	1.5
Head: Total area	21.4	1.1
Tail: Lengths	117.8	7.5
Tail: Distance neck-to-droplet	61.7	2.9

Table 2: Measured Values on Mouse Sperm

Parameter	Mean	SD
Head: Circumference	20.5	1.1
Head: Total area	20.8	0.5
Tail: Lengths	110.0	5.4
Tail: Distance neck-to-droplet	15.2	1.3

Table 3: Measured Values on Rabbit Sperm

Parameter	Mean	SD
Head: Circumference	27.1	2.9
Head: Total area	37.4	8.8
Head: Lengths	7.8	0.9
Head: Widths	4.8	0.8
Tail: Lengths	43.1	3.7

Table 4: Measured Values on Cynomolgus monkey (Macaca fascicularis) Sperm

Parameter	Mean	SD
Head: Circumference	18.2	0.3
Head: Total area	17.2	1.6
Head: Lengths	4.8	0.3
Head: Widths	3.6	0.1
Tail: Lengths	73.6	1.0

Units:
Length [µm]
Area [µm²]

Figure 1: Rat Sperm, 3D. a) measurements on head (x8567), b) spermia (x1822), c) sperm head (x8567), d) droplet (x14954)
(Magnification on all figures as orginal magnification)

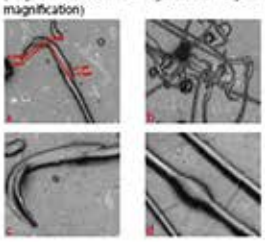


Figure 2: Mouse Sperm. a) measurements on head 3D, (x8545), b) note one sperm without droplet (x2136)

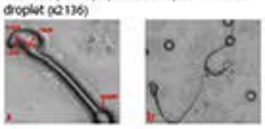


Figure 3: Rabbit Sperm. a) measurement modus: note angle head-neck (x8545), b) coil tail (3D) (x8545)

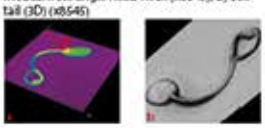
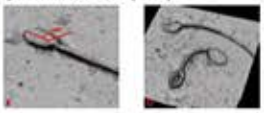


Figure 4: Cynomolgus monkey (Macaca fascicularis) Sperm. a) measurement modus: note angle head-neck, 3D (x8545), b) note one sperm with coil tail, 3D (x8545)



Treatment

- Only naive animals were used.

Fixation

- Samples were placed into 2.5% glutaraldehyde solutions prior to evaluation.

Sample Preparation

- Rinsing with Aqua dest. followed by 5 minutes staining with non-diluted modified May-Grünwald (Merck) (1:1) and subsequent further 3x rinsing with Aqua dest.
- Preparation of smears

Evaluation by LEXT

- LEXT 3D Measuring Laser Microscope OLS4000 (Olympus Schweiz AG)

Measurements

- 10 sperms per 3 animals/species

DISCUSSION

Laser Scanning Microscopy is usually not a tool for biological materials and was never applied to tissues or cells. The reason is simply that a laser is reflected by wet surfaces and hence, imaging is impossible. However, using a fixative for electron microscopy evaluation and handling dried slides of cell smears without coverslipping, the application of LEXT OLS 4000 technology was deemed to be possible. An important issue is to achieve the contrast of the cells under investigation to the mounting base, i.e. the glass slide by a previously applied staining of the cells.

An attempt was made to use LEXT OLS4000 for the morphological analysis on sperm of rats, mice, rabbits and cynomolgus monkeys. The magnification of up to x17'090 provided excellent images similar to scanning electron microscopy (figures 1-4). Measurements on sperm parameters included circumference, length, widths, and area of head; distance neck to droplet in mice and rats (tables 1-4); tail lengths, and angles head to neck in monkeys and rabbits (figures 3, 4). The length parameters obtained were comparable to published values 2. Abnormalities were easily detectable.

CONCLUSION

Laser Scanning Microscopy by LEXT OLS4000 is a promising technology for sperm evaluation. 3D pictures are similar to those obtained by scanning electron microscopy with highest measuring accuracy. The application provides results within minutes once a preparation undergo evaluation. The images and related image analyses are useful tools for the interpretation of induced sperm injury.

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