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MORPHOLOGIC AND MORPHOMETRIC ANALYSIS OF MANUALLY COLLECTED RHINOCEROS (*Ceratotherium simum simum*) SPERMATOZOA

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Abstract

Sperm morphology and morphometry are related to fertility. Semen is routinely assessed for morphologic anomalies. Detailed spermatologic analysis also requires morphometric data. In this study morphologic and morphometric characteristics of manually obtained white rhinoceros spermatozoa (*Ceratotherium simum simum*) are presented.

Zusammenfassung

Spermienmorphologie sowie –morphometrie stehen in Zusammenhang mit der Fertilität eines Individuums. Eine morphologische Ejakulatanalyse ist wesentlicher Bestandteil der spermatologischen Routinediagnostik. In einer umfassenden Fertilitätsuntersuchung werden zudem morphometrische Kriterien berücksichtigt. In der vorliegenden Studie werden die morphologischen und morphometrischen Eigenschaften eines manuell gewonnenen Breitmaulnashornejakulats (*Ceratotherium simum simum*) präsentiert.

Résumé

La morphologie et la morphométrie du sperme sont liés à la fertilité. La présence d'anomalies morphologiques du sperme est contrôlé en routine. Des analyse spermatologiques détaillées requièrent aussi des données morphométriques. Dans cette étude, les caractéristiques morphologiques et morphométriques de spermatozoïdes de rhinocéros blanc (*Ceratotherium simum simum)* obtenus manuellement sont présentés.

Key words : White rhinoceros, *Ceratotherium simum simum* , semen collection, semen assessment, sperm morphology, sperm head morphometry

Poster Abstract

The demographic crisis of the captive southern white rhinoceros (*Ceratotherium simum simum*) population has led to a multi-institutional research project in order to overcome reproductive problems (5). Semen assessment is an important tool to evaluate the reproductive soundness of male individuals as a possible contributing factor to the poor reproductive rate (3). As basic information on rhinoceros spermatozoa in literature is scarce, in this study, stained preparations of a manually obtained semen sample were assessed for morphologic and morphometric characteristics.

Semen collection was conducted on a thirty-year-old white rhinoceros at Salzburg Zoo. Since his arrival at the zoo in 1991, no copulation was observed and no offspring has yet been sired. The male was conditioned to enter an indoor restraint chute and to tolerate manual stimulation of penis and preputium (8). The ejaculate was obtained by penile massage with pharmacologically induced erection onset (6). The sample consisted of three fractions which were collected separately into pre-warmed graduated containers.

In addition to a morphologic and computer assisted morphometric analysis, the three ejaculatory fractions were assessed for volume, pH, sperm concentration, percentage of motile spermatozoa and vitality (Table 1). The total volume of the ejaculate was 23 ml. It contained $1{,}26 \times 10^9$ spermatozoa. The collected sample's appearance was characterised by an ivory coloured homogenous fluid with no specific odour and was not contaminated by urine or other substances.

Table 1: Seminal parameters of a manually collected white rhinoceros ejaculate

Ejaculatory Fraction	Volume (ml)	pH[1]	Sperm concentration[2] ($\times 10^6$/ml)	($\times 10^9$/fraction)	Motility[3] (%)	Vitality[4] (%)
1	5	7.5	96	0.48	70	84
2	15	7.7	44	0.66	80	83
3	3	7.7	39	0.12	75	81
Mean ± S.D.		7.6 ± 0.1	60 ± 26		75 ± 5	83 ± 2

[1]measured by colour change of an indicator strip
[2]determined by haemocytometer
[3]visual estimation to the nearest 5%
[4]assessment by vital staining with bromphenolblue-nigrosin

The morphologic analysis was performed on mounted bromphenolblue-nigrosin smears. 300 spermatozoa were examined (100 from each fraction) by phase-contrast microscopy using a Leitz Laborlux S microscope (Leica Microsystem Holdings GmbH, 35578 Wetzlar, Germany) at 1000x under oil immersion. Head, mid-piece region and tail of structural abnormal spermatozoa were subjectively classified according to the origin of their pathological deformation (4). 85% (mean) of the analysed spermatozoa were morphologically normal – abnormal spermatozoa mainly revealed primary anomalies; for detailed results see table 2.
All examined spermatozoa showed an asymmetrical tail insertion, which was considered as normal. Cytoplasmatic droplets were absent in the assessed ejaculate.
Morphometric analysis of the sperm head dimensions was performed using the Hamilton Thorne Morphology Analyser Version 10.7F IVOS (integrated visual optical system) (Hamilton Thorne Research, Beverly, MA 01915, USA). Air dried smears were prepared from the fresh semen sample, stained with a modified Farelly procedure (1) and mounted. Aim of this study was to determine head size parameters of normal appearing cells. 200 sperm heads of classified as normal spermatozoa were measured using the 100x objective magnification under oil immersion. In the examined ejaculate normal appearing spermatozoa were 5,8µm in length and 3,2 µm in width (mean). All evaluated head dimensions are given in table 3. In a future investigation, also sperm head size parameters of abnormal appearing cells will be determined in order to provide a complete quantitative and reproducible computer assisted morphometric rhinoceros semen analysis.

Table 2 : Morphological analysis results of manually collected white rhinoceros spermatozoa (%)

	Fraction 1	Fraction 2	Fraction 3
Morphologically abnormal	18	15	13
Primary anomalies	**14**	**13**	**10**
Specific head defects	7	6	5
Acrosomal defect	7	4	4
Amorphous head	0	2	1
Unspecific head defects	4	6	3
Tapering head	3	1	2
Broad head	0	3	1
Small oval head	1	0	0
Pear-shaped head	0	2	0
Unspecific mid-piece defects	0	1	1
Unspecific tail defects (coiled tail)	3	0	1
Secondary anomalies	**4**	**2**	**2**
Bent tail	4	0	1
Absent tail ("detached" head)	0	2	1
Tertiary anomalies (broken tail)	**0**	**0**	**1**

Table 3: Head dimensions of as normal classified white rhinoceros spermatozoa (n=200)

Head size parameter	mean ± S.D.
Major axis (µm)	5.8 ± 0.2
Minor axis (µm)	3.2 ± 0.2
Elongation (%)	54.8 ± 2.4
Area (μm^2)	15.2 ± 1.0
Perimeter (µm)	15.9 ± 0.5
Symmetry (%)	93.0 ± 2.4

In view of the fact that no detailed morphologic and morphometric data for white rhinoceros (*Ceratotherium simum* simum) exists, our findings were compared to ejaculate reference data of the fertile stallion (2, 7, 9) as the closest domestic relative. The results resemble those of the horse. Interpreting our findings in relation to fertility remains speculative until further data on white rhinoceros spermatozoa can be available.

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