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Sperm attributes and morphology on *Rusa timorensis*: Light and scanning electron microscopy

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ABSTRACT

This study provides standard information on the attributes of sperm and describes the surface structure of normal and abnormal spermatozoa of *Rusa timorensis*. Two fertile stags were used as the source of semen collected during the first breeding season commencing from April 5 to July 2, 2012. Another five stags were used as the source of semen collected during the second breeding season commencing from April 1 to June 27, 2013. Semen samples were collected from the stags using an electro-ejaculator. The ejaculate was processed and samples prepared for light and scanning electron microscopy (SEM) according to standard methods. No significant difference ($P > 0.05$) was found between sperm attributes in comparison between different stags and different months of the fertile seasons. The results of this study have also demonstrated that there are no differences in size, shape and surface structure between spermatozoa of the different stags and different months of the fertile seasons. Sperm attributes (volume, pH, sperm concentration, general motility, progressive motility and viability) were 2.2 ± 0.29 ml, 7.2 ± 0.17, $886.3 \pm 39.7 \times 10^6$ spermatozoa/ml, $78.7 \pm 2.01\%$, $80.8 \pm 1.85\%$ and $83.2 \pm 0.85\%$, respectively. Morphological analysis showed low percentage of abnormal spermatozoa $13.9 \pm 2.88\%$. Scanning electron microscopy revealed spermatozoa which consisted of a flat paddle-shaped head, short neck and a tail, which was subdivided into midpiece, principal piece and endpiece. The average spermatozoon was 66.2 ± 0.69 μm in total length. The flat paddle-shaped head was 7.8 ± 0.28 μm long, 4.2 ± 0.15 μm at its widest width, 2.4 ± 0.18 μm basal width and 0.7 ± 0.02 μm thick. As for the tail, the midpiece length was 13.2 ± 0.14 μm, 0.6 ± 0.04 μm in diameter; the principal piece was 42.6 ± 0.04 μm, and 2.8 ± 0.06 μm for the endpiece. Abnormal spermatozoa such as tapered head, microcephalic head, decapitated spermatozoa and bent tails were observed. Results provide standard information useful for development of strategies for semen cryopreservation and assisted reproductive technology in this species.

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1. Introduction

Examination of the structure and function of mammalian spermatozoa was conducted with the aid of light and scanning electron microscopies. Microscopic description of normal and abnormal sperm cells of some deer species have been reported by Wislocki (1949); Bierschwal et al. (1970); Dott and Utsi (1971); Andersen (1973); Gosch and Fischer (1989); Wahid et al. (2000) and Amare (2009). However, studies on spermatozoa of *Rusa timorensis* are limited.

The understanding of reproductive characteristics, especially of semen, is important for development of strategies for semen cryopreservation and assisted reproductive technology in deer species. Semen quality is highly species-specific and requires in-depth knowledge of the basic aspects of reproductive biology of the targeted species. Routine semen analysis provides standard information on the attributes of sperm (Barrat, 2007). This assay reveals useful information for the evaluation of male infertility. As semen samples can show substantial variation, a minimum of two properly collected samples over two spermatogenic cycles should be examined at 37 °C (Vasan, 2011). Computer-assisted spermatozoa morphometric analysis helps to improve sperm assessment and allows for the identification of sperm attributes which cannot be detected by visual evaluation (Rijsselaere et al., 2004).

To date, there is no information on the microscopic description of normal and abnormal sperm cells of Rusa deer (*R. timorensis*). Therefore, the aim of this study was to build standard information on the attributes of sperm and description of the surface structure of normal and abnormal spermatozoa of Rusa deer (*R. timorensis*).

2. Materials and methods

2.1. Animals and management

Two fertile stags, 4 (#S_1) and 5 years (#S_2) old, with a live body weight of 76.9 and 80.6 kg, respectively, were used as the source of semen collected during the first breeding season commencing from April 5 to July 2, 2012. Another five stags aged between 4 and 6 years, with an average live weight of 66.7 kg, were used as the source of semen collected during the second breeding season commencing from April 1 to June 27, 2013. The stags were aged using their date of birth records. All stags had good body condition and a pair of symmetrical and normal testicles. The stags were mixed with the female deer at the University's Deer Unit (2.99° N, 101.7° E). Food and water were available ad libitum.

2.2. Semen collection

Prior to semen collection, the stags were anaesthetized using a combination of 1 mg of xylazine hydrochloride and 2 mg of ketamine hydrochloride kg^{-1} body weight (Fletcher, 2001). A standard electro-ejaculation protocol by Wahid et al. (2000) was adopted for semen collection. The semen was collected using an electro-ejaculator (P-T Electronics, Boring, USA) every 2 weeks from April 5 to July 2, 2012 for the first breeding season and from April 1 to June 27, 2013 for the second breeding season. The hand-held electrical probe was specifically designed for small ruminants. The electrical probe was inserted into the rectum and the probe electrodes oriented towards the accessory sex glands. Electric stimulation was increased gradually from 2 to 18 V with a maximum interval of 5 s (on–off) to obtain an ejaculate. Erections occurred during electrical stimulation, and the semen was collected into a graduated conical tube to determine the volume of the ejaculate. After electro-ejaculation was completed, each animal was given Yohimbine hydrochloride intravenously at a dose rate of 2 mg kg^{-1} body weight to reverse the anaesthetic effect.

2.3. Semen evaluation

Immediately after collection, the semen sample was transferred to the Laboratory and placed into a water bath (37 °C) throughout the semen evaluation process. The semen was determined macroscopically for its volume, colour and consistency. Five microlitres of fresh undiluted semen was placed on a pre-warmed glass slide for wave pattern examination. The wave pattern was observed under a warmed stage light microscope at 200× magnification and a score between 0 and 5 was given. Light microscopic evaluation was also conducted on the percentage of motile sperm by mixing one drop of semen with four drops of physiological saline and then the mixture was homogenized. One drop of the mixture was transferred onto a clean warm glass slide and covered with a cover slip. The percentage of motile spermatozoa was estimated by rapid observation of five separate fields at 400× magnification. This examination was done with a good light microscope by racking down the condenser and reducing the light. General motility was measured as the percentage of sperm that were moving (all motile sperm cells). Progressive motility was measured as the percentage of sperms with productive flagella motion such as rapid, linear and forward progression. Sperm viability was assessed by mixing one drop of semen and stained with four drops of eosin-nigrosin (Barth and Oko, 1989), smeared and placed on a warmer stage for 10 s fixation. A total of 200 sperms were counted and viable sperm were determined. Sperm heads which absorbed the stain were classified as dead sperm and unstained heads as viable sperms. Sperm morphology was classified as either normal or abnormal at 1000× magnification (Barth and Oko, 1989). The sperm concentration was calculated using a Neubauer counting chamber as described by Kirkman-Brown and Bjorndahl (2009). The pH was determined by pH indicator paper (Whatman International Ltd., Maidstone, UK).

2.4. Spermatozoa morphometric analysis

For morphometric study, the semen sample and eosin-nigrosin stain were mixed at 1:3, v/v for 30 s. A smear of the mixture was made on a pre-warmed glass slide and air-dried. All slides were mounted and examined under a light microscope at 1000× magnification. One hundred viable spermatozoa from random fields were selected