

BIOMETRY OF FROZEN-THAWED SPERM FROM INDIAN JAISELMERI CAMEL

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ABSTRACT

The objective of the present study was to measure various biometric end points of frozen-thawed sperm in Indian Jaiselmeri camel (*Camelus dromedarius*), as sperm morphometry in combination with other objective traits which can be useful for developing a fertility index. The sperm head greatest length varied from 8.18 to 8.84 μm , whereas its width ranged from 4.82 to 5.58 μm . The mean value of sperm head area and perimeter for 7 breeding males was 39.75 μm^2 and 28.44 μm , respectively. The ratio of sperm width to length varied from 0.56 to 0.67 with a mean value of 0.63. Based on mean values of sperm tail length, mid-piece length and its width, the 7 bulls were categorised into 3, 4 and 2 groups, respectively.

Key words: Biometry frozen-thawed, camel, Jaiselmeri, morphometry, sperm

Evaluation of sperm concentration and motility is frequently used to assess semen quality, but provides limited information regarding potential fertility of sires (Brahmkshtri *et al*, 1999; Correa *et al*, 1997; Zhang *et al*, 1998). Other criteria, including computerised analysis of motility and acrosome integrity, have also been used to assess semen quality; however, associations with non-return rates of bulls are not high or even consistent (Farrell *et al*, 1998; Januskauskas *et al*, 2000; Kjaestad *et al*, 1993). Non-return rates are not generally explained by routine semen analysis, and therefore, subfertile sires with apparently normal semen provide impetus for further studies to elucidate other markers of fertility. Sperm head morphology has been suggested as an indicator of fertility (Casey *et al*, 1997). Combining head shape and sperm morphometry, as well as other objective traits into an overall fertility index, could have the potential to rank sires according to their fertilising capability.

India possesses 6 breeds of camel (*Camelus dromedarius*) and Jaisalmeri camels are well known among them for their riding and race potential. The exploitation of such potential can be enhanced by producing sires with better reproductive efficiency and undertaking such endeavours to improve the fertility. Due to the potential role of sperm biometry in assessment of sire fertility (Casey *et al*, 1997), the present study was undertaken to evaluate sperm biometry of breeding males belonging to Jaiselmeri camel breed of India.

Materials and Methods

Sperm biometry was done on frozen-thawed semen of 7 breeding males of Jaiselmeri camel, which were cryoprocessed and prepared at National Research Centre on Camel at Bikaner (Rajasthan, India). Slide preparation and morphometric evaluation were performed as described by Aggarwal *et al* (2007). Briefly, 3 straws of each male (from different ejaculates) were thawed in a water bath (37°C for 30 s) and pooled. A drop of the pooled semen was mixed with 2 or 3 drops of eosin-nigrosin stain, placed on a clean glass slide at 37°C and spreaded into a thin smear. One hundred live sperms were used for sperm biometry estimation from various fields on 3 slides prepared for each male using a Trinocular Research Phase Contrast Microscope (1000 x magnification) fitted with a high-resolution digital CCD camera (still image resolution= 768 x 576 pixels) and analysed with imaging software. The system was first calibrated with images of standard length for known magnifications and measurement accuracy of $\pm 0.1 \mu\text{m}$. Various measurements of the sperm head (length, width, perimeter, and area) and sperm tail (length and width of mid-piece and length of tail) were recorded. The ratio of width to length for sperm head was also calculated.

All values for a parameter were categorised into groups using the value of critical difference (CD) for each end point; males within a group had similar values, but these were significantly different from males of other groups. The CD was estimated by first

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Table 1. Biometry of the sperm head in camel.

| Animal No. | Maximum length (µm) | Maximum width (µm) | Area (µm ²) | Perimeter (µm) | Max.width/ Max.length |
|------------|--------------------------|-------------------------|--------------------------|--------------------------|-------------------------|
| 1 | 8.23 ^{a*} ±0.52 | 5.51 ^d ±0.34 | 40.86 ^d ±2.21 | 28.59 ^c ±1.10 | 0.67 ^b ±0.05 |
| 2 | 8.32 ^a ±0.50 | 5.58 ^d ±0.37 | 40.42 ^c ±2.35 | 29.01 ^d ±1.53 | 0.67 ^b ±0.06 |
| 3 | 8.66 ^b ±0.80 | 4.82 ^a ±0.69 | 38.53 ^a ±2.71 | 28.19 ^a ±1.03 | 0.56 ^a ±0.12 |
| 4 | 8.18 ^a ±0.74 | 5.12 ^b ±0.63 | 38.05 ^a ±2.95 | 27.82 ^a ±1.11 | 0.63 ^a ±0.11 |
| 5 | 8.68 ^c ±0.87 | 5.35 ^c ±0.70 | 41.00 ^d ±3.14 | 28.24 ^b ±1.06 | 0.62 ^a ±0.12 |
| 6 | 8.84 ^c ±0.78 | 5.17 ^b ±0.58 | 39.51 ^b ±2.72 | 28.45 ^b ±1.03 | 0.59 ^a ±0.09 |
| 7 | 8.45 ^b ±0.87 | 5.20 ^b ±4.94 | 39.84 ^b ±3.35 | 28.78 ^c ±1.16 | 0.67 ^b ±0.12 |
| Average** | 8.48±0.56 | 5.32±0.07 | 39.75±3.11 | 28.44±0.70 | 0.63±0.03 |
| CD at 5 % | 0.24 | 0.19 | 0.92 | 0.38 | 0.07 |

Each value is a mean ± S.E. of 100 sperms of a breeding male. *Values within columns with different superscripts differ (P < 0.05). **Average value is a mean ± S.E. of 700 sperms from 7 males.

calculating the value of standard error of difference (SED) through ANOVA, performed using the PC-2 version of fixed model least squares and maximum likelihood (LSML) computer program (Harvey, 1990), which included the effect of males as sources of variation. The SED was then multiplied with the t-value for P=0.05 at error degree of freedom to arrive at the value of CD (Panse and Sukhatme, 1985).

Results and Discussion

Based on average values of sperm head length and the CD value, the 7 males were categorised into 3 groups (Table 1) and the values ranged from 8.18 to 8.84 µm. Sperm width varied from 4.82 to 5.58 µm with an average of 5.32 µm and there were 4 distinct groups among breeding males. The greatest sperm head area and perimeter was 41 µm² and 29.01 µm with an average value of 39.75 µm² and 28.44 µm, respectively for these 2 parameters. The ratio of sperm head width to length varied from 0.56 to 0.67 enabling the 7 breeding males to be allocated into 2 groups.

Maximum midpiece length, width and length of sperm tail was 13.21 µm, 0.84 µm and 44.56 µm, respectively (Table 2), whereas the average values for these 3 parameters of sperm tail were 12.46 µm, 0.81 µm and 43.58 µm, respectively.

We determined average values for sperm biometry end points in 7 breeding males of Indian Jaiselmeri camel and there were several unique and significant differences among males for each end point. There are some reports regarding sperm biometry in buffaloes (Aggarwal *et al*, 2007), stallions (Casey *et al*, 1997), boars (Hirai *et al*, 2001) and rams (Gravance *et al*, 1998). However, data generated in our study reflects that sperm biometry of male camel is unique and may form the basis for correlating its fertility status, as the sperm morphometry has been correlated with fertility in stallion (Gravance *et al*,

Table 2. Biometry of the sperm tail in camel.

| Animal No. | Length of midpiece (µm) | Width of midpiece (µm) | Length of tail (µm) |
|------------|---------------------------|-------------------------|--------------------------|
| 1 | 12.74 ^{c*} ±0.48 | 0.79 ^a ±0.04 | 44.05 ^b ±2.34 |
| 2 | 13.21 ^d ±0.61 | 0.80 ^a ±0.06 | 43.80 ^b ±1.90 |
| 3 | 12.04 ^a ±0.74 | 0.81 ^a ±0.05 | 44.56 ^c ±3.92 |
| 4 | 12.72 ^c ±0.75 | 0.79 ^a ±0.06 | 43.20 ^a ±4.15 |
| 5 | 12.33 ^b ±0.67 | 0.81 ^a ±0.04 | 43.20 ^a ±4.12 |
| 6 | 12.33 ^b ±0.71 | 0.80 ^a ±0.05 | 42.14 ^a ±3.85 |
| 7 | 11.86 ^a ±0.99 | 0.84 ^b ±0.06 | 44.08 ^b ±4.13 |
| Average** | 12.46±1.52 | 0.81±0.01 | 43.58±3.60 |
| CD at 5 % | 0.24 | 0.02 | 1.19 |

Each value is a mean ± S.E. of 100 sperms of a breeding male. *Values within columns with different superscripts differ (P < 0.05).

**Average value is a mean ± S.E. of 700 sperms from 7 males.

1996; Casey *et al*, 1997) and boars (Hirai *et al*, 2001). It has also been postulated that sperm head area and shape effects total sperm volume and in turn sperm freezability by influencing sperm cryoresistance (Esteso *et al*, 2006).

This study reports biometry of live sperm for Indian Jaiselmeri camel and this data provide a preliminary basis for assessing sires with better fertilising potential in combination with other morphological and reproductive traits.

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