Journal of Equine Veterinary Science 35 (2015) 244–249

Contents lists available at ScienceDirect

Journal of Equine Veterinary Science

journal homepage: www.j-evs.com

Original Research

Morphometric Characteristics of Spermatozoa in the Arabian Horse With Regard to Season, Age, Sperm Concentration, and Fertility

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ARTICLE INFO

Article history:
Received 17 October 2014
Received in revised form 8 December 2014
Accepted 4 January 2015
Available online 9 January 2015

Keywords:
Arabian horse
Sperm morphometry
Sperm concentration
Fertility

ABSTRACT

One hundred eighty ejaculates were collected from 15 healthy Arabian horses (6–26 years old) for morphometric evaluation of their spermatozoal dimensions. The progressively motile sperm percentage, sperm abnormalities percentage, and sperm cell concentration ($\times 10^6$ sperm/mL) were determined. Stained slides with nigrosin–eosin solution were prepared for sperm morphometric analysis using an eye-piece micrometer. The sperm measurements were sperm head length, head maximum breadth, head base breadth, acrosome length, midpiece and breadth, tail length and breadth, sperm head area, and perimeter. Data were divided according to season, stallions' age, and fertility. Results revealed that mensuration of sperm head length, head maximum breadth, midpiece length, and tail length were 5.96 ± 0.004, 3.06 ± 0.004, 10.17 ± 0.008, and 48.88 ± 0.022 μm, respectively. The head area and perimeter were 14.33 ± 0.022 μm^2 and 14.16 ± 0.009 μm, respectively. The total number of sperm per ejaculate in spring and summer was significantly ($P < .05$) higher than in autumn and winter. The lowest sperm concentration (153.61 ± 26.75 × 10^6/mL) and the longest head length (6.00 ± 0.01 μm) were found during winter. Sperm head length, head maximum breadth, head base breadth, head area, and perimeter were the lowest in group A (<10 years). Sperm morphometry was highly significantly ($P < .001$) affected by the stallion. The head maximum breadth, head base breadth, sperm head area, and perimeter were ($P < .001$) smaller in stallions with high fertility (69%–79%) compared with those of low fertility (50%–59%). In conclusion, stallion sperm morphometry was affected by the season, stallions' age, and stallions. Sperm head maximum breadth, head area, and perimeter were smaller in stallions of high fertilizing capacity.

1. Introduction

Fertilizing ability of spermatozoa depends on the status of their shapes and sizes, which affect the course of the acrosomal reaction and sperm penetration of the ovum [1–6]. Sperm head size and shape result from the size and shape of the nucleus and acrosome [4], and it is also possible that sperm concentration in an ejaculate influences sperm shape and dimensions and, as a result, motility characteristics and fertilizing ability [4,7,8]. Some authors suggest a correlation between the morphometric characteristics of spermatozoa and sperm concentration in the ejaculates of stallions [9], dogs [10], and boars [4,6,11,12]. Some studies have indicated an existence of an association between the sperm head morphometry and fertility in bulls [1], horses [13], humans [2,14,15], boars [16,17], and canines [18]. The frequency of an incidence of

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http://dx.doi.org/10.1016/j.jevs.2015.01.005

sperm morphologic defects decreases fertility in boars [19,20], stallions [21], bulls [22], bucks and rams [23,24], and humans [25–27].

Male age is one of significant causes of variation in spermatozoa morphology and morphometric dimensions [28,29].

It has been found that sperm dimensions are highly variable in males belonging to different animal species [15,30], breeds within a species [31], and between individuals of one and the same population [5,32]. Spermatozoa head dimensions of males with decreased fertility differ from the head dimensions of spermatozoa of highly fertile men [33,34] and stallions [3,35]. Morphometric measurements of sperm heads (length, width, area, and perimeter) have been significantly higher in the subfertile than fertile stallions [13]. There has been a confirmed association of sperm head dimensions and shape with stallion, ejaculate characteristics within stallion, and date of staining [4,10,12,36]. The variation between stallions was generally greater than variation between ejaculates within stallion [37].

The total length of normal stallion sperm is about 55 μm (head length, 5 μm; middle piece, 8 μm; tail, 42 μm) [38]. The sperm head length and head breadth of clinically normal fertile stallions is 5.88, 5.49, and 5.96 μm, and 2.97, 2.65, and 2.95 μm [9,35,39], respectively. The mean sperm head area of stallions is 13.22, 11.22, and 13.31 μm^2 [9,35,39], respectively. In addition, the mean stallion sperm head perimeter is 14.92, 13.79, and 15.54 μm [9,35,39], respectively. The mean measurements of sperm head length, breadth, area, and perimeter in the subfertile versus fertile stallions are 5.77 versus 5.33 μm, 2.89 versus 2.75 μm, 12.66 versus 11.37 μm^2, and 14.59 versus 13.64 μm, respectively [13].

The present study is an attempt to investigate the sperm morphometric characteristics of Arabian horses in relation to seasons, stallions' age, concentration of spermatozoa in the ejaculates, and stallions fertilizing capacity.

2. Materials and Methods

2.1. Stallions

A total of 15 healthy Arabian stallions (*Equus ferus caballus*), between 6 and 26 years old, were involved in this study during a period of 12 months. These stallions belonged to El-Zahraa Governmental Stud, nearby Cairo, Egypt, and they have been used as sires in the regular natural breeding program of the stud. The fertility of stallions was determined by the percentages of mares (20–26 mares were mated per stallion) that conceived on the first cycle mating by the stallion, retrospectively. The fertility data were collected during two breeding seasons (each season is 7 months).

2.2. Semen Collection and Evaluation

One hundred eighty ejaculate samples were collected from the stallions (12 ejaculates were collected per stallion) while they mounted a mare in estrus using Equine Artificial Vagina kit CSU (Colorado State University Model). Immediately after collection, semen samples were transferred to a well-equipped laboratory and evaluated by conventional methods. The percentages of progressively motile sperm and sperm abnormalities, as well as sperm cell concentration ($\times 10^6$ sperm/mL) were determined objectively using Sperm Vision version 3.5 software (Minitub of America, Inc). Stained slides with nigrosin-eosin solution were prepared for sperm morphometric analysis [40]. A semen sample of 50 μL was mixed with 50 μL of eosin (Eosin G stain, 2% solution; Minitub, Ref. 15405/0025) and 100 μL nigrosin (Nigrosin stain, 4% solution; Minitub, Ref. 15405/0029). Only unstained (living) spermatozoa with clear outline of the head and tail fixed straight were selected. Twenty-five spermatozoa per slide (300 spermatozoa per individual stallion) were measured with a total of 4,500 spermatozoa from all ejaculates. Mensuration was carried out with an eye-piece micrometer (Filar balloted; Bausch and Lomb, Lancaster, PA) which was calibrated with a stage micrometer scale. Every pixel of the micrometer scale represented 0.085 μm when using an oil immersion lens ×1,000. The following sperm morphometric measurements were taken: sperm head length—the distance between the point of head junction with the midpiece and the furthest point in the front part of the sperm head, sperm head maximum breadth—the distance between the furthest points located on the sperm head perimeter, measured perpendicularly to the long axis of the sperm head, sperm head base breadth—the distance between the two points located at the head base where the head is connected with the midpiece, acrosome length—the distance between the point at the middle of acrosome base and the furthest point in the front part of the sperm head, midpiece length—the distance between the midpiece junction with the head and the beginning of the principal piece of the tail, midpiece breadth—the distance between the two sides of the midpiece at its midpoint, tail length—the distance measured along the long axis of the tail and limited by the point where the head is connected with the midpiece and the point of tail's end, tail breadth—the distance between the two sides of the tail at its midpoint, sperm head area—area limited by a curve running along the perimeter of the sperm head, and sperm head perimeter—length of the boundary limiting the sperm head (adapted from Banaszewska et al). The head

Table 1
Sperm mensuration traits (μm) in the fertile Arabian horse.

Sperm Mensuration Traits	Mean ± SEM	Range
Sperm head length (μm)	5.96 ± 0.004	5.95–5.96
Sperm head maximum breadth (μm)	3.06 ± 0.004	3.05–3.14
Sperm head base breadth (μm)	1.40 ± 0.003	1.38–1.49
Sperm acrosome length (μm)	3.81 ± 0.005	3.77–3.91
Sperm midpiece length (μm)	10.17 ± 0.008	10.08–10.22
Sperm midpiece breadth (μm)	0.67 ± 0.002	0.72–0.66
Sperm tail length (μm)	48.88 ± 0.022	48.52–49.08
Sperm tail breadth (μm)	0.48 ± 0.002	0.43–0.51
Sperm head area (μm^2)	14.33 ± 0.022	14.05–14.54
Sperm head perimeter (μm)	14.16 ± 0.009	13.98–14.46

Abbreviation: SEM, standard error of the mean.
Number of ejaculates = 180.