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Original Research

Evaluation of Sperm Characteristics in Caspian Stallions Using Computer-Assisted Sperm Analysis

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ABSTRACT

Knowledge of reference values for seminal parameters is a prerequisite for stallion breeding management. The present study was conducted to determine seminal characteristics of Caspian stallions. Fifty-two ejaculates were collected from seven healthy Caspian stallions (age: 8–16 years old) during the breeding season. Thirty-five ejaculates (five ejaculates per stallion) were used for calculation of mean values of seminal characteristics. After evaluation of pH and osmolality in initial collections, the semen was analyzed for volume, motility, and some morphometric parameters using computer-assisted sperm analysis (CASA). The overall mean ± standard deviation values for volume of ejaculate, concentration, total motility, progressive motility (PMS), curvilinear velocity (VCL), straight-line velocity (VSL), average path velocity, straightness (STR), linearity (LIN), amplitude of lateral head displacement, beat cross frequency, elongation, and area were 19.89 ± 10.65 mL, 234.82 ± 143.14 × 10^6 sperm/mL, 87.06 ± 7.62%, 44.54 ± 14.65%, 189.33 ± 30.64 μm/s, 82.88 ± 11.49 μm/s, 113.52 ± 13.02 μm/s, 71.20 ± 8.38%, 45.57 ± 9.12%, 6.68 ± 1.05 μm, 34.15 ± 9.37 Hz, 61.77 ± 4.85%, and 4.70 ± 1.25 μm^2, respectively. There were significant differences among stallions in sperm velocity and head-related parameters ($P < .05$). Sperms with greater elongation and lower area had greater PMS, VSL, STR and LIN ($P < .05$), and lower VCL ($P < .05$). In conclusion, the present study provided the mean values for seminal parameters of Caspian stallions. Additionally, the results revealed that sperms with more elongated heads had greater straight velocity and were, in turn, more progressive.

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1. Introduction

The Caspian miniature horse is one of the oldest horse breeds in the world, which was originated from the southern coast of Caspian Sea and northern areas of Alborz Mountains, and is believed to be the first horse to be used in the Middle East [1]. Interestingly, it was rediscovered in 1965 [2]. This breed is considered critically endangered with the registered population of approximately 150 horses in Iran [2]. This small population resulted in inbreeding [3,4], which in turn could adversely impact fertility in this breed [5–7]. Therefore, the limited population of Caspian horses requires intensive attention in terms of breeding management. Undoubtedly, reproductive management of stallions would be one of the cornerstones of breeding management programs for preservation of Caspian horses. In this regard, the variation in sperm characteristics of stallions needs to be assessed to define the standards for selection of a stallion based on his sperm characteristics [8].

The present study was conducted to determine the seminal characteristics of Caspian stallions including computer-assisted sperm analysis (CASA), which provides the opportunity to analyze several seminal parameters

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including semen concentration, and seminal velocity and morphometric characteristics avoiding the subjective optical microscopic evaluation of semen and the resultant variability in sperm analysis, although the appropriate settings of CASA play a significant role in the accuracy of the outcomes [9,10].

2. Materials and Methods

2.1. Animals

This study was a cross-sectional study, which was performed during the breeding seasons (March–July) of 2011 and 2012 at the Animal Breeding Center, Karaj, Iran (latitude: 35° 46′ N; longitude: 50° 58′ E). Seven healthy Caspian stallions (age: 8–16 years old) received alfalfa hay and concentrate since 3 months before the beginning of the study and had free access to water.

2.2. Semen Collection and Evaluation

Before the commencement of semen collection for analysis, semen was collected daily from each stallion for five consecutive days for excluding of extragonadal sperm. Semen was collected twice a week using Missouri artificial vagina (IMV, France) with in-line filter for collecting the gel-free fraction. The period of sexual rest was not more than 4 days before the semen collection. In total, 52 ejaculates were collected from the stallions (7, 6, 7, 13, 5, 8, and 6 ejaculates from stallions S1, S2, S3, S4, S5, S6, and S7, respectively). Then, semen was diluted with prewarmed (37°C) INRA 96 extender (IMV, France) at the ratio of 1:1. After equilibration for 30 minutes at room temperature, the semen concentration was estimated using hemocytometer. Accordingly, the semen was further diluted to obtain the concentration of 25−50 × 10^6 sperm/mL to be analyzed by CASA (HTM-CEROS version 12.3; Hamilton-Thorne Research, Danvers, MA). Prewarmed Leja slide (20 μm in depth; Leja Amsterdam, the Netherlands) was loaded with extended semen. Four microscopic fields were randomly selected and scanned five times. The CASA analysis was setup at 60 Hz (frame per second), 45 frames, minimum contrast of 70, and minimum cell size of four pix. Cells were considered progressive with at least 50 μm/s average path velocity (VAP) and 75% straightness (STR). Spermatozoa with VAP of <20 μm/s were considered immotile. Total motility, progressive motility (PMS), curvilinear velocity (VCL), straight-line velocity (VSL), VAP, linearity (LIN; calculated by dividing VSL to VCL), STR (calculated by dividing VSL to VAP), amplitude of lateral head displacement (ALH), beat cross frequency (BCF), elongation (calculated by dividing the minor axis of sperm head to the major axis of sperm head multiplied by 100; ELON), and area (calculated by multiplying the minor axis of sperm head by the major axis of sperm head).

2.3. Statistical Analysis

Initially, data sets were tested for normal distribution using Kolmogorov–Smirnov test (UNIVARIATE procedure). Positively skewed data were normalized using log transformation. The intra- and inter-stallion variation in volume and concentration of ejaculate, VCL, VSL, VAP, STR, LIN, ALH, BCF, ELON, and area was analyzed using generalized linear model (GLM) procedure. The intra- and inter-stallion variation in total motility and PMS was analyzed using GENMOD procedure including function link logit in the model. LSMEANS statement was used to perform multiple comparisons among stallions. The relationships between sperm head-related variables including ELON and area and sperm velocity parameters including PMS, VCL, VSL, STR, and LIN were analyzed using correlation and regression procedures. All analyses were conducted in SAS [11].

3. Results

All semen collected in this study contained gel. The mean ± standard deviation (SD) for osmolarity and pH of collected semen was 292.71 ± 2.81 mOsm/kg H_2O and 7.28 ± 0.21, respectively.

Values (mean ± SD) for volume of ejaculate, concentration, total motility, PMS, VCL, VSL, VAP, STR, LIN, ALH, BCF, ELON, and area were 19.89 ± 10.65 mL, 234.82 ± 143.14 × 10^6 sperm/mL, 87.06 ± 7.62%, 44.54 ± 14.65%, 189.33 ± 30.64 μm/s, 82.88 ± 11.49 μm/s, 113.52 ± 13.02 μm/s, 71.20 ± 8.38%, 45.57 ± 9.12%, 6.68 ± 1.05 μm, 34.15 ± 9.37 Hz, 61.77 ± 4.85%, and 4.70 ± 1.25 μm^2, respectively (Table 1). There were intra-stallion variation in total motility and PMS ($P < .05$); however, no intra-stallion variation was observed in VCL, VSL, VAP, STR, LIN, ALH, BCF, ELON, and area ($P > .05$). There were significant differences among stallions in some of the seminal parameters ($P < .05$; Table 1).

Elongation was positively correlated with PMS ($r = 0.59$; $P < .0001$), VSL ($r = 0.32$; $P < .05$), STR ($r = 0.64$; $P < .0001$), and LIN ($r = 0.69$; $P < .0001$) and negatively correlated with VCL ($r = -0.55$; $P < .0001$; Fig. 1). Area was positively correlated with VCL ($r = 0.52$; $P < .0001$) and negatively correlated with PMS ($r = -0.62$; $P < .0001$), VSL ($r = -0.40$; $P < .01$), STR ($r = 0.70$; $P < .0001$), and LIN ($r = 0.72$; $P < .0001$; Fig. 2).

4. Discussion

The objective of this study was to investigate the semen characteristics in Caspian stallions. In the present study, semen in Caspian stallions contained gel, in agreement with the report by Tajik et al [12]. However, Dastafshan and Dordari [13] reported that in Caspian miniature horses, the semen is free of gel fraction. The mean gel-free volume and concentration of semen in Caspian stallions were comparable with those reported in other breeds [14,15]. Motility parameters including VAP, VCL, and VSL in Caspian stallions (114.48 ± 11.51, 188.62 ± 26.61, and 84.97 ± 11.81, respectively) seemed to be similar to those reported in horses (128.44 ± 27.21, 224.44 ± 39.23, 78.06 ± 17.08, respectively) in the study by Albrizio et al [16], who used the same CASA system, settings, and chambers as we did.

The present study revealed significant individual variation among stallions in sperm velocity parameters, which agrees with findings of other studies in equine [17], caprine [18], and ovine [19]. Moreover, there was significant

Table 1
Seminal parameters of fresh ejaculates of seven different Caspian stallions.

Parameter	Stallions							Overall
	S1	S2	S3	S4	S5	S6	S7	
Volume (mL)	13.20 ± 4.97	28.40 ± 13.15	15.60 ± 5.98	18.20 ± 5.02	13.20 ± 2.17	24.40 ± 12.30	26.20 ± 15.66	19.89 ± 10.65
Concentration ($\times 10^6$/mL)	339.08 ± 201.05	147.58 ± 73.89	379.48 ± 132.19	237.02 ± 110.36	153.15 ± 130.84	242.41 ± 101.54	145.02 ± 58.65	234.82 ± 143.14
Total motility (%)	92.60 ± 5.94[a]	90.40 ± 6.39[bg]	80.40 ± 7.09[c]	89.00 ± 4.64[dg]	82.80 ± 11.37[e]	84.80 ± 5.76[f]	89.40 ± 5.94[g]	87.06 ± 7.62
PMS (%)	46.20 ± 10.03[a]	32.60 ± 7.57[bg]	53.20 ± 7.40[c]	61.60 ± 7.02[d]	28.40 ± 6.09[e]	57.60 ± 9.45[f]	32.20 ± 7.69[g]	44.54 ± 14.65
VCL (μm/s)	177.94 ± 19.89[a]	243.76 ± 27.18[b]	185.56 ± 34.05[a]	163.94 ± 4.64[a]	197.82 ± 11.69[a]	174.34 ± 18.52[a]	181.96 ± 7.47[a]	189.33 ± 30.64
VSL (μm/s)	73.34 ± 7.93[a]	85.28 ± 10.40[a]	92.34 ± 12.51[b]	91.50 ± 4.77[b]	73.92 ± 6.66[a]	91.16 ± 8.10[b]	72.60 ± 4.34[a]	82.88 ± 11.49
VAP (μm/s)	98.36 ± 12.10[a]	132.10 ± 12.32[b]	115.38 ± 16.76[ab]	110.66 ± 2.42[a]	114.90 ± 6.42[ab]	113.86 ± 4.86[ab]	109.38 ± 5.71[a]	113.52 ± 13.02
STR (%)	71.80 ± 5.76[ab]	62.60 ± 5.03[b]	77.20 ± 5.76[ac]	80.20 ± 4.09[c]	63.20 ± 4.76[b]	78.40 ± 5.18[ac]	65.00 ± 2.55[b]	71.20 ± 8.38
LIN (%)	41.40 ± 4.16[ac]	35.80 ± 5.02[ab]	50.20 ± 5.97[cd]	56.60 ± 5.27[de]	39.40 ± 5.41[a]	54.20 ± 8.87[e]	41.40 ± 3.29[abc]	45.57 ± 9.12
ALH (μm)	6.78 ± 0.38[ab]	8.28 ± 1.16[a]	6.80 ± 0.89[ab]	6.28 ± 0.44[b]	6.58 ± 0.52[b]	6.14 ± 1.28[b]	5.88 ± 0.58[b]	6.68 ± 1.05
BCF (Hz)	35.44 ± 4.04[abc]	39.60 ± 4.73[a]	34.82 ± 11.17[abc]	24.32 ± 4.77[b]	39.30 ± 4.57[abc]	24.92 ± 11.96[ab]	40.68 ± 6.53[c]	34.15 ± 9.37
ELON (%)	61.00 ± 2.65[abc]	58.00 ± 5.39[ac]	63.40 ± 3.78[abc]	67.60 ± 1.82[b]	57.20 ± 2.49[a]	65.00 ± 4.64[bc]	60.20 ± 3.49[ac]	61.77 ± 4.85
Area (μm^2)	5.24 ± 1.04[a]	5.88 ± 0.71[a]	4.34 ± 1.29[ab]	3.32 ± 0.19[b]	5.30 ± 1.19[a]	3.44 ± 0.30[b]	5.38 ± 0.88[a]	4.70 ± 1.25

Abbreviations: ALH, amplitude of lateral head; BCF, beat cross frequency; ELON, elongation; LIN, linearity; PMS, progressive motility; STR, straightness; VAP, average path velocity; VCL, curvilinear velocity; VSL, straight-line velocity.
Data are presented as mean ± standard deviation.
[a,b,c,d,e,f,g]Values with different superscript letters within rows differ ($P < .05$).

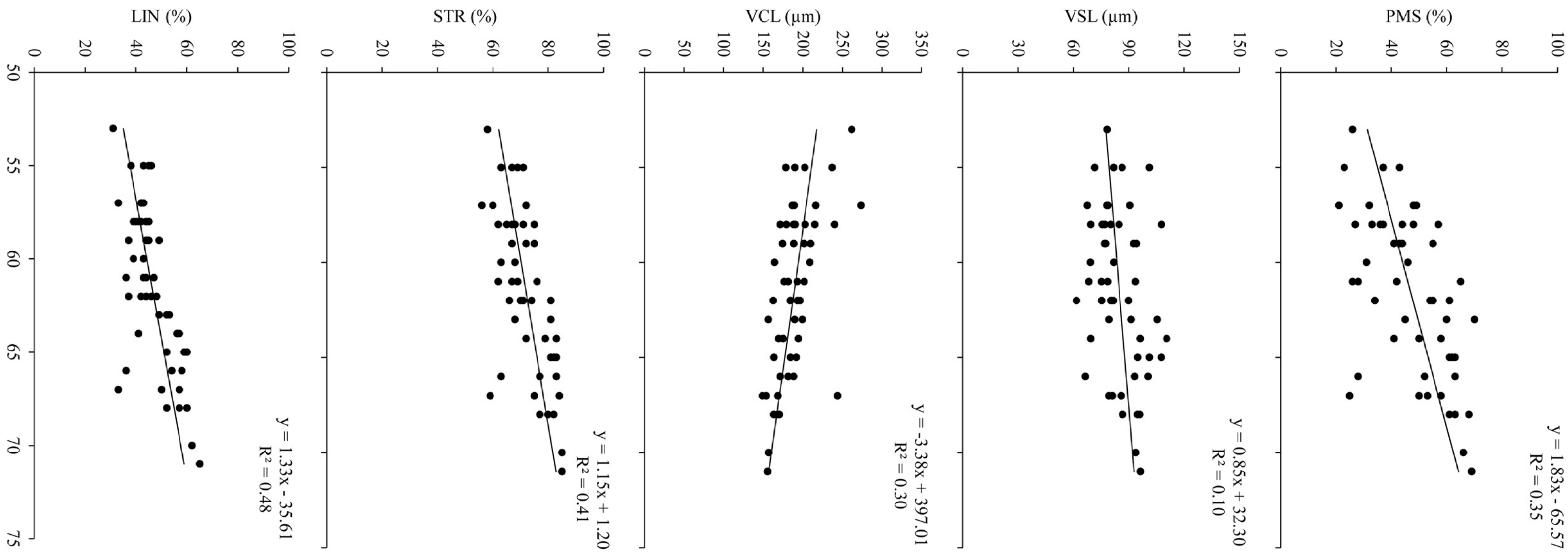

Fig. 1. Correlation of ELON with PMS, VSL, VCL, STR, and LIN in Caspian stallions. ELON, elongation; LIN, linearity; PMS, progressive motility; STR, straightness; VCL, curvilinear velocity; VSL, straight-line velocity.

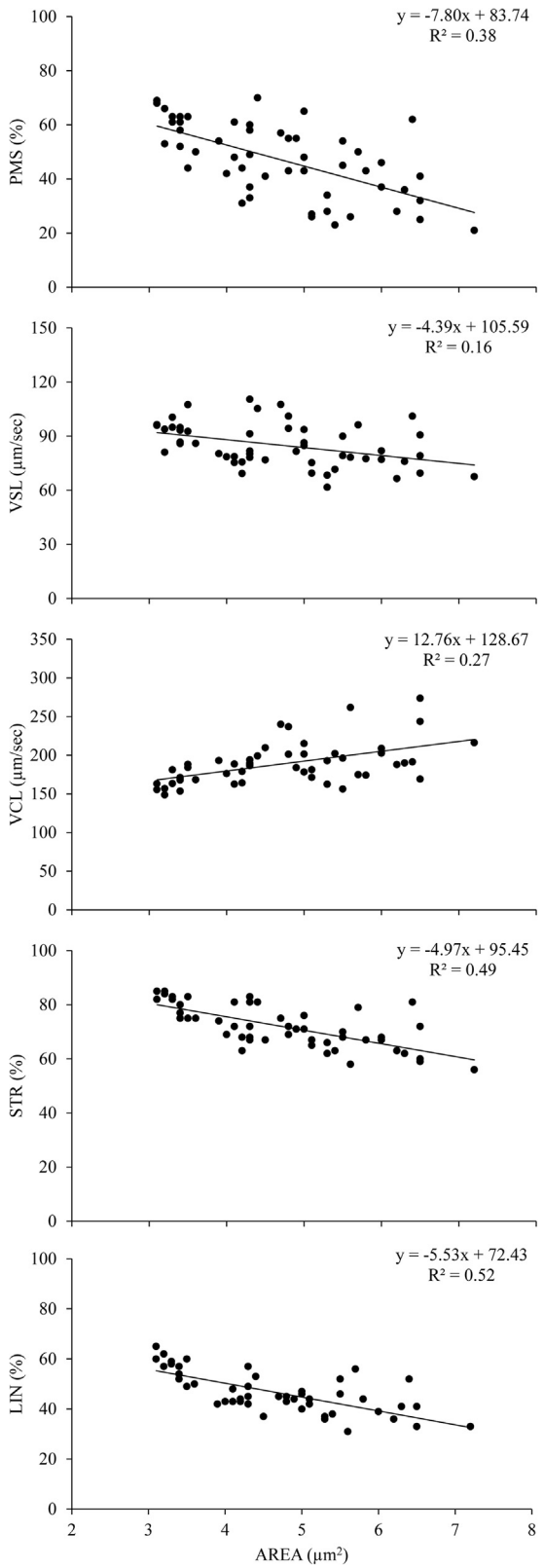

Fig. 2. Correlation of area with PMS, VSL, VCL, STR, and LIN in Caspian stallions. LIN, linearity; PMS, progressive motility; STR, straightness; VAP, average path velocity; VCL, curvilinear velocity; VSL, straight-line velocity.

difference among Caspian stallions in sperm head parameters including elongation and area. Sperms with greater elongation and lower area had greater PMS, VSL, STR, and LIN. In contrast, sperms with lower elongation and greater area had greater VCL. Similarly, individual variation in morphometric measures of sperm head among males has been reported in animals [20], birds [21], and fish [22]. Individual variation among stallions with respect to fertility has also been indicated [23]. Sperm swimming velocity has been indicated as a major determinant of the male fertilization success in a wide range of species including cattle [24], red deer [25], pigs [26], humans [27], rats [28], domestic fowl [29,30], Atlantic salmon [31], and sea urchin [32]. Subfertile stallions had wider sperm heads and higher sperm head dimensions compared with fertile stallions [33]. Malo et al [20] found positive correlation between sperm head length and overall sperm velocity in red deer. Furthermore, the ratio of sperm head length to sperm head width was correlated with overall velocity and VAP of sperm [20]. It has been inferred that more elongated sperms with higher ratio of head length to head width would be more hydrodynamically efficient [20] with reduced drag [34] and, in turn, would pass through the female genital tract with more velocity and STR. Therefore, possible positive relationship among velocity parameters [35], morphometric measures, and fertility remained to be investigated in Caspian stallions.

In conclusion, the present study demonstrated mean values for seminal parameters of Caspian stallions emphasizing the presence of individual variation among stallions and correlation between sperm head-related and velocity parameters.

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