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A Study of Variation in Semen Quality of Dromedary Camel Bulls

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ABSTRACT

The aim of the present study was to determine the sperm parameters of dromedary camels used for artificial insemination. A total of 200 ejaculates was collected using an artificial vagina from 6 males and throughout 4 years (2012/2013; 2013/2014; 2014/2015 and 2015/2016). Ejaculates were analyzed according to the following parameters: color (grey, white, white milky), viscosity (liquid, viscous, very viscous), volume (direct observation), mass motility (scale from 0 to 5), viability (eosin/ nigrosin stain), and sperm concentration (Thoma cell). Data were statistically analyzed by the GLM procedure of SAS with three factors (season, month and bulls) and the difference was examined using Duncan test (α = 5%). χ^2 test was used for color and viscosity. The Results showed that volume, percentage of viable sperm and total sperm were significantly higher in the three last years compared with the first one. There were also significant monthly changes in semen characteristics with maximum values registered in January and February (winter). However, no significant variation in viscosity was found during months and years. Concentration, viability, mass motility, total sperm and total viable sperm viable were varied significantly between dromedary bulls. This study showed that the effects of climatic parameters (hot temperature, less rainfall) and housing conditions (social isolation, limitation in movement…) could explain the yearly variation in sperm parameters of dromedary camel bulls.

Keywords: Year, month, sperm quality, dromedary camel

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INTRODUCTION

The introduction of assisted reproductive technologies (artificial insemination, embryo transfer, etc.) remains one of the most important factors in the improvement of certain production and reproduction parameters in camel species (Skidmore *et al*., 2013; Skidmore and Billah, 2016). However, these biotechnologies are not widely applied as routine methods for these species (Wani *et al*., 2010) and the knowledge in this area is still far from that known in other domestic species. This is due to difficulties in sperm collection, evaluation of its quality given the viscous nature and due to a lack of standard techniques of semen freezing (Tibary and Anouassi, 1997; Skidmore *et al*., 2013).Increasing the benefits of

these techniques depends on a strict selection of good dromedary camel bulls based on an examination of the genital tract, assessment of mating capacity (behaviours and libido) and an evaluation of semen quality.

Sperm quality is extremely important because each male participates in a large number of services throughout the breeding season. Thus, a good evaluation of the quality of the semen of each male is imperative for ensuring the production of valuable offspring for camel and could help identify causes of low fertility. Dromedary camel has seasonal reproductive behavior and the Tunisian breeding season occurs during December to March (Hammadi, 2003). During this period, sperm quality varies and these possible fluctuations are associated with several factors such as (i.e.: breed, age, libido, etc.; El-Bahrawi, 2005; Al-Bulushi *et al.*, 2016), climatic conditions (Yagil and Etzion, 1980; Den, 2008), photoperiod (Musa *et al*., 1993), and other factors of different etiology. All of these factors require careful control to attain better semen quality for artificial insemination. In a previous study (El-Bahrawi, 2005), sperm quality in dromedary camel was found to vary among months, with the sperm quality including sperm volume and motility whiche highest during the middle of the breeding season and lowest in the beginning. It is worth noting that although males show all signs of sexual behavior, but inter-male individual variations were observed for sperm parameters in several species for example, alpaca (Bravo *et al*., 1997), llama (Giuliano et al., 2008), dromedary bulls (El-Bahrawi, 2005; Al-Bulushi *et al*, 2016)and stallion (Dowsett and Knott, 1996). In general, only little information is available about variations in semen quality of dromedary camel. Therefore, the objective of this study was to evaluate the semen of dromedary and to examine the sources of variation (years, months and males) of its quality.

MATERIALS AND METHODS

Animals and management: this study was carried out during Tunisian breeding season, starting from December to March, throughout 4 consecutive years (2012/2013; 2013/2014; 2014/2015 and 2015/2016)at the Arid Lands Institute's experimental station in Médenine, Tunisia (33° 30′ N, 10° 40′ E and 18 m above sea level).Six clinically healthy male dromedary camels, ranging in age from 6 to 17 years, with a mean body weight of 545 ± 63 kg and good body condition score (3.8 ± 0.7 arbitrary units from 0 to 5, according to Faye et al., 2001), were used in this study. The camels were housed in individual boxes (5 ×3 m with 3-m-high solid walls), fed with 5 kg oat hay and 3 kg concentrate supplement and they were watered once every 2 days.

Semen collection and evaluation: Bulls were used for semen collection twice weekly. Semen was collected using bull artificial vagina (30 cm long, 5 cm internal diameter) and a female maintained in couched position. After collection, ejaculates were immediately placed in water bath at 36 °C and were subjected to the following tests as described by Monaco et al. (2015). In brief, color (grey, white or white milky) and ejaculate volume (from a graduated tube; ml) were directly evaluated after semen collection. Viscosity was assessed as liquid, viscous, very viscous. Gross activity or mass motility (scale: 0 – 5) was examined under a phase contrast microscopy by placing a drop of semen on a pre-warmed slide. The viable number of sperm viable and

concentration were determined using eosin/nigrosine stain and a thoma cell after dilution in NaCl (3%), respectively.

Statistical analyses: Data was normally distributed (Kolmogorov-Simirnov test, P> 0.05). Sperm parameters data were statistically analyzed by the GLM procedure of SAS (SAS v 9.3; 2012) with three factors (season, month and bulls), and the interaction between those variable (year x month). The difference between these factors was examined by Duncan test. X^2 test was used for color and viscosity. The the p-level was set at 0.05. All data were expressed as mean and standard deviation.

RESULTS

***Mean ejaculate characteristics*:** Results of semen parameters are reported in table 1. During the mating session, the ejaculate volume was 12.6 ± 0.6 ml. The concentration of spermatozoa as 450.6 ± 28.9 x 10^6SPZ/ml, with a percentage of live spermatozoa was 47.8 ± 1.3% and sperm mass motility of 2.4 ± 0.1.

Table 1: Semen characteristics of dromedary camel bulls

Variables	Mean ± SD	Min	Max
Volume (ml)	12.6 ± 0.6	1.0	42.5
Concentration (10^6 SPZ/ml)	450.6 ± 28.9	6.0	2360.0
Viability (%)	47.8 ± 1.3	0.0	88.8
Mass motility (0 – 5)	2.4 ± 0.1	0.0	5.0
Total SPZ (10^6)	5122.4 ±445.4	90.0	50464.7
Total SPZ viable (10^6)	2602.0 ± 239.8	0.0	21912.5

Total SPZ: total number of spermatozoa per ejaculate.
Total viable SPZ: total number of viable sperm per ejaculate.

Yearly and monthly changes of semen parameters: The variation of semen parameters between years and months are summarized in Table (2). The ejaculate volume increased significantly between years (P<0.0001) and months (P=0.0032), with maximum values recorded throughout the last 3 years and during the months January, February and March. The volume show an upward trend (P=0.0722) within the same year and between months of collection.

Furthermore, no significant difference of the mass motility was observed during the 4 years (P=0.2552) and months (P = 0.0468). However, the interaction between years and months had a significant effect (P<0.0001) on mass motility.

Then, the percentage of sperm viable, concentration, total number of spermatozoa and the total number of viable spermatozoa did not differ significantly among months. Significant changes were observed for both viability (P = 0.0002) and total number of viable spermatozoa per ejaculate (P = 0.0383) between the four studied years, while no significant difference was found for the concentration. A highly significant effect of year x month interaction was noticed for the following parameters: viability, concentration, total number of spermatozoa and total number of viable spermatozoa per ejaculate.

Table 2: Variation of sperm parameters between years (2012/2013; 2013/2014; 2014/2015 and 2015/2016) and months of collection (December, January, Februray, March) in dromedary camels

Variables	Month	Year 1 2012/2013	Year2 2013/2014	Year 3 2014/2015	Year 4 2015/2016	Mean ± SD
Volume (ml)	December	1.9 ± 0.3^{Bb}	UND	UND	8.0 ± 1.3^{Ba}	4.7 ± 1.1^{b}
	January	3.4 ± 1.0^{Bb}	11.8 ± 3.1^{Ba}	11.3 ± 1.6^{Ba}	13.0 ± 2.8^{ABa}	10.8 ± 1.2^{a}
	February	4.5 ± 0.6^{ABc}	15.0 ± 1.3^{ABb}	19.5 ± 1.8^{Aa}	13.4 ± 1.8^{ABb}	13.8 ± 0.9^{a}
	March	6.9 ± 1.0^{Ab}	20.5 ± 2.3^{Aa}	14.8 ± 1.7^{Ba}	18.4 ± 3.5^{Aa}	14.1 ± 1.2^{a}
	Mean ± SD	5.0 ± 0.6^{b}	15.4 ± 1.2^{a}	14.8 ± 1.0^{a}	14.3 ± 1.5^{a}	
Mass motility (0 – 5)	December	1.3 ± 0.3^{B}	UND	UND	2.5 ± 0.7^{A}	1.8 ± 0.4^{b}
	January	1.7 ± 0.4^{ABb}	2.4 ± 0.3^{ab}	2.3 ± 0.3^{Bab}	3.3 ± 0.3^{Aa}	2.5 ± 0.2^{a}
	Februay	2.2 ± 0.3^{ABb}	2.4 ± 0.2^{b}	3.2 ± 0.2^{Aa}	2.3 ± 0.3^{Ab}	2.5 ± 0.1^{a}
	March	2.7 ± 0.3^{Aa}	1.9 ± 0.3^{bc}	2.5 ± 0.3^{ABab}	1.2 ± 0.2^{Bc}	2.2 ± 0.2^{ab}
	Mean ± SD	2.2 ± 0.2	2.3 ± 0.1	2.6 ± 0.2	2.3 ± 0.2	
Concentration (10^6 SPZ/ml)	December	593.5 ± 129.9^{A}	UND	UND	527.6 ± 138.4^{AB}	567.1 ± 91.0
	January	218.5 ± 45.8^{Bb}	512.5 ± 130.5^{ab}	624.2 ± 97.6^{a}	713.7 ± 202.4^{Aa}	554.1 ± 67.3
	Februay	228.2 ± 25.9^{Bb}	386.7 ± 44.2^{b}	552.4 ± 109.3^{a}	336.2 ± 109.4^{ABb}	389.6 ± 38.5
	March	660.6 ± 100.9^{Aa}	395.8 ± 61.5^{a}	419.9 ± 102.8^{a}	114.1 ± 39.5^{Bb}	425.8 ± 54.0
	Mean ± SD	457.9 ± 55.8^{ab}	415.2 ± 39.7^{ab}	522.1 ± 60.1^{a}	370.7 ± 71.3^{b}	
Viability (%)	December	24.3 ± 5.3^{Bb}	UND	UND	72.8 ± 3.6^{Aa}	46.4 ± 8.3
	January	26.5 ± 4.8^{Bb}	54.8 ± 3.5^{Aa}	49.3 ± 4.1^{a}	61.2 ± 8.1^{ABa}	49.4 ± 3.0
	Februay	37.3 ± 4.2^{Bb}	45.2 ± 2.6^{Bab}	50.5 ± 3.1^{a}	46.2 ± 5.7^{BCab}	45.2 ± 1.8
	March	57.5 ± 4.4^{Aa}	40.6 ± 5.1^{Bb}	51.1 ± 4.4^{ab}	42.4 ± 2.9^{Cb}	49.8 ± 2.4
	Mean ± SD	42.4 ± 3.2^{b}	46.4 ± 2.1^{ab}	50.4 ± 2.3^{a}	51.7 ± 3.2^{a}	
Total SPZ (10^6)	December	1213.7 ± 352.2^{Bb}	UND	UND	3859.6 ± 1106.6^{a}	2272.1 ± 625.5^{b}
	January	904.2 ± 391.9^{Bb}	4356.9 ± 1392.8^{Bab}	6574.6 ± 941.0^{ABab}	10331.6 ± 5330.1^{a}	5951.0 ± 1213.0^{a}
	Februay	1033.7 ± 178.9^{Bc}	6075.8 ± 1030.7^{ABab}	9583.3 ± 2076.5^{Aa}	4226.5 ± 1426.1^{bc}	5695.2 ± 768.6^{a}
	March	4469.3 ± 1035.8^{Ab}	8268.6 ± 1646.8^{Aa}	4140.3 ± 629.4^{Bb}	1995.6 ± 855.3^{b}	4379.1 ± 527.7^{ab}
	Mean ± SD	2488.2 ± 508.3^{b}	6129.9 ± 759.1^{a}	6453.4 ± 759.4^{a}	4888.0 ± 1445.3^{a}	
Total SPZ viables (10^6)	December	300.7 ± 102.8^{Bb}	UND	UND	2868.3 ± 934.5^{ABa}	1327.7 ± 543.8
	January	306.4 ± 156.9^{Bb}	2562.4 ± 853.8^{ab}	2994.1 ± 490.2^{Bab}	5022.3 ± 1816.8^{Aa}	2829.7 ± 482.6
	Februay	389.9 ± 70.7^{Bc}	2763.9 ± 531.0^{b}	5492.0 ± 1396.3^{Aa}	1788.7 ± 576.0^{Bbc}	2833.3 ± 459.5
	March	2628.5 ± 627.0^{Aab}	3692.7 ± 1212.6^{a}	2533.5 ± 629.4^{Bab}	951.3 ± 459.1^{Bb}	2405.3 ± 358.8
	Mean ± SD	1310.2 ± 312.8^{c}	2898.9 ± 425.8^{ab}	3535.7 ± 524.2^{a}	2324.8 ± 528.4^{bc}	

UND: values undetermined
Total SPZ: total number of spermatozoa per ejaculate.
Total viable SPZ: total number of viable sperm per ejaculate.

Table 3: Inter-males (n =6) variation of sperm parameters

	#3	#373	#504	#514	#515	#808
Volume (ml)	11.5 ± 1.4 ^{ab}	12.2 ± 1.8^{ab}	12.9 ± 1.4^{ab}	16.6 ± 1.7^{a}	10.2 ± 1.1^{b}	13.6 ± 1.5^{ab}
Concentration (10^6SPZ/ml)	449.1 ± 123.9^{b}	830.4 ± 96.8^{a}	664.1 ± 68.9^{a}	290.3 ± 34.8^{b}	293.4 ± 33.2^{b}	326.8 ± 60.2^{b}
Viability (%)	47.2 ± 2.5^{ab}	57.2 ± 2.1^{a}	57.0 ± 2.6^{a}	38.5 ± 3.1^{b}	43.0 ± 2.6^{b}	45.5 ± 3.4^{b}
Mass motility (0 à 5)	2.5 ± 0.2b^{c}	3.4 ± 0.2^{a}	3.0 ± 0.2^{ab}	1.9 ± 0.2^{c}	2.1 ± 0.2^{c}	1.9 ± 0.2^{c}
Total SPZ (10^6)	4667.3 ± 1528.7^{bc}	9825.5 ± 1959.1^{a}	7696.9 ± 1267.4^{ab}	4734.9 ± 644.9^{bc}	2757.8 ± 365.4^{c}	3204.0 ± 552.9^{c}
Total SPZ viables (10^6)	2339.0 ± 846.2^{b}	5821.1 ± 1312.8^{a}	4296.6 ± 610.4^{a}	1862.0 ± 292.1^{b}	1239.5 ± 184.2^{b}	1461.4 ± 243.0^{b}

Total SPZ: total number of spermatozoa per ejaculate.
Total viable SPZ: total number of viable sperm per ejaculate.
Between bulls, values with different superscripts within a column differ significantly

The colour (P= 0.1644) and viscosity (P = 0.7761) did not vary between the years (Figure 1). A number of 116 ejaculates amongst 205 were characterized by a white milky color and 110 ejaculates were very viscous.

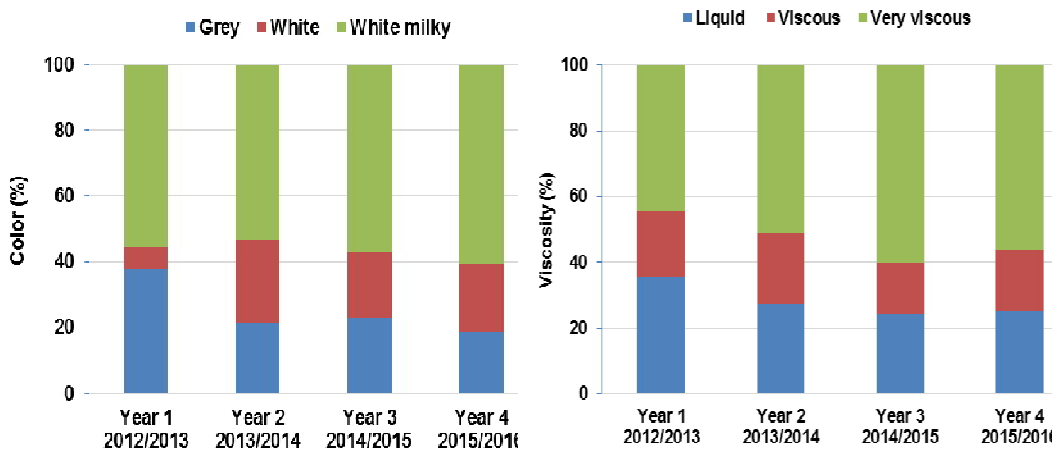

Figure 1. Yearly variation in relative frequency of color and viscosity of dromedary camel semen

Likewise, color and viscosity did not change (P>0.05) between months. During the rutting season, ejaculates of Maghreb dromedary camels were known by a color varying from grey to white milky or creamy (Figure 2), with an abundance of milky white color (more than 50%).

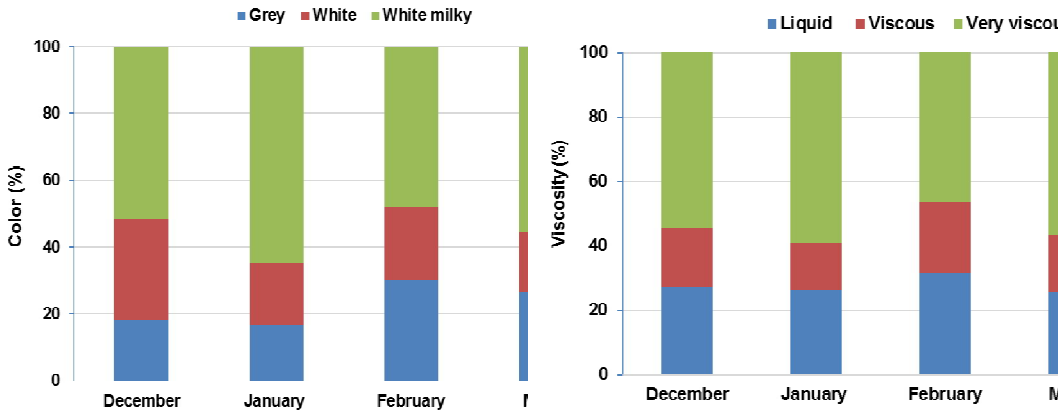

Figure 2. Monthly variation in relative frequency of color and viscosity of dromedary camel semen

***Variation of sperm parameters among camel bulls*:** There was a significant difference between bulls in all studied parameters (Table 2). The volume varied between the six bulls, with camel #514 was distinguished with the highest volume value (16.6 ± 1.7 ml) and the lowest one (10.2 ± 1.1 ml) was recorded for the camel #515. Mass motility varied (F = 9.33, $P<0.0001$) among the six camel bulls. Indeed, the maximum value was recorded in dromedary # 373 followed by # 504, while the minimum value was found in dromedaries # 514 and # 808. The viability, concentration, total number of SPZs and total number of viable SPZs varied ($P<0.0001$) between dromedaries.

Moreover, a highly significant difference ($P<0.0001$) was found among camels in color of ejaculates. The camels #373 and #504 were distinguished from the others by the abundance of the white milky color with a percentage of 88.2% and 86.3%, respectively. Similarly, there was a significant difference in sperm viscosity between males (Figure 3).

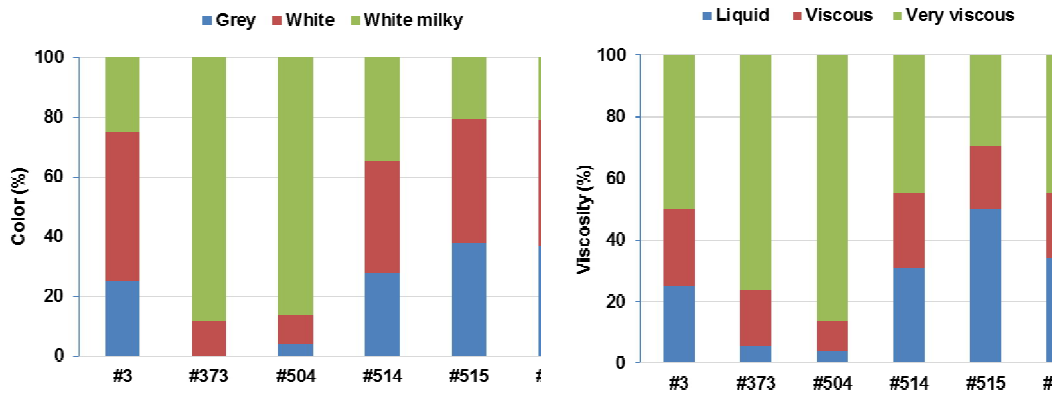

Figure 3: Variations in relative frequencies of color and viscosity of sperm between dromedary came Bulls

DISCUSSION

The present work describes dromedary ejaculate characteristics and examines the variations of sperm quality among years, months and between bulls.

The mean ejaculate volume (12.6 ± 0.6 ml) was comparable to that observed by Al-Eknah *et al*., (2004). Based in previous works, the ejaculate volume varies from 1.2 to 26 ml (Mosaferi *et al*., 2005) and from 1 to 15 ml (Hammadi *et al*., 2008). In our present study, dromedary camel bulls gave an ejaculate with a very large volume, which varies from 1.0 - 42.5ml, producing several doses of semen with a sufficient quality. These deviating results obtained by various researchers may be explained by differences between males in sexual behaviors, libido and mating capacity. The sperm concentration described in our study was similar to that found by El-Bulushi et al.(2016) in dromedary camel (430 ± 60 x 10^6 SPZ/ml) and by Mosaferi et al. (2005) in Bactrian camel (414.8 ± 25.0 ×10^6 SPZ/ml). It was higher to that reported previously in numerous studies (El-Hassanein, 2003; Ziapour et al., 2014). Sperm motility and total sperm are known to be important indicator of the reproductive potential of bulls; they also used as a parameters for breeding soundness evaluation (Hurtgen, 1992). The mass motility in the present study is higher (1.2 ± 1.6) than that cited by Monaco et al. (2015). Furthermore, the percentage of viable spermatozoa varies greatly; it fluctuates from 0.0 to 88.8% with an average of 47.8 ± 1.3%. This value is close to that reported byHassan *et al*. (1995), but it is less than 54.1 ± 22.1% (Monaco *et al*., 2015) and 60.9 ± 19.2% (Hammadi *et al*., 2008). In this study, we evaluate all ejaculates without selecting the best one; this is the reason behind the low percentage of viability.

Sperm parameter varies from year to year and among months of sperm collection. The significant increase in volume, viability, total number of SPZ and total viable SPZ during the last 3 years is explained in part by the implantation of a new strategy of semen collection, with a maximum mating time of 45 min and on the other hand by the sexual arousal of bulls through the exposure to female herds. The maximum values of these parameters (volume, mass quality, viability, total number of SPZ and total viables SPZ) were registered in January and February. However, at the beginning of the breeding season (December), sperm quality remained low. This is probably due to climatic conditions since dromedary camel bulls prefer to mate females during the coldest and the rainiest period of the year (Yagil and Etzion, 1980) and it may also due to the duration of spermatogenesis, which lasts from 30 to 75 days in mammals (França and Russell, 1998; Hess and França, 2007). In this context, El-Bahrawi (2005) reported that camels are able to emit good ejaculates around mi-December or sometimes in January until the end of February. Moreover, in 2003 Hammadi found that mating occurred at the beginning of the season do not induce ovulation and only 1/3 of fertilized females become pregnant.

The results of the present study indicated that semen parameters vary significantly between individual dromedary camel bulls. Compared to the others bulls, males #373 and #504 showed marked superiority in motility, viability, concentration, total number and total viable spermatozoa. This is a comparable to that observed in other species like alpaca (Bravo *et al*., 1997; Vaughan *et al*., 2003) llama (Giuliano *et al*., 2008) and dromedary camel (Al-Bulushi *et al*., 2016). These last authorsreported an inter-male variation

in some semen characteristics (viscosity, percentage of viable intact-acrosomes, dead intact and non-acrosome intact spermatozoa, total motility, progressive motility, path velocity, progressive velocity and track speed). The variation in sperm quality is commonlyencountered in all animal species, and it may be related to various factors such as age, testicular size, plasma testosterone level and accessory sex gland activity. Furthermore, El-Bahrawi (2005) attributed this variability to the effects of race, age and collection method (female vs. dummy). According to Bravo et al. (1997), the variation between males of alpacas is due to the frequency of mating, which negatively affects sperm quality and particularly concentration and percentage of abnormal spermatozoa.

In conclusion, our results demonstrate the existence of considerable variation in semen quality not only among years and months but also between dromedary camel bulls. Ejaculates collected in January and February demonstrated good quality, especially regarding sperm motility, and was more suitable for further program of cryopreservation or artificial insemination.

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