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Reproductive seasonality of male dromedary camels

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A B S T R A C T

Reproductive seasonality has been reported in numerous species, including male dromedary camels, yet investigations into seasonal changes in camel semen quality have yet to be conducted. The aim of this study was to characterise the seasonal changes in camel semen quantity and quality as well as correlate these changes to testis and accessory sex gland morphology, sexual behaviour, libido and environmental factors such as day length and ambient temperature in Oman. Semen was collected twice a month for a year and testicular and accessory sex organ biometry recorded once a month via ultrasonography (n = 8 bulls). Blood samples were collected monthly to assess testosterone levels. Results indicated that testes and accessory sex glands size increased during October-April, peaking with testosterone concentrations during January ($P < 0.05$). The sexual behaviour and libido of camels was also greater during the months of October-April ($P < 0.05$). Attempts to collect semen were 100% successful during November-February. Semen volume, as well as sperm gross activity, concentration, motility, average path velocity and percentage with intact acrosomes were the greatest during January and decreased from May-September ($P < 0.05$). Changes in values for semen variables, testosterone concentrations and sex organ anatomy were also highly correlated with seasonal changes in day length and ambient temperatures. In conclusion, a clearly defined reproductive season was observed in male camels in Oman ranging from December-March, with peak reproductive function occurring during December-January. To increase the success of breeding programs, matings or semen collections should be timed to occur when reproductive function is maximal.

1. Introduction

Seasonality underpins reproduction in most species, with breeding occurring during those times of the year to ensure offspring are born in conditions optimal for survival. Seasonality is primarily mediated by changing photoperiod length, however other environmental factors such as temperature and climate variation and availability of quality feed can also affect seasonal patterns of reproduction. Changing photoperiod length stimulates the pineal gland to secrete the neuro-hormone, melatonin. A decrease in the length of the photoperiod results in an increase in the amount of melatonin secreted, which in turn influences the hypothalamic-reproductive axis causing pulsatile secretions of gonadotrophin releasing hormone (GnRH) and the release of reproductive androgens from the gonads. Seasonality is usually more evident in females, such as in the ewe, where oestrus and ovulation are tightly controlled to occur during several months of the year (Rosa and Bryant, 2003), yet seasonality in males can also occur.

Seasonality in males is often observed by changes to testosterone concentrations, with a peak resulting in increased testes and

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accessory sex gland size and ultimately an increase in sperm quantity and quality. Seasonal changes in semen quality have been observed in males of many species including rams (Ibrahim, 1997), bulls (Koivisto et al., 2009), buffalo (Bhakat et al., 2015), and stallions (Waddington et al., 2017). Seasonality in the male dromedary camel and its influence on reproductive variables has been reported in several studies (Deen et al., 2005; Deen, 2008; El-Harairy and Attia, 2010; Swelum et al., 2018b).

Spermatogenesis in the camel has been reported to continue throughout the year, but there are greater rates during the colder months of the year, such as November to March (Abdel-Raouf et al., 1975; Osman et al., 1979; Tingari et al., 1984; El-Kon et al., 2011). An increase in concentrations of reproductive steroids and gonadotropins during the portion of the year when photoperiod is decreasing is also indicative of a breeding season (Azouz et al., 1992; Tibary and Anouassi, 1997), with variable testosterone concentrations reported as a result of findings in several studies throughout the year (Yagil and Etzion, 1980; Deen et al., 2005; Deen, 2008; El-Kon et al., 2011). Similarly, seasonal changes in testicular biometry and echogenicity of the testicular parenchyma have been observed (Pasha et al., 2011). Differences in the breeding behaviour of male camels throughout the year have also been described (Padalino et al., 2015). In India, the duration of copulation was shorter during the early breeding season (December), increased during the peak season (January to April) and decreased as photoperiod decreased towards the end of the breeding season, with there being similar trends for expression of libido (Deen, 2008). Results from studies in Saudi Arabia, indicate that in the non-breeding season, male camels will not express symptoms of sexual behaviour and even refuse to approach an oestrous female (Swelum et al., 2018a,b). Even though there are these reports of a seasonal loss of libido in male dromedary camels, pregnancies following natural matings in the non-breeding season have still occurred (Vyas et al., 2004), indicating that perhaps the quantity of spermatozoa produced during the non-breeding season is still adequate for fertilisation to occur during natural breeding with continuance throughout the year.

From these previously described studies, we hypothesised that the dromedary camel has a distinct seasonal profile in the amount of semen produced and changes to testicular function, however the quality of semen produced and the changes to accessory sex gland size observed via ultrasonography have yet to be reported. Furthermore, using advanced objective measures of semen assessment such as computer assisted sperm analysis (CASA) and correlating these measurements with changes in testosterone concentration and seasonal weather conditions (i.e., ambient temperature) has yet to be used to describe seasonality in dromedary camels in the Gulf regions of the Middle Eastern countries. As such, the present study was conducted to characterise the seasonal changes in; testis, prostate and bulbourethral glands (BUG) morphology, sexual behaviour and libido as well as to establish reference values for semen characteristics during different seasons in Omani male dromedary camels.

2. Materials and methods

2.1. Study area climate and animals

This study was conducted between January and December (2015) at the Animal Research Center, Royal Camel Corps, Muscat, Sultanate of Oman. The Climate is subtropical, hot and humid in the summer (May to October) and cool in the winter (November to April). There is an annual rainfall between 20 and 300 mm/year during the middle to the end of the winter (FAO, 2009). The meteorological data on mean monthly variables for the study period were obtained from the local meteorological centre (Directorate General of Meteorology, Public Authority for Civil Aviation, Muscat, Oman).

Eight male dromedary camels aged 10–14 years, with a history of normal fertility in natural breeding programs were selected for this study. These animals were kept in individual pens and had free access to water, were fed fresh green grass/dry fodder, and were also fed commercial concentrate pellets supplemented with minerals daily. The experimental animals were not used for natural mating during the study period and were trained for semen collection using an artificial vagina (AV).

2.2. Experimental design

This study was conducted to characterize seasonal changes on the; morphology of testes and accessory sex organs (prostate and BUG), sexual behaviour, libido and sperm quality in male dromedary camels. The size of the genital organs (testes, prostate and BUG) and testosterone profiles were recorded once a month in the eight male dromedary camels from January to December. Semen collection was attempted twice a month at weekly intervals throughout the study period. The sexual behaviour and libido of each camel during the period of semen collection were examined as well as the ejaculate volume, concentration, morphology, kinematics and membrane status of spermatozoa.

2.3. Testicular volume

Testicular length (TL) and width (TW) were measured by calipers. Testicular volume (TV) was determined using the mathematical formula of the prolate sphere using procedures that were previously described (Morrill et al., 1972; Watson-Whitmyre and Stetson, 1985). The total volume of semen was the average of both testes.

2.4. Ultrasonographic measurement of prostate and bulbourethral glands

Ultrasonographic evaluations were conducted using a Mindray M5 portable veterinary ultrasonic machine equipped with 5–8 MHz linear transducer (Mindray, China). The experimental animals were restrained in suitably designed crates in standing position. The prostate and BUG were examined using transrectal ultrasonography and dimensions of these were determined using previously described procedures (Derar et al., 2012). The body of the prostate and BUG were ultrasonically assessed, and the images were collected from the monitor of the ultrasonic device and sizes of these organs were quantified based on the plane of the maximum diameter of the testes. The size of the BUG was calculated to be the average of both BUG.

2.5. Radioimmunoassay

Blood samples (10 mL) were collected prior to each ultrasonographic session, by jugular venepuncture, into heparinized tubes. Plasma was separated by centrifugation at 3000 rpm for 15 min at 4 °C and stored at −20 °C until assessment. Plasma testosterone concentration was quantified using a commercially available RIA kit (Testosterone RIA, DE4386, Demeditec Diagnostics GmbH, Kiel, Germany). The intra- and inter-assay coefficients of variations were 8.1% and 10.8%, respectively. The minimum detection limit for testosterone was 0.03 ng/dL.

2.6. Semen collection

Semen was collected as described previously (Al-Bulushi et al., 2018) using an AV that was developed for semen collections from bulls (30 cm length and 5 cm internal diameter). A smooth latex liner was mounted and fixed at both ends of the AV and a transparent graduated glass water-jacketed semen collection vessel was attached to the apex of the internal latex liner. The inner chamber of the AV was filled with water (45 to 48 °C) to give an internal AV temperature of 41 to 42 °C. The water-jacketed semen collection vessel was then filled with warm water (37 °C) and the inner surface of the AV was lubricated. Prior to semen collection, male camels were exposed to a sexually receptive female for a period of 10 min, after which the camel was allowed to approach and mount a female camel restrained in sternal recumbency. The technician positioned on the left side of the female then grasped the male's prepuce, cleaned the preputial orifice and directed the erect penis into the AV for copulation. During copulation, the pressure sensation of penis penetration into the cervix was imitated manually by holding the latex liner between the AV and semen collection vessel.

2.7. Classification of sexual behaviour and libido

The following sexual behaviours; sniffing the female, flehmen, grinding of teeth/whistling, yawning, restlessness, urination, standing with spread hind legs, poll gland secretion, neck rubbing, blathering, and dulla were recorded during the initial "teasing" period as described previously (Fatnassi et al., 2014). Sexual behaviour was scored from 1 to 5: 1 (poor), not expressing any signs of sexual behaviour; 2 (fair), expressing relatively infrequent sniffing and flehmen responses; 3 (good), expressing sniffing, flehmen, grinding of teeth and yawning responses; 4 (very good), expressing sniffing, flehmen, grinding of teeth, yawning, urination, standing with spread legs, restlessness, poll glands secretion, neck rubbing and dulla responses; 5 (excellent), same as 4, very excited physical actions with frequent neck rubbing and dulla responses.

Libido was described as the male camels' willingness and eagerness to mount and copulate into the AV (Al-Bulushi et al., 2018) and was scored from 1 to 4: 1 (poor), not willing to mount and copulate; 2 (fair), willing to mount and copulate for a period of 2 to 3 min with or without ejaculation; 3 (good), eager to mount and copulate for a period of 4 to 6 min with ejaculation; 4 (excellent), eager to mount and copulation was terminated forcefully after completing the ejaculation.

2.8. Semen evaluation

2.8.1. Initial characteristics

The volume of the ejaculates was recorded immediately following collection and semen was transferred to a 35 °C water bath. Gross sperm activity was considered as the oscillatory activity of spermatozoa in an undiluted semen sample and was assessed by examining a drop of neat semen on a pre-warmed slide using a phase contrast microscope (magnification: x100, Olympus BX20, Tokyo, Japan). Gross sperm activity was scored from 0 to 3: 0 (poor), no oscillatory activity; 1 (fair), slow oscillatory activity; 2 (good), moderate oscillatory activity; 3 (very good), rapid oscillatory activity.

Semen viscosity was measured as described previously (Morton et al., 2008) and scored from 0 to 4 (0 - no viscosity; 1 - 0.1 to 2 cm; 2–2.1 to 4 cm; 3–4.1 to 6 cm; 4 - > 6 cm). Briefly, 50 μL of semen was drawn into a pipette and 25 μL was pipetted onto a warm glass slide (37 °C). The pipette was slowly raised to form a thread of semen which was pulled slowly until it separated from the 50 μL semen pool. The length (mm) of the thread of semen was considered a direct indicator of viscosity (Bravo et al., 2000).

Ejaculates were then diluted 1:1 with pre-warmed Triladyl (Minitub, Tiefenbach, Germany) extender containing 20% fresh egg yolk and incubated in a water bath for liquefaction. Samples were pipetted at 5 min intervals to assess liquefaction. After complete liquefaction, sperm concentration was recorded using a Makler Counting Chamber (Sefi-Medical Instruments, Haifa, Israel) (Evans and Maxwell, 1987).

2.8.2. Sperm morphology

To examine sperm morphology, smears were applied to glass slides from liquefied semen, air-dried and stained with Farrelly stain according to manufacturer's instructions (Minitube, Germany). At least 200 spermatozoa were examined under a phase contrast microscope (Magnification: x1000, Olympus BX20) under oil immersion. Spermatozoa with protoplasmic droplets, abnormal head, mid-piece and tail shapes were categorized as abnormal spermatozoa (Ziapour et al., 2014).

2.8.3. Assessment of motion characteristics

Motion characteristics of spermatozoa were evaluated using the computer assisted sperm assessment (CASA; CEROS, Version12, Hamilton Thorne Biosciences, USA). The following CASA variables were used; frame rate = 60 Hz; number of frames acquired = 45; minimum contrast = 55; minimum cell size (pixels) = 6; medium average path velocity cutoff = 30 μm/s; medium threshold straightness = 60%; slow average path velocity cutoff = 10 μm/s; slow straight line velocity cutoff = 5 μm/s; static average path velocity cutoff = 4 μm/s; static straight line velocity cutoff = 1 μm/s; non-motile head size (pixels) = 0.5–4.8; non-motile head intensity = 0.25–1.8; magnification = 1.87x; video frequency = 60; illumination intensity = 2300; temperature = 37 °C.

Liquefied, diluted semen samples were further diluted with Triladyl extender to 50 × 10^6 spermatozoa/mL. Three microliters of semen were placed on a pre-warmed 20 μm standard count analysis chamber (Leja, Nieuw-Vennep, The Netherlands). Five randomly selected microscopic fields were assessed five times each and approximately 500 spermatozoa counted. The total motility (TM), progressive motility (PM), average path velocity (VAP), progressive velocity (VSL) and track speed (VCL), lateral head amplitude (ALH), beat cross frequency (BCF), straightness (STR) and linearity (LIN) of spermatozoa were analyzed. Ejaculates with < 100 × 10^6 spermatozoa/ml or with a total motility of less than 60% were classified as a non-qualified ejaculate.

2.8.4. Viability and acrosome integrity

Sperm viability and acrosome integrity were evaluated as described previously (Kershaw-Young et al., 2013). Briefly, 90 μL of semen (50 × 10^6 spermatozoa/mL) were mixed with 10 μL of 1% neutral buffered formalin to fix and immobilize spermatozoa. Fixed spermatozoa (100 μL) were stained with 6 μL of fluorescent isothiocyanate-conjugated lectin from Arachis hypogaea (working concentration 40 μg/mL; FITC-PNA; Sigma) and incubated at 37 °C for 10 min in the dark. Propidium iodide (0.5 μL; working concentration 0.6 mM; PI, Sigma) was then added and the sample incubated for another 5 min. Stained spermatozoa (20 μL) were then placed onto a glass slide and covered with a 22 × 40 mm coverslip. At least 200 spermatozoa per sample were examined using an Olympus BX51 epifluorescence microscope (magnification: x400). Both PI and FITC-PNA stained spermatozoa were classified as non-viable with damaged membranes and a non-intact acrosome. Spermatozoa stained with only PI were considered as non-viable with intact acrosomes. Unstained spermatozoa were classified to be viable if there were intact membranes and acrosomes. Spermatozoa stained with FITC-PNA alone were classified as viable but with a non-intact acrosome.

2.9. Statistical analysis

All statistical analyses were performed in GENSTAT (Version 17, VSN International, Hemel Hempstead, UK). Data relating to the percentage of successful semen collection attempts and qualified ejaculates were statistically analysed using a generalized linear mixed model (GLMM) by Poisson distribution with log binomial. Count data collected on the sexual behaviour and libido of male camels as well as the gross motility of ejaculates were analysed using an ordinal linear regression (ORL) model. Data recorded on the accessory sex glands sizes and testosterone concentrations were analysed using a linear mixed mode. Data relating to semen motion characteristics and membrane acrosome status of each ejaculate were treated as a separate observation and were analysed using a linear mixed model. For all models, month was specified as a fixed effect, while male camel was defined as a random effect. With use of all models, $P < 0.05$ was considered statistically significant.

Correlations between testosterone concentration and average day length, aerial temperature, size of accessory sex glands, sexual behaviour, libido, initial semen characteristics and sperm quality (motility kinematics and membrane status) were determined using Genstat. An r2 coefficient > 0.8 was considered highly correlated.

3. Results

3.1. Temperature and day length

Mean temperature and day length varied among the months of the study period ($P < 0.001$; Fig. 1). There were the least mean

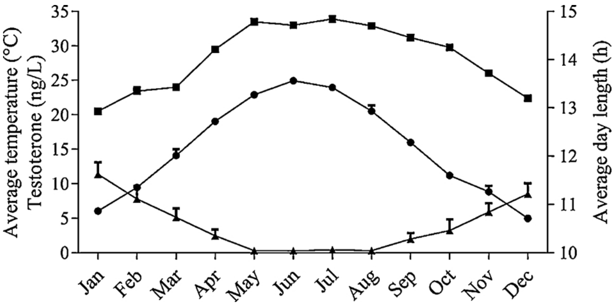


Fig. 1. Mean (± SEM) monthly temperature (°C, ■) and photoperiod length (h, ●) recordings for Oman and serum testosterone concentrations (ng/L, ▲) of male dromedary camels (*n* = 8); Data for temperature and photoperiod length were obtained from the local meteorological centre, Directorate General of Meteorology, Public Authority for Civil Aviation, Muscat, Oman.

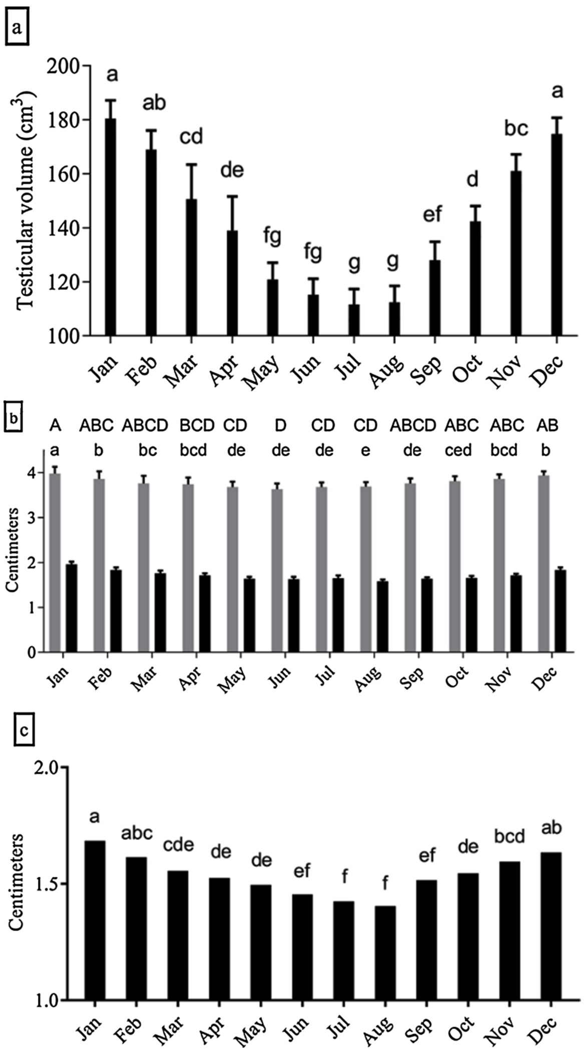


Fig. 2. Monthly mean (± SEM) testicular volume (a), length (light grey bars) and width (black bars) of prostate (b) and size of BUG (c) in male dromedary camels (n = 8); Bars without common superscripts differ ($P < 0.05$); Differences between prostate length and width are indicated using upper case and lower case letters, respectively (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

temperatures (20.5 ± 0.2 °C) during January, while there were the greatest temperatures in July (33.9 ± 0.4 °C). The day with the shortest photoperiod was in December (10.71 ± 0.01 h) whereas the day with the longest photoperiod was in June (13.56 ± 0.01 h). Mean temperature was also highly correlated with photoperiod length (r = 0.90, Table 3).

3.2. Sex organs and accessory sex glands

The testicular volume, prostate and BUG dimensions varied during the study period ($P <$ 0.001; Fig. 2). Testicular volume was less during the months of June to August compared to October to April ($P <$ 0.001; Fig. 2a). The prostate dimensions (length and width; Fig. 2b) and BUG size (Fig. 2c) varied among the months during which the study was conducted ($P <$ 0.05).Furthermore, the values for these variables were least during the months when the ambient temperature was greatest from June to August in comparison to the other months of the year with the largest prostate size being in January. The values for mean testicular volume (r = −0.94), prostate length (r = −0.97) and width (r = −078) and BUG (r = −0.91) size were negatively correlated with values for day length (Table 3).

3.3. Testosterone profiles, sexual behaviour and libido

The mean testosterone concentrations of camels are depicted in Fig. 1, and values for sexual behaviour and libido during the study period are depicted in Fig. 3. There was the peak in testosterone concentration in January (11.4 ± 1.74 g/mL), while a concentration

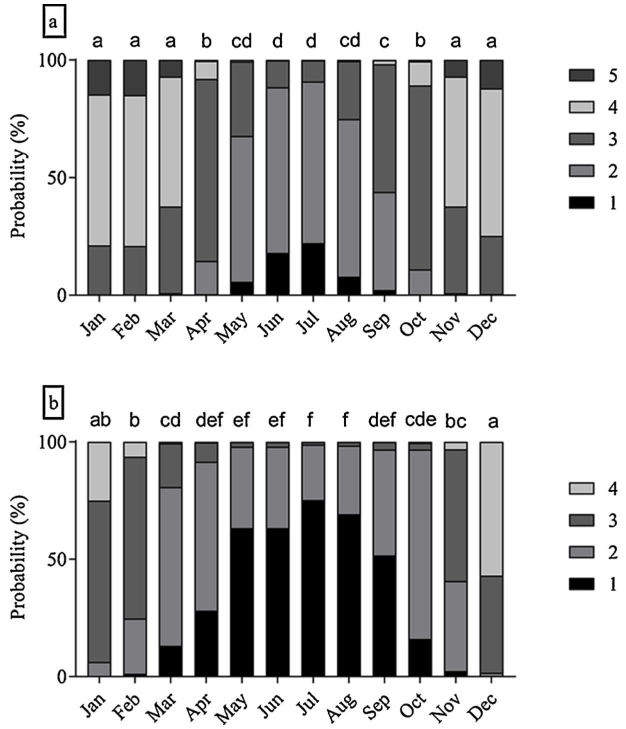


Fig. 3. The monthly predicted likelihood ratios that a male dromedary camel will have a 1 to 5 score for sexual behaviour (a) or 1 to 4 score for libido (b) during the time period when semen collection occurs; Data are generated from an ordinal logistic regression in Genstat; Bars without common superscripts differ between months ($P < 0.05$).

of less than 1 ng/mL was recorded from May to August (Fig. 1). Values for testosterone concentration were highly correlated with values for average testicular volume (r = 0.98), sexual behaviour (r = 0.93), libido (r = 0.97), ejaculate volume (r = 0.67) and percentage of qualified ejaculates (r = 0.90).

The values for sexual behaviour and libido varied among months ($P <$ 0.001). The values for sexual behaviour and libido of camels were greater during the months of October to April ($P <$ 0.05). Findings resulting from the use of the ORL analysis indicated that the likelihood of having a sexual behaviour or libido score of 4 and 5, respectively, was less during the months of May to August ($P <$ 0.05; Fig. 3b and c), whereas likelihood of having a sexual behaviour and libido score of more than 3 was greater during the months when the photoperiods were less peaking in December.

3.4. Initial semen characteristics

The percentages of qualified ejaculates, average semen volume, gross sperm activity, sperm concentration and percentage of abnormal spermatozoa are presented in Table 1. There was < 50% success rate for semen collection from June to August and a 100% success rate for semen collection during November to February (Table 1). From October to March, 93.3% of the successful collection attempts were qualified, whereas only 42.4% were qualified from April to September (P < 0.001).

The volume of ejaculates was larger during November to March compared with May to September ($P <$ 0.001; Table 1). The ejaculates with the largest volumes were collected in February (4.03 ± 0.36 mL), whereas those with the least volume were collected in July (2.40 ± 0.14 mL). Gross sperm activity was greater during October to April compared to May to September ($P <$ 0.001). Sperm concentration was greater in January and February compared to April to November ($P <$ 0.001). There was the greatest sperm concentration in January, while there was the least concentration in June (436 ± 24.6 and 109 ± 7.97 mL x 10^6 spermatozoa/mL, respectively). There was no significant difference in the percentage of abnormal spermatozoa in the qualified ejaculates collected throughout the study.

3.5. Sperm Motion characteristics, viability and acrosome integrity

The data for mean motion kinematics, viability and acrosome integrity of spermatozoa collected during the current study are presented in Table 2. The TM, PM, VAP, VSL, VCL, ALH and LIN varied among months ($P <$ 0.05), whereas BCF and STR were relatively consistent among months. The TM, PM and VAP were greatest during January (87.1 ± 1.1%, 27.0 ± 0.72% and 108 ± 4.11 μm/s, respectively) and least during June (29.7 ± 6.59%, 6.64 ± 1.73% and 44.5 ± 8.72 μm/s, respectively; $P <$

Table 1
Characteristics of semen (mean ± SEM) collected from male dromedary camels (n = 8) throughout the year; Values without common superscripts differ.

Variables	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	*P* value
Successful semen collection attempts (%)	100 ^{ab}	100 ^{a}	87.5 ^{abc}	87.5 ^{abc}	62.5 ^{bd}	43.8 ^{de}	31.3 ^{e}	43.8 ^{de}	56.3 ^{cdf}	75^{af}	100 ^{a}	100 ^{a}	< 0.001
Semen volume (ml)	3.78 ± 0.34 ^{a}	4.03 ± 0.36 ^{a}	3.82 ± 0.39 ^{a}	3.21 ± 0.41 ^{ab}	2.80 ± 0.24 ^{b}	2.50 ± 0.20 ^{b}	2.40 ± 0.14 ^{b}	2.57 ± 0.18 ^{b}	2.72 ± 0.17 ^{b}	3.50 ± 0.27 ^{ab}	4.03 ± 0.22 ^{a}	3.56 ± 0.27 ^{ab}	0.005
Gross sperm activity (0-3)	2.56 ± 0.13 ^{a}	2.63 ± 0.15 ^{a}	2.57 ± 0.16 ^{a}	2.14 ± 0.27 ^{a}	1.10 ± 0.18 ^{b}	0.86 ± 0.17 ^{b}	1.20 ± 0.21 ^{b}	1.43 ± 0.32 ^{b}	1.22 ± 0.17 ^{b}	2.17 ± 0.18 ^{a}	2.25 ± 0.19 ^{a}	2.44 ± 0.13 ^{a}	< 0.001
Concentration (10^6 sperm/mL)	436 ± 24.6 ^{a}	410 ± 14.2 ^{a}	362 ± 27.5 ^{abc}	308 ± 47.5 ^{bc}	171 ± 34.3 ^{e}	109 ± 7.97 ^{e}	190 ± 15.9 ^{de}	155 ± 22.9 ^{e}	196 ± 24.3 ^{de}	280 ± 19.5 ^{cd}	317 ± 24.1 ^{bc}	379 ± 22.4 ^{ab}	< 0.001
Qualified ejaculates* (%)	100 ^{a}	100 ^{a}	92.9 ^{ab}	64.3 ^{ab}	50 ^{bc}	14.3 ^{c}	60 ^{bc}	42.9 ^{bc}	25 ^{bc}	83.3 ^{ab}	81.3 ^{a}	100 ^{a}	< 0.001

* Ejaculates with total motility ≥ 60% on CASA evaluation and/or ≥100 × 10^6 spermatozoa/mL; Values without common superscripts differ.

0.001; Fig. 4). There were the greatest percentages of viable, and acrosome intact spermatozoa in December (62.9 ± 1.12%), compared with the least percentages in June (43.1 ± 4.92%; $P < 0.001$).

4. Discussion

In the present study there were assessments and comparison of seasonal changes of testes functions and accessory sex organ (prostate and BUG) sizes, sexual behaviour, libido, sperm quantity and quality of dromedary camels throughout the year in the Gulf regions of the Middle Eastern countries. Overall, results indicate that large quantities of camel sperm with relatively greater gross sperm motility, viability, total motility and velocity can be collected in Oman from December to March, with a peak in the values for these variables occurring during December to February. There was greater testes, prostate and BUG size during December to February, as was there relatively greater testosterone concentrations, sexual behaviour and libido. The values for these variables began to decrease from April to September, with there being the least values for these variables during June and July. The changes in reproductive and endocrine functions were associated with changes in length of the photoperiod and ambient temperature, with there being a greater quality semen produced during the period when ambient temperatures were less and photoperiod was shorter in December and January. To our knowledge, this is the first study where there was assessment of the relationship of semen characteristics, testosterone concentrations, sex organ morphology and libido of semen collected throughout the year to length of the photoperiod and ambient temperature, confirming the optimal time for the breeding season of male dromedary camels in the Gulf regions of the Middle Eastern countries is December to March.

Table 2
The mean (± SEM) motion characteristics, viability and acrosome integrity of spermatozoa of male dromedary camels (n = 8) throughout the year; Values without common superscripts differ.

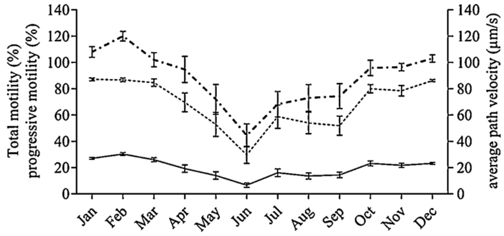
Variables	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	*P* value
TM (%)	87.1 ± 1.05 ^{a}	86.7 ± 1.40 ^{a}	84.6 ± 2.81 ^{ab}	69.6 ± 7.13 ^{bcd}	52.8 ± 9.16 ^{e}	29.7 ± 6.59 ^{f}	58.8 ± 8.94 ^{cde}	53.9 ± 8.21 ^{de}	51.9 ± 7.27 ^{e}	80.0 ± 3.17 ^{abc}	78.5 ± 4.01 ^{abc}	86.2 ± 0.89 ^{a}	< 0.001
PM (%)	27.0 ± 0.72 ^{ab}	30.3 ± 1.08 ^{a}	26.0 ± 1.57 ^{abc}	19.2 ± 2.80 ^{de}	13.9 ± 2.88 ^{e}	6.64 ± 1.73 ^{f}	16.0 ± 2.93 ^{de}	13.6 ± 2.30 ^{ef}	14.4 ± 2.13 ^{e}	23.2 ± 1.85 ^{bcd}	21.7 ± 1.53 ^{cd}	23.2 ± 0.75 ^{bcd}	< 0.001
VAP (μm/s)	108 ± 4.11 ^{ab}	120 ± 3.65 ^{a}	102 ± 5.38 ^{ab}	94.7 ± 9.97 ^{bcd}	71.9 ± 11.3^{efg}	44.5 ± 8.72 ^{fh}	68.2 ± 9.75 ^{defgh}	73.0 ± 10.2 ^{cdef}	74.4 ± 9.42 ^{cde}	95.8 ± 5.84 ^{bcd}	96.4 ± 2.82 ^{bc}	103.8 ± 2.80 ^{ab}	< 0.001
VSL (μm/s)	55.4 ± 2.30 ^{b}	68.0 ± 2.41 ^{a}	51.4 ± 3.47 ^{bc}	47.3 ± 5.38 ^{bcd}	36.7 ± 5.69 ^{de}	24.3 ± 4.60 ^{e}	36.5 ± 5.50 ^{cde}	38.4 ± 5.43 ^{cde}	44.8 ± 6.28 ^{bcd}	53.0 ± 3.61 ^{b}	48.9 ± 1.54 ^{bcd}	52.9 ± 1.68 ^{b}	< 0.001
VCL (μm/s)	201 ± 9.67 ^{b}	242 ± 8.72 ^{a}	194 ± 10.4 ^{b}	183 ± 19.1 ^{bcd}	130 ± 19.9 ^{ef}	90.3 ± 16.91 ^{f}	132.2 ± 20.1 ^{def}	143 ± 18.6 ^{cdef}	146 ± 17.3 ^{de}	182.9 ± 10.2 ^{bcd}	189 ± 5.09 ^{bc}	205 ± 4.59 ^{b}	< 0.001
ALH (μm)	11.3 ± 0.39 ^{abc}	12.6 ± 0.41 ^{a}	9.99 ± 0.33 ^{cdefg}	10.1 ± 0.83 ^{cdefg}	7.81 ± 1.05 ^{egh}	6.97 ± 1.20 ^{h}	8.18 ± 1.15 ^{defgh}	9.2 ± 1-.05 ^{cdefgh}	10.2 ± 1.10 ^{bcde}	10.3 ± 0.39 ^{bcd}	10.2 ± 0.44 ^{cdef}	12.3 ± 0.61 ^{ab}	< 0.001
BCF (Hz)	29.6 ± 0.69	28.8 ± 0.49	29.8 ± 0.65	27.7 ± 2.06	24.0 ± 3.20	22.3 ± 3.86	23.8 ± 3.33	25.3 ± 2.81	27.1 ± 2.62	28.4 ± 0.55	28.0 ± 0.62	28.3 ± 0.56	0.391
STR (%)	49.8 ± 0.66	54.3 ± 0.92	49.2 ± 0.79	45.4 ± 3.33	39.6 ± 5.27	38.4 ± 6.68	41.4 ± 5.89	42.4 ± 4.80	47.3 ± 4.64	50.3 ± 1.21	47.8 ± 1.32	48.4 ± 1.54	0.095
LIN (%)	26.3 ± 0.55 ^{a}	27.3 ± 0.32 ^{a}	26.4 ± 0.52 ^{a}	24.1 ± 1.76 ^{ab}	20.5 ± 2.73 ^{b}	19.6 ± 3.37 ^{b}	21.2 ± 3.01 ^{ab}	22.3 ± 2.47 ^{ab}	24.4 ± 2.52 ^{ab}	27.1 ± 0.43 ^{a}	26.3 ± 0.53 ^{a}	26.8 ± 0.32 ^{a}	0.045
Viable-intact acrosome (%)	57.2 ± 1.10 ^{abcd}	59.8 ± 1.38 ^{abc}	58.4 ± 1.03 ^{abcd}	53.0 ± 4.03 ^{bcde}	43.5 ± 5.92 ^{e}	43.1 ± 4.92 ^{e}	47.6 ± 6.72 ^{cde}	51.9 ± 1.39 ^{bcde}	49.0 ± 4.86 ^{de}	61.7 ± 1.50 ^{ab}	57.8 ± 1.11 ^{abcd}	62.9 ± 1.12 ^{a}	< 0.001
Abnormal Spermatozoa (%)	10.7 ± 0.45	10.6 ± 0.53	11.2 ± 0.39	10.1 ± 0.79	8.77 ± 0.95	12.1 ± 0.47	10.8 ± 0.53	11.5 ± 0.53	11.2 ± 0.31	10.2 ± 0.57	10.1 ± 0.43	10.6 ± 0.44	0.198

Values without common superscripts differ.

Table 3

Correlations coefficients among day length, average temperature, testosterone concentration, size of accessory sex glands, sexual behaviour, libido and semen variables of ejaculates collected from male dromedary camels (n = 7) throughout the year.

Correlation	Average temperature (°C)	Average day length (h)	Length of prostate (mm)	Width of prostate (mm)	BUG (mm)	Testicular volume (cm^3)	Testosterone (ng/mL)	Sexual behaviour (1-5)	Libido (1-4)	Semen Volume (mL)
Average temperature (°C)	1.00									
Average day length (h)	0.90	1.00								
Length of prostate (mm)	−0.90	−0.97	1.00							
Width of prostate (mm)	−0.93	−0.78	0.86	1.00						
BUG (mm)	−0.93	−0.91	0.93	0.90	1.00					
Testicular volume (cm^3)	−0.97	−0.94	0.95	0.91	0.98	1.00				
Testosterone (ng/mL)	−0.97	−0.92	0.96	0.96	0.95	0.98	1.00			
Sexual behaviour (1-5)	−0.96	−0.93	0.90	0.84	0.93	0.97	0.93	1.00		
Libido (1-4)	−0.97	−0.93	0.94	0.92	0.95	0.99	0.97	0.96	1.00	
Semen Volume (ml)	−0.72	−0.70	0.67	0.63	0.76	0.78	0.67	0.84	0.74	1.00
Sperm Concentration (x10^6/mL)	−0.83	−0.73	0.77	0.81	0.81	0.84	0.79	0.86	0.82	0.85
TM (%)	−0.86	−0.86	0.84	0.77	0.81	0.87	0.83	0.91	0.87	0.89
PM (%)	−0.86	−0.82	0.81	0.80	0.80	0.86	0.83	0.90	0.86	0.90
VAP (μm/s)	−0.86	−0.85	0.82	0.77	0.81	0.88	0.83	0.92	0.87	0.90
VSL (μm/s)	−0.80	−0.83	0.80	0.71	0.78	0.83	0.78	0.87	0.82	0.85
VCL (μm/s)	−0.85	−0.85	0.82	0.75	0.80	0.87	0.82	0.92	0.87	0.90
ALH (μm)	−0.83	−0.89	0.87	0.73	0.79	0.86	0.83	0.87	0.87	0.76
BCF (Hz)	−0.86	−0.87	0.83	0.73	0.81	0.85	0.81	0.91	0.82	0.87
STR (%)	−0.80	−0.86	0.80	0.68	0.77	0.82	0.78	0.86	0.80	0.84
LIN (%)	−0.83	−0.92	0.85	0.67	0.80	0.86	0.80	0.91	0.83	0.88
Viable-intact acrosome (%)	−0.79	−0.89	0.81	0.61	0.72	0.81	0.75	0.87	0.81	0.83



	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
TM (%)	a	a	ab	bcd	e	f	cde	de	e	abc	abc	a
PM (%)	ab	a	abc	de	e	f	de	ef	e	bcd	cd	bcd
VAP (μm/s)	ab	a	ab	bcd	efg	fh	defgh	cdef	cde	bcd	bc	ab

Fig. 4. Monthly mean (± SEM) total sperm motility (TM; —), progressive sperm motility (PM; ⸺) and average sperm path velocity (VAP; -·-) of camels (n = 8 bulls) spermatozoa; Data include 'non-qualified ejaculates' (ejaculates with no or few or spermatozoa < 100 × 10^6 spermatozoa/mL and/or total motility < 60% on CASA).

In the present study, values for testosterone concentration were negatively correlated with values for ambient temperatures and length of the photoperiod, that is as the length of the photoperiod became shorter and ambient temperatures less, testosterone concentration increased. This relationship between environmental conditions, and testosterone concentrations has been well documented in previous camel studies (Yagil and Etzion, 1980; Deen et al., 2005; Deen, 2008; El-Kon et al., 2011) as well as other males of species such as bucks (Delgadillo et al., 2004), stallions (Dhakal et al., 2011) and rams (Milczewski et al., 2015). Variation in testosterone concentration throughout the year, however, has been reported to be associated with the abundance of 3β-hydroxysteroid dehydrogenase in Leydig cells (Derar et al., 2005). El Allali et al. (2005) reported that dromedary camels respond to photoperiodic changes which alters melatonin secretory pattern. Melatonin functions to regulate the secretion of GnRH which in turn via the release of luteinising hormone (LH) pulses regulate testosterone secretions from the testis. In the current study, it is likely that a reduction in photoperiod during December led to an increase in the release of melatonin, which resulted in the increased release of GnRH, the simulation of the reproductive axis and an increase in the secretion of testosterone. This endocrine and physiological cascade was previously reported (Vyas et al., 2008) as a result of a study where there was application of eye masks to sexually quiescent female camels to inhibiting the sensing of these animals to changes in the photoperiod. This resulted in the stimulation of ovarian functions during the non-breeding season. Similarly, an improvement in the reproductive performance of male camels during the non-breeding season was reported when there was a similar imposing of an eye masking treatment (Swelum et al., 2018a) and insertion of melatonin implants (Swelum et al., 2018b). Although melatonin concentrations were not quantified in the current study, there was quantitation of melatonin concentrations in a previous study (Qarawi and El-Mougy, 2008) and during the periods when there were greater testosterone concentrations there were also greater melatonin concentrations in camels during the breeding season compared to the non-breeding season. As such, it is likely that the seasonality observed in camels is due to responses to changes in photoperiod and temperature.

Values for ambient temperatures and photoperiod lengths were also negatively correlated with the values of the dimension and size of camel testes and accessory sex glands in the present study. There have been similar seasonal variations reported in testicular dimensions in previous studies (Tingari et al., 1984; Pasha et al., 2011; Swelum et al., 2018b). The increase in volume of testes and Leydig cell numbers during the breeding season of camels is likely due to the corresponding greater concentrations of testosterone during the breeding season, increasing the efficiency of spermatogenesis (Johnson and Thompson, 1987; Simoni et al., 1999; Al-Qarawi et al., 2001). Increases in testosterone were also associated with an increase in sexual behaviour and libido, which has been well documented. Deen (2008) reported that relatively greater testosterone concentrations were associated with an increased duration of copulation and greater libido scores in camels during January to April compared to what occurred during the other months of the year. Similarly, when there were natural mating conditions, Rai et al. (1988) observed that the duration of copulation was shorter during the initial part of the breeding season, increased during peak rutting season and decreased as ambient temperatures increased near the end of the breeding season in April or May. Indeed, the results of present study clearly indicate that changing environmental stimuli such as a shortened photoperiod, induces increases in testosterone production which leads to improvement in sexual behaviour, and libido as well as to larger testes and accessory sex glands. Most importantly, these anatomical changes resulted in an increase in the amount of semen produced as well as the quality of semen produced during the months of December to January.

Values for camel semen variables varied during the course of the year. For the first time in the present study, an advanced semen analysis occurred for assessment as to whether camel semen quality changed with season of the year and whether there were correlations with values for changes in testosterone, anatomical morphology and environmental conditions. In the present study, 93% of ejaculates collected during December and January qualified as being of sufficient quality for use of animals for breeding, whereas from May to August, the percentage of ejaculates with the qualified classification decreased to 37.3%. Semen volume, as well as sperm concentration, motility and viability were also greater compared with values for these variables during the months when ambient temperatures were greater. Additionally, male camels refused to mount a female and copulate into the AV during the months of June to August when photoperiods were longer and ambient temperatures were relatively greater. The increase in the number of qualified ejaculates during December to February is likely associated with a corresponding increase in testosterone concentrations and libido. Similarly, from studies conducted in India (Deen, 2008) there was also a lesser semen volume and greater number of failed semen collection attempts during the months of the non-breeding season when ambient temperatures were greater. Interestingly, in the current study, the increase in semen volume was positively correlated with accessory sex gland size. The primary function of the accessory sex glands is production of seminal fluid as components of ejaculates, with larger sex glands potentially contributing larger amounts of fluid to the ejaculate (Setchell, 1991). Seminal fluid is known to contain many beneficial components for spermatozoa including energy sources, antioxidants, and proteins necessary for final maturation and fertilisation within the female reproductive tract (Juyena and Stelletta, 2012). Consequently, it is not surprising that semen quality, including sperm motility, velocity and viability was also greater during the months of the year when photoperiod was shorter. This may have been a consequence of the greater volumes of accessory sex gland fluid and the beneficial constituents for sperm viability. Seasonal variation in semen quality has also been reported in several males of other domestic species, including bulls (Koivisto et al., 2009; Bhakat et al., 2015), bucks (Zarazaga et al., 2009), stallions (Gamboa et al., 2010) and rams (Malejane et al., 2014). Interestingly, in the present study, sperm morphology was not altered by seasonality, indicating that the production of morphologically normal sperm cell structures remains constant throughout the year. These findings are consistent with those previously reported (Swelum et al., 2018b) where there were not any changes to sperm morphology even after camel bulls were implanted with exogenous melatonin. The proportions of live morphologically normal spermatozoa in the current study ranged from 91% to 88% which was similar to that previously reported (Shekher et al., 2012) but greater than those previously reported for alpacas (58% to 83%; Bravo et al., 1997) and dromedary camels (71% to 84%; Zeidan et al., 2001). These differences in morphological abnormalities could be attributed to the age of camels used, as older males are reported to have greater percentages of sperm abnormalities (Tibary and Anouassi, 1997).

In conclusion, the results of the study has clearly indicate there are effects of seasonality on the reproductive capacity of male dromedary camels in the Gulf regions of the Middle Eastern countries of the world. Large quantities of semen with relatively greater sperm gross motility, viability, total motility and velocity were collected during the months when photoperiod was shorter and ambient temperatures were less from December to February, yet the breeding season can be extended from November through to March. In response to decreases in photoperiodic stimuli, camels produced more testosterone during these months, which resulted in increased testes and accessory sex gland size as well as increased sexual behaviour and libido. When there was a stimulated reproductive axis during the months when photoperiod was decreasing there was a consequent increase in amounts of high quality semen. The information obtained from conducting this study has contributed to the knowledge of reproductive biology and physiology of the male dromedary camel. It suggests that the optimal time to collect high quality semen from dromedary camels in Oman is December to January and this could ultimately result in improved reproductive breeding programs for camels if there is consideration given for altering reproduction management practices as a consequence of the findings in the present study.

Conflict of interest

The authors confirm the following statements:

1 That there has been no duplicate publication or submission elsewhere of this work
2 That all authors have read and approved the manuscript, are aware of the submission for publication and agree to be listed as co-authors
3 That there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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