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BIOPHYSICAL AND BIOCHEMICAL CHARACTERISTICS OF EJACULATED SEMEN OF DROMEDARY CAMEL (*Camelus dromedarius*) AND LLAMA (*Llama glama*)

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ABSTRACT

Experiments were conducted to study the morphology of ejaculated spermatozoa and some biophysical and biochemical characteristics of dromedary and llama seminal plasma. Semen collected by artificial vagina was evaluated for its biophysical characteristics, seminal pH and sperm morphology. The osmolarity of seminal plasma was measured by an automatic osmometer and its biochemical constituents were measured by an automatic biochemistry analyser. The average volume of an ejaculate in dromedary camels was 4.3 ± 0.4 ml whiles it was 0.93 ± 0.2 ml in llamas. In dromedaries, the proportion of normal spermatozoa was 86.05 ± 2.7%, while in llamas it was 64.32 ± 2.3%. The pH of both dromedary (7.98 ± 0.1) and llama semen (7.8 ± 0.04) was alkaline and the average osmolarity of seminal plasma in dromedary camels was 392.4 ± 7.8 while in llamas it was 384.9 ± 11.4 mOsm/Kg. The concentration of glucose was 0.02 ± 0.01mM in dromedary camels while no glucose was observed in llama seminal plasma. The total protein concentration for dromedary and llama seminal plasma were 6.93 ± 0.8 and 12.82 ± 2.0 g/l, respectively, while albumin concentrations were 1.64 ± 0.2 and 2.73 ± 0.81 g/l, respectively. Triglycerides were considerably lower in the seminal plasma of llamas when compared with dromedaries (0.41 ± 0.24 vs. 15.6 ± 2.3 mM, respectively), whereas the calcium concentration was considerably higher in the seminal plasma of llamas when compared with dromedaries (4.97 ± 0.26 vs. 2.14 ± 0.17 mM). The concentration of phosphate was higher in dromedary (2.65 ± 0.72 mM) when compared with llama (1.57 ± 0.29mM) seminal plasma. The concentration of sodium, in the present study were 154.5 ± 0.9 and 141.4 ± 5.4 mM, while chloride were 137.93 ± 2.2 and 128.36 ± 6.78 mM for dromedary and llama seminal plasma, respectively.

Key words: Biochemical constituents, dromedary camel, llama, semen, storage

Artificial insemination (AI), which is the most powerful tool for livestock improvement, has not been developed as a routine method for breeding camelids. The judicious use of AI, and ET could be used to increase overall reproductive efficiency and accelerate genetic improvement in these species. However, semen, which has been studied extensively in other mammalian species, has not been studied so well in camelids and there is only limited knowledge available on the biochemical properties of the camelid semen (El-Manna *et al,* 1986; Mosaferi *et al,* 2005, Morton *et al,* 2007). The ejaculated semen in camelids is highly viscous and needs to be liquified before its evaluation and processing for preservation. In dromedaries, diluted semen (1:1) can be liquified within 90 min at 37°C and can be stored for 48h at 4°C, (Wani *et al,* 2008). However, liquifaction of alpaca (Garnica *et al* 1993; Morton *et al* 2007) and llama (unpublished observations) is considerably longer, ranging from 8–48h. Information about the osmolarity and biochemical constituents of seminal plasma are lacking in both the dromedary camel and llama, which are crucial for improving the current state of processing and preservation of semen for these species.

Mammalian sperm are ejaculated in a heterogenous environment composed of testicular and epididymal fluid together with the secretory products of male accessory glands. Biochemical characteristics of seminal fluid are different in the various portions of the male genital tract especially when considering ionic concentrations and pH (Tuck *et al,* 1970; Hinton *et al,* 1981). Such differences could play an important role in the regulation of important sperm functions as well as motility, capacitation and fertilising ability acquisition (Eliasson, 1970; Eliasson & Lindholmer, 1972). Therefore, studies on biophysical and biochemical characteristics of semen

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are essential for semen processing, its preservation and AI.

This study was, therefore, conducted to evaluate the morphology of ejaculated spermatozoa and to extend the knowledge about biophysical and biochemical characteristics of dromedary and llama semen in order to achieve some information for its preservation and processing.

Materials and Methods

Semen was collected at weekly intervals from 3 adult dromedary and 3 llama males housed at the Camel Reproduction Centre, Dubai, UAE, during the breeding season. They were maintained on commercially formulated camel rations and hay and exposed to natural day length and ambient temperatures.

Semen was collected from dromedary bulls using a receptive female and artificial vagina (AV). Briefly, olfactory contact was made by leading a receptive female past the male's pen. The female was then restrained in sternal recumbency and the male allowed to mount the female. The penis was deflected into the AV and the male allowed to copulate. The AV consisted of a modified bull artificial vagina (30 cm in length with a 5 cm internal diameter, Minitüb, Tiefenbach, Germany) with a rubber AV liner (Minitüb, Tiefenbach, Germany) a cervix-like stricture, and a camel collecting glass (IMV Technologies, L'Aigle, France) filled with water heated to 38-40°C. In llamas, semen was collected in a similar way as in dromedaries using a modified sheep AV (a steel tube 20 cm in length and an internal diameter of 5.5 cm, with a thermostatically controlled electric coil wrapped around the outside, covered with insulating material to maintain water temperature, as llamas can mate even up to 40 min) with a silicone liner. The AV was filled with warm water (38 - 40°C) and connected to a heating box to maintain the temperature during semen collection. Silicone AV liners were constructed in-house as described by Morton *et al* (2007). Briefly, medical grade silicone (Prosil® 20; Barnes Pty Ltd; Bankstown, NSW, Australia) was mixed according to the instructions, painted onto aluminium piping (46 cm length, 3.3 cm internal diameter) and dried (140°C) for 30 min. Ten additional layers of silicone were added and dried (140°C; 15 min) prior to final drying for 1 h (140°C).

The volume of semen was noted from the graded collecting vessel and its colour and consistency were assessed by direct visual examination. Seminal pH was measured by an electronic pH meter (Cyberscan 500, Eutech Instruments, Holland) by placing the probe directly into the semen. The semen was then transferred into a 15 ml tube and kept undiluted in an incubator at 37°C until its liquifaction. The seminal plasma was separated by centrifugation at 3500 x g for 30 min and stored at –20°C until further analysis.

For morphological studies, smears of neat semen were made on glass slides and dried quickly under a hot stream of air. Slides were stained with freshly prepared 10% (v/v) Giemsa stain for 35-40 min, rinsed with distilled water, air-dried and covered with DPX mountant and coverslips. A minimum of 200 spermatozoa per slide were evaluated under a phase contrast microscope (Olympus, Japan) at 400X. Spermatozoa were classified as normal, with protoplasmic droplets (proximal or distal droplets), abnormal mid-piece (curved, bent, double), abnormal tail (curved/bent/coiled, double, short) and others (all other abnormalities not included under the categories mentioned above).

The osmolarity of seminal plasma was measured by an automatic osmometer (Knauer, No A0300, Berlin, Germany) and its biochemical constituents were measured by an automatic chemistry analyzer (Roche Hitachi 912) using kits, reagents and instructions provided by the manufacturer. The test principle is colorimetric assay where the photometric measuring system detects color or turbidity produced by the chemical reactions between reagents and analyte of interest in the sample while in the reaction cells. This system is capable of monochromatic and bichromatic photometry of end point; kinetic, ultraviolet and visible light chemistry determinations.

Results

The average volume of an ejaculate in dromedary was 4.3 ± 0.4 ml while it was 0.93 ± 0.2 ml in llamas. Dromedary semen was milky white to creamy white and llama semen was predominantly grayish white in colour. Both dromedary and llama semen had a very viscous consistency and the spermatozoa were entrapped in a fibrinous network, but were released slowly during the liquifaction process. The pH, osmolarity and biochemical parameters of seminal plasma are summarised in Table 1.

In dromedaries, the proportion of normal spermatozoa was 86.05 ± 2.7%, while 1.02 ± 0.2, 2.7 ± 0.6 and 9.7 ± 2.9% had protoplasmic droplets, mid piece abnormalities and tail abnormalities,

respectively. In llamas the proportion of normal sperm was 64.32 ± 2.3%, while 7.6 ± 2.0, 6.26 ± 0.8, 15.9 ± 1.8 and 5.8 ± 0.7% of sperm had protoplasmic droplets, mid-piece, tail and other abnormalities, respectively.

Table 1. Osmolarity, pH and some biochemical constituents of ejaculated dromedary camel and Llama semen.

Parameters	***Camelus dromedarius*** **Mean ± SEM (Range)**	***Llama glama*** **Mean ± SEM (Range)**
pH	7.98 ± 0.1 (7.7 – 8.4)	7.8 ± 0.04 (7.6 – 8.0)
Osmolarity (mOsm/Kg)	392.4 ± 7.8 (367 – 414)	384.9 ± 11.4 (358 – 471)
Sodium (mM)	154.5 ± 0.9 (147 – 159)	141.4 ± 5.4 (101 – 155)
Potassium (mM)	12.7 ± 0.3 (10.6 – 14.9)	23.4 ± 1.65 (15.1 – 30.85)
Magnesium (mM)	1.16 ± 0.1 (0.5 – 2.1)	1.7 ± 0.1 (1.2 – 2.1)
Calcium (mM)	2.14 ± 0.17 (1.6 – 3.8)	4.97 ± 0.26 (3.7 – 6.0)
Phosphate (mM)	2.65 ± 0.72 (0.15 – 6.9)	1.57 ± 0.29 (0.24 – 2.72)
Chloride (mM)	137.9 ± 2.2 (129 – 164)	128.36 ± 6.78 (85 – 146)
Triglycerides (mM)	15.6 ± 2.3 (6.4 – 23.7)	0.41 ± 0.24 (0.0 – 2.6)
Total proteins (g/l)	6.9 ± 0.8 (3.0 – 13.0)	12.82 ± 2.02 (5.0 – 23.0)
Albumin (g/l)	1.64 ± 0.2 (1.0 – 4.0)	2.73 ± 0.81 (0.0 – 7.0)
Glucose (mM)	0.02 ± 0.01 (0.0 – 0.1)	0.0

Discussion

The average volume of the dromedary semen (4.3 ± 0.4 ml) collected in the present study is in agreement with previous studies, which have reported an average volume of 3 ml (Billah and Skidmore, 1992) and 4.3 ml (Aminu-deen and Sahani, 2000), but are lower than 8.5 (Abdel-Raouf and El-Naggar, 1976) and 8.49 (Taha Ismail, 1988) reported in other studies. Interestingly, the volume of llama semen collected in the present study (0.93 ± 0.2 ml) was also reduced compared with more than 2.0 ml reported in previous studies (Gauly and Leidinger, 1996; Lichtenwalner *et al,* 1996; Von Baer and Hellemann, 1998; Giuliano *et al,* 2007). Reasons for the reduced volume of semen obtained in the present study may include the age of the animals, agro climatic conditions, number of collection per week and the collection procedure. Rutting season (Aminu-deen *et al,* 2003) and health status of the male (Sieme *et al,* 1990) have also been seen to influence the quality and the quantity of semen in dromedary males. The number of males used for semen collection in a study may also affect the average semen volume and other parameters.

The pH of dromedary semen (7.98 ± 0.1) was found to be alkaline in the present study, which confirms the earlier report of a pH of 7.8 (Tingari *et al,* 1986) but is lower than 8.6 (Abdel-Raouf and El-Naggar, 1976) and higher than 7.2 – 7.4 (Agarwal *et al,* 2004) reported for the same species. The seminal plasma of Bactrian camels is also slightly alkaline with a pH of 7.4 ± 0.03 (Mosaferi *et al,* 2005) and 7.37 ± 0.06 (Zhao *et al,* 1992). Moreover, the pH of llama semen in the present study was also alkaline (7.8 ± 0.04) which is similar to 8.16 ± 0.04 found in alpaca semen (Morton *et al,* 2007) and also corresponding with the alkaline nature of semen from old world camelids. The absence of seminal vesicles and high prostate secretion in seminal plasma may be the reason for alkaline nature of camel semen. This might be helpful in creation of an alkaline-buffered milieu in the vaginal surroundings that is normally maintained acidic (unpublished observations) for better survival of spermatozoa.

The average osmolarity of dromedary camel (392.4 ± 7.8 mOsm/kg) and llama semen (384.9 ± 11.4 mOsm/kg) in the present study was similar to the osmolarity of 316 ± 1.48 mOsm/kg in bactrian camel (Mosaferi *et al,* 2005) and 336.9 ± 3.3 mOsm/kg in alpaca semen (KM Morton, unpublished data). To the best of our knowledge there are no earlier reports on the osmolarity of semen in these species. When compared to the serum of same species, the osmolarity was very high in seminal plasma, which confirms earlier reports in other species that the seminal plasma osmolarity is higher than that of blood (Tuck *et al,* 1970; Hinton *et al,* 1981; Polak and Daunter, 1984). It has been reported that seminal osmolarity influences sperm motility both in invertebrates and vertebrates including mammals. Mammalian spermatozoa motility is influenced by seminal osmolarity as demonstrated in bull (Liu and Foote, 1998). Besides influencing sperm motility, seminal osmolarity variations have been demonstrated to be able to regulate Ca^{2+} influx, acrosome reaction and fertilising ability acquisition in human sperm (Rossato *et al,* 1996). Finally, to study the effects of sperm exposure to a hypo-osmotic medium, the hypo-osmotic swelling-test is known as

an important test of plasma membrane functionality (Jeyendran *et al,* 1984) and it is considered one of the most important laboratory parameter to be evaluated during semen analysis in humans as suggested by the WHO (1999). Suggest that seminal osmolarity plays an important role in the regulation of sperm functions and should be taken into account in the preparation of medium for sperm washing and incubation during assisted fertilisation techniques in order to preserve and possibly enhance sperm fertilising ability.

The total protein concentration observed in the present study for dromedary (6.93 ± 0.8 g/l) and llama (12.82 ± 2.0 g/l) seminal plasma is higher than 0.92 g/l reported for dromedary (Agarwal *et al,* 2004), 3 - 4 g/l for alpaca (Garnica *et al,* 1993) and 4.0 ± 0.1 g/l for llama seminal plasma (Bravo *et al,* 2000), but lower than that reported for Bactrian camel seminal plasma (22 ± 0.1 g/l; Mosaferi *et al,* 2005). Our observations on total protein concentration are, however, similar to 7.75 g/l observed by El-Manna *et al,* 1986) in dromedary camels. The role of proteins in seminal plasma is mainly to regulate osmolarity (Agarwal *et al* 2004) and protect sperm during high dilution (Garnica *et al,* 1993). Several protein factors have been identified that promote sperm viability and fertilisation. These include heparin binding proteins in boar and bull seminal plasma (Sanz *et al,* 1993; Miller *et al,* 1990), a major protein from bovine seminal plasma that induces hyper activation of the spermatozoa (Aumuller *et al,* 1988), and boar spermadhesins, which are a class of proteins that have been postulated to play a role in sperm-egg interaction as well as in sperm capacitation (Sanz *et al,* 1993). The differences in the values of total proteins in llama, dromedary and Bactrian camels might be due to sub-species differences. The concentration of albumin in the present study for dromedary (1.64 ± 0.2 g/l) and llama (2.73 ± 0.81 g/l) seminal plasma is similar to 1-3 g/l reported for alpaca (Garnica *et al*, 1993) but lower than 11 ± 0.1 g/l reported for Bactrian (Mosaferi *et al,* 2005) seminal plasma.

The mean concentration of triglycerides observed in the present study was 15.6 ± 2.3 mM and 0.41± 0.24 mM for dromedaries and llamas, respectively. While, we are not aware of any previous reports regarding this parameter in dromedary camels and llamas, in Bactrian camels triglycerides concentrations of 101 ± 5.5 mg/dl, (Mosaferi *et al,* 2005) have been reported, which is lower than the concentration we observed in dromedary but higher than that observed for llama seminal plasma. Interestingly, the triglyceride, concentration was considerably lower in the seminal plasma of llamas when compared with dromedaries, highlighting differences between the New and Old World camelids.

The concentration of glucose in dromedary seminal plasma (0.02 ± 0.01mM) observed in the present study was considerably lower than the 35.8 ± 0.9 mg/dl (Mosaferi *et al,* 2005) and the 12.15 ± 9.59 mg/dl (Zhao *et al,* 1992) reported for Bactrian camels, and the 4.41 ± 0.28 mg/dl reported previously for dromedary camel seminal plasma (Agrawal *et al,* 2004). A relatively low concentration of glucose has been reported previously in the seminal plasma of alpacas (4-8 mg/dl; Garnica *et al,* 1993) and no glucose was observed in llama seminal plasma in the present study. The seminal plasma of most domestic animals contains fructose as the principal sugar. However, metabolism of glucose as the primary sugar has also been reported in some species. Given the relatively low concentrations of glucose in the seminal plasma of alpaca, dromedary camel and llamas, this suggests that the sperm from these species does not metabolise glucose. However, Bravo (2002) reported that while fresh alpaca semen metabolises both glucose and fructose, glucose is metabolised in preference to fructose, and conclude that glucose should be included in semen extenders. Interestingly though, when comparing the longevity of liquid stored alpaca semen, sperm survival was better in Tris, citric acid diluents, which contained fructose compared with glucose (KM Morton, unpublished data). Despite these contrasting results, it is likely that the small fraction of glucose present in the seminal plasma of dromedaries serves as an alternative source of energy for spermatozoa under critical circumstances (i.e. the absence of fructose).

The concentration of calcium in the seminal plasma of dromedary camels in the present study is similar to that of 8.2 ± 0.1 mg/dl (Mosaferi *et al,* 2005) and 12.65 ± 4.7 mg/dl (Zhao *et al,* 1992) reported in Bactrian camels whiles the concentration observed in llama seminal plasma is similar to 18 mg/dl reported in alpacas (Garnica *et al,* 1993). Together, these observations suggest that, the calcium concentration in the seminal plasma of New World camelids (alpacas and llamas) is considerably higher than that of dromedaries and Bactrian camels, again highlighting differences between the New and Old World camelids. We are not aware of any previous reports on different cations in the seminal plasma of dromedary and llama seminal plasma. A negative correlation between calcium concentration

and abnormal sperm rate and positive correlations between magnesium level and sperm concentration and calcium level and motility rate has been observed in humans (Rossato *et al,* 2002); However, in the present study it was not done.

The concentration of phosphate observed in the present study for dromedary (2.65 ± 0.72 mM) is similar to 12.6 ± 1.2 mg/dl observed in Bactrian camels (Zhao *et al,* 1992) and 10 mg/dl reported in llama (Bravo *et al,* 2000) seminal plasma. In llama seminal plasma, however, a lower concentration of phosphate (1.57 ± 0.29mM) was observed in the present study when compared with the observations of the aforementioned studies. In alpacas, the inorganic phosphate has been seen to be in between 7-17 mg/dl (Garnica *et al,* 1993) with higher values in 3-year-old males (12 ± 2) when compared with 6-year-old males (8 ± 0.4).

The concentration of chloride observed in dromedary (137.93 ± 2.2 mM) and llamas (128.36 ± 6.78 mM) seminal plasma, in the present study, are higher than 97.09 ± 0.2 mEq/l (Mosaferi *et al* 2005) but lower than 173.2 ± 59.11 mEq/l (Zhao *et al* 1992) reported in Bactrian camels. Our observations are also, considerably lower than 348-404 mEq/l observed in alpacas (Garnica *et al,* 1993) and 402 ± 10 mEq/l observed in llama seminal plasma (Bravo *et al,* 2000).

The concentration of sodium, in the present study were 154.5 ± 0.9 and 141.4 ± 5.4 mM, for dromedary and llama seminal plasma, respectively, which is similar to that of 158.6 ± 1.6 mEq/l reported earlier in dromedary camel (Agarwal *et al,* 2004) and 163.8 ± 13.2 mEq/l in Bactrian camel (Zhao *et al,* 1992) seminal plasma. The concentration of potassium in the present study for dromedary (12.7 ± 0.3 mM) and llama (23.4 ± 1.65 mM) seminal plasma was also similar to that of 16.68 ± 0.72 mEq/l reported earlier (Agrawal *et al,* 2004) for dromedary seminal plasma. A major change in the concentration of sodium or potassium ions in the seminal plasma has been reported as an indication of disturbed sperm motility and reduced viability in humans (Skandhan and Mazumdar, 1981). Also, a positive correlation has been observed between sodium concentration and sperm motility in humans (Gusani *et al,* 1992).

In conclusion, the results of the present study have elucidated several important biochemical factors in camelid seminal plasma, which provide important new information for the preservation of semen from these species. Furthermore, for the first time, the results of the present study have demonstrated differences in the biochemical characteristics of seminal plasma from New and Old World camelids. Moreover, these results highlight the evolutionary differences between the New and Old World camelids, which should be incorporated when designing semen preservation procedures.

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