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Morphometric Analysis of the Testes and Characteristics of Epididymal Sperm of One Humped Camel (*Camelus dromedarius*) with Special Reference to Using Oviplus® Extender

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Abstract: The study aimed to determine the morphometric dimensions of the testes and characteristics of epididymal sperm in one-humped camel (*Camelus dromedarius*) using oviplus® semen extender. A total of 15 sexually matured camel bulls brought for slaughter to the Maiduguri central abattoir were used. The thoracic and scrotal circumferences were measured prior to slaughter. The testes were collected within one hour of slaughtering and transported in ice box to the laboratory where the testes and the epididymis were separated from the adhering tissues. The epididymis was flushed in a sterile container and 2mls of oviplus® semen extender was added to it. The paired testicular weight and length, paired testis volume; weight of testicular parenchyma and weight of tunica albuginea were all measured. Results revealed that for most morphometric dimension there was a significant difference between the left and right variable, except cauda epididymis which was considered extremely significant ($P<0.005$). The semen characteristics ; motility, pH, concentration, morphology, live and dead ratio were taken for both the right and left epididymis. However, there was no significant between the left and right epididymal parameter. All morphometric and semen characteristics are strongly correlated with the average weight of the testis and the scrotal circumference (r=0.62). Also, a strong correlation existed between the testes volume and the scrotal circumference (0.72). Furthermore, there was a perfect correlation between average semen concentration and average motility (1.0000). It is therefore concluded that this study has established a baseline data on the morphometry of the testis, epididymis and semen characteristics of one humped camel slaughtered at Maiduguri Abattoir. It was also concluded that oviplus semen extender has preserved the viability of the spermatozoa collected.

Key words: Camel (*Camelus dromedarius*) · Spermatozoa · Epididdymes · Testis · Oviplus®

INTRODUCTION

Camels are domesticated animals which play a vital socio-economic role and support millions of human beings in arid and semi-arid zones of Africa and Asia [1]. It is fully adapted to rigours of extreme diurnal variation of temperature and has the ability to produce and reproduce in harsh weather conditions [2]. Camels have provided man with animal protein and energy and have given nomads immense mobility [3]. Daylight and environmental temperature are the two major climatic conditions that influence the physiological and biochemical changes which have affected their sexual behavior [4]. The seasonality of reproduction is also evident in the form of an endocrine surge reproductive steroids and gonadotrophs [5, 6].

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Morphometric dimensions and the changes that occur during growth of the testes from birth to maturity, morphometric analysis on the testes of any breed or specie is necessary for predicting not only sperm production but also storage potential and fertilizing ability of the breeder male that the testes size is a good indicator of the present and future sperm production in the bulls [7, 8] Hassan *et al*. [9] reported that the testicular parameters should suggest the level of sexual activity and semen production from the daily sperm production potential. The growth and development of the testicular organs in various species of domestic animals has been well documented [10, 11].

There is a general agreement that the semen characteristics is not the only criteria to take into cognizance while evaluating the reproductive potential of a breeder male, therefore the study of the gonadal and extra gonadal sperm reserve is essential for the assessment of male fertility [12]. The use of epididymal sperm is important in preservation of animal species and have been used in many laboratories because it is easier to harvest in some animals of proven fertility when found dead or animals that are at the risk of extinction [13-15]. Cryopreserved epididymal sperm is presently used for intracytoplasmic sperm injection (ICSI) in human and animal insemination [16].

The use of epididymal spermatozoa from several animal species such as horses [17] goats [14] , dogs [18] and dromedary camel [19] have been preserved or used with great success for artificial insemination or for *in vitro* fertilization. Furthermore, it is reported that extender type used for dilution of semen is an important and effective factor for successful storage and survival rates of frozen and non-frozen spermatozoa [20]. Various types of semen extenders existed and many of them are commercially available [21, 22]. Whereas, there is paucity of information on the testicular morphometric characteristics and semen characteristics of the one humped camels. Thus the aim of this study is to evaluate if viable of sperm cells can beharvested from the epididymis of one humped camel.

MATERIALS AND METHODS

Animals and Sampling Technique: The study was conducted in Maiduguri, Nigeria, situated at an altitude of 354m above sea level, between latitude 10.2°N and 13.4°N and longitudes 9.8°E and 14.4°E. The abattoir is located within the metropolis and is situated in the cattle market. A total of 15 matured slaughter camel bulls brought for slaughter to the Maiduguri Central Abattoir were selected using purposive sampling Technique. Samples were collected between October to December.

Sample Collection: Testicles were collected from camels destined for slaughter at the Maiduguri Abattoir. All animals had well descended testes. The Thoracic Circumference (TC) and Scrotal Circumference (SC) were measured using a non-stretchable tape at the greatest circumference of the scrotum. The testes were collected within 1 hours of slaughter .Collections were done between7.30 and 9.00am.

Preparation of Extender: OviPlus is a liquid concentrated semen extended and was used for the preparation of 200 ml ready to use extender containing antibiotics in which semen can be preserved. For the preparation of the stock solution, 136 ml of bi-distilled water was added to the contents of the bottle. It was then stirred carefully into 40 ml of egg yolk. In order to obtain the optimal semen preservation properties of OviPlus, it was necessary to add the stock solution to the egg yolk not vice versa as indicated by the manufacturer.

Epididymal Semen Collection: Semen was collected using retrograde flushing method with Oviplus semen extender [23]. The collection of semen involved the infusion of 1ml normal saline into the lumen of the deferens with a 21 gauge syringe, oviplus semen extender was also infused. The content of the epididymis was emptied into clean test tube.

Morphometric Dimensions

Paired Testes and Epididymal Length: The length of the testis and epididymis were measured using a non stretchable tape.

Paired Testes and Epididymal Weight: The testes and epididymis was separated free of adhering connective tissues and fats. The left and right testes and epididymis were measured separately and their weight recorded. The epididymis was then divided into caput, corpus and cauda segment. All weighing of samples was done using a highly sensitive balance in the laboratory.

Testes Volume: The volume of the testes and epididymis were measured volumetrically using the Archimedes principles of water displacement in a measuring cylinder and result recorded.

Tunica Albuginae Weight: The tunica albuginae was then peeled off each testis after cutting the testes in halves and the weight value record.

Evaluation of Semen Characteristics: The Hydrogen-ion concentration (pH) of the semen was measured using universal indicator paper and standard commercial stains. Generally, camel sperm motility (%) detected as an oscillatory motion of the sperm flagellum, Sperm motility was estimated by adding one drop of the diluted fresh semen with physiological saline (0.9% Sodium Chloride) on the dry, clean and pre-warmed (37°C) glass slide. Sperm motility was estimated by observing the approximate percentage of spermatozoa moving forward motion across the field of vision with a normal vigorous swimming motion. About 10 microliters of the extended semen was pippeted onto a clean, pre-warmed microscope slide. A cover slip was lowered onto the sample, avoiding formation of air bubbles, and the slide was examined using a microscope with a 40X objective.

The determination of sperm cell viability was done as described by Melissa [24]. About 0.1ml of the semen was dropped on a glass slide by the help of a Pasteur pipette and a drop of Eosin-negrosin was also placed on the glass slide. Another glass slide was then used to make a smear and then allowed to dry. After which the slide was viewed under the microscope at X1000 magnification using oil immersion to view the sperm cells and the battlement method of counting. The cells that appeared pink were counted as dead due to loss of membrane integrity, while the white cells were counted as live. The live dead ratio was noted and recorded and the percentage abnormalities were also recorded. The abnormalities noted were; detached heads, Narrow heads, coiled tail, bent tails and double tails. The epididymal sperm concentration was determined by methods described by Melissa [24]. A hand dilution was made by diluting 1 part semen with 9 parts formal-buffered saline to make a 1:9 dilution, then took 1 part of the 1:9 dilution and added 9 parts of formal-buffered saline to make a 1:9 dilution. The Neubauer counting chamber (Haemocytometer) was used to count the spermatozoa. A clean cover slip was placed over the counting chamber and then a negative pressure was created by blowing air into it and then sliding the cover slip over the counting chamber. 10ul was then pippeted into the space between the cover slip and the microscope glass slide, which then spread by capillary action to fill up the chamber. The counting chamber, was then mounted on the microscope and viewed using X40 objective lens. Five large boxes were used within which the sperm cells were counted. The boxes were the four boxes at the four angles, and the centre box.

Statistical Analysis: All data generated were expressed as mean ± standard error (mean ±SEM) and were subjected to student t-test and correlation analysis. Graph pad prism7 was employed to access statistical differences between left and right/ epididymal parameter ($p< 0.05$).

RESULTS

Table 1: Mean± standard error of the mean (SEM) Thoracic and Scrotal circumference of Camel bull

Variables	Mean±SEM
Thoracic circumference	183.4 ±3.6
Scrotal circumference	30.9± 0.8

The result revealed a significant difference between the left, right testicular and epididymal variables except for cauda epididymis which was considered extremely significant ($P<0.005$).

Table 2: Ean±SEM Morphometric characteristics of Camel bull slaughtered at the Maiduguri abattoir

Variables	Left	Right	P-value
Weight of testis(g)	80.4±5.3	77.2±5.8	0.672
Length of testis (cm)	9.2±5.3	9.1±5.8	0.9899
Volume of testis (ml)	83.1±5.5	83.1±5.5	0.9899
Weight of tunica albugenia (g)	13.2±0.6	14.0±1.2	0.55555
Weight of parenchyma (g)	65.1±4.7	60.7±4.9	0.52223
Weight of epididymis (g)	9.2±0.5	9.2±0.7	0.52223
Length of epididymis (cm)	15.1±0.5	14.5±0.6	0.52727
Weight of caput (g)	7.4±0.4	7.0±0.6	0.5835
Weight of corpus (g)	5.2±0.3	6.2±0.4	0.055528
Weight of cauda	3.7±0.4	6.4±0.4	0.055529

There was no significant difference ($p<0.005$) between the left and right testis/ epididymal parameters.

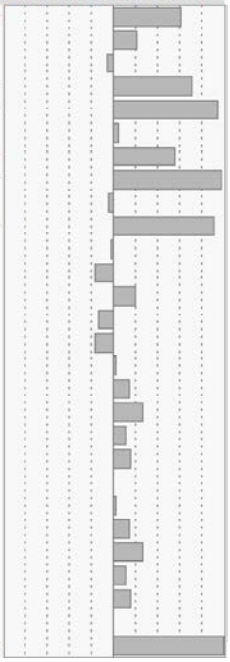
Table 3: Mean±SEM Semen characteristics of epididymal reserve of one humped camel slaughtered in Maiduguri Abattoir

Variables	Left	Right	p-value
Semen Motility (%)	48.0±6.8	48.0±6.8	0.99999
Semen pH	5.6±0.1	5.5±0.1	0.485344
Semen Concentration (10^8)	8.93 ±5.0	10.46 ±1.4	0.770422
Live sperm (%)	73.7±8.7	76.2 ± 8.7	0.840455
Dead sperm (%)	13.4±4.3	10.7±3.8	0.641636

Table 4: Correlation analysis of Morphometric dimensions and semen characteristics of one humped camel

Pairwise Correlations

Variable	by Variable	Correlation	Count	Lower 95%	Upper 95%	Signif Prob
AWT	SC	0.6163	15	0.1520	0.8577	0.0144*
AWE	SC	0.2164	15	-0.3328	0.6559	0.4386
AWE	AWT	-0.0598	15	-0.5550	0.4668	0.8325
AVT	SC	0.7164	15	0.3225	0.8988	0.0027*
AVT	AWT	0.9490	15	0.8498	0.9833	<.0001*
AVT	AWE	0.0434	15	-0.4795	0.5436	0.8778
AWTP	SC	0.5552	15	0.0600	0.8311	0.0317*
AWTP	AWT	0.9861	15	0.9575	0.9955	<.0001*
AWTP	AWE	-0.0479	15	-0.5467	0.4760	0.8654
AWTP	AVT	0.9213	15	0.7745	0.9739	<.0001*
APH	SC	-0.0208	15	-0.5274	0.4968	0.9414
APH	AWT	-0.1715	15	-0.6286	0.3736	0.5410
APH	AWE	0.2045	15	-0.3437	0.6488	0.4646
APH	AVT	-0.1315	15	-0.6031	0.4083	0.6405
APH	AWTP	-0.1689	15	-0.6269	0.3759	0.5474
AM	SC	0.0225	15	-0.4955	0.5287	0.9366
AM	AWT	0.1469	15	-0.3951	0.6130	0.6014
AM	AWE	0.2681	15	-0.2830	0.6861	0.3340
AM	AVT	0.1068	15	-0.4289	0.5870	0.7048
AM	AWTP	0.1511	15	-0.3915	0.6157	0.5909
AM	APH	-0.0046	15	-0.5157	0.5088	0.9869
ASC	SC	0.0225	15	-0.4955	0.5287	0.9366
ASC	AWT	0.1469	15	-0.3951	0.6130	0.6014
ASC	AWE	0.2681	15	-0.2830	0.6861	0.3340
ASC	AVT	0.1068	15	-0.4289	0.5870	0.7048
ASC	AWTP	0.1511	15	-0.3915	0.6157	0.5909
ASC	APH	-0.0046	15	-0.5157	0.5088	0.9869
ASC	AM	1.0000	15	.	.	<.0001*



For all morphometric and semen characteristic correlated. There was no significant statistical change in most parameters except AWT vs SC, AVT vs SC, AVT vs AWT, AWTP vs SC and AWTP vs AVT $P< 0.05$ at 95% C.I.

DISCUSSION

The dimension of the thoracic circumference of the camel recorded in this study was similar to the findings of Chinter *et al*. [25] was carried out on Maghrebi camels in Tunisia. Our study recorded higher values of Scrotal circumference for camels Slaughtered in Maiduguri with that slaughtered in Kano Central Abattoir which was reported by Ibrahim *et al*. [11]. This could be attributed to rutting season which commences from October to January during which sexual activity is at its peak. The mean testis, epididymal weight recorded in this study were within the normal range as previously reported by Osman and Polen [26] who reported that the mean testis of camel weigh between 80g and 140g which is comparable with the findings of the present study. This present study also recorded that the testis weight was similar to that of Osman and Polen [26] which agreed with Okwun *et al*. [27] who reported that males with larger testis tend to produce more sperm therefore it is from these results that testicular morphometric characteristic could be used to predict the sperm production capacity in live camel, and reliable selection for breeding based on the testicular morphometry is possible.

The present study revealed that the caput epididymis is the largest part of the epididymis, this is in agreement with reports by Ibrahim *et al*.[11]who also recorded that the caput epididymis was the largest part of the epididymis. On the contrary, the corpus epididymis was reported to be the largest part of the epididymis [26] which could be attributed to breed differences, because the two humped camel was used in the previous study whereas the one humped camel was used in this current study.

The result of the present study revealed that for most of the morphometric dimensions, there was no significant differences between the left and right testicular as well as epididymal parameters ($P<0.05$).

Earlier studies by Nwakalor and Obasi [28] and Bitto and Okpale [29] had reported several relationships between the scrotal circumferences, testis and epididymis weight in other species of animals, this is in agreement with the result of the present study which showed significant correlation between the scrotal circumference and weight of the testes, but however, disagrees with the relationship between the scrotal circumference and the average weight of the epididymis. The present study showed or found a positive correlation between weight

of testicular parenchyma, average weight of the epididymis and average weight of the testes, scrotal circumferences, volume of the testes and average weight of the testis. This would be reliable predictor of sperm producing ability and fertility of camels, since testis weight is known to be correlated with sperm reserves in the testis or epididymis, and this is a direct reflection of testicular integrity for sperm production [30].

Maiada *et al*. [2] reported an increased sperm concentration during rutting season. However the present study reveals a weak correlation between scrotal circumference and semen concentration, this could be attributed to the method of semen collection. The present study reveals that the hydrogen- ion concentration (pH) is greatly reduced. This could be attributed to the fact that alkalinity reaction of camel is higher during sexual activity than during sexual rest.

The present study revealed that the sperm motility was within the acceptable range. The present study shows that the total number of live sperm was more than that of dead sperm; also percentage of acrosome damage is less. This agrees with findings by Maiada *et al*. [2].

It was concluded from this study that oviplus semen extender has preserved the viability of sperm cells collected using retrograde flushing method and has also provided baseline information on morphometry and semen characteristics from the Extragonadal sperm reserve in one humped camel (*Camelus dromedarius*) in Maiduguri, Nigeria.

REFERENCES

1. El-Hariri, M.A. and A.K.A. Atti, 2010. Effect of age, pubertal stage and season on testosterone concentration in male dromedary camel. Journal of Biological Science, 17: 227-230.
2. Maiada, W.A., E.B. Allam, A.E.B. Abdalla, M.H. Zeidan and A.M. Abd El-Salaam, 2013. Morphological and Histological changes in the camel testis in relation to semen characteristics during breeding and non-breeding seasons. National Science, 11: 129-137.
3. Ali, A.M.I., H. Al-hassan and A. Jibrin, 2012. Scrotal circumference and testicular morphometric characteristic of the camel (*Camelus dromedarius*) in the semi arid enviroment of northern Nigeria. International Journal Morphology, 30: 1369-1372.
4. Hafez, S.E.S., 2000. Reproduction in Farm Animal. (Lea and Febiger, Philadelphia), pp: 55-67.
5. Anouassi, A., M. Adnani and A. El Raed, 1992. Artificial insemination in the camel requires induction of ovulation to achieve pregnancy. In: Proceeding of 1st international camel conference, Dubai, UAE, pp: 175-177.
6. Azouz, A.M., ZH.D. Ateia and Z. AShawky, 1992. Hormonal changes during rutting and the non-breeding season in male dromedary camels. In: Proceedings of the 1st International Camel Conference, pp: 169-171.
7. Togun, V.A. and G.N. Egbunike, 2006. Seasonal variations in the sperm production characteristicof Zebu (White Fulani) cattle genitalia in the humid tropical environment. Middle East Journal Science, 1: 87-95.
8. Leal, M.C., S.C. Becker-Silva, H. Chiarini-Garcia and L.R. Franca, 2004. Sertol cell efficiency and daily sperm production in goats (*Capra hircus*). Anim. Reprod., 1(1): 122-128.
9. Hassan, M.R., S. Pervage, M. Ershaduzzaman and M.A. Talukder, 2009. Influence of age on the spermiogramic parameters of native sheep. J. Bangladesh Agric. Univer., 7: 301-4.
10. Orlu, E.E. and G.N. Egbunike, 2010. Breed and seasonal variation in the testicular morphometry, gonadal and extragonadal. Sperm reserves of barred Plymouth rock and Nigerian indigenous breed Plymouth rock and Nigerian indigenous breeds of the domestic fowl. Pakistan J. Biol. Sci., 13(3): 120-5.
11. Ibrahim, A.A., J. Aliyu, R.M. Ashiru and J. Jamilu, 2012. Biometric study of three breeds of sheep in Nigeria. Int. J. Morphology, 30(4): 1597-1603.
12. Riaz, H.P., A.S. Qureshi, Z. Ur-Rehma and A. Wael Kamal, 2013. Seasonal anatomical changes in the testis of the one-humped camel.
13. Jansen, N., Goldstein, M. Schiegel, P. Palermo and G. Rosenwaks, 2000. Use of electively cryopreserved microsurgically aspirated epididymal sperm with IVF and intracytoplasmic sperm injection for obstructive azoospermia. Fertil. Steril, 74: 696-701.
14. Blash, S., D. Melican and W. Gavin, 2000. Cryopreservation of epididymal sperm obtained at necropsy from goats. Theriogenology, 54: 899-905.
15. Cary, J.A.M., S. Blash, D. Melican and W. Gavin, 2002. Cryopreservation of epididymal sperm obtained at necropsy from goats. Theriogenology, 54: 899-905.

16. Patrizio, P., 2000. Cryopreservation of epididymal sperm. Molcecular Cell Endocrinology, 169: 11-14.
17. Morris, L., C. Tiplady and W.R. Allen, 2002. The in vivo fertility of caudaepididymal spermatozoa in the horse. Theriogenology, 58: 643-646.
18. Marks, S., J. Dupuis, D. Mickelsen, M. Memon and C. Platz, 1994. Conception by use of postmortem epididymal semen extraction in a dog. J. Am. Vet. Med. Assoc, 205: 1639-1640.
19. Wani, N.A., 2008. In vitro embryo production in camel (*Camelus dromedarius*) from in vitro matured oocytes fertilized with epididymal spermatozoa stored at 4°C. Animal Reproduction Science, 111: 69-79.
20. Paulenz, H., L. Söderquist, R. Pérez-Pé and K.A. Berg, 2002. Effect of different extenders and storage temperatures on sperm viability of liquid ram semen. Theriogenology, 57: 823-836.
21. Yamashiro, H.K., H. Wang, Y. Yamashita and T. Terada, 2006. Effect of semen collection in extender solution on the characteristics of goat spermatozoa. J. \Reprod. Dev., 52: 397-406.
22. Kasimanickam, R., V. Kasimanickam and A. Tibracy, 2011. Effect of semen extenders on sperm parameters of ram semen during liquid storage at 4°C. Small Rumin. Res., 99: 208-213.
23. Minitube, 2015. Manual 13610/0025. Hauptstrasse 41: 84184 Trefenbach, Germany, pp: 4-5.
24. Melissa, R., 2004. collection and evaluation of semen.production in goats (*Capra hircus*). Anim. Reprod., 1(1): 122-128.
25. Chinter, M., M. Hammodi, T. Khorchani, R. Knit, C. Cherni and H.M. Ben, 2009. Body Measurements of Maghrebi Camel types (*Camelus dromedarius*) in the southern Tunisia. In The second conference of the international society of camelid research and development. Djerba, Tunisia (Tunisia).
26. Osman, D.I. and I. Polen, 1986. Spermatogenesis in the camel (*Camelus dromedarious*). Animal Reproduction Science, 10: 23-36.
27. Okwun, O.E., G. Igboeli, J.J. Lunstra and D.D. Johnson, 1996. Number and function of Sertoli cells, number and yield of spermatogonia, and daily sperm production in three breeds of boar. Reproduction Fertility, 107: 137-149.
28. Nwarkalor, L.N. and U.O. Obasi, 1991. Relationship of Testicular Size, Body Measurements and Age in the White Fulani (Bunaji) and Ndama bulls. . East Africa Agric Jornal, 57: 23-31.
29. Bitto, I.I. and M.I. Okpale, 2006. Gonadal and extragonadal sperms reserves in slaughtered White Fulani (Bunaji) bulls in a lowland tropical environment. Nigerian Journal of Animal Production, 33: 300-307.
30. Adeyemo, G.O., O. Longe and D.O. Adejumo, 2007. The Reproductive performance of Broiler Cocks fed cottonseed cake- based diets. International Journal of Poultry Science, 6: 140-144.