

Testicular and epididymal morphometry of one-humped camel (*Camelus dromedarius*) in Maiduguri Nigeria, and characteristics of their chilled and frozen-thawed epididymal spermatozoa extended with Oviplus[®] and coconut milk

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Abstract

This study assessed the testicular and epididymal morphometry of one-humped camel (*Camelus dromedarius*) in Maiduguri Nigeria, as well as the characteristics of their epididymal spermatozoa extended with Oviplus[®] and coconut milk based extenders. The study involved samples collected from 50 slaughtered camels (100 epididymides) in Maiduguri central abattoir. Retrograde flushing technique was used to harvest the semen using two different extenders (Oviplus[®] and coconut milk). The flushed samples were analyzed for progressive motility, livability and concentration. Samples from both extenders were divided into two aliquots; one aliquot was stored in a refrigerator at 4°C and evaluated each day for 72 hours, the other aliquot was cryopreserved in liquid nitrogen (-196°C) and evaluated after 24 hours. The left and right testicular and epididymal morphometry were not significantly ($p > 0.05$) different in the camels sampled. The progressive motility in samples extended with Oviplus[®] was $67.9 \pm 21.6\%$, $47.6 \pm 22.7\%$, $27.1 \pm 17.0\%$ and $7.7 \pm 9.4\%$ at 0, 24, 48, and 72 hours of chilling, respectively; while in coconut milk, the motilities were $67.0 \pm 22.6\%$, $49.6 \pm 24.5\%$, $31.5 \pm 18.8\%$ and $13.2 \pm 11.9\%$ at 0, 24, 48, and 72 hours of chilling, respectively. Similarly, the percentage livability in Oviplus[®] was $79.3 \pm 18.8\%$, $65.3 \pm 8\%$, $42.6 \pm 18.4\%$ and $16.7 \pm 14.9\%$ at 0, 24, 48, and 72 hours, respectively; while in coconut milk, the livability was $78.2 \pm 19.1\%$, $64.2 \pm 20.8\%$, $44.4 \pm 19.6\%$ and $22.4 \pm 18.5\%$ at 0, 24, 48 and 72 hours, respectively. There were no significant differences ($p > 0.05$) in the post-thaw motility and livability between cryopreserved samples extended with coconut milk and those extended with Oviplus[®]. Progressive motility in chilled and cryopreserved epididymal spermatozoa of the camels sampled can be preserved for 24 hours in coconut milk as well as in the commercially available Oviplus[®].

Keywords: Camel; Coconut milk, Epididymal semen, Retrograde flush, Oviplus[®], Maiduguri Nigeria.

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Introduction

The dromedary or one-humped camel (*Camelus dromedarius*) is one of the two species within the genus *Camelus*. They are multipurpose animals used for milk, meat, wool, transport, race, tourism, agricultural work and beauty contests (Alaskar et al., 2021). The total number of camels in the world is estimated at 37.51 million, of which, 32.67 million live in Africa and a population of 289,794 are found in Nigeria (FAOSTAT, 2019). Camels are capable of long active reproduction despite late puberty (6 years) and 2-year calving interval. In addition, camels could produce as many or more calves as most indigenous cattle in arid environments (Morton, 1984). Properly managed, and with use of modern technology, camel reproduction can be efficiently optimized for maximum benefit to the poor communities that typically rear them (Gherissi and Lamraoui, 2021). Camels in Nigeria are concentrated in the semi-arid northern part of the country and owned mostly by nomadic tribes (Umaru and Bello, 2013). They exhibit high genetic diversity, probably because of continuous gene flow with other populations during the annual transhumant migration by pastoralists across borders, hence cannot be distinguished into distinct breeds (Abdussamad et al., 2015).

Lack of significant advancement in application of artificial reproduction technology (ART) in camel is attributed partly to difficulties in semen collection and the highly viscous nature of the semen. The widely used semen collection methods in domestic ruminants need to be carefully evaluated for applicability in the dromedary camel (Waheed et al., 2011). There are reports of successful collection and preservation of epididymal sperm which were effectively used to produce viable offspring in dromedary camel (Skidmore et al., 2018). However, majority of the studies reported low post-thaw motility and few pregnancies when chilled or frozen semen are used for artificial insemination (Gherissi and Lamraoui, 2021). In order to enhance artificial insemination (AI) in camel production, availability of viable and functional camel semen, preserved in a suitable extender is necessary. It was found that success

of camel AI is highly dependent on many factors, but there was reduced fertility of preserved semen despite apparent high *in vitro* quality (Al-Bulushi et al., 2019a). A number of different diluents have been tried for short term liquid-storage of camel spermatozoa (Al-Bulushi et al., 2019b). However, further research is required to determine the most appropriate diluent for semen extension because the extenders differ in content and complexity.

The egg yolk based extenders are used for camel semen extension but with challenges in cryopreservation (Swelum et al., 2019). Commercial extenders are expensive and not readily available for farmers in many developing countries. Consequently, there is need to evaluate the efficacy of some readily available plant based extender sources such as coconut milk as alternative for camel semen preservation. Coconut milk contains energy compounds such as glucose and fructose, fatty acids, proteins and some essential vitamins such as ascorbic acid, folic acid, biotin and pantothenic acid, antioxidant enzymes and phospholipids (DebMandal and Mandal, 2011). These components enhance the good preservation of semen in different domestic and wild animal species (Bedair et al., 2020; Asuku et al., 2021). The objectives of this study were to determine the testicular and epididymal morphometry of the dromedary camel slaughtered in Maiduguri Nigeria, and evaluate the effects of Oviplus® and coconut milk based extenders on post-thaw qualities of their chilled and cryopreserved epididymal spermatozoa.

Materials and Methods

Study design: The research was conducted in Maiduguri, Nigeria. Testicles from 50 matured slaughtered dromedary camels were collected from Maiduguri central abattoir. The cross-sectional study design involved convenience sampling of only apparently healthy male camels that were 3 – 7 years of age. The age and weight were estimated using dentition and weighing tape respectively, (Mungai et al., 2010; Bello et al., 2013).

Sample collection from testicles: Testicles from slaughtered and skinned camels included in the study were removed from the scrotal sac and immediately taken to the Theriogenology Laboratory, University of Maiduguri. The length and width were measured using a vernier caliper. Each epididymis was separated from the surrounding connective tissues and the length and width also measured using the vernier caliper. The lumen of the *ductus deferens* was cannulated and flushed with a blunt 22-gauge needle in a retrograde direction with syringe loaded with warm (37°C) extenders as described by Monaco *et al.* (2020). Each testicle was flushed with different extenders (Oviplus® or coconut milk) into a clean calibrated test tube for analysis.

Semen evaluation: The flushed semen collected from the epididymis was extended in the two different extenders (Oviplus® + Egg yolk or Sodium citrate + coconut milk) in ratio 2:1. The samples were evaluated microscopically for sperm motility, concentration and livability according to (Hafez and Hafez, 2001). After the initial evaluation of the samples, each sample that met the criteria of not less than 85% active sperm motility was then divided into two (2) aliquots. One part was stored in refrigerator at 4°C for the period of 72 hours and the motility and livability evaluated while the other aliquot was processed for cryopreservation.

Extender formulations: Oviplus® (Minitub GmbH, Tiefenbach, Germany) semen extender was prepared according to the manufacturer's instruction. Briefly, 136 ml of bi-distilled water was added to 24 ml concentrate (composition: Oviplus®, citric acid, sugar, buffer, antibiotic, and ultra-pure water) for a preparation of 200 ml ready-to-use extender; this mixture was then added to 40 ml of egg yolk as the working solution and refrigerated until use. The coconut milk based extender (100 ml) is a solution of 50 ml of tri-sodium citrate (2.9%) + bi-distilled water + 1g of antibiotic (crystalline penicillin and streptomycin) + 50 ml coconut milk.

Semen cryopreservation and post-thaw evaluation: The method of cryopreservation adopted was as described by Skidmore *et al.* (2013). The semen was diluted with extenders incorporated with 9% glycerol at room temperature. The semen was then loaded in to mini straws (0.25ml) and cooled to 4°C for 4 hours in a refrigerator. The straws were then vaporized at -120°C by suspending them in liquid nitrogen vapor (4 cm above liquid nitrogen level) for 10 minutes. The straws were plunged into liquid nitrogen and frozen at -196°C. Twenty four hours after storage in the liquid nitrogen, the frozen semen were thawed in a water bath at 37°C for 30 seconds. The post-thaw sperm motility and livability were then evaluated.

Statistical analyses: Data obtained were analyzed using IBM SPSS (version 20.0) software and results were presented in tables as mean \pm SD. Independent t-test was used to compare the means of parameters. P-values less than 0.05 were considered statistically significant.

Results

Testicular and Epididymal Morphometry: There were no significant differences ($p > 0.05$) between the length and width of the right and left testes of the camels slaughtered in Maiduguri central abattoir (Table 1). Similarly, there were no significant differences ($p > 0.05$) between the length and width of the left and right epididymis (Table 1).

Epididymal Sperm Characteristics: The mean spermatozoa concentration of the camels sampled was 138.3 ± 21.8 million per ml, while the mean motility and livability of epididymal spermatozoa were $67.5 \pm 22.0\%$ and $78.8 \pm 18.9\%$, respectively (Table 2).

Progressive Individual Motility: Immediately after flushing and extension at 37°C (0 hour), the percentage motility was not significantly ($p > 0.05$) different between samples extended with Oviplus® (67.9 ± 21.6) and coconut milk ($67.0 \pm$

22.6) [Table 3]. Similarly, no significant difference ($p > 0.05$) was observed in same parameter after 24 and 48 hours of storage at 4°C, until 72 hours post-dilution when the motility of the spermatozoa extended in coconut milk had a significantly ($p < 0.05$) higher motility than the one extended with Oviplus® (Table 3).

Percentage Livability: The effects of Oviplus® and coconut milk extenders on the livability of the spermatozoa analyzed at 0, 24, 48 and 72 hours is presented in Table 4. There were no significant differences ($p > 0.05$) in the percentage livability of the spermatozoa in both Oviplus® and coconut milk extenders on hour 0,

24 and 48, but on hour 72, the percentage livability of the spermatozoa extended with coconut milk was significantly higher ($p < 0.05$) than that of spermatozoa extended with Oviplus® (Table 4).

Post-thaw motility and livability of the extended and cryopreserved epididymal spermatozoa: The post-thaw motility and livability of the extended and cryopreserved spermatozoa in both extenders (Oviplus® and coconut milk) were not significantly ($p > 0.05$) different from each other (Table 5).

Table 1: Testicular and epididymal morphometry (mean ± SD) of camels slaughtered in Maiduguri Central Abattoir, Nigeria (n = 50).

Parameters	Left (cm)	Right (cm)	P-value
Length of Testis	8.7 ± 0.8	8.6 ± 0.7	0.460
Width of Testis	3.9 ± 0.6	3.8 ± 0.6	0.459
Length of Epididymis	12.9 ± 1.4	12.7 ± 1.4	0.574
Width of Epididymis	2.2 ± 0.7	2.3 ± 0.8	0.860

Table 2: Epididymal spermatozoa characteristics (mean ± SD) of one-humped camel slaughtered in Maiduguri, Nigeria.

Parameters	Concentration ($10^6/ml$)	Motility (%)	Livability (%)
Mean ± SD	138.3 ± 21.8	67.5 ± 22.0	78.8 ± 18.9
Confidence interval	132.1 – 144.5	63.1 – 71.8	75.0 – 82.5

Table 3: Individual motility (mean ± SD) of camel epididymal spermatozoa in Oviplus® and coconut milk based extenders evaluated daily for 72 hours post extension.

Extender type	37°C		4°C	
	0 hour	24 hours	48 hours	72 hours
Oviplus®	67.9 ± 21.6 ^a	47.6 ± 22.7 ^a	27.1 ± 17.0 ^a	7.7 ± 9.4 ^a
Coconut milk	67.0 ± 22.6 ^a	49.6 ± 24.5 ^a	31.5 ± 18.8 ^a	13.2 ± 11.9 ^b

^{a, b} Values with different superscripts in a column are significantly different ($p < 0.05$)

Table 4: Percentage livability (mean ± SD) of extended camel epididymal spermatozoa evaluated daily for 72 hours post-extension.

Extender type	37 °C		4 °C	
	0 hour	24 hours	48 hours	72 hours
Oviplus®	79.3 ± 18.8 ^a	65.3 ± 19.8 ^a	42.6 ± 18.4 ^a	16.7 ± 14.9 ^a
Coconut milk	78.2 ± 19.1 ^a	64.2 ± 20.8 ^a	44.4 ± 19.6 ^a	22.4 ± 18.5 ^b

^{a, b} Values with different superscripts within a column are significantly different ($p < 0.05$)

Table 5: Post-thaw motility and livability (mean ± SD) of extended and cryopreserved epididymal spermatozoa of camel in Maiduguri, Nigeria.

Extender type	Motility (%)	Livability (%)
Oviplus®	29.3 ± 17.6 ^a	48.1 ± 19.8 ^a
Coconut milk	33.4 ± 19.0 ^a	48.7 ± 20.5 ^a

Discussion

The findings in this study that the left and right testes and epididymis were not significantly different from each other in length and width contrasts with the findings of Ibrahim *et al.* (2012), who reported a significant difference between the right and left testes and epididymis of camels. In some mammals, the left testes had been reported to be larger than the right testes, and this difference was attributed to the enlarged pampiniform plexus that is on the left side (Mahmud *et al.*, 2015).

The mean epididymal sperm concentration of the camels sampled in this present study ($138.3 \times 10^6/\text{ml}$) is lower than the 296.76×10^6 spermatozoa/ml reported by El-Bahrawy *et al.* (2006) for dromedary camels found in Cairo and the 312.36×10^6 spermatozoa/ml reported by Zeidan *et al.* (2001) for camels in Dubai. It is thought that the differences between the sperm concentration recorded in this study and those reported by earlier researchers may partly be due to differences in the method of sperm collection. Zeidan *et al.* (2001) and El-Bahrawy *et al.* (2006) both employed artificial vagina as method of semen collection in their respective work. A much higher concentration (332×10^6 spermatozoa/ml) was however reported by Monaco *et al.* (2020) who used the same

retrograde method of semen collection as used in this present study; it is thought in this case that the differences might be due to breed variation, state of nutrition and other environmental factors. Earlier studies have shown that a minimum concentration of 100×10^6 spermatozoa/ml is needed for a successful AI in camel and fertility can be improved if 300×10^6 spermatozoa/ml is inseminated (Bravo *et al.*, 2000). This may suggest that, although the epididymal spermatozoa had lower concentration in the current study, the spermatozoa recovered using the retrograde flushing technique in this study can be used for successful insemination. In addition, the higher recovery obtain by Monaco *et al.*, (2020) compared with the current study could be due to significantly better welfare conditions of the camel bulls used for tourism purposes which were used for their study. The camels used in the current study were most likely culls, brought in for slaughter.

The lower progressive forward motility of epididymal spermatozoa that was recorded in this present study compared to that earlier reported by El-Bahrawy *et al.* (2006) could also be due to differences in method of semen

collection. Furthermore, the variation when compared to that of Monaco *et al.* (2020) could also be attributed to the fewer number of animals used by the latter author, although with better welfare conditions at the time of sample collection. Skidmore *et al.* (2013) reported an average motility of 75 – 80 % of fresh dromedary semen as appropriate for AI and a frozen-thaw motility of about 40 %. The mean livability obtained in the current study was not far different from those reported earlier by Al-Qarawi and El-Belely (2004) and Monaco *et al.* (2020) who documented 84% livability in epididymal semen collected using retrograde flushing method.

The effects of the two different extenders used in the current study and time related changes on sperm characteristics showed that immediately after extension, the motility was not different between Oviplus® and coconut milk, and the mean values recorded in this study were not different from those reported by Abdoon *et al.* (2013) who compared Ovicell® and tris-fructose egg-yolk extenders on sperm characteristics of camel. The significantly higher sperm motility obtained in coconut milk after 72 hours when compared with that of Oviplus® could be due to the inclusion of a buffer (sodium citrate) in addition to the earlier reported anti-oxidant characteristic of coconut milk (Karunasiri *et al.*, 2020). The current study also revealed that the viability of spermatozoa in both Oviplus® and coconut milk did not differ significantly. This concurs with the findings of Khalifa and Lymberopoulos (2013). Moreover, Oopik *et al.* (2003) reported that buffers neutralize acids and prevent changes in pH which then enhances the livability of spermatozoa. The post-thaw qualities were significantly lower than the pre-freezing values in both extenders.

Conclusion

The left and right testicular and epididymal morphometry do not differ significantly in one-humped dromedary camel found in Maiduguri. The progressive motility in chilled and cryopreserved epididymal spermatozoa of camel can be preserved for 24 hours in coconut milk

extender as well as the commercial Oviplus® extender.

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