

Abattoir Study on Testes and Epididymal Sperm of Somali Camels in Eastern Ethiopia

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Abstract

Testicular function and health parameters were evaluated in Somali camels slaughtered at Jigjiga abattoir. Scrotal sac contents were harvested from 74 animals. Gross scrotal and/or testicular lesions (GSTL) were noted and testicular circumference (TC) was measured using a tape. Epididymal tail flushing was collected from 20 testes and microscopically evaluated for mass activity (poor, moderate or strong) as well as % of live and morphologically normal or defective (head, tail and protoplasmic droplet) sperm. Testicular circumference of Somali camels averaged 19.0 ± 0.5 cm and varied ($p < 0.05$) with age (5 - 8 years (15.7 ± 0.9 cm's) to 9 - 12 years (20.05 ± 0.5 cm's) and > 12 years (23.7 ± 0.4 cm's)) and body condition (thin (16.4 ± 1.1 cm's), medium (18.8 ± 0.7 cm's) and good (21.1 ± 0.7 cm's) of animals. Twenty two (29.7%) camel bulls exhibited GSTL including; scrotal scar (18.9%), testicular atrophy (5.4%), orchitis \pm wound (4.1%), and unilateral partial cryptorchidism (1.4%). 20%, 55% and 20% of epididymal flushing samples showed poor, moderate and strong mass activity, respectively ($p > 0.05$). Average frequency (%) of normal morphology sperm (63.8 ± 1.5) exceeded ($p < 0.05$) that of live sperm (33.1 ± 2.4) whereas that of sperm head defects (20.7 ± 1.1) was higher ($p < 0.05$) than that of protoplasmic droplets (8.5 ± 1.05), and tail defects (7 ± 0.6). Mass activity and average live sperm % were better amongst camels aged >12 years. Average % of total ($p < 0.01$) and tail ($p < 0.05$) sperm defects varied relative to study months. The study indicates that camel testicular parameters were influenced by physical maturity, season, and traumatic injuries. Deeper studies are needed to characterize the breeding potential and problems of sexually active male camels from different local breeds.

Keywords: Camels; Ethiopia; Gross lesions; Sperm; Testis

Introduction

The one humped camel (*Camelus dromedarius*) is multi-purpose animal endowed with unique biological adaptations to hot and arid environments. Around 60% of the worlds' total dromedary population is found in east African pastoralist and agro-pastoralist areas where they serve as a vital source of food (milk and meat) and cash income; means of transportation; and other social - economic - ecological services [1-3]. Developing camel production offers a logical strategy for alleviating dry-land food and livelihood insecurity in the face of deteriorating climate and declining natural resources [4-6].

Productivity of camels reared under traditional pastoralist and agro-pastoralist systems is very low owing to; inefficient husbandry, feed shortage, diseases, and lack of services and technologies [7,8]. Despite existence of substantial phenotypic variation, genetic improvement of dromedaries for marketable production traits is negligible [9-11]. Efficient reproduction is crucial for facilitating genetic improvement as well as optimizing milk and meat production [11]. Camels have a naturally slow reproductive cycle [8,12-14], which is further compromised by different forms of infertility [13-15]. The species has physiological peculiarities which hindered development and wide application of artificial insemination (AI) to speed up genetic improvement [16-19].

Traditional camel breeding management consists of selection of sires and controlled breeding. Pastoralists select camel breeding sires based on performance on criteria such as; color, conformation, fitness, docility as well as disease and drought tolerance. Majority of herds

keep a single outstanding breeding bull [12,13,20], which can serve 10 - 14 females per day [13]. Latter numbers add-up over a mating season and male’s reproductive life-span of 16 - 17 years [13,20], or in AI breeding programs [16,21]. Hence, it is essential to conduct proper breeding soundness evaluation (BSE) of sires to ensure optimum physical maturity and health; normal structure and function of reproductive organs; optimum sperm production and quality; and adequate sexual desire [22,23].

In dromedary, examination of testicular size and consistency; ability to extend penis; and ability to ejaculate and produce viable semen were recommended for infertility diagnosis [17,24]. However, physiological male camel BSE parameters are not well established for different breeds and production environments. Likewise, epidemiology and impact of disorders affecting breeding potential of camel sires are poorly understood. Corresponding research knowledge on Ethiopian camels is even more rudimentary. Therefore, this study attempted to 1) describe testicular circumference and associated variations in Somali camel bulls, 2) describe the major types and distribution of gross gonadal disorders in Somali camel bulls and 3) describe basic epididymal flushing sperm qualities and associated variation in Somali camel bulls.

Materials and Methods

Study animals

The study was conducted on Somali camel bulls slaughtered at Jigjiga abattoir over eight months (January to May) during 2016 and 2018. Each month three busy slaughter days were selected (total 24 working days) and all mature healthy, camel bulls encountered on latter days were sampled giving a total of 74 study animals.

Abattoir examination and sampling

Study animals were visually inspected ante-mortem to subjectively classify body condition in to three categories (thin, medium and fat) based on flesh cover of vertebral and thoracic prominences. Post-mortem, age of camel bulls was estimated based on dentition as described by Dioli, *et al.* [14] and classified in to three categories i.e. 5 - 8 years, 9 - 12 years and ≥ 13 years (Figure 1). Scrotal sac was visually inspected to note any gross lesions (abnormal size, asymmetry, skin scarring and/or open wound). Finally, testes were harvested along with surround integument put in a labeled (date and animal #) plastic bag, placed in bucket filled with warm water (+40°C) and quickly (< 1 hour) transported to the laboratory at Jigjiga University (JJU).



Figure 1: Camel dental age and testicular investigation activities.

Testicular measurement and epididymal sperm flushing

At the laboratory, testes and spermatic cord were carefully separated from surrounding integuments. Testes were palpated to note abnormal consistency (hard - fibrosis or flabby - degeneration) of tissue. Testicular circumference (TC) was measured as described by others [23,26] (Figure 1). Epididymal sperm flushing was performed on larger testis from 20 healthy Somali camel bulls by modifying the protocol described by others [27,28]. Briefly, a tubular segment extending from distal body of epididymis to broad upper section of ductus deferens was severed without causing too much bleeding. Open tip of epididymis was placed in sterile petri-dish and that of ductus deferens was raised to a height that allows straight suspension. A sterile syringe filled with 5 ml of 0.9% sterile saline solution (SSS) warmed to 35°C was fixed to open tip of ductus deferens and fluid was infused down by digital pressure. The dissected tubular segment was digitally milked until ≥ 2 ml of fluid was recovered. The flushed fluid was transferred using another sterile syringe to a sterile glass centrifuge tubes and incubated at 35°C until analysis.

Microscopic sperm quality analysis

Epididymal flushing sperm quality was evaluated as described for camel semen [23,24,26]:

- **Mass activity:** A drop of sperm sample was placed in the middle of a warm (33 - 35°C) slide, covered with cover slip and microscopically examined at x 100 - 400 magnifications. Based on paired tests, sperm mass activity was subjectively graded as strong (wave like movement), medium (some vibrating movement) or weak/ absent (scare - no movement activity).
- **Sperm viability and morphology:** Proportion (%) of live sperm and sperm having different morphology were calculated out of 200 cells from duplicate stained smears. For this, one drop of epididymal flushing was mixed with two drops of Eosin - Nigrosin stain solution on warm slide +33/35°C, smeared like a thin blood film, air dried, covered with cover slip, and examined at x 400 to 1000 (oil emersion) magnification. Based on cytoplasmic staining, spermatozoa were classified as live (clear) or dead (red colored). Sperm morphology was classified as normal, head defects, tail defects and protoplasmic droplet.

Statistical analysis

Data collected from abattoir and laboratory examination was coded and entered on Microsoft Excel spreadsheet. Statistical analysis was performed on SPSS-20 software. Descriptive statistics were used to summarize categorical variables (n (%)) and numerical variables (means \pm standard error (SE)). Association of categorical variables was analyzed by Chi-square and Fishers exact tests. Relationship of numerical variables was evaluated by Pearson's or Spearman's bivariate correlation tests. Variation of numerical variables relative to different factors were tested by parametric (95% confidence interval (CI), independent t and one way-ANOVA) and/or non-parametric (Kruskal Wallis H or Man-Whitney U) comparison of mean. Statistical significance was determined at $p < 0.05$.

Results

gross scrotal and testicular examination findings

Abattoir investigation of camel TC and gonadal lesions was conducted in months of January (24.3%), February (20.3%), March (29.7%), April (13.5%) and May (12.2%). Majority ($p < 0.05$) of camels were aged 9 - 12 years (54.1%) followed by 5 - 8 years (33.8%) and > 12 Years (12.2%). Meanwhile, body condition of study animals was thin (24.3%), medium (40.5%) and good (35.1%) ($p > 0.05$).

Testicular circumference of Somali camel bulls varied from 6 to 30.4 cm's and averaged 19.0 ± 0.5 cm's (95% CI; 18.01 - 20.04 cm). Average TC varied with age ($p = 0.000$) and body condition ($p = 0.001$) group camels (Table 1). Overall, 22 (29.7%) Somali camel bulls exhibited gross scrotal and/or testicular lesions GSTL.

The later mainly comprised scrotal skin scars (18.9%), atrophied testes (5.4%), orchitis \pm skin wound (4.1%), and unilateral (left) undescended (inguinal) testis (1.4%) ($p < 0.05$). Abattoir workers indicated that unilateral undescended testis was a common finding. Gross scrotal and/or testicular lesions involved right (45.5%), left 7 (22.7%) or both 5 (31.8%) gonadal sides ($p > 0.05$). Combined frequency of GSTL was higher in January ($p = 0.010$) but showed limited variation with age and body condition (Table 1).

Factors	Categories	TC (cm's)		GSTL n (%)	
		Mean (SE)	95 % CI LB-UB	Total	Scar
Study months	January	18.8 (1.1)	16.4 - 21.1	11 (61.1)*	8 (44.4) *
	February	17.8 (1.5)	14.6 - 21.0	3 (20.0)	1 (6.7)
	March	19.0 (0.8)	17.4 - 20.6	6 (27.3)	4 (18.2)
	April	21.8 (0.7)	20.3 - 23.3	1 (10.0)	-
	May	18.7 (1.3)	15.6 - 21.8	2 (22.2)	1 (11.1)
Age group	5 - 8 Years	15.7 (0.9) ^c	13.8 - 17.3	9 (36)	5 (20)
	9 - 12 Years	20.05 (0.5) ^b	19.3 - 21.2	12 (30)	8 (20)
	> 12 Years	23.7 (0.5) ^a	22.4 - 24.9	1 (11.1)	1 (11.1)
Body condition	Poor/Thin	16.4 (1.1) ^c	14.1 - 18.7	5 (27.8)	1 (5.6)
	Medium	18.8 (0.7) ^b	17.3 - 20.3	7 (23.3)	6 (20)
	Good	21.1 (0.7) ^a	19.6 - 22.6	10 (38.5)	7 (26.9)

Table 1: Summary of TC and GSTL relative to months, age group and body condition. Superscript * denotes significant difference at $p < 0.05$, superscripts a b and c denote decreasing order of group means, Gross scrotal and/ or testicular lesions = GSTL, Testicular circumference = TC.

Average TC (cm's) of camels exhibiting scrotal scar (20.75 ± 0.9), normal testes (19.25 ± 0.5), orchitis (18.1 ± 6.2), atrophied testes (12.4 ± 2.4) and undescended/inguinal testis [12] showed significant variation ($p = 0.028$).

Epididymal flushing sperm quality

Overall, 20%, 55% and 20% epididymal flushing samples exhibited poor, moderate and strong sperm mass activity, respectively ($p > 0.05$). Live sperm % varied from 12 - 52 and averaged 33.1 ± 2.4 (95% CI; 28.1 - 38.1) whereas morphologically normal sperm % ranged from 48 to 74 and averaged 63.8 ± 1.5 (95% CI; 60.6 - 67.0). Average % of sperm head defects (detached > small = tapered heads) $20.7 \pm 1.1\%$ (95% CI; 18.4 - 23) was higher than that of protoplasmic droplets (distal > proximal) $8.5 \pm 1.05\%$ (95% CI; 6.3 - 10.7) and tail defects (folded > curled > detached) $7 \pm 0.6\%$ (95% CI; 5.7 - 8.3).

Epididymal flushing sperm mass activity was better ($X^2 = 9.5$, $p = 0.029$) in camel bulls aged > 12 years (50% moderate and 50% strong) than 9 - 12 years (40% poor and 60% moderate) and 5 - 8 years (50% poor and 50% moderate). Average live sperm % was higher ($p = 0.001$) in epididymal flushing samples showing strong (47 ± 2.4 , 95% CI: 39.4 - 54.6) than moderate (32.4 ± 2.2 , 95% CI; 27.4 - 37.3) and poor (23.6 ± 3.9 , 95% CI; 12.9 - 34.3) mass activity. Average live sperm % was higher in camels older than 12 years ($p < 0.01$). Average % sperm total morphological defects and tail defects were higher in February and March compared to April (Table 2).

Average TC (cm's) showed relative variation in camels with poor (18.4 ± 2.1), moderate (21.1 ± 0.9) and strong (23.85 ± 0.7) epididymal flushing sperm mass activity ($p = 0.068$). Meanwhile, TC showed strong positive correlation to % of live sperm ($r = 0.535$, $p = 0.015$) and % sperm showing tail defect ($r = 0.511$, $p = 0.021$).

Discussion

Traditional Somali camel pastoralists select a single bull based on locally desirable phenotypic traits and exclusively use this animal as a breeding sire for 16 -17 years [8,13,20]. A superior camel breeding bull can mate with 50 to 120 females in one breeding season [14] and father 850 to 2,040 potential offspring's over a reproductive life of 17. This figure becomes several folds higher if said sire is used in AI breeding program. Therefore, reproductive efficiency of camel breeding sires is critical for optimizing productivity and speeding-up genetic improvement. However, research on male camel BSE parameters and fertility disorders is limited at global level [29] and almost non-existent in Ethiopia. This study attempted to provide baseline information on the TC and gross lesions (acquired and congenital disorders) and epididymal sperm quality of Ethiopian Somali camels.

Factors	Categories	Live-Sperm	Morphological Types			
			Normal	Head defect	Tail defect	Protoplasmic droplets
Study Months	February	37.3 (2.4)	55.3 (3.7)	26.7 (1.8)	8.7 (1.3)*	9.3 (1.3)
	March	26.0 (5.7)	60.5 (4.3)	21.0 (2.9)	9.5 (1.9)*	9.0 (1.9)
	April	34.4 (4)	68.0 (1.2) *	19.2 (1.2)	6.2 (0.5)	6.6 (1.4)
	May	34.0 (2)	62.7 (1.3)	19.3 (3.5)	4.7 (1.8)	13.3 (3.7)
	Sig.	p = 0.519	p = 0.009	p = 0.230	p = 0.049	p = 0.169
Age group	5 - 8 Years	36.0 (0)	62.0 (2)	20.0 (6)	5.0 (1)	13.0 (5)
	9 - 12 Years	25.4 (2.6)	64.6 (2)	19.8 (1.5)	6.6 (0.9)	9.0 (1.5)
	> 12 Years	42.0 (2.3) *	63.3 (2.9)	22.0 (1.8)	8.0 (0.9)	6.8 (1.3)
	p = 0.063	p = 0.001	p = 0.860	p = 0.655	p = 0.341	p = 0.221
Body condition	Poor/Thin	17.0 (5)	64.0 (2)	24.0 (2)	6.0 (2)	6.0 (2)
	Medium	35.8 (2.7)	63.8 (2.2)	20.8 (1.6)	7.2 (0.8)	8.2 (1.6)
	Good	33.8 (3.9)	63.8 (2.7)	19.8 (1.9)	7.0 (1.2)	9.5 (1.5)
	Sig.	p = 0.063	p = 0.999	p = 0.578	p = 0.870	p = 0.638

Table 2: Epididymal flushing sperm quality relative to months, age and body condition. Superscript * indicates significant difference at $p < 0.05$.

Estimation of TC is a highly repeatable non-evasive livestock BSE parameter. The parameter has strong correlation to total daily sperm output reflecting ability to sire a large number of progeny. Generally, TC of can be influenced by species, breed, age and body size of animals as well as by breeding seasons [22,23]. Currently, TC of Somali camels varied from 6 to 30.4 cm's and averaged 19 cm's. Average TC increased with age (5 - 8 < 9 - 12 < 13 - 15 years) and body condition (poor/thin ≤ medium < good) of camel bulls. Israeli camels aged 6 years and older were reported to have higher average TC of 30 cm's [30]. This study only examined animals destined for slaughter (unwanted for breeding) and took internal measurement of TC after removing scrotal integuments. These factors could contribute to lower TC estimates in addition to discrepancies attributed to breed and management differences. In line with present findings, others have indicated that TC of dromedary increased with age after onset of puberty (3 to 5 years) and influenced by nutritional status as well as rut season [21,30,31]. In eastern Africa, seasonality of camel breeding is less pronounced but rutting generally tends to increase during rainy/growing months [14]. The current study didn't include rainy months which could explain lack of significant temporal TC variability.

Information on reproductive health problems of male camels is generally limited. Few clinical and abattoir studies have documented conditions such as: phimosis, paraphimosis, orchitis, testicular hypoplasia and unilateral cryptorchidism, testicular degeneration and fibrotic atrophy of testes due to filariasis [2,32]. Currently, 29.7% Somali camel bulls exhibited gross evidence of prior or active gonadal lesions including; scrotal scars (18.9%), atrophied testes (5.4%), orchitis ± scrotal wound (4.1%) and unilateral (left) undescended testis (1.4%). Hence, gonadal trauma appears to be a relatively common problem in camels and in severe cases can impair breeding potential in the immediate (Orchitis) or long (Fibrosis/Atrophy) term. Combined frequency of GSTL was higher in January which could reflect rut related aggression/assault as previously indicated by others [14,21,30]. Common incidence of unilateral cryptorchidism reported by abattoir personnel could suggest an inbreeding problem due to prolonged use of single breeding sires in traditional Somali pastoralist camel herds [8,12,13,20].

Billions of reasonably fertile (mature and motile) camel spermatozoa spend 1.5 days in the distal body and tail of epididymis. Studies had confirmed that such gametes can be harvested and used for experimental and salvage breeding purposes in dromedaries [27,28] and other livestock [25]. In contrast, research on seminal or epididymal flushing sperm quality of Ethiopian camels was thus far lacking. Currently, Somali camel epididymal flushing samples exhibited moderate (55%) to strong (20%) sperm mass activity and average live and normal sperm contents of 33% and 64%, respectively. Strong mass activity and live sperm % were positively correlated to each other as

well as to older age (> 12 years) and larger TC. This probably reflects effect of advanced age on physical maturation including testicular development and by extension on viable sperm production potential. Mass activity is logically expected to improve as quantity of viable sperm increases. Somali camel epididymal flushing sperm showed higher average head defects (20.7%) than protoplasmic droplets (8.5%) and tail defects (8.5%). Frequency of total and tail defects was higher during February and March than in April. The major types of sperm head (detachment) and tail (folded) defects observed along with increased frequency in colder months is consistent indication of cytological injury associated with thermal fluctuation.

Present epididymal flushing sperm quality estimates were below the acceptable level of motile ($\geq 50\%$), live ($\geq 82.0\%$) and morphologically normal ($\geq 72.3\%$) sperm for camel AI semen [24]. However, current mass activity and normal sperm % were comparable to previous semen quality estimates of 64.2% and 85.8% [16] as well as 60 - 80% and 60 - 92% [30]. Low level of strong mass activity and live sperm % in this study probably reflect thermal damage resulting from sub-optimal sample handling and delays in sample collection and/or analysis. In agreement to current assertions, camel sperm qualities including motile and normal sperm % have been shown to vary relative to season and semen collection technique [27,28,30]. Other study conducted by Senan., *et al.* [33] also reported that proportion of motile and defective sperm in camel semen varied depending on types of extenders (70.6 - 81.6%) and stains (13.7 - 25.8) employed. Meanwhile, low % of proximal protoplasmic droplets indicates advanced stage of epididymal tail sperm maturation [25].

Conclusion

The study offered useful baseline information on gross disorders and functional parameters of testes in Somali camels. Physical testicular trauma was a common problem particularly in younger camel bulls during rutting months. This reflects need for proper rearing and protection of replacement breeding bulls. Testicular circumference and epididymal flushing sperm quality parameters showed strong inter-relations and variations relative to age of camels and examination period. Hence, male camel breeding soundness evaluation findings should be interpreted with due considerations to physical development and health status of animals as well as season of year. More comprehensive investigation is required to document male reproductive health problems and fertility parameters of different Ethiopian camel populations.

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