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ORIGINAL ARTICLE

Effect of Normal Saline and Oviplus® on Epididymal Sperm Quality and Relationship with Testicular Morphometry in Sahel Bucks

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SUMMARY

This study was designed to determine the effect of normal saline infusion into the epididymis on epididymal sperm quality in Sahel bucks postmortem and relationship between testicular and epididymal morphometric parameters with characteristics of the recovered sperm. A total of 60 slaughtered Sahel bucks were used. The bucks were randomly divided into two equal groups and spermatozoa collected using retrograde flushing method. In group 1, Normal Saline (1 ml) was infused into the epididymis, followed up with 1 ml of Oviplus® semen extender. In the second group, 2 mls of Oviplus semen extender was used. It was found that the epididymal sperm concentration was significantly lower ($P < 0.05$) in group 1 compared with group 2. The epididymal sperm concentration in group 2 (1760×10^6 sperm/mL) was significantly higher ($P<0.05$) compared with group 1 (338×10^6 sperm/mL). The weight of the testes, testicular and epididymal parameters were significantly positively correlated with each other in both group 1 and group 2. However, in group 1, motility and concentration were significantly positively correlated ($r= 0.450$; $P=0.012$) while all the other parameters studied were not significantly different with each other. Some parameters that were significantly positively correlated with epididymal sperm concentration included: Scrotal Circumference ($r = 0.602$; $P = 0.000$), paired testicular weight ($r = 0.412$; $P = 0.013$), Epididymis ($r = 0.377$; $P = 0.024$) and Cauda Epididymis ($r = 0.372$; $P = 0.026$). In conclusion, epididymal sperm recovery was successful using Oviplus® semen extender with and without normal saline in Sahel Bucks. However, the spermatozoa concentration and motility was significantly higher using Oviplus® semen extender alone and is hence recommended for epididymal sperm recovery in Sahel bucks.

Key words: Sahel bucks, spermatozoa, epididymis, testes, oviplus®, normal saline.

INTRODUCTION

Morphological characteristics of Sahel goats (*Capra hircus*) has been well described and are found across the Sahel zone, in the southern fringe of the Sahara Desert from northern Nigeria northwards (Devendra and Solaiman, 2010).

Genetic improvement of the Sahel goat is yet to commence due to several factors including absence of any breeding program.

Successful collection and storage of semen is essential in reproductive biotechnology methods such as Artificial Insemination, Embryo transfer Cryopreservation and cloning for ex-situ management of genetic diversity (Jimenez-Rabadan *et al.,* 2016).

It has been shown that strong relationship existeds between the scrotal circumference and the testicular as well as the epididymal parameters in Sahel goats (Oyeyemi *et al.,* 2012). In addition, it was found that size variables of the scrotum and testis positively correlated with one another and with cauda epididymal sperm counts in Sahel goats examined postmortem (Abba and Igbokwe, 2015).

Collection and use of epididymal sperm is an important technique in the propagation and conservation of animal specimens with high genetic values after serious injury or from dead animals (Blash *et al.*, 2000, Cary *et al.,* 2002).

Previous studies have shown that it was possible to recover viable sperm with 41 % forward motility from the cauda epididymis of bulls stored at room temperature of 18-20°C for 30 h. (Bertol *et al.*, 2013). Furthermore, the epididymal sperm remain viable with adequate fertilizing ability up to 48 h after the death of the animal (Cary *et al.*, 2004, Turri *et al.*, 2014).

Turri *et al.* (2014) investigated the pre-freeze/post-thaw quality of goat epididymal sperm as a function of testicle storage temperature (environment or +5°C) and time elapsed between animal's death and sperm recovery (0, 24, 48, 72 h) to establish the optimal protocols for the recovery and cryopreservation of epididymal sperm in 50 mature goats. They found that, for periods up to 48 hours, postmortem maintenance of refrigeration temperature (+5°C) is fundamental to reduce epididymal sperm quality decay. Furthermore, testicle refrigeration also had a positive effect on post-thaw samples, reducing the rate of decline of spermatozoa motility, sperm viability and sperm abnormalities.

Semen is collected, processed and used immediately for Artificial Insemination or *In-vitro* fertilization or stored for later use (Gillan *et al.*, 2004). Several types of semen extenders exist and many of them are commercially available (Yamashiro *et al.,* 2006, Kasimanickam *et al.,* 2011).

Semen collection, extension and preservation in live goats and at postmortem has been described (Blash *et al.*, 2000, Cary *et al.*, 2002, Jimenez-Rabadan *et al.*, 2016).

Detrimental effect of seminal plasma on sperm viability has been described in goats and previous studies suggested that the initial pH of an extender was crucial to sustain high sperm motility. Liu *et al.* (2016) found a strong negative correlation between long-term liquid storage of goat semen and sperm motility due to decreased semen pH. Furthermore, increasing the initial extender pH from 6.04 to 6.25 or storage with stabilized pH improved sperm motility, storage with artificially lowered pH impaired sperm motility (Liu *et al.*, 2016).

MATERIALS AND METHODS

Animals and Sampling Technique

A total of 60 mature Sahel bucks weighing 18-20 kg, aged approximately 1.5 years with body condition score of 3 (on a scale of 1-5) as described by (Burkholder, 2000, Bukar *et al.*, 2012) were used for this study. Purposive sampling technique was employed for the study.

Testicles were collected from bucks presented for slaughter at the Maiduguri Metropolitan Abattoir. All the goats had well descended and intact testes. The animals were weighed using a measuring scale (Kg) and aged by dentition as described by (Matika *et al.*, 1992) before slaughter. Thereafter, Scrotal Circumference (SC) was measured at the greatest circumference of the scrotum using flexible tape as described by (Oyeyemi *et al.,* 2012).

Dressing of the bucks is usually concluded within 1 hr of slaughter and testicles removed from the dressed carcass. The right

and left testes were incised and removed from the scrotum. The epididymis was separated from the testes and sperm collected using the retrograde flushing method as described (Cary *et al.,* 2004, Bertol *et al.*, 2013) with modification of sperm collection at the corpus-cauda junction.

Epididymal semen collection and evaluation

Semen was collected using two different flushing methods with Oviplus semen extender (Minitube 2015) with or without Normal Saline. OviPlus is a liquid concentrate for the preparation of 200 ml ready to use extender in which semen of small ruminants can be preserved. OviPlus contains antibiotics (Minitube 2015).

In the first method, each epididymis was flushed using the retrograde flushing method as described by Cary *et al.* (2004) and Bertol *et al.* (2013). The collection involved infusion of normal saline (1ml) into the lumen of the vas deferens using a 21 gauge syringe and needle of pre-constituted Oviplus® extender and 1 ml of normal saline (total of 2 mls infused). The epididymis was then incised at the corpus-cauda junction. The content of the epididymis (extended semen) was collected into a clean test tube through the incision.

In the second method, 2mls of Oviplus® extender was infused into the lumen of the vas deferens and collected through an incision at the corpus-cauda junction as described in the first method. The 2 collection methods were used in a random and systematic manner for each successive buck sampled, alternating between the 2 methods.

The testes were labeled in collecting bags and transported at +5 °C to the Andrology Laboratory of the Department of Theriogenology, University of Maiduguri and epididymal sperm collected and analyzed within 6 h of slaughter.

After the epididymal sperm was collected, the epididymis, *Tunica albuginea*, and facia were separated and weighed using electronic weighing balance (XY Electronic Balance; XY2000JB). The right and left testicular length (cm) were measured using a flexible tape as described by (Toe *et al.*, 2000).

The paired epididymides length and weight were measured using a flexible tape and electronic weighing balance respectively. The caput, corpus and cauda epididymis were also separated and weighed.

Epididymal semen evaluation

The pH of the sperm was determined using a pH meter. Spermatozoa motility and morphology were determined according to methods described by Gillan *et al.* (2004). A drop of the extended epididymal semen was pipetted onto a clean, prewarmed slide, to determine microscopic gross semen motility. The drop was covered by a slip and viewed under a field microscope at x40 magnification.

For determination of epididymal sperm concentration, epididymal semen was diluted into 1:10 with buffered-formal saline. One part of the 1:10 dilution was added to 9 parts of formal-buffered saline to obtain 1:100 dilution. The epididymal semen concentration was determined using the hemocytometer method (Bearden *et al.,* 2004). The mixture was dropped and allowed to spread under the cover slip, placed tightly on the hemocytometer. The cells were allowed to settle and then counted at x40 magnification. The cells were counted diagonally from top left to right bottom in 5 small squares of the improved Neubauer hemocytometer.

Percentage live epididymal sperm cells and abnormalities were determined according to the method described by (Bearden *et al.,* 2004). A thin smear drop of the epididymal semen sample was made on clean grease-free glass slides and stained with Eosin-Nigrosin stain before a smear was made. Thereafter, sperm cells were counted using

Table 1: Morphometric dimensions of the testis and epididymis and characteristics of semen collected using oviplus® semen extender with and without normal saline in sahel bucks

Parameters	Oviplus® + Normal Saline (n=60)	Oviplus® only (n=60)	P-value
Weight of bucks (Kg)	19.47 ± 2.06	19.11 ± 1.30	0.398
Scrotal circumference (Cm)	20.93 ± 1.69	20.36 ± 1.65	0.171
Weight of left and right Testis (Kg)	52.91 ± 12.61	52.86 ± 8.02	0.984
Length of left and Right Testis (Cm)	6.45 ± 0.89	6.18 ± 0.34	0.099
Weight of Right and Left Testicular Parenchyma (g)	49.05 ± 11.48	49.02 ± 7.59	0.991
Weight of Right and Left Testicular *Tunica albuginea* (g)	3.26 ± 0.67	3.14 ± 0.57	0.441
Weight of Right and Left Epididymis (g)	7.86 ± 1.63	7.81 ± 1.49	0.901
Length of Right and Left Epididymis (g)	11.69 ± 1.35	11.54 ± 0.74	0.567
Weight of Right and Left Caput Epididymis (g)	3.86 ± 0.80	3.70 ± 0.77	0.411
Weight of Right and left Corpus of Epididymis (g)	1.18 ± 0.88	1.05 ± 0.30	0.220
Weight of right and left Cauda Epididymis (g)	3.11 ± 0.71	3.01 ± 0.65	0.521
Volume for Right and Left Testis	51.08 ± 10.25	52.67 ± 7.79	0.477
Motility (%)	75.08 ± 14.98	83.88 ± 5.51	0.004
pH	5.55 ± 0.10	5.62 ± 0.06	0.004
Concentration ($\times 10^6$ spermatozoa/mL)	338 $\times 10^6$ ± 196	1760 $\times$ 10^6 ± 399	0.000
Live spermatozoa (%)	90.51 ± 6.70	92.37 ± 5.27	0.083
Abnormality (%)	3.15 ± 3.00	2.12 ± 2.37	0.156

values ($P<0.05$) are significantly different

light microscope at x100 magnification for live and dead ratio. For the semen abnormalities, thin smear of the semen sample were made on clean glass slides, fixed with buffered-formal saline and examined at x100 magnification.

The data generated on left and right testicular and epididymal morphometry as well as spermatozoa characteristics were pooled and subjected to descriptive statistics using SPSS (Version 16). Student t-test was used to determine the difference between parameters in Oviplus combination with normal saline and Oviplus only groups. Correlation analysis and their significance were conducted for the 2 groups and the result presented in tables. Values at $p<0.05$ were considered significant.

RESULTS

The morphometric dimensions of the testis and epididymis and characteristics of sperm collected using Oviplus semen extender with and without normal saline in Sahel Bucks are shown in Table 1. Furthermore, the weight of the Sahel bucks, the scrotal circumference, weight, length and volume of left and right testicular and epididymal parameters, pH concentration and the proportion of live and abnormal spermatozoa are shown in table 1.

Table 2a: Matrix of correlation coefficients (*r*) of body weight, scrotal, testicular and epididymal morphometric parameters with epididymal sperm motility and concentration correlates collected using oviplus® semen extender and normal saline

	W	SC	TW	TL	TP	TTV	EW	EL	COEW	CDEW	TV	MOTILITY	CONC
W	1.000	0.347	.363*	0.035	.364*	.413*	.393*	0.187	0.168	0.321	0.227	0.064	0.196
		0.060	0.048	0.854	0.048	0.023	0.032	0.323	0.376	0.084	0.227	0.738	0.298
SC	0.347	1.000	.779**	.404*	.778**	.781**	.629**	0.334	.557**	.394*	.757**	0.102	0.101
	0.060	.	0.000	0.027	0.000	0.000	0.000	0.072	0.001	0.031	0.000	0.591	0.596
TW	.363*	.779**	1.000	.634**	.995**	.694**	.824**	.578**	.672**	.623**	.892**	0.116	0.103
	0.048	0.000	.	0.000	0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.542	0.588
TL	0.035	.404*	.634**	1.000	.620**	.453*	.552**	.660**	.592**	.472**	.518**	0.050	-0.281
	0.854	0.027	0.000	.	0.000	0.012	0.002	0.000	0.001	0.008	0.003	0.792	0.132
TP	.364*	.778**	.995**	.620**	1.000	.677**	.832**	.571**	.657**	.636**	.884**	0.155	0.106
	0.048	0.000	0.000	0.000	.	0.000	0.000	0.001	0.000	0.000	0.000	0.415	0.577
TTV	.413*	.781**	.694**	.453*	.677**	1.000	.615**	0.277	.527**	.448*	.715**	0.168	0.153
	0.023	0.000	0.000	0.012	0.000	.	0.000	0.139	0.003	0.013	0.000	0.374	0.418
EW	.393*	.629**	.824**	.552**	.832**	.615**	1.000	.482**	.699**	.880**	.770**	0.165	-0.030
	0.032	0.000	0.000	0.002	0.000	0.000	.	0.007	0.000	0.000	0.000	0.383	0.873
EL	0.187	0.334	.578**	.660**	.571**	0.277	.482**	1.000	.395*	.436*	.389*	-0.175	-0.228
	0.323	0.072	0.001	0.000	0.001	0.139	0.007	.	0.031	0.016	0.034	0.355	0.227
COEW	0.168	.557**	.672**	.592**	.657**	.527**	.699**	.395*	1.000	.510**	.550**	0.021	-0.198
	0.376	0.001	0.000	0.001	0.000	0.003	0.000	0.031	.	0.004	0.002	0.914	0.295
CDEW	0.321	.394*	.623**	.472**	.636**	.448*	.880**	.436*	.510**	1.000	.590**	0.128	-0.114
	0.084	0.031	0.000	0.008	0.000	0.013	0.000	0.016	0.004	.	0.001	0.500	0.549
TV	0.227	.757**	.892**	.518**	.884**	.715**	.770**	.389*	.550**	.590**	1.000	0.156	0.057
	0.227	0.000	0.000	0.003	0.000	0.000	0.000	0.034	0.002	0.001	.	0.409	0.766
MOTILITY	0.064	0.102	0.116	0.050	0.155	0.168	0.165	-0.175	0.021	0.128	0.156	1.000	.450*
	0.738	0.591	0.542	0.792	0.415	0.374	0.383	0.355	0.914	0.500	0.409	.	0.012
CONC	0.196	0.101	0.103	-0.281	0.106	0.153	-0.030	-0.228	-0.198	-0.114	0.057	.450*	1.000
	0.298	0.596	0.588	0.132	0.577	0.418	0.873	0.227	0.295	0.549	0.766	0.012	

*Correlations (*r*) are significant at $P < 0.05$

Table 2a and 2b show the correlation coefficients and significance Oviplus semen extender alone and in combination with normal saline respectively. The results of group 1 (Table 2a) clearly showed that the weights of the bucks as well as the testicular and epididymal parameters were significantly positively correlated with each other. However, the morphometric parameters were not correlated with sperm concentration and motility. Motility and concentration were significantly positively correlated in group 1 (r= 0.450; P=0.012). Similarly, results of group 2 (Table 2b) indicated that the weights of the bucks as well as the testicular and epididymal parameters were significantly positively correlated with each other. The parameters studied that were significantly positively correlated with epididymal sperm concentration were: Scrotal circumference (r = 0.602; P = 0.000), Weight of left and

Table 2b: Matrix of correlation coefficients (*r*) of body weight, scrotal, testicular and epididymal morphometric parameters with epididymal sperm motility and concentration correlates collected using oviplus® semen extender only

	W	SC	TW	TL	TP	TTV	EW	EL	COEW	CDEW	TV	MOTILITY	CONC
W	1.000	.602**	.412*	.477**	.407*	0.318	.377*	0.034	0.158	.372*	0.295	0.061	1.000
		0.000	0.013	0.003	0.014	0.059	0.024	0.845	0.357	0.026	0.081	0.750	
SC	.602**	1.000	.817**	.650**	.817**	.448**	.547**	0.327	0.324	.469**	.585**	0.155	.602**
	0.000		0.000	0.000	0.000	0.006	0.001	0.052	0.054	0.004	0.000	0.415	0.000
TW	.412*	.817**	1.000	.808**	.995**	.633**	.593**	.519**	.424**	.509**	.789**	0.129	.412*
	0.013	0.000		0.000	0.000	0.000	0.000	0.001	0.010	0.002	0.000	0.496	0.013
TL	.477**	.650**	.808**	1.000	.802**	.545**	.689**	.567**	.411*	.627**	.726**	-0.049	.477**
	0.003	0.000	0.000		0.000	0.001	0.000	0.000	0.013	0.000	0.000	0.798	0.003
TP	.407*	.817**	.995**	.802**	1.000	.573**	.601**	.512**	.436**	.524**	.809**	0.103	.407*
	0.014	0.000	0.000	0.000		0.000	0.000	0.001	0.008	0.001	0.000	0.590	0.014
TTV	0.318	.448**	.633**	.545**	.573**	1.000	0.285	.329*	0.112	0.242	.348*	0.193	0.318
	0.059	0.006	0.000	0.001	0.000		0.093	0.050	0.514	0.154	0.038	0.307	0.059
EW	.377*	.547**	.593**	.689**	.601**	0.285	1.000	.536**	.554**	.950**	.379*	-0.031	.377*
	0.024	0.001	0.000	0.000	0.000	0.093		0.001	0.000	0.000	0.022	0.870	0.024
EL	0.034	0.327	.519**	.567**	.512**	.329*	.536**	1.000	0.194	.482**	0.289	0.138	0.034
	0.845	0.052	0.001	0.000	0.001	0.050	0.001		0.257	0.003	0.088	0.469	0.845
COEW	0.158	0.324	.424**	.411*	.436**	0.112	.554**	0.194	1.000	.490**	.426**	-0.098	0.158
	0.357	0.054	0.010	0.013	0.008	0.514	0.000	0.257		0.002	0.010	0.608	0.357
CDEW	.372*	.469**	.509**	.627**	.524**	0.242	.950**	.482**	.490**	1.000	.376*	-0.161	.372*
	0.026	0.004	0.002	0.000	0.001	0.154	0.000	0.003	0.002		0.024	0.395	0.026
TV	0.295	.585**	.789**	.726**	.809**	.348*	.379*	0.289	.426**	.376*	1.000	-0.248	0.295
	0.081	0.000	0.000	0.000	0.000	0.038	0.022	0.088	0.010	0.024		0.187	0.081
MOTILITY	0.061	0.155	0.129	-0.049	0.103	0.193	-0.031	0.138	-0.098	-0.161	-0.248	1.000	0.061
	0.750	0.415	0.496	0.798	0.590	0.307	0.870	0.469	0.608	0.395	0.187		0.750
CONC	1.000	.602**	.412*	.477**	.407*	0.318	.377*	0.034	0.158	.372*	0.295	0.061	1.000
		0.000	0.013	0.003	0.014	0.059	0.024	0.845	0.357	0.026	0.081	0.750	

*Correlations (*r*) are significant at P < 0.05

right Testis (r = 0.412; P = 0.013), length of left and right testis (r = 0.477; P = 0.003), weight of right and left testicular parenchyma (r = 0.407; P = 0.014), weight of right and left epididymis (r = 0.377; P = 0.024) and weight of right and left cauda epididymis (r = 372; P = 0.026). However, none of the parameters was significantly correlated with motility of epididymal sperm collected using Oviplus® alone.

Key for TABLES 2a and 2b:

W:	Weight of Buck (Kg)
SC:	Scrotal Circumference (Cm)
TW:	Weight of Testes (Kg)
TL:	Length of Testes (Cm)
TP:	Weight of Testicular Parenchyma (g)
TTV:	Weight of *Tunica vaginalis* (g)
EW:	Weight of Epididymis (g)
EL:	Length of Epididymis (g)
COEW:	Weight of Corpus Epididymis (g)
CDEW:	Weight of Cauda Epididymis (g)
TV:	Volume of Testes (mLs)
MOILITY:	Epididymal Sperm Motility (%)
CONC:	Epididymal Sperm Concentration $\times 10^6$ spermatozoa/mL

DISCUSSION

An evenly matched and homogenous group of Sahel bucks were used in the current study. This was apparent in the sameness ($P>0.05$) of the Sahel buck weight, scrotal circumference, weight, length and volume of left and right testicular and epididymal parameters in the Oviplus + Normal Saline compared with the Oviplus® only group.

However, the epididymal sperm pH was significantly lower ($P < 0.05$) in the Oviplus® + Normal Saline group compared with the group Oviplus semen extender alone. However, pH in both groups was within normal range as reported previously. Normal semen pH in bucks ranged from 5.9 to 7.3 (Garner and Hafez, 2000). Previous studies suggested that the initial pH of an extender was crucial to sustain high sperm motility. Liu *et al.* (2016) found that increasing the initial extender pH from 6.04 to 6.25 improved sperm motility. Thus, the significantly higher pH of the Oviplus® only group indicates likelihood of better survivability and quality of the extended sperm.

The epididymal sperm concentration in group 1 (338×10^6 /mL) was lower than the normal range 2000-3000 $\times 10^6$/mL reported by Garner and Hafez (2000). Nevertheless, the five-fold increase in the epididymal sperm concentration in the Oviplus ® only (1760×10^6 /mL) suggested that it was a much more effective flushing method. This was important because the aim of epididymal sperm recovery is often salvage of the male gamete. The result of the current study agree with a previous study by Abba and Igbokwe (2015) where they reported a normal cauda epididymal sperm head count of $1940 \pm 670 \times 10^6$/mL in homogenized testicular and cauda epididymal tissues from 66 Sahel bucks. Furthermore, the result of the current study showed that progressive forward spermatozoa motility was also significantly higher in the Oviplus only group indicating better quality harvest of epididymal sperm.

The very high proportion of live spermatozoa (>90 %) in both groups and very low proportion of abnormal spermatozoa indicates that the epididymal sperm collection was successful. In addition, both parameters were not significantly different ($P>0.05$) between the 2 groups indicating the usefulness of normal saline when epididymal sperm is needed to be collected urgently but with insufficient Oviplus® semen extender.

In the current study, the weights of the bucks as well as the testicular and

epididymal parameters were significantly positively correlated with each other. This agrees with the report of Abba and Igbokwe (2015) who similarly found that the weight of Sahel bucks correlated (r = 0.49–0.70) with testicular and epididymal size variables (Scrotal Circumference, Scrotal length, Testicular weight and gonadosomatic index) although they did not correlate significantly (P > 0.05) with epididymal sperm counts (Abba and Igbokwe, 2015). It was also motility and concentration were significantly positively correlated in the group with Oviplus® and Normal Saline (r= 0.450; P=0.012).

Similarly, results from epididymal sperm collected using Oviplus alone indicates that the weights of the bucks as well as the testicular and epididymal parameters were significantly positively correlated with each other. In addition and unlike in the group with Normal Saline, some of the morphometric parameters were correlated with sperm concentration. These agrees with previous studies which correlated semen collected using artificial vagina or electroejaculator with body weight testicular and epididymal parameters (Daudu 1984, Agga *et al.*, 2011, Oyeyemi *et al.*, 2012). However, none of the parameters was significantly correlated with motility of epididymal sperm collected using Oviplus alone probably because the epididymal spermatozoa are part of the extragonadal reserve. Whereas, semen is the liquid ejaculate containing spermatozoa in suspension with the male gametes and secretions from the male accessory glands (Garner and Hafez, 2000).

observed in the current study that only

CONCLUSION

Epididymal sperm recovery was successful using Oviplus® semen extender with or without normal saline in Sahel Bucks. However, the spermatozoa concentration and motility were significantly better using Oviplus semen extender alone and is hence the recommended method for epididymal sperm recovery in Sahel bucks.

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