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Research Paper

Comparative Study on Characteristics of Semen Collected from Sahel Bucks using Slicing Swim up and Mincing Flushing Technique

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This study was conducted to determine morphometric dimensions of the testis and semen characteristics of Sahel bucks using slicing swim up (SST) and mincing flushing (MFT) technique. A total of 40 matured bucks slaughtered at Maiduguri Central Abattoir were selected for the study. The scrotal circumference was measured with tape prior to slaughter. The testis was collected within 30 min of slaughter and transported to the artificial insemination Laboratory University of Maiduguri on ice-pack. The weight and length of the testis, epididymal weight and length, and weights of tunica albuginea and testicular parenchyma were measured, while SST and MFT were used to harvest epididymal spermatozoa. There was no significant difference ($p > 0.05$) between the right and left testes. The SST method recorded motility of 83 ± 1.18 and MFT recorded 79.50 ± 1.01. For sperm concentration, SST was 82 ± 1.28 and MFT was 80 ± 1.27 respectively, there was significantly different ($p<0.05$) in motility while for concentration, was not significantly difference ($p>0.05$). Live sperm of 87.48 ± 0.10 and 84.40 ± 0.88 was obtained using SST and MFT respectively. There were no significant differences between dead sperm recorded by SST and MFT ($p>0.05$). The pH of the semen 5.46 ± 0.01 using SST was not significantly different from 4.55 ± 0.2 using MFT. It was therefore concluded from this study that both methods are efficient for sperm recovery. The STT allows for better population of motile and live sperm than MFT. It is therefore recommended that further studies should be carried out to determine the fertilizing capacity through Artificial insemination.

Keywords: Sahel bucks, testes, epididymis, slicing and swim up, mincing and flushing

INTRODUCTION

Goats are the most important domestic animals in tropical livestock production. They are kept as a source of income, social human means of livelihood mostly by the rural small scale farmers (Alphonsus *et al.*, 2010). In goat husbandry setting, male fertility generally has the greater influence on the herd performance than the fertility of individual does (Yoseph, 2004).

Morphometric dimensions are the changes that occur

during growth of the testes from birth to maturity (Gautane *et al*, 2016). 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 bulls (Togun and Egbunike, 2006). Leal *et al*. (2004) and Hassan *et al.* (2009) reported that testicular parameters should suggest the level of sexual activity and semen production from the daily sperm production potential. Oyeyemi *et al. (*2012) reported that a strong correlation exists between scrotal circumference and testes. More so Abba and Igbokwe, (2015) reported that scrotum and testis have positive correlation with one another and cauda epididymal sperm counts in Sahel goats examined post slaughter. The growth and development of the testicular organs in various species of domestic animals has been well documented (Orlu and Egbunike, 2010; Ibrahim *et al*., 2012). Post mortem male gonads or testicles collected from slaughtered matured bucks, is a viable option for sperm recovery for basic research in preparation for germplasm conservation (Gautane *et al*., 2016). Collection and preservation of epididymal spermatozoa is an important method for the propagation and conservation of animal's specimens with high genetic values after getting injured or from dead animals (Blash *et al.,* 2000; Cary *et al.,* 2002) endangered species of animals (Santiago-Moreno *et al*., 2006) and pets (Leibo and Songsasen, 2002).

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 (Paulenz *et al*., 2002). Various types of semen extenders exist and many of them are commercially available (Yamashiro *et al.,* 2006, Kasimanickam *et al.,* 2011). Sperm from the epididymis has been productively used for artificial insemination (AI) and for in vitro production of embryos in goat and several species (Barati *et al*., 2009). Gautane *et al. (*2016) reported the recovery of sperm cells using two methods of sperm recovery, the slicing swim up and the mincing and flushing technique in non descript native goats in the Philippines with more sperms cells in volume and concentration using mincing and flushing technique. The aim of the study is to determine the recovery of viable spermatozoa in the epididymis of Sahel Bucks using two different methods of sperm recovery; the mincing and flushing technique and slicing and swim up technique.

MATERIALS AND METHODS

Study animal and sampling technique

The study was conducted in Maiduguri, Borno State, Nigeria, situated at an altitude of 354 m 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. The study involves a total number of forty mature Sahel bucks weighing averagely 20 kg were used. Most animals were between 1-3 years and aging was determined by dentition (Matika *et al.,* 1992). Purposive sampling technique was used for this study. Testicles were collected from bucks brought to the slaughter house (abattoir) and were randomly selected from the ones destined for slaughter. The scrotal circumference was measured using a non-stretchable tape at the greater circumference of the scrotum (Oyeyemi *et al*., 2012). The testes of slaughtered animals were collected after dressing the carcass through incising the scrotum where the left and right testes were removed and labeled in collecting bag separately and transported to the Artificial Insemination Laboratory, University of Maiduguri laboratory immediately.

Epididymal semen collection

Semen were collected using two methods of sperm recovery the mincing and flushing and slicing and swim up technique, regardless of right and left, one testicle from each pair was processed using slicing and swim up technique, while the other one was processed using mincing and flushing technique. As described by Ehling, (2006).

Oviplus semen extender was used to preserve the semen until assay. Oviplus a liquid concentrate for the preparation of 200 ml ready to use extender containing antibiotics in which semen can be preserved was used. 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 (Minitube, 2005).

In the first method (Slicing and swim up technique) the parietal tunic was removed and the tail (caudal) of the epididymis was sliced longitudinally half way using sterile razor blade and immediately drop in to a 50 ml volume Petri dish containing 15 ml of oviplus solution. The solution was allowed to stand for 5 – 10 min to allow epididymal sperm to swim- up to the upper part of the oviplus solution. The flushed solution was emptied and transferred in a 10ml conical tube as described by Ehling, (2006).

The second method (Mincing and flushing technique) the cauda epididymis was minced in to 4 – 8 pieces in a sterile Petri dish and the exposed surface areas were flushed with oviplus solution. The solution was allowed to stand for 5 – 10 min, to allow the epididymal sperm to move out by themselves. The flush solution was emptied and transferred in to a clean conical tube as described by Ehling, (2006).

Evaluation of semen characteristics

The motility of spermatozoa was determined by method described by (Mamuad *et al*., 2005). A droplet of about 10 microliters of the sperm suspension (extended semen) was placed in a clean pre-warmed (37^{o}C) microscope slide using a Pasteur pipette. A cleaned pre-warmed cover slip was later placed gently to avoid air bubble formation. At least, nine (9) microliters of the extended semen microscopic fields were examined in other to evaluate the percentage of motile sperm it was viewed at X40 magnification. The sperm concentration was determined according to method described by Melisa, (2004). Serial dilution was made by diluting 1 part semen with 9 part formal buffered saline to make a 1:10 dilution, then took 1 part of the 1:10dilution and added 9 part of formal-buffered saline to make a 1:100 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. 10 ul was then pipette into the space between the cover slip and the microscope glass slide which then spread by capillary action to fill up the chamber. Five large boxes were used within which the sperm cells were counted. The counting chamber was then mounted on the microscope and viewed using x40 objective lens. For the determination of sperm cell morphology 0.1 ml 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 viewed under the microscope at x 1000 magnification using oil immersion to view the sperm cells using the battlement method of counting and viewed under the microscope at X100 magnification.

Morphometric dimension

Testicular morphometry: Scrotal circumference was evaluated using a measuring tape.

Paired testes weight

The testes and epididymis were separated free of adhering connective tissue and fats. The left and right testes and epididymis were measured separately and their weight recorded. Corpus and cauda epididymis were identified and their weight were taken and recorded separately using a general purpose weighing balance.

Tunica albuginea weight

The tunica albuginea was then peeled off from each testis after cutting the testes in two halves and the weight value recorded.

Statistical analysis

All data generated were reported as mean ± SEM and significant differences were statistically analyzed by Student's t-test at ($p<0.005$)

RESULTS AND DISCUSSION

The semen characteristics using the slicing and swim-up and the mincing and flushing technique reveals that there is significant differences sperm motility ($p<0.05$), semen pH ($p<0.05$), live and dead sperm ($p<0.05$) whereas there was no significant differences in sperm concentration ($p>0.05$). From (Table 1), the dimension of scrotal circumference recorded in this study was 19.55 ± 0.19 which was similar to the findings of Bukar *et al. (*2017) who reported similar finding in bucks slaughtered at Maiduguri central abattoir with a scrotal circumference of 20.93 ± 0.19. Table 2 shows for morphometric dimensions, there was no significant difference between the left and right variables ($p> 0.05$). Table 3 shows the % abnormality of sperm cells in Sahel Bucks used in the study, the result reveals that there was no significant differences ($p>0.05$) in both S&ST and M & FT, except for detached head that showed significant difference ($P< 0.05$) (Figure 1). The weight of the right and left testis recorded in this study was 60.44 ± 1.84 and 61.79 ± 1.69 respectively, there was no significant difference between the right and left testes ($p>0.05$), the values obtained in this study is greater than that of Oyeyemi *et al*. (2012) who reported the mean testis weight was 52.16 ± 10.29, Bukar *et al. (*2017) recorded 51.95 ± 12.15 and 53.83 ± 13.20, Shujat *et al. (*2016) reported that the mean weight of right and left testis was 35.96 and 69.80 and it shows significant differences ($p<0.05$). On the other hand Raji *et al. (*2012) stated that mean testicular at age one, two, and three years were 55.00 ± 2.87, 77.28 ± 1.84 and 103.01 ± 2.23 gm respectively in red Sokoto bucks, however the values in their study was higher than the present study, this could be attributed to age, breed differences and nutritional level. The mean length of the right and left testis in this study is 9.79 ± 0.24 and 9.81 ± 0.22, there was no significant difference ($p<0.005$). The results of this study is higher than that reported by Shujat *et al.* (2016) who reported the mean testis length of pubertal teddy bucks was 6.97 cm and 7.14 cm. Nimase, (2008) reported mean testicular length in pubertal bucks has 5.03 ± 0.13 which was lower than what was obtained in this present study. This difference may be due to season of sample collection.

The mean weight of the right and left epididymis recorded in this study was 7.31 ± 0.17 and 7.37 ± 0.16, there was no significant difference between the means ($p<0.05$). The present study recorded higher values when compared with the values obtained by Wares, (2013) who recorded mean epididymal weight as 6.1 ± 0.0 gm in

Table 1. Scrotal Circumference of Sahel bucks slaughtered at Maiduguri Abattoir.

Dimension	Mean ± SEM	p-value
Scrotal Circumference	19.55 ± 0.19	0.19

Table 2. Morphometric dimension of Testis and Epididymis of Sahel Bucks Slaughtered at Maiduguri Abattoir.

Morphometric Dimension	Mean ± SEM		
	Right	Left	p-value
Weight of Testis	60.44 ± 1.84	61.79 ± 1.69	0.59
Weight of Epididymis	7.31 ± 0.17	7.37 ± 0.16	0.80
Weight of caput epididymis	1.11 ± 0.03	1.14 ± 0.03	0.48
Weight of corpus epididymis	3.14 ± 0.14	3.23 ± 0.14	0.65
Weight of Cauda epididymis	3.11 ± 0.15	3.17 ± 0.14	0.77
Weight of testicular parenchyma	47.83 ± 1.82	45.78 ± 1.62	0.40
Weight of tunica Albuginea	3.86 ± 0.11	3.84 ± 0.10	0.89
Length of Testis	9.79 ± 0.24	9.81 ± 0.22	0.95
Length of epididymis	10.88 ± 0.16	10.85 ± 0.15	0.89
Volume of the Testis	51.89 ± 0.12	51.72 ± 0.13	0.34

Table 3. % Abnormality of sperm cells obtained using S&ST and M&FT.

Sperm Abnormalities	Method 1 (SST)	Method 2 (M&FT)	P VALUE
Detach head	4.93 ± 0.86	7.98 ± 0.97	0.02
Detach tail	4.83 ± 0.76	3.93 ± 0.54	0.34
Bent tail	3.45 ± 0.68	5.28 ± 0.90	0.11
Coiled tail	2.08 ± 0.42	2.98 ± 0.58	0.21
Narrow head	9.24 ± 1.02	8.90 ± 1.09	0.82

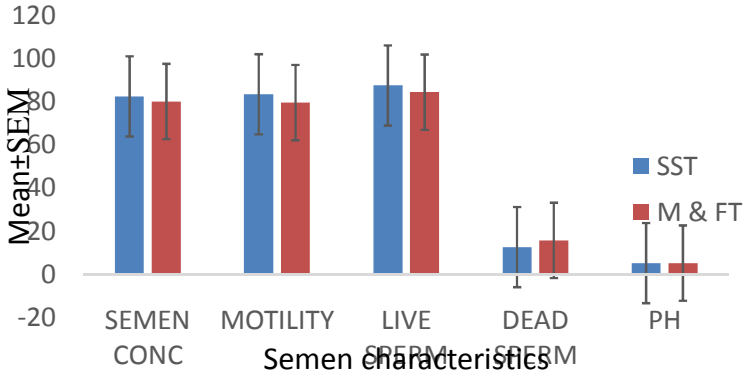

Figure 1. Semen characteristics of Sahelian bucks obtained using different methods of sperm recovery.

pubertal bucks. These discrepancies could be attributed to breed differences and time of sample collection. The mean length of the right and left corpus epididymis recorded in this study was 3.14 ± 1.4 and 3.23 ± 0.14, there was no significant difference ($p<0.05$). This is in agreement with the findings of Devi, (2013) who reported the mean length of corpus epididymis to be 3.80 ± 0.01.

For the Semen characteristics two methods of sperm recovery were employed the slicing and swim up technique and mincing and flushing technique. The present study recorded a percentage sperm motility of 83.38 ± 1.18 there was no significant difference between the two methods of recovery. Ehling *et al. (*2006) isolated ram epididymal sperm slicing the cauda epididymis and suspending it in a one step freezing medium; they recovered an average sperm motility of 79.7% and 93% acrosome intact sperm. Also a similar experiment was reported by Gautane, (2016) where native goat epididymal sperm were isolated by the slice and swim up technique.

The result of the present study reveals that the percentage of live sperm was 87.48 ± 0.10 using the slicing and swim up technique and 84.40 ± 0.88 using mincing and flushing technique, this indicates that there was a significant differences between the methods of sperm recovery ($p<0.05$) (Figure 1). This result is comparable with the findings of Gautane *et al.* (2016) who reported live sperm to be 88.9 ± 2.76 using slicing and swim up technique and 81.7 ± 2.8 using mincing and flushing method, the mean values were statistically significant ($p>0.005$). The present study recorded a sperm concentration of 82.43 ± 1.28×10^7 using slicing and swim up technique and 80.0 ± 1.37×10^7 using mincing and flushing method, however there was no significant differences between the two methods of recovery ($p>0.005$). This finding does not agree with that of Gautane et al. (2016) who reported higher concentration using mincing and flushing technique. The value of pH recorded in this study was 5.46 ± 0.01 using slicing and swim up technique and 4.55 ± 0.0 using mincing and flushing technique. There was no significant differences between the two methods of sperm recovery ($p<0.005$).

Conclusion

This study has provided information on the use of oviplus semen extender in maintaining the viability of sperm cells in Sahel bucks collected using slicing swim up and mincing and flushing technique in sahel bucks slaughtered in Maiduguri central Abattoir. The conclusion of this study is that both slicing swim up and mincing and flushing technique are acceptable methods of sperm recovery which enhances the viability of sperm cells.

Authors` Declaration

We declare that this study is an original research by our research team and we agree to publish it in the journal.

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