International Journal of Agricultural Technology 2017 Vol. 13(7.2):1645-1657
Available online http://www.ijat-aatsea.com
ISSN 1686-9141

Evaluation of Epididymal Sperm from Post-Mortem Derived Cauda Epididymides of Ram (*Ovis aries*)

Shesea Mae A. Marata[1*], Darlene Fe P. Castro[1], Lerma C. Ocampo[2,3], Marlon B. Ocampo[1,2] and Maureen B. Gajeton[2]

[1]College of Veterinary Science and Medicine, Central Luzon State University, [2]Reproductive Biotechnology and Physiology Unit, Philippine Carabao Center; [3]Department of Biological Sciences, College of Arts and Sciences, Central Luzon State University, Science City of Munoz, Nueva Ecija, Philippines 3120.

Shesea Mae A. Marata, Darlene Fe P. Castro, Lerma C. Ocampo, Marlon B. Ocampo and Maureen B. Gajeton (2017). Evaluation of Epididymal Sperm from Post-Mortem Derived Cauda Epididymides of Ram (*Ovis aries*). 13(7.2):1645-1657.

Cauda epididymides (CEs) collected post-mortem could be an important source of genetic material from male for conservation and use for assisted reproductive technologies. In this study, we characterize the epididymal sperm (ES) collected post-mortem from CEs of ram raised locally. Testicles (n=12) were sliced off from the donor ram post-mortem, placed in a styropore box at ambient temperature and transported to the laboratory within 2 hr. The mean weight of the testicle was 130.2 g with a mean length of 11.2 cm. The epididymis and scrotal sac had a mean weight of 11.5 g and 92.1 g, respectively. The CEs were detached from the testicle and the tail were sliced longitudinally and dropped into a 50-ml conical tube with 15 ml TRIS buffer medium. The ES was collected after 10 min, washed through centrifugation and measured. The mean head area was 380.3 μm^2 and tail length of 183.5 µm. The mean sperm volume was 0.92 ml, pH of 6.6 and a sperm concentration of $2.02x10^9$ cells/ml. Conventionally, the sperm motility rate was between 63-65%, whereas through CASA, the sperm motility reading was 65-69%. The mean progressive motility was 44.5%. The mean viability rate was 80.3% with 63.2% morphologically normal sperm. The results provided basic informations on the sperm head and tail morphometry, motility and viability of epididymal sperm collected post mortem from CEs of ram.

Keywords: Epididymal sperm, motility, viability, cauda epididymides, post mortem

Introduction

In the Philippines, sheep industry is gaining popularity for having more biological advantages compared to goats (Lambio *et al.*, 1988). Sheep is less destructive to crops, grazed in flocks hence, easier to manage, less discriminating, had a wider range of herbage preference, more resistant to

[*] **Coressponding Author:**SheseaMae A. Marata1; **E-mail address:** ocampomarlon29@yahoo.com

parasitism and respiratory diseases. Despite these advantages however, sheep industry also suffers from production losses including unexpected loss of breeding animals of high genetic value. This can be due to loss of libido, reproductive tract injury or death, and can be costly in terms of the potential loss of genetically valuable germplasm. ES is an important source of germplasm for animal genetic resource conservation. In situations wherein semen collection through standard procedures becomes problematic and/or impossible, the post-mortem recovery of ES can be the only viable option to preserve male gametes from animal of high genetic value or from endangered species. As such, measures should be undertaken to employ more efficient assessment/evaluation of reproductive parameters of breeding animals. For instance, morphological characteristics of spermatozoa could affect the ability to effect fertilization, therefore semen analysis is of high diagnostic value in assessing testicular and epididymal function, and should be considered a prerequisite for selecting superior breeding bucks (Enos *et al.*, 2014).

The post-mortem recovery of ES and their use in sheep has received much less attention than other domestic species (Kaabi *et al.*, 2003; Ehling *et al.*, 2006) despite the fact that it can be cryopreserved and used for artificial insemination, in vitro fertilization and related ARTs (Soler *et al.*, 2003, 2005, 2008; Fernandez-Santos *et al.*, 2006). In this study, evaluation of the sperm head and tail morphometry, motility and viability of ram ES were conducted to provide basic informations on ES quality which is needed for use in genebanking. Sperm morphology for example, has been identified as a characteristic that can be useful in the prediction of fertilizing capacity (Morales *et al.*, 2010). It is widely accepted that sperm morphology is a good indicator of fertility and it has been proposed that sperm quality may be related to subtle changes in sperm head morphology. The minimum acceptable standards are fair gross motility or 30 percent individual motility and 70 percent normal morphology (Schoenian, 2016).

Materials and Methods

Animals and reagents

Six matured non-descript rams (age > 3 yr) were used. The rams came from backyard raisers who sold it for slaughter (meat purposes), a practice common in the Philippines to supplement income for use of the family. Age estimation was based on animal dentition. The rams were temporarily maintained inside the abattoirs compound to recover before slaughter. These rams were not trained to artificial vagina and are used for natural mating.

All the chemicals used were of reagent grade and purchased from Sigma - Aldrich Chemical Company (St Louis, MO, USA) except for Tris-base (Promeg Corp., Madison, WI, USA). Tris-citric acid-lactose-raffinose buffer (TLB medium) was prepared a day before collection of cauda epididymides. A one liter preparation composed of 15.7 g Tris-hydroxymethyl amino methane, 8.8 g citric acid monohydrate, 14.1 g lactose, 25.4 g raffinose and gentamycin solution (50 µg/ml) using an ultra-pure water (Milli-Q, Integral 5). Before use, the medium was sterilized by filtration using a 0.2 µm syringe filter.

Testicle collection and transport

Scrotum intact testicles were cut immediately after the animal's death, covered in paper towels and placed in a styropore box at ambient temperature and transported to the laboratory within 2 hr (Fig. 1). Each testicle was individually weighed before and after detachment of CEs using an analytical balance. Length (cm) and circumference (cm) of each testicle were measured. The weight (grams) of the CEs was also taken for recording purposes.

Preparation of CEs and ES recovery

CEs were aseptically excised from the testicles of rams, washed aseptically and incised longitudinally using a sterile scalpel blade and placed in a 50 ml conical tube with 25 ml TLB medium for 30 min to allow ES to swim-up. Thereafter, the upper 2/3 of the medium were collected, transferred to a 15 ml conical tube, centrifuged at 4,000 rpm for 5 min to form a pellet and the volume assessed against the graduated lines in µl units (Hirai *et al.*, 2011). The acidity of the ES was examined using Brom Thymol Blue pH paper.

Sperm motility

Sperm motility evaluation was done through conventional means using an inverted microscope (Nikon Eclipse Tx10i) at 40-100x magnification and by using a computer assisted sperm analyzer (CASA; HTM-IVOS-Ultimate, Hamilton Thorne BioSciences, Beverly MA, USA). Briefly, a sample of semen was diluted with TLB medium and about10-20 µl was pipetted into a clean pre-warmed (37℃) microscope slide. A coverslip was carefully lowered into the sample, avoiding formation of air bubbles before examination. Visual motility was recorded using the imaging software (NIS elements) for at least ten widely-spaced fields to provide an estimate of percentage motility using the scoring system (Table 1). Through CASA, each sample were diluted (25×10^6 cells/ml)

in TLB medium and kept at 38℃ water bath pending analysis. Then pre-warmed (38℃) chamber slide (SC20.01FA; LejaR, Nieuw-Vennep, The Netherlands) was loaded with 10 µl sample, allowed to settle for 1 min on MiniThermc stage warmer before analysis. At least 5 fields per sample were selected and observed for motility or progressive motility and expressed in percentage.

Sperm concentration

The sperm concentration was determined using a haemocytometer. Briefly, the sperm sample was diluted at 1:200 (5 µl sperm sample + 995 µl saline solution) in an RBC pipette and the sperm counted in the central large area of the Neubauer haemocytometer which consists of 25 squares and each square consists of 16 smaller squares. The dimensions of the large central area of the Neubauer counting chamber are 1 mm (width) x 1mm (height) x 0.1 mm (depth) for a volume of 0.1 cubic millimeter (mm3) or 0.1 µl. Since the sperm concentration is expressed in number per cubic centimeter (cm3), the sperm count must be multiplied by a factor of 10,000. The sperm (n) was counted in five (5) squares. The sperm concentration was computed using the following formula:

Sperm concentration per ml = n x dilution factor x 50,000
= n x 200 x 50,000
= n x 10,000,000
= n x 10^7

Table 1. Scoring system for the motility of sperm cells (Mamuad *et al.*, 2005)

Motility(%)	Grade	Characteristics
91-100	Excellent Motility	90% or more of the spermatozoa is very rigorous in motion. Swirls caused by the movement of the sperm are extremely rapid and constantly going forward progressively.
76-90	Very Good Motility	Approximately 75-90% of the spermatozoa is in vigorous rapid motion. Waves and eddies form and rapidly but not so rapid as in excellent motility.
60-75	Good Motility	About 60-75% of the spermatozoa is in motion. Motion is vigorous but waves and eddies formed move slowly across the field of vision.
40-59	Fair Motility	From 40-55% of the sperm is in motion. The movements are largely vigorous or eddies are formed.
Less than 40	Poor Motility	Less than 40% of the sperm is in motion. The motion is not progressive but mostly weak and oscillary.
0	Zero Motility	No motility is discernable.

Morphological assessment

The ES viability (percentage live and dead sperm) and morphology (percentage with normal shape) were evaluated using a 1:2 dilution of semen sample and eosin-nigrosin stain. Briefly, 5 µl ES sample was dropped in a clean glass slide and added with 10 µl eosin-nigrosin stain before mixing gently using the tip of the pipette to minimized secondary abnormalities. After mixing, both edge of another glass slides was dipped into the mixture and smeared throughout another glass slide, forming a feather like smear (thin smear) and air-dried for 15-30 min. Nikon imaging software was used in examining the percentage viability and morphology of ES. The nigrosin stain created a dark background for the stained samples under the microscope whereas, the eosin stain penetrated the head of dead sperm due to the degradation of their cell membrane resulting to either pink or dark violet coloration. Live sperm appeared colorless or translucent Fig. 2). Percent live and dead sperm were determined from 10 separate fields under a magnification of 40x. The following equation was used in the percentage estimation:

$$\%\ Live = \frac{no.of\ live\ sperm\ counted}{total\ no.of\ sperm\ counted} x100$$

$$\%\ Dead = \frac{no.of\ dead\ sperm\ counted}{total\ no.of\ sperm\ counted} x100$$

$$\%\ Normal = \frac{no.of\ normal\ sperm\ counted}{total\ no.of\ sperm\ counted} x100$$

$$\%\ Abnormal = \frac{no.of\ abnormal\ sperm\ counted}{total\ no.of\ sperm\ counted} x100$$

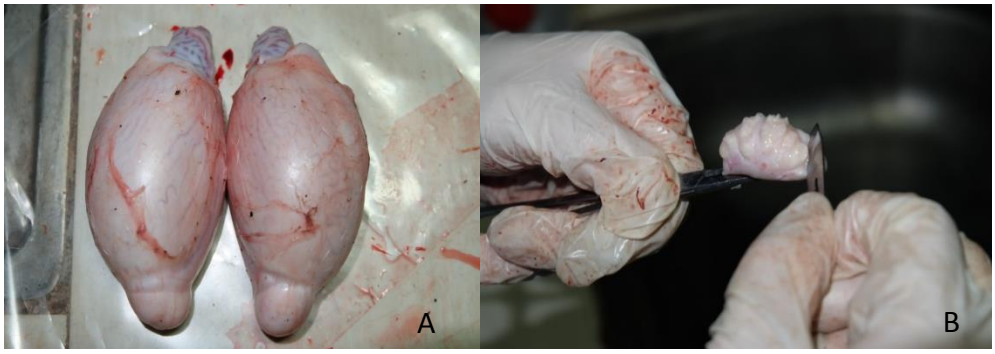

Fig. 1. Ram testicle collected at post mortem (A) and CEs slicing (B).

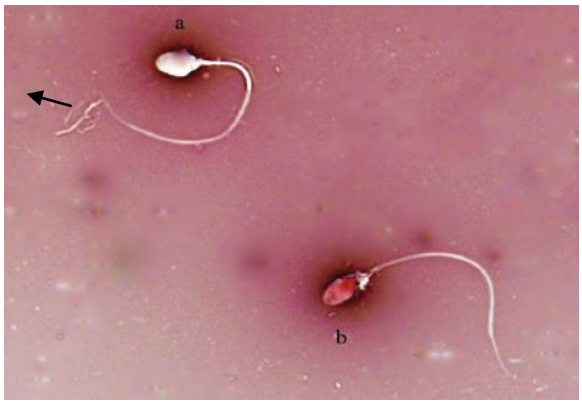

Fig. 2. Live (a) and dead (b) sperm after staining.

Sperm morphometry

The computer software (version 2.0) with the NIS-Elements application was used to calculate the following morphometric parameters: Area of sperm heads was determined using micron square (u^2) unit while the tail measurement was micron (u). A total number of 300 sperms was measured and analyzed for each sample (Hirai *et al.*, 2001).

Results and Discussion

The mean weight of right and left testicles (n=6 rams) were 132.8 gm and 127.6 gm, respectively. Also, the mean weight of CEs and scrotal sac were 11.5 gm and 92.1gm, respectively. The mean length of right and left testicles

was 11.1 cm. In Churra breed of ram, the mean testicular and epididymal weight reported was 191.11 gm and 34.4 gm, respectively (Kaabi *et al.*, 2008), higher than the values we obtained. In indigenous goats found in Nigeria, the testicular biometry reported was lower than non-descript rams we used (Raji *et al.*, (2008). For mature ram (≥ months old), a scrotal circumference of 32-40 cm could be considered ideal/satisfactory for breeding. Any measurement < 32 cm of the scrotum should not be used for breeding. Similarly, the weight of the testicle and the CEs does matter in relation to the volume and quality of semen produced (Curry *et al.*, 1996; Hassan *et al.*, 2009; Schoenian, 2016). Therefore, the scrotal circumference should be measured as it gives a good indication of rams breeding ability.

Table 2. Morphometry of ram testicles, CEs and scrotal sac.

Ram	Weight (gm)				Length (cm)	
No.	Right testicle	Left testicle	Epididymis tail	Scrotal sac	Right testicle	Left testicle
1	106.5	117.2	10.6	85.6	11.0	10.5
2	126.4	121.9	10.4	112.7	11.0	10.5
3	181.1	158.8	12.6	121.0	14.0	13.0
4	133.0	130.2	13.1	66.0	11.0	10.5
5	139.7	131.5	9.2	111.2	11.5	11.0
6	109.9	106.0	12.8	55.8	10.0	10.5
Mean	132.8	127.6	11.5	92.1	11.1	11.0

The basic parameters observed on the ES of rams was summarized in Table 3. The mean pH was 6.6 (6.4 – 7.0), sperm volume was 0.9 ml (0.7 – 1 ml), sperm concentration of 2.1 x 10^9/ml and 19.7 x 10^9/ml from right and left CEs, respectively. The % motility estimate through conventional means ranged from 63 - 64 %. Through CASA, the % motility reading was 77 -82 % with progressive motility rate of 30-32 % (Table 6).

The pH of CEs fluid is acidic and could have a profound effect on semen quality. In most mammalian species, the inhibition of ES motility is due to acidic pH, maintained by the presence of lactate (Babcock *et al.*, 1983; Carr *et al.*, 1985). In ram, ES motility is activated by dilution to alkaline solution and reached its peak at pH of around 7-8 (Gatti *et al.*, 1993b). In this study, the pH observed was between 6.4 – 7.0, still within the optimal range needed to support ES motility (Bartoov, *et al.*, 1980; Zhou *et al.*, 2015). Also, the mean sperm volume obtained was 0.9 ml using the slicing method. It was similar to the ES volume recovered using the slicing method in goat (Gautane *et al.*, 2016; Gajeton *et al.*, 2017) and ram (Kaabi *et al.*, 2003). Whereas, retrograde flushing

from ductus deferens through CEs yielded a mean ES recovery of 0.3 ml at 0 hr when the testicle was transported at AT and 0.4 ml at RT (Turri *et al.*, 2014). The differences in ES volume might reflect their different genetic potentiality and genetically superior bucks could produce higher volume of semen. Volume is an important criterion in semen evaluation to spermatogenic activity in adult male sheep, whereas in younger male sheep, testicular volume measurement is mainly important in assessing the onset of puberty or puberty development and it is sometimes used to evaluate testicular abnormalities such as cryptorchidism (Sultana *et al.*, 2013).

The sperm concentration obtained from right CEs (20.7 x 10^9/ml) was higher than left CEs (19.7 x 10^9/ml), which is similar to other studies. In Churra breed of ram, the mean ES concentration obtained was 1.4 x 10^9/ml (Kaabi *et al.*, 2003) and 35.0 x 10^9/ml in Angora goat (Ritar *et al.*, 1992). In Alpine bucks, the ES concentration obtained was 0.9 - 1.2 x 10^9/ml, suggesting that the yield of ES is also influenced by the breed (Turri *et al.*, 2014). The mean motility rate through conventional and CASA was 63.2 % and 80.0%, respectively with a mean progressive motility of 31.5 %. This findings were similar to Black Manchega ram (Garcia-Alvarez *et al.*, 2009) and 16 other breeds (Ehling *et al.*, (2006) where postmortem ES were collected through the slicing method and the CEs kept at ambient temperature. Sperm motility is an important requirements of fertile semen and a positive correlation between sperm concentration at semen collection, motility and viability has been well established.

Table 3. ES parameters observed from non-descript rams collected post-mortem and transported at ambient temperature .

Ram	Parameters						
	pH	Sperm volume		Sperm concentration*		% Motility	
No.	right	left	right	left	right	left	
1	7.0	1	0.5	31.4	36.8	70	65
2	7.0	0.7	0.7	31.7	26.4	60	65
3	6.5	1	1	14.2	15.3	70	65
4	6.4	1.5	1	13.3	8.9	60	60
5	6.4	0.8	0.8	12.9	10.7	65	60
6	6.4	1	1	20.8	20.1	60	65
Mean	6.6	1	0.8	20.7	19.7	64.2	63.3

Sperm volume (ml), sperm concentration (x 10^9/ml).

Our results showed a mean viability rate of 80.3 % with a mean normality rate of 63.2 % (Table 4). This was lower compared to the ES

collected from Black Manchega ram (Garcia-Alvarez *et al.*, 2009) but similar to Iberian red deer (Martinez *et al.*, 2008; Karja *et al.*, 2010). In related studies, ES viability in ram, red deer and mouflon decreases progressively as the time between the animals death and sperm collection increases (Aguado *et al.*, 1994; Garde *et al.*, 1994).

The mean ES abnormal rate observed was 36.9 %. The occurrence of a specific structural abnormality of sperm affecting the whole population or a high percentage of the populations might be associated with infertility. Sperm morphology is impaired by cold shock, rough semen handling, prolonged sexual inactivity, and abnormal heat or cold stress during sperm storage in the epididymis. Also, higher proportion of abnormal spermatozoa could be an indicator of genetically heritable fertility problems. Sperm cellular abnormalities are categorized as primary and secondary abnormalities. Primary abnormalities (eg., coiled tails, double forms (two heads, midpieces, or tails, abaxial midpieces (midpieces that attach to the head off-center), medusa formations (ciliated globular masses), abortive tails and spheroids (round headed sperm) are those that have occurred during spermatogenesis. Secondary abnormalities (eg., loose heads, droplets of cytoplasm on the tail or midpiece, bent tails, detached heads, and bodies in the sample other than spermatozoa (red blood cells, white blood cells, bacteria, squamous epithelial cells or spermatozoal clumps) are those that have occurred during the later stages of development, during ejaculation, or during the collection process (Herrick *et al.*, 1962). The percentage of morphological abnormalities in the semen with normal fertility should be less than 5% during the breeding season. If collections are made during the summer, the percentage of abnormalities can be expected to be higher. In this study, CEs collection was made during the summer months, explaining why the % ES abnormalities observed was more than 5%.

Table 4. ES viability assessment using eosin-nigrosin stain.

Ram No.	Parameters							
	Right (%)				Left (%)			
	Live	Dead	Normal	Abnormal	Live	Dead	Normal	Abnormal
1	89.0	11.0	62.9	37.1	84.3	15.7	65.4	34.6
2	87.0	13.0	71.0	29.0	82.7	17.3	70.1	29.9
3	94.3	5.7	58.9	41.1	92.0	8.0	72.8	27.2
4	65.7	34.3	49.4	50.6	68.0	32.0	58.2	41.8
5	76.0	24.0	63.3	36.7	70.7	29.3	51.8	48.2
6	71.0	29.0	63.3	36.7	82.7	17.3	70.7	29.3
Mean	80.5	19.5	61.5	38.5	80.1	20.0	64.8	35.2

In this study, the calculated mean head area of ES from right and left CEs were 37.92 and 38.14 μm^2, respectively. Also, the mean tail length of ES from both right and left CEs was 18.35 µm. The result was in conformity with the automatic evaluation of ram (29.7-31.3 μm^2) and pig (35.7 ± 3.74 μm^2) sperm head morphometry (Yaniz *et al.*, 2012; Kondracki *et al.*, 2017). In some breed of pig (27.2 ± 0.2 μm^2) and goat (25.5 ± 0.2 μm^2), the sperm head morphometry was smaller (Vicente-Fiel *et al.*, 2013). The tail length of ES from rams examined was shorter than pig (44.7 ± 1.8 μm^2) and Zebu (±35 µm) (Kondracki *et al.*, 2017). It has been suggested that spermatozoa with large and long heads are more fertile than those with smaller heads, and that this morphometric parameter could be an indicator of fertility and viability.

Table 5. Morphometry of ES head (μm^2) and tail (µm).

Ram No.	Right testicle		Left testicle	
	Head area	Tail length	Head area	Tail length
1	29.74	17.28	31.45	17.70
2	42.74	18.81	41.19	18.70
3	35.22	18.45	36.86	18.47
4	39.15	18.63	40.22	18.35
5	43.13	19.22	42.45	18.59
6	37.56	16.35	36.66	19.66
Mean	37.92	18.12	38.14	18.58

ES progressive motility is attained during epididymal maturation but the sperm does not become motile until it is released from the epididymis. Its assessment is important because it is an essential prognostic fertility factor, especially when the proportion of motile spermatozoa is below 40.0 %. Our results showed that the mean progressive motility of ES collected from individual non-descript rams had no difference with a mean of 31.5 %. It was higher compared to earlier reports on ram mean percentage progressive motility of 11.5 ± 6.9 % (Bohlooli *et al.*, 2012) but lower in Black Manchega, Churra and 16 other breed of rams (Kaabi *et al.*, 2003; Ehling *et al.*, 2006; Garcia-Alvarez *et al.*, 2009).

Table 6. Percent ES motility reading through conventional and CASA.

Ram No.	Conventional		CASA			
	Motility		Motility		Progressive Motility	
	Right	Left	Right	Left	Right	Left
1	70.0	65.0	64.2	69.1	28.5	27.9
2	60.0	65.0	93.6	99.1	13.6	29.3
3	70.0	65.0	62.2	76.6	28.0	25.3
4	60.0	60.0	67.3	83.1	31.0	43.5
5	65.0	60.0	79.9	66.2	40.3	24.6
6	60.0	65.0	99.1	98.0	50.9	33.9
Mean	64.2	63.3	77.7	82.0	32.1	30.8

In conclusion, the results of the study provided additional informations on the ES characteristics collected post mortem from non-descript rams. That, CEs maintained at ambient temperature for a few hours post mortem could still yield viable ES with potentials for use in related ARTs .

References

Aguado, MJ., Garde, JJ., Madriadano JM., Perez S., Garrido D. and Montero V. (1994). Congelacion post mortem de semen de epididimo de morueco. In: Proceedings of the 7th Jornadas Int Reprod Anim IA, Murcia, Spain, p. 283.

Babcock, D.F., Rufo G.A. and Lardy, H.A. (1983). Potassium-dependent increases in cytosolic pH stimulate metabolism and motility of mammalian sperm. Proc. Natl. Acad. Sci. USA, 50, 1327-1331.

Bartoov, B., Bar-Sagie, D. and Alayevsky, A. (1980). Effect of pH on ram semen collective motility driven by mitochondrial respiration. Intl J Androl., 3:602-612.

Bohlooli, S., Cedden, F., Bozo, Ş., Razzaghzadeh, S. and Pishjang, J. (2012). Correlation between conventional sperm assay parameters in cryopreserved ram semen. 3, 884–889.

Carr, D.W., Usselman, M.C. and Acott, T.S. (1985). Effect of pH, lactate, and viscoelastic drag on sperm motility: a species comparison. Biol Reprod., 30, 913-925.

Curry, M. R., Millar, J. D., Tamuli, S.M., And Watson, P. F. (1996). Surface area and volume measurements for ram and human spermatozoa. Biology of reproduction. P. 1327.

Ehling, C., Rath, D., Struckmann, C., Frenzel, A., Schindler, L. and Niemann, H. (2006). Utilization of frozen-thawed epididymal ram semen to preserve genetic diversity in Scrapie susceptible sheep breeds. Theriogenology, 66:2160-2164.

Enos, P. and Tapiwa, L. (2014). Caudal epididymal sperm morphology and body measurements relationships of the Gwembe dwarf bucks, 2(October), 346–351.

Fernandez-Santos, MR., Esteso, MC., Montoro, V., Soler, AJ. and Garde, JJ. (2006). Cryopreservation of Iberian red deer epididymal spermatozoa: effects of egg yolk, glycerol and cooling rate. Theriogenology, 66:1931-1942.

Garde, J., Perez, S., Aguado, MJ., Garrido, D., Ayllon, E., Montero, V. and Diaz, JD. (1994). Evolucion post mortem de la viabilidad y de la capacidad fecundante del semen de

ciervo y de muflon. In: Proceedings of the XIX Jornadas de la SEOC, Burgos, Spain, pp. 542-545.
Gatti, JL., Chevrier, C. and Paquignon, M. (1993b). Relation between internal pH (pHj) and motility of ram and boar spermatozoa. In Baccetti, B. (ed.), Comparative Spermatology 20 Years After. Raven Press, New York, USA, pp. 369-372.
Gajeton, MB., Tomas, JV., Cruz, KJ., Ocampo, MB. and Ocampo, LC. (2017). Assessment of post mortem epididymal sperm from non-descript buckling (*Capra hircus*). Entomol Appl Sci Lett., 4(1):20-25.
Garcia-Alvarez, O., Maroto-Morales, A., Garde, JJ., Esteso, MC., Ramon, M. and Soler, AJ. (2009). Sperm characteristics and in vitro fertilization ability of thawed spermatozoa from Black Manchega ram: electroejaculation and postmortem collection. Theriogenology, 72:160-168.
Gautane, JJ., Balagan, EJY., Ocampo, MB. and Ocampo, LC. (2016). Characteristics ofepididymal sperm recovered from slaughterhouse derived testes of nondescript / native goats in the Philippines, 12(2), 215–228.
Hassan, MR., Pervage, S., Ershaduzzaman, M. and Talukder, MAI. (2009). Influence of age on the spermiogramic parameters of native sheep, 7(2), 301–304.
Hirai, M., Boersma, A., Hoeflich, A., Wolf, E., Ller, RA. and Braun. (2001). Objectively measured sperm motility and sperm head, 22(1), 104–110.
Herrick, John, and H.L. Self. 1962. Evolution of fertility in the bull and boar. Iowa state university press. Pp. 17-40; 59-68; 99-101.
Kaabi, M., De Paz, P., Alvarez, M., Anel, E., Boixo, JC. and Roussi, H. (2003). Effect of epididymis handling conditions on the quality of ram spermatozoa recovered postmortem. Theriogenology, 60:1249-1259.
Karja, NWK., Respaty, EMA., Nuraini, I., Prihatno, SA. and Gustari, S. (2010). Characteristics of frozen-thawed epididymal spermatozoa and refrigerated storage of ram spermatozoa. J Indonesian Trop Anim Agric., 35:63-67.
Kondracki, S., Wysokinska, A., Kania, M. and Gorski, K. (2017). Application of two staining methods for sperm morphometric evaluation in pig. J Vet Res., 61:345-349.
Lambio, AL., Parawan, OO., Sevilla, CC., Quirante, V. and Villar, E. (1988). Sheep Production Systems (Philippines). Los Banos, Laguna: PCARRD, 1988. 152p.
Mamuad, FV., Venturina, EV. And Saito, H. (2004). Collection, Processing and HandlingBuffalo Semen. Water Buffaloes and Beef Cattle Improvement Project (WBBCIP). AJoint-JICA Assisted Project of the Philippine Carabao Center and the Bureau of Animal Industry, Philippines.
Martinez, F., Martinez-Pastor, F., Alvarez, M., Fernandez-Santos, MR., Esteso, MC. and De Paz, P. (2008). Sperm parameters on Iberian red deer: electroejaculation and post mortem collection. Theriogenology, 70:216-226.
Morales, MA., Ramon, M., Alvarez, GO., Soler, AJ., Esteso, MC., Pastor, MF., Guzman, MD., and Garde, JJ. (2009). Characterization of ram (ovis aries) sperm head morphometry using the sperm-class analyzer.
Raji *et al.*, Igwebuike, JU. and Aliyu, J. (2008). Testicular biometry and its relationshipwith body weight of indigenous goats in Nigeria. ARPN J Agric Biolog Sci., 3:1-4.
Ritar, AJ., Mendoza, G., Salamon, S. and White IG. (1992). Frequent semen collection and sperm reserves og male Angora goat. J Reprod Fertil., 95:97-102.
Schoenian, S. (2016). Sheep 201: A Beginner's Guide to Raising Sheep.
Soler, AJ., Garcia, AJ., Fernandez-Santos, MR., Esteso, MC. and Garde, JJ. (2003). Effects of thawing procedures on post-thawed in vitro viability and in vivo fertility of red dear epididymal spermatozoa cryopreserved at -196 ℃. J Androl., 24:746-756.

Soler, AJ., Esteso, MC., Fernandez-Santos, MR. and Garde, JJ. (2005). Characteristics ofIberian red deer spermatozoa cryopreserved after storage at 5℃ in the epididymis forseveral days. Theriogenology, 64:1503-1517.

Soler, AJ., Poulin, N., Fernandez-Santos, MR., Cognie, Y., Esteso, MC., Garde, JJ. andMermillod, P. (2008). Heterologous in vitro fertility evaluation of cryopreserved Iberianred deer epididymal spermatozoa with zona- intact sheep oocytes and its relationship withthe characteristics of thawed spermatozoa. Reprod Domest Anim., 43:293-298.

Sultana, F., Hussain, SS., Khatun, A., Apu, AS. and Khandoker, M. (2013). Study on buck evaluation based on semen quality and fertility. Bangladesh animal husbandry association; buck; j; non-return rate; sci; semen quality. pp. 101-108.

Turri, F., Madeddu, M., Gliozzi, TM., Gandini, G. and Pizzi, F. (2014). Effect of testiclepostmortem storage on goat frozen-thawed ES quality. Animal, 8:440-447.

Vicente-Fiel, S., Palacin, I., Santolaria, P., Hidalgo, CO., Silvestre, MA., Arrebola, F. and Yaniz, JL. (2013). A comparative study of the sperm morphometry in cattle, sheep, ram and pigs using a CASMA-F. Theriogenology, 79:436-442.

Yaniz, JL., Vicente-Fiel, S., Palacin, I., Capistros, S. and Santolaria, P. (2012). Automatic evaluation of ram sperm morphometry. Theriogenology, 77:1343-1350.

Zhou, J., Chen, L., Li, J., Li, H., Hong, Z., Xie, M., Chen, S. and Yao, B. (2015). The semen pH affects sperm motility and capacitation. http: //journals. plos.org/plosone/article?id=10. 11371/journal.pone.0132974.

(Received 22 October 2017 ; accepted 25 November2017)