Journal of Reproduction and Infertility 3 (3): 58-66, 2012
ISSN 2079-2166

DOI: 10.5829/idosi.jri.2012.3.3.66216

Effects of Buck Age, Storage Duration, Storage Temperature and Diluent on Fresh West African Dwarf Buck Semen

Ngoula Ferdinand, Tebug Tumasang Thomas, Kenfack Augustave, Defang Fualefac Henry, Tendonkeng Fernand and Pamo Tendonkeng Etienne

Department of Animal Science,
Faculty of Agronomy and Agricultural Sciences, University of Dschang-Cameroon

Abstract: West African Dwarf (WAD) goats serve an important role in the rural village economy of West and Central Africa, especially among small-holder livestock owners; however, its productivity is low. The use of artificial insemination (AI), which is the most important animal reproductive biotechnology, can greatly contribute to improve this productivity. AI in goat with fresh semen yields a much higher pregnancy rate than when frozen-thawed semen is used. The short life span of fresh semen is a major constraint on the use of AI in genetic improvement programs for goat. In this study, we investigated the effects of age, temperature, diluents and storage time on WAD buck semen. Such information will be a useful guide in semen collection and processing for artificial insemination in WAD bucks. Semen was collected weekly for four weeks from sixteen healthy WAD bucks aged 1-5 years old using an electro-ejaculator and evaluated according to buck age, storage duration, storage temperature and diluent. The mean semen volume and concentration were 0.56 ± 0.08 mL and 1.09±1.24x10^9 sperms/mL, respectively. 71.43% of ejaculate was classified as whitish, viscous and thick creamy. The mass motility of sperms was 4.00 ± 0.53 on a scale from 0 to 5 and classified as good for AI. The sperm morphology was acceptable in 87.00±5.71% of freshly collected sperms. The mean percentage of abnormal tails and heads was 11.58±1.56% and 1.56±0.52%, respectively. In tris-based extender, individual sperm motility declined progressively from score 4 directly after collection to score 0 on a scale from 0 to 5 after 97 h of incubation at room temperature and 105 h at 4°C. In skimmed milk-base diluent, the decrease was more rapid: from “score 3” to “score 0” in 45 h after collection at room temperature and 65 h at 4°C. In tris-based extender, the sperm motility was fairly good for AI (score 3) 36 and 45 hours after collection at room and refrigerated temperature, respectively. In skimmed milk-based extender, this parameter was fairly good for AI 5 and 17 hours after collection at room and refrigerated temperature, respectively. It was concluded that semen characteristics of WAD bucks were good for AI at the collection time. Tris-based extender can preserve buck semen for longer periods (36 at room temperature and 45 hours at refrigerated temperature) for AI than skimmed milk-based extenders (5 at room temperature and 17 hours at refrigerated temperature).

Key words: WAD Buck % Sperm Characteristics % Semen Preservation % AI

INTRODUCTION

The West African Dwarf (WAD) goat is an indigenous trypanosoma tolerant small ruminant found throughout West, Central and East Africa, India and Fiji [1-3]. WAD goat is known to have a remarkable ability to utilize roughages [4]. It is the lowest in milk and meat production in Africa [5]. The low production of WAD goat is the main constraint to its production [6]. Selective breeding to develop this animal requires the implementation of Artificial Insemination (AI) program.

AI may be regarded as a first generation assisted reproductive technology that is the most widely used and the one that has made the most significant contribution to genetic improvement worldwide [7-9]. The use of AI has the potential to greatly improve the production and

Corresponding Author: Ngoula Ferdinand, Animal Physiology Laboratory, Department of Animal Science, Faculty of Agronomy and Agricultural Sciences, Universi tyof Dschang, P.O. Box: 188 Dschang-Cameroon.
Tel: +237 97941676.

productivity of WAD goat. Compared with natural mating, AI gives an increase in the number of offspring per sire and allows a spatial and temporal dissociation between collection of spermatozoa and fertilization [9]. AI limits the disease spread by the use of healthy male semen and decreases the interval between generations.

The success of AI is based on the ability to efficiently collect, evaluate and preserve semen from quality bucks for use in inseminating does over generations. Studies related to semen characteristics have been documented [7, 9-16]. The increasing need for the use of AI in WAD goats has created a greater demand for semen from superior progeny tested sires. However, very few relevant studies are available on semen characteristics of the WAD buck. In addition, AI using buck frozen semen is a problem because of the high production coast and poor productivity often obtained [17, 18] which does not allow its widespread use. Blash *et al*. [19] reported that the process of freezing and thawing of buck semen reduces the percentage of live sperm cells and acrosomal integrity. Another limiting factor in semen preservation is its exposure to light during manipulation before storage, leading to formation of reactive oxygen species with damage to sperm cell motility and genomic integrity [20, 21]. Therefore, using fresh semen stored at room temperature or between 0 to 5°C could be a good alternative to improve AI technic [22] and therefore improve the productivity of inseminated does. The buck age, type of diluent used and the storage time also affect semen quality [9, 23-25]. A tris-based extender was recommended by Evans and Maxwell [7]. However, there are few studies that have used liquid semen stored for different periods of time in tris-based extender in field conditions in Cameroon. This technology could be considered a useful tool to improve production and productivity of WAD goat. The objectives of this study, therefore, were to determine the characteristics of fresh WAD buck semen with regard to buck age, storage temperature, storage duration and semen extender. It is hoped that such information will be a useful guide in semen collection and processing for artificial insemination in WAD goat.

MATERIALS AND METHODS

Location of Study Area: The study was carried out at the Research and Teaching Farm (FAR) of the Faculty of Agronomy and Agricultural Sciences of the University of Dschang, Cameroon from April to October 2008. The University of Dschang is located in the Menoua Division, West region of Cameroon. This division is situated between latitudes 5° and 6° north and between longitudes 10° and 11° east. Its relief is hilly with altitudes ranging from 700 to 1500m above sea level, with the Sudano-Guinean type of climate made up of two seasons: the dry season (from mid-November to mid-March) and the rainy season (from mid-March to mid-November). The annual rainfall is estimated at 1700mm. Temperatures vary between 14.0 to 28.3°C throughout the year.

Selection and Managements of Bucks: The study was carried out on 16 West African Dwarf Bucks aged from 1 to 5 years at the Teaching and Research Farm of the University of Dschang, Cameroon. The bucks were housed isolated from does and identified by ear notching, tethered each day from 8am to 5pm in a pasture dominated by *Brachiariaruziziensis* and *Pennisetumclandestinum*. The goats also received a multi mineral bloc with a chemical composition of: organic matter (70.97 %), crude protein (1.00 %), lipids (37.43 %), ashes (29.03%), neutral detergent fiber (25.03), acid detergent fiber (19.92 %), acid detergent lignin (14.92 %), cellulose (4.92%) and hemicellulose (5.35%).

Before the semen collection process, some physical characteristics of the bucks were measured and these included: age, scrotal volume and the rectal temperature. The scrotal volumes (V) were measured using a formulae proposed by Hanzen [26]: Volume (cm^3) = 0.0396 x Length (cm) x Scrotal Perimeter (cm).

Semen Collection and Preparation: Before use, all equipments were washed with a 1% sodium carbonate solution, rinsed with distilled water, sanitized with 70% alcohol and then dried for use overnight in an oven at 50°C [27]. Semen was collected weekly for four weeks from 16 healthy WAD bucks aged 1-5 years old using an electro-ejaculator method [28]. Through a funnel held by an assistant, the ejaculate was collected into a graduated tube heated at 36°C and labeled according to buck age (1 to 2 years and 2 to 5 years).

Semen Evaluation

Macroscopic Examination of the Semen: The macroscopic assessments, including the color, volume, pH, viscosity and consistency were carried out on freshly collected semen.

The consistency was assessed as described by [27] and used to visually assess the semen concentration in sperms.

The viscosity of the semen was assessed by drawing 10 µL of the ejaculate with a micropipette and extruding twice on the sides of a test tube in a stand. When the sample was not running down, it was marked as viscous [29].

A universal pH paper was used to approximate the pH of the semen. A drop of pure and undiluted semen was placed in the middle of the pH paper and the color resulting from it matched to the standard colors.

Microscopic Examinations: These included wave motion, sperm motility, semen concentration and sperm morphology [27,30]. To evaluate the mass activity of sperms (wave motion), a drop (20 µL) of semen was placed on a prewarmed slide (36°C) without a cover-slip and examined under a phase contrast microscope (100 X). The mass activity was scored as 1 = no perceptible motion, 2 = weak motion without forming any waves, 3 = small, slow moving waves, 4 = vigorous movement with moderately rapid waves and eddies and 5 = dense, very rapidly moving waves and eddies.

For motility, a sample of diluted semen was removed to a prewarmed slide and covered with a cover-slip. Motility was subjectively assessed using a phase contrast microscope at X 200 magnification and a 6-point scale, (0 = No sperm motion, 1 = Very slow motion, trembling, or oscillation of the tail, 2 = Slow motion, trembling, uncoordinated motion, with a few rapid motions, 3 = Circular motions without trembling, 4 = Rapid motion, some with linear motion and some without linear motion, 5 = Rapid and linear motion of sperms) [30].

Morphological aberrations were determined from a total count of 400 spermatozoa in smears Colored with Nigrosin and Eosin. Sperm abnormalities were classified as described by Baril *et al*. [30]. The sperm concentration was determined using Neubauer Haemocytometer as described by Hafez [29].

Extension and Preservation of Buck Semen

Preparation of Diluents

Two Types of Extenders Were Used in this Study:

- tris-based extender consisted of 8.3 3g Tris, 2.5g D-glucose, 4.5 g citric acid, antibiotics (250 mg streptomycin, 2.000.000 IU penicillin) and 250 mL distilled water;
- skimmed milk-based extender consisted of 11 g skim milk, antibiotics (250 mg streptomycin, 2.000.000 IU penicillin) and 100 mL distilled water.

Tris and skimmed milk-based extenders were prepared according to the methods described by Baril *et al*. [30]. The final solutions were stored in a refrigerator between 4-8°C for at most two days. Before use, it was re-warmed to 35°C in a water bath.

Dilution and Preservation of Semen: Only ejaculates with a wave motion scoring > 3 on scale of 0 to 5 and with a sperm concentration of more than 2.00×10^9 per mL were accepted.

After collection, each semen sample was diluted in the ratio allowing having a required temperature and divided into two parts. One part preserved at room temperature and the other at refrigerated temperature (4°C). These samples were put into light proved bottles (5 ml), corked and dropped in opaque cups with warm water (36°C). The warm water was to prevent rapid change in temperature and to allow a gradual change from 35°C to 22°C at room temperature and from 36°C to 4°C at refrigerated temperature.

Control and Assessment Schedule: The wave motion, sperm concentration, all the macroscopic and sperms morphology assessments were carried out once for each ejaculate immediately after collection. Observations for individual sperms motility and live sperms were done twice a day, between 8 and 9 am in the mornings and 5 and 6 pm in the evenings and these assessments were done every day until the individual motility scored “0”.

Statistical Analysis: Data obtained was subjected to student’s t-test and chi-square test for the establishment of significance. Descriptive statistics was also used in some cases. SPSS 19.0 software of IBM was used for analysis. A p value < 0.05 was considered as significant.

RESULTS

Effects of Buck Age on Semen Characteristics, Scrotal Volume and Rectal Temperature: The effects of buck age on semen characteristics are summarized in Table 1. Independently of the buck age, the mean volume of ejaculate was 0.56 ± 0.08 mL and the mean pH was slightly acidic (6.73 ± 0.25). The buck ejaculates were dominated by the whitish color, thick creamy consistency and was more viscous. The mean scrotal volume and mean rectal temperature were 98.26±17.41 cm^3 and 39.28±0.39°C respectively. The scrotal volume, pH and sperm volume

Table 1: Effects of buck age on scrotal volume, rectal temperature and some semen characteristics

	Bucks ages (years)	Overall mean	
	---	---	
Parameters	1-2(n=8)	2-5(n=8)	1-5(n=16)
Semen			
Volume (mL)	0.55 ± 0.09[a]	0.58 ± 0.07[a]	0.56 ± 0.08[a]
pH	6.75 ± 0.28[a]	6.72 ± 25[a]	6.73 ± 0.25[a]
Colour (%)			
- Yellowish	50	0	28.57
- whitish	50	100	71.43
Consistency (%)			
- thin creamy	50	0	28.57
- thick creamy	50	100	71.43
Viscosity (%)			
- non viscous	50	0	28.57
- viscous	50	100	71.43
Sperm cell			
Mass mobility (0-5 scale)	4.50 ± 0.58[a]	4.67 ± 0.58[a]	4.57 ± 0.53[a]
Concentration (x 10^9)	3.90 ± 0.96[a]	4.73 ± 1.33[a]	
4.26	1.12[a]		
Morphology			
-Normal sperms (%)	87.64 ± 4.64	86.00 ± 7.93	87.00 ± 5.71
-Abnormal heads (%)	1.29 ± 1.27	2.01 ± 1.13	1.56 ± 0.52
-Abnormal tails (%)	11.04 ± 1.12	12.00 ± 1.27	11.58 ± 1.56
Scrotal Volume (cm)	98.26 ± 20.11[a]	97.88 ± 19.33[a]	98.26 ± 17.41[a]
Rectal Temperature (°C)	39.25 ± 0.28[a]	39.33 ± 0.58[a]	39.28 ± 0.39[a]

n= number of buck used

a= data with no significant differences between the two age groups.

were not significantly affected by buck age ($p>0.05$). Half of the ejaculates collected from 1-2 years bucks were yellowish, viscous and thin-creamy while in 2-5 years bucks, they were entirely whitish, thick creamy and viscous.

In general, the percentage of normal sperm was non significantly more elevated in 1-2 years bucks as compared to the one of 2-5 years bucks ($p>0.05$).

When considering the abnormalities, the 2-5 years bucks has more abnormalities than the younger ones bust no significant difference was recorded for this parameter. The mean sperm concentration was 4.26±1.12 x 10^9/mL and was not significantly affected by bucks age ($P>0.05$).

The buck age did not have any significant effects on sperm mass motility, although the value of this parameter seemed more elevated in 2-5 years bucks as compared to 1-2 years bucks.

Effects of Storage Temperatures on the Motility of Sperms Stored in Tris-based Extender: The sperm mobility declined with storage time, from score 4 (rapid motion) to score 0 (no mobility) after 95 and 105 hours of preservation at room and refrigerated temperature respectively (Figure 1).

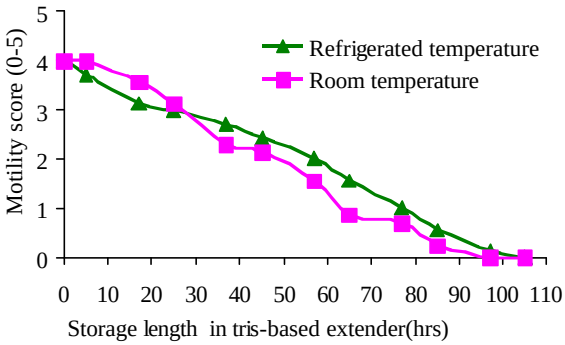

Fig. 1: Change of motility of sperm stored in tris-based extender with storage time

The effects of storage temperatures on the motility of sperms stored in tris-based extender as illustrated in Figure 1 shows that, the motility of the sperms declined progressively from a score of "4" (rapid motion of sperms in a linear projection) to a score of "0" (when the sperms showed no motility) after 97 hour for room temperature and 105 hour for refrigerated temperature. It was also observed that, from dilution time (0 hour) to the 25th hour of preservation, the sperms mobility was more elevated at room temperature than at refrigerated temperature and from the 25th hour of preservation, the motility of sperms

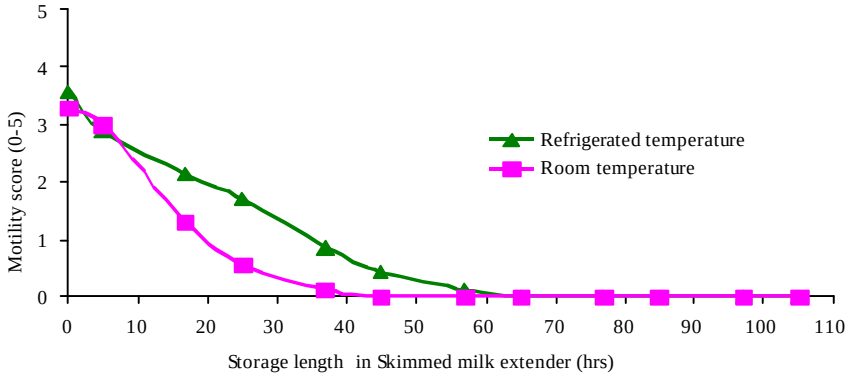

Fig. 2: Changes in motility of sperm stored in skimmed milk-based extender with storage time

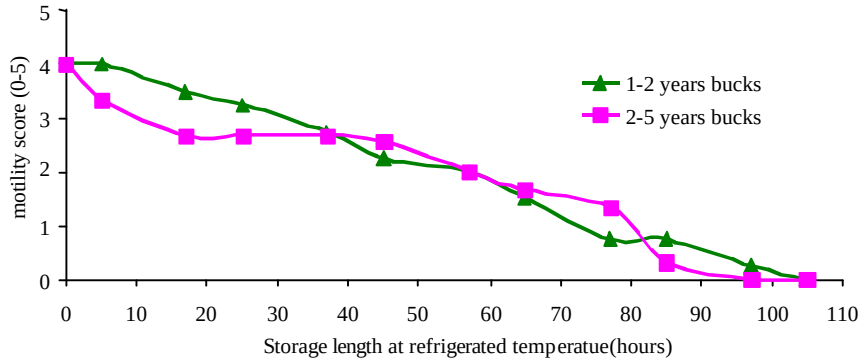

Fig. 3: Changes in function of buck age of the motility of sperm stored in tris-based extender at refrigerated temperature with time

at the refrigerated temperature was better than the motility at the room temperature up till when the scores at both temperatures turned to "0". No significant difference between the motility of sperms at both temperatures (P>0.05) was recorded.

Effects of Storage Temperature on the Motility of Sperms Stored in Skimmed Milk-Based Extender: The effects of storage temperature on the motility of sperms stored in a skimmed milk-based extender are illustrated in Figure 2.

In the skimmed milk-based extender, the motility of the sperms declined progressively and rapidly with time of storage at both temperatures from a 3.57 and 3.29 motility scores to a "0" score at the 45th and at the 65th hours at room and refrigerated temperatures respectively. From dilution time to the 5th hour of preservation, the motility scores were "Fair to Fairly-Good" for AI, but beyond the 5th hour up to the 45th and 57th of preservation ("0" score) respectively, the scores were "Poor" at both temperatures. Although motility at the refrigerated temperature was better than the one recorded at room temperature, statistically the differences observed were not significant (P> 0.05).

Effects of Bucks Age on the Motility of Sperms Stored in a Tris-based Extender at Refrigerated Temperature (4°C): As illustrated in Figure 3, the motility of sperms in the two buck age groups (2-5 and 1-2 years buck age groups) at refrigerated temperature declined progressively from the same score (4.0) at dilution time to a scores of "0" at the 97th hour and the 105th hour of storage respectively. The results also showed that, the motility of sperms in the two age groups declined from a motility classification of "Good" from the time of dilution to a "Fair" classification at the 37th hour of storage. After the 37th hour of preservation, the sperm motility became "Poor" up to the "0" score. There was no significant difference (P> 0.05) observed between the motility of sperms in both age groups.

Effects of Buck Age on the Motility of Sperms Stored in a Tris-based Extender at Room Temperature: As illustrated in Figure 4, from the 5th hour, the sperm motility gradually declined from a score of 4.0 ("Good" class) to a score of 2.5 ("Fair" class) at the 37th hour of storage in both age groups. The motility of the sperms of the two age groups was "Fair"

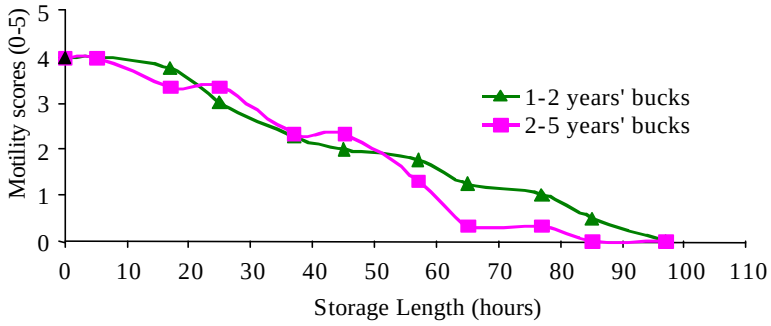

Fig. 4: Change in function of buck age of the motility of sperm stored in tris-based extender at room temperature with time

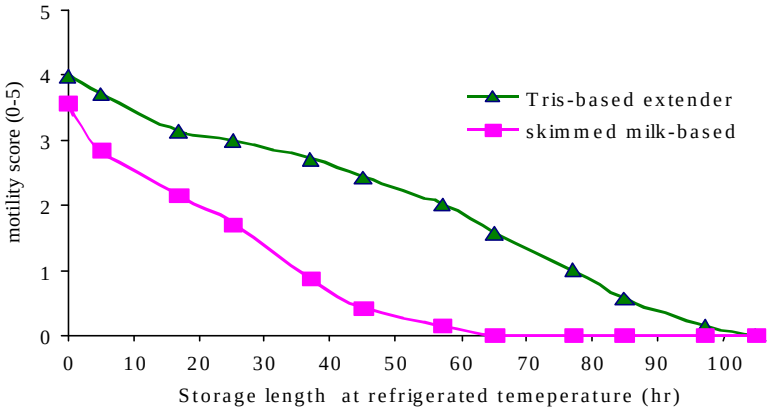

Fig. 5: Change in motility of sperm stored in tris and skimmed milk-based extender at refrigerated temperature with storage time

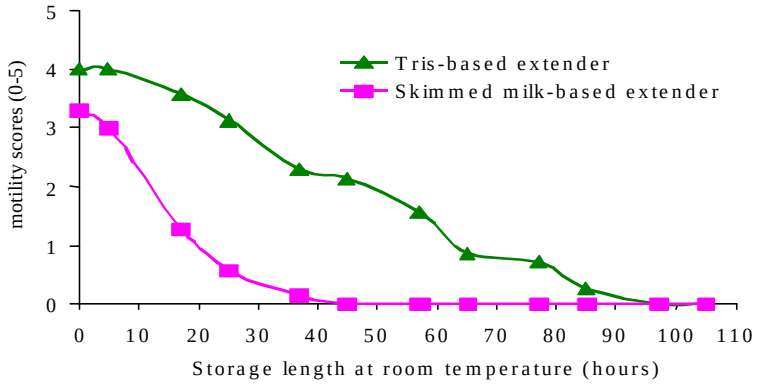

Fig. 6: Change in motility of sperm stored in tris and skimmed-milk based extender at room temperature with storage time

from the 37th hour of storage (score of 2.5) up to the 97th and the 85th hour of storage when the scores were "0" in both groups respectively. No significant difference (P>0.05) was observed between the sperm motility of the buck age groups at room temperature.

Effects of Extenders on the Motility of Sperms Stored at Refrigerated Temperature: Figure 5 illustrates the effects of extenders on the motility of sperms stored atrefrigerated temperature.

Without considering the bucks age, the motility of sperms stored in the tris-based extender at refrigerated temperature decreased from the time of dilution (4.0 score) to a score of "0" at the 105th hour of storage. In the skimmed milk-based extender, the sperm motility decreased rapidly from a lower score of 3.57 at the time of dilution to a "0" score at the 65th hour of storage (Figure 5). Comparing the motility of sperms in the extenders, the tris-based extender was better than the skimmed milk-based extender, although the differences were not significant (P>0.05).

Effects of Extenders on the Motility of Sperms Storage at Room Temperature: Figure 6 illustrates the motility of sperms stored in tris and skimmed milk-based extenders at room temperature. The sperm motility in the tris and the skimmed milk-based extenders scored 4.0 and 3.29 at dilution time (0 hour) and "0" at the 97th and 45th hour of storage respectively. These scores classify the tris-based extender better than the skimmed milk extender at room temperature, although the differences were not statistically significant (P>0.05).

DISCUSSION

This study showed that, the volumes of semen produced were within the range mentioned for bucks 0.6-1.0 [5, 30] and 0.1-2.66 mL [5]. Compared to other breeds, the mean quantity of ejaculate produced by the WAD buck in this study was the smallest in volume among the known African breeds (0.56 ± 0.09 mL/ejaculate) but greater in volume as compared to the Black Bengal bucks of India and Pakistan which range from 0.355-0.433 mL [31]. The small volume of the ejaculate can also be attributed to the low quantity and quality of feed on which WAD buck feed and the management conditions which are still lacking [5]. Considering that the production of every animal is a function of the animal's breed, size, management and the environment in which it is found [29] and the WAD buck being one of the dwarf goat breeds of the world and the smallest of the domestic African goat breeds [5, 32]. It is normal that they produce small quantities of ejaculates which should also be lower in sperms/mL.

Comparing the consistency method in assessing the ejaculate concentration in sperms to the haemocytometer method, this study confirms reports that, the consistency method was good for field assessment, because it is fast but not precise. The consistency method is better in the pre-selection on field of the best bucks that can be used for AI or for an AI centre. It is also good for use where there is no laboratory nearby for immediate quality and quantity evaluation of the semen.

The pH and the color of the semen of most livestock species is slightly acidic (6.2-6.8) and with a yellowish-white color [29, 33]. Independently of buck ages, the WADB used in this study produced ejaculates with a pH of 6.73 ± 0.23 confirming that they have the same pH as other buck breeds. Comparing the buck ages, there was no significant difference (P> 0.05) between the pHs of the two buck groups (1-2 years bucks 6.75 ±0.25 and 2-5 years bucks 6.72 ± 0.25).

The study showed that, the percentage of abnormal sperms from the freshly collected and undiluted ejaculates of the WAD buck used in this trial was 13%, better than the 15% for Boer bucks [27]. Considering the buck ages and grouping the abnormalities into head and tail, the sperm abnormalities in the head portion were below 5% and the tail abnormalities below 15%, so considered "Good" for AI [27].

The result of the wave motion of the ejaculates used in this study shows that, the mean score of the wave motion was 4.25 ± 1.11 and classified the ejaculate wave motion as "Very Good" for AI. When the 2-5 years bucks were compared to the 1-2 years bucks, it was found out that, they had a better mass motility (4.73 ± 0.33) than 1-2 years bucks and also classified "Very Good" for AI

The motility of sperms at refrigerated and room temperatures in the tris-based extender was classified as "Very Good" from dilution to "Fair" at the 45th hour for AI no matter the buck age group considered. Between the 25 and the 45th hours of preservation, the motility of the sperms at both temperatures was classified as "Fair" and only good for uterine AI and beyond the 45th hour, the sperms were no longer good for AI in goats at all [27, 30, 34].

In the skimmed milk-based extender, the sperms motility at refrigerated and room temperatures showed that, the refrigerated temperature had a better sperm motility from dilution time with a classification of "Fairly Good" to the 17th hour with a "Fair" classification than at room temperature which had a "Fairly Good" classification for 5 hours of preservation only. These results confirm reports of other studies [29, 30, 34, 35] which reported that milk extenders cannot preserve semen for AI for more than 24 hours and should be prepared prior to use and preserved for at most two days in the refrigerator between 4-8°C. Comparing the effects of the buck ages on the motility of sperm in the tris-based extender at refrigerated temperature, the 1-2 years bucks had a better motility of the sperms than the 2-5 years bucks from dilution time to the 37th hour of preservation. This study confirms reports that motility is less vigorous in older than younger bucks' semen and this is because, the older bucks have a higher percentage of abnormal sperms than younger bucks and that the quality of the prostate gland secretions or juice deteriorate with age [27, 30]. The difference could also be due to the sperm producing germinal and the Sertoli cells of the testes in the 1-2 years bucks which still have the capacity of producing vigorous sperms and enough sperm nutrients respectively. The results of the effects of buck age on the motility of sperms in the tris-based

extender at refrigerated and room temperatures showed that, the motility of sperms from the two buck age groups were statistically similar and could be used for cervical and deep intra-vagina insemination in goats. This can be used from dilution time to the 37th or 45th hour of preservation for goat cervical AI and intra-uterine AI (laparoscopic AI in goats). Beyond the 37th hour of preservation, the ejaculate was classified "Poor" and not fit for AI at all.

Comparing the effects of the tris-based and the skimmed milk-based extenders on the motility of sperms at refrigerated temperatures, the trial showed that motility in the tris-based extender was better than the skimmed milk-based extender whatever the preservation temperature. The results confirmed that, tris-based extender can preserve buck semen for longer periods (36-45hours) for AI than skimmed milk-based extenders (5-17 hours).

These trials confirm work carried out by Ghalsasi [27], Hafez [29], Baril *et al*. [30], Derivaux and Ectors [33] and Baldassarre and Karatzas [34] who reported that the tris-based extender can preserve buck sperms longer than the milk-based extenders. These trials in addition showed that, the tris-based extender is better in preserving buck ejaculate at refrigerated and room temperatures than the skimmed milk-based extender.

CONCLUSION

In conclusion, Tris-based extender is better than the skimmed milk-based extender in preserving buck semen whatever the preservation period. This extender can be used to preserve semen up to 4 hours after dilution in the refrigerator and for 36 hours at room temperature to be used for AI in goats.

REFERENCES

1. Oppong, E.N.N., 1965. A note on goats in Ghana with reference to the need to develop goat husbandry to improve the nation's diet. Ghana Fme, 9: 144-148.
2. Sacker, E.D. and J.C.M. Trail, 1966. Production characteristics of head of East African Mubende goats. Trop. Agric. Fri., 43: 43-51.
3. Upton, M., 1985. Returns from small ruminant's production in South West Nigeria. Agric. Syst., 17: 65-84.
4. Oyeyemi, M.O., M.O. Akusu and O.E. Ola-Davies, 2000. Effect of successive ejaculation on the spermiogram of West African dwarf goats (Capra hircus L) Vet. Arch., 70: 215-221.
5. Steele, M., 1996. The tropical agriculturalist: Goats. London: CTA MacMillan education, Ltd. London, pp: 1-133.
6. Manjeli, Y., A. Téguia, R.M. Njwe, J. Tchoumboué and E.E. Ayong, 1996. Enquête sur l'élevage caprin dans les hauts plateaux de l'Ouest-Cameroun. Small Ruminant Research and Development in Africa. Proceedings of the Third Biennial Conference of the African Small Ruminant Research Network, UICC, Kampala, Uganda, 5-9 December 1994. ILRI (International Livestock Research Institute) Nairobi, Kenya, pp: 326.
7. Evans, G. and W.M.C. Maxwell, 1987. Salamon's artificial insemination of sheep and goats. Butterworths, Sydney.
8. Chemineau, P., Y. Cognié, Y. Guérin, P. Orgeur, J.C. Vallet, 1991. Collection and preservation of spermatozoa, in: Training manual on artificial insemination in sheep and goats, Rome, FAO Animal Production and Health Paper, 83: 115-130.
9. Leboeuf, B., B. Restall and S. Salomon, 2000. Production and storage of goat semen for artificial insemination. Animal Reproduction Science, 62: 113-141.
10. Oyeyemi, M.O., M.O. Akusu and O.E. Ola-Davies, 2001. Effect of successive ejaculation on the spermiogram of West African Dwarf goats. Israel journal of veterinary medicine, 56(4), www.isrvma.org/.../.
11. El-kon, I.I. and S.A. Darwish, 2011. Effect of glutathione (GSH) on microscopic parameters and DNA integrity in Egyptian buffalo semen during liquid and frozen storage. Journal of Reproduction and Infertility, 2(3): 32-40.
12. El-Sisy, G.A., W.S. El-Nattai and R.J. El-Sheshtawy, 2008. Effect of superoxide dismutase and catalaseon viability of cry opreserved buffalos permatozoa. Global Veterinaria, 2(2): 56-61.
13. El-Sisy, G.A., R.I. El-Sheshtawy A.A. Mohamed and W.S. El-Nattat, 2010. Correlations between semen parameters and conception rate in buffaloes. Global Veterinaria, 5(1): 15-21.
14. Mansour, M.M., 2009. Modification of hypo-osmotic swelling test to evaluate the integrity of stallion sperm plasma membrane Global Veterinaria, 3(4): 302-307.
15. Badr, M.R., M.G.A. El-Malak and M.H. Hassan, 2010. Effect of trehaloseon cry opreservation, oxidative stress and DN Aintegrity of buffalos pennatozoa. Journal of Reproduction and Infertility, 1(2): 50-57.

16. Olayemi, F.O., D.A. Adeniji and M.O. Oyeyemi, 2011. Evaluation of spennmotility and viabilityinhoney-include deggyolk based extenders. Global Veterinaria, 7(1): 19-21.
17. Langford, G.A., G.J. Marcus, A.J. Hackett, L. Ainsworth, M.S. Wolynetz and H.F. Peters, 1979. A comparison of fresh and frozen semen in the insemination of confined sheep housed in total confinement. Canadian Journal of Animal Science, 59(4): 686-691.
18. Hackett, A.J. and M.S. Wolynetz, 1981. Comparison of Natural mating and Artificial Insemination on reproductive performance of three strains of sheep housed in total confinement. Canadian Journal of Animal Science, 61(4): 907-912.
19. Blash, S., D. Melican and W. Gavin, 2000. Cryopreservation of epididymal sperm obtained at necropsy from goats. Theiriogenology, 54: 899-905.
20. Foote, R.H., 1967. Influence of light and agitation on bovine spermatozoa stored with protective agents. Journal of Dairy Science, 50: 1468-1474.
21. Aitken, R.J. and J.S. Clarkson, 1989. Significance of reactive species and antioxidants in defining the efficacy of sperm preparative techniques. Journal of Andrology, 9: 367-376.
22. Salamon, S. and W.M. Maxwell, 2000. Storage of ram semen. Animal Reproduction Science, 62: 77-111.
23. Sule, W.F., M.O. Oyeyemi and M.O. Akusu, 2007. Coconut milk-citrate as extender for West Africa dwarf buck spermatozoa at room temperature. Biokemistri, 19(2): 65-73.
24. Donoghue, A.M. and G.J. Wishart, 2000. Storage of poultry semen. Animal Reproduction Science, 62: 213-232.
25. Mara, L., M. Dattena, S. Pilichi, D. Sanna, A. Branca and P. Cappai, 2007. Effect of different diluents on goat semen fertility. Animal Reproduction Science, 102: 152-157.
26. Hanzen, C., 2009. La propédeutique de l'appareil reproducteur et l'examen du sperme des ruminants. Faculté de Médecine Vétérinaire. Service de Thériogenologie des animaux de production, Retrieved on 25/09/2011 fromwww. therioruminant. ulg.ac.be/.../200809/R06_Propedeutique_male_...
27. Ghalsasi, P.M., 2004. Technical training manual on artificial insemination in Goats. Maharashtra Goat and Sheep Research and Development Institute, Phlatan. Nimbkar Agricultural Research Institute, Phlatan, India, pp: 41.
28. Oyeyemi, M.O., M.O. Akusu and O.E. Ola-Davies, 2000. Effect of successive ejaculation on the spermiogram of West African dwarf goats (*Capra hircus* L). Veterinary Archives, 70: 215-221.
29. Hafez, E.S.E., 1987. Reproduction in Farm Animals. 5th Ed. Lea and Febiger, Philadelphia, USA, pp: 315-481.
30. Baril, G., P. Chemineau, Y. Cognie, Y. Guerin, B. Leboueuf, P. Orgeur and J.C. Vallet, 1993. Manuel de formation pour l'insémination artificielle chez les Ovins et les caprins. Etude FAO Production et Santé Animale, 83: 231.
31. Biswas, D., F.Y. Bari, M. Samsuddin and M.M. Rahman, 2002. Determination of Percentages for preserving the Black Bengal buck (Carprahircus) Spermatozoa for long time. Pakistan Journal of Biological Science, 5(6): 715-718.
32. Ndamukong, K.J.N., M.M.H. Sewell and M.F. Asanji, 1989. Traditional sheep and goat production in the North West Province, Cameroon. In: R.T. Wilson and A. Melaku (eds.). African Small Ruminant Research and Development. ILCA, Addis Ababa, Ethiopia, pp: 129-144.
33. Derivaux, J. and F. Ectors, 1989. Reproduction Chez les Animaux Domestiques. Troisième Edition revue, Cabay, Belgique, pp: 1141.
34. Baldassarre, H. and C.N. Karatzas, 2004. Advanced assisted reproduction technologies (ART) in goats. Animal Reproduction Science, 82-83: 255-266.
35. Melissa, R., 2004. Sperm motility: Collection and Evaluation of Semen. Reproduction Index. Retrieved on 25/09/2011 from http://www.vivo.colostate.edu/hbooks/pathphys/reprod/semeneval/motility.html.