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Original research

Evaluation of whole and skimmed cow milk extenders on West African Dwarf goat Semen

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ABSTRACT

This study evaluated the efficacy of whole and skimmed cow milk as extenders for the semen of West African Dwarf (WAD) goat bucks. Semen was obtained from two adult WAD bucks by electro-ejaculation method and maintained in an insulator at 37℃. Pre-extension and post-extension evaluation of the semen was carried out immediately, 15, 30, 45 and 60 minutes post collection. Whole and skimmed cow milk were used to extend semen obtained from the black and brown bucks respectively. The semen characteristics evaluated included mass activity, percentage progressive motility, normal livability and normal morphology. Results revealed that whole cow milk effectively maintained the WAD buck semen characteristics post extension for a period of 30 minutes. Thereafter, values of each semen characteristic decreased progressively as the period of storage increased. There was rapid decrease in the characteristics of the buck semen extended with skimmed cow milk immediately post extension, the sperm cells were dead 15 minutes post extension. These findings suggest that WAD bucks can be extended with whole cow milk for 30 minutes at 37℃ successfully and not with skimmed milk.

Keywords: Cow milk, extender, goat buck, semen characteristics.

INTRODUCTION

The West African Dwarf (WAD) goats are the most predominant breeds of goat in the Southern part of Nigeria. They have stocky body and short legs. They are very hardy as they are trypanotolerant and resistant to humid environment (Adebayo & Chineke, 2011). They are commonly found among household and small-scale farmers where they serve as source of meat, milk, skin, fiber and for generation of income (Idiong & Udom, 2011).

The WAD does have high fertility, prolificacy and fecundity rates and can be used for breeding programs in order to transfer their hardiness and survivability in extreme conditions to the next generation (Daramola *et al*., 2007). However, the recent increase in the demand for goat meat surpasses the traditional method of reproduction in goat and has emphasized the need to adopt and adapt assisted reproduction technology that is capable of maximizing its reproductive potentials (Ajala *et al*., 2012; Raji *et al*., 2015).

Assisted reproduction technologies are manipulated reproductive processes used to improve genetic improvements of animals (Vikrama & Balaji, 2010). They include artificial insemination (AI), *in vitro* production of embryo, oestrous synchronization, superovulation, Multiple Ovulation and Embryo Transfer (MOET) and cryopreservation of embryos. Artificial insemination (AI) is the most universally practiced ART that has been used in almost all domestic species (Daly *et al*., 2020).

Artificial insemination plays an important role in goat breeding where it has been used as a tool to improve and control reproduction. It may equally extend the usefulness of proven sires that are unable to mate normally either due to age or other physical reasons. The success of any artificial insemination program depends on the appropriate management of collected semen which includes its extension, storage and use (Leboeuf *et al*., 2008).

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The use of suitable semen extenders is one of the most important factors in the success of artificial insemination in goats. The extended semen can be used fresh, chilled or frozen but the best fertility and conception rate is achieved when fresh semen is used (Olurode & Ajala, 2016). Freshly collected buck semen can survive for a period of less than 24 hours post collection at 37°C (Leboeuf *et al*., 2003).

For metabolic maintenance of sperm cells for optimum performance, Sugars, antibiotics and buffers are usually incorporated into to the extenders to improve their quality. They are also used extensively to preserve both sperm motility and sperm viability for an optimal period of time. However, availability and cost of semen extenders are important factors usually considered in the practice of semen extension and AI (Kulaksiz & Daskin, 2012).

Cow milk is readily available at cheap cost in livestock farms in Nigeria. However, there is paucity of information of its use as an extender for goat semen. Therefore, this study was carried out to investigate the efficacy of using whole and skimmed cow milk as semen extender for WAD goat bucks at room temperature.

MATERIALS AND METHODS

STUDY AREA

The experiment was carried out in the laboratory of the Department of Theriogenology and Production, at the Veterinary Teaching Hospital, Faculty of Veterinary Medicine, University of Ilorin, Kwara State, Nigeria. University of Ilorin is sited in Ilorin, the capital city of Kwara State on latitude 8.4799° N and longitude 4.5418° E. It covers approximately a land mass of 5,000 hectares. It is located strategically at the geographical and cultural confluence of the North and South. It is located at about 500km away from Nigeria's national capital; Abuja and about 300km away from the nation's economic capital, Lagos (Abiodun & Gbenga, 2016).

EXPERIMENTAL ANIMALS AND MANAGEMENT

Two apparently healthy adult WAD bucks of the average age of two (2) years were used for the experiment. Goat 1 was tagged 'Black' while goat 2 was tagged 'Brown'. The scrotal circumference of the black and brown bucks was measured to be 18.2cm and 17.9cm wide respectively. The bucks were housed in the same pen in the goat unit of the Veterinary Teaching Hospital, Faculty of Veterinary Medicine, University of Ilorin, Ilorin. Pen was of standard size, well ventilated and the floor was covered with concrete. The bucks were allowed to graze from 7 am to 3 pm every day on pastures consisting of carpet grass (*Axonopus compressors*), guinea grass (*Panicum maximum*) and elephant grass (*Pennisetum purpreum*). Dry cassava peels and bean chaff rations were provided in the evening as supplements. Clean water was provided *ad libitum*. The goats were dewormed using Ivermectin at a dosage of 0.04mg / kg body weight.

COW MILK COLLECTION

The cow was restrained in a standing position and the udder was cleaned with warm water and a clean towel. A clean transparent white bowl was placed below the udder. Then, the teats were squeezed and milk was collected into the bowl and divided into two portions. The first portion was used as fresh whole while the second portion was skimmed, transferred into a sterilized glass test tube and stored in a refrigerator.

PREPARATION OF SKIMMED COW MILK

Skimmed cow milk was prepared by storing the cow milk in freezer for 24 hours after which it was taken out and allowed to thaw. After thawing, the milk was dispensed into clean sterilized centrifuge test tubes, tightly capped and centrifuged at 1000 rpm for 5minutes. The supernatant which is a fatty layer was then discarded.

SEMEN COLLECTION

Semen was collected by electro-ejaculator method. Prior to semen collection, the bucks' preputial areas were shaved and prepuces were properly cleaned with tissue paper soaked with warm water. Faeces were evacuated from the rectum using a gloved index finger; the rectal probe was lubricated thoroughly with petroleum jelly and gently inserted into the rectum of the bucks by a slow rotating movement with the electrodes on both sides in contact with the rectum. The hind legs of the bucks were held firmly in a standing position by two attendants. The electro-ejaculator was then plugged to a source of electricity and switched on. Voltage was applied to the animal through the lubricated rectal probe inserted into the rectum, by rotating the knob of the variable resistor, increasing the stimulating power from zero to 20MA, held for 3-5 secs and then brought back to zero. This process was repeated three to five times by gradually increasing the applied voltage after a short resting period. By regulating the voltage, the nerves responsible for ejaculation were stimulated, erection took place and this was followed by ejaculation. The ejaculate was collected into a collection instrument comprising of nylon cone funnel, fitted into a calibrated test tube which was inserted into an insulator. The ejaculate was maintained in a water bath at 37℃ (Raji *et al*., 2015).

SEMEN EXTENSION

SEMEN EXTENSION WITH WHOLE COW MILK EXTENDER

Buck semen was extended with whole cow milk extender at a ratio of 1:1. A volume of 0.1ml of semen was aspirated using a clean warm pipette and dispensed into a clean glass test tube containing 0.1ml of the whole cow milk extender

which was kept at 37℃ and was gently mixed together. The extended semen was kept in a water bath at temperature 37℃ in order to maintain the viability of the sperm cells.

SEMEN EXTENSION WITH SKIMMED COW MILK EXTENDER

Buck semen was extended with skimmed cow milk extender at a ratio of 1:1. A volume of 0.1ml of semen was aspirated using a clean warm pipette and dispensed into a clean glass test tube containing 0.1ml of the skimmed cow milk extender which was kept at 37℃ and was gently mixed together. The extended semen was kept in a water bath at temperature 37℃ in order to maintain the viability of the sperm cells.

SEMEN EVALUATION

EVALUATION OF SEMEN IMMEDIATELY AFTER COLLECTION

Color and consistency were determined by visual assessment immediately after collection of semen. The volume of ejaculate was read and recorded from a calibrated collecting tube. Sperm mass activity, progressive motility, live-dead ratio and percentage normal morphology were determined by conventional methods (Oyeyemi, *et al.,* 2000). For motility determination, a drop of semen was aspirated using a sterilized warm pipette and dispensed onto a clean warm microscope slide, covered with a cover slip and observed under a light microscope. The results were then recorded. For live-dead ratio, a drop of semen was aspirated using a sterilized warm pipette and dispensed onto a clean warm microscope slide, a drop of warm eosin-nigrosin stain was then added and mixed together. Afterwards, a smear was made and allowed to dry at room temperature. The slide was then viewed under a light microscope and results were recorded. Sperm motility and live-dead ratio of sperm cells were re-evaluated after 15, 30, 45 and 60 minutes of semen collection and recorded.

EVALUATION OF SEMEN EXTENDED WITH WHOLE COW MILK

Sperm mass activity, progressive motility and live-dead ratio of whole cow milk extended semen were determined by conventional methods (Oyeyemi, *et al.,* 2000). For motility determination, a drop of extended semen was aspirated using a sterilized warm pipette and dispensed onto a clean warm microscope slide, covered with a cover slip and observed under a light microscope. The results were then recorded.

Live-dead ratio was estimated by aspirating a drop of semen using a sterilized warm pipette and dispensed onto a clean warm microscope slide, a drop of warm eosin-nigrosin stain was then added and mixed together. Afterwards, a smear was made and allowed to dry at room temperature. The slide was then viewed under a light microscope and results were recorded.

Sperm motility and live-dead ratio of sperm cells were re-evaluated after 15, 30, 45 and 60 minutes of semen collection and recorded.

EVALUATION OF SEMEN EXTENDED WITH SKIMMED COW MILK

Sperm mass activity, progressive motility and live-dead ratio of skimmed cow milk extended semen were also determined by conventional methods as earlier described (Oyeyemi, *et al.*, 2000)

RESULTS

MASS ACTIVITY

The mean mass activity of semen immediately and after the first 30 mins were the same (4.0±0.0) which significantly reduced to 3.0±0.0 and 2.0±0.0 at 45 and 60 mins respectively (Table I). Extended semen with whole milk showed mean mass activity of 4.0±0.0 at immediate and 15 mins PSE that got significantly reduced to 3.0±0.0 and 2.0±0.0 at 30 and 45 mins respectively.

On the other hand, the mean mass activity of semen post collection in the brown WAD buck immediately (0 min) and 15 mins after PSC were the same (4.0±0.0) (Table II). At 30 mins and 45 mins post collection, the value significantly reduced to 3.0±0.0 and 2.0±0.0 respectively. Extended semen with whole milk showed mean mass activity immediately after extension to be 2.0±0.0 and 1.0±0.0 with significance difference ($P \leq 0.05$) between the two values. The values of mean mass activity after this time (30, 45 and 60 mins) significantly reduced to 0.

PROGRESSIVE SPERMATOZOA MOTILITY

Progressive motility percentage of plain semen of WAD buck and extended semen with whole milk are presented in Table I. The progressive motility immediate PSC (95.2±3.8), 15 mins (93.8±4.7%) and 30 mins (90±5.2%) PSC were statistically similar (Table I). This was significantly reduced to 76.5±3.1% at 45 mins PSC and 51.0±2.6% at 60 mins PSC. The progressive motility of extended semen followed the same pattern similar to that of plain semen from the immediate PSE to 60 mins PSE.

The progressive motility of the brown WAD buck semen was statistically similar to that of black WAD buck. The values at immediate PSC (93.1±3.4), 15 mins (91.2±4.0) and 30 mins (90.5±5.1) were statistically similar but reduced significantly to 71.5±3.4 and 63.2±4.3 at 45 and 60 mins PSC respectively. On the contrary, extension of semen with skimmed milk caused a significant reduction in progressive motility right from 51.7±2.3% obtained at immediate PSE to 22.5±2.9 at 15 mins PSE and then to 0.0 after this time point.

Table I. Semen Analysis of black West African Dwarf goat buck semen post semen collection for a duration of 60 minutes post semen collection (PSC ☐ and Post semen extension (PSE ☐ with whole milk.

Semen Characteristics	Number of trials	Immediately PSC/PSE	15 mins PSC/PSE	30 mins PSC/PSE	45 mins PSC/PSE	60 mins PSC/PSE
Mass activity	4	4.0±0.0^{a}	4.0±0.0^{a}	4.0±0.0^{a}	3.0±0.0^{b}	2.0±0.0^{c}
	4	4.0±0.0^{x}	4.0±0.0^{x}	3.0±0.0^{y}	2.0±0.0^{y}	2.0±0.0^{y}
% Progressive motility	4	95.2±3.8^{a}	93.8±4.7^{a}	90±5.2^{a}	76.5±3.1^{b}	51.0±2.6^{c}
	4	92.6±3.0^{x}	90.5±3.8^{x}	89.7±5.3^{x}	70.6±4.1^{y}	60.2±3.1^{z}
% Normal morphology	4	94.8±2.7^{a}	91.75±4.2^{a}	89.3±4.7^{a}	75.8±3.3^{b}	52.5±2.1^{c}
	4	93.0±4.2^{x}	91.5±5.4^{x}	88.6±6.4^{x}	65.6±4.3^{y}	51.5±2.4^{z}
% Liveability	4	96.0±2.4^{a}	94.3±3.7^{a}	92.1±5.2^{a}	78.7±2.8^{b}	53.2±4.1^{c}
	4	94.5±3.8^{x}	91.9±5.1^{x}	89.2±5.8^{x}	62.7±4.0^{y}	50.6±3.9^{z}

Means with different superscript$^{(a,b,c\ or\ x,y,\ z)}$ along the same row are statistically different at P≤0.05

Table II. Semen analysis of brown West African Dwarf goat buck semen immediately after collection for 60 minutes post semen collection (PSC ☐ and post semen extension (PSE ☐ with skimmed cow milk.

Semen Characteristics	Number of trials	Immediately PSC/PSE	15 minutes PSC/PSE	30 minutes PSC/PSE	45 mins PSC/PSE	60 mins PSC/PSE
Mass activity	4	4.0±0.0^{a}	4.0±0.0^{a}	3.0±0.0^{b}	2.0±0.0^{c}	2.0±0.0^{c}
	4	2.0±0.0^{x}	1.0±0.0^{y}	0.0^{z}	0.0^{z}	0.0^{z}
% Progressive motility	4	93.1±3.4^{a}	91.2±4.0^{a}	90.5±5.1^{a}	71.5±3.4^{b}	63.2±4.3
	4	51.7±2.3^{x}	22.5±2.9^{y}	0.0^{z}	0.0^{z}	0.0^{z}
% Normal morphology	4	94.2±3.1^{a}	92.5±4.2^{a}	90.7±5.0^{a}	69.2±3.8^{b}	52.7±3.4^{c}
	4	41.5±2.3^{x}	21.25±1.5^{y}	0.0^{z}	0.0^{z}	0.0^{z}
% Liveability	4	96.1±2.5^{a}	93.5±4.3^{a}	90.6±6.2^{a}	70.5±2.8^{b}	51.2±3.0^{c}
	4	55.7±4.5^{x}	27.75±2.22^{y}	3±0.8^{z}	0.0$^{\epsilon}$	0.0$^{\epsilon}$

Means with different superscript $^{(a,b,c\ or\ x,y,z,\epsilon)}$ along the same row are statistically different at P≤0.05

PERCENTAGE SPERMATOZOA NORMAL MORPHOLOGY

There was no significant (P>0.05) difference in normal spermatozoa morphology between plain semen and the semen extended with whole milk at the five time points of semen evaluation (Table I). In the two groups (PSC and PSE), the spermatozoa normal morphology ranged between 51 to 99.0%. However, extension with skimmed milk caused deterioration in spermatozoa morphology and hence a significant reduction in percentage normal morphology (Table II). The percentage normal morphology of plain semen of brown buck at immediate PSC, 15 mins and 30 mins were 93.1±3.4, 91.2±4.0 and 90.5±5.1 respectively which were statistically similar but higher than 71.5±3.4 and 65.2±4.3 obtained at 45 mins and 60 mins PSC respectively. On the contrary, the immediate percentage normal morphology of extended semen with Skimmed milk at immediate PSE and 15 mins PSE were 51.7±2.3 and 22.5±2.9 respectively. These values were lower than the 60 mins PSE obtained for semen extended with whole milk. Then after, the percentage normal morphology PSE (skimmed milk) drastically reduced to 0.0.

PERCENTAGE SPERMATOZOA LIVEABILITY

The percentage spermatozoa liveability in the black WAD buck semen at immediate PSC was 96.0±2.4 which was statistically similar to the values obtained at 15 mins (94.3±3.7) and 30 mins (92.1±5.2) PSC. The latter was significantly reduced to 78.7±2.8 and 53.2±4.1 at 45 mins and 60 mins respectively. Similarly, the pattern of change with semen extended with whole milk followed the same trend of changes observed with plan semen. The percentage normal spermatozoa morphology obtained at immediate (94.5±3.8%), 15 mins (91.9±5.1), 30 mins (89.2±5.8%), 45 mins (62.7±4.0%) and 60 mins (50.6±3.9%) PSE were statistically similar to the values obtained with plain semen.

The percentage spermatozoa liveability in the brown WAD buck semen at various time points was similar to that of black WAD buck semen. These values were 96.1±2.5, 93.5±4.3 and 90.6±6.2% at immediate, 15 mins and 30 mins PSC respectively before significantly reduced to 70.5±2.8% and 51.2±3.0% at 45 mins and 60 mins PSC respectively. On the contrary, extension with skimmed milk significantly (P<0.05) reduced the normal percentage spermatozoa liveability to 55.7±4.5% at immediate PSE and 27.75±2.2% PSE. The value at 30 mins was 3±0.8%, and then after, drastically reduced to 0.0 at 45 mins and 60 mins PSE respectively.

DISCUSSION

In this study, the efficacy of whole and skimmed cow milk as semen extender was evaluated in WAD bucks. Also, the survivability of fresh and extended WAD bucks' semen for duration of 60 minutes was evaluated. The semen characteristics evaluated were mass activity (MA), %

progressive motility (PM), % normal morphology (NM) and % livability.

The color of semen which indicates sperm cell concentration was milky-white for both black and brown buck and this connotes high sperm concentration as described by Ajala *et al*., (2012).

The semen characteristics of both bucks evaluated immediately after collection were within normal values and acceptable when compared to values reported by Raji *et al*., (2015) for the same breed. We observed that the values were not significantly different when re-evaluated 30minutes post semen collection and following extension with whole cow milk. However, gradual decrease in the assayed seminal parameters was observed 45- 60 minutes post extension. This gradual decrease in semen characteristics is in tandem with the report of Ngoula *et al*., (2012) that increase in duration of storage of semen causes gradual deterioration in the quality and viability of sperm cells. The ability of the whole cow milk to maintain the semen characteristics of the WAD bucks excellently for about 30 minutes post collection could be attributed to casein contained in cow milk which has been suggested earlier by Bergeron *et al*., (2007) to be responsible for the protective ability of milk on sperm cells. Also, lactose which is an energy source present in cow milk has been reported to maintain the motility of sperm cells (Bustani & Baiee,2021). The progressive and significant drop in the values of seminal parameters of the WAD bucks 45minutes and 60minutes post semen extension with whole cow milk could be as a result of energy exhaustion during metabolism (MdGulshan *et al*., 2013). This could also be due to non-addition of antibiotics to the whole cow milk extender thereby, causing rapid depletion of sugar by competing micro-organisms (Santos & Silva, 2020).

We also observed that 15minutes post extension of the WAD bucks with skimmed cow milk, the values of semen characteristics reduced drastically below the recommended and acceptable values earlier reported for these breed of goats (Oyeyemi *et al.*, 2000; Ajala *et al.*, 2012; Raji *et al.*, 2015). In fact, 30 minutes post extension of the WAD bucks semen with skimmed milk, the values of the semen characteristics had reduced to zero. The drastic sperm cell mortality is in correlation with the report of Pellicer-Rubio & Combarnous, (1998) that the bulbourethral secretion of buck promotes a decrease in the percentage of motile spermatozoa, a deterioration in the quality of sperm movement, breakage of acrosomes, and cellular death of goat epididymal spermatozoa diluted in skim milk and incubated at 37°C. However, contrary to the observations of our present study, Foote *et al*., (2002) reported that skimmed milk is as effective as whole milk in protecting spermatozoa during storage of semen at 48°C because it is nearly devoid of lipids (0.1%, mostly triglycerides).

CONCLUSION

In conclusion, this study shows that extension of West African Dwarf buck semen using whole cow milk in the ratio 1:1 maintained sperm cell motility, liveability, mass activity and normal morphology for a period of 30 minutes when incubated at 37°C. Therefore, we suggest that WAD buck semen can be safely extended in whole cow milk. Also, extension of WAD buck semen in skimmed cow milk at ratio 1:1 caused a rapid sperm cell mortality and decrease in mass activity, normal morphology and progressive motility and at 15 minutes post extension, all sperm cells were dead. Therefore, we suggest that skimmed cow milk has a deleterious effect on WAD buck semen and may not be used as semen extenders for goat semen, while whole milk is suitable within the first 30 mins PSE. However, further studies should be carried out to substantiate these findings.

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