Nig. J. Anim. Prod. 2023, 50(6):87- 104 https://doi.org/10.51791/njap.v50i6.8023 Nigerian Journal of Animal Production© Nigerian Society for Animal Production.

Impact of *Aloe vera* leaf extracts on semen quality and fertilizing potential in red Sokoto bucks

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Abstract

Due to some bacteria's resistance to conventional antibiotics, it is necessary to continuously look for alternatives when replacing conventional antibiotics in commercial extenders with herbal antimicrobials. The purpose of this study was to evaluate the antibacterial activity of Aloe Vera Leaf Methanolic Extracts (AVLME) on semen quality and spermatozoa fertilisation potential in extended caprine semen. The Aloe barbadensis Miller leaf extracts was carried out using methanol. Fresh semen was collected from a healthy Red Sokoto buck using electro- ejaculator method. The experiment was divided into five treatments, the first treatment (control) had the appropriate concentration of penicillin and streptomycin in a Tris-based extender, and the remaining treatments had the antibacterial agents replaced with AVLME using the following concentrations; 0.25, 0.50, 0.75 and 1.0g/L and evaluated at 0, 24, 48 and 72 hours at 5℃ for bacterial load (x cfu/mL), morphology (%), liveability (%), pH, acrosome integrity (%) and spermatozoa morphology classification. The experimental design was a Completely Randomized Design. Data obtained were analysed using ANOVA (SAS). Bacterial load was suppressed best at 0 hour (0.75g/L) and 24 hours (0.25g/L). Significant decline was observed in semen quality parameters and fertilising potential during storage. Proportions of spermatozoa cell morphology classification were within expected limits of abnormality. Aloe vera leaf methanolic extracts at the optimum concentration between 0.25 and 0.75, has the potential to replace conventional antibacterial agents in commercial Red Sokoto caprine extenders.

Keywords: *Aloe barbadensis*, methanolic extracts, antibacterial, artificial insemination, Red Sokoto buck

Impact des extraits de feuilles d'Aloe vera sur la qualité du sperme et le potentiel fertilisant des bouc Red Sokoto

Résumé

En raison de la résistance de certaines bactéries aux antibiotiques conventionnels, il est nécessaire de rechercher en permanence des alternatives lors du remplacement des antibiotiques conventionnels dans les extenseurs commerciaux par des antimicrobiens à base de plantes. Le but de cette étude était d'évaluer l'activité antibactérienne des extraits méthanoïques de feuilles d'Aloe Vera (AVLME) sur la qualité du sperme et le potentiel de fertilisation des spermatozoïdes dans le sperme caprin étendu. Les extraits de feuilles d'Aloe barbadensis Miller ont été réalisés à l'aide de méthanol. Du sperme frais a été collecté à partir d'un bouc Red Sokoto sain en utilisant la méthode de l'électro-éjaculateur. L'expérience a été divisée en cinq traitements, le premier traitement (témoin) avait la concentration appropriée de pénicilline et de streptomycine dans un extenseur à base de Tris, et les traitements restants avaient les agents antibactériens remplacés par AVLME en utilisant les concentrations suivantes ; 0,25, 0,50, 0,75 et 1,0 g/L et évaluées à 0, 24, 48 et 72 heures à 5? pour la charge bactérienne (x cfu/mL), la morphologie (%), la viabilité (%), le pH, l'intégrité de l'acrosome (%) et la classification de la morphologie des spermatozoïdes. La conception expérimentale était une conception complètement randomisée. Les données obtenues ont été analysées à l'aide d'ANOVA (SAS). La charge bactérienne a été supprimée au mieux à 0 heure (0,75 g/L) et 24 heures (0,25 g/L). Une baisse significative a été observée dans les paramètres de qualité du sperme et le potentiel

fertilisant pendant le stockage. Les proportions de classification de la morphologie des cellules des spermatozoïdes se situaient dans les limites d'anomalie attendues. Les extraits méthanoïques de feuilles d'Aloe vera à la concentration optimale entre 0,25 et 0,75 ont le potentiel de remplacer les agents antibactériens conventionnels dans les extenseurs caprins Red Sokoto commerciaux.

Mots-clés : Aloe barbadensis, extraits méthanoliques, antibactérien, insémination artificielle, bouc Red Sokoto

Introduction

The importance of goat farming in the food and nutritional security of rural dwellers cannot be overemphasized, more importantly, it is a key agricultural sector in developing countries, especially in the rain-fed regions where crop production is uncertain, and rearing large ruminants is restricted by acute scarcity of feed and fodder (Kumar *et al*., 2010; Sow *et al*., 2021; Patel *et al*., 2022). Considering the production potential of goats and as well as the importance of their milk, genetic improvement via the implementation of Artificial Insemination (AI) programme through semen preservation is crucially required. This is possible only with semen preservation of superior bucks with suitable extenders for an extended period, as it is essential for the success of the AI programme. Goat semen can be preserved either at room temperature, at refrigerated temperature for 24–48 hours or cryopreserved for long-term storage. Preservation of goat semen at refrigerated temperature is cheaper and more feasible compared to cryopreservation, making it an attractive option for small-scale goat breeding programmes. (Dorado *et al*., 2010) It is well known that bacteriospermia is detrimental to spermatozoa quality (Andrabi *et al*., 2001). Irrespective of the extender and the storage conditions used, semen handling and preservation negatively affect sperm quality. Bacteria transmitted through natural mating or Artificial insemination can impair the reproductive function of females and thus lowering fertility rates (Andrabi *et al*., 2001). The female genital tract is physiologically designed to destroy infections introduced during mating but the mechanism may be overwhelmed by a large number of pathogens in the semen (Morrell and Wallgren, 2011). The presence of micro-organisms, especially bacteria in the ejaculates can affect fertilisation directly, by adhering to spermatozoa, impairing their motility and inducing acrosome reaction (Khalid *et al*., 2010). Antibacterial resistance is now a major global issue. Strategies to improve the current situation include research to find new and innovative antibiotic alternatives. Antibiotics and chemotherapeutic agents have been of value in controlling many infections but they depend on judicious use to minimise the incidence of resistant forms (Danso, 2002). Approximately 20% of the plants found in the world have been submitted to pharmacological or biological tests, and a substantial number of new antibiotics introduced on the market are obtained from natural or semi-synthetic resources (Mothana and Lindequist, 2005).

The use of antibiotics in semen extenders to check the growth of several organisms originating from donors or contamination during semen processing provides a major contribution to the development of Artificial Insemination (AI) (Andrabi, *et al*., 2001). Traditionally, at different Semen Production Units (SPUs), Streptomycin and Penicillin (SP) are the antibiotic combinations that are being added to the semen extender (Raheja, *et al*., 2008).

Plants have been used as a source of medicines for both humans and animals since time immemorial in crude forms such as decoctions, syrups, powders, infusions and ointments (Ghorbani, 2014). Many plants and plant parts have been shown to exhibit antibiotic and

antimicrobial properties. Anosa and Okoro, (2011) reported anticoccidial activity of the methanolic extract of *Musa paradisica* root in chickens while, Ademola and Eloff, (2011) reported anthelminthic activity of acetone extract and fractions of *Vernonia amygdalina* (bitter leaf) against *Haemonchuscontortus* eggs and larvae in small ruminants to mention but a few.

Aloe vera, a cactus-like perennial plant belonging to the family liliaceae, widely distributed in the tropical and subtropical regions of the world has been chosen as a source of antibiotics (Lanjhiyana *et al*., 2011). Most of the Aloe species are indigenous to Africa and are grown in warm climates, both as wild and cultivated plants, in countries in southern, eastern and northern Africa, India and in China (Bozzi, 2007). *Aloe vera* is a succulent plant that stores water in its roots and leaves. Of the approximately 350 species of aloe vera that exist in the world, *Aloe Barbadensis* Miller is noted for its therapeutic properties. Hence, it is known as Aloe Vera**,** the "True Aloe". *Aloe vera* contains saponin, a chemical compound that acts as an antimicrobial agent (Sharma *et al.,* 2013).

Many of the medicinal effects of aloe leaf extracts have been attributed to the polysaccharides found in the inner leaf parenchymatous tissue (Ni *et al*., 2004). Aloe vera is the oldest medicinal plant ever known and the most applied medicinal plant worldwide. In the pharmaceutical industry, it has been used in the manufacturing of topical products such as ointments and gel preparations, as well as in the production of tablets and capsules (Eshun *et al*., 2004).

Materials and Methods

Experimental Site

The semen collection was carried out in the small ruminant unit of the teaching and research farm at, the University of Ibadan. Further laboratory analyses were carried out at the Animal Microbiology and Physiology Laboratory of the Department of Animal Science, University of Ibadan, Ibadan ($7^{0}20^{1}$N, $3^{0}50^{1}$E; 200m above sea level).

Collection of Plant Material

Fresh healthy and uninfected leaves of *Aloe barbadensis* Miller were collected from the aloe vera plantation of the Agronomy Department, University of Ibadan. The leaves were washed under running tap water to eliminate dust and other foreign particles and to cleanse the leaves thoroughly.

Processing of Methanolic Aloe Vera Extract

The extraction process was carried out using the Soxhlet extraction method. Collected *Aloe barbadensis miller* leaves were chopped into smaller parts and 250g each of prepared aloe vera leaf was weighed into three different reflux apparatus sets up, and were extracted using 500mL each of methanol at 64 ^{o}C. The extraction process was carried out between 12-14 hours until the extraction was completed (the refluxing solvent became clear). Extract was collected by evaporating the solvent using rotary evaporators, centrifuged and sieved to obtain a clear *aloe vera* extract which was poured into an air-tight container and stored in the freezer until required for use.

Semen Collection, Dilution and Preservation

Semen was collected using the electro-ejaculator method (Oyeyemi *et al*., 2000) from a healthy Red Sokoto buck maintained at the small Ruminant Unit, Teaching and Research Farm, University of Ibadan. Before semen collection, the buck prepuce was properly cleaned; the rectal probe was lubricated thoroughly with petroleum jelly and gently inserted into the rectum of the buck by a slow rotating movement with both electrodes down. The buck was 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 by rotating the knob of the variable resistor, turning it back to zero, and repeating the process several times by gradually increasing the applied voltage. By regulating the voltage, the

nerves responsible for ejaculation were stimulated and erection took place. This was followed by ejaculation. The ejaculate was collected into a graduated tube (bathed in warm water at 37 ºC) through a funnel held by an assistant.

The composition of Tris-based extenders was used as the experimental extenders. It consisted of 1.0g of fructose, 1.40g citric acid, 2.40g of Tris (Hydroxy methyl amino methanol), 0.01g of Penicillin and 0.10g of Streptomycin in 100 mL of distilled water. Another Tris-based extender was prepared without streptomycin and penicillin which was as well dissolved in 100mL of distilled water. Each treatment was replicated thrice and each treatment with their replicates was in three places, 0, 24, 48 and 72 hours. The relative composition of the diluents is presented in Table 1.

Semen aliquots were diluted at 37 ºC with the prepared extenders. Diluted semen was cooled at 5 ºC and refrigerated at this temperature for assessment every 24 hours for 4 days. After 24 hours of refrigeration, the semen straws were thawed at 37 ºC in a water bath for post-thaw semen evaluation. The design for this study was Complete Randomized Design (CRD).

Bacterial Count

For the determination of viable bacterial count, the medium was prepared by dissolving 23g of nutrient Agar (Gilco lab USA) in 1000 mL of distilled water and heated to boiling point. The solution was autoclaved at 121 ºC under 15lb/inch pressure for 20mins and was allowed to cool to about 35 ºC.

Salmonella-Shigella (SS) agar and Eosin Methylene Blue (EMB) agar medium were used for the isolation of microorganisms. SSA and EMB were prepared according to the manufacturer's instructions as follows;

Salmonella-Shigella (SS) agar: 60g of the medium was suspended in 1000 mL of distilled water. Homogenized and sterilized using an autoclave at 121 ºC for 15mins and allowed to cool to 35 ºC before use.

Eosin Methylene Blue (EMB) agar: 36g of EMB Agar was suspended in 1000mL of distilled water. Heated to dissolve the medium completely. Dispensed and sterilized by autoclaving at 15lbs. pressure (121 ºC) for 15 mins.

Serial dilution was carried out to produce the bacterial load in the samples. 9 mL of distilled water were pipette into a clean test tube covered with cotton wool and foil and was autoclaved at 121ºC. 1mL of each sample was dispensed into the test tubes containing 9 mL of sterile distilled water and were serially diluted. 1mL of the last dilution factor for each treatment with replicates was inoculated into well-labelled empty sterile petri dishes. Nutrient Agar was added to each sample in the petri dishes and incubated at 37ºC. This was done for 0-hour, 24-hour, 48-hour and 72-hour samples. Manual counting was performed with the aid of magnification under uniform and properly controlled artificial illumination

(**American Public Health Association (**APHA) 1992.

Semen Quality Assessment

The spermatozoa quality assays (morphology, liveability and pH) were performed at 0, 24, 48 and 72 hours of refrigeration. A drop of thawed semen sample was placed on a pre-warmed glass slide and covered with a cover slip. Progressive motility was assessed with a phase contrast microscope at X400 at 37ºC. The final motility was examined at least with 200 spermatozoa, selected randomly from a minimum of four microscopic fields.

Eosin-Negrosin stain was used to examine the morphologically normal spermatozoa. Spermatozoa liveability was assessed by placing a smear of the semen on a glass slide and staining it with Eosin-Negrosin for microscopic evaluation. Dead sperm cells absorbed the purple

stain while live sperm cells appeared colourless.(Stanistaw *et al*., 2017).

Semen Fertilizing Potential

Morphology classification and acrosome integrity were performed at 0, 24, 48 and 72 hours of refrigeration. The acrosome integrity percentage was determined from sperm smears stained with eosin–nigrosin examined under a phase contrast microscope at 1000x magnification under an oil immersion objective and bright-field (Yildiz *et al*., 2000). A total of 200 spermatozoa were counted in at least four microscopic fields. Classification of sperm cells morphologically into primary and secondary abnormalities was done as well.

Statistical Analysis

Data obtained was subjected to analysis of variance (ANOVA) using the SAS (1999) package. Significant differences occurred when means were separated using Duncan Multiple Range Test of the same package.

Results

Table 1: Composition of Experimental Extender per 100 mL distilled water.

Extender Components	0AVLME	0.25AVLME	0.50AVLM	0.75AVLME	1.0AVLME
Tris (g)	2.40	2.40	2.40	2.40	2.40
Citric Acid (g)	1.40	1.40	1.40	1.40	1.40
Fructose (g)	1.00	1.00	1.00	1.00	1.00
Penicillin (g)	0.0	0.00	0.00	0.00	0.00
Sreptomycin (g)	0.10	0.00	0.00	0.00	0.00

AVLME = Aloe Vera Leaf Methanolic Extract

Table 2: Effect of Aloe Vera Leaf Methanolic Extract on Bacterial Load in Caprine Semen

Period (hr)	Concentration (g/L)	Bacterial Load(X10 ^{5} cfu/mL)	Minimum	Maximum
0	0	0.08 ± 0.05	0.05	0.15
	0.25	0.05 ± 0.00	0.05	0.05
	0.50	0.27 ± 0.10	0.15	0.35
	0.75	0.00 ± 0.00	0.00	0.00
	1.0	0.02 ± 0.03	0.00	0.05
24	0	0.07 ± 0.06	0.00	0.10
	0.25	0.00 ± 0.00	0.00	0.00
	0.50	0.70 ± 0.75	0.00	1.50
	0.75	0.07 ± 0.08	0.00	0.15
	1.0	0.10 ± 0.10	0.00	0.20
48	0	2.75 ± 2.62	0.95	5.75
	0.25	1.68 ± 1.88	0.45	3.85
	0.50	0.93 ± 0.13	0.80	1.05
	0.75	2.07 ± 0.16	1.95	2.25
	1.0	0.68 ± 0.25	0.40	0.85
72	0	3.82 ± 0.25	3.65	4.10
	0.25	3.45 ±0.28	3.20	3.75
	0.50	4.12 ± 0.43	3.70	4.55

0.75	6.90 ± 1.20	5.55	7.85
1.0	9.05 ± 0.41	8.70	9.50

Effect of AVLME on bacteria load in extended caprine semen

Table 2 shows the effect of aloe vera leaf methanolic extract on the bacterial load (X 10^5cfu/mL) of buck semen. At 0 hour, least bacterial load was observed in 0.75g/L with no bacterial load followed by 1.0g/L (0.02 x 10^5cfu/mL), 0.25g/L (0.05 x 10^5cfu/ml), 0.0g/L (0.08 x10^5cfu/ml) and 0.5g/L (0.27 x 10^5cfu/mL). At 24 hours, 0.25g/L had the least bacterial load (0.00) followed by 0.0g/L (0.07), 0.75g/L (0.07), 1.0g/L (0.01) and 0.5g/L (0.70). At 48 hours, an increment was observed in the bacterial load, compared to the values obtained at 24 hours, least bacterial load was observed in 1.0g/L (0.68 x 10^5cfu/mL) followed by 0.50g/L (0.93 x 10^5cfu/ml), 0.25g/L (1.68 x 10^5cfu/mL), 0.75g/L (2.07 x 10^5cfu/mL) and 0.0g/L (2.75 x 10^5cfu/mL). At 72 hours, further increase was observed in all the concentration levels (treatments) with 1.0g/L having the highest bacterial load followed by 0.75g/L, 0.50g/L, 0.0g/L and the least bacterial load was observed in 0.25g/L.

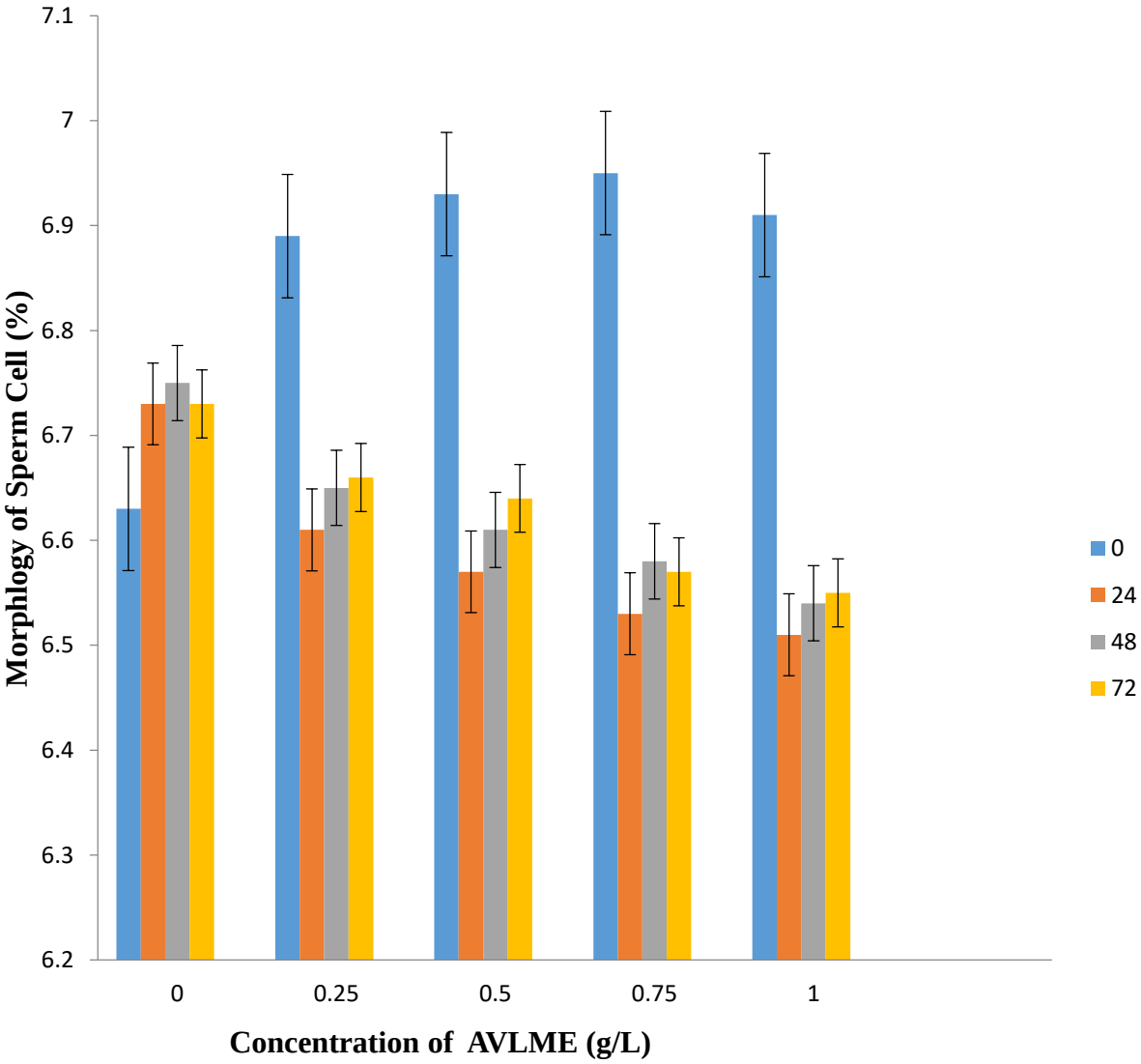

Fig 1: Effect of AVLME on the Morphology of spermatozoa in caprine semen

Effect of AVLME on semen quality in extended caprine semen

Morphology of Sperm Cells in Extender Containing AVLME as a source of Antibiotics

Fig 1 reveals the mean values of sperm cell morphology. At 0hour, mean values were significantly similar among treatments (p>0.05). At 24 hours, significant effect was observed with 0.0g/L having the highest value (94.00%) though the value was significantly similar to the values of 0.5g/L (85.00%) and 0.75g/L (84.00%) but significantly different from 0.25g/L and 1.0g/L (76.33% and 76.67% respectively) which were similar to 0.5g/L and 0.75g/L no significant effect at 48 and 72 hour (p>0.05).

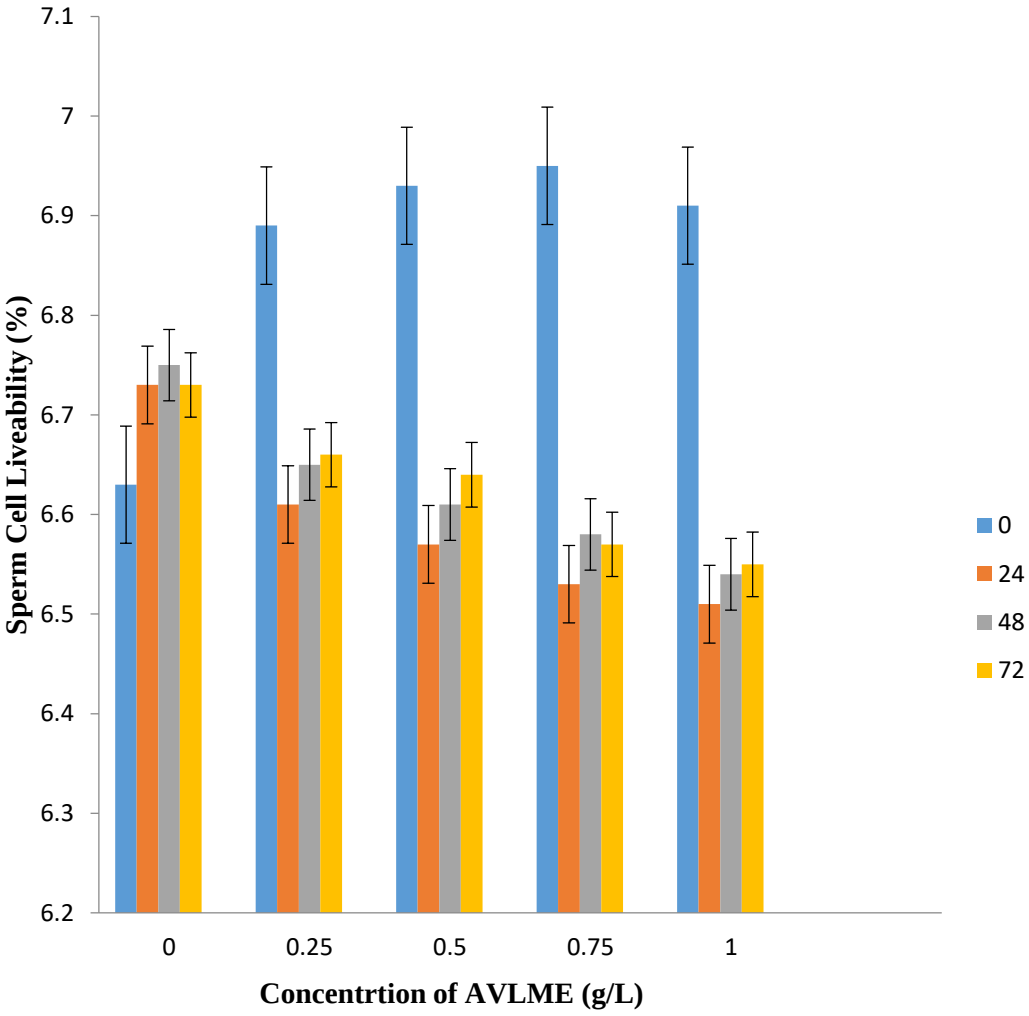

Fig 2: Effect of AVLME on sperm cell liveability of caprine semen

Sperm Cell Liveability in Extender Having AVLME as source of Antibiotics

Figure 2 showed sperm cell liveability in extender containing AVLME as source of antibiotics. At 0, 24, 48 and 72 hours of refrigeration, no significant difference was observed ($p>0.05$) in sperm cell liveability.

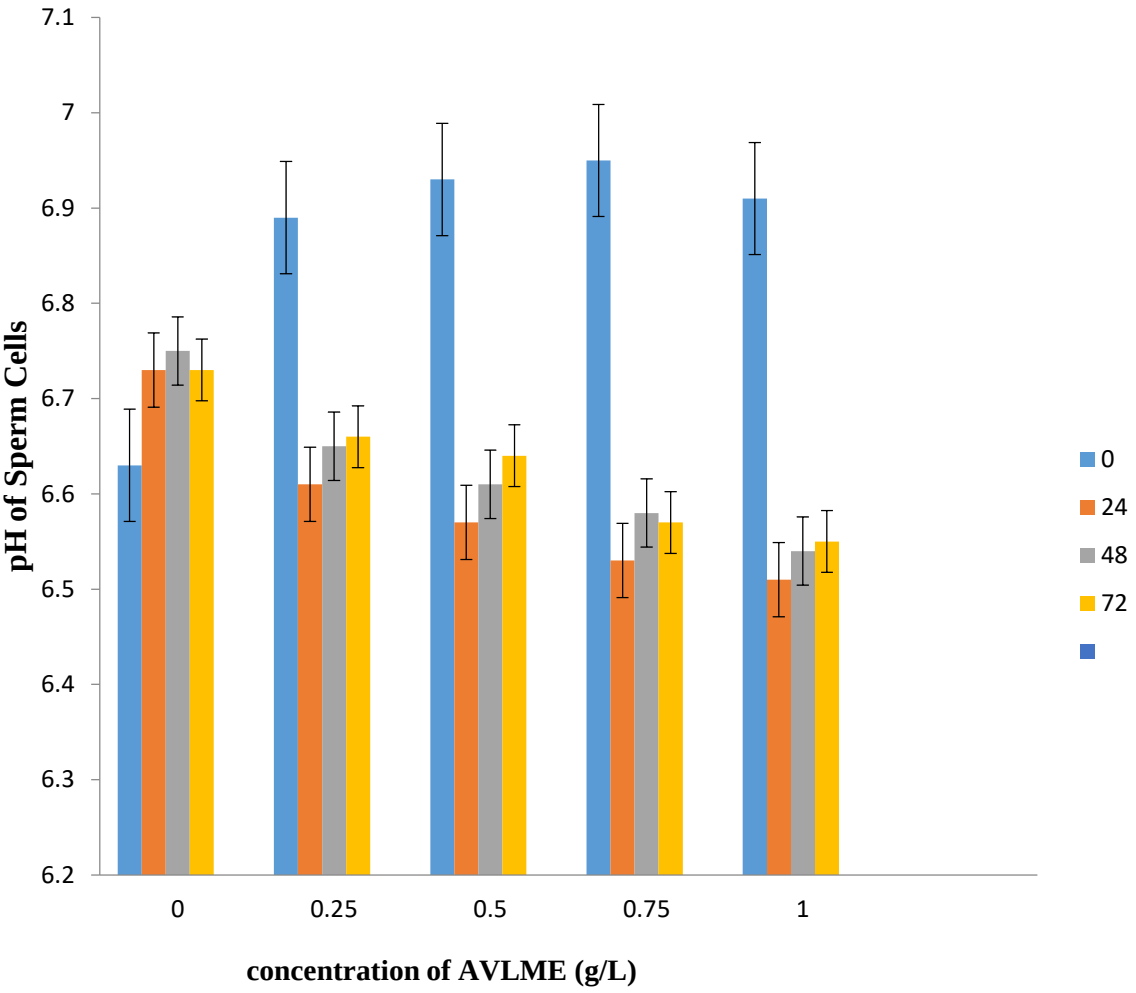

Fig 3: Effect of AVLME on the pH of caprine semen

pH of Semen in Extender Having AVLME as Source of Antibiotics

Fig 3 showed the pH of semen. At 0hour, pH values ranged between 6.63 in 0.0g/L and 6.95 in 0.75g/L no significant difference ($p>0.05$). At 24 hours, significant difference was observed with 0.0g/L having the highest pH (6.73) while 1.0g/L had the least pH (6.51). At 48 hours, 0.0g/L had the highest pH (6.75) while 1.0g/L had the least value of 6.54, mean values were significantly different ($p<0.05$). At 72 hours, 0.0g/L also had the highest value (6.73) and 1.0g/L was observed with the least value of 6.55. Values were significantly different ($p<0.05$).

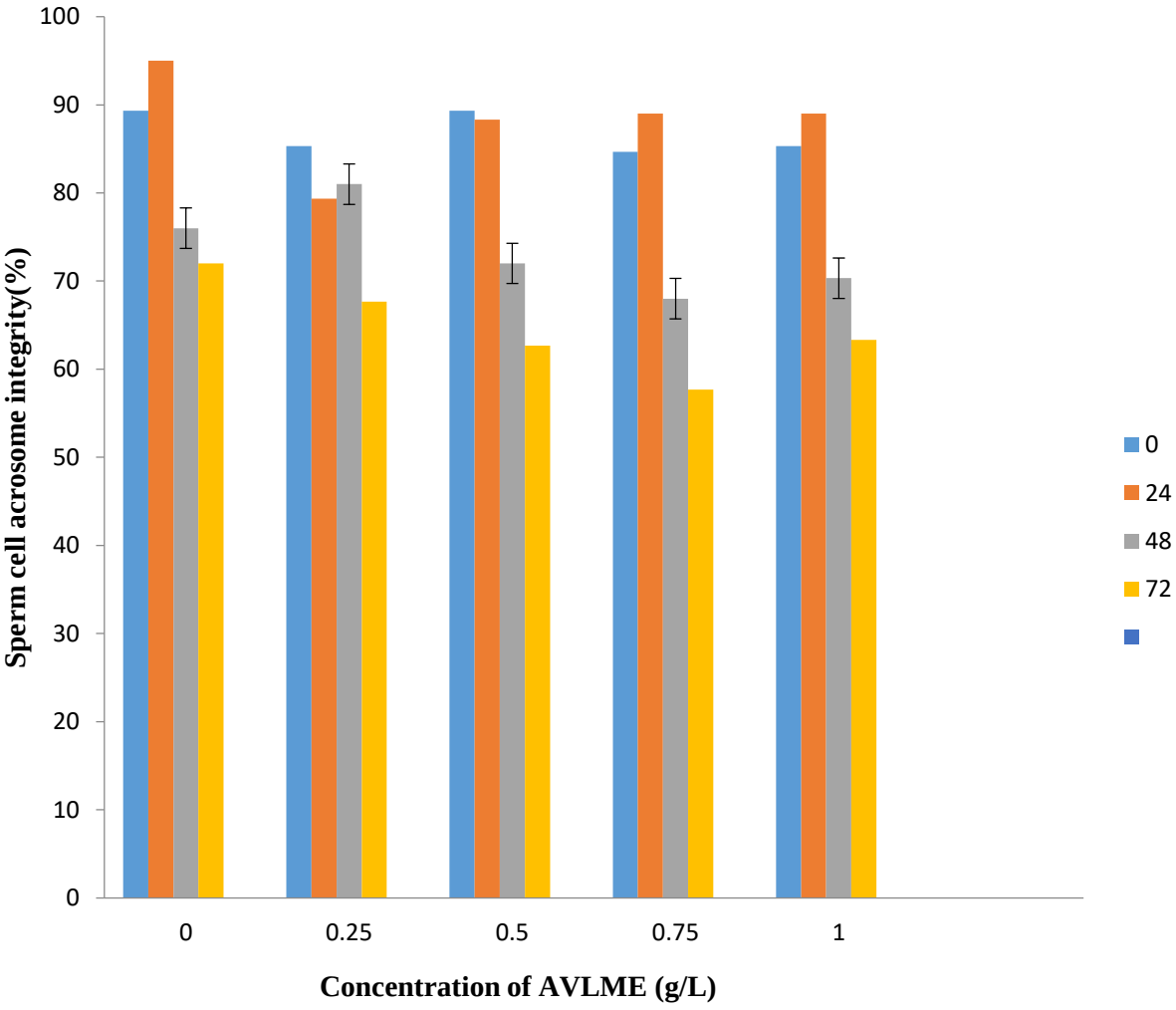

Fig 4: Effect of AVLME on Acrosome Integrity of sperm cells in caprine semen

Effect of AVLME on fertilizing potential in extended caprine semen

Acrosome Integrity of Sperm Cells in Extender with AVLME as source of Antibiotics.

Fig 4 showed the mean values of acrosome integrity of sperm cells. At 72 hrs similar mean values ($p>0.05$) were observed with 0.0, 0.25. 0.5 and 1.0g/L. Value from 0.75g/L was the lowest (57.67%) and it was significantly similar to 0.25, 0.5 and 0.75g/L but significantly different from 0.0g/L (72.00%).

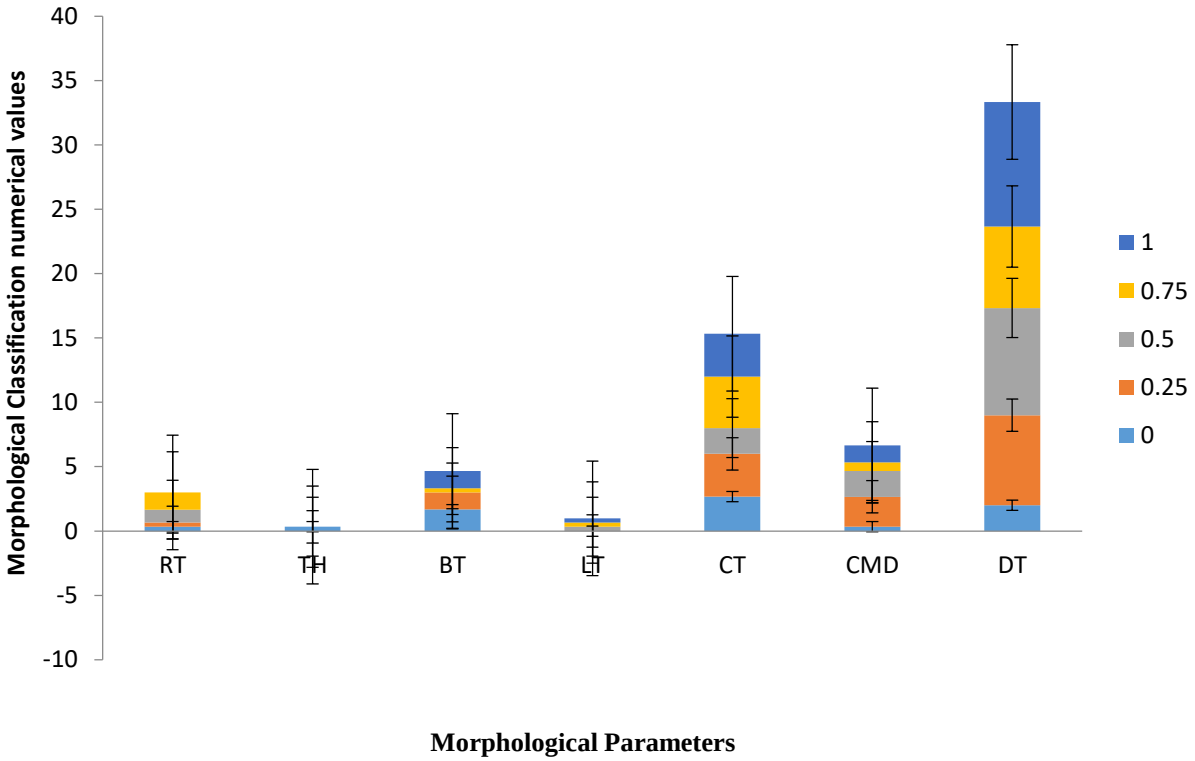

Fig 5: Effect of AVLME on caprine semen morphological classification at 0 hour

Morphological Classification of Sperm Cells in Extender with AVLME as source of Antibiotics. Secondary abnormalities observed were Bent Tail (BT), Looped Tail (LT), Curved Mid-Piece (CMP), Detached Tail (DT) and Coiled Tail (CT). Primary abnormalities observed were Rudimentary Tail (RT) and Twin Head (TH).

Effects of AVLME on the Morphological Classification of sperm cells in extended caprine semen at 0 hour

Fig 5 revealed the mean values for sperm cells with Rudimentary Tails (RT) to be significantly different ($p<0.05$), 0.75g/L (1.33) had the highest proportion followed by 0.5g/L (1.00), 0.25g/L (0.33), 0.0g/L (0.33) and 1.0g/L (0.00). Sperm cells proportion having Twin Head (TH), Bent Tail (BT), Looped Tail (LT), Coiled Tail (CT) and Curved Mid Piece were significantly similar ($p>0.05$). among treatments. However, sperm cells proportion with Detached Tail (DT) was significantly different with 1.0g/L (9.67) having the greater proportion and the least value was observed in 0.0g/L (2.00).

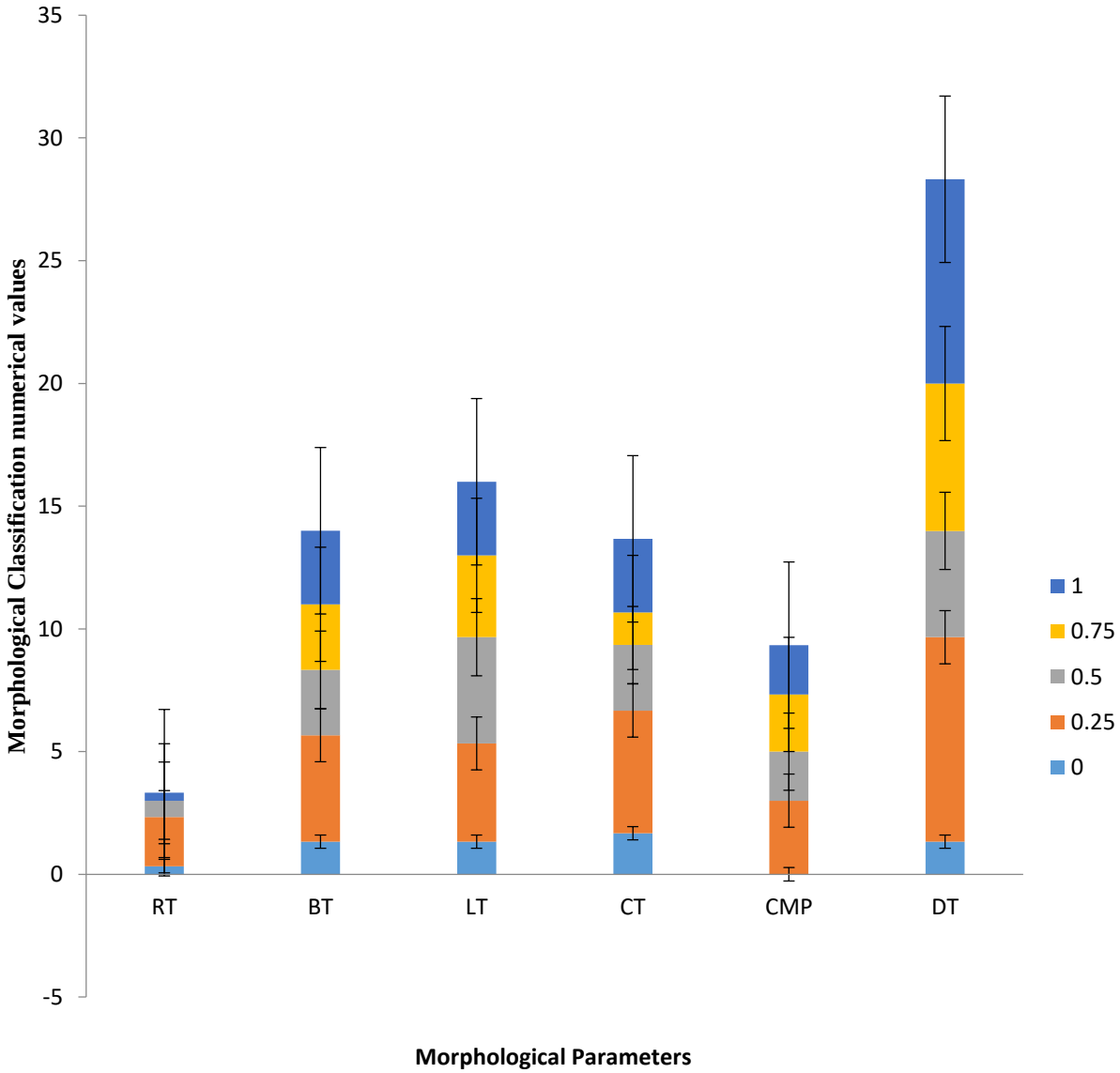

Fig 6: Effect of AVLME on caprine semen morphological classification at 24 hours

Effects of AVLME on the Morphological Classification of sperm cells in extended caprine semen at 24 hours

Fig 6 showed that sperm cells with RT proportion was higher in 0.25g/L followed by 0.5g/L, 0.0g/L, 1.0g/L and 0.75g/L, values were significantly different ($p<0.05$). Significant differences were observed among treatments with BT, LT, CT, CMP and DT sperm cell defects. No significant effect on TH sperm cells as observed in 0.0g/L, 0.25g/L, 0.5g/L, 0.75g/L and 1.0g/L level of Methanolic AVE inclusion.

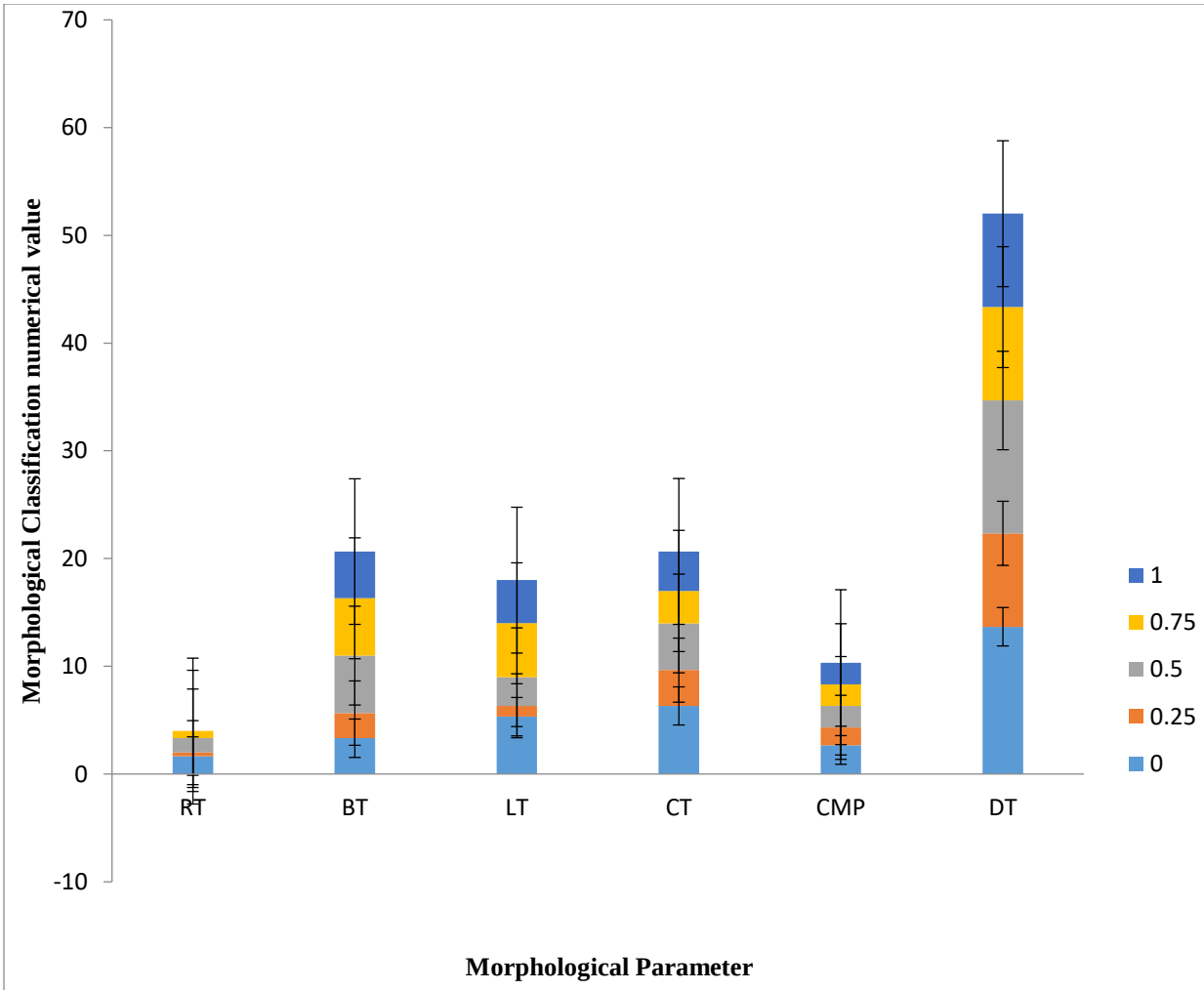

Fig 7: Effect of AVLME on caprine semen morphological classification at 48hours

Effects of AVLME on the Morphological Classification of sperm cells in extended caprine semen at 48 hours

There was an increment in the proportion of sperm cells with defects as compared to 0hour and 24 hours. No significant effect on RT, BT, CT, CMP and DT proportion of sperm cells at 0.0g/L, 0.25g/L, 0.5g/L, 0.75g/L and 1.0g/L. No sperm cell with TH as observed at 24 hours. Proportion of Sperm cells with Looped Tail was significantly different ($p<0.05$), 0.0g/L had the highest proportion followed by 0.75g/L, 1.0g/L, 0.5g/L and the least was observed in 0.25g/L as shown in Fig 7.

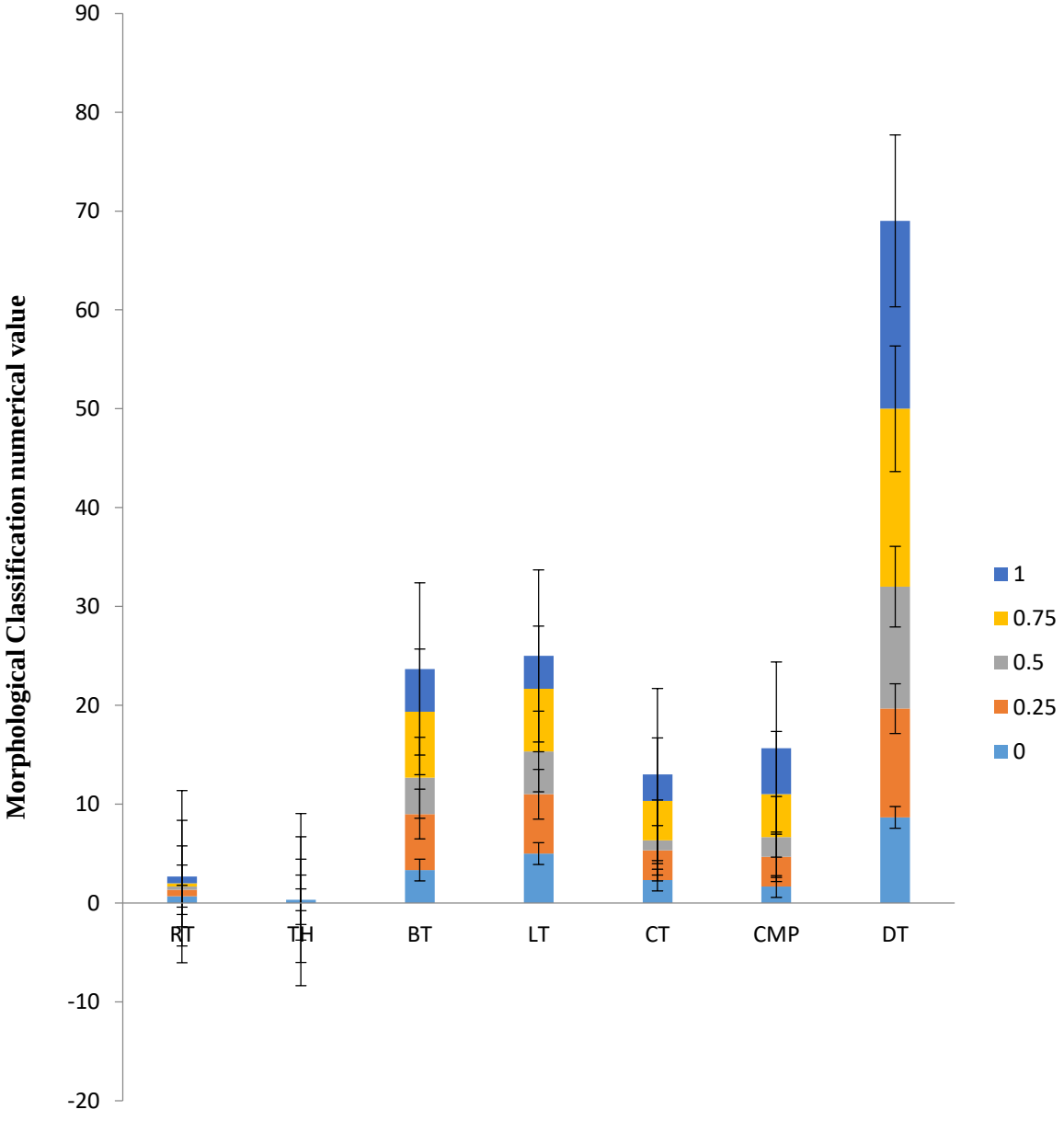

Fig 8: Effect of AVLME on caprine semen morphological classification at 72hours

Effects of AVLME on the Morphological Classification of sperm cells in extended caprine semen at 72 hours

Fig 8 revealed that sperm cells with RT, BT, TH, LT, CT and CMP had no significant different ($p>0.05$). DT sperm cells numerical values had significant effect; 1.0g/L had the highest value 19.00, followed by 0.75g/L (18.00), 0.5g/L (12.33), 0.25g/L (11.00) and 0.0g/L (8.67). However, proportion of sperm cell defect was higher at 72 hours compared to 0, 24 and 48 hours

Discussion

Effect of AVLME on bacterial load in extended caprine semen

The bacterial load in extended caprine semen with AVLME as source of antibiotics was presented in Table 2. At 0 hour the bacterial load ranged between 0.00 x 10^5cfu/mL (0.75g/L) and 0.27 x 10^5cfu/mL (0.5g/L). At 24 hours, 0.25g/L had no bacterial load, highest bacterial load was observed at 1.0g/L (0.10 x 10^5cfu/mL) observation at 0 and 24 hour might result from a better release of the secondary metabolites in AVLME at 0.75g/L of 0 hour and 0.25g/L of 24 hours of semen extension and storage as reported by Adeshina *et al.*, (2010) that the presence of secondary metabolites in plant is responsible for their antibacterial properties.

At 48 hours and 72 hours, an increase in bacterial load was observed compared to values at 0 and 24 hours. At 72 hours, least bacterial load was observed in 0.25g/L, followed by 0.0g/L, 0.5g/L, 0.75g/L and 1.0g/L with values of 3.45, 3.82, 4.12, 6.90 and 9.05 x 10^5cfu/mL respectively. This might result from reduction or depletion of the secondary metabolites over time in the extended semen, this is in agreement with the report of Lenta *et al*., (2007) that crude extracts are liable to contamination and deterioration which reduces their susceptibility activities.

Effect of AVLME on semen quality in extended caprine semen

At 24 hours, significant effect was observed with 0.0g/L having the highest value (94.00%) to values obtained in 0.25g/L (76.33%) and 1.0g/L (76.67%). However, mean value at 0.0g/L was significantly similar with mean values at 0.50g/L (85.00%) and 0.75g/L (84.00%). No significant effect was observed at 0 hour, 48 and 72 hours of refrigeration. Mean values were within acceptable range of morphology for high quality semen which implies sperm cell morphology is not negatively affected based on the report of John *et al*., (2003) that high quality semen contains a minimal number (5 to 15%) of abnormal sperm cell, whereas low quality semen frequently contains a large number (30% or more) morphologically abnormal sperm cell.

At 0, 24, 48 and 72 hours of refrigeration, no significant difference was observed in sperm cell liveability. This result shows that AVLME had no effect on sperm cell liveability. However, it was observed that liveability mean values decreases with time, which implies that refrigeration of AVLME had negative effect on the sperm cells liveability with time.

pH is a very important factor in maintaining the integrity of biomolecules and physiological functions. It is the same for semen, wherein pH plays an essential role in maintaining the functionality of spermatozoa during fertilization. (WHO, 2010). At 0-hour, pH value was highest in 0.75g/L (6.95) and lowest in 0.0g/L (6.63). pH value at 24,48 and 72 hours was highest in 0.0g/L and lowest in 1.0g/L. At 24 hours, values ranged from 6.73 to 6.51. at 48 hours, values ranged from 6.75 to 6.54 and pH values at 72 hours ranged from 6.73 to 6.55. This implies that pH value throughout the period of storage was slightly acidic, and this was in line with the report of Oyeyemi *et al*., (2001) that buck semen is slightly acidic. Changes in the pH of semen may be regarded as a rough measure of the metabolic activity of spermatozoa, producing lactic acid from glycolysis. Mukai and Okuno 2017.

Effect of AVLME on fertilizing potential in extended caprine semen

No significant difference was observed among the treatments at 0 hour and 48 hours. At 24 hours, 0.25g/L had the least acrosome integrity value of 76.33% which was significantly different from values in 0.0g/L (95.00%), 0.50g/L (88.33%), 0.75g/L (89.00%) and 1.0g/L (89.00%). at 72 hours, least acrosome integrity value was observed in 0.75g/L (57.67%), which was significantly similar to value observed in 0.25g/L (67.67%), 0.5g/L (62.67%) and 1.0g/L (63.33%) and significantly different from 0.0g/L (72.00%). This implies that AVLME had the potential of protecting the acrosome from

undergoing capacitation during refrigeration. Capacitation is a very important process that the sperm cell undergoes in order for the acrosome reaction to take place before fertilization (Curry, 2000).

The mean values of sperm cell having Twin Head (TH), Looped Tail (LT), Coiled Tail (CT), Curved Mid-Piece (CMP) were significantly similar at 0.0g/L,0.25g/L, 0.5g/L ,0.75g/L and 1.0g/L level of AVLME inclusion. Rudimentary Tail (RT) and Detached Tail (DT) sperm cell mean values observed were significantly different. The presence of abnormal forms of sperm cells at 0 hour was in consistent with the report of WHO, 2010 that a number of abnormal forms of spermatozoa are normally encountered in all ejaculate. However, according to Bartha and Oko (1989) the proportion of defects was within expected limit of normality (less than 10%).

There was significant difference between mean values of sperm cells with RT, Bent Tail (BT), LT, CT, CMP and DT. 0.25g/L of extract inclusion had the highest proportion of sperm cells with RT, BT, CT, CMP, and DT. Mean values of sperm cells with twin head was significantly similar. The proportion of defects was within the expected limit as stated by Laing WHO, 2010 that the maximum allowed frequency of sperm head, mid-piece and tail abnormalities have been put at 20%, 5% and 5% respectively.

Sperm cells had mainly secondary sperm abnormalities as observed at 0 and 24 hours. Mean values of sperm cells with RT, BT, CT, CMP and DT were significantly similar. Proportion of sperm cells with LT differed significantly, hest proportion was observed at 0.0g/L (5.33), and 0.25g/L had least value of 1.00. These abnormalities were too small to impede sperm motility (WHO, 2010).

Sperm cells at 72 hours as well had mainly secondary sperm abnormalities as observed at 0, 24 and 48 hours. Mean values of sperm cells having RT, TH, BT, LT, CT and CMP at 0.0g/L, 0.25g/L, 0.5g/L, 0.75g/L and 1.0g/L were significantly similar (P>0.05). There was a significant increase in the proportion of DT sperm cells. Morphological spermatozoa defects that were prominent were mainly secondary sperm cell abnormalities, which according to Blom (1983), are defects which arise after spermatogenesis and during epididymal transit of spermatozoa. They could be classified as minor sperm abnormalities which may not be associated with infertility (WHO, 2010).

Conclusion

This study revealed the effect of aloe vera leaf methanolic extract on caprine semen bacterial load having better potency compared to synthetic antibiotics without impairing (Streptomycin and Penicillin) in extension and storage.

Recommendation

With respect to the response of bacterial and caprine spermatozoa to aloe vera leaf methanolic extracts used in this study, it is recommended that aloe vera leaf methanolic extract can be used in place of penicillin and streptomycin in caprine semen short term extender.

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