IJSAR ISSN: 2504-9070, Vol. 6, Issue. 1 2023 (www.ijsar.org)

PRESENT OF BACTERIAL CONTAMINATION ON SEMEN QUALITY OF SELECTED NIGERIAN BREEDS OF RAMS

[*1]GARBA S. I., [3]ABUBAKAR M. M., [3]MACHA Y. P., [3]MAI M. H., AND [2]GARBA I.

[1] Department of Animal Production, Federal College of Animal Health and Production Technology, Vom, Plateau State.
[2] Artificial Insemination Laboratory, Livestock Investigation Division, National Veterinary Research Institute, Vom, Plateau State.
[3] Department of Animal Production, Faculty of Agriculture, Abubakar Tafawa Balewa University Bauchi, Bauchi State.

[*]**Corresponding author**: sirajowase@gmail.com, 08036071717

Abstract
ThOshibanjoe study was carried to determine the present of bacterial contamination on semen quality of selected Nigerian breeds of sheep at the Livestock Investigation Division of National Veterinary Research Institute, Vom with the objective to presence of bacterial load on semen and necessitate the harvest of quality ejaculate. A total of Nine (9) rams of three different breeds; Balami, Uda and Yankasa were used for the experiment in a complete randomized design. Six (6) Semen ejaculates were collected from each ram for six (6) weeks using artificial vagina and assessed for semen quality and bacterial contamination. From the result, the volume varied from 0.80±0.13-1.04±0.16mL, colour from 3.20±0.13-4.50±0.67 (milky to creamy), mass activity from 4.10±0.28-4.50±0.16, sperm individual motility from 75.30±9.01-91.20±1.69%, live spermatozoa determine (viability) 95.40±1.28-97.10±0.61%, and concentration from, 1981.60±357.31-3545.60±453.92 x 106/mL. The concentration in Uda was significantly (P<0.05) lower (1981.60±357.31 x 106/mL) compared with Yankassa (3039.40±333.34 x 106/mL) and Balami (3545.60±453.92 x 106/mL). Escherichia coli, Bacillus spp and Staphylococcus spp. were found in semen of all breeds except Proteus spp found only in Yakassa. Ram semen can be contaminated by bacterial with subsequent effect on semen quality as observed on sperm cell concentration in this study and Escherichia coli and Bacillus spp are the most common bacterial contaminants in ram semen. Despite hygienic measures during collection, processing, and storage of semen, several opportunistic microbes find their ways into semen. Therefore, stringent sanitary precautions are required at every step of semen collection and processing to achieve success in AI programmes.
Keyword: *bacterial contamination, semen quality, Yankassa, Uda and Balami rams*

INTRODUCTION

Semen quality is a major of fertility in male (ram) animals and is indicative of fertilizing potential of semen. Semen quality is adversely affected by bacterial contamination (Diemer *et al.,* 1996). Presence of bacteria, fungi and viruses has been detected in semen samples that deteriorate semen quality as well as transmit the pathogens to next generation. Infection in animals, environment, preputial sheath, entry of organisms during semen collection, processing and packaging, contribute to the microbial load of semen, as a consequence, bacteria might contaminate the female's reproductive tract which in turn leads to negative effects on subsequent fertility (Ochsendrof and Fuchs 1993; Griveau *et al.,* 1995). The role of specific microbes in semen leading to reproductive disorder among dairy animals is well established. Roberts, (1971) observed deleterious effect of bacterial load on fertility of semen. Sperm cells are said to be adversely affected by bacteria due to their direct spermicidal effect there by affecting motility, morphology and various semen quality parameters (Najee *et al.,* 2012). Bacteria like *E. coli* significantly decrease sperm motility and lead to increased agglutination of spermatozoa. Additionally, *E. coli* has been determined to adhere to the sperm membrane/surface via mannose binding sites (Monga and Roberts, 1994; Wolffet *et al.,* 1993). The microbial flora of semen may cause reproductive disorders in females, increase embryonic mortality, lower conception rates, and cause abortion and other complications at a large scale Abro *et al.,* (2015), as AI is widely practiced in livestock production and improvement. This study was designed to evaluate fresh ram semen and to determine presence of bacterial load on semen quality of three Nigerian breeds of rams.

MATERIALS AND METHOD

STUDY AREA

This study was carried out at Livestock Investigation Division, National Veterinary Research Institute (NVRI), Vom, Jos South Local Government Area, Plateau state. It is located on latitude 9°44N and longitude 8º45E with a population of 306,716 people as (NPC census, 2006) and a total area of 510km^2. It has an annual rainfall of 131.75cm and a temperature range 12ºC-33ºC with physical feature of rocky granites of old volcanoes (Knubsen and sohel, 1970, NVRI, 2006).

Housing and management of animals

The rams were kept in individual pens and exercised once a day, the day before semen collection so as to maintain the sexual vigor of the animals and ensure quality semen production. Vaccination, deworming, screening of sexually transmitted diseases and other health programs was routinely maintained to ensure good health.

Experimental design

A complete randomized design was used for this study. Briefly, a total number of Nine (9) rams of three different breeds; Balami, Uda and Yankasa were sourced from small ruminants Unit of Livestock Investigation Division, National Veterinary Research Institute, Vom, Plateau State.

Sample collection and handling

Semen was collected aseptically from the ram weekly for six (6) weeks using an artificial Vagina. The Rams was trained for semen collection during the acclimatization period. The semen was collected into graduated test tube and immediately taken to the laboratory and placed in a water bath at 37^{0}C. A sterilized swap stick was dipped into the semen collected and placed on ice pack to the laboratory for microbial evaluation.

Semen quality assessment

The ejaculates were kept in a water bath at 37^{0}C to access volume, colour, mass activity, individual motility, sperm

concentration, morphology and live spermatozoa.

Volume: volume of semen was measured directly from the calibrated tube used for the collection.

Colour: scoring of the semen colour was done as described by Zemjanis (1970).

Semen pH: this was determined by dipping a litmus paper into the ejaculate and corresponding colour changes observed and recorded.

Gross motility: was examined as quickly as possible after collection, by placing a drop of the semen sample on a pre-warmed at 37 ^{o}C glass slide, covered with cover slip and examined at ×10 magnification.

Progressive sperm motility (individual motility): semen is diluted in citrate buffer in the ratio of 1:10. Place a very small drop of diluted semen on a clean greese free microslide. The microslide is pre-warmed at 37 ^{o}C. The semen drop is then covered with coverslip. At least 4-5 fields was examined (×40 magnification) for assessment of progressive sperm motility. The number of progressively motile sperms and non motile sperms in each field was counted and the average taken.

Spermatozoa concentration: was determined as described by Azawi and Ismaeel (2012).

Live sperm cells: this was determined as described by Esteso *et al.* (2006).

Sperm abnormalities: was determined by making a thin smear of the semen sample, on clean grease-free glass slide and stained with eosin-negrosin. Sperm cells will counted per slide using hand counter under light microscopy at ×40 magnification.

Culture, isolation and identification

A total of 42 swabs were taken accordingly with a sterile swab (Nicecare®) from each semen sample and take to microbiology laboratory for bacterial culture, isolation and identification on icepacks. Accordingly, samples for bacterial isolation were processed by methods described by Cheesbrough (1985) and Cowan and Steel (1993). Biochemical test were performed according to Forbes *et al.* (2007).

Statistical analysis

Data collected were expressed as mean ± standard error of mean (SEM) using SPSS version 25. Repeated measure one-way analysis of variance (ANOVA) was used to test for differences between groups, followed by Duncan multiple comparison Test. Values of $P<0.05$ were considered significant.

RESULT

The baseline mean values for semen colour, volume, pH, mass activity of spermatozoa individual motility, concentration, live sperm percentage and normal sperm percentage are presented in Table 1. There were significant ($P<0.05$) differences in the mean ejaculate volume between Uda (0.80±0.13mL), Yankassa (0.98±0.50 mL) and Balami (1.04±0.16 mL). However, the value obtain from Balami was higher compared to Uda and Yankassa. The overall seminal colour (3.37±0.90) of the three breeds was milky. The mean pH of Yankassa (6.30±0.15) was higher than Uda (6.22±0.28) and Balami (6.10±0.10), respectively. It was observed that the rams seminal pH (6.21±0.77) was lower than normal pH values of 6.5 to 7.7, however, no significant difference of pH between the breeds. There was no significant ($P>0.05$) difference in the mass activity of semen in the three breeds. Although Balami (4.50±0.16%) had a higher mass activity compared to Uda (4.10±0.28%) and Yankassa (4.50±0.16%). The result shows an overall moderately distinct swirl movement (4.27±0.14%). On the overall, the individual sperm cell motility was 84.47±3.52%. There was no significant ($P>0.05$) difference in the individual motility Although Balami (91.20±1.69%) showed a high motility compared to Yankassa (86.90±4.56%) and Uda (75.30±9.01%). The overall concentration of the ram spermatozoa was observed to be (2834±53±248.91 x 10^{6}/mL). There was a significant ($P<0.05$) difference in the

concentration of sperm cells in Uda (1981.60±357.31 x 10^6/mL) than those seen in Balami (3545.60±453.92 x 10^6/mL) and Yankassa (3039.40±333.34 x 10^6/mL). There was no significant ($P>0.05$) difference in the morphology and live spermatozoa. However, the higher values of live spermatozoa was observed in Yankassa (97.10±0.61%) than in Balami (96.80±1.07%) and Uda (95.40±1.28%), respectively.

Microbiological evaluation of fresh ram semen

The bacteria identified from ram semen, showed cultural, morphological, biochemical and staining characteristics. The results are presented in Table 2. *Staphylococcus spp* produced TW colonies on blood ager (BA). The TLF colony is lactose fermenting on MacConkey agar (MCA). They are Gram+ve cocci arranged in clusters, catalase +ve and coagulase +ve. *Escherichia coli* appear as SG colony on BA and SLF colony, lactose fermenter on MCA. They are Gram-ve motile bacilli, indole -ve, catalase +, oxidase –ve, urease -ve, citrate -ve, and ferment glucose, sucrose, lactose with gas production on TSI. *Proteus spp* appear as swarming gray cololny on BA and pink non-lactose fermenter on MCA with swarming motility. They are Gram-ve motile rods, oxidase-ve, catalase –ve, urease +ve, citrate +ve, indole +ve, and ferment glucose only with H_2S gas production on TSI. *Bacillus spp* produces typical characteristics of medusa head type DF colony and grey haemolytic colonies on BA and LLF colony on MCA. The frequency of bacterial isolates is presented in Table 3. A total number of 25 bacteria were isolated from 42 semen samples taken. Of the total samples, *Escherichia coli* and *Bacillus* spp have the highest isolate rate of 9 (21.43%), respectively, with *Proteus spp* 1 (2.38%) with least isolation rate. The result showed that Yankassa had the highest genera of bacteria isolates (4).

DISCUSSION

The colour of the ram semen was creamy to milky-creamy in this study. This result agrees with the earlier reports of Bag *et al.,* 2002; Azizunnesa *et al.,* 2014. Bag *et al.* (2002) stated that the colour of ram semen varied from milky to creamy. There was no significant difference in semen volume and sperm morphology. The ejaculate volume varied in this study which agrees with previous report by Moss *et al.* (1988). Azizunnesa *et al.* (2014) stated that the normal ejaculated volume of semen of ram was 1.2 ± 0.0 mL.

The pH of ram semen range from this study was normal which agrees with earlier report of Patel (1967) and Huat (1975) who reported an average pH range 6.5 and 7.7. Similarly, the mass activity was higher in Yankassa compared to Balami and Uda, however, no significant difference was seen in the rams. The mass activity varied in this study which matches with the report of Cunha *et al. (*2012) who reported that the mass motility varied from 0-5 depending on species. Also the live spermatozoa of Yankassa were higher compared to Uda and Balami which is in line with the report of Mahmuda *et al.* (2015). The result of this study showed that the sperm concentration is in congruence with the report of Azizunnesa *et al.* (2014) and Mahmuda *et al.* (2015) who stated that the sperm concentration of indigenous ram lay between 3900 to 4500 x 10^6/mL.

From the result obtained in this study, the bacteria isolated from semen samples are similar to those reported by Adil *et al.* (2018), Meena *et al.* (2015). The bacteria isolated are mostly commensal which is in line with earlier studies carried out by Aurich and Spergser, (2007). Most bacteria can be declared as commensals, but there are also potentially pathogenic ones that can contaminate collected semen. Several of these bacteria have been identified in association with breeding failure in cattle and warrants precautionary and preventive measures for successful breeding program

Mitra *et al.,* (2016).

Semen samples most frequently are found to be contaminated with *Staphylococci, Coliforms, Streptococci,* etc, Corona *et al.* (2005) which negatively affects the motility and viability of bovine semen. Microbial contamination affects motility, morphology and various semen quality parameters (Najee *et al.,* 2012). This agrees with the findings of the present study where the sperm cell concentration was significantly ($P<0.05$) affected with *Escherichia coli, Staphylococcus spp* and *Bacillus spp* isolated from the ram semen. Sperm cells are said to be adversely affected by bacteria due to their direct spermicidal effect. For example, early studies concluded concentration-dependent spermicidal effects of *Escherichia coli* and can decrease sperm motility and lead to increased agglutination of spermatozoa. Additionally, *Escherichia coli* has been determined to adhere to the sperm membrane/surface via mannose binding sites (Monga and Roberts, 1994; Wolffet *et al.,* 1993). Similarly, *Staphylococcus spp* isolated in this study was similar to earlier reported by Ahmed *et al.* (2017) from rams in Bangladesh. Microbial contaminants lead to decrease in motility due to adherence with sperm cells and as the total microbial number varies in semen samples, so does the type of microorganisms. Yaniz *et al.* (2010) reported the presence of aerobic bacteria in almost all the harvested samples of semen.

The production of reactive oxygen species (ROS) by macrophages and polymorphonuclear granulocytes due to microbial contamination can compromise sperm functioning and potential to fertilize. ROS at higher levels may affect sperm quality by compromising membrane integrity, chromatin integrity, blocking oxidative metabolism, and reducing chances of fertilization and thereby subsequent conception (Lone *et al.,* 2016). The microbial flora of semen may cause reproductive disorders in females, increase embryonic mortality, lower conception rates, and cause abortion and other complications at a large scale.

Table 1: Table 1: Semen characteristics of Balami, Yakassa and Uda rams (Mean±SD)

Breed	Colour	Volume (mL)	pH	Mass activity	Individual motility (%)	Concentration ($x10^6$/mL)	Normal sperm Morphology (%)	Live spermatozoa (%)
Uda	3.40±0.63	0.80±0.13	6.22±0.28	4.10±0.28	75.30±9.01	1981.60±357.31[a]	97.30±1.00	95.40±1.28
Yankassa	4.50±0.67	0.98±0.50	6.30±0.15	4.20±0.29	86.90±4.56	3039.40±333.34[b]	97.30±1.13	97.10±0.61
Balami	3.20±0.13	1.04±0.16	6.10±0.10	4.50±0.16	91.20±1.69	3545.60±453.92[b]	97.70±0.84	96.80±1.07
Overall mean	3.37±0.90	0.94±0.07	6.21±0.77	4.27±0.14	84.47±3.52	2834±53±248.91	97.43±0.56	96.43±0.59

The mean values with different superscript within the same column differ significantly ($P<0.05$). Color (1-5 grades): 1 = watery, 2 = yellowish, 3 = milky, 4 = creamy. Mass activity (1-5 grades): 1= no swirl, 2 = very slow distinct swirl, 3 = slow distinct swirl, 4 = moderately fast distinct swirl, 5 = fast distinct swirl.

Table 2: Cultural characteristics of bacterial isolates from preputial washing of Balami, Yakassa and Uda rams.

Cultural characters			Biochemical tests													Bacteria identified
Blood agar	MacConkey agar	Gram reaction	catalase	coagulase	oxidase	Indole	Citrate	TSI	urease	Glu	Mal	Suc	Sor	Lac	Xyl	
SG colonies	SLF Colonies	G –ve	+		-	-	-	AG	-	+	+	+	d	+	+	Escherichia coli
DF colonies	LLF Colonies	G +ve	+		-	-	+	AA	-	+	+	+	+	-	+	Bacillus spp
TW colonies	TLF Colonies	G +ve	+	+										+		Staphylococcus spp
Swarming gray colonies	NBG Colonies	G –ve	-		-	+	+	H_2S	+	+	+	+	+	-	d	Proteus spp

TSI: Triple Sugar Iron. Glu=glucose, Lac=lactose, Suc=sucrose, Mal= maltose, Sor= sorbitol, Xyl= Xylose. (-) negative reaction, (+) positive reaction.

Table 3: Bacterial Isolates from semen of Yankassa, Balami and Uda rams

Breed	Bacterial Isolates				total
	Escherichia Coli	***Staphylococcus Spp***	***Proteus spp***	***Bacillus Spp***	
Yankassa (n=15)	3	2	1	3	9
Balami (n=13)	1	2	0	1	4
Uda (n=14)	5	2	0	5	12
Total	9 (21.43%)	6 (14.24%)	1 (2.38%)	9 (21.43%)	25

n= number of semen swaps collected.

CONCLUSION AND RECOMMENDATION

Bacterial contamination of ram semen can have profound effect on semen quality as observed on sperm cell concentration in this study. *Escherichia coli* and *Bacillus spp* are the most bacterial contaminants in semen of Yankassa, Balami and Uda rams. Despite hygienic measures, several opportunistic microbes find their ways into semen during collection, processing, and storage of semen. Stringent sanitary precautions are therefore required at every step of collecting semen and it's processing to achieve success in AI programmes.

ACKNOWLEDGMENTS

The authors are thankful to staff of Artificial Insemination Laboratory, Livestock Investigation Division and Central Diagnostic Laboratory, NVRI, Vom, for providing necessary technical support to carry out the present work.

Conflict of Interests

The authors declare that they have no conflicting interests.

REFERENCE

Abro SH, Abro R, Tunio M, Rind R, Bughio S. (2015) Evidence of bacterial contamination in the frozen bovine semen, *Pak Journal of Agri Agril Engg Veterinary Science*; 31(1):102-108.

Adil Rasool Paray, Mukesh Bhakat, Shabir Ahmad Lone, Tushar Kumar Mohanty, Ranjana Sinha, Javid Ur Rahman, Zahid Bashir Khanday, Ziaullah Danish (2018). Role of preputial washing in reducing microbial load and improving bovine semen quality. Asian Pacific Journal of Reproduction; 7(3): 97-102.

Ahmed, E., Islam MS, Alam MGS, Jha PK, Ghosh, S, Naher N and Bari FY (2017). Bacterial contamination of ram semen used for artificial insemination in indigenous ewes. The Bangladesh *Veterinarian* (2017) 34(1): 20 – 26.

Aurich C, Spergser J (2007): Influence of bacteria and gentamicin on cooled-stored stallion spermatozoa. Theriogenology 67 912–918.

Azawi, O.I. and Ismaeel, M.A. (2012).

Effects of seasons on some semen parameters and bacterial contamination of *Awassi ram* semen. *Reproduction of Domesticated Animal,* 47: 403-406.

Azizunnesa, Zohara BF, Bari FY, Alam MGS (2014): Effects of proportion of egg yolk and preservation time on chilled Semen from Indigenous Rams. GSTF International Journal of Veterinary Science 1 18-26.

Bag S, Joshi A, Rawat PS, Mittal JP (2002): Effect of initial freezing temperature on the semen characteristics of frozen thawed ram spermatozoa in a semiarid tropical environment. Small Ruminant Research 43 23-29.

Cheesbrough, M. (1985) Culturing of Anaerobes. In: Medic al Laboratory Manual for Tropical Countries, Butterworth Co., Kent, 248-264.

Corona, A. and Cherchi, R. (2009). Microbial quality of equine frozen semen. Anim. Reprod. Sci., 115: 103-109.

Cowan, S.J. and Steel, K.J. (1993) Manual of Identification of Medical Bacteria. Third Edition, Cambridge University Press, Cambridge, 22-126.

Diemer T, Weidner W, Michelmann HW, Sciefer HG, Rovan E, Mayer F. (1996). Influence of Eschericia coli on motility parameters of human spermatozoa in vitro. Int J Andorl; 19: 271-277.

Esteso, M. C., soler A., Fernandez-santos, M. Quinteromoreno. A, A. And Garde, J.J (2006). Functional significance of the sperm head morphometric size and shape for determining freezability in Iberian Red Deer (Cervuselaphushipanicus) epididymal sperm samples. *Journal of Andrology*, 27 (5): 662-670.

Forbes, B.A, Sahm D.F, and Weissfeld A.S. (2007). Bailey and Scotts' Diagnostic microbiology 12th ed. Elsevier . Anon., 1994. The Microbiology of Water 1994. Part 1: Drinking Water, Report on Public Health and Medical Subjects, No. 71. Methods for the Examination of Waters and Associated Materials. London: HMSO.

Griveau, J.F., Domount, E., Renard, P., Challegani, J.P. and Lelannou, D. (1995). Reactive oxygen species lipid peroxidationand enzymatic defense system in human spermatozoa. J. Reprod. Fertil., 103: 17-26.

Huat, K.S. 1973. Semen characteristics of crossbred goats. (Kambing Kajong Jamnapari) Kajian Vet. (Malaysia Singapore) 7(2):63-66. Ref. Nov. 1975.

Lone SA, Prasad JK, Ghosh SK, Das GK, Kumar N, Balamurugan B, (2016). Effect of cholesterol loaded cyclodextrin (CLC) on lipid peroxidation and reactive oxygen species levels during cryopreservation of buffalo (Bubalus bubalis) spermatozoa. Asian Pac J Reprod; 5(6): 476-480.

Mahmuda BBA, Azizunnesa, Zohara BF, Alam MGS, Bari FY (2015): Effect of preservation time on the quality of frozen semen in indigenous rams. Bangladesh Journal of Animal Science 44 10-15.

Meena GS, Raina VS, Gupta AK, Mohanty TK, Bhakat M, Abdullah M,Bishist R. (2015). Effect of preputial washing on

bacterial load and preservability of semen in Murrah buffalo bulls. Vet World; 8 (6): 798-803.

Mitra J, Chowdhury S, Panda S, Chakraborty M, Singha A. (2016). Microbiological evaluation of bovine frozen semen samples in West Bengal, India. Explor Anim Med Res; 6 (2): 185-191.

Monga M, Roberts J. A. (1994). Sperm agglutination by bacteria: Receptorspecific interactions. J Androl; 15: 151-158.

Moss TA, Melrose DR, Reed HCB, Vendeplasche M 1988: Spermatozoa semen and artificial insemination. Fertility and Infertility in Domestic Animals 4 132-154.

Najee H. B, Al-Shawii AM, Abd-Al Rahman LY. (2012). Bacterial contamination of imported bulls frozen semen. Al-Anbar J Vet Sci; 5 (1): 19996527.

Ochsendrof F. R, and Fuchs J. (1993). Oxidative imbalances in male fertility. In: Fuchs J, Packer L, editors. Oxidative stress in dermatology. New York: Marcel Dekker; p. 489-531.

Patel, J. (1967). Artificial Insemination in goats. Indian Vet. J. 44:509-511.

Roberts S. J. (1971). Veterinary obstetrics and genital disease. 2nd ed. Indian: CBS Publishers and Distributors;, p. 612-750.

Wolff H, Panhans A, Stolz W, Meurer M. (1993). Adherence of Escherichia coli to sperm: A mannose mediated phenomenon leading to agglutination of sperm and E. coli. Fertil Steril; 60: 154-158.

Yaniz, J.L., Marco-Aguado, M.A., Mateos, J.A. and Santolaria, P. (2010). Bacterial contamination of ram semen, antibiotic sensitivities, and effects on sperm quality during storage at 15°C. Anim. Reprod. Sci., 1-2: 142-149.

Zemjanis, R. (1970). Collection and evaluation of semen. In: Diagnostic and Therapeutic techniques in Animal Reproduction. 2nd Edition, Williams and Wilkins Co. Baltimore MD.139-156.