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# Spermiogram, microbial contaminants and sensitivity to antibiotics in fresh semen of two poultry lines

SO Asuku[1]*, RI Ali[1], IM Alkali[1], UM Bamanga[2], AR Mustapha[1], ZA Algoni[3], MU Abdulrahman[3] & A Abba[2]

1. *Department of Theriogenology, Faculty of Veterinary Medicine, University of Maiduguri, Borno State, Nigeria*
2. *University of Maiduguri Veterinary Teaching Hospital (UMVTH), Maiduguri, Borno State, Nigeria*
3. *Faculty of Veterinary Medicine, University of Maiduguri, Borno State, Nigeria*

*Correspondence: Tel.: +2347060692699; E-mail: suleimanomeiza06@gmail.com



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**Abstract**

This study aimed to isolate bacteria flora in fresh semen of helmeted guinea cock *(Numidia meleagris)* and Nigeria local turkeys *(Meleagris gallopavo)* and determine the sensitivity of the semen microbes to antibiotics with further assessment of sperm quality characteristics in the freshly collected semen. Five matured guinea cocks weighing approximately 3 – 4kg and five matured turkey toms of 10 – 14kg body weights were selected for the study as sperm donors. Semen collection was done twice a week for five weeks using a modified abdominal massage method and ejaculates were pooled independently based on the animal group. The pooled ejaculates in each species were further divided into aliquot A for spermiogram analysis and aliquot B for microbes and antimicrobial sensitivity analysis using standard methods. Results showed that freshly collected semen from both donor birds were contaminated; although, presented good qualities when evaluated for sperm characteristics such as progressive motility (%), livability (%), morphologic abnormalities (%) and concentration (cell×$10^9$/ml). Isolated contaminants included *Escherichia coli, Shigella spp, Staphylococcus aureus and Bacillus spp* in fresh semen of the helmeted guinea cocks, similar in turkeys except for *Shigella spp.* The organisms were all sensitive to streptomycin, gentamycin and pefloxacin except the *Staph aureus* which showed resistance to pefloxacin and *Bacillus spp* which was resistant to gentamycin. It was then concluded, that whereas freshly collected semen of guinea cock and turkey toms were contaminated during collection, they still possessed good quality traits when evaluated for semen characteristics and antibiotics like streptomycin, pefloxacin or gentamycin are recommended for use during *in-vitro* processing and extension of semen from both poultry species.

*Keywords*: Antibiotics, Bacterioflora, Cloaca, Helmeted guinea cock, Semen quality, Turkey toms

## Introduction

In Nigeria, the poultry industry is expanding and along with domestic chicken *(Gallus gallus domesticus)*, helmeted guinea fowls *(Numidia meleagris)* and domestic turkeys *(Meleagris gallopavo)* are front-line avian species believed to impact on poultry agricultural subsector. While both species are

important in egg and meat production, guinea fowl are seasonal breeders whose semen quality decreases in the dry season but are more prolific during the rainy season (Nwakalor *et al.,* 1988). Domestic turkeys depend almost entirely on Artificial Insemination (AI) but their semen cannot be kept *in-vitro* for long without irreparable loss in potential fertility index (Iaffaldano *et al.,* 2005). Meanwhile, biotechnology like AI is used widely for the effective propagation of desirable traits in livestock (Ax *et al.,* 2000; Dhama *et al.,* 2014), the technique can only benefit the poultry industry through the successful collection and efficient maintenance of semen quality characteristics (Donoghue & Wishart, 2000; Dumpala *et al.,* 2006).

Poultry semen gets contaminated with harmful microbes via contact with the papillae in the walls of the avian cloaca during collection and can be transferred to hens or progeny (Lierz, 2008). While the cloaca is a unique outlet for the passage of intestinal and reproductive tract secretions in birds (Ax *et al.,* 2000), these dual functions appeared indispensable in the poor outcome of AI in poultry breeding (Dhama *et al.,* 2014; Alkali *et al.,* 2020). Previous studies revealed *Salmonella spp*, *Mycoplasma spp, Clostridium, Campylobacter, Escherichia coli, Corynebacterium, Enterococcus faecalis and Candida albicans* as common microbes identified in the cloaca and semen of birds (Donoghue *et al.,* 2004; Kabir, 2010; Alkali *et al.,* 2020). Whereas it is evident that these organisms affect semen quality and can be transmitted in flocks through semen contamination (Haines *et al.,* 2013), other findings showed *campylobacter and salmonella species* as seldom a cause of apparent clinical diseases in poultry flocks (Donoghue *et al.,* 2004).

The populations of wild Galliformes have declined including those in captivity (Hennache, 2009), which underscores the need for efficient methods to preserve and improve semen storage of some domestic poultry described as closely related genetically (Saini *et al*., 2007). In the last few decades till date, significant progress has been made including the pre-dilution of semen with extenders fortified with antibiotics (Sexton *et al.,* 1980; Iafalldano *et al.,* 2005; Dumpala *et al.,* 2006). Antibiotics and sometimes antimycotics are often incorporated in extenders without prior knowledge of the type of microorganisms that is prevalent in the semen. Donoghue *et al.* (2004) reported the inconsistency in the reduction of microbial concentration in turkey semen following pre-dilution with extenders incorporated with antibiotics. Although extensive reports on semen contaminants of most avian species are published, only a few are reported for turkeys and none for the helmeted guinea fowl *(Numidia meleagris).* This study was designed therefore to isolate bacteria flora in fresh semen of two poultry lines; the helmeted guinea cock *(Numidia meleagris)* and Nigeria local turkey toms *(Meleagris gallopavo)* and to determine the sensitivity of the semen microbes to antibiotics with further assessment of sperm quality characteristics in the freshly collected semen.

**Materials and Methods**

*Research laboratory and experimental animals*

This is an experimentally designed study conducted in the Andrology and Microbiology Laboratories of the Faculty of Veterinary Medicine, University of Maiduguri, Nigeria. The birds were purchased from poultry breeders within the Maiduguri metropolis and housed in individual cages at the Faculty of Veterinary Medicine Large Animal Clinic Complex, University of Maiduguri. Five matured guinea cock weighing approximately 3 – 4 kg and five matured turkey toms weighing 10 – 14 kg were used for the study as sperm donors. They were acclimatized and trained for semen collection for two weeks, fed with commercial poultry feed and given clean water *ad libitum.*

*Semen collection and evaluation*

Semen was collected twice a week (Mondays and Thursdays) for 5 weeks using the modified abdominal massage methods as described by Asuku *et al.* (2022). Ten replicates were made for each guinea cock and the turkey tom respectively, summing up a total of fifty ejaculates for each animal group. Ejaculates from all donors were pooled independently based on the group and immediately divided into aliquots A and B in graduated 1ml Eppendorf tubes. Aliquot A was evaluated for both macroscopic (volume, colour, consistency) and microscopic characteristics (progressive motility, morphologic abnormality, livability and concentration) while aliquot B was evaluated for microbial contaminants and antibiotic sensitivity. Semen volume (ml), colour (white, creamy or milky) and consistency (thick or thin) were observed via a graduated collection vial and the microscopic changes were evaluated as follows:

*Progressive motility:* This was performed by adding a drop of freshly collected semen on a clean glass slide and diluted with a drop of normal saline. The mixture was then covered with a glass cover slip and estimated subjectively by two operators when slides were examined at x40 magnification under the light

microscope. Motility was expressed as a percentage of motile spermatozoa observed in the microscopic fields.

*Viability and morphologic abnormality:* The live/dead ratio of spermatozoa and the morphology were determined using nigrosin-eosin (5% eosin, 10 % nigrosin) stained slides as reported in the work of Yahaya *et al.* (2013). Briefly, a drop of eosin-nigrosin solution was made on a clean glass slide followed by a drop of semen and mixed gently. Thereafter, a smear was made and allowed to air dry for a few minutes, then viewed with immersion oil at x100 magnification under the light microscope. The viability was recorded as the number of unstained spermatozoa (live) to the stained (dead) spermatozoa expressed in percentage after a differential count of 100 sperm cells observed at different microscopic fields. Similarly, the morphologic abnormalities were also evaluated and expressed as a percentage of abnormal cells against the normal sperm cells (Yahaya *et al.,* 2013).

*Concentration:* Sperm counts were determined with a Neubauer counting chamber (hemacytometer) as performed in the work of Ngu *et al.* (2014). Semen was diluted with formal saline at 1:400 and used to charge the counting chamber through the capillary action of the red cell pipette. The hemacytometer was mounted and sperm cells within five large diagonal squares were counted and presented as the number of cells x $10^9$/ml (Mohan *et al.,* 2016).

*Isolation and identification of microbial contaminants in the semen of helmeted guinea fowl and domestic turkeys*

All semen samples were properly labelled; inoculated into the nutrient broth and incubated at 37°C for 24 hours to obtain the bacteria growth. Those that indicated growth were further sub-cultured into various selective/differential media to obtain a pure culture. The selective and differential media were the Mannitol Salt Agar (MSA) for the isolation of *Staph aureus*, MacConkey Agar for the isolation of lactose and non-lactose fermenters, Salmonella Shigella Agar (SSA) for the isolation of *Salmonella* and *Shigella species,* Eosin Methylene Blue agar (EMB) for the isolation of *Escherichia coli* and Nutrient agar for identification of *Pseudomonas*. Colonial growth and morphology; characterized by size, shape and colour were presumptively identified on the various media after 18 – 24 hours and further subjected to Gram's staining technique to differentiate the gram-positive and gram-negative organisms when examined under x100 objective of a light microscope. The pure cultures were further transferred onto nutrient agar (Mueller Hinton agar) and subjected to a anti-microbial sensitivity test.

*Sensitivity of semen microbes to antibiotics*

Using a sterile wire loop, streaking of the test organism was done according to methods described by Harrigan & McCance (1976) in standard medical laboratory technique. The Disc diffusion antibiotic susceptibility test was performed using a similar method reported by Alkali *et al.* (2020). It involved fourteen (14) commercially prepared gram-positive and gram-negative antibiotic-impregnated discs such as; Ciprofloxacin (10µg), Gentamycin (10µg), Pefloxacin (5µg), Rifampin (10µg), Amoxycilin (30µg), Erythromycin (30µg), Chloramphenicol (20µg), Ampiclox (30µg), Ceporex (10µg), Augmentin (30µg), Nalidixic acid (30µg), Tarivid (10µg), Streptomycin (30µg), Septrin (30µg) and Ampicillin (30µg). A colony from the identified culture were inoculated into Mueller-Hinton Broth (MHB) and incubated for an hour. Thereafter, the broth culture was inoculated onto Mueller-Hinton agar (MHA) plates using a sterile swab. Antibiotic-impregnated paper discs were then placed on each MHA plate and pressed gently to ensure contact. The plates were incubated at 37°C for 24 hours and the diameter of the visible zone of inhibition was measured and compared to reference values. The results were interpreted qualitatively as resistant, intermediate and susceptible. Organisms were considered sensitive if the zone of inhibition was greater than a reference value (16mm), intermediate (-) where the kill zone was between 12mm – 16mm and non-sensitive or resistant where the kill zone was greater than 12mm (Ngu *et al.,* 2014).

*Statistical analysis*

Data were analyzed using an unpaired sample *t*-test and presented as Mean ± SD. *P*-values of less than 0.05 were considered statistically significant and *GraphPad InStat®* was the statistical software used for the analysis.

**Results**

During the period of acclimatization, turkey toms got accustomed to procedures of semen collection faster than the guinea cocks and consequently, quality traits such as semen volume, livability and sperm concentration appeared significantly lower ($p < 0.05$) in the semen of guinea cock than that of the turkey toms; there was no significant difference in mean progressive motility of semen from both donor birds (Table 1). The morphologic abnormality was significantly higher ($p < 0.05$) in the semen of the

guinea cock (12.8 ± 2.1%) than that of the turkey toms (8.4 ± 2.3%). Meanwhile, the mean semen volume was 0.03 ± 0.1mls and 0.20 ± 0.03ml in guinea cocks and the turkey toms respectively; they both had a concentration greater than 3 billion spermatozoa per ml (table 1).

In this study, four isolates which include *Escherichia coli, Shigella spp, Staphylococcus aureus* (Plate 1 & II) *and Bacillus spp* were identified in fresh semen of guinea cock and turkey toms with the exception of *Shigella spp* in fresh semen of turkey toms (Table 2). The most frequently identified organism was *Escherichia coli* in samples from both donor birds (Plate III). All the organisms were sensitive to

**Table 1**: Sperm characteristics in fresh semen of helmeted guinea cock and local turkey toms in Nigeria

| Parameters | Semen volume (mls) | | Progressive motility (%) | | Live/dead ratio (%) | | Morphologic abnormalities (%) | | Sperm Conc. (cell $\times 10^9$/ml) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Guinea Cock | Turkey Tom | Guinea Cock | Turkey Tom | Guinea Cock | Turkey Tom | Guinea Cock | Turkey Tom | Guinea Cock | Turkey Tom |
| Week 1 | 0.03 | 0.19 | 70.0 | 90.0 | 70.0 | 80.0 | 15.0 | 10.0 | 3.5 | 5.6 |
| Week 2 | 0.03 | 0.21 | 85.0 | 95.0 | 70.0 | 85.0 | 10.0 | 10.0 | 3.8 | 6.0 |
| Week 3 | 0.02 | 0.25 | 80.0 | 80.0 | 75.0 | 80.0 | 12.0 | 07.0 | 3.1 | 5.1 |
| Week 4 | 0.05 | 0.16 | 85.0 | 85.0 | 70.0 | 90.0 | 12.0 | 10.0 | 2.9 | 6.0 |
| Week 5 | 0.03 | 0.20 | 85.0 | 80.0 | 75.0 | 85.0 | 15.0 | 05.0 | 3.5 | 6.2 |
| Mean ± SD | 0.03± 0.01[a] | 0.20 ± 0.03[b] | 81.0 ± 6.5 | 86.0 ± 6.5 | 72.0 ± 2.7[a] | 84.0 ± 4.1[b] | 12.8 ± 2.1[a] | 8.4 ± 2.3[b] | 3.3 ± 0.3[a] | 5.7 ± 0.4[b] |

Mean values in the last column with different superscripts differ significantly at $p < 0.05$

**Table 2**: Cultured and isolated microorganisms in fresh semen of guinea cock and turkey toms

| Sample | Nutrient Agar | | Salmonella-Shigella agar | | Macconkey agar | | Mannitol salt agar | | Eosin methylene blue | |
|---|---|---|---|---|---|---|---|---|---|---|
| | A | B | A | B | A | B | A | B | A | B |
| *Shigellaspp* | - | - | + | + | + | - | - | - | - | - |
| *Salmonella spp* | - | - | - | - | - | - | - | - | - | - |
| *Staph aureus* | - | - | - | - | - | - | + | + | - | - |
| *Escherichia coli* | - | - | - | - | + | + | - | - | + | + |
| *Bacillus spp* | - | - | - | - | - | - | + | - | - | - |
| *Proteus* | - | - | - | - | - | - | - | - | - | - |
| *Pseudomonas* | - | - | - | - | - | - | - | - | - | - |

Keys: A: semen sample from guinea cocks B: semen sample from turkey toms
+: identified -: not identified

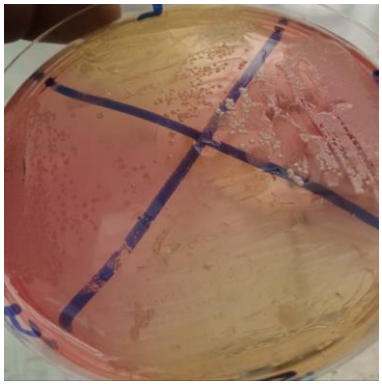

**Plate I**: Colonies appeared golden yellow on Mannitol Salt Agar (MSA) indicative of the growth of *Staphylococcus aureus*

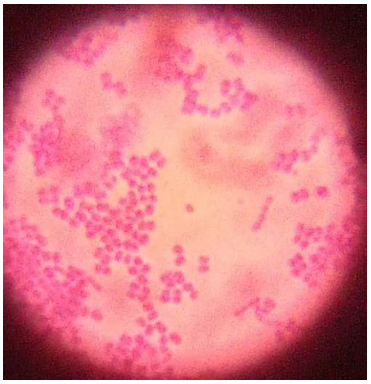

**Plate II**: Gram stain showing Gram +ve cocci in clusters and purple colouration indicative of *Staph spp*

**Plate III**: Growth of *E. coli* indicated by green metallic sheen in Eoisin Methylene Blue

**Table 3**: Antibiotic sensitivity of bacteria organisms isolated in fresh semen of guinea cock and turkey toms

| Antibiotics | *Escherichia coli* | *Shigella spp* | *Staph. aureus* | *Bacillus spp* |
|---|---|---|---|---|
| Streptomycin | Sensitive | Sensitive | Sensitive | Sensitive |
| Gentamycin | Sensitive | Sensitive | Sensitive | Resistant |
| Pefloxacin | Sensitive | Sensitive | Resistant | Sensitive |
| Ciprofloxacin | - | - | Sensitive | Sensitive |
| Augmentin | - | - | - | Resistant |
| Tarivid | Sensitive | - | Sensitive | Sensitive |
| Ceporex | Resistant | - | Sensitive | - |
| Nalidixic Acid | Resistant | Resistant | - | Sensitive |
| Septrin | - | - | Resistant | Sensitive |
| Ampicillin | Sensitive | Resistant | Resistant | Sensitive |

*****Organisms were sensitive if the kill zone > 16mm, intermediate (-) if kill zone is between 12mm – 16mm and non-sensitive or resistant if the kill zone < 12mm

streptomycin, gentamycin and pefloxacin except for *Bacillus spp* which was resistant to gentamycin and *Staph aureus* which showed resistance to pefloxacin (Table 3).

**Discussion**

Poultry semen gets contaminated with microbes via contact with the papillae in the walls of the avian cloaca during collection (Ngu *et al.,* 2014). Although earlier studies reported the presence of millions of microbes in artificial ejaculates of chicken semen (Sexton *et al.,* 1980) and that the endotoxins from most bacteria and fungi organisms are believed to pose detrimental effects on sperm survival (Watson, 1990), the freshly collected semen from both donor birds in this study showed good quality characteristics but contaminated with microbes such as *Escherichia coli, Shigella spp, Staphylococcus aureus and Bacillus specie* similar to the findings of Ngu *et al.* (2014) and Alkali *et al*. (2020). The turkey toms being more easily accustomed to procedures of semen collection than the guinea cocks may be due to their large body size with sufficient area, available for massage around the dorsolateral lumbosacral region of the vent in the toms.

In the present study, the yield of semen outputs from both the guinea cock and the turkey toms differed significantly ($p < 0.05$) which may be attributed to breed, season and species variability. This finding agrees with the reports of Etchu *et al.* (2013) and Mohan *et al.* (2016). The authors noted the influence of breed, nutrition and season on the variability of semen qualities in different birds. The overall mean volume of guinea cock semen (0.03 ± 0.01mls) obtained in this work was similar to previous reports by Nwakalor *et al.* (1988) but lower than reports of Mohan *et al.* (2016) in different breeds of guinea fowl. The disparity may be due to the variety in types of guinea fowl used for both studies. Progressive motility of spermatozoa, being the most reliable quality indicator of good semen (Ax *et al.,* 2000) was evaluated in the current study. It was greater than 70% in fresh semen of guinea cock and turkey toms and showed no statistical difference ($P > 0.05$) between the two sperm donors. Meanwhile, semen characteristics which were significantly lower in guinea cock when compared with qualities of the fresh semen in turkey toms were still within the normal range acceptable for successful AI in avian specie (Yahaya *et al.,* 2013).

The morphologic abnormality was higher in the guinea cock semen (12.8 ± 2.1%) when compared with the turkey semen, but showed a lower mean value when compared to previous abnormality reported by Nwakalor *et al.* (1988) in guinea cocks. The higher number of dead and abnormal spermatozoa in the semen of the guinea cocks obtained in this study may be attributed to the collection procedure which appeared difficult in the *Numidia meleagris*. Furthermore, the live spermatozoa (Plate IV) were still within the range (> 70 – 75 %) acceptable for avian AI (Zahradden *et al.,* 2005; Yahaya *et al.,* 2013) and abnormalities were mostly secondary type (Plate V) similar to previous reports by Mohan *et al.* (2016).

Microbes inhabiting avian cloaca are correlated strongly with semen contamination in poultry during collection procedures (Ngu *et al.,* 2014). In this study, the frequent isolation of *Escherichia coli* in fresh semen of guinea cock and turkey toms agreed with previous reports of Haines *et al.* (2013) and Ngu *et al.* (2014). *Escherichia coli* which are gram-negative, rod-shaped facultative anaerobic bacteria produce endotoxin capable of impairing the normal functions

of spermatozoa. Alkali *et al*. (2020) reported a similar observation in both fresh and extended turkey semen which makes clear the need for pre-dilution of semen with extenders fortified with known sensitive antibiotics. Also revealed in this work is that *E. coli* is sensitive to streptomycin, pefloxacin and gentamycin which accords with earlier reports by Alkali *et al*. (2020) except where the latter recognized the resistance of *E. coli* to streptomycin when in combination with penicillin. Other gram-negative organisms that were tested include *Salmonella spp and Pseudomonas* but were not identified in any of the semen samples.

*Shigella spp* a non-lactose fermenter was isolated only in guinea cock semen and also showed sensitivity to streptomycin, pefloxacin and gentamycin. This may be due to the poor efficiency in restraint and semen collection method that was not able to preclude semen contact with faecal deposits around the cloaca in most collection trials in the *Numidia Meleagris* specie. *Shigella* is normally isolated in the faeces of infected animals or surfaces contaminated with such faeces (Butler *et al.,* 1986). In this study, it was concluded, that whereas freshly collected semen of guinea cock and turkey toms were contaminated during collection, they still possessed good quality traits when evaluated for semen characteristics. Antibiotics such as streptomycin, pefloxacin or gentamycin can be used during *in-vitro* processing and extension of semen from both poultry species.

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**Conflict of Interest**

The authors declare that there is no conflict of interest.

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