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Semen Characteristics and Spermatozoa Biometry of Different Varieties of Guinea Fowls

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ABSTRACT

A total of twelve male guinea fowls, from each of pearl, white, white breasted and lavender varieties aged eight months were selected based on their phenotypic characters, and trained for semen collection by abdominal massage technique. The semen was analyzed for colour, volume, spermatozoa motility (%), spermatozoa concentration, live and abnormal spermatozoa(%). Further, spermatozoa biometry was studied by using transmission electron microscopy. Significantly higher semen volume (0.041±0.005 ml) and spermatozoa concentration (2497.78±87.17×10^6) was found with white breasted variety of guinea fowl. Higher percent live spermatozoa (88.03±0.93) and lower spermatozoa abnormality (10.11±1.36) were observed in semen of pearl guinea fowls but the volume and spermatozoa concentration were the least among the four varieties. Spermatozoa biometry studies showed significantly higher head midpiece and tail length in white breasted variety as compared to other varieties. The total length of spermatozoa of white breasted variety was more than double the length, than observed with the spermatozoa of white and lavender variety of guinea fowls. The study concluded that the spermatozoa of white breasted variety were robust with better seminal parameters, long head, comparable longer midpiece and strikingly longer tail.

Keywords: Guinea fowl, spermatozoa biometry

Fertility is one of the major components that determine the profitability of any commercial poultry breeding operation. Guinea fowls are semi domesticated birds where the fertility and hatchability are less compared to other poultry species. Further, seasonality of breeding (Ayorinde, 1989; Konlan *et al.,* 2011; Premavalli, 2013), sexing problems (Rahman *et al.,* 2015) and low semen volume (Thurston *et al.,* 1982a) may also contribute to lower fertility rates. Assessment of semen quality characteristics of poultry birds is an excellent indicator of their reproductive potential and is the major determinant of fertility and subsequent hatchability of eggs (Peters *et al.,* 2008). Further assessment of basic seminal parameters will help in deciding the suitability of semen for further processing for short term or long term storage without loss in its quality. Genetically superior sires can be selected based on the seminal parameters and the germplasm can be disseminated to large number of females which will speed up the genetic progress by artificial insemination technology. Hence, the present study was carried out to access the seminal parameters and spermatozoa biometry of pearl, white, white breasted and lavender varieties of guinea fowl. The present study was carried out during October-December months where the maximum and minimum temperature in the study area (13.1623° N, 80.2433° E) was 31.8 and 21.2ºC. The results of this study may throw light on any specific approach in processing of semen of different varieties for short term and long term storage which maintains its quality parameters in optimum levels thereby, improving fertility by assisted reproductive technology in guinea fowls.

MATERIALS AND METHODS

Semen collection

Twelve healthy, mature male guinea fowls aged eight months were selected based on their phenotypic characters from each variety namely pearl, white, white breasted and lavender. The birds were then housed in individual cages providing a floor space of 1sq.ft per bird. Standard breeder ration containing 17% crude protein and 2700 kcal of Metabolisable Energy, was provided *ad libitum* with free access to drinking water. The male guinea fowls were trained for semen collection by abdominal massage technique (Burrows and Quinn, 1937). The semen was collected during the early hours of the day twice a week. The pearly white drop of ejaculate coming out of the papillae was directly aspirated into a sterile tuberculin glass syringe to prevent mixing of the neat semen with the natural contaminants like transparent fluid, faeces and urates. Later, it was transferred into a sterile eppendorf tube and kept in a water bath at 18˚C for further evaluation.

Semen evaluation

Immediately after each collection, the semen from each variety was evaluated for volume, colour, per cent motility, concentration of spermatozoa, per cent live and abnormal spermatozoa.

The volume of the semen collected was measured directly in the glass tuberculin syringe with 0.01ml accuracy. For examination of pH, a drop of freshly collected semen was placed on a strip of limited range pH paper (Merck India 6.5 – 9.0) with an accuracy of 0.5 and the colour developed was compared with the standards given.

The motility of spermatozoa in each variety were assessed by placing a small drop of semen in the middle of a clean grease free slide which was covered with a cover glass slip and examined under simple light microscope (10x). The overall motility was assessed and expressed as percentage as described by Parker *et al*. (1942).

The concentration of spermatozoa in the fresh semen of each variety of guinea fowl was determined using

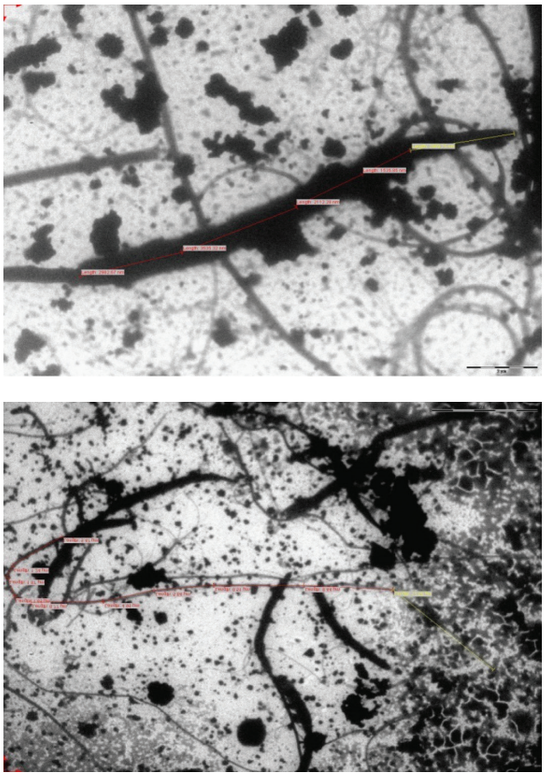
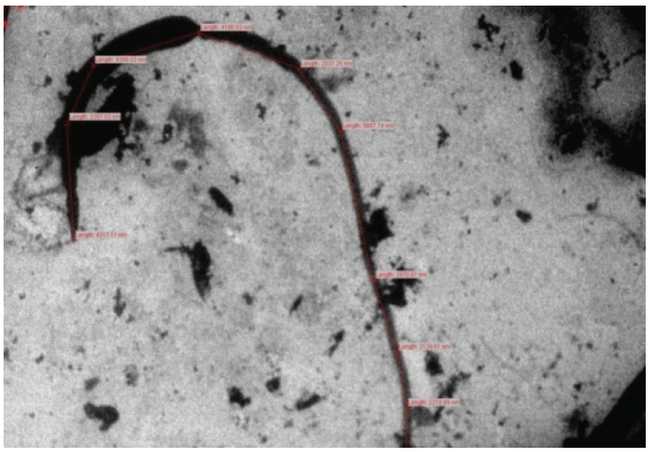


Fig. 1: Ultrastructure and biometry of pearl guinea fowl spermatozoa using Transmission Electron Microscopy

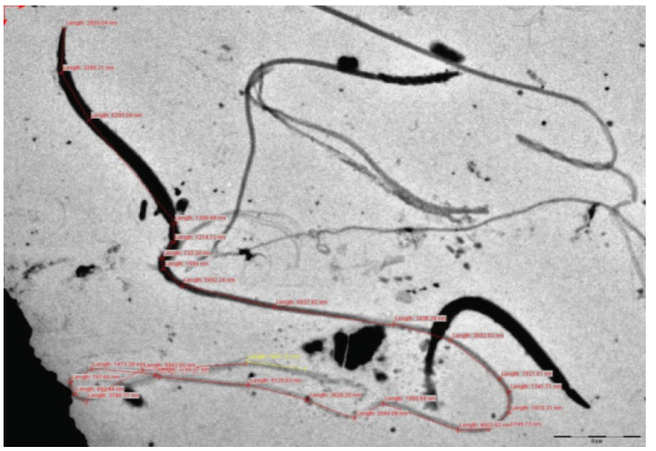


Head – 11.03µm

Mid piece – 4.1µm

Tail – 17.9µm

Magnification x 3700

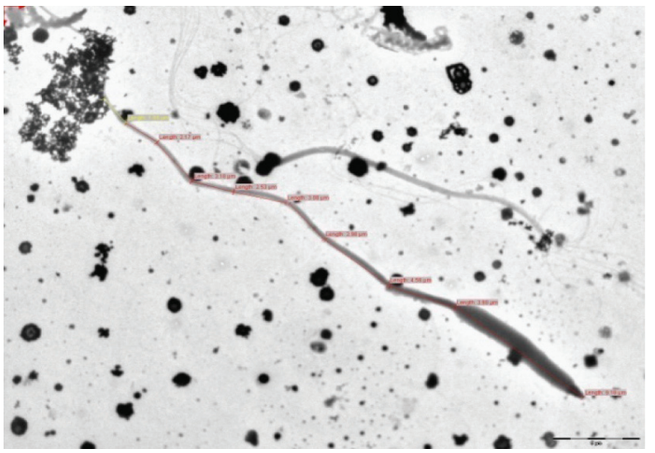


Head – 14.45µm

mid piece – 4.74µm

Tail – 63.60µm

Magnification x 2550



Head – 9.18µm

mid piece – 3.98µm

Tail – 20.51µm

Magnification x 2550

Fig. 2: Ultrastructure and biometry of white, white breasted and Lavender guinea fowl spermatozoa using Transmission Electron Microscopy

a "NEUBAUER" type hemocytometer and the final concentration of spermatozoa expressed as millions ($\times 10^6$) per ml according to the procedure of Allen and Champion (1955).

The viability of spermatozoa in the fresh semen of each variety of guinea fowl was determined by Eosin-Nigrosin staining procedure as described by Bakst and Cecil (1997). Smears were prepared carefully, stained and a maximum of 200 spermatozoa were counted to assess the per cent live spermatozoa and expressed as percentage. Unstained spermatozoa were counted as live and partially or totally stained ones were considered as dead. The same slides, stained for the assessment of live spermatozoa count were used for the abnormal spermatozoa count. Spermatozoa abnormalities within the fraction of live spermatozoa were considered and maximum of 200 spermatozoa were counted and expressed as per cent.

Spermatozoa biometry

The morphology of spermatozoa in undiluted raw semen of different guinea fowl varieties were studied in Transmission Electron Microscope (TEM). A volume of 20 µl of raw semen sample was added to 10 µl of 1 per cent phosphotungstic acid and mixed well. On the surface of mixture, copper coated grid was placed and by using blotting paper the excess fluid was absorbed and the grid was transferred to the petridish and incubated at 37˚C for 15–30 minutes. Then the gird was viewed under TECHNAI 10 Transmission electron microscope.

Statistical Analysis

The data recorded in this study were analyzed by one way ANOVA method as per Snedecor and Cochran (1994) using SPSS.20 Software.

RESULTS AND DISCUSSION

Seminal attributes of each variety (pearl, white, white breasted and lavender varieties) of guinea fowl were assessed along with biometry of spermatozoa.

Semen colour

Most of the guinea fowl semen ejaculates were pearly white in colour. Zelleke and Ayalew (2003) reported that the most obvious evaluation of semen quality is colour and the poultry semen should be pearly white in colour. Appearance of any other color is indicative of the presence of a contaminant.

Semen volume

The mean semen volume (ml) observed in this study were 0.027±0.002, 0.029±0.003, 0.041±0.005 and 0.035±0.005 in pearl, white, white breasted and lavender varieties of guinea fowls respectively. The values obtained were within the range observed by Nwakalor *et al*. (1988) in golden sovereign guinea fowl (0.032 ± 0.001 ml) and in pearl guinea fowl (0.048 ± 0.002). However, Mohan *et al*. (2013) and Hudson (2015) observed higher semen volume in pearl variety guinea fowls with corresponding values of 0.048 ± 0.002 and 0.04 ml respectively. Higher semen volume than observed in this study, was also observed by Mohan *et al.* (2016) with significantly higher volume in pearl variety (0.055±0.003) compared to lavender (0.051±0.003) and white varieties (0.035±0.004). Further, the increase or decrease in the output of semen recorded in this study compared to the reports of previous authors, might be due to differences in genetic makeup of the birds, collection time preferably cool hours of early morning or late evening, seasonality etc.

Per cent motility

The mean per cent motility observed in this study were 83.47±1.75, 77.86±2.06, 82.50±1.59 and 81.39±1.63 in pearl, white, white breasted and lavender varieties of guinea fowls respectively. No significant differences were observed in motility between the varieties. The values obtained were comparable with the earlier findings of Mohan *et al.* (2016) who reported the percent motility to be 87.00±4.40, 84.34±5.11 and 90.11±3.70 % in pearl, white and lavender varieties of guinea fowl, respectively. Whereas, Hudson (2015) and Mohan *et al*. (2013) observed motility percentages of 81.47±1.52 and 85.00 ± 5.76% respectively, in pearl guinea fowls. The previous reports indicate the numerical differences between the varieties, which were also observed in the present study. In addition lower motility percentage than observd in the current study (37.10±0.1 %) were also reported by Nwakalor *et al.* (1988) in golden sovereign guinea fowl. Except lavender variety, all other varieties had shown motility percentages

of above 80% which is suitable for further dilution and Artificial Insemination (AI) in guinea fowls.

Concentration of spermatozoa

Significantly higher (P≤0.01) mean spermatozoa concentration (×10^6/ml) was observed in semen of white breasted variety guinea fowls (2497.78±87.17) followed by lavender (2236.94±47.80) white (2101.94±87.18) and pearl varieties (1780.56±61.03). Previous report by Mohan *et al.* (2016) showed significantly higher spermatozoa concentration (x10^9) (P≤0.05) in lavender varieties (3.67±0.31) of guinea fowl compared to pearl (3.51 ±0.22) and white varieties (3.05 ±0.17). The concentration of spermatozoa observed in this study was also lower than those observed by Hess *et al*. (1986) in eight months old guinea fowls (5.265±0.326x10^9), Nwakalor *et al*. (1988) in golden sovereign guinea fowl (2.62±0.01x10^9), Lavor *et al*. (2012) in Helmeted guinea fowl (8.34±12.41×10^9) and Mohan *et al*. (2013) in pearl guinea fowl (3.27±0.14x10^9). The lower values observed in the current study may be attributed to difference in the place of study, genetic makeup of the birds, nutrition etc.

Per cent live spermatozoa

The per cent live spermatozoa observed in this study were 88.03±0.93, 82.44±1.40, 85.47±1.56 and 87.11±1.23 in pearl, white, white breasted and lavender varieties of guinea fowls respectively where the white variety had shown significantly lower values. Mohan *et al*. (2016) reported that the per cent live spermatozoa of pearl, white and lavender varieties of guinea fowls were 89.52±3.97, 86.19±4.91 and 90.71±4.11 respectively however no significant differences were observed between the varieties. The values observed in the current study were comparable with earlier reports of Mohan *et al*. (2013) and Hudson (2015) in pearl guinea fowl semen who observed a live spermatozoa percentage of 87.22±3.17 and 87.73±0.56 in undiluted fresh semen. However, Nwakalor *et al*. (1988) in golden sovereign guinea fowl (91.6±0.1) have recorded higher live spermatozoa. In contrast, Seigneurin and Blebois (2005) in guinea fowl and Blebois *et al*. (2005) in Galor G55 guinea fowl recorded lower live spermatozoa count with corresponding values of 55 % and 64 % respectively. The previous works discussed above, have also shown differences between the varieties, which were also observed in the present study.

Per cent abnormal spermatozoa

The abnormal spermatozoa noticed in the present study were between 10- 13 %, no significant difference were observed between the guinea fowl varieties. Complementary results were also observed by Hudson (2015) in pearl variety guinea fowls (12.05±0.24). However, Nwakalor *et al.* (1988) reported relatively higher percent abnormal spermatozoa in golden sovereign guinea fowl, Hess *et al*. (1986) in guinea fowl with corresponding values of 23.1 % and 50 % respectively. Relatively better semen quality in terms of lower abnormal spermatozoa were reported by Mohan *et al.* (2016) in pearl white and lavender guinea fowls, with corresponding values of 4.44±0.13, 6.79±0.15 and 4.08±0.21 respectively. In addition, Mohan *et al.* (2013) also reported lower abnormal spermatozoa count in pearl guinea fowls (5.11 ± 0.14). The varying values observed might be due to varying season between the study areas, variations in the genetic makeup of the birds, nutritional impact etc. The semen from all the guinea fowl varieties in the study have shown more than 80 % of morphologically normal spermatozoa which is sufficient for further processing and storage.

Spermatozoa Biometry

The spermatozoa head length (µm) had shown highly significant (P≤0.01) difference between different varieties of guinea fowls. The head length of spermatozoa was significantly highest in white breasted variety (13.29±0.38) compared to pearl variety (12.06±0.47). The length of spermatozoa head in white variety fell in between (12.51±0.40) and had no significant difference with either variety. Among all, the lowest length of spermatozoa head was recorded in lavender variety (9.18±0.26).

There was significant (P≤0.01) difference observed in terms of length of spermatozoa midpiece (µm) among the varieties. The length of spermatozoa midpiece was significantly (P≤0.01) higher in white breasted (4.49±0.08) compared to pearl (4.13±0.07) and white (4.10±0.07) varieties. The lavender variety had lowest length of spermatozoa midpiece (3.68±0.09) among all the four varieties.

The length of spermatozoa tail (µm) also had shown

significant (P≤0.01) difference among the varieties. Highest value of length of the spermatozoa tail was observed in white breasted (60.48±0.90) variety of guinea fowl, which was significantly different from that of Pearl (54.30±2.23), lavender (20.86±0.32) and white (20.18±0.56) varieties.

With respect to total length, the white-breasted variety registered significantly highest ((P≤0.01) length (78.25±0.82) followed Pearl (70.49±2.42), white (36.80±0.90) and lavender varieties (33.71±0.59). Comparing with the earlier works, Thurston *et al*. (1982b) obtained average head length of 14.6 µm, midpiece length of 3.9 µm, tail length of 59 µm and total length of 77.5 µm in guinea fowl. The authors did not mention about the variety, but the length of head, tail and total length is in concurrence with the values obtained with semen of white breasted variety and midpiece length in concurrence with all four varieties of guinea fowl under study. However, Thurston and Hess (1987) reported a total spermatozoa length of 75-80 µm which was in accordance with the white breasted variety of guinea fowl. Etches (1996) reported a higher tail length of 85 µm in turkey spermatozoa compared with the present study where a maximum length of 60.48±90 µm was observed with white breasted variety. Further, Korn *et al.* (2000) reported overall length of Japanese quail spermatozoa as 230 and 250 µm, with the higher mid piece length of 160 to 170 µm compared with the current study. Significant (P≤0.01) differences were obtained between four varieties for which earlier works could not be traced for comparison and discussion.

From the current study, it is evident that the seminal parameters vary between the varieties of guinea fowl. The best male guinea fowl from each variety can be selected based on results obtained by macroscopic and microscopic evaluation of semen. Selecting elite males will ensure better dissemination of their superior germplasm through AI, thereby improving fertility and speeding up the genetic gain in guinea fowls.

REFERENCES

Abdul-Rahman, I.I., Awumbila, B., Jeffcoate, I.A., Robinson, J.E. and Obese, F.Y. 2015. Sexing in guinea fowls (*Numida meleagris*). *Poultry Sci*., **94** (2): 311-318.

Allen, C.J., and Champion, C.H. 1955. Competitive fertilization in fowl. *Poultry Sci*., **34**: 1332-1342.

Ayorinde, K.L. 1989. Effect of semen dosage and insemination frequency on the fertility of local pearly guinea fowl in Nigeria. *Trop. Agr.,* **66** (2): 135-136.

Bakst, M.R. and Cicil, H. 1997. Spermatozoa viability, Eiosin/ Nigrosin stain for determining Live dead and abnormal spermatozoa counts. In: techniques for semen evaluation, semen storage and fertility determination. Bakst M.R and H. Cicil, Ed, *Poultry SciAssociation.* Inc, savory, Illinois.

Blesbois, E., Grasseau, I. and Seigneurin, F. 2005. Membrane fluidity and the ability to survive cryopreservation in domestic bird spermatozoa. *Reproduction*, **129**: 371-378.

Burrows, W.H. and Quinn, JP. 1937. The collection of spermatozoa from the domestic fowl and turkey. *Poultry Sci*., **16**: 19-24.

Etches, R.J. 1996. Reproduction in poultry. CAB International, Cambridge University, UK.

Hess, R. A, Hughes, B. L. and Thurston, R. J. 1986. Frequency and structure of macrophages and abnormal spermatozoa cells in guinea fowl semen. *Reprod. Nutri. Dev.,* **26** (1A): 39-51

Hudson, G.H. 2015. Fertility Parameters through Artificial Insemination in Guinea Fowls. *M.V.Sc. Thesis submitted to the Tamil Nadu Veterinary and Animal Sciences University*, Chennai.

IBM Corp. 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.

Konlan, S.P., Avornyo, F.K., Karbo. N. and Sulleyman, A. 2011. Increasing guinea fowl eggs availability and hatchability in the dry season. *J. World Poult. Res.,* **1**(1): 1-3.

Korn, N., Thurston, RJ., Poser, B.P. and Scott, T.R. 2000. Ultrastructure of spermatozoa from Japanese quail. *Poult. Sci*., **79** (3): 407-414.

Lavor, C.T.B., Câmara, S.R., Bandeira, B.M., Salgueiro, C.D.M., Castro, E.V. and Nunes, J.F. 2012. Powdered coconut water as preservation medium of guinea fowl (*Numida meleagris*) spermatozoa. *Ciência Anim*., **22**(1): 79-81.

Mohan, J., Khanday, J.M., Sigh, R.P. and Tyagi, J.S. 2013. Effect of storage on the Physico-Biochemical characteristics and fertility of guinea fowl semen. *Adv. Anim. Vet. Sci.,* **1**(2): 65–68.

Mohan, J., Sharma, S.K., Kolluri, G., Singh, R.P., Tyagi, J.S., and Kataria, J.M. 2016. Semen quality characteristics and seasonality in different varieties of male Guinea fowl. *Adv. Anim. Vet. Sci.,* **4**(6): 320-326.

Nwakalor, L.N., Okeke, G.C. and Njoku, D.C. 1988. Semen characteristics of guinea fowl *Numida meleagris meleagris*. *Theriogenology*, **29**: 545-554.

Parker, J.E., McKenzie, F.F. and Kempster, H.L. 1942. Fertility in the male domestic fowl. *Res.. Bull*., **347:** 1-50.

Peters, S.O., Shoyebo, O.D., Ilori, B.M., Ozoje, M.O., Ikeobi, C.O.N. and Adebambo, O.A. 2008. Semen quality traits of seven strains of chickens raised in the humid tropics. *Int J. Poult. Sci.,* **7**(10): 949-953.

Premavalli, K. 2013. Influence of strain, age and system of management on the productive and reproductive performance of guinea fowl. *Ph.D Thesis submitted to the Tamil Nadu Veterinary and Animal Sciences University*, Chennai.

Seigneurin, F. and Blesbois, E. 2005. The first method of cryopreservation of guinea fowl semen. Proc. of the French poultry research days. Cited in *cabi-org*.

Snedecor, G.W. and Cochran, W.G. 1994. Statistical Methods. IX Edition. Oxford and IBH Publishing co., Kolkatta.

Thurston, R.J. and Hess, R.A. 1987. Ultrastructure of spermatozoa from domesticated birds: comparative study of turkey, chicken and guinea fowl. *Scanning Microscop...* **1**(4):1829-1838.

Thurston, R.J., Hess, R.A., Hughes, B.L. and Froman, D.P. 1982a. Seminal plasma free amino acids and seminal and blood plasma proteins of the guinea fowl (*Numidia meleagris*). *Poult. Sci*., **61**(8): 1744-1747.

Thurston, R.J., Hess, R.A., Hughes, B.L. and Froman, D.P. 1982b. Ultrastructure of the guinea fowl (*Numidia meleagris*) spermatozoon. *Poult.. Sci*., **61**: 1738-1743.

Zelleke, G. and Ayalew. W. 2003. Application of Artificial Insemination on Poultry in Ethiopia. *Proceedings of 11th annual conference of the Ethopian society of animal production*, pp. 215-222.