Semen Quality Parameters of Three Duck Genotypes in the Humid Tropics

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Abstract

Semen quality parameters are central to the assessment of the breeding value of male animals in respect of their suitability for breeding purposes and evaluation of their reproductive performances. Semen samples collected from the epididymis of five (5) adult males each of Muscovy, Mallard and their *intergeneric* hybrid Mule ducks were analysed for their semen quality indices (mass motility, percentage motility, semen concentration, live/dead ratio, percentage normal and abnormal sperm morphology). The result of the analysis of variance indicated significant ($P < 0.05$) genotype effect on all the semen parameters and absence of sperm cells in the semen of the Mule ducks. Muscovy duck had significant ($P < 0.05$) higher mass motility, individual motility and sperm concentration than Mallard and Mule ducks while percentage live/dead ratio, normal and abnormal sperm morphology were similar ($P > 0.05$) for Muscovy and Mallard ducks. The absence of sperm cells in the semen of the male Mule ducks is a pointer to their sterility. Though genetic variation influenced some semen quality parameters of Muscovy and Mallard ducks; nevertheless, semen from the two waterfowl genotypes is suitable for breeding purposes either for backyard or commercial duckling production.

***Key words*:** Ducks, Sperm Quality Parameters, Epididymis, Breeding value, Sperm morphology, Sterility

Introduction

The application of artificial insemination (A.I.) technology has been considered as a valuable technique in the poultry industry by ensuring effective selection of male animals and better management of the breeding stock (Das et al., 2004). This reproductive technique has contributed immensely to the mass production of livestock and also helps in efficient utilization of both fresh and stored semen of good sires. The reproductive performance of the male animal is hinged on production of semen containing normal sperm (quality) in adequate numbers (quantity), together with the desire (libido) and mating ability (servicing capacity) (Molekwa and Umeisobi, 2009). Nevertheless, the first step in assessing the genetic worth of a male animal for breeding purpose is the semen evaluation.

The desired genetic attributes of male animals are encrypted in DNA codes and are transferred to the next generation via the sperm cells. Therefore, semen quality evaluation is central to identification and selection of good sires capable of transferring the economic genes to the next generation and whose semen are suitable for stor-

age for future breeding purposes through cryopreservation. The assessment of semen quality characteristics of poultry gives an excellent indicator of their reproductive potential and has been reported to be a major determinant of fertility and hatchability of eggs (Peters et al., 2008) and is also crucial for reproduction success, both in natural mating and A. I. (Zawadzka et al., 2015). Among indicators used to evaluate semen quality includes; ejaculate volume, semen color, sperm concentration, sperm motility, sperm viability, and percent sperm deformity (Mocé and Graham, 2008).

Poultry production in Nigeria and Africa at large irrespective of the scale of production or management system adopted is synonymous with chicken while other available native species such as guinea fowl, duck and pigeon are utterly neglected and seldom exploited for economic or nutritional purposes (Oguntunji, 2013). Duck is one of the rarely exploited livestock in Nigeria despite the presence of congenial environment for its husbandry across all agro-ecological zones and a readily available large market (Oguntunji and Ayorinde, 2015). This waterfowl ranked third among domesticated avian species in Nigeria with an estimated population of 9 553 911 after chicken (101 676 710) and guinea fowl (16 976 907), respectively (NBS, 2012).

Empirical reports on semen evaluation of poultry species in Nigeria are meagre and the few available ones are heavily skewed to the indigenous chickens (Machebe and Ezekwe, 2002; Ajayi et al., 2011; Ajayi et al., 2014). Comparative semen evaluation of the available duck genotypes (local, exotic and crossbred mule ducks) in Nigeria is scarce and the only reported one was on Muscovy duck (Etuk et al., 2006). Several reports on semen characteristics of ducks (Cyriac et al., 2013; Zawadzka et al., 2015) and domestic fowls (Peters et al., 2008; Adeoye et al., 2017) indicated that breeds and strains significantly affected semen quality and quantity. Against this background, the objectives of the present study were to evaluate and compare the semen quality parameters of three Nigerian duck genotypes (Muscovy, Mallard and their intergeneric cross Mule ducks).

Materials and Methods

Location of the experiment

The experiment was conducted at the Duck Unit of the Teaching and Research Farm of Bowen University, Iwo, Osun state, Nigeria in April, 2018. The study area is located in a Derived Savanna agro-ecological zone and the coordinate of the study area is Latitude 7° 38' 6.97" N and Longitude 4° 10' 53.62" E. The climate and vegetation were interphase between Rain Forest and Savanna Grassland and are characterized with double maxima rainfall and mixture of deciduous trees and tall grasses. Wet (April–September) and dry (October–March) seasons are the principal seasons in the area.

Experimental animals

Fifteen (15) adult drakes comprising 5 Muscovy, 5 Mallard and 5 hybrid Mule ducks were used for this experiment. They were brought from North-west Nigeria and sourced from a reputable poultry market at Shasha, Ibadan, Oyo State, Nigeria.

The ducks were conditioned for three weeks in order to acclimatise them to their new environment and to alleviate any form of stress that might influence results before semen samples were taken. They were fed *ad libitum* with commercial layers' mash containing 18% CP and 2600 Kcal ME / kg. The birds were also dewormed and antibiotics (LA – oxytet) was administered to prevent and control diseases.

Data collection

The experimental birds were sacrificed in the morning (07.00–09.00 hours) by severing the jugular vein. Dissection of the birds followed immediately and 1 ml semen samples were taken from left and right epididymis of each drake. In order to avoid post-harvest stress effect on semen parameters, one bird at a time was sacrificed, dissected and semen sample analysed immediately.

Sperm motility

An incision was made on the surface of the caudal of the epididymis and a drop of semen was mixed with a drop of 2.9% warm sodium

citrate buffer placed on grease-free pre-warm glass slide and covered with a warm cover slide. The proportion of the motile sperm cells moving in a progressively forward unidirectional manner was counted using a light microscope (x 400 magnification). A minimum of five microscopic fields were assessed to evaluate sperm motility on at least 300 sperms for each sample. Mass motility was subjectively estimated depending on the rate of motile sperm cells. The scoring system was from 1 to 5 where the bottom, the middle and the top of the scale represents poor, good and excellent motility, respectively (Etches, 1996) while percentage motility was subjectively calculated ranging from 0 to 100%.

Sperm livability and morphology

A drop of semen from the caudal epididymis with the aid of a micropipette was gently placed on a grease-free warm glass slide and mixed with a drop of warm Eosin-Nigrosin stain. A thin smear was made from the mixed solution on a clean warm glass slide and air-dried under room temperature for 10 minutes. The slide was then microscopically (x 400 magnification) examined. The dead sperm cells (eosin-permeable) appeared pink because they pick up the stain due to compromised plasma membrane while the live ones (eosin-impermeable) appeared colourless, that is they did not pick up the stain. The viable sperm cells were then classified into normal and abnormal depending on the presence of sperm defects or not. Percentage morphologically normal and deformed sperm cells were estimated by observing 300 spermatozoa in different microscope fields. Besides, the sperm morphology defects were classified according to the region/segment of the sperm cell where defects were observed.

Sperm concentration

Sperm concentration was counted by haemocytometer using the improved Neubauer (deep 1 / 10mm, LABART, Germany) chamber according to Pant and Srivastave (2004).

Statistical analysis

Data obtained from the semen parameters were analysed with Statistical Package for the Social Science (SPSS, 2001) version 16 using a one-way analysis of variance (ANOVA) with genotype as the fixed effect:

$Y_{ij} = \mu + G_i + e_{ij}$

Y_{ij} = individual observation of the dependent variable

μ = Population mean

G_i = Effect of i^{th} genotype on semen parameters ($_i$ = Muscovy, Mallard and Mule ducks)

e_{ij} = The random error associated with each observation, assumed to be normally and independently distributed, with a mean of zero and homogeneity of variance.

Significant differences between the means were assessed using New Duncan's Multiple Range Test at 5% probability level.

Results and Discussion

Gross motility and individual motility

There was a significant ($P < 0.05$) difference in the mass and percentage sperm motilities of the three duck genotypes (Table 1). The Muscovy ducks had significantly higher values in the two semen parameters (3.90 and 96.50%) compared to the Mallard (2.00 and 42.50%) and Mule (0.00; 0.00%) ducks.

The mass motility for Muscovy duck (3.90) in the present study was higher than the values reported for Muscovy ducks in Israel (Gvaryahu et al., 1984), Nigeria (Etuk et al., 2006) and India (Cyriac et al., 2013). The higher mass motility score for Muscovy duck (3.90) compared to Common duck (2.00) was consistent with the report of Cyriac et al., (2013) who reported higher motility score of 3.54 for Muscovy duck compared to Common duck breeds (Kuttanad, 3.42 and Pekin, 3.38) in India. The motility score (2.00) reported for Mallard ducks in this study was lower than 2.80 and 3.50 reported for yearling and captive Mallard ducks, respectively (Stunden et al., 1998). The sperm cell motility (96.50%) for Muscovy duck in this study was higher than the related reports on Muscovy ducks (Etuk et al., 2006; Azim et al., 2011; Cyriac et al., 2013; Chen et al., 2016) and Common duck breeds (Penfold et al., 2000; Cyriac et al., 2013; Zawadzka et al.,

Table 1. Semen characteristics of three duck genotypes

	Genotype		
Semen parameter	Muscovy duck	Mallard duck	Mule duck
Mass motility	3.90 ± 1.00[a]	2.00 ± 2.32[b]	0.00 ± 0.00[c]
Motility (%)	96.50 ± 0.71[a]	42.50 ± 3.54[b]	0.00 ± 0.00[c]
Sperm Concentration (x10^9)	5.20 ± 2.12[a]	4.27 ± 3.11[b]	0.00 ± 0.00[c]
Livability (%)	95.50 ± 0.71[a]	90.00 ± 0.90[a]	0.00 ± 0.00[b]
Normal morphology (%)	99.29 ± 1.40[a]	99.30 ± 0.61[a]	0.00 ± 0.00[b]
Abnormal morphology (%)	0.71 ± 0.01[a]	0.78 ± 0.98[a]	0.00 ± 0.00[a]

Means with different superscripts along the row are significantly different at 5% probability level

2015; Nahak et al., 2015) but similar to 91.67–96.67% reported for Domyati drakes (Ghonim et al., 2010) in Egypt. In addition, 42.50% reported in the present study for Mallard ducks was much lower than 88.67% reported for captive Mallard ducks (Denk, 2005).

Considering the highest mass motility and percentage motility of the sperm cells of the indigenous Muscovy ducks in contrast to the non-indigenous Mallard ducks; it seems that genotype plays a significant role on the reported values. This assertion was buttressed by the earlier report of Kamar (1962) that *Sudani* duck, the Egyptian local Muscovy duck had highest mass motility compared to the exotic Rouen and Pekin drakes. A major possible reason for the observed differences between the two duck genotypes could be attributed to their differential adaptation to the environmental factors, most especially thermal stress. The Nigerian local Muscovy ducks and Egyptian indigenous *Sudani* ducks have been reported to be more thermal-tolerant than their thermal-susceptible non indigenous counterparts by Oguntunji et al. (2019) and Makram (2015), respectively. Similarly in chickens, higher motility of sperm cells have been documented for strains of chicken carrying heat-tolerant naked neck (Na) and frizzle (F) genes compared to their normal and exotic strain counterparts (Ajayi et al., 2011; Peters et al., 2008; Machebe and Ezekwa, 2002).

Spermatozoa motility is central to reproductive efficiency of male animals. Sperms are vehicles which carry DNA to the ovum (Zahraddeen et al., 2005); therefore, avian spermatozoa must be motile to migrate from the site of insemination (cloaca or vagina) to the area of sperm storage (uterovaginal sperm storage tubules) (Bakst et al., 1994; Ashizawa et al., 2000) prior to fertilization.

Sperm Concentration

Muscovy ducks had significantly ($P < 0.05$) higher spermatozoa concentration than Mallard ducks (5.20 x 10^9 vs. 4.27 x 10^9) while no (0.00 x 10^9) spermatozoon was detected in the semen of their hybrid Mule ducks (Table 1).

The sperm cell concentrations for both local Muscovy and exotic Mallard ducks in the present study were much higher than 1.32 x 10^9 / ml reported for yearling and captive Mallard ducks (Stunden et al., 1998); 3.58 x 10^9 / ml reported for Pintail ducks (Penfold et al., 2000), 1.70 – 1.80 x 10^6 / ml reported for Nigerian Muscovy ducks (Etuk et al. 2006), 2.48 x 10^9 / ml in Dumyati drakes in Egypt (Ghonim et al., 2010), 3.03, 3.22

and 1.94 x 10^9 / ml reported for White Pekin, *Kuttanad* and Muscovy ducks, respectively in India (Cyriac et al., 2013). However, the values reported for the two genotypes were lower than 8.5 x 10^9 / ml and 6.9 x 10^9 / ml reported for two conserved strains of Polish *Anas platyrhynchos* (Zawadzka et al., 2015).

The higher values reported for conserved Polish duck strains compared to the values reported for the understudied genotypes could be attributed to the intensive selection those strains have undergone over the years. A possible major reason for the higher sperm concentration of local Muscovy and exotic Mallard ducks compared to results of other investigators could be attributed to the semen collection method. Semen samples used by previous researchers were obtained through various semen collection methods prone to the influenced of various factors influencing ejaculate quality. Conversely, semen samples used for this study were obtained directly from the epididymis known for storage of mature spermatozoa awaiting ejaculation; thus, circumventing factors influencing ejaculatory process and ejaculate quality; hence higher sperm cell concentration.

Furthermore, since most factors influencing ejaculate qualities were eliminated by collecting semen samples directly from epididymis, it can be concluded that significant difference in the semen concentrations of the studied genotypes could be attributed to genetic differences. Similarly, Peters et al. (2008) and Ajayi et al. (2011) adduced variation in semen concentration of different genotypes of Nigerian local chickens to genetic variation and natural tendencies existing among them.

Another possible factor responsible for significantly higher sperm concentration in Muscovy ducks compared to Mallard duck could be attributed to genetic variation in their thermotolerance or heat stress adaptation. The study area where the experiment was conducted was a humid tropical environment characterized with high environmental temperature and humidity. Reports of various researchers on semen quality on chickens in the same environment where the present study was conducted followed a similar trend whereby strains of chicken carrying thermal-tolerant naked-neck (Na) and frizzle-feather (F) genes had higher sperm cell concentrations than their normal and exotic counterparts and most normally-feathered local chickens also had better sperm counts than the exotic stocks (Machebe and Ezekwe, 2002; Ajayi et al., 2011; Peters et al., 2008; Ajayi et al., 2014; Adeoye et al., 2017). Previous reports by Oguntunji et al. (2019) have shown that indigenous Muscovy ducks were more heat-tolerant than exotic Mallard ducks.

Furthermore, the absence of sperm cells in the semen of Mule ducks is consistent with the earlier reports of Coombs and Marshall (1956) who reported absence of sperm cells in the semen of hybrid Mule ducks produced from the intergeneric crossing of the male Muscovy and female Mallard ducks. The reports of Snapir et al. (1998) corroborated further that semen of Mule ducks was clear under microscopic analysis and neither spermatozoon nor spermatids cells were present in the center of seminiferous tubules in Mules in contrast to the white semen and high semen motility in Muscovy ducks.

Absence of spermatozoa in the semen of the hybrid Mule duck is a pointer to the widely reported sterility in this duck. Sterility of the intergeneric hybrid male is a common phenomenon in avian and mammalian species. Similarly, Mammalian male Mules (male donkey x female horse) or Hinnies (male horse x female donkey) have been reported sterile and did not produce spermatozoa (Short, 1972). Conversley, Marchant and Gomot (1972) reported presence of sperm cells in the semen of male Mule duck produced from the cross of Pekin male and Muscovy female while Zong and Fan (1989) also reported the presence of immotile sperm cells in the semen of a three year old Mule produced by crossing of male donkey and female horse.

Percentage livability or Live / dead ratio

There was no striking ($P > 0.05$) difference in the livability of the sperm of the Muscovy 95.00%) and Mallard (90.00%) ducks. Mule ducks had no sperm cell, therefore, the values recorded for the parental species were significantly

higher ($P < 0.05$) than 0.00% recorded for Mule ducks.

The non-significant percentage livability reported for the local Muscovy and exotic Mallard ducks is consistent with the reports of the previous investigators on semen quality of Muscovy and Common duck breeds (Cyriac et al., 2013; Zawadzka et al., 2015). The range (90.00–95.00%) of percentage live / dead ratio observed for the two genotypes were comparable with the values reported for Muscovy duck and other breeds of Common duck (Penfold et al., 2000; Cyriac et al., 2013; Nahak et al., 2015; Zawadzka et al., 2015). However, the proportion of the live sperm reported for Muscovy ducks in the present study was higher than 51.5–74.4% and 80.81% reported by Chen et al. (2016) and El Azim et al. (2011) respectively, for Muscovy ducks.

In contrast to sperm motility and concentration, the non-significant effect of genotype on this parameter in the present and previous studies on ducks is suggestive that this semen parameter is likely to be more influenced by the environment than genotype, hence no genetic effect.

The number of live and dead sperm cells is a good predictor of the reproductive potential of the male animal and could also help in determining suitability of sire for breeding purposes. The significant importance of this sperm quality index to fertility was elucidated by the report of Cyriac et al. (2013) that semen samples with less than 60% live cells cannot be used for A I purpose and percentage dead sperm cells of less than 10% can be considered to be of superior quality. It could be deduced from the recommended standard for breeding purpose that semen samples of both Muscovy and Mallard ducks in the present study are suitable for both A I programmes and cryopreservation.

Sperm morphology

The percentage normal and abnormal sperm cells for Muscovy (99.20%; 0.80%) and Mallard (99.30%; 0.70%) ducks were similar ($P > 0.05$) (Table 1). Further analysis of sperm defect morphology (Table 2) revealed only two major morphological abnormalities in the tail and mid-piece.

The two abnormalities observed in the tail region were bent and rudimentary tails while only curved mid-piece was observed in the mid-piece. Nevertheless, preponderance of abnormality was detected in the tail compared to the mid-piece for both Muscovy (66.20% vs. 33.80%) and Mallard (66.66% vs. 33.33%) ducks.

Similar to the results of the present study, non-significant differences in both normal and defective sperm cell morphologies were reported in three duck genotypes (Cyriac et al., 2013) and different strains and breeds of chicken (Machebe and Ezekwe, 2002; Ajayi et al., 2011; Tarif et al., 2013; Adeoye et al., 2017). On the contrary, a significant difference was reported for percentage normal cells in two *Anas platyrhynchos* strains of duck in Poland (Zawadzka et al., 2015). In addition, the percentage normal sperm cells reported for the understudied duck genotypes is comparable

Table 2. Morphological defects in the semen of three duck genotypes

		Genotype		
Sperm region	Specific Defect	Muscovy duck	Mallard duck	Mule duck
Head (%)	None	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Mid-piece (%)	Curved mid-piece	33.80 ± 0.01	33.33 ± 1.01	0.00 ± 0.00
Tail (%)	Rudimentary Tail	0.00 ± 0.00	33.33 ± 0.76	0.00 ± 0.00
	Curved tail	66.20 ± 0.01	33.33 ± 0.96	0.00 ± 0.00

with 91.67–95.64% reported for Dumyati ducks in Egypt (Ghonim et al., 2010) but higher than the values reported for Muscovy and Common ducks in related studies (Etuk et al., 2006; Penfold et al., 2000; Cyriac et al., 2013; Zawadzka et al., 2015; Chen et al., 2016). Conversely, the number of deformed sperm cells in the present study was much lower compared to 8.19 to 9.63% reported for White Pekin ducks (Nahak et al., 2015), 18% in Pintail duck (Penfold et al., 2000), 33.6–40.3% in two strains of Polish conserved stocks (Zawadzka et al., 2015) and 10.46%. 12.04 and 11.22% reported for Muscovy, Kuttanad and White Pekin ducks (Cyriac et al., 2013), respectively.

A major possible factor responsible for the observed higher normal and negligible morphologically abnormal sperms in the present study in contrast to the related studies could be linked to the semen collection method. Semen samples used in this study were collected directly from the epididymis in contrast to the related studies where semen samples were collected through different collection methods subject to the influence of ejaculatory process and handling methods. Improper handling of ejaculates during microscopic examination could greatly influence values obtained for sperm abnormality (Machebe and Ezekwe, 2002). In addition, the differences in normal and defective sperm morphology in the present and other related studies on ducks could also be attributed to factors such as genetic variation, age, season, nutrition, management, health status, handling stress, heat stress among others.

Related studies on types of sperm morphological defects of ducks and other waterfowl species are meager. Similar to the report of this study, tail defects constituted majority of sperm anomalies observed in Muscovy drakes (El Azim et al., 2011) while Zawadzka et al. (2015) reported bent neck as the principal sperm defect in conserved Polish duck strains. Contrary to the two morphological defects observed in the semen samples of Muscovy and Mallard ducks in this study, Penfold et al. (2000) reported six sperm abnormalities for Pintail duck, a member of the *genus Anas.*

Sperm morphology affects the motility of sperm cells in the female reproductive tract and is also central to the fertilizing ability of male animal. This assertion was supported by the report of Bask and Brillard (1994) that only sperm cells with normal morphology can ascend through the vaginal of the hen to the sperm storage tubules.

It is noteworthy that the percentage normal and abnormal sperm morphology documented for the investigated duck genotypes were outside the range capable of adversely influencing reproductive efficiency. Ajayi et al. (2011) suggested that sperm cell deformity of less than 10% cannot influence fertility of cocks while Cyriac et al. (2013) submitted that if the head abnormality is more than 3 to 5%, the semen sample is not suitable for A I and the total abnormality permitted are 15 to 20% (Cyriac et al., 2013). In view of the foregoing, the high percentage of normal and low percentage of sperm abnormality of local Muscovy and exotic Common ducks portend a great hope for good fertilizing capacity.

Conclusion

The findings in the present study suggested that semen quality of local Muscovy ducks was superior to that of their exotic Mallard duck counterparts in the humid tropical environment. The better sperm motility and higher sperm cell concentration of Muscovy ducks is an indication that their semen can compare favourably with their exotic counterparts in small scale or commercial production of ducklings. Nevertheless, the semen of both Muscovy and Mallard ducks are suitable for natural and commercial production of ducklings through A I programmes. The absence of sperm cells in the semen of Mule ducks is a pointer to their sterility.

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