



Assessment of Quality Characteristics of Raw Semen in Pearl Guinea Fowls

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

A study was conducted to evaluate the semen quality parameters of individual and pooled guinea fowl semen at Poultry Research Station, Madhavaram Milk Colony, Tamil Nadu Veterinary and Animal Sciences University, Chennai- 51. Twenty-two male pearl guinea fowls, aged 12 months, chosen randomly were used for the study. The birds were trained for semen collection by abdominal massage technique and the semen samples were collected Weekly twice. Samples were evaluated for volume, color, consistency, pH, motility, concentration, viability, and morphology. Six birds were excluded from pooled semen analysis due to either the absence of semen production or low semen quality. The mean semen pH, spermatozoa motility (%),

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spermatozoa concentration ($10^6/\text{ml}$), per cent live spermatozoa and per cent abnormal spermatozoa in pooled raw semen of selected males were 7.19 ± 0.01 , 81.47 ± 1.52 , 1408.75 ± 83.21 , 85.82 ± 0.88 and 13.75 ± 0.65 respectively. The semen quality parameters were within the normal range for optimum fertility by artificial insemination. This study insights into the screening process for selecting males for inclusion in breeding programs and factors affecting semen quality in this species.

Keywords: Guinea fowl; spermatozoa; semen; concentration; motility; livability; abnormality.

1. INTRODUCTION

In India, guinea fowls are popular among marginal farmers and other vulnerable groups as small-scale poultry enterprise. It is being reared in semi-arid pockets of many northern regions mainly in states of Punjab, Uttar Pradesh, Assam, Odisha and Madhya Pradesh and also in the south mainly in states of Andhra Pradesh and Tamil Nadu mostly for their meat and egg. Guinea fowls are semi wild birds that are yet to be genetically improved for commercial meat and egg production. However, their fertility and hatchability rates are comparatively lower than those of other poultry species, resulting in slower genetic progress and a restricted scope for improvement. This may be due from factors such as a narrow sex ratio, challenges in sexing the birds [1], breeding seasonality [2,3], and potential inbreeding. In addition, the male gonads in guinea fowl are small and semen volume is scanty. Only viable and morphologically normal spermatozoa carrying intact genetic material can contribute to egg fertility. Artificial insemination emerges as a promising solution to enhance fertility rates in Guinea fowls. However, it is well known that optimal fertility through artificial insemination necessitates the insemination of quality spermatozoa in the female reproductive tract. Consequently, the semen evaluation stands as crucial parameter in fertility assessment. Therefore, the research was conducted to assess the semen quality parameters of individual guinea fowl birds which will proffer useful information in selection of birds with higher semen quality for breeding program or Artificial Insemination.

2. MATERIALS AND METHODS

Twenty-two male guinea fowls of the pearl variety, aged 12 months, were chosen randomly and housed individually in cages with one square foot of floor space per bird. They were provided with standard guinea fowl breeder ration and unrestricted access to drinking water. The birds were then given a period of one week to adopt in

the new cage environment during which their feeding and drinking behaviors were monitored to ensure normalcy. The birds were then trained for semen collection by abdominal massage technique by Burrows and Quinn [4]. Feathers around the vent region were regularly trimmed to facilitate easy semen collection.

Semen collection was done twice a week during the early hours of the day, following the procedures outlined by Burrows and Quinn [4]. The soft part of the abdomen and bilateral region of the pygostyle were massaged rapidly and continuously until the guinea fowls responded by protruding the papillae. Upon protrusion, semen was gently squeezed out using the thumb and index fingers and directly aspirated into sterile glass tuberculin syringes to avoid contamination. The collected ejaculate, characterized by its pearly white appearance, was then transferred into sterile Eppendorf tubes and placed in a water bath maintained at 18°C to 20°C for subsequent evaluation.

2.1 Macroscopic Evaluation

The volume of semen collected was measured directly in the tuberculin syringe with 0.01 ml accuracy. The colour [5] and consistency of the semen was evaluated by visual assessment. Semen consistency was estimated under daylight scored as 1 = clear (watery), 2 = cloudy, 3 = milky (Moderate) and 4 = creamy (Thick) as described previously [6].

Semen pH was accessed by placing a drop of freshly collected semen on a strip of limited range pH paper (Merck India 6.5-9.0) with an accuracy of 0.5 and the color developed was compared with standards given [7].

2.2 Microscopic Evaluation

The motility of spermatozoa in individual and pooled semen samples were assessed [8] by placing a small drop of semen in the middle of clean grease free glass slide, which was covered

with a cover slip and examined under microscope.

The spermatozoa concentration was determined using Neubauer type haemocytometer and the final concentration of spermatozoa was expressed as millions per ml [9].

The spermatozoa viability was determined in fresh and diluted semen by eosin - nigrosin staining procedure [10], with staining solution containing one per cent eosin and five per cent nigrosin. Smears were carefully stained and a maximum of 200 spermatozoa were counted in each smear under oil immersion. The unstained spermatozoa were counted as live and expressed as percentage. The same slides stained for the assessment of live and dead spermatozoa were used for abnormal spermatozoa and a maximum of 200 spermatozoa were counted and expressed in percentage.

3. RESULTS AND DISCUSSION

The semen characteristics of 22 numbers of individual guinea fowl males trained for semen collection and pooled raw semen were presented in Tables 1 & 2 respectively. Birds numbered 10, 15, 17, and 19 have not yielded any semen. Birds 14 and 18 were excluded from pooled semen analysis due to low sperm concentration and decreased motility. Only 16 birds with suitable seminal attributes were chosen for further evaluation of pooled samples.

3.1 Macroscopic Characteristics

The mean semen volume of guinea fowl observed in this study was 0.04 ± 0.01 ml. This is in accordance with the previous studies in pearl guinea fowls [11] and in white breasted (0.041 ± 0.005) guinea fowls [5]. The volume observed in this study was higher than those reported [5] in pearl guinea fowls (0.027 ± 0.002), lavender (0.035 ± 0.005) and White guinea fowls (0.029 ± 0.003). Marginally lower values than observed in this study was also reported [12] wherein the semen volume in Golden Sovereign guinea fowl was 0.032 ± 0.001 ml. Higher semen volume of 0.073 ± 0.003 ml and $94.97\pm 10.91\mu\text{l}$ was observed by Singh [13] and Lavor *et al.* [14] in indigenous guinea fowls and helmeted guinea fowls respectively. As reported by Thurston *et al.* [15], the semen volume in guinea fowls was less compared to chicken and turkeys. Higher semen

volume of 0.130 ml, 0.28 ± 0.01 and 0.16 ± 0.01 ml was observed [16,17,18] respectively in Beltsville Small White turkeys. The difference observed in this study may be due to seasonality of breeding [13], variation in climate [19], place of the study [5], time of semen collection [5] and difference in genetic makeup of the birds [5].

The selected guinea fowl males yielded pearly white semen which was in agreement with previous studies [20,21,5]. Off-color or watery semen and semen contaminated with blood or droppings / urates debris was not used for analysis [21].

The mean semen pH observed in this study were 7.23 ± 0.03 and 7.19 ± 0.01 in trained individual males and pooled semen samples from selected males respectively. Similar results were also obtained in Italian partridge [22]. Moderately lower (7.15 ± 0.01) and higher (8.10 ± 0.03) values were observed by Mohan *et al.* [11] and Keerthy [5] respectively in pearl guinea fowls. The pH of the guinea fowl semen observed in this study was moderately higher than that of White Leghorns [23,24]. In contrast, higher seminal pH of 7.4 was observed in Hubbard broiler breeder chicken [25] and Gramapriya hybrid cocks [26] respectively. The variation may be due to differences in genetic makeup of the birds, breeder nutrition, environment and method of analysis.

3.2 Microscopic Characteristics

The mean per cent spermatozoa motility of trained individual and pooled semen from selected males observed in this study were 74.28 ± 3.09 and 81.47 ± 1.52 respectively. The values were higher than the value (37.1 ± 0.1) reported in Golden Sovereign guinea fowl [12] and lower than that reported in pearl variety guinea fowls (85.00 ± 5.76 , 83.47 ± 1.75) [11,5]. Relatively lower spermatozoa motilities than obtained in pooled semen were observed by in Beltsville Small White (76.40 ± 0.40), crossbred (75.75 ± 0.40) and native turkeys (66.10 ± 0.58) [16]. Higher per cent spermatozoa motility than observed in this study was reported in chicken, with values of 85.60 ± 1.38 [23], 87.35 ± 10.12 [24] and 84.01 ± 0.04 [26] in White Leghorn, Naked neck and Gramapriya hybrid cock's semen respectively. In contrast, lower spermatozoa motilities were also observed in Red Jungle fowl with corresponding values of 55.2 ± 6.67 [27] and 63.5% [28] respectively.

Table 1. Semen quality parameters of trained individual males (Mean± SE) n=6

Bird No.	Colour	Consistency	Volume (ml)	pH	Motility (%)	Concentration (10 ⁶ /ml)	Live Spermatozoa (%)	Abnormal Spermatozoa (%)
1	Pearly White	Thick	0.04±0.009	7.17±0.02	79.17±1.56	3350.00±267.90	90.91±1.12	7.95±0.85
2	Pearly White	Thick	0.02±0.004	7.20±0.03	76.67±3.56	1322.16±212.20	80.30±2.63	12.43±1.84
3	Pearly White	Thick	0.05±0.009	7.28±0.02	80.83±3.53	1627.88±387.40	86.76±1.76	13.68±0.71
4	Pearly White	Thick	0.03±0.007	7.28±0.04	61.67±2.38	1428.83±267.00	88.72±1.89	12.24±1.22
5	Pearly White	Thick	0.03±0.003	7.23±0.03	76.67±4.21	1664.73±264.88	88.53±1.43	13.96±2.50
6	Pearly White	Thick	0.05±0.012	7.34±0.03	77.00±2.12	1317.50±251.82	87.88±1.56	11.00±0.56
7	Pearly White	Thick	0.04±0.007	7.15±0.03	78.33±3.42	1693.33±262.16	86.21±1.94	13.69±0.31
8	Pearly White	Thick	0.04±0.005	7.20±0.03	72.50±2.60	1163.33±235.26	84.97±1.73	14.08±0.30
9	Pearly White	Thick	0.05±0.009	7.28±0.02	72.50±3.57	1060.73±192.47	86.53±1.67	15.72±1.24
10			-	-	-	-	-	-
11	Pearly White	Moderate	0.08±0.007	7.15±0.02	75.83±2.33	1063.75±166.44	90.43±1.45	13.22±1.62
12	Pearly White	Thick	0.04±0.006	7.27±0.03	75.83±2.10	1347.78±282.26	90.00±0.86	16.03±0.96
13	Pearly White	Thick	0.03±0.008	7.25±0.04	69.17±4.52	1501.37±375.66	91.36±0.77	13.07±1.43
14	Pearly White	Thick	0.02±0.003	7.27±0.03	75.83±3.53	605.83±132.44	85.60±1.61	11.42±0.60
15			-	-	-	-	-	-
16	Pearly White	Thick	0.03±0.004	7.30±0.03	70.83±2.90	1112.92±251.62	83.84±1.89	12.66±1.20
17			-	-	-	-	-	-
18	Pearly White	Thick	0.03±0.005	7.22±0.03	64.17±4.41	762.92±137.20	83.39±2.38	10.12±0.60
19			-	-	-	-	-	-
20	Pearly White	Moderate	0.10±0.007	7.27±0.04	77.50±3.28	764.17±127.94	89.26±1.35	9.25±0.86
21	Pearly White	Thick	0.04±0.008	7.13±0.02	69.17±2.33	803.33±108.00	84.53±1.01	11.86±0.59
22	Pearly White	Moderate	0.08±0.008	7.13±0.02	83.33±3.27	980.42±208.65	87.04±1.39	11.69±1.16
Overall Mean			0.04±0.01	7.23±0.03	74.28±3.09	1309.50±229.52	87.01±1.58	12.45±1.03

Table 2. Semen quality parameters of pooled raw semen from selected males (Mean± SE) n=10

Sample No.	pH	Motility (%)	Concentration (10⁶/ml)	Live Spermatozoa (%)	Abnormal Spermatozoa (%)
1	7.2	75	1059	89.75	12.67
2	7.2	80	1213	90.05	11.1
3	7.2	85	1340	86.50	11.3
4	7.2	80	2016	85.55	11.87
5	7.2	75	1407	86.00	12.85
6	7.2	80	1528	86.52	13.04
7	7.2	80	1203	88.83	12.05
8	7.1	85	1311	89.79	10.92
9	7.2	90	1553	88.15	12.73
10	7.1	85	1457	86.11	11.97
Overall Mean	7.19±0.01	81.47±1.52	1408.75±83.21	87.73±0.56	12.05±0.24

The mean spermatozoa concentration of trained individual and pooled semen from selected males observed in this study was 1309.50 ± 229.52 and $1408.75 \pm 83.21 \times 10^6$ respectively, which was lower than the earlier reports with values of $3.087 \pm 0.398 \times 10^9$ [29], $2.62 \pm 0.01 \times 10^9$ [12], 3.18×10^9 [13], $3.27 \pm 0.14 \times 10^9$ [11] and $3.51 \pm 0.22 \times 10^9$ [30] in 8 months old guinea fowl, Golden Sovereign guinea fowl, indigenous guinea fowls and pearl guinea fowls respectively. However, comparable spermatozoa concentration of $1780.56 \pm 61.30 \times 10^6$ cells/ml as observed in this study was also reported in pearl guinea fowls [5]. Lower spermatozoa concentration than obtained in this study was reported in Red jungle fowls (800 million) [28]. The difference observed in this study may be due to seasonality of breeding [13], variation in climate [19], place of the study, time and frequency of semen collection and difference in genetic makeup of the birds.

The mean per cent live spermatozoa observed in this study were 87.01 ± 1.58 and 87.73 ± 0.56 , which is in accordance with the earlier findings in pearl guinea fowls [11,5]. In contrast, noticeable lower percentage of live spermatozoa was reported with of 64% [31] and 55% [32] respectively in guinea fowls. Marginally higher value was reported in Golden Sovereign guinea fowl (91.6 ± 0.1) [12]. Comparable values as in the present study were also observed as 85.30 ± 0.65 [16], 86.91 ± 0.67 [17] and 85.38 ± 1.70 [18] in Beltsville Small White turkeys. Higher per cent live spermatozoa is vital attribute having strong bearing with fertility parameters indicating the superiority of semen samples obtained in the study.

The mean per cent abnormal spermatozoa observed in this study were 12.45 ± 1.03 and 12.05 ± 0.24 in trained individual males and pooled semen from selected males respectively. The values observed were in accordance with the earlier works [5] and higher than that reported [11] in pearl guinea fowls. Higher values of 26% and 23.1% were reported in eight months old guinea fowl [29] and Golden Sovereign guinea fowls [12] respectively. The lower abnormal spermatozoa found in this study may be due to ideal age of the breeder males used for this study.

4. CONCLUSION

In conclusion, the study underscores the importance of evaluating semen characteristics

in guinea fowls before implementing breeding programs. Only 16 out of 22 birds (72%) displayed good semen quality in individual semen analysis. Therefore, screening male guinea fowls for semen quality prior to breeding programs can enhance reproductive performance while minimizing resource wastage. Additionally, screening can facilitate culling of birds with poor semen quality, expediting genetic improvement. Factors such as age of breeder males, breeding season, and environmental conditions play significant roles in semen quality and should be considered in future breeding and management practices. Further research may be warranted to explore the effects of additional factors on semen quality and fertility outcomes in guinea fowl.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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