International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2019; 7(2): 1838-1842

Received: 07-01-2019
Accepted: 09-02-2019

R Richard Churchil
All India Co-ordinated Research Project on Poultry for Eggs, Kerala Veterinary and Animal Sciences University, Mannuthy, Thrissur, Kerala, India

Lijo John
All India Co-ordinated Research Project on Poultry for Eggs, Kerala Veterinary and Animal Sciences University, Mannuthy, Thrissur, Kerala, India

P Ezhil Praveena
All India Co-ordinated Research Project on Poultry for Eggs, Kerala Veterinary and Animal Sciences University, Mannuthy, Thrissur, Kerala, India

Stella Cyriac
All India Co-ordinated Research Project on Poultry for Eggs, Kerala Veterinary and Animal Sciences University, Mannuthy, Thrissur, Kerala, India

Correspondence
R Richard Churchil
All India Co-ordinated Research Project on Poultry for Eggs, Kerala Veterinary and Animal Sciences University, Mannuthy, Thrissur, Kerala, India

Strain and age related changes of semen attributes in white leghorn roosters

R Richard Churchil, Lijo John, P Ezhil Praveena and Stella Cyriac

Abstract
The present study was conducted to assess the semen quality parameters at young (32 weeks) and old (64 weeks) age in two pedigreed populations of single comb White Leghorn selected for egg production for 21 generations. The roosters of IWP strain had yielded significantly ($p<0.05$) high volume of semen with better ($p<0.05$) appearance score at 32 weeks of age and significantly ($p<0.05$) high motility at both the ages compared to their counterparts in IWN strain. The percent live sperm was significantly ($p<0.05$) more in IWN strain at 64 weeks of age. A clear interaction of age was evident in this study as all the semen quality parameters deteriorated significantly ($p<0.01$) at older age in both IWN and IWP strains. On phenotypic scale, appearance score was having high positive correlation with motility, concentration and percent live sperms in both the strains but negative correlation with percent abnormal sperms. Semen volume was having significant positive correlation with motility in IWN ($p<0.05$) and IWP ($p<0.01$) strains but strong ($p<0.01$) negative association with concentration in both the strains. The sperm motility had a strong ($p<0.01$) association on positive direction with live sperms in both the strains.

Keywords: Cocks, sperm variables, genetic strain, age, correlation

Introduction
The assessment of semen quality characteristics of poultry birds gives an excellent indicator of their reproductive potential and is a major determinant of fertility and subsequent hatchability of eggs (Jarinkovicova *et al*. 2012) [8]. The parameters commonly used to evaluate semen quality are ejaculate volume, semen colour, sperm concentration, sperm motility, sperm viability and percent sperm deformity (Moce and Graham 2008) [16].
Several earlier studies clearly indicate the influence of age, breed, seasonal variation, time of collection and nutrition on the quality of semen. Season (Saeed and Al Soudi, 1975) [21] and breed (Egbunike and Oluyemi, 1979) [4] differences in production and quality of semen of roosters has been reported. The environmental temperature at ejaculation found to have profound effect on avian sperm motility (Ashizawa and Sano, 1990; Wishart and Wilson 1999) [1, 28]. There are reports indicating influence of breed and age on the quality of fresh and stored semen in broiler breeders (Kelso *et al*. 1996) [9]. The semen of older turkey birds had significantly lower motility, viability and mass movement than younger birds (Kotlowska *et al*. 2005) [10]. Reports on indigenous and broiler (Tabatabaei *et al*. 2009) [24] and pedigreed lines (Long *et al*. 2010) indicated that the semen motility was high in younger chickens. Malik *et al*. (2013) [14] found that the semen concentration, individual motility, volume and total abnormalities were significantly different among different genotypes of bantam chicken, domestic chicken, and red jungle fowl.
The individual sperm motility of indigenous cocks was found to have significant ($p<0.01$) positive correlation with live sperm, sperm concentration and sperm output (Bah *et al*. 2001) [2]. Peters *et al*. (2008) [20] observed that the volume and motility of seven sire lines had significant ($p<0.05$) positive correlation. In a study with broiler breeders, Modupe *et al*. (2013) [17] found very high degree of positive correlation between semen volume and concentration.
Barring a very few (Murugesan *et al*. 2013) [18], the information on the semen quality parameters in White Leghorn strains of chicken long term selected for egg production at older age is scanty in the literature. There is dearth of information on semen quality parameters of young pedigreed White Leghorn roosters and also on the nature of association between various semen quality traits in pedigreed layer populations. This study was therefore carried out to evaluate the semen quality of roosters parameters at young and old age and their associations in two strains of White Leghorn selected for 21 generations for egg production.

Materials and Methods

Experimental Birds: The roosters of IWN and IWP strains of single comb White Leghorn maintained at All India Coordinated Research Project on Poultry for Eggs of Kerala Veterinary and Animal Sciences University, Mannuthy, Thrissur, Kerala, India were utilized for the study. These strains had a history of being selected long term of 21 generations for egg production on the basis of an index that takes into account individual production and sire and dam family averages (Osborne 1957) [19]. Egg weight at 28 weeks as an independent culling level was superimposed over the combined selection for egg number. Sib selection was practiced for the selection of males for breeding based on a sire index (Osborne 1957) [19] constructed using their full and half sib family averages.
The roosters under study were reared in deep litter up to 16 weeks and thereafter kept in individual cages under photoperiod of 16h and ad libitum feeding. The semen quality was studied from 160 roosters, 80 from each strain at 32 and 64 weeks of age.

Semen Collection: The semen was collected by abdominal massage method in the morning as per Lake and Stewart (1978) [11].

Macroscopic Examination: Immediately after collection, individual ejaculate was examined onto the glass funnel to study the appearance score and semen volume. The appearance of semen was scored 1 to 5 by visual examination following the method described by McDaniel and Craig (1959) [15]. Semen volume was measured by a tuberculin syringe graduated up to an accuracy of 0.01 ml. The semen was then shifted on ice to the laboratory. The semen samples were individually subjected to study other physical parameters.

Motility: The microscopic evaluation of sperm motility was done by placing a drop of the diluted semen on a slide and a cover slip over it to form a uniform film for examination and also to prevent quick drying of semen. Motility was assessed on the basis of percentage of sperm showing forward motion (Birkhead *et al.* 1999) [3].

Sperm Concentration: The sperm concentration was estimated as per the procedure suggested by Taneja and Gowe (1961) [25]. A sample of well mixed semen was drawn up to the 0.5 mark of the R.B.C. pipette. The pipette was filled up to 101 mark using formal saline (Saline with few drops of formalin) as diluting fluid and agitated sufficiently to mix the contents. A thick cover glass was placed over the well cleaned counting chamber of 'Neubaur' haemocytometer and a drop of diluted semen was added to the counting chamber. The sperm cells were allowed to settle, chamber was focused under high power objective and a total of five large squares each consisting of 16 small squares were focused to count the number of sperms. In each square, all the sperms in the centre and those touching the upper and right hand borders were counted and those straddling the other two edges were ignored. The number of sperm cells counted in 80 small squares was divided by 100 to give the concentration of spermatozoa in million/ ml or $\times 10^9$/ ml of semen.

Percent Live Sperms: To estimate the percentage of live and dead spermatozoa, the staining method described by Lake and Stewart (1978) [11] was adopted with some modifications. A solution of nigrosine (0.6g) and eosin (0.16 g) was made in 100 ml of 2.9% sodium citrate solvent. Two hundred µl of eosin-nigrosine stain was placed in a clean test tube and 10-15 µ1 of semen was mixed well with the stain immediately after collection. After allowing the stain-semen mixture to stand for about one minute at room temperature, a drop of it was placed on a clean grease-free slide to prepare a thin smear and examined with an oil immersion objective. A differential count on a sample of 300 spermatozoa (50 spermatozoa in each of 6 microscopic fields) was made categorizing the stained and partially stained sperms as 'dead' and unstained sperms as 'live'.

Percent Abnormal Sperms: The smears as prepared for live sperm estimation were used to study the percentage of abnormal spermatozoa (Lake and Stewart 1978) [11]. A total of 200 spermatozoa were counted under the oil immersion objective of the microscope and classified as either normal or abnormal and the total percentages of abnormal spermatozoa were calculated.

Statistical Analysis: Statistical analysis of the semen characteristics to find the strain difference between IWN and IWP and age difference between 32 and 64 weeks was performed by the SPSS program using t-test.

Results and Discussion

The mean values of semen quality parameters of IWN and IWP strains at two different ages are given in Table 1. The overall semen volume of White Leghorn roosters subjected to the present study was 0.45 and 0.33 ml respectively at 32 and 64 weeks of age. The volume of semen in White Leghorn roosters observed in the study was in general, lower than the earlier report of Peters *et al.* (2008) [20] for the same breed. At the age of 64 weeks, both the strains in the present study yielded higher volume compared to 0.16 to 0.25 ml of semen reported by Murugesan *et al*. (2013) [18] in four strains of White Leghorn aged between 62 and 68 weeks of age. The present study on semen volume at 32 weeks of age revealed strain difference, although, the information on strain difference in young White Leghorn roosters is scarce in the literature. On the other hand, no strain difference persisted at 64 weeks of age in the present study contrary to the earlier observation of Murugesan *et al*. (2013) [18]. The present study revealed significant ($p<0.01$) decrease in semen volume at older age. However, the information in the literature on the effect of age on semen volume of White Leghorn breeder roosters is scanty.

Table 1. Semen quality attributes of IWN and IWP strains of White Leghorn at 32 and 64 weeks of age

Parameters	Age	Strains		Overall
		IWN	IWP	
Volume (ml)	32 weeks	0.42^{bx}±0.02	0.47^{ax}±0.02	0.45±0.01
	64 weeks	0.32^{y}±0.02	0.34^{y}±0.02	0.33±0.01
Appearance score	32 weeks	4.35^{bx}±0.04	4.52^{ax}±0.04	4.44±0.03
	64 weeks	3.62^{y}±0.05	3.68^{y}±0.05	3.65±0.04
Motility (%)	32 weeks	80.83^{bx}±1.62	87.73^{ax}±2.09	84.28±1.35
	64 weeks	55.81^{by}±2.25	62.13^{ay}±2.25	58.97±1.60
Concentration (10^9 sperms/ ml)	32 weeks	3.72^{x}±0.11	3.75^{x}±0.10	3.73±0.07
	64 weeks	2.81^{y}±0.10	2.79^{y}±0.09	2.80±0.07
Live sperms (%)	32 weeks	83.49^{y}±1.71	80.91^{y}±2.14	82.20±1.37
	64 weeks	69.63^{ax}±1.56	64.56^{bx}±2.00	67.09±1.28
Abnormal sperms (%)	32 weeks	5.64^{y}±0.26	6.04^{y}±0.25	5.84±0.18
	64 weeks	10.24^{x}±0.58	9.68^{x}±0.58	9.96±0.41

^{a,b} Mean values bearing different superscripts within the row differ significantly ($p<0.05$)

^{x,y} Mean values bearing different superscripts within the column for each quality parameter differ significantly ($p<0.01$)

The overall appearance score of semen of White Leghorn roosters was 4.44 and 3.65 at 32 and 64 weeks of age respectively. The appearance score of semen of IWN and IWP strains at 32 weeks was marginally better than naked neck (3.94) and dwarf (3.84) genetic lines of same age reported by Shanmugam *et al*. (2012) [2]. At 64 weeks of age, the strains under study showed intermediate values which were lower than that of IWC (4.0) and IWK (4.11) but higher than that of IWH (3.34) and IWI (3.21) strains of White Leghorn reported earlier by Murugesan *et al*. (2013) [18]. The appearance score of IWP was significantly ($p<0.05$) better than that of IWN at 32 weeks of age but became comparable at 64 weeks of age. There is dearth of information on strain difference for appearance score of semen of young White leghorn breeders. However, contrary to the present findings, strain difference in long term selected White Leghorn breeders at older age has already been reported (Murugesan *et al*. 2013)) [18]. Moreover, age has significant ($p<0.01$) deteriorative effect on appearance score in White Leghorn breeders. This was in agreement with the reports of Shanmugam *et al*. (2012) [2] in naked neck and dwarf genetic lines.

The strain-pooled sperm motility was 84.28% at 32 weeks of age with IWP registering significantly ($p<0.05$) higher value than IWN strain. The strain difference was consistent in 64 weeks of age also. The semen motility observed in this study at younger age compares favourably with earlier results of 82.5% in White Leghorn (Peters *et al*. 2008) [20], 83.5% in broiler breeder cocks (Modupe *et al*. 2013) [17] and 80.5% in dwarf chicken (Shanmugam *et al*. 2012) [2] but much higher than that of RIR cocks (66.8%) observed by Machal and Kfiivanek (2002) [13]. At older age, both IWN and IWP strains in the present study had similar motility to that of IWK, IWH and IWI strains of White Leghorn but lower than IWC strain as reported by Murugesan *et al*. (2013) [18]. Although, influence of genetic strain on sperm motility and also interaction of age on sperm motility has been reported in indigenous chicken by Shanmugam *et al*. (2012) [2], there is dearth of information for White Leghorn breeder cocks.

The overall sperm concentration observed in the present study at 32 weeks of age (3.73 billion per ml) was in line with that of Peters *et al*. (2008) [20] in White leghorn (3.5) and Modupe *et al*. (2013) [17] in Hubbard broilers (3.9) but much higher than that of RIR (0.75) and BPR (1.05) cocks recorded by Machal and Kfiivanek (2002) [13]. At older age of 64 weeks, the sperm concentration was similar to that of earlier observations of Elagib *et al*. (2012) [5] in White Leghorn cocks (2.85) but much lower than the findings of Murugesan *et al*. (2013) [18] in three different strains of White leghorn cocks. Both the strains under study showed a significant ($p<0.01$) thinning of semen at older age compared to younger age. This age dependant reduction in sperm concentration was similar to the findings of Shanmugam *et al*. (2012) [2] in native chicken. The strain difference on live sperm percentage was discernible only in the older age in this study with IWN recording significantly ($p<0.05$) high sperm concentration only at 64 weeks of age.

The percent live sperm of White Leghorn roosters recorded in the study at 32 weeks of age was in agreement with that of 80.7 to 84% in White Rock lines (Tarif *et al*., 2013) [26] and 85.94 % in indigenous naked neck chicken (Shanmugam *et al*. 2012) [2] but higher than 71.1% percent live sperms in Beijing-You chickens (Hu *et al*. 2013) [7] of same age group. At older age of 64 weeks both the strains under study had poor sperm viability than earlier report on IWC (88.84%) and IWK (76.91%) strains but comparable to that of IWI strain (65.71%) of White Leghorn (Murugesan *et al*. 2013) [18]. Strain difference in percent live sperm was evident at 64 weeks of age in the present study. Consistent to the present findings, Murugesan *et al*. (2013) [18] also reported this kind of strain difference in White Leghorn cocks selected for egg production. The percent live sperm showed a significant ($p<0.01$) negative shift in older roosters from younger ones in both the strains contrary to the findings of Sonseeda *et al*. (2013) [23], who observed no breed or age interaction on live sperm concentration in Thai native cocks.

The present study revealed that the percent abnormal sperm in White Leghorn roosters at younger age was similar to that of indigenous roosters (6.32%) reported by Tuncer *et al*. (2008) but lower than the range of values (10.17 – 16.58%) observed by Galal (2007) [6] in seven genotypes of indigenous chicken. At older age of 64 weeks, the White Leghorn roosters showed higher percent abnormal sperms than the range of value (1.54 to 7.04) recorded in four strains of same breed at same age (Murugesan *et al*. 2013) [18]. Although there was no strain difference in percent abnormal sperms, interaction of age was found in both IWN and IWP strains with significant ($p<0.01$) increase in the percentage of abnormal sperms at older age. Although earlier reports on White Leghorn roosters are limited, Shanmugam *et al*. (2012) [2] has reported interaction of age with abnormal sperm concentration in naked neck and dwarf gene lines.

The phenotypic correlation of various semen characteristics studied in IWN and IWP strains are given in Table 2. The significant positive correlation of appearance score with other vital fertility characteristics like motility, concentration and live sperms in both IWN and IWP strains indicates that the appearance score can be taken as an indicator of fertility in field

conditions where microscopic evaluation facility is a limiting factor. The volume of semen had significant high positive association with motility in both the strains. But a significant ($p<0.05$) correlation on negative direction between volume and concentration was recorded in both the strains. Negative correlation between volume and concentration similar to the earlier report of Bah *et al*. (2001) [2] could be an indication of semen collected without lymphatic fluid should contain higher concentration than those diluted with the fluid. The highly motile semen samples ought to have more viable sperms as it was evident from the present results of motility having a very high ($p<0.01$) positive correlation with percent live sperms, which is also in conformation with the earlier findings of Hu *et al*. (2013) [7] in Beijing-You chickens and Bah *et al*. (2001) [2] in local chickens of Nigeria. The negative association of percent abnormal sperms with motility and percent live sperms in IWN and IWP strains was strongly predictive of semen samples with high live motile sperms of having less abnormal sperms, as expected and in line with the earlier observation on local chickens of Nigeria (Bah *et al*. 2001) [2]. On the other hand, since the motility is measured as percent sperms having progressive motility, the very high ($p<0.01$) positive correlation between motility and live sperms observed in both the strains in the present study is also bound to occur.

Table 2: Correlation among different semen quality characteristics in IWN (upper diagonal) and IWP (lower diagonal) strains

	Appearance score	Volume (ml)	Motility (%)	Concentration (10^9 sperms/ ml)	Live sperms (%)	Abnormal sperms (%)
Appearance score		-0.064	0.551*	0.470*	0.303*	-0.138
Volume (ml)	0.218*		0.097*	-0.227*	0.093	-0.182
Motility (%)	0.589**	0.143**		0.052	0.872**	-0.374**
Concentration (10^9sperms/ ml)	0.494**	-0.176*	0.088		0.096	0.013
Live sperms (%)	0.208*	0.120*	0.788**	-0.109		-0.281 **
Abnormal sperms (%)	-0.244*	0.095	-0.271*	-0.031	-0.113*	

* Significant ($p<0.05$)
** Significant ($p<0.01$)

In conclusion, the present study revealed that young White Leghorn chicken have good semen attributes in terms of volume, appearance, motility, concentration, viability and structural integrity at younger age with marked deterioration of these parameters as age advanced. Influence of genotype on semen attributes of single comb White Leghorn roosters was evident in this study with IWP strain yielding semen of higher volume, motility and better appearance compared to IWN strain. Appearance score was having high positive correlation with other vital semen quality parameters; therefore it could be considered as simple criteria to determine the quality of semen in farm conditions.

References

1. Ashizawa K, Sano R. Effects of temperature on the immobilization and the initiation of motility of spermatozoa in the male reproductive tract of the domestic fowl, *Gallus domesticus*. Comparative Biochemistry and Physiology. 1990; 96:297-01.
2. Bah GS, Chaudhari SUR, Al-Amin JD. Semen Characteristics of Local Breeder Cocks in the Sahel Region of Nigeria. Revue d'Elevage et de Medecine. Veterinaire des Pays Tropicaux (Paris). 2001; 54:153-58.
3. Birkhead TR, Martinez JG, Burke T, Froman DP. Sperm mobility determines the outcome of sperm competition in the domestic fowl. Proceedings of the Royal Society B: Biological Sciences. 1999; 266:1759-64.
4. Egbunike CN, Oluyemi JA. Comparative studies of the reproductive capacity of the Nigerian and Exotic poultry breeds. Nigerian Journal of Animal Production. 1979; 6:47-51.
5. Elagib HAA, Musharaf NA, Makawi SA, Mohamed HE. The effect of age and season on semen characteristics of White leghorn cocks under Sudan conditions. International Journal of Poultry Science. 2012; 11:47-49.
6. Galal A. Predicting semen attributes of naked neck and normally feathered male chickens from live performance traits. International Journal of Poultry Science. 2007; 6:36-42.
7. Hu J, Chen JL, Wen J, Zhao GP, Zheng MQ, Liu RR, et al. Estimation of the genetic parameters of semen quality in Beijing-You chickens. Poultry Science. 2013; 92:2606-12.
8. Jarinkovicova L, Machal L, Machal J, Filipcik R, Tumova E, Horsky R. Relationship of ejaculate quality and selected biochemical parameters of blood in cockerels of three laying lines. Czech Journal of Animal Science. 2012; 57:370-76.
9. Kelso KA, Cerolini S, Noble RC, Sparks NHC Speake B K. Lipid and antioxidant changes in semen of broiler fowl from 25 to 60 weeks of age. Journal of Reproduction and Fertility. 1996; 106:201-06.
10. Kotlowska M, Glogowski JM, Dietrich GJ, Kozlowski K, Faruga A, Jankowski J, Ciereszko A. Biochemical characteristics and sperm production of turkey semen in relation to strain and age of the males. Poultry Science. 2005; 84:1763-68.
11. Lake PE, Stewart JM. Artificial insemination in poultry. Bulletin, Ministry of Agriculture, Fisheries and Food, Her Majesty's Stationery Office, Belfast, Great Britain, 1978, 34.
12. Long JA, Bongalhardo DC, Pelaez J, Saxena S, Settar P, Sullivan, NPO *et al*. Rooster semen cryopreservation: Effect of pedigree line and male age on post-thaw sperm function. Poultry Science. 2010; 89:966-73.
13. Machal L, Kfiivanek I. Indicators of semen quality of roosters of three parental layer lines and specific conductivity of the semen. Acta Veterinaria Brno. 2002; 71:109-16.
14. Malik A, Haron AW, Yusoff R, Nesa M, Bukar M, Kasim A, Evaluation of the ejaculate quality of the red jungle fowl, domestic chicken and bantam chicken in Malaysia. Turkish Journal of Veterinary and Animal Sciences. 2013; 37:564-68.
15. McDaniel GR, Craig IV. Behaviour traits, semen measurements and fertility of White Leghorn males. Poultry Science. 1959; 38:1005-14.
16. Moce E, Graham JK. *In vitro* evaluation of sperm quality. Animal Reproduction Science. 2008; 105:104-18.
17. Modupe O, Livinus AC, Ifeanyi NB. Semen quality characteristics and effect of mating ratio on reproductive

performance of Hubbard broiler breeders. Journal of Agricultural Science. 2013; 5:154-59.
18. Murugesan S, Matam N, Kulkarni R, Bhattacharya T K and Chatterjee RN. Semen quality in white leghorn chicken selected for egg production traits. Turkish Journal of Veterinary and Animal Sciences, 2013, 37:747-49.
19. Osborne R. Family selection in poultry. The use of sire and dam family averages in choosing male parents. Proceedings of Royal Society of Edinburgh. 1957; 66: 374-93.
20. Peters SO, Shoyebo OD, Ilori BM, Ozoje MO, Ikeobi CO N, Adebambo OA. Semen quality traits of seven strain of chickens raised in the humid tropics, International Journal of Poultry Science. 2008; 7:949-53.
21. Saeed JM, Al-Soudi KA. Semen quality traits of seven strains of chickens raised in the humid tropics. Journal of Animal and Veterinary Advances. 1975; 7:848-49.
22. Shanmugam M, Rajkumar AU, Reddy MR Rao SVR. Effect of age on semen quality in naked neck and dwarf chicken under tropical climatic conditions. Animal Production Science. 2012; 52:964-68.
23. Sonseeda P, Vongpralub T, Laopaiboon B. Effects of environmental factors, ages and breeds on semen characteristics in Thai indigenous chickens: A one-year study. Thai Journal of Veterinary Medicine. 2013; 43:347-52.
24. Tabatabaei S, Batavani RA, Talebi AR. Comparison of semen quality in indigenous and Ross broiler breeder roosters. Journal of Animal and Veterinary Advances. 2009; 8:90-93.
25. Taneja GS, Gowe RS. Spermatozoa concentration in the semen of two breeds of fowl estimated by three different methods. Poultry Science. 1961; 40:608-615.
26. Tarif AMM, Bhuiyan MMU, Ferdousy RN, Juyena NS Mollah MBR. Evaluation of semen quality among four chicken lines. IOSR Journal of Agriculture and Veterinary Science. 2013; 6:7-13.
27. Tuncer PB, Kinet H, Ozdogan N. Evaluation of some spermatological characteristics in Gerze cocks. Ankara Üniversitesi Veteriner Fakültesi Dergisi. 2008; 55:99-102.
28. Wishart GJ, Wilson YI. Temperature dependent inhibition of motility in spermatozoa from different avian species. Animal Reproduction Science. 1999; 57:229-235.