Comparative studies of semen quality traits and sperm kinematic parameters in relation to fertility rate between 2 genetic groups of breed lines

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ABSTRACT Semen quality is important for roosters' fertility. The correlations between semen quality traits and fertility has less been analyzed, impeding the selection of effective parameters for roosters selection. This research aimed to investigate the variation in semen quality traits in relation to fertilization capacity between 2 chicken breeds. A total of 156 Rhode Island Red (n = 78) and White Leghorn (n = 78) roosters of 53 wk were selected for semen quality estimation including semen volume, pH, sperm concentration, motility, viability, abnormal sperm percentage, and sperm kinematic parameters. Individual fertility was measured by artificial insemination using each 30 birds from 2 breeds. Significant variations were observed between breeds in semen volume, pH, sperm motility (**MOT**), viability, and abnormal sperm percentage ($P < 0.05$). The volume, MOT, and viability in Rhode Island Red were higher than those of White Leghorn roosters ($P < 0.001$). In addition, sperm kinematic parameters such as curvilinear velocity (**VCL**), straight line velocity (**VSL**), amplitude lateral head displacement (**ALH**), and average path velocity (**VAP**) in Rhode Island Red were higher than those of White Leghorn ($P < 0.001$). Fertility rate was positively correlated with MOT (r = 0.57), concentration (r = 0.43), viability (r = 0.39), VSL (r = 0.36), ALH (r = 0.43), and ALH (r = 0.38) for Rhode Island Red roosters ($P < 0.05$). Fertility rate of White Leghorn roosters was positively correlated with MOT (r = 0.71), concentration (r = 0.39), VCL (r = 0.52), ALH (r = 0.50), and VAP (r = 0.39) ($P < 0.05$). Principal component analysis of sperm kinematic descriptors revealed 2 principal components explaining more than 65% of total variance. In addition, for both genetic lines, the whole population was divided into 3 independent clusters. These results indicated that selection of roosters based on semen quality traits for may improve the fertility, and multivariate analysis may help to precise selection by comprehensive usage of different measures of sperm quality.

Key words: rooster, semen quality, sperm kinematics, fertility, computer-assisted semen analysis

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INTRODUCTION

Selection of males for reproduction is of great importance for poultry industry. It is therefore mandatory to monitor semen quality traits routinely to evaluate their reproductive capacity (Banaszewska et al., 2015). Assessing semen quality traits and fertilization capacity can be performed using different options (Froman and Feltmann, 1998), such as sperm live/dead percentage analysis and morphological evaluation (Lukaszewicz et al., 2008). Even with this, standard staining techniques used to evaluate semen quality and spermatozoa are usually inadequate to recognize abnormalities in the morphological structure of sperm cells (Andraszek and Smalec, 2011). Some scholars recommended that the fundamental semen analysis must be dealt deeply to include cytogenetic and molecular techniques, because many sperm imperfection cannot be distinguished at the morphological level, as they usually involve changes in chromatin structure (Andraszek and Smalec, 2011). The 3-dimensional organization of sperm chromatin determines its potential capacity to fertilize an egg cell and also affects embryo development (Ward, 2009).

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Most of the times for identifying roosters' fertility, initial sperm motility (**MOT**) is considered as a single dependable trait of semen. Other parameters such as viability and spermatozoa morphology also have great contribution to fertility rate, which confirms the previous study in local Iraq and ISA brown cross-bred lines (Hermiz et al., 2016). Sperm metabolism, sperm concentration, motility, and percentage of deformed or dead sperm are some of the parameters strongly related with individual male fertility (Wilson et al., 1979). In chicken and turkey industries, analysis of MOT and mobility is a basic part of selecting males with outstanding fertility rate and culling out males which have poor fertility performance (Donoghue, 1999). From the view point of animal genetics and enhancement, multivariate analysis like principal components analysis (**PCA**) and cluster analysis simultaneously considers a group of traits which may be used for selection intention (Pinto et al., 2006). Therefore, the attached kinematic parameters used as one issue in the assessment of fertility in poultry and the potential selection of roosters for artificial insemination programs are crucial. This information is essential to provide comparative data for the accurate identification of abnormal forms and the differential subpopulation structure in different breed lines. Many studies have focused more on age and strain-related semen quality trait analysis (Shanmugam et al., 2014; Mugiyono et al., 2015), whereas correlations between the various semen quality traits and fertility of Rhode Island Red (**RIR**) and White Leghorn (**WL**) roosters has been less analyzed, which impeded the identification of effective parameters for roosters selection. Hence, the aim of present study was to assess and compare the relationships between semen quality traits, sperm kinematic parameters, and fertilization capacity between 2 different genetic groups of chicken breed lines.

MATERIALS AND METHODS

Statement of Ethics

The study was performed in accordance with local ethical guidelines and met the requirement of the animal care and use committee (No. IAS2020-05) of Institute of Animal Science of Chinese Academy of Agricultural Sciences, Beijing, China.

Experimental Roosters and Experimental Design

A total of 60 pure parent stocks of 2 genetic lines of RIR (n = 78) and WL (n = 78) of 53 wks of age from experimental farm of Institute of Animal Science of Chinese Academy of Agricultural Sciences were randomly selected for this study. Each rooster was individually cage-housed under a 16L:8D photoperiod. A standard breeder diet that met nutrient requirements was provided for free access. The conventional semen quality traits and sperm kinematic parameters evaluation were performed for these roosters at 53 wk of age. The individual fertility was also estimated for each 30 birds randomly selected from the 2 breeds using artificial insemination at 55 wk of age. Parameters appropriate for roosters selection were identified by correlations analysis and multivariate analysis of semen quality traits.

Semen Quality Traits and Sperm Kinematic Parameters Estimation

Semen samples of the selected roosters were collected 3 times at a 2-d interval by the dorsal abdominal massage method described by Burrows and Quinn (1936). Semen collection was performed in the morning from 8:00 am up to 11:30 am and in the afternoon from 2:00 pm up to 4:00 pm by the same trained technician. Immediately after the collection, the semen samples were transferred to the heat bath of 37°C and then evaluated for primary criteria including semen volume, pH, MOT, concentration, viability, and abnormal sperm percentage. The semen volume was measured by weighing following the described by WHO. (2010). Semen pH was measured with a pH meter within 5 min after the semen collection (Seven Compact S210, Mettler-Toledo instruments Co., Ltd., Schwerzenbach, Switzerland). A drop of 10 μL of diluted semen (1:100 in 0.9% NaCl) was placed on a prewarmed special slide of 20 μm deep to estimate the MOT, concentration, and viability by a computer-assisted semen analysis system (**CASA**, ML-608JZII, Nanning Songjingtianlun Biotechnology Co., Ltd., Guangxi, China). This system consists of an optical phase-contrast microscope (Olympus, Tokyo, Japan) at 400× magnification, camera, minitherm heating stage, image digitizer, and computer saving and analyzing. In addition, sperm kinematic parameters including average path velocity, velocity straight line, curvilinear velocity, amplitude lateral head displacement, beat cross frequency, straightness, wobble, and linearity were also obtained from CASA. Sperm morphology was determined by in vivo staining with the crystal violet (Santiago-Moreno et al., 2009). After staining, the slides were air-dried and examined under a light microscopy (Olympus, Tokyo, Japan) at 400× magnification. The abnormal sperm percentage was calculated as the percentage of abnormal spermatozoa of around 500 spermatozoa analyzed per sample.

Fertility and Hatching Performance

A total of 60 roosters (30 from each genetic group) were randomly selected to be used as semen donors to inseminate hens of the same line. In total, 450 hens were used for this experiment. The first round artificial insemination was performed in the afternoon on 2 consecutive days and at a 3-d interval thereafter. Depending on the sperm production, male to female ratio were 1:7 or 1:8, and a fixed volume of 20 μL semen was inseminated for each hen. Egg were marked by the roosters' ID and collected daily from day 2 to 14 following the first insemination and stored at temperature of 18°C and relative humidity of 75% until incubation. Before the incubation, abnormal,

Table 1. Effects of breed lines and time of semen collection on semen quality and sperm motion kinematic parameters of 2 genetic groups breed lines.

	Breed		Time of semen collection		*P*-value	
Item	RIR	WL	Morning	Afternoon	Breed	Time
VOL (mL)	0.52 ± 0.03	0.24 ± 0.02	0.43 ± 0.04	0.32 ± 0.03	<0.001	0.027
MOT (%)	67.44 ± 3.23	49.66 ± 5.50	54.61 ± 5.15	62.77 ± 4.23	0.007	0.230
CON ($\times 10^9$/mL)	5.61 ± 0.03	5.04 ± 0.03	5.53 ± 0.03	5.11 ± 0.03	0.178	0.319
VIA (%)	74.48 ± 2.80	56.09 ± 5.41	62.23 ± 5.00	68.56 ± 4.07	0.004	0.335
DEF (%)	14.93 ± 0.89	18.85 ± 1.21	16.29 ± 0.91	17.54 ± 1.31	0.012	0.433
Semen pH	6.85 ± 0.09	7.59 ± 0.10	7.19 ± 0.09	7.16 ± 0.15	<0.001	0.832
VCL (μm/s)	61.37 ± 1.17	55.13 ± 1.18	57.35 ± 1.13	59.22 ± 1.46	<0.001	0.310
BCF (Hz)	0.78 ± 0.01	0.89 ± 0.04	0.84 ± 0.03	0.83 ± 0.04	0.006	0.997
STR (%)	0.59 ± 0.02	0.60 ± 0.01	0.61 ± 0.01	0.58 ± 0.02	0.855	0.111
VSL (μm/s)	25.49 ± 0.71	22.89 ± 0.46	24.58 ± 0.59	23.78 ± 0.68	0.003	0.386
ALH (μm)	17.97 ± 0.34	16.38 ± 0.44	16.79 ± 0.33	17.59 ± 0.49	0.006	0.183
LIN (%)	0.42 ± 0.01	0.42 ± 0.01	0.43 ± 0.01	0.41 ± 0.01	0.905	0.116
VAP (μm/s)	43.37 ± 0.82	38.94 ± 0.81	40.55 ± 0.79	41.79 ± 1.02	0.001	0.335
WOB (%)	0.84 ± 0.02	0.89 ± 0.01	0.87 ± 0.01	0.87 ± 0.01	0.007	0.763

Data are means ± SEM.

Abbreviations: ALH, amplitude lateral head displacement; BCF, beat cross frequency; CON, sperm concentration; DEF, abnormal sperm percentage; LIN, linearity; MOT, sperm motility; RIR, Rhode Island Red; SCT, semen collection time; STR, straightness; VAP, average path velocity; VCL, curvilinear velocity; VIA, live sperm percentage; VOL, ejaculate semen volume; VSL, straight line velocity; WL, White Leghorn; WOB, wobble.

unclean, and over- and under-weight eggs were discarded. In total, 65 to 72 eggs per roosters were incubated in the same condition. The eggs were candled on day 11 postincubation. Those eggs without clear viable embryos were opened to determine whether they contained an early dead embryo or were an unfertilized oocyte. Fertility was determined for each rooster as the percentage of fertile eggs of the total number of setting eggs. The hatchability of fertile eggs was calculated as the percentages of hatched eggs of the total number of fertile eggs. The hatchability of setting eggs was calculated as the percentage of hatched eggs of the total number of setting eggs.

Data Analysis

Data were analyzed using SAS software (version 9.2; SAS Inst. Inc., Cary, NC). Significance level was set at $P < 0.05$. Model used for the variance components estimation for semen quality traits were:

$$Y_{ij} = \mu + B_i + T_j + e_{ij},$$

Where Y_{ij} = the observed value of the ij^{th} for the relevant traits; μ = overall mean; B_i = breed effect (RIR and WL); T_j = time of semen collection effect (Morning and Afternoon); and e_{ij} = residual effect. Student t test was performed to assess the significance of difference of the fertility and hatching performance data. Correlation of various semen quality traits with fertility were estimated by the Pearson product moment correlation. In addition, PCA on the sperm kinematic parameters was performed for each genetic group.

RESULTS

Semen Quality Traits and Sperm Kinematic Parameters of RIR and WL Roosters

Semen quality characteristics and sperm kinematic parameters obtained from RIR and WL roosters are presented in Table 1. Significant variations were found between RIR and WL roosters in semen volume, pH, MOT, viability, and abnormal sperm percentage. The semen volume, MOT, and viability in RIR roosters (0.52 ± 0.03 mL, 67.44 ± 3.23%, and 74.48 ± 2.80%, respectively) were higher ($P < 0.001$) than those of WL roosters (0.24 ± 0.02 mL, 49.66 ± 5.50%, and 56.09 ± 5.41%, respectively). In contrary, higher abnormal sperm percentage and semen pH values were observed in WL (18.85 ± 1.21% and 7.59 ± 0.01, respectively) ($P < 0.001$) as compared with RIR roosters (14.93 ± 0.89% and 6.85 ± 0.09, respectively). In

Table 2. Fertility and hatchability of Rhode Island Red and White Leghorn roosters of 55 wk age.

Item	RIR (N = 30)	WL (N = 30)	*P*-value
Fertility (%)	89.46 ± 1.55	81.73 ± 3.06	0.028
Hatchability from the fertilized eggs (%)	71.73 ± 2.67	72.44 ± 2.38	0.845
Hatchability from the total setting eggs (%)	66.79 ± 3.14	60.85 ± 3.54	0.215

Data are means ± SEM.

Fertility (%) = (Fertilized eggs number/Total setting eggs number) × 100, Hatchability from the fertilized eggs (%) = (Hatched chick number/Fertilized eggs number) × 100, Hatchability from the total setting eggs (%) = (Hatched chick number/Total setting eggs number) × 100.

Abbreviations: RIR, Rhode Island Red; WL, White Leghorn.

Table 3. Pearson's correlation coefficient between semen quality traits, sperm kinematic parameters, and fertility rate of Rhode Island Red (above the diagonal) and White Leghorn roosters (below diagonal).

Item	VOL	MOT	CON	VIA	DEF	VCL	BCF	STR	VSL	ALH	LIN	VAP	WOB	FR
VOL	1.00	0.49[1]	0.26	0.29	0.01	0.13	−0.44[1]	−0.12	0.37[1]	0.17	0.29	−0.00	−0.15	0.20
MOT	0.07	1.00	0.56[2]	0.72[2]	−0.004	0.25	−0.29	−0.27	0.62[2]	0.64[2]	0.12	0.40[1]	−0.07	0.57[2]
CON	0.36[1]	0.36[1]	1.00	0.54[2]	0.20	0.18	−0.13	−0.14	0.16	0.24	−0.01	0.33	0.19	0.39[1]
VIA	0.21	0.31	0.05	1.00	0.16	0.20	−0.11	−0.26	0.39[1]	0.66[2]	−0.17	0.39[1]	−0.10	0.43[1]
DEF	−0.08	0.16	−0.20	0.18	1.00	0.20	0.02	0.24	0.10	−0.04	0.18	0.14	0.19	0.01
VCL	0.06	0.61[1]	0.32	0.05	−0.16	1.00	−0.03	−0.19	0.09	0.43[1]	−0.28	0.16	−0.19	0.13
BCF	−0.11	−0.17	−0.14	0.07	0.10	−0.15	1.00	−0.05	−0.31	−0.17	−0.20	−0.15	−0.04	−0.33
STR	0.17	−0.47[2]	0.02	−0.07	−0.06	−0.64[2]	−0.39[1]	1.00	0.18	−0.16	0.38[1]	0.07	0.23	−0.22
VSL	0.31	−0.01	0.30	−0.04	−0.20	0.03	−0.62[2]	0.73[2]	1.00	0.62[2]	0.63[2]	0.42[1]	−0.01	0.36[1]
ALH	0.05	0.58[2]	0.26	−0.02	−0.19	0.82[2]	−0.15	−0.52[2]	0.05	1.00	−0.22	0.56[2]	−0.19	0.43[1]
LIN	−0.13	−0.45[1]	−0.16	−0.35	−0.06	−0.32	−0.15	0.50[2]	0.38[1]	−0.32	1.00	−0.03	0.16	0.01
VAP	0.04	0.47[2]	0.24	0.09	−0.11	0.89[2]	−0.09	−0.56[2]	0.03	0.74[2]	−0.17	1.00	0.71[1]	0.38[1]
WOB	−0.03	−0.19	−0.12	0.14	0.06	−0.04	0.13	0.06	0.01	−0.01	0.17	0.41[1]	1.00	0.08
FR	0.15	0.71[2]	0.39[1]	0.03	−0.20	0.52[2]	−0.26	−0.39[1]	−0.02	0.50[2]	−0.41	0.39[1]	−0.20	1.00

Abbreviations: ALH, amplitude lateral head displacement; BCF, beat cross frequency; CON, sperm concentration; DEF, abnormal sperm percentage; FR, fertility rate; LIN, linearity; MOT, sperm motility; STR, straightness; VAP, average path velocity; VCL, curvilinear velocity; VIA, live sperm percentage; VOL, ejaculate semen volume; VSL, straight line velocity; WOB, wobble.
[1]$P < 0.05$.
[2]$P < 0.001$.

addition, the average value of sperm kinematic parameters, such as curvilinear velocity, straight line velocity, amplitude lateral head displacement, and average path velocity were higher ($P < 0.001$) in RIR roosters than those of WL roosters. However, beat cross frequency was higher in WL ($P < 0.05$), whereas sperm concentration did not differ ($P > 0.05$) between RIR and WL roosters (5.61 ± 0.03 vs. 5.04 ± 0.03 × 10^9/mL). There was no statistical significance because of the effect of time of semen collection ($P > 0.05$) except for semen volume. Semen volume in the morning (0.43 ± 0.04 mL) was higher than that collected in the afternoon (0.32 ± 0.03 mL) ($P < 0.05$).

Fertility and Hatching Performance

As shown in Table 2, fertility was lower in WL roosters (81.73%) than RIR (89.46%) ($P < 0.05$). However, the hatchability from the fertilized eggs and hatchability from the setting eggs were similar in the 2 genetic groups (71.73% and 66.79 vs. 72.44% and 60.85%, respectively).

Correlations of Semen Quality Traits and Sperm Kinematic Parameters With Fertility Rate of Roosters

The phenotypic correlation of various semen quality traits and sperm kinematic parameters studied in RIR and WL roosters are given in Table 3. Fertility rate was positively correlated with MOT (r = 0.57, $P < 0.001$), sperm concentration (r = 0.39, $P < 0.05$), sperm viability (r = 0.43, $P < 0.05$), straight line velocity (r = 0.36, $P < 0.05$), amplitude lateral head displacement (r = 0.43, $P < 0.05$), and average path velocity (r = 0.38, $P < 0.05$) in RIR roosters. While in WL roosters, MOT (r = 0.71, $P < 0.001$), sperm concentration (r = 0.39, $P < 0.05$), curvilinear velocity (r = 0.52, $P < 0.001$), amplitude lateral head displacement (r = 0.50, $P < 0.001$), and average path velocity (r = 0.39, $P < 0.05$) were positively associated with fertility rate. On the other hand, abnormal sperm percentage did not have any correlation with MOT or other sperm motion kinematic parameters in both genetic groups.

PCA of Sperm Kinematic Parameters

The PCA data matrix gives 2 principal components (**PC**) with eigenvalues greater than one, which accounted for 69.60 and 65.04% of the cumulative variance from the initial parameters for RIR and WL roosters, respectively (Table 4). In both breeds, PC1 and PC2 were used to differentiate each sperm kinematic parameter and grouped into subcluster analysis. For RIR roosters, PC1 was positively associated with curvilinear velocity, beat cross frequency, amplitude lateral

Table 4. Principal component analysis on the computerized sperm kinematic parameters obtained from Rhode Island Red and White Leghorn roosters at 53 wk of age.

		Initial eigenvalues			Eigenvectors							
Breed	PC	Eigen values	Variance (%)	Cumulative variance (%)	VCL	BCF	STR	VSL	ALH	LIN	WOB	VAP
RIR	PC1	4.09	51.09	51.09	0.89	0.28	−0.82	−0.13	0.94	−0.83	0.31	0.93
	PC2	1.48	18.52	69.60	0.29	−0.56	0.18	0.83	0.27	0.36	−0.26	0.29
WL	PC1	3.38	42.28	42.28	0.94	−0.49	−0.32	0.50	0.95	−0.39	0.01	0.92
	PC2	1.82	22.27	65.04	0.08	−0.34	0.84	0.47	0.12	0.89	0.14	0.01

Abbreviations: ALH, amplitude lateral head displacement; BCF, beat cross frequency; LIN, linearity; PC, principal component; RIR, Rhode Island Red; STR, straightness; VAP, average path velocity; VCL, curvilinear velocity; VSL, straight line velocity; WL, White Leghorn; WOB, wobble.

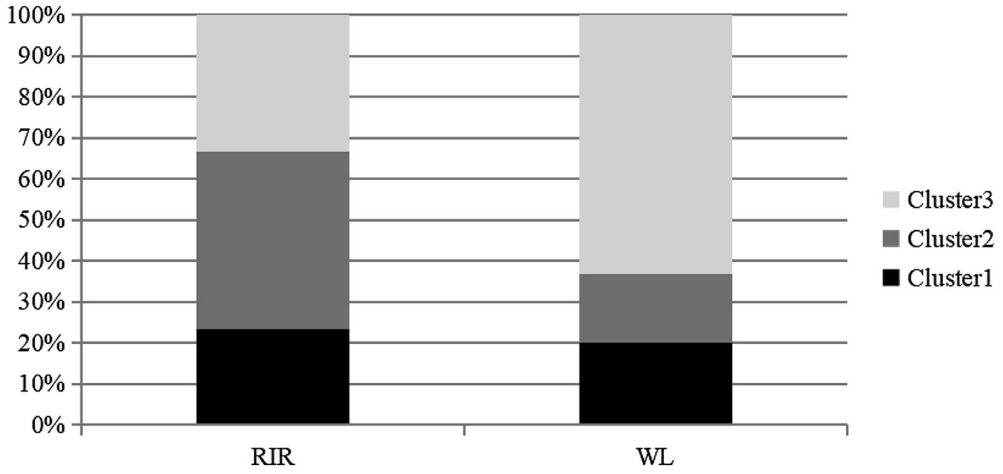

Figure 1. Frequency of distribution within each cluster, as defined after the clustering and discriminant analyses of sperm kinematic parameters in Rhode Island Red (RIR) and White Leghorn (WL) roosters.

head displacement, wobble, and average path velocity and negatively related to straightness, straight line velocity, and linearity; PC2 was positively related to all the sperm kinematic parameters except for beat cross frequency and wobble. Similarly for WL roosters, PC1 was positively related to curvilinear velocity, straight line velocity, amplitude lateral head displacement, wobble, and average path velocity and negatively linked to beat cross frequency, straightness, and linearity; PC2 was positively related to all the sperm motion parameters except for beat cross frequency.

For both genetic lines, the whole population was divided into 3 independent subpopulations or clusters (Figure 1 and Table 5). For RIR, Cluster 1 comprised 23.3% of the cells and was defined by both linear and oscillatory movement (with slow curvilinear velocity, average path velocity, and straight line velocity); Cluster 2 comprised 43.3% of the cells and characterized by the highest sperm kinematic motility; and Cluster3, with 33.3% of the cells, was defined by both linear and oscillatory movement (with medium curvilinear velocity, average path velocity, straight line velocity, and amplitude lateral head displacement). Cluster 1 was predominant in 10 birds with a low averaged fertility of 86.64%, Cluster 2 in 7 with a high averaged fertility of 91.84%, and Cluster 3 in 13 with a medium averaged fertility of 89.01%. In all case, Cluster 3 was clearly greater than the others. For WL roosters, Cluster 1 included 20% and characterized by medium linear and oscillator sperm kinematic movements (with medium curvilinear velocity, average path velocity, straight line velocity, and amplitude lateral head displacement); Cluster 2 with the highest frequency at 63.3% by slow sperm kinematic motility; Cluster 3 was less frequent at 16.7% characterized by high MOT. Cluster 1 was predominant in 6 birds with a medium averaged fertility of 82.89%, Cluster 2 in 19 with a low averaged fertility of 62.18%, and Cluster 3 in 5 with a high averaged fertility of 94.90%.

DISCUSSION

Measuring the fertility of individual male is more difficult as compared with females, but there is an option to quantify the fertility potential of roosters by assessing semen quality traits (Tabatabaei et al., 2009; Froman and Rhoads, 2013). In the present study, semen quality of RIR and WL roosters related to fertility potential were focused by evaluating conventional semen quality characteristics such as semen volume, pH, MOT, concentration, viability, and abnormal sperm percentage, and sperm kinematic parameters like curvilinear velocity, straight line velocity, amplitude lateral head displacement, straightness, linearity, beat cross frequency, wobble, and average path velocity.

Table 5. Subclustering distribution of sperm kinematic parameters in respective of fertilizing capacity of from Rhode Island Red and White Leghorn roosters at 55 wk of age.

		Sperm kinematic parameters (centroids)								
Breed	Cluster	VCL	VAP	ALH	VSL	STR	LIN	WOB	BCF	Fertility (%)
RIR	Cluster1	55.10	38.96	16.14	25.72	0.68	0.46	0.81	0.78	86.64
	Cluster2	63.66	45.31	18.83	26.41	0.58	0.41	0.85	0.77	91.84
	Cluster3	60.48	42.90	18.08	23.45	0.55	0.38	0.87	0.81	89.01
WL	Cluster1	55.15	38.83	16.34	25.98	0.66	0.47	0.91	0.80	82.89
	Cluster2	52.77	37.75	15.22	22.73	0.60	0.43	0.88	0.83	62.18
	Cluster3	65.48	47.14	19.45	26.69	0.56	0.40	0.89	0.79	94.90

Abbreviations: ALH, amplitude lateral head displacement; BCF, beat cross frequency; LIN, linearity; RIR, Rhode Island Red; STR, straightness; VAP, average path velocity; VCL, curvilinear velocity; VSL, straight line velocity; WL, White Leghorn; WOB, wobble.

Significant variations were found between RIR and WL roosters in semen volume, pH, MOT, sperm viability, and abnormal sperm percentage, which confirms a previous study by Mavi et al. (2019) in RIR, Punjab Red, and their crossbreed. On the contrary, higher semen volume and MOT were reported by Tarif (2013) in Sasso roosters and Adeoye et al. (2018) in Nigerian local chickens. Particularly semen volume in WL roosters observed in this study was in general lower than the earlier report for the same breed (Peters et al., 2008; Churchil et al., 2019). Both genetic groups had lower sperm viability than earlier report on indigenous and Ross broiler breeders (Tabatabaei et al., 2009) but are comparable to that of WL strain (Murugesan et al., 2013). It is obvious that semen quality traits are affected by both genetic and nongenetic factors. The reasons for lower value in this study may be a result of continuous strain selection for higher egg production which may bring a depressing impact on semen quality traits and reproductive efficiency (Murugesan et al., 2013). Shanmugan et al. (2016) demonstrated lower semen volume in contrast with the present study for the pure WL line. Abnormal sperm percentage and semen pH were higher in WL roosters compared with RIR roosters. The average abnormal sperm percentage recorded in the present study was higher compared with previous reports from indigenous Beijing-You roosters at 43 wk of age (Hu et al., 2013), brown tinted layer pure line chickens at 62 wk of age (Shanmugam et al., 2014), Isa-brown by Azubuike et al. (2017), indigenous and Ross broiler breed Tabatabaei et al. (2009), and 7 genotype of indigenous chicken by Galal (2007).

The semen pH recorded was to some extent close to alkaline in RIR breed, whereas neutral in nature in WL roosters. The semen pH in the present study was within the range from the reported data of poultry semen (Hu et al., 2013; Mavi et al., 2019). There was no difference in sperm concentration between the 2 genetic groups. The average values were in accordance with that for Hubbard broilers (Modupe et al., 2012), and 2 strains of WL roosters at 32 and 64 wk of age reported by Churchil et al. (2019).

Significant higher fertilization capacity was observed in RIR roosters. This shows that spermatozoa from RIR are more effective than those from WL roosters in terms of fertility. On the basis of total eggs set, hatchability between RIR and WL breeds did not differ significantly. Obviously fertility of layer strains (97%) is in general better than that of the broiler strains (92%) (Froman et al., 2016). However, in the present study, the fertility and hatchability of the 2 genetic groups were lower as compared with the previous studies by Islam et al. (2002) in WL and White Rock, Zelleke et al. (2005) in WL, and Wondmeneh and Adey (2011) in Fayoumi but higher than that of Brahma and Cochin reported by Hrnčár et al. (2015) and Orpington chickens by Askarianzadeh et al. (2018). The reasons behind for lower result in this study could be different factors including age and maternal factors including egg shell thickness, egg shell porosity, and egg shape index.

Sperm motility and other sperm motion parameters are considered to be the most important characteristics associated with fertilizing capacity (Verstegen et al., 2002). In this study, MOT, concentration, viability, straight line velocity, amplitude lateral head displacement, and average path velocity showed positive correlations with fertility rate in RIR roosters. In WL roosters, MOT, concentration, curvilinear velocity, amplitude lateral head displacement, and average path velocity had positive correlations with fertility rate. This suggested that fertility potential of RIR and WL are influenced by genotype and that MOT, concentration, viability, curvilinear velocity, straight line velocity, amplitude lateral head displacement, and average path velocity may be crucial parameters for evaluating breeding soundness of cocks. This is consistent with a previous study in Japanese quails (Farooq, 2014). A strong positive correlation between MOT and fertility were also reported in different chicken breeds (Sun et al., 2019; Wolc et al., 2019). Abnormal sperm percentage did not have any correlation with MOT and fertility, which in contrary with the previous study reported by Ansah et al. (1985). These data implied that assessment of semen quality and sperm motion kinematic parameters might be used as an indicator of RIR and WL roosters' fertility.

The CASA technology has permissible achievement of sperm kinematic parameters that can be used for advanced multivariate statistics (Agarwal et al., 2003). A combination of computerized and statistical techniques has permitted to classify the overall sperm population of semen samples into homogeneous, separate subpopulations by grouping spermatozoa with similar sperm kinematic parameter characteristics. From the 2-step cluster procedures, different sperm subpopulations with different fertilizing capacities were obtained and their distribution varied significantly between breeds, providing more information than the traditional analysis data that are based on the mean values. The different sperm subpopulations could be assumed synergistically to maximize fertilization achievement (Quintero-Moreno et al., 2003). For improving reproductive management and spermatozoa characterization in poultry industry, applying modern technologies is mandatory in understanding the biological basis of roosters fertility difference (Parker and McDaniel, 2003). Therefore, the objective analysis of sperm motion subpopulations could be contributed on screening roosters efficiently at the onset of semen production for certain sperm phenotypes which are indicative of their reproductive potential (Barbato, 1999). In this study, both genetic population can be divided into 3 independent clusters based on the cluster analysis of sperm motion parameters. The 3 clusters did show difference in fertility and further confirmed the effectiveness of multivariate analysis in precise selection of roosters of high fertility by comprehensive usage of different measures of sperm quality.

CONCLUSION

In conclusion, the conventional semen quality traits including MOT, concentration, viability, and sperm kinematic parameters including curvilinear velocity, straight line velocity, amplitude lateral head displacement, and average path velocity are key important traits which provided voluble information for comprehensive evaluation of RIR and WL roosters' fertility. Selection of roosters on the basis of semen quality traits especially MOT and sperm kinematics parameters for artificial insemination may improve fertility rate. Further studies are needed to elucidate the association of the phenotypes to genotypes and to explain how sperm kinematic characteristics is related to fertility. Multivariate analysis may help to precise selection of breeder roosters by comprehensive usage of different measures of sperm quality.

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