Evaluation Semen Characterization of Roosters Resulted from Different Local Lines and Their Crosses with ISA Brown

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Abstract: - This study conducted at Gardarash station, College of Agriculture in collaboration Directorate of Agricultural Research–Erbil during June and July, 2014. A total of 92 roosters were belongs to three local lines and their main and reciprocal crosses with ISA brown. The semen collected with abdominal massage technique into tube maintained at 38-39^{o}C. Volume, pH and colour score of the semen were recorded. The wave patterns, individual motility, percentages of live/dead and abnormal/normal sperms were evaluated by using Eosin/Nigrosine stains under light microscope. Length and width of wattles were measured using Digital Caliper while the colour of wattles score recorded visually. SAS program used to study the effect of genetic groups and age of roosters on studied traits. The above traits were reanalyzed to study their affects by colour of semen and by colour of wattles. Duncan Multiple Range Test conducted to diagnosing the significance differences between the means of the levels of each factor. Correlation coefficients among the studied traits were calculated.

Overall mean of ejaculate volume, sperm concentration, live sperms, abnormal sperms, mass motility, individual motility and pH value were 0.369 (ml), 4.47 (n*10^{9})/ml, 91.35%, 12.01%, 68.40%, 75.60% and 7.12 respectively. There were significant differences among genetic groups in ejaculated volume, sperm concentration, abnormal sperm%, mass motility% and individual motility% only. Effect of age of roosters was significant on mass and individual motility only. Higher negative and significant correlation was between abnormal sperm with live sperm (-0.49), while the higher positive and significant correlation was between mass motility and individual motility (0.95). Overall means of wattle length and width were 55.61 and 46.46 (mm) respectively. The genetic group (local black with brown neck) excelled the other pure and crossed genetic groups significantly in their wattle length and width. Effect of age of roosters found to be not significant on both traits. The correlation between length and width of the wattles was highly significant (0.79). Roosters with creamy semen was significantly better than roosters with watery semen in ejaculate volume, sperm concentration, abnormal sperms and mass motility, while the roosters with milky semen had moderate values. The differences in all semen parameters according to the colour of wattles were not significant. Roosters having brown wattles had significantly greater length and width comparing with those having redden white wattles.

Conclusion: The semen parameters must be evaluated individually. The results obtained from this study will help to do some further researches on freezing of semen roosters and artificial insemination.

Key-Words: - Poultry Genetic Groups, Age, Semen Parameters, Wattles Measurements.

1 Introduction

The assessment of semen quality characteristics of poultry birds gives an excellent indicator of their reproductive potential and has been reported to be a major determinant of fertility and subsequent hatchability of eggs [21]. Fertility and hatchability on the other hand are the major determinant of profitability in the hatchery enterprise [22]. Moreover, volume and color of semen sample are also evaluated to determine the teasing of male and presence of any lesion or infection in genital tract [23]. Infertility problems in poultry have often been blamed on the male [8]. The differences in semen per ejaculate volume was affected by differences in the strains, breeds, age, body size, nutrient feed, temperature environment, as well as the differences in types of chicken have different semen qualities which affect the production and quality of the breeding programs [1]. The biological performance of chickens was influenced by three factors, namely genetic factors, environmental factors, and the interaction between them [26]. Improvement of reproductive capacity of local roosters by crossbreeding with exotic stock has been reported

by [6]. The semen characteristics of some breeds of roosters have been described by [2]. The relationship between secondary sexual characters including wattle measurements and fertility was previously confirmed in domestic fowl [20]. Earlier study conducted by [29] reported that the degree of development of the secondary sexual characters including measurements of wattle could also affect the reproductive potential of an individual.

Although both sexes have to cooperate to pass their genes to their progenies and according to the sexual ratio in the flock (1 male: 6-8 females), selecting the roosters according to their semen quality will optimize their reproduction output. There are many local genetic lines had selected according to their plumage colour [14]. Since, no researches found on evaluating semen parameters as well on wattle traits of local roosters in Kurdistan region, this study conducted to evaluate the mentioned traits of roosters belongs to local genetic groups and their crosses with the commercial strain ISA brown. Testing of semen resulted from males of local lines as well from that of their crosses with ISA brown were the best option to determine the quality and make it easy to predict the outcome of the breeding programs.

2 Materials and Methods

The present study conducted at Gardarash station, College of Agriculture, University of Salahaddien in collaboration with Hawler Research Station, Directorate of Agricultural Research–Erbil Ministry of Agriculture during June and July, 2014. A total of 92 roosters aged 6 or 7 months were belongs to lines 1 (Local pure black with brown neck), 3 (Local pure white), 5 (Local pure black) and their main and reciprocal crosses with 2 (ISA brown). The roosters were fed according to Isa brown guide [13], and bred in a clean well ventilated hall and belonged to ordinary management.

The roosters were given preliminary training for two weeks to enable them producing enough semen during actual semen collection exercise. The semen collected with abdominal massage technique into tube maintained at 38-39^{o}C using water bath [17]. Care was taken before semen collection to minimize contamination by faeces and urines. The volume of the semen was recorded to the nearest 0.1 ml. The pH value of the fresh semen was measured by using a colour-scaled pH meter (5.5 – 9.0, Merck) [8 and 16]. Semen colour score (where 1 = watery, 2 = milky and 3 = creamy) were recorded visually. The wave pattern, individual motility, percentages of live/dead and abnormal/normal sperms was evaluated as described by [30]. Eosin/ Nigrosine stains were used on smears for live/dead and abnormal/normal sperm counts. Dead sperms picked up the eosin stain, while live sperms did not. Sperm count was done as described by [11] with a light microscope (x 400). Sperm concentration was calculated by multiplying the number of sperms counted by the multiplying factor of the chambers counted and the dilution factor of the semen. The length and width of wattles were measured using Digital Caliper while the colour of wattles score (Where 1 = red, 2 = redden white and 3 = brown) recorded visually.

General Linear Model (GLM) within the statistical program [24] was used to study the effect of genetic groups and age of roosters on semen parameters as well on wattle traits. The above traits were reanalyzed to study their affects by colour of semen and by colour of wattles. Duncan Multiple Range Test [5] was conducted to diagnosing the significance differences between the means of the levels of each factor. Correlation coefficients among the studied traits were calculated.

3 Results and Discussion

Tables 1 and 2 summarize the results including the differences among genetic groups and age of roosters of sperm parameters. The overall mean of ejaculate volume, sperm concentration, live sperms, abnormal sperms, mass motility, individual motility and pH value were 0.369 (ml), 4.47 (n*109)/ml, 91.35%, 12.01%, 68.40%, 75.60% and 7.12 respectively.

It was found that there were significant differences among genetic groups in ejaculated volume. The greater amounts of ejaculated volume recorded for Isa brown (L2) and Local pure white (L3) and were 0.523 and 0.515 (ml) respectively (Table 1). The variation in semen volume among genetic groups may be explained by the normal physiological processes regulating spermatogenesis and respond to the massage technique during semen collection [27]. Also Peters et al. [22] found that there were differences in strain with respect to semen volume, where the exotic strain of White Leghorn and Giriraja had the highest semen volume probably because they had been selected for high reproductive efficiency. Harris et al. [12] reported that poultry lines with heavier body weights and larger testes produce more sperms during spermatogenesis and thus may yield bigger semen volume. [4 and 8] stated that ejaculation volume, which depends on breed, age, individual, season, light and many other environmental factors, is

averagely 0.7 ml and increase for heavy breeds. Lake and Stewart [18] found the average ejaculate volume for broiler cocks 0.35 ml, for light-weight egg layer 0.15 ml, for medium-weight egg layer 0.2 ml. Iskandar et al. [15] noticed that the volume of semen per ejaculate was 0.26 ml in Arabian males.

In the present study, the sperm concentration differed significantly among genetic groups of roosters. Highest (5.89 n*109/ml) and lowest (3.65 n*109/ml) sperm concentration were measured for L3 and L1 x L2 respectively (Table 1). Hafez [10] stated that the differences in volumes and sperm concentration of the domestic fowl semen depends largely on the relative contribution of the various reproductive glands, the number of sperms that could be obtained from a breed/strain and the extent to which the genetic potentials can be exploited, as well to the fact that the strains are from different genetic back grounds and that the indigenous chickens are fully adapted to this environment [22]. The results of [6], [22] and [28] showed that there were differences in strain with respect to concentration. [4 and 8] observed the sperms concentration decrease for heavy breeds. Tarif et al. [27] noticed that sperm concentration significantly varied among line of roosters and varied from 9.6×10^9 to 7.5×10^9 per ml.

The differences among genetic groups in their live sperm % were not significant, while the differences were significant in their abnormal sperms %. However the crossed roosters (L2 x L5) recorded the highest percentage of live sperms (93.08%), while the crossed roosters (L3 x L2) recorded the lowest percentage (8.19%) of abnormal sperms (Table 1). Banarjee and Katpatal [3] determined the rate of abnormal sperms for White Leghorn, Rhode Island Red, Leghorn x Rhode Island Red and Deshi roosters breeds were 23.3%, 23.2%, 24.2% and 25.9% respectively. According to the research of [15], the percentage of abnormal sperms of fresh semen Arabic males was 14.75. The differences in the abnormal sperms among the collected semen were considered statistically important, while no difference was found in the results of dead-live sperms [28]. Many researchers including [18] indicate that defects depend on rooster semen acrosome from the point of its effect on fertility, are the most important ones than the motility can do in the evaluation process of semen quality. Lastly, Tarif et al. [27] stated that the proportion of live sperms significantly varied from 82.2 to 87.3% among line of roosters, while normal sperms did not differ significantly among line of roosters and varied from 87.2to 90.1%. Nevertheless, the high percentage of live sperms and low percentage of abnormal sperms in the present study was good enough for routine AI in poultry.

Mass and individual motility percentages were significantly affected by genetic groups. The greatest percentages of mass motility 75.75 and 75.71% were found for the genetic groups (L3xL2) and L3 respectively, and individual motility (82.71 %) was measured for L3 (Table 2). The variation in semen motility among genetic groups could be due to the genetic potential of individual line. Also previously, results of [6], [22], [27] and [28] showed that there were differences in strain with respect to motility. By using fresh semen Arabic males, [15] stated that mass movement of sperms ranged from good to very good.

The results in Table (2) showed that there were no significant differences between the genetic groups in pH values of their semen, which ranged between 7.08 and 7.18. The pH values found in this study lays within the range (6.95-7.5) reported earlier in different strains by [4], [8] and [15]. On the other hand, Tuncer et al. [28] stated that the differences in the sperms pH values among the collected semen were considered statistically important.

The effect of age of roosters noticed in this study was not significant on all studied semen parameters except those on percentages of both mass and individual motility where the roosters aged 6 months recorded significant superiority on those aged 7 months by 5.92% and 6.89% in their mass and individual motility respectively (Tables 1 and 2). Earlier studies conducted by [7] and [9] reported that age of rosters had a highly significant effect on sperm concentration, abnormal sperms, and sperm mobility, while, its effect on semen ejaculate volume and semen pH were insignificant.

Person correlation coefficients between the semen quantity and quality traits are also calculated and some of them were negative especially those between abnormal sperm with each of live sperm (-0.49), mass motility (-0.39) and individual motility (-0.31) and the correlations were highly significant. Other correlations were positive and highly significant especially those between mass motility and individual motility (0.95) also those between live sperm and both mass and individual motility (0.35) (Table 3). It was shown that positive and highly significant correlation value obtained between semen volume and sperm concentration (0.29) indicated that the increase in volume might translate to higher sperm concentration. The correlation estimates obtained for semen volume and each of mass and individual motility were low, positive and expected because the more the volume of fluid, the more space is available for sperms to

move easily. Also selecting roosters for higher semen volume could also mean selecting them for high sperm motility. The positive correlation between semen volume and sperm concentration in this study is consistent with the reports of [25]. Previously, Peters, et al. [22] stated that the coefficients between semen quality and quantity traits were generally very low to medium with positive values ranging from 0.01-0.35, and sire strain variation was found to be significant on semen volume, sperm concentration, sperm motility and percentage of active and sluggish sperms.

This study revealed that the overall means of wattle length and width were 55.61 and 46.46 (mm) respectively. The genetic group (local pure black with brown neck) excelled the other pure and crossed genetic groups significantly in their wattle length (71.88 mm) and width (57.75 mm). The effect of age of roosters found to be not significant on both traits (Table 4). The correlation between length and width of the wattles was highly significant (0.79). McGary et al. [19] compare the sizes of the secondary sexual characters including wattle length and wattle width of two strains, and reported that the differences were significant ($P<0.001$). Also noticed that males with greater WL tended to have higher fertility ($r=0.346$; $P<0.01$) and sperm penetration ($r=0.383$).

The results in Table (5) showed that roosters with creamy semen was significantly better than roosters with watery semen in some of semen parameters including ejaculate volume, sperm concentration, abnormal sperms and mass motility, while the roosters with milky semen had moderate values. The differences in other traits including live sperm, individual motility, pH value, wattle length and wattle width were not significant according to colour of semen. Peters et al. [22] reported that the most obvious evaluation of semen quality is colour, which affected significantly by strain. The results of a study conducted by [15] using fresh semen Arabic males, the semen was white and consistency ranged from slightly thickened until thick.

Although the differences in all semen parameters according to the colour of wattles were not significant, the roosters with red wattles recorded higher values of their ejaculate volume (0.382 ml) and sperm concentration (4.75 $n*10^9$/ml), while the roosters with brown wattles had higher values of live sperms (94.31%), mass motility (75.00%), individual motility (80.25%), pH value (7.15), and less abnormal sperms (8.86%). Roosters having brown wattles had significantly greater length and width comparing with those having redden white wattles, while roosters with red wattles had moderate measurements (Table 6).

4 Conclusion

It can be concluded that the semen parameters of local genetic groups and their crosses with Isa brown must be evaluated individually in order to select the best to be used in mating. The outcomes obtained from this study, which was considered as a pre-study, will help to do some further researches on freezing of semen roosters and artificial insemination. The present study provides further evidence suggesting the potential for secondary sexual characters including length and width wattles, to indicate male fertility level.

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Table 1. Means ± S.E. for the factors affecting Ejaculate Volume, Sperm Concentration, Live Sperm and Abnormal Sperm of different genetic groups of roosters:

Effects	No.	Ejaculate Volume (ml)	Sperm Concentration ($n*10^9$)/ml	Live Sperm %	Abnormal Sperm %
Overall mean	92	0.369±0.02	4.47±0.18	91.35±0.44	12.01±0.52
Genetic Group (♂×♀)					
Local Pure Black with Brown Neck (L1)	13	0.340±0.03 b	3.49±0.37 c	90.08±0.74 a	12.73±0.83 abc
L2 x L1	7	0.391±0.04 ab	4.33±0.34 abc	92.81±1.73 a	12.04±1.45 abc
L1 x L2	12	0.378±0.05 ab	3.65±0.30 c	90.80±1.15 a	11.27±1.15 ab
Isa Brown Pure (L2)	7	0.523±0.06 a	5.55±0.79 ab	91.60±0.96 a	10.92±1.40 ab
Local Pure White (L3)	7	0.515±0.10 a	5.89±0.63 a	90.63±1.32 a	13.94±1.51 bc
L2 x L3	6	0.263±0.02 b	4.67±0.94 abc	91.47±1.64 a	9.99±0.95 ab
L3 x L2	8	0.324±0.05 b	5.29±0.65 abc	90.91±2.07 a	8.19±0.67 a
Local Pure Black (L5)	9	0.322±0.05 b	5.00±0.75 abc	89.46±1.84 a	16.84±2.44 c
L2 x L5	12	0.401±0.04 ab	4.36±0.46 abc	93.08±1.05 a	11.90±1.60 abc
L5 x L2	11	0.288±0.04 b	3.99±0.51 bc	92.77±1.50 a	11.47±2.13 ab
Age (month)					
6	59	0.379±0.02 a	4.52±0.24 a	91.89±0.44 a	11.36±0.56 a
7	33	0.353±0.03 a	4.37±0.29 a	90.39±0.91 a	13.17±1.04 a

Means not having a common letter within each column differ significantly (P<0.05).

Table 2. Means ± S.E. for the factors affecting Mass Motility, Individual Motility and pH value of different genetic groups of roosters:

Effects	No.	Mass Motility %	Individual Motility %	pH value
Overall mean	92	68.40±1.46	75.60±1.43	7.12±0.02
Genetic Group (♂×♀)				
Local Pure Black with Brown Neck (L1)	13	70.69±2.45 abc	78.92±2.64 ab	7.14±0.04
L2 x L1	7	68.29±1.90 abc	75.00±1.29 ab	7.10±0.07 a
L1 x L2	12	69.42±3.33 abc	77.42±4.32 ab	7.13±0.05 a
Isa Brown Pure (L2)	7	72.86±1.96 ab	79.71±1.91 ab	7.10±0.07 a
Local Pure White (L3)	7	75.71±5.71 a	82.71±5.71 a	7.09±0.06 a
L2 x L3	6	72.00±5.57 abc	79.00±5.57 ab	7.08±0.08 a
L3 x L2	8	75.75±4.77 a	79.25±4.69 ab	7.11±0.06 a
Local Pure Black (L5)	9	59.67±2.48 bc	67.11±2.78 b	7.11±0.04 a
L2 x L5	12	57.67±5.26 c	66.67±5.15 b	7.18±0.04 a
L5 x L2	11	68.73±6.16 abc	75.09±5.48 ab	7.13±0.05 a
Age (month)				
6	59	70.53±1.76 a	78.07±1.69 a	7.13±0.02 a
7	33	64.61±2.47 b	71.18±2.44 b	7.12±0.03 a

Means not having a common letter within each column differ significantly (P<0.05).

Table 3. Simple correlation coefficient between semen parameters:

Traits	Sperm Concentration	Live Sperm	Abnormal Sperm	Mass Motility	Individual Motility	pH value
Ejaculate Volume	** 0.29	0.12	-0.12	0.11	0.13	** -0.29
Sperm Concentration		-0.03	-0.17	0.15	0.11	-0.11
Live Sperm			** -0.49	** 0.35	** 0.35	-0.18
Abnormal Sperm				** -0.39	** -0.31	0.09
Mass Motility					** 0.95	-0.17
Individual Motility						-0.18

Table 4. Means ± S.E. for the factors affecting wattle traits (mm) of different genetic groups of roosters:

Effects	No.	Wattle Length	Wattle Width
Overall mean	92	55.61±1.10	46.46±0.88
Genetic Group (♂×♀)			
Local Pure Black with Brown Neck (L1)	13	71.88±2.73 a	57.75±2.73 a
L2 x L1	7	58.34±3.14 b	47.34±3.09 b
L1 x L2	12	53.88±1.63 bc	45.85±2.19 b
Isa Brown Pure (L2)	7	55.89±2.91 b	45.97±2.51 b
Local Pure White (L3)	7	44.88±2.89 d	41.85±1.99 b
L2 x L3	6	46.77±2.28 cd	41.17±1.28 b
L3 x L2	8	56.15±2.41 b	47.47±1.82 b
Local Pure Black (L5)	9	54.05±1.35 bc	45.40±1.45 b
L2 x L5	12	54.51±2.42 bc	41.44±1.78 b
L5 x L2	11	50.14±3.01 bcd	44.97±2.61 b
Age (month)			
6	59	55.66±1.50 a	46.17±1.18 a
7	33	55.53±1.53 a	46.98±1.27 a

Means not having a common letter within each column differ significantly ($P<0.05$).

Table 5. Means ± S.E. for colour of semen affect on semen parameters of different local genetic groups of roosters and their crosses with Isa Brown:

Semen Parameters	Colour of Semen		
	Watery	Milky	Creamy
	No. (33)	No. (46)	No. (13)
Ejaculate Volume (ml)	0.308±0.02 b	0.405±0.02 a	0.400±0.07 a
Sperm Concentration ($n*10^9$)/ml	3.37±0.24 b	4.92±0.25 a	5.66±0.39 a
Live Sperm %	91.72±0.62 a	90.99±0.64 a	91.70±1.41 a
Abnormal Sperm %	13.55±0.93 b	11.80±0.70 b	8.82±0.84 a
Mass Motility %	65.64±2.49 b	68.65±2.01 ab	74.54±3.63 a
Individual Motility %	72.76±2.41 a	77.00±1.98 a	77.85±3.92 a
pH value	7.14±0.03 a	7.12±0.02 a	7.10±0.05 a
Wattle Length (mm)	54.06±1.85 a	57.55±1.56 a	52.71±2.75 a
Wattle Width (mm)	45.51±1.46 a	47.28±1.29 a	45.98±2.23 a

Means not having a common letter within each row differ significantly ($P<0.05$).

Table 6. Means ± S.E. for colour of wattles affect on semen parameters of different local genetic Groups of roosters and their crosses with Isa Brown:

Semen Parameters	Colour of Wattles		
	Red	Redden White	Brown
	No. (60)	No. (28)	No. (4)
Ejaculate Volume (ml)	0.382±0.02 a	0.344±0.03 a	0.370±0.08 a
Sperm Concentration ($n*10^9$)/ml	4.75±0.24 a	3.90±0.27 a	4.28±0.76 a
Live Sperm %	90.61±0.56 a	92.54±0.67 a	94.31±1.70 a
Abnormal Sperm %	12.07±0.58 a	12.33±1.16 a	8.86±1.48 a
Mass Motility %	67.93±1.66 a	68.46±3.18 a	75.00±3.14 a
Individual Motility %	75.47±1.69 a	75.21±2.99 a	80.25±3.25 a
pH value	7.12±0.02 a	7.13±0.03 a	7.15±0.09 a
Wattle Length (mm)	56.75±1.26 ab	51.81±2.16 b	65.29±3.84 a
Wattle Width (mm)	46.92±1.08 ab	44.47±1.57 b	53.48±4.12 a

Means not having a common letter within each row differ significantly ($P<0.05$).