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Correlation Between Age of Rooster and Semen Quality in Iranian Indigenous Broiler Breeder Chickens

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Abstract: The goal of present study was to evaluate the effect of Indigenous broiler breeder rooster age on some of the semen quality parameters. For this research, 15 Indigenous broiler breeder Roosters were classified in three treatment groups according their ages: 26, 34 and 45 weeks and evaluation of semen repeated 4 times for each group. Semen was collected from all roosters by abdominal massage method. After dilution, semen samples were examined microscopically for quality parameters (concentration, motility, viability and morphological defect rates of spermatozoa). The difference of spermatozoa concentration between 26 and 34 weeks roosters was not significant; but sperm concentration reduced significantly in 45 weeks roosters. Sperm motility and viability rates reduced significantly with ageing of roosters. Morphological defect rates of spermatozoa. Morphological defect rates of spermatozoa increased significantly with ageing of roosters. Among observed morphological defects, Larger head, smaller head and 180° bent head, increased significantly with ageing of roosters. While, tail knotting and 180° bent tail decreased significantly with ageing of roosters. The differences of other defects between groups were not significant. It is concluded that concentration, motility and viability rates of spermatozoa in indigenous broiler breeder roosters reduced with increasing the age from 26-45 weeks. While in this period, morphological defect rates of spermatozoa increased. Therefore, present study confirmed that semen quality reduced with ageing of indigenous broiler breeder roosters.

Key words: Cock, age, indigenous, sperm quality, morphological defects

INTRODUCTION

Age has an adverse effect on the reproductive success of birds. However, the factors that influence the age-related decline in avian reproduction are still poorly understood. In hens, reduction in fertility occurs by the middle of reproduction period and is characterized, among other things, by a decrease in the Duration of Fertility (DF) (Gumulka and Kapkowska, 2005). For good results in the artificial insemination of chickens, the quality of semen should be ensured (Tabatabaei *et al*., 2009). The importance of semen evaluation in poultry breeding for selecting breeding males or for routinely monitoring their reproductive performance are well recognized (Cheng *et al*., 2002). Studies on turkey showed that the age of the toms affected the sperm quality of both fresh and stored semen. Ageing was accompanied by a reduction in the number of spermatozoa in the ejaculate and in semen volume and by a decrease in motility, viability and membrane integrity of spermatozoa. Consequently, these changes led to a progressive decline in the fertilizing ability of turkey semen and may also affect its preservability during storage (Iaffaldano *et al*., 2007). Fertilizing ability of the semen can be made by motility, live-dead and morphological evaluations. In addition to hereditary traits, live-weight and semen collection techniques are known to affect semen quality. There is a significant positive correlation between body weight and seminal volume, pH and abnormal spermatozoa rate, whereas there is a negative correlation between body weight and motility, sperm concentration and viability of sperm in poultry (Alkan *et al*., 2002). Therefore, for evaluation the age effect on semen quality, the strain and weight of roosters must be equal. So far, no comprehensive studies have been carried out to account for the age effect of Indigenous roosters on sperm quality.

MATERIALS AND METHODS

For this study, 15 Indigenous broiler breeder Roosters with nearly the same weights (3 kg) were used. These roosters were classified in to three treatment

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groups (each group contain 5 cocks) according their ages. Group 1: 26 weeks, Group 2: 34 weeks and group 3: 45 weeks. All roosters were maintained in enclosed houses and were fed with standard breeder diet (2700 kcal Kg^{-1}, 13% protein, 1% calcium, 0.45% phosphorous). All males received 16 h light day^{-1} throughout the experiment. The roosters were trained to give semen 7 days before the collection began. Semen was collected by the abdominal massage method (Lake, 1957). Semen evaluation for each treatment group, repeated 4 times with 3 days intervals. Therefore, total of 60 semen samples were collected and analyzed in this study. Volume of semen was measured when aspirated from the cloacal vent by using insulin syringes, while their needles were exit. Modified Ringer's solution (sodium chloride: 68 g, potassium chloride: 17.33 g, calcium chloride: 6.42 g, magnesium sulphate: 2.5 g, sodium bicarbonate: 24.5 g, distilled water: 10,000 mL) were used as a diluent of semen (Martin, 2004). Glass test tubes were used for semen collection. After exciting of roosters with abdominal massage, the male organ became swell and protrude outwards and downwards and white semen seen in the central furrow of the organ. The semen was milked down by firm finger pressure either side of the vent in to the collecting tube. If the semen, which should be white, was discolored due to contamination by fecal material or blood, it was useless and was eliminated. The glass funnel was used for semen collection in some roosters that projected their semen. Immediately after collection, semen was diluted (1: 200) and examined. The temperature of dilution was about 15°C and this temperature was kept during the examination. The parameters that were analyzed for semen quality included: concentration, motility, viability and morphological defect rates of spermatozoa. For evaluation of motility, one drop of the diluted semen was placed on the slide and covered with glass cover. The sperm motility was estimated by microscopic observation (400x magnification). Motility was expressed as the percentage of motile spermatozoa with moderate to rapid progressive movement. At least 10 microscopic fields were examined for each sample. Sperm concentration was calculated with a hemocytometer slide. A phase-contrast microscope with immersion was used for morphological examinations. Sperm morphology was examined in smears stained with eosin and nigrosin. At each preparation, 300 cells were counted and the percentage of various defects calculated. The proportions of live (eosin-impermeable) and dead (eosin-permeable) spermatozoa in a sample were assessed on the basis of 300 spermatozoa. The morphological defects of acrosome, head, mid-piece, tail and their proportions were evaluated.

Data were analyzed by using statistical program (SPSS, version 16). The data between treatment groups were analyzed by one-way analysis of variance and subsequent Duncan's multiple comparison test (post-hoc). The results were given as Mean±SEM (Petrie and Watson, 2006).

RESULTS AND DISCUSSION

Comparison of semen quality in different ages of Indigenous broiler breeder roosters are presented in Table 1 and comparison of morphological defects rates of acrosome, head, mid-piece and tail of spermatozoa in different ages of roosters are shown in Table 2.

As shown in Table 1, sperm concentration between treatment group 1 (26 weeks) and group 2 (34 weeks) roosters was not significant ($p>0.05$), but it's concentration reduced significantly ($p<0.05$) in group 3 (45 weeks) roosters. Sperm motility and viability rates reduced significantly ($p<0.05$) with ageing of roosters. Morphological defect rates of spermatozoa increased significantly ($p<0.05$) with ageing of roosters. In Table 2, different morphological defects observed in spermatozoa and their rates in all groups were presented. As shown in

Table 1: Comparison of semen quality in different ages of Indigenous broiler breeder roosters

Age of rooster (weeks)	Sperm concentration (10^9 ML^{-1})	Sperm motility (%)	Sperm viability (%)	Morphological defects (%)
26	3.41±0.23^a	85.67±0.69^a	90.64±1.47^a	7.12±0.25^a
34	3.28±0.48^a	80.10±0.46^b	82.30±1.62^b	10.54±0.83^b
45	2.17±0.76^b	74.5±0.27^c	74.11±1.35^c	15.10±0.50^c

Means within a column with different superscripts differ significantly ($p<0.05$)

Table 2: Comparison of morphological defect rates of acrosome, head, mid piece and tail of spermatozoa in different ages of indigenous broiler breeder roosters

	Age of roosters (weeks)		
Morphological defects	26%	34%	45%
Acrosome swelling	3.43±0.12^a	3.54±0.17^a	3.51±0.62^a
Acrosome detachment	10.86±0.10^a	11.14±0.09^a	11.35±0.62^a
Coma shaped acrosome	0.81±0.23^a	0.78±0.85^a	0.83±0.05^a
90° bent head	21.14±0.23^a	23.17±0.10^a	22.84±0.16^a
180° bent head	2.85±0.15^a	8.20±0.14^b	11.12±0.21^c
Head detachment	2.84±0.08^a	2.91±0.07^a	3.05±0.13^a
Knotted head	2.53±0.10^a	2.72±0.06^a	2.80±0.04^a
Larger head	2.05±0.12^a	5.86±0.13^b	9.62±0.21^c
Smaller head	1.35±0.10^a	4.17±0.23^b	6.47±0.32^c
Mid-piece bending	4.66±0.07^a	4.38±0.11^a	4.42±0.06^a
Mid-piece swelling	1.44±0.13^a	1.51±0.08^a	1.49±0.50^a
Knotting at head mid-piece-border	3.14±0.08^a	3.08±0.82^a	3.12±0.67^a
90° bent tail	7.12±0.12^a	7.27±0.05^a	7.43±0.24^a
180° bent tail	19.64±0.20^a	9.23±0.24^b	4.21±0.27^c
Tail knotting	11.42±0.12^a	6.85±0.72^b	3.10±0.43^c
Tail detachment	3.43±0.40^a	3.48±0.12^a	3.53±0.07^a
Curled tail	1.09±0.7^a	1.12±0.04^a	1.04±0.43^a

Means within a row with different superscripts differ significantly ($p<0.05$)

Table 2, 180° bent head, Larger head and smaller head increased significantly ($p<0.05$) with ageing of roosters. Vice versa, 180° bent tail and tail knotting decreased significantly ($p<0.05$) with ageing of roosters. Acrosome swelling, Acrosome detachment, Coma shaped acrosome, 90° bent head, Head detachment, Knotted head, Mid-piece bending, Mid piece swelling, Knotting at head mid-piece-border, 90° bent tail, Tail detachment and Curled tail rates were not significant with ageing of roosters ($p>0.05$).

The purpose of present research was to evaluate the effect of Indigenous broiler breeder rooster age on semen quality parameters. For success in artificial insemination of chickens, the semen quality should be well (Alkan *et al*., 2002). The importance of semen evaluation in poultry breeding for selecting breeding males or for routinely monitoring their reproductive performance are well recognized (Cheng *et al*., 2002). In this study, the difference of spermatozoa concentration between 26 weeks and 34 weeks roosters (3.41±0.23 and 3.28±0.48×10^9 ML^{-1}, respectively) was not significant; but, it's concentration reduced significantly in 45 weeks roosters (2.17±0.76×10^9 ML^{-1}). Kotlowska *et al*. (2005) and Rosato *et al*. (2006) reported that with ageing of tomes, spermatozoa concentration reduced. Also Fuerst-Waltl *et al*. (2006) reported that sperm concentration was lower in higher age bulls. The cause for not reducing the sperm concentration in group 2 roosters was not clear. In present study, sperm motility (85.67±0.69, 80.10±0.46 and 74.5±0.27% for 26, 34 and 45 weeks roosters, respectively) and viability (90.64±1.47, 82.30±1.62 and 74.11±1.35% for 26, 34 and 45 weeks roosters, respectively) rates were reduced with ageing of indigenous roosters. These results were according to findings of Rosato *et al*. (2006) in Turkey that there were negative, correlation between age of toms and motility and viability of spermatozoa. Also in honey bee, Sperm viability decreased significantly with increasing drone age, but motility patterns did not change (Locke and Peng, 2008). In this study, morphological defect rates of spermatozoa (7.12±0.25, 10.54±0.83 and 15.10±0.50% for 26, 34 and 45 weeks roosters, respectively) were increased with ageing of indigenous roosters. On previous study, morphological defects of spermatozoa in indigenous roosters with 28 weeks ages were Acrosome swelling, Acrosome detachment, Coma shaped acrosome, 90° bent head, 180° bent head, Head detachment, Knotted head, Larger head, Smaller head, Mid-piece bending, Mid-piece swelling, Knotting at head mid-piece-border, 90° bent tail, 180° bent tail, Tail knotting, Tail detachment and Curled tail (Tabatabaei *et al*., 2009). In present research, with ageing of roosters, total of these abnormalities observed; but, rates of some abnormalities changed significantly with ageing of roosters. Larger head, smaller head and 180° bent head, increased significantly ($p<0.05$) with ageing of roosters. While, tail knotting and 180° bent tail decreased significantly ($p<0.05$) with ageing of roosters. Other defects were not significant with ageing of roosters ($p>0.05$). Unfortunately, there were not available reports about effect of rooster age on different defects rates of spermatozoa, to compare with the results. In general, present study indicated that reproductive performance reduced with increasing the age of indigenous broiler breeder roosters. In turkey, quality of fresh and stored semen was affected by age (Douard *et al*., 2003). Furthermore, in another study on turkey, the quality of semen decreased during the second part of the reproductive period but in different manner according the strain (Rosato *et al*., 2005). The exact reason for reduce semen quality with ageing is not clear. The peroxidation in PUFAs of the n-3, n-9 and n-6 series due to the ageing may be responsible of the changes of viability, motility and fertilizing ability of spermatozoa (Rosato *et al*., 2006). Also another study indicated that changes in the proportions of the various lipid components in spermatozoa, may be associated with reduce the fertility of male chickens. Free fatty acids and cholesterol esters increase continuously with ageing. Of the various PL (total phospholipids) classes, phosphatidylserine and phosphatidylcholine display a pattern of changes with age positively and negatively, respectively, in relation to the changes of fertility. The proportion of phosphatidylethanolamine significantly decrease by the end of the reproductive period (Cerolini *et al*., 1997).

The study confirm that semen quality reduce with ageing of indigenous broiler breeder roosters. It is also according to findings of Kelso *et al*. (1997), Ciereszko *et al*. (2000) and Iaffaldano *et al*. (2007) in other species and mammalians.

CONCLUSION

Spermatozoa quality can be affected by age of Indigenous broiler breeder roosters. With increasing the age of roosters from 26-45 weeks, concentration, motility and viability rates of spermatozoa reduced, while in this period, morphological defect rates of spermatozoa increased. Therefore, the study confirmed that semen quality reduced with ageing of indigenous broiler breeder roosters.

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