

## Comparison of Semen Quality in Indigenous and Ross Broiler Breeder Roosters

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**Abstract:** The aim of this study, was to evaluate the effect of rooster strain (Indigenous and Ross-308 broiler breeder roosters) on semen quality. Total of 90 semen samples were collected from 15 Ross-308 and 15 Indigenous roosters at 3 days interval. Semen was collected from roosters by abdominal massage. After dilution, semen samples were examined microscopically for quality parameters (sperm concentration, live-dead proportion, motility rate and morphological defects of sperm). The concentration of spermatozoa in Indigenous and Ross broiler breeder roosters were  $3.3 \pm 0.38 \times 10^9$  and  $6.6 \pm 0.53 \times 10^9$   $\text{ML}^{-1}$ , respectively ( $p < 0.01$ ). The motility rate of spermatozoa in ross roosters were  $78 \pm 1.7\%$  and significantly ( $p < 0.01$ ) differed from those of indigenous roosters ( $86.5 \pm 0.78\%$ ). The viability rate in ross males were  $82.25 \pm 2.04\%$  and in indigenous roosters were  $89.63 \pm 1.32\%$  ( $p < 0.01$ ). The percentage of morphological defects in ross and indigenous males were  $10.13 \pm 0.40$  and  $7.00 \pm 0.53\%$ , respectively ( $p < 0.01$ ). Total acrosome, head, mid-piece and tail abnormalities in ross roosters were  $12.55 \pm 0.33$ ,  $25.91 \pm 0.13$ ,  $20.55 \pm 0.07$  and  $41.04 \pm 0.19\%$ , while in Indigenous roosters were  $14.31 \pm 0.18$ ,  $31.4 \pm 0.24$ ,  $10.15 \pm 0.11$  and  $44.1 \pm 0.26\%$ , respectively and the differences were significant ( $p < 0.01$ ). It is concluded that semen quality could be different between genetically improved (Ross-308 strain) and indigenous roosters.

**Key words:** Cock, strain, breeder, sperm quality

### INTRODUCTION

For good results in the artificial insemination of chickens, the quality of semen should be ensured (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 artificial insemination, often the semen of all roosters was mixed together. Because of this pooling, roosters are often not evaluated individually for reproductive potential at the time of collection beyond visual assessment of ejaculate color and volume (Holsberger *et al.*, 1998). 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. The semen collector can affect semen quality by contamination of semen with feces, urine and blood (Alkan *et al.*, 2002). Dilution of chicken semen resulted in a significant decrease in the percentage of dead sperm in stored semen. Sperm motility from both undiluted and

diluted chicken semen is lowest when stored at  $41^\circ\text{C}$ , which is near the body temperature of the hen. This is in contrast to semen stored at 25, 15 or  $5^\circ\text{C}$  (Dunpala *et al.*, 2006). It is very important to inform the proportion of abnormal spermatozoa in a semen sample to determine fertility (Alkan *et al.*, 2002).

The aim of our study, was to evaluate the effect of rooster strain (Ross and Indigenous broiler breeder fowls) on semen quality parameters.

### MATERIALS AND METHODS

The study was carried out, at department of Clinical Science, Faculty of Veterinary Medicine, University of Urmia, from 21 November 2007. Fifteen Ross broiler breeder strain 308 and 15 indigenous cocks with the same ages (30 weeks) and nearly the same weight (3.2 kg) were used at the beginning of this study. The indigenous roosters were obtained from reproduction and breeding center of indigenous broiler breeder in Urmia province in west Azerbaijan of Iran. All roosters were maintained in enclosed houses and were fed with standard breeder diet ( $2700 \text{ kcal kg}^{-1}$ , 13% protein, 1% calcium, 0.45% phosphorous). All males received 16 h light/day throughout the experiment. The roosters were trained to give semen 10 days before the collection began. Semen

was collected by the abdominal massage method (Lake, 1957). Total of 90 semen samples were collected from 15 Ross and 15 Indigenous roosters at 3 days interval and the mean of 3 evaluation of semen quality for each rooster were obtained. 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. 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 (400 × 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. 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 14). Student's t-test was carried out to find the differences in semen quality (concentration, motility, viability and morphological defects) between Ross and Indigenous broiler breeder roosters. The results were given as Mean±SEM.

## RESULTS AND DISCUSSION

Comparison of semen quality in indigenous and Ross-308 broiler breeder roosters are presented in Table 1 and comparison of total morphological defect

rates of acrosome, head, mid-piece and tail of spermatozoa in indigenous and Ross-308 broiler breeder roosters are presented in Table 2. As shown in Table 1, sperm concentration and morphological defects in Ross roosters were significantly higher than those of indigenous, while motility and viability rates in Indigenous roosters were significantly higher than those of Ross ( $p < 0.01$ ). As shown in Table 3, the acrosome abnormalities observed in Ross and Indigenous semen samples were comma shaped acrosome, acrosome detachment and acrosome swelling. The head abnormalities observed in Ross-308 samples were 90 and 180° bent head and head detachment, while in indigenous samples were 90 and 180° bent head, head detachment, knotted head, smaller and larger heads. The mid-piece abnormalities observed in Ross -308 samples were mid-piece knotting and mid-piece detachment and in indigenous samples were mid-piece swelling, mid-piece bending and knotting at head-mid piece border. The tail abnormalities observed in Ross -308 samples were 90 and 180° bent tail, tail knotting and tail detachment, while in

Table 1: Comparison of semen quality in indigenous and ross-308 broiler breeder roosters

Strain	Sperm concentration ( $10^9 \text{ML}^{-1}$ )	Serm motility (%)	Sperm viability (%)	Morphological defects (%)
Ross	6.6±0.53*	78±1.70*	82.25±2.04*	10.13±0.40*
Indigenous	3.3±0.38	86.5±0.78	89.63±1.32	7.00±0.53

\*Significant at  $p < 0.01$

Table 2: Comparison of total morphological defect rates of acrosome, head, mid-piece and tail of spermatozoa in indigenous and ross-308 broiler breeder roosters

Strain	Acrosome (%)	Head (%)	Mid-piece (%)	Tail (%)
Ross	12.55±0.33*	25.91±0.13*	20.55±0.07*	41.04±0.19*
Indigenous	14.31±0.18	31.4±0.24	10.15±0.11	44.1±0.26

\*Significant at  $p < 0.01$

Table 3: The morphological defects in acrosome, head, mid-piece and tail of spermatozoa

Sperm segment	Defects	Ross (%)	Indigenous (%)
Acrosome	Acrosome swelling	1.64±0.07*	3.32±0.05
	Acrosome detachment	3.19±0.06*	10.22±0.09
	Coma shaped	7.72±0.17*	0.78±0.07
Head	90° bent head	21.33±0.12*	20.82±0.10
	180° bent head	0.82±0.11*	2.20±0.07
	Head detachment	3.74±0.09*	2.80±0.06
	Knotted head	0±0*	2.40±0.07
	Larger head	0±0*	1.97±0.10
	Smaller head	0±0*	1.12±0.08
Mid-piece	Mid-piece bending	19.37±0.07*	5.38±0.06
	Mid-piece detachment	1.18±0.04*	0±0
	Mid-piece swelling	0±0*	1.52±0.11
	Knotting at head-mid-piece-border	0±0*	3.25±0.04
Tail	90° bent tail	5.91±0.22*	7.27±0.05
	180° bent tail	19.13±0.14*	21.17±0.20
	Tail knotting	8.40±0.09*	11.31±0.08
	Tail detachment	7.60±0.13*	3.22±0.03
	Curled tail	0±0*	1.10±0.06

\*Differences in defect between strains are significant at  $p < 0.01$

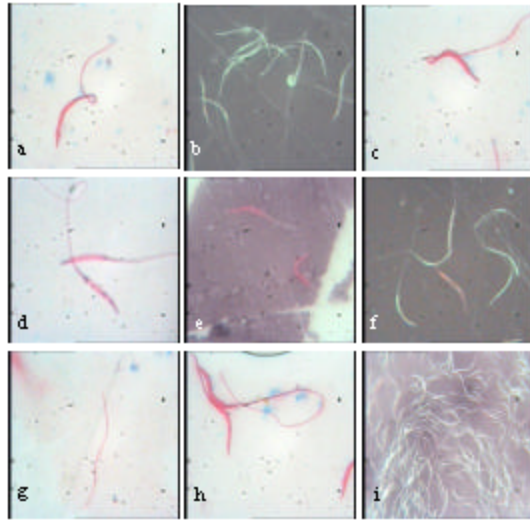


Fig. 1: Some morphological defects of rooster sperm, a: Knotted tail at mid-piece-tail border, b: Mid-piece knotting, c: 180° bent tail at mid-piece-tail border, d: Tail knotting, e: 90° bent head, f: live and dead sperms. The red sperm is died, while whites are live, g: Tail detachment, h: 180° bent tail and i: Population of live sperms

indigenous samples were tail detachment, 90 and 180° bent tail, tail knotting and curled tail. Some of morphological defects are shown in Fig. 1.

The purpose of this study, was to evaluate the effect of rooster strain (Indigenous and Ross-308 broiler breeder fowls) on semen quality parameters. The mean concentration of spermatozoa in Ross broiler breeder roosters was similar to results of Parker and McDaniel (2006). In another study by McDaniel *et al.* (2004), the concentration of spermatozoa was  $7.5 \pm 0.21 \times 10^9 \text{ ML}^{-1}$  in Ross roosters. Gumulka and Kapkowska (2005) reported that average concentration of spermatozoa in broiler breeder roosters at 29-60 weeks of age, ranged from  $4.68 \pm 0.13 \times 10^9$ - $5.53 \pm 0.11 \times 10^9 \text{ ML}^{-1}$ . Unfortunately, there were no written reports about semen analysis of Iranian indigenous roosters to compare our results with them. In one study by Selvan (2007) in Rock fowls, sperm motility was 78.28-85.31% based on the influence of age and dietary protein, calcium and vitamin-E. McDaniel *et al.* (2004) reported that the dead sperm in control group of Ross rooster samples was  $8 \pm 0.7\%$  and Neuman *et al.* (2002) also reported  $10.3 \pm 0.5\%$  dead sperm in control group of white leghorn layer breeder roosters. Dumpala *et al.* (2006) reported  $17.5 \pm 1.29\%$  dead sperm in undiluted neat sperm of Ross broiler breeder roosters. Selvan (2007) reported that the percent of live spermatozoa in Rock fowls was 81.71-87.61% with

different dietary regimen. In this study, the mean morphological defects of spermatozoa in Ross-308 and Indigenous broiler breeder rooster samples were  $10.13 \pm 0.40$  and  $7 \pm 0.53\%$ , respectively. In one study, morphological defect rate in leghorn and New Hampshire roosters were  $5.44 \pm 0.95$  and  $6.76 \pm 0.95\%$ , respectively and abnormalities in acrosome, head, mid-piece and tail region were 6.81, 60.2, 26.98 and 6.01% in leghorn roosters and 0.37, 4.98, 1.69 and 3.17% in New Hampshire cocks, respectively (Alkan *et al.*, 2002). An abnormal spermatozoon of Rock roosters in study of Selvan (2007) was 3.09-5.75% dependent on influence of age, dietary protein, vitamin-E and calcium. Alkan *et al.* (2002) determined  $17 \pm 0.06\%$  morphological defects in spermatozoa samples of American Bronz male turkeys. Prostaglandins are present in turkey seminal plasma and sperm and cyclooxygenase (COX) inhibitors decrease sperm motility (Kennedy *et al.*, 2003). Schlegel *et al.* (1981) found that  $\text{PGF}_2\alpha$  has negatively correlation with human sperm motility. High levels of  $\text{PGF}_2\alpha$  were also found to suppress bull sperm motility and induce membrane damage (Fayed, 1996). Prostaglandins of the E series stimulate sperm motility, whereas  $\text{PGF}_2\alpha$  inhibits motility (Colon *et al.*, 1986; Gottlieb *et al.*, 1988). One reason for different percentage of sperm motility in Ross and Indigenous roosters could be difference in the prostaglandin contents of seminal plasma and spermatozoa that needs to further research. Another reason for difference in sperm motility of Ross and Indigenous roosters could be difference in the proportions of arachidonic and docosahexaenoic acids in the phosphatidylethanolamine fraction of the sperm phospholipids (Cerolini *et al.*, 1977; Darin-Bennett *et al.*, 1974; Kelso *et al.*, 1996; Ravie and Lake, 1985; Surai *et al.*, 1998).

## CONCLUSION

It is concluded that sperm concentrations and morphological defects were significantly higher in Ross roosters than those of Indigenous males ( $p < 0.01$ ), while sperm motility and viability rates were significantly higher in Indigenous roosters than those of Ross males ( $p < 0.01$ ).

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## REFERENCES

- Alkan, S., A. Baran, O.B. Ozdas and M. Evecen, 2002. Morphological defects in turkey semen. *Turk. J. Vet. Anim. Sci.*, 26: 1087-1092. <http://journals.tubitak.gov.tr/veterinary/issues/vet-02-26-5/vet-26-5-16-0107-40.pdf>.
- Cerolini, S., K.A. Kelso, R.C. Noble, B.K. Speake, F. Pizzi and L.G. Cavalchini, 1997. Relationship between spermatozoa lipid composition and fertility during aging of chickens. *Biol. Reprod.*, 57: 976-980. PMID: 9227357.
- Cheng, F.P., T.J. Guo, J.T. Wu, T.E. Lin, P.J.F. Ursem, B. Colenbrander and H.P. Fung, 2002. Annual variation in semen characteristics of pigeons (*Columba livia*). *Poult. Sci.*, 81: 1050-1056. PMID: 12162344.
- Colon, J., F. Ginsburg, J. Lessing, C. Schoenfeld, L.T. Goldsmith, R.D. Amelar, L. Dublin and G. Weiss, 1986. The effect of relaxin and prostaglandin E2 on the motility of human spermatozoa. *Fertil. Steril.*, 46: 1133-1139. PMID: 3536606.
- Darin-Bennett, A., A. Poult and I.G. White, 1974. The Phospholipids and phospholipids-bound fatty acids and aldehydes of dog and fowl spermatozoa. *Reprod. Fertil.*, 471-474. PMID: 4452987.
- Dumpala, P.R., H.M. Parker and C.D. McDaniel, 2006. The effect of semen storage temperature and diluent type on the sperm quality index of broiler breeder semen. *Int. J. Poult. Sci.*, 5 (9): 838-845. <http://www.pjbs.org/ijps/fin707.pdf>.
- Fayed, A.H., 1996. Effect of PGF<sub>2</sub> $\alpha$  and methylxanthins on enzymatic release of bull epididimal spermatozoa *in vitro*. *Contraception*, 53: 181-184. PMID: 8689884.
- Gottlieb, C., K. Svanborg, P. Eneroth and M. Bygdeman, 1988. Effect of prostaglandins on human sperm function and seminal adenosine triphosphate content. *Fertil. Steril.*, 49: 322-327. PMID: 3338588.
- Gumulka, M. and E. Kapkowska, 2005. Age effect of broiler breeders on fertility and sperm penetration of the perivitelline layer of the ovum. *Anim. Reprod. Sci.*, 90: 135-148. PMID: 16257603.
- Holsberger, D.R., A.M., Donghue, D.P. Froman and M.A. Ottinger, 1998. Assessment of ejaculate quality and sperm characteristics in turkeys: Sperm mobility phenotype is independent of time. *Poult. Sci.*, 77: 1711-1717. PMID: 9835348.
- Kelso, K.A., S. Cerolini, R.C. Noble, N.H.C. Sparks and B.K. Speake, 1996. Lipid and antioxidant changes in semen of broiler fowl from 25-60 weeks of age. *Reprod. Fertil.*, 106: 201-206. PMID: 8699402.
- Kennedy, J.H., N. Korn and R.J. Thurston, 2003. Prostaglandin levels in seminal plasma and sperm extracts of the domestic turkey and the effects of cyclooxygenase. *Reprod. Biol. Endocrin.*, 1: 74. PMID: 14613550.
- Lake, P.E., 1957. The male reproductive tract the fowl. *J. Anat.*, 91: 116-129. PMID: 13405819.
- Martin, R.D., 2004. Artificial insemination of poultry. *Poultry Essays*, Essay 14, Bernal Publishing. [www.bernalpublishing.com/poultry/essays/essay14.shtml](http://www.bernalpublishing.com/poultry/essays/essay14.shtml).
- McDaniel, C.D., J.E. Hood and H.M. Parker, 2004. An attempt at alleviating heat stress infertility in male broiler breeder chickens with dietary ascorbic acid. *International J. Poult. Sci.*, 3 (9): 593-602. <http://www.pjbs.org/ijps/fin271.pdf>.
- Neuman, S.L., T.L. Lin and P.Y. Hester, 2002. The effect of dietary carnitine on semen traits of white Leghorn roosters. *Poult. Sci.*, 81: 495-503. PMID: 11989749.
- Parker, H.M. and C.D. McDaniel, 2006. The immediate impact of semen diluent and rate of dilution on the sperm quality index, ATP utilization, Gas exchange and Ionic balance of broiler breeder sperm. *Poult. Sci.*, 85: 106-116. PMID: 16493953.
- Ravie, O. and P.E. Lake, 1985. The phospholipids-bound fatty acids of fowl and turkey spermatozoa. *Anim. Reprod. Sci.*, 9: 189-192. DOI: 10.1016/0378-4320(85)90023-5.
- Schlegel, W., S. Rotermund, G. Farber and E. Nieschlag, 1981. The influence of Prostaglandins on sperm mobility. *Prostaglandins*, 21: 87-99. PMID: 7208955.
- Selvan, S.T., 2007. Influence of dietary protein, calcium and vitamin-E on the semen quality in broiler breeder males. *Tamilnadu J. Vet. Anim. Sci.*, 3 (2): 60-64. [www.tanuvastn.nic.in/tanjvas/vol3\(2\)/60-64.pdf](http://www.tanuvastn.nic.in/tanjvas/vol3(2)/60-64.pdf).
- Surai, P.F., E. Blesbois, I. Grasseau, T. Chalah, J.P. Brillard, G.J. Whishart, S. Cerolini and N.H.C. Sparks, 1998. Fatty acid composition, glutathione peroxidase and superoxide dismutase activity and total antioxidant activity of avian semen. *Comp. Biochem. Physiol. Part B*, 120: 527-533. DOI: 10.1016/S0305-0491(98)10039-1.