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Semen quality characteristics of Aseel, Kadaknath and native chicken of Kerala#

D. Akshay[1*], S. Harikrishnan[2], Hiron M. Harshan[3], P. Anitha[4] and P.G. Vineetha[5]
Department of Poultry Science
College of Veterinary and Animal Sciences, Mannuthy, Thrissur – 680 651
Kerala Veterinary and Animal Sciences University
Kerala, India

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Abstract

The present study was conducted to compare the microscopic and macroscopic parameters of semen of three Indian indigenous chicken breeds viz. Aseel, Kadaknath and native chicken of Kerala. The semen volume in native chicken of Kerala was significantly higher compared to that of Aseel and Kadaknath roosters. Significantly ($p<0.05$) higher appearance score was observed in native chicken of Kerala and Kadaknath roosters compared to that of Aseel roosters. The pH of semen was found slightly alkaline in all the three breeds. Most of the semen samples were white in colour. Most of the semen samples were thick in Kadaknath roosters, thick and medium thick in native chicken of Kerala and thick, medium thick and watery in Aseel roosters. The major contaminants of semen observed were faeces, blood and uric acid crystals. Significantly ($p<0.01$) higher mass activity score and initial progressive motility was measured in native chicken of Kerala compared to Aseel roosters and the values of Kadaknath roosters were comparable to both. There was no significant difference in sperm concentration and sperm abnormality among three indigenous breeds. The sperm viability percentage was significantly ($p<0.05$) higher in native chicken of Kerala compared to Aseel and Kadaknath roosters. The characteristics of fresh semen samples collected from all the three breeds revealed that all three were fit for artificial insemination.

Keywords: *Semen, Aseel, Kadaknath, native chicken, Kerala, artificial insemination*

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1. *MVSc. Scholar, Department of Poultry Science, CVAS, Mannuthy*
2. *Assistant Professor and special officer, CASM Thiruvazhamkunnu*
3. *Associate professor, Department of Animal reproduction Gynaecology and Obstetrics*
4. *Professor and Head, Department of Poultry Science, CVAS, Mannuthy*
5. *Assistant Professor, CASM Thiruvazhamkunnu*

**Corresponding author: akshayraghu15@gmail.com, Ph. 9656578057*

The contribution of backyard poultry production comprising of native chicken is declining over the past years and the loss of genetic diversity is a major concern as they are being replaced with high yielding varieties for improving the productivity. However, the hardiness, disease resistance and tolerance for heat stress of native chicken and increasing demand for their meat and eggs command attention. Native chicken is a major component in well-balanced farming system that provides rural households with animal protein, emergency cash flow, socio-cultural life and women's empowerment. Out of 19 recognised indigenous poultry breeds in India, Aseel and Kadaknath are the prominent breeds which gained much attention of the public. Meanwhile, it is essential to study the genetic and breeding potential of non-descriptive chicken like native chicken of Kerala.

The poultry industry has gradually achieved intensive battery rearing of breeding roosters due to increase in AI. Semen traits were ignored in comparison to other economically important traits in both commercial and local breeds. Considerable genetic improvement has been achieved for growth traits in broilers during the past decades. This has been accompanied by a steady decrease in the reproductive quality of the roosters. This may be due to low fertility caused by poor semen quality. Decreasing the number and increasing the quality of breeding males in local breeds should be achieved by improving semen quality of the roosters. Semen quality is also an important factor determining the breeding value of males, because it influences the fertilisation rate of the eggs for hatching as well as the reproductive efficiency of their progeny (Mavi *et al.,* 2017). Many indicators are currently used to evaluate semen quality including ejaculate volume, semen colour, sperm density, sperm motility, sperm viability and sperm deformity (Getachew, 2016). Functional tests *i.e*., membrane and acrosome integrity can also be included to assess the quality of semen, as these are more related to fertility.

In breeding system, male birds are as important as female birds. Assessment and establishment of semen quality parameters of the native chicken breeds are essential for selection and breeding programmes. The selection of males based on semen evaluation along with quantitative traits will improve fertility in the population and helps in defining their breeding value. The semen quality indices are also required for calculation of extension rates during preservation of semen and artificial insemination (AI). The growing prevalence of artificial insemination (AI) in the poultry sector highlights the importance of distributing high-quality sperm.

The aim of the study was to compare the semen attributes like pH, colour of semen, appearance score, mass motility score, initial progressive motility, sperm abnormality and concentration in Aseel, Kadaknath and native chicken of Kerala chickens raised under intensive management system.

Materials and methods

Twenty-four adult roosters of 30 weeks of age (eight each from Aseel, Kadaknath and native chicken of Kerala) procured from Kerala Veterinary and Animal Sciences, University Poultry and Duck farm, Mannuthy were utilised for the study. The roosters were maintained in individual cages with the dimension of 39 cm × 46 cm × 44 cm. All birds were provided with male breeder diet containing 16 per cent crude protein, 2600 kcal metabolisable energy /kg (BIS, 2007) and *ad-libitum* water.

The semen was collected from roosters at four days interval by abdominal massage method (Lake *et al.,* 1985) and the semen volume was measured immediately after collection. Based on colour, the semen samples were classified as yellowish white, white and chalky white and the semen samples were categorised in to watery, medium thick, thick and very viscous based on consistency. The appearance of semen was scored from 1 to 5 by visual examination as described by McDaniel and Craig (1959). The pH of fresh semen was measured using narrow range pH paper [Merck pH indicator paper with colour scale (pH 6.5-9)]. The semen samples were examined for the presence of possible contaminants like blood, faecal matter or uric acid crystals by visual examination and the per cents of samples with the different contaminants

were calculated (Stella, 2011).

A drop of undiluted semen was taken on a dry, clean, grease free glass slide with a micropipette and the mass activity was estimated on the basis of swirling oscillation of semen and score as per Wheeler and Andrews (1943), where '0' (zero) score indicated no activity and '5' (five) was the highest possible score. The mean progressive motility of sperms in per cent was calculated after placing a drop of undiluted semen over dry, clean, grease free glass slide with a cover slip and observing quickly under 40× objective of the microscope (Stella, 2011).

The density of spermatozoa was estimated as per Raghavendra *et al.* (2022) and Sutiyono *et al.* (2021) by using Neubauer's hemocytometer. Fresh neat semen was filled into the RBC pipette up to '0.5' mark, followed by eosin-formal-saline up to '1.01' mark. After thorough mixing, two drops of semen-solution mixture were discarded. A cover glass was placed over counting chamber of 'Neubauer' hemocytometer and a drop of diluted semen was added to the counting chamber. The sperms were allowed to settle for one to two minutes. The counting 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/mm^3 or $\times 10^9$/ ml of semen. The mean concentration was calculated for each breed separately.

The sperm viability was estimated as per Raghavendra *et al.* (2022) in which one drop of two per cent eosin and four drops of 10 per cent nigrosine were mixed on a clean grease free glass slide. One drop of semen sample was added to it and mixed gently and uniformly and from this mixture, a moderately thick smear was made on a clean, grease free glass slide, air-dried and examined under oil immersion objective of the microscope. About 300 spermatozoa (about 50 spermatozoa each in six random microscopic fields) were counted to estimate sperm viability. Unstained spermatozoa were categorised as live and stained or partially stained spermatozoa were counted as dead.

The smear prepared for sperm viability was used to estimate sperm abnormality (Raghavendra *et al.,* 2022). A total of 200 spermatozoa were counted under oil-immersion objective of the microscope and estimated the abnormal sperms and the abnormality percentage was calculated.

The data obtained from three indigenous breeds were assessed statistically by one-way ANOVA and Friedmann test using SPSS version 24.0.

Results and discussion

Macroscopic parameters

The mean semen volume was significantly ($P<0.05$) higher in native chicken of Kerala compared to Aseel and Kadaknath roosters (Table 1). The semen volume observed for Aseel and Kadaknath in the present study was lower than that reported by Haunshi *et al*. (2011) and Mavi *et al.* (2017), but higher than that observed by Biswas *et al.* (2009) and Mohan *et al.* (2011). However, a more or less similar volume of semen in Kadaknath birds was reported by Mohan *et al.* (2011). The semen volume of native chicken of Kerala obtained in the present study was in accordance with the findings of Sutiyono *et al.* (2021) in native roosters of Indonesia. However, the volume of semen in all the three breeds was in the normal range and in accordance with the findings of Lake and Stewart, (1978) and Bah *et al.* (2001).

The appearance score of semen was significantly ($p<0.05$) higher in native chicken of Kerala and Kadaknath roosters, compared to Aseel rooster (Table 1). The findings on appearance score of native chicken of Kerala and Kadaknath are in close agreement with Churchil *et al.* (2019) who reported a score of 3.62 and 3.68 in IWN and IWP strains of White Leghorn roosters, respectively and Inyawilert *et al.* (2019) who reported an appearance score

of 3.80 in Thai native roosters. Contrary to the present findings, Haunshi *et al.* (2011) reported a higher appearance score of 4.43 and 4.05 in Aseel and Kadaknath roosters. These variations may be due to the presence or absence of excess seminal fluid or due to individual variation among the roosters. The appearance score in general indicates the quality of semen and could be used to determine the rate of extension. The appearance score also depends on the concentration of spermatozoa and volume of the seminal fluid.

The mean pH of semen in all the three breeds were slightly alkaline and ranged from 7.24 – 7.25 (Table 1). These findings are in agreement with the findings of Mussa *et al.* (2021) in Thai Native chicken and Rhode Island red roosters. Also, a similar pH was reported by Chauhan *et al.* (2017) in Gramapriya roosters and by Siudzinska and Lukaszewicz, (2008) in Italian Partridge and White Crested Black Polish roosters. According to Latif *et al.* (2005) semen pH of chicken was slightly alkaline in nature (7.0 – 7.4) and varies between breeds. Alkaline pH was associated with higher sperm motility, fertilising ability and plasma membrane integrity. The pH of the semen measured in all the three breeds in the present study was within the range typically reported for chicken semen.

The major semen colours in Aseel, Kadaknath and roosters of native chicken of Kerala (Table 2) were white (80.95, 90.48 and 91.67 per cent, respectively), chalky white (4.76, 9.52 and 4.17 per cent, respectively) and yellowish white (14.29, 0.00 and 4.17 per cent, respectively). The current findings were in agreement with Peters *et al.* (2008) who reported creamy white colour semen for various chicken breeds such as Giriraja, Frizzled feather, Naked neck, Nera black and normal feathered roosters.

The consistency of raw semen in Aseel, Kadaknath and roosters of native chicken of Kerala (Table 3) was viscous (0.00, 0.00 and 4.17 per cent, respectively), thick (57.14, 80.95 and 70.83 per cent, respectively), medium thick (19.05, 9.52 and 20.83 per cent, respectively) and watery (23.81, 9.52 and 4.17 per cent, respectively). According to Peters *et al.* (2008), the consistency of domestic poultry semen ranged from a dense, opaque suspension to a liquid fluid. The variations from the normal white colour were due to the contamination or due to the presence of excess transparent fluid. Majority of the semen samples collected in the present study were white in colour with thick consistency and hence it was considered of good quality.

The semen contaminants observed in Aseel, Kadaknath and native chicken of Kerala are presented in Table 4. The major semen contaminant in all three breeds was faeces. In Aseel roosters, 4.76 per cent of the ejaculates were contaminated with faeces but none had blood or uric acid crystals as contaminants. The predominant semen contaminants in Kadaknath roosters were faeces (9.52 per cent) followed by 4.76 per cent each of blood and uric acid crystals. In native chicken of Kerala, 4.17 per cent each of the ejaculates were contaminated with faeces and blood. However, uric acid crystals were absent. In the present study, Aseel roosters yielded more clear semen (95.24 per cent) followed by native chicken of Kerala (91.67 per cent) and the same was lowest in Kadaknath (80.95 per cent) roosters. The presence of blood in semen was indicated by a brownish red pigment or reddish discolouration and blood contamination might be due to the excess force applied or injury during the semen collection as per several authors (Etches, 1996; Tabatabaei *et al*., 2009; Getachew, 2016). The semen contaminants reduce the fertilising capacity of spermatozoa and hence the contaminated semen with faeces, blood and urate crystals should be eliminated from further evaluation or insemination.

Microscopic Parameters

The mean mass activity score and progressive motility of sperms were significantly ($p<0.05$) higher in native chicken of Kerala compared to Aseel roosters and the value of Kadaknath rooster was comparable to both (Table 5). The mass activity score observed in the present study was in agreement with the findings of Tarif *et al.* (2013) in Sasso and synthetic roosters and Mussa *et al.* (2021) in Thai native and commercial roosters. Contrary

Table 1. Macroscopic semen quality attributes of Aseel, Kadaknath and native chicken of Kerala

Parameters	Breeds			
	Aseel (n=21)	Kadaknath (n=21)	Native chicken of Kerala (n=24)	p-value
Semen volume (mL)	$0.27^{b} \pm 0.03$	$0.26^{b} \pm 0.03$	$0.38^{a} \pm 0.04$	0.02*
Appearance Score	$2.81^{b} \pm 0.28$	$3.71^{a} \pm 0.14$	$3.75^{a} \pm 0.12$	0.02*
Semen pH	7.24 ± 0.11	7.25 ± 0.15	7.24 ± 0.11	0.96^{ns}

Mean values bearing different superscripts in the same row differ significantly

*significant (p<0.05), ns-non-significant

Table 2. Frequency distribution of semen colour in Aseel, Kadaknath and native chicken of Kerala, per cent

Breed	n	Yellowish white	Chalky white	White
Aseel	21	14.29	4.76	80.95
Kadaknath	21	0	9.52	90.48
Native chicken of Kerala	24	4.17	4.17	91.67

Table 3. Frequency distribution of semen consistency in Aseel, Kadaknath and native chicken of Kerala, per cent

Breed	n	Watery	Medium thick	Thick	Very viscous
Aseel	21	23.81	19.05	57.14	0
Kadaknath	21	9.52	9.52	80.95	0
Native chicken of Kerala	24	4.17	20.83	70.83	4.17

Table 4. Frequency distribution of semen contamination in Aseel, Kadaknath and native chicken of Kerala roosters, per cent

Breed	n	Contaminants			Clear semen
		Faeces	Blood	Uric acid crystals	
Aseel	21	4.76	0	0	95.24
Kadaknath	21	9.52	4.76	4.76	80.95
Native chicken of Kerala	24	4.17	4.17	0	91.67

to the present findings, lower mass activity score was observed in native and improved chicken varieties (Haunshi *et al*., 2010), in Aseel × RIR and White Plymouth rock roosters (Tarif *et al*., 2013) and in Gramapriya roosters (Chauhan *et al.* 2017). The initial motility of sperms of Kadaknath breed observed in the present study was in agreement with the findings of Biswas *et al.* (2009), Haunshi *et al.* (2011) and Mohan *et al*. (2011). However, the motility obtained for Aseel breeds was slightly lower than that of the findings of Haunshi *et al.* (2011), Mohan *et al*. (2011), Jabbar *et al*. (2015) and Yousaf e*t al.* (2016) but was in par with Mavi *et al.* (2017). The initial progressive motility value for native chicken of Kerala in the present study was significantly (p<0.01) better and was comparable with the findings of Al-Daraji *et al.* (2002) in Barred Plymouth Rock and New Hampshire, Churchil *et al.* (2019) in IWP strain of White Leghorn, Mussa *et al.* (2021) in RIR and Thai native roosters. The sperm motility is primarily the determinant of fertility and serves as an indicator for both the quantity and quality of live spermatozoa in a semen sample. The present study revealed that the mass activity score and the initial sperm motility of native chicken of Kerala were good, followed by Kadaknath roosters. The lower initial sperm motility observed in Aseel might be due to the proportionately higher watery consistency of semen recorded in the present study. Though the motility estimation could be used to detect

Table 5. Microscopic semen quality attributes of Aseel, Kadaknath and native chicken of Kerala

Parameters	Breeds			p-value
	Aseel (n=21)	Kadaknath (n=21)	Native chicken of Kerala (n=24)	
Mass activity score	$4.14^{b} \pm 0.21$	$4.33^{ab} \pm 0.17$	$4.75^{a} \pm 0.09$	0.042*
Initial progressive motility (%)	$77.19^{b} \pm 3.79$	$82.05^{ab} \pm 2.91$	$89.71^{a} \pm 1.20$	0.006**
Sperm density (billions/mL)	4.41 ± 0.47	4.15 ± 0.16	4.03 ± 0.19	0.66^{ns}
Sperm viability (%)	$84.40^{b} \pm 2.14$	$87.26^{b} \pm 1.23$	$91.66^{a} \pm 0.64$	0.002**
Sperm abnormality (%)	9.60 ± 1.06	9.57 ± 0.51	7.91 ± 0.52	0.16^{ns}

Mean values bearing different superscripts in the same row differ significantly

** highly significant (p<0.01), * significant (p<0.05), ns-non-significant

gross differences in semen quality, sperm motility is a key factor affecting fertility among chickens (Donoghue *et al.,* 1998).

The mean sperm density in Aseel, Kadaknath and native chicken of Kerala was 4.41, 4.15 and 4.03 billion/mL, respectively without any statistical difference (Table 5). The sperm density observed in Kadaknath roosters was in agreement with the findings of Biswas *et al*. (2009) and Mohan *et al.* (2011) and that observed in Aseel was in agreement with Mohan *et al*. (2011). Contrary to the present findings, Haunshi *et al.* (2011) documented higher sperm density in Aseel and Kadaknath roosters while Jabbar *et al.* (2015) and Yousaf *et al*. (2016) measured lower sperm density in Aseel roosters. The present finding in native chicken of Kerala was similar with the finding of Sutiyono *et al.* (2021) in native roosters of Indonesia. Tabatabaei *et al.* (2010), Rakha *et al*. (2015) and Mussa *et al*. (2021) measured a lower sperm concentration in indigenous broilers, red jungle fowl and Thai native roosters. Similarly, Al-Daraji *et al.* (2002), Tuncer *et al.* (2006), Santiago-Moreno *et al.* (2009), Yousaf *et al.* (2016), Chauhan *et al.* (2017) and Churchil *et al.* (2019) also reported lower sperm concentration in various chicken breeds. Assessing sperm concentration is critical towards estimating the number of sperm doses and for evaluating the quality of semen. The domestic cockerel semen had an average sperm density of 3-7 billon sperm / mL (Gordon, 2005; Hafez and Hafez, 2013). Hence it can be concluded that the sperm density observed for all the three breeds under the present study are in normal range.

The sperm viability was significantly (p<0.05) higher in native chicken of Kerala (91.66 per cent) compared to Aseel (84.40 per cent) and Kadaknath (87.26 per cent) roosters (Table 5). The per cents of viable sperm observed in Aseel and Kadakanth were in close agreement with the findings of Mohan *et al.* (2011). Contrary to the findings, Jabbar *et al.* (2015), Yousaf *et al.* (2016) and Mavi *et al*. (2017) reported lower sperm viability in Aseel roosters while, higher viable sperms were observed in both indigenous breeds by Haunshi *et al.* (2011). The mean sperm viability observed in native chicken of Kerala was in accordance with the findings of Mussa *et al.* (2021) in Thai native roosters. According to Shukla (2011), an average of 70 per cent live spermatozoa is needed for successful AI. As the per cent of live sperms in the three breeds under study was well above 70, it could be concluded that the semen from all the three indigenous breeds under study are of good quality.

The proportion of abnormal sperms in Aseel, Kadaknath and native chicken of Kerala were 9.60, 9.57 and 7.91 per cent, respectively, without any statistical difference. The proportion of sperm abnormalities observed in Aseel roosters are in close agreement with the findings of Yousaf *et al.* (2016). Contrary to the present findings, Haunshi *et al.* (2011) and Mohan *et al*. (2011) documented a relatively lower number of abnormal sperms in Aseel and Kadaknath and Biswas *et al.* (2009) in Kadaknath roosters. The per cent sperm abnormality obtained for native chicken of Kerala in the present study is comparable with the findings of Tuncer *et al.* (2006), Tabatabaei *et al.* (2009) and Sun

et al. (2021) in various native chicken breeds. Ramamurthy *et al.* (1986), Ramamurthy *et al.* (1998), Abaza *et al.* (2003) and Haunshi *et al.* (2011) reported higher abnormal sperm in roosters of different chicken breeds than the present findings. Getachew (2016) opined that good quality semen ejaculate should contain 85-90 per cent morphologically normal sperm hence, the semen collected from three indigenous breeds is within the normal range.

Conclusion

The present study revealed that the semen attributes in terms of semen volume, semen appearance score, mass activity score, initial motility, and sperm viability were higher in native chicken of Kerala compared to other indigenous breeds *viz.* Aseel and Kadaknath. The sperm density and per cent of abnormal sperms were comparable in all the three indigenous breeds. It was also concluded that the characteristics of fresh semen samples collected from all the three breeds were fit for artificial insemination.

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Conflict of interest

The authors declare that they have no conflict of interest

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