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Comparison of Sperm Attributes in two Indigenous Layer Breeds and their Relationship with Fertility

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ABSTRACT

To compare Aseel and Kadaknath breeds, 50 roosters per breed were evaluated for colour, volume, pH, motility, viability, membrane and acrosome integrity. Ten roosters / breed were also evaluated for fertility rate. There was no significant difference in colour, pH, volume and motility of semen among both breeds. However, percentage of viable spermatozoa and spermatozoa with intact membrane was non-significantly ($P > 0.05$) higher in ejaculates of Kadaknath (82.04 ± 4.47, 44.17 ± 3.96 than Aseel (78.35 ± 5.37, 30.98 ± 9.02) roosters. Significant positive correlation was observed among different sperm attributes, but correlation between motility / viability and membrane integrity / acrosome damage was higher in Aseel breed (0.86 and 0.60) as compared to Kadaknath (0.40 and 0.05). A very weak positive correlation was also observed between fertility rate and sperm traits in both breeds. Although, viability, membrane integrity and acrosome integrity were higher in semen of Kadaknath than Aseel breed, but both breed were almost equivalent in their fertility. Selection of roosters on the basis of sperm attributes may be useful in AI practices aimed at genetic improvement for breeds.

Keywords: Semen evaluation, Kadaknath, Aseel, fertility, relationship

The chick's reproductive potential is assessed from the semen quality. The semen quality and quantity are affected by the breed and strain of chicken (Peters *et al.*, 2008; Prieto *et al.,* 2011; Shanmugam *et al.,* 2012). Genetic selection for higher egg production affects semen quality (Shanmugam *et al.*, 2013). Semen is evaluated by macroscopic (color, consistency, appearance score and volume) and microscopic (concentration, initial motility, abnormal sperms and percent dead sperms) methods in Roosters (Peter *et al.*, 2004 and Moce and Graham, 2008). Initial motility is considered as the single reliable characteristic of semen for identifying the fertility of roosters, but other attributes like viability, membrane and acrosome integrity also contributes to the fertility of semen. The semen quality parameters reported for White Leg horn (Tarif *et al.*, 2013), Plymouth Rock (Elagib *et al.,* 2012), Rhode Island Red (RIR) (Kabir *et al.,* 2007) and indigenous Roosters (Hu *et al.*, 2013), Synthetic, White Rock and Assel RIR lines (Abu Md *et al.,* 2013), Rhode Island Red roostes (Churchil *et al.*, 2014), Lingnan, Bangkok, Kedu and Arabic (Almahdi et al, 2014), Nigerian indigenous (NI) and Isa White (IW) chickens Mkpughe *et al.*, 2015), Vanaraja and Indigenous Chicken of Assam (Das *et al.*, 2015), local Iraq breeds and ISA brown crosses (Hermiz *et al.*, 2016) demonstrated high degree of variation.

Aseel and Kadaknath are two important indigenous chicken breeds of India. Aseel is a meat type game bird with brown feathers and long

shanks. Kadaknath is a dual purpose bird with fibromelanosis, non-inhibitor dermal melanin and slow feathering characters. Consumers and farmers are interested in native gerplasm due to unique hardness of these breeds, their ability to thrive under adverse climate conditions and desirable taste and flavor of eggs and meat. There is a significant demand for the products of native chickens like Kadaknath and Aseel. Literature reveals that a considerable variation exists in the production traits of these native chicken breeds. It was pointed out that investigations are still required to establish baseline values for production parameters of these breeds and characterize their performance. These breeds differ on various growth, production, egg and semen quality traits (Huanshi *et al.*, 2010). Aseel breed with significantly ($p \leq 0.001$) higher body weights had significantly higher semen volume ($p \leq 0.05$) and sperm motility ($p \leq 0.01$) but had lower seminal plasma cholesterol ($p \leq 0.05$) as compared to Kadaknath. There is scarcity of information on semen attributes, inter-relationship among the sperm parameters and with fertility of Aseel and Kadaknath breed Roosters. This study was aimed to evaluate the roostes semen quality parameters, their inter-relationship and relationship with fertility of Aseel and Kadaknath breed.

MATERIALS AND METHODS

Study area and period

The experiment was conducted at Poultry Farm, Directorate of Livestock Farms and Reproductive Biology Lab, Department of Veterinary Gynaecology and Obstetrics, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, India during the period from December, 2016 to February, 2017.

Experimental Roosters: Aseel and Kadaknath (32-44 weeks old)

Housing and feeding of the Roosters: All the roosters were kept in individual cages and were given poultry feed and water ad libitum.

Collection of semen: Single ejaculate of semen was collected from each of roosters twice a week by abdominal massage method. Three replicates of semen samples were collected and evaluated from each of the Roosters.

Semen Evaluation roosters: Three ejaculates of each of 50 Aseel and Kadaknath breeds were evaluated for macroscopic (colour, volume, pH) and microscopic (Concentration, motility, viability, membrane integrity and acrosome integrity) attributes.

Macroscopic: Colour and volume were observed after collecting the semen in a 5.0 ml graduated centrifuge tube. pH was noted by putting a drop of semen on a pH strip kept on a glass slide and was matched with pH chart.

Microscopic

Individual Motility: Semen was diluted with phosphate buffered saline in a ratio of 1:4, mixed, incubated at 37ºC. A drop of diluted semen was placed on a pre-warmed slide, covered with a cover slip and observed under a light binocular microscope at 400X. About 200 motile and im-motile spermatozoa were counted in different fields using attached CCTV system and percentage of motile spermatozoa was calculated.

Viability (Blom, 1977): About 10 µl of diluted semen was thoroughly mixed with 10 µl of eosin-nigrosin stain at 37°C and a thin smear was prepared. About 200 spermatozoa were counted under an oil immersion objective lens (1000 X) of light microscope in different fields and classified as live spermatozoa with white bright head and dead spermatozoa with light or dark pink stained head. Percentage of live spermatozoa was calculated.

Membrane Integrity (Jeyendran *et al.*, 1984): Functional integrity of the sperm was evaluated by hypo-osmotic swelling test (HOST). Briefly, 10 µL of semen was incubated in 100 µL of hypo-osmotic solution (50 mosm of trisodium citrate-fructose solution) at 37°C for 30 min. A total

of 150 spermatozoa with coiled and un-coiled tails were counted in different fields. A control was also run in PBS (pH 7.4). The number of spermatozoa with coiled tailed/swollen heads in PBS was deducted from the number in hypo-osmotic solution and the resultant figure was taken as the HOS (Hypo-osmotic swelling.)-reactive spermatozoa.

Acrosome integrity (Watson 1975): Acrosomal integrity of spermatozoa was assessed using Giemsa stain. A smear (10 µl) of washed semen was prepared on a clean glass slide, air dried and fixed in 2 % glutaraldehyde for 30 minutes. After drying, the smear was stained in Giemsa working solution (stock Giemsa stain, 3 ml; 0.1 M phosphate buffer, 2 ml; pH 7.4 and DDW, 35 ml) for 2 h. Smears were air dried and examined under oil immersion (1000x) of the bright field microscope. At least 200 spermatozoa were counted from each slide and classified into two categories viz. intact acrosome and damaged acrosome (partially or completely) for determining acrosome damage.

Fertility trial: Ten roosters per breed selected on the basis of semen traits were evaluated for fertility. Five hens were inseminated with ejaculated semen of one chick. About 20-25 eggs / hen / chick / trial were incubated at 37°C for 21 days. On the 18th day of incubation, eggs were candled using a bright electric bulb. The number of fertile eggs was recorded and percent fertility was calculated as follows. Four hatches were set for each chick.

$$\text{Percent Fertile} = \frac{\text{Total number of fertile eggs}}{\text{Total number of eggs set}} \times 100$$

Statistical Analysis

The mean and SE were calculated using Microsoft excel programme. Significant differences between the two breeds of various semen attributes were tested by one way ANOVA using CPCS1 programme (Statistical Department, PAU, Ludhiana, Punjab). Pearson correlation among different semen traits was calculated using SPSS programme.

RESULTS AND DISCUSSION

Macroscopic and microscopic attributes of semen of Aseel and Kadaknath breeds are shown in Table 1.

Macroscopic Evaluation of semen: Colour of semen was either white or creamy white. It was white and creamy white in 21.5%, 78.5% and 19%, 81% in Aseel and Kadaknath breeds, respectively. Average volume of ejaculate was non-significantly (P>0.05) higher in Aseel (0.36 ± 0.08) as compared to Kadaknath (0.30 ± 0.06 ml) breed. Haunshi *et al.* (2010) also did not observe significant difference in volume of these two breeds. There was no significant differences in pH of ejaculates of Kadaknath (7.17±0.11) and Aseel (7.11±0.15) breeds, which ranged from 6.8±0.33-7.33±0.16 and 6.66±0.17-7.33±0.17, respectively.

Microscopic Evaluation of semen: Fig. 1 depicts viability and membrane integrity of chick spermatozoa. Dead spermatozoa were stained pink, whereas, live ones remained white. Hypo-osmotic swelling test resulted in coiling either near the mid piece or end piece (Fig. 1 b). There was no significant difference in percentage of motile spermatozoa in ejaculates of Aseel (75.87±5.73) and Kadaknath (75.78±4.08) breeds as also observed by Haunshi *et al.* (2010). However, percentage of viable spermatozoa and spermatozoa with intact membrane was non-significantly (P > 0.05) higher in ejaculates of Kadaknath (82.04 ± 4.47, 44.17 ± 3.96 than Aseel (78.35 ± 5.37, 30.98 ± 9.02) roosters. Giemesa staining of sperm smears resulted in dark purple intact, dark pink irregular and light pink distorted head, indicating spermatozoa with intact acrosome, partial and completely damaged acrosome, respectively. Spermatozoa with fully damaged acrosome was non-significantly (P > 0.05) higher in semen of Aseel (53.07±13.91) as compared to Kadaknath (36.30.±12.82) roosters. A higher concentration of spermatozoa, greater viability and lower

abnormal sperm percentage was also reported in Kadaknath than Aseel breed by Biswas *et al.* 2009 and Haunshi *et al.* 2010.

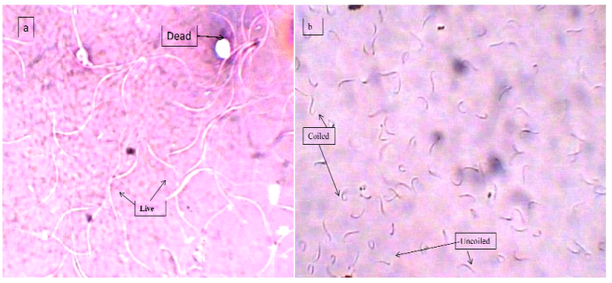

Fig. 1: Showing live/dead (a) and coiled / un-coiled spermatozoa (b) indicating viability and membrane integrity of Aseel and Kadaknath breeds.

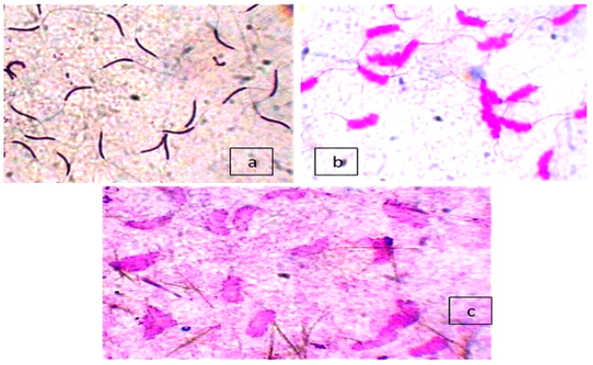

Fig. 2: Showing acrosome integrity of spermatozoa of Aseel and Kadaknath breeds. Spermatozoa with intact (a), partial damaged (b) and completely damaged acrosome (c).

Inter-relationship among sperm attributes: Pearson coefficient correlation was calculated among different semen traits, all of these were very significantly positively correlated, as shown in Table 2. Some of the correlations were weak, especially among motility (0.02) /viability (0.015, 0.04) / membrane integrity (0.001, 0.05) and acrosome damage in Kadaknath breed. Correlation between motility / viability and membrane integrity / acrosome damage was higher in Aseel breed (0.86 and 0.60) as compared to Kadaknath (0.40 and 0.05). It indicated that intactness of acrosome depends upon motility, viability and membrane integrity of spermatozoa. Similar to our observations positive significant correlations were also observed among semen traits of seven strains of chicken (Peter *et al.*, 2008), Indian red jungle fowl (Rakha *et al.*, 2015), local lines of Iraq and their crosses (Hermiz *et al.*, 2016).

Table 1: Comparison between macroscopic and microscopic semen attributes (Mean ± SE) of Aseel and Kadaknath breeds

Sl. No	Semen attributes	Aseel	Kadaknath
Macroscopic attributes			
1.	Colour	Creamy white	Creamy white
2.	Volume (ml)	0.36±0.08[a]	0.30±0.06[a]
		(0.17±0.7-0.53±0.4)	(0.2±0.05-0.43±0.06)
3.	pH	7.11±0.15[a]	7.17±0.11[a]
		(6.8±0.33-7.33±0.16)	(6.66±0.17-7.33±0.17)
Microscopic attributes			
1.	Individual motility (%)	75.87±5.73[a]	75.78±4.08[a]
		(53.33±26.69-86.67±1.67)	(62.5±4.33-82.5±1.45)
2.	Viability (%)	78.35±5.37[a]	82.04±4.47[a]
		(44.0±22.09-85.67±0.88)	(52.92±27.63-92.5±0.50)
3.	HOST (%)	30.98±9.02[a]	44.17±3.96[a]
		(8.0±1.15-51.67±8.67)	(22.73±6.01-64.80±1.23)
4.	Acrosome damage (%)		
a)	Partial	39.07±11.63[a]	45.69±11.34[a]
		(21.88±13.0-55.40±8.23)	(15.50±0.87-64.50±5.49)
b)	Complete	53.07±13.91[a]	36.30.±12.82[a]
		(22.33±15.35-86.67±4.89)	(02.00±0.0-71.0±5.72)

Figures in parentheses represent range of semen traits.

Superscripts indicate non - significant difference at 5 % level with in the columns (a,a)

Correlation between fertility rate and sperm

Table 2: Pearson coefficient correlation among different sperm traits and fertility rate of Aseel and Kadaknath breeds

Sl. No.	Semen attributes	Motility		Viability		HOST		Acrosome damage			
								Partial		Complete	
		A	KN	A	KN	A	KN	A	KN	A	KN
1.	Motility	1.0	1.0								
2.	Viability	0.40	0.86	1.0	1.0						
3.	Membrane Integrity	0.14	0.06	0.18	0.10	1.0	1.0				
4.	Acrosome Damage										
a.	Partial	0.000	0.18	0.015	0.21	0.001	0.17				
b.	Complete	0.022	0.04	0.04	0.06	0.05	0.60				
5.	Fertility rate	0.08	0.01	0.03	0.04	0.03	0.04	0.02	0.04	0.02	0.03

Table 3: Sperm traits and fertility rate of selected roosters of Aseel and Kadaknath

Chick No					Acrosome Integrity	
	Fertility rate (%)	Motility (%)	Viability (%)	Membrane Integrity (%)	Partial (%)	Complete (%)
			Aseel			
1.	66.31 ±10.65	86.67 ± 1.67	85.67 ± 0.88	55.40 ± 8.23	8.00 ± 1,15	22.33± 15.40
2.	58.50± 12.34	83.33 ± 3.33	82.67 ± 4.18	52.30 ± 4.74	10.33 ± 0.33	28.00± 19.50
3.	64,41 ± 8.56	78.33 ± 7.27	83.33 ± 4.34	48.86± 12.12	21.67 ± 5.79	37.67± 16.40
4.	67.28 ± 1.77	76.67 ± 1.68	82.67 ± 4.18	45.03± 10.77	29.33±19.91	41.67± 16.10
5.	60.67 ± 5.23	83.33 ± 1.67	80.33 ± 3.18	40.92 ± 3.14	36.3 ± 2.67	45.33± 20.77
6.	62.82± 13.39	76.67 ± 8.34	83.67 ± 3.18	38.13 ± 3.91	30.00 ± 3.06	49.33± 13.20
7.	38.07± 15.89	71.67±10.94	78.67 ± 6.07	35.21± 12.13	29.33±19.91	50.67± 15.40
8.	72.53 ± 8.53	80.00± 5.78	77.67 ± 2.19	33.02± 10.56	25.33 ± 6.67	55.33± 18.70
9.	66.16± 10.59	65.00± 2.60	73.33 ± 7.76	30.23 ± 1.16	10.33 ± 0.33	71.67 ± 2.34
10.	56.85± 10.63	53.33±12.69	44.00±22.09	21.88± 13.00	8.00 ± 1.16	86.67 ± 4.49
Mean	61.35 ± 2.96	75.50±7.99	77.20±5.81	40.09±7.98	20.87±6.09	48.88±14.09
			Kadaknath			
1.	50.70±9.85	85.00 ± 2.89	92.50 ± 0.29	69.34 ± 0.00	69.00 ± 3.79	8.00 ± 0.58
2.	72.56±5.83	83.33 ± 4.44	88.67 ± 0.33	65.19 ± 0.00	66.00 ± 5.78	12.00±1.73
3.	60.36±2.93	82.50 ± 1.45	87.67 ± 0.88	63.85 ± 1.73	64.50 ± 5.50	17.00±7.51
4.	67.90±11.52	81.67 ± 1.67	85.67 ± 1.45	61.70 ± 6.63	62.33 ± 2.61	25.00±5.78
5.	62.62 ± 7.84	78.33 ± 4.41	81.33 ± 4.18	60.77±10.64	61.00±17.02	24.67±17.21
6.	57.51 ± 8.91	75.00 ± 5.78	78.67 ± 2.19	51.20 ± 6.02	58.67±20.48	37.00 ± 2.89
7.	72.46 ± 9.18	73.33 ± 3.34	76.33 ± 5.05	47.76±13.11	52.67±18.91	46.00± 19.08
8.	65.44 ± 5.91	71.67 ± 7.64	75.50±10.12	46.96 ± 6.82	50.00±10.41	55.33± 20.29
9.	65.38±15.60	60.00±11.56	63.00±19.16	27.24±11.46	26.67±13.21	71.00 ± 4.05
10.	63.33 ± 7.06	42.50±24.57	59.00±15.03	22,73 ± 6.01	20.33 ± 6.02	79.00 ± 0.00
Mean	63.82 ± 3.95	73.33±23.22	78.83±20.94	51.66±16.35	35.60±11.26	27.80±9.95

attributes of roosters selected for artificial insemination: Fertility evaluation of Aseel roosters exhibited an average fertility rate of 61.36 ± 9.75% (Table 3). Average percentage of motile, viable and spermatozoa with intact membrane, partially and completely damaged acrosome was 71.55 ±3.15 %; 77.20 ± 3.86 %; 41.00 ± 3.33 %; 20.86 ± 3.40 % and 48.87 ± 6.08 %, respectively. A significant weak correlation (0.02 – 0.08) was observed between fertility rate and sperm attributes of Aseel breed.

Kadaknath roosters revealed an average fertility rate of 63.82 ± 3.94 % (Table 4). However, average percentage of motile, viable and spermatozoa with intact membrane, partially and completely damaged acrosome was 73.33 ± 23.22 %; 78.83 ± 20.94 %; 51.66 ± 16.35; 35.60 ± 11.26 and 27.80 ± 9.95, respectively. A significant low correlation (0.01-0.04) of fertility rate was obtained with sperm traits of Kadaknath breed. It indicated that fertility rate of Aseel breed was more correlated to sperm motility, viability, membrane integrity and acrosome damage.

Since only roosters with higher sperm attributes were selected for artificial insemination, therefore, fertility trial results suggests that selection of roosters exhibiting higher motility, viability, membrane and acrosome integrity leads to significant improvement in the fertility of artificially inseminated hens. Although, viability, membrane integrity and acrosome integrity were higher in semen of Kadaknath than Aseel breed, but both breed were almost equivalent in their fertility breeds. Selection of roosters on the basis of sperm attributes may be one of the useful in AI practices aimed at genetic improvement for breeds.

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