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Influence of Indigenous Nicobari and Ankleshwar Chicken Breeds on Physical and Biochemical Attributes of Semen

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A B S T R A C T

The objective of the current experiment was to investigate the various semen characteristics of two indigenous breeds of chicken namely Nicobari and Ankleshwar, as such data were not yet reported. Freshly ejaculated semen samples were collected from twenty breeder roosters of each breed and subjected to physical and biochemical analysis. Immediately after collection, the semen volume of individual birds was recorded using tuberculin syringe and further analysed for seminal motility, concentration and percentage of live, dead and abnormal spermatozoa using standard procedures. After recording the hydrogen ion concentration in semen, other biochemical attributes such as total protein, triglyceride, cholesterol, transaminase enzymes, alkaline phosphatase, calcium, phosphorus and uric acid concentration were estimated in the seminal plasma of both the native breeds. The physical characterization of semen showed significant ($p<0.05$) breed differences in seminal volume and per cent morphologically abnormal spermatozoa. Significant ($p<0.05$) biochemical changes were seen in the concentrations of triglyceride, cholesterol, Glutamic oxaloacetic transaminase and uric acid. The study showed significant breed variations in the seminal attributes of the selected native breeds of chicken.

Introduction

For the success of artificial insemination in poultry, examination of the semen characteristics of different breeds of poultry and its preservation is the need of the day. The evaluation of semen characteristics emulates the reproductive potential of the cock and hence the major determinant of fertility and subsequent hatchability of eggs (Zahraddeen *et al*., 2005). Such types of studies are lacking in indigenous chicken breeds such as Nicobari and Ankleshwar. Nicobari fowl is an endangered and endemic breed of poultry in the islands of Andaman and Nicobar and produces highest number of eggs among all the indigenous chicken breeds of India (Ahlawat and Chatterjee, 2002). Ankleshwar breed of chicken belong to the 'Ankleshwar' area of Bharuch district of Gujarat and are reared mainly by the tribal population under backyard poultry farming as

a livelihood source of income. These birds are maintained without vaccination and medications, and have reasonable feed efficiency and excellent fertility (GAU Report, 2003). In spite of the distinctive characteristics of indigenous chicken, there is a lack of concern for the conservation and improvement of these breeds under field conditions (Pandey *et al*., 2005). However, there is worldwide recognition of the need for the conservation of livestock diversity and for characterization of breeds and populations (FAO, 1995). Hence, findings on physical and biochemical characteristics of semen of these indigenous breeds will be immensely helpful in future research and breeding strategies for the conservation and improvement of native breeds.

Materials and Methods

Experimental birds and husbandry practices

The proposed experiment was conducted in the Division of Avian Physiology and Reproduction, Central Avian Research Institute, Izatnagar, Bareilly, U.P. The institute is situated at an altitude of 169 m above the mean sea level, at latitude of 28°N and longitude of 79°E. The place experiences extreme hot (45°C approx) and cold (5°C approx) conditions with the relative humidity ranging between 15 to 85%. Twenty healthy and adult males from each breed i.e. Nicobari and Ankleshwar chicken were taken randomly and maintained in individual cages under uniform husbandry conditions. They were given normal breeder ration with maize, de-oiled rice bran (DORB), soyabean, oyster shell, marble chips, limestone, dicalcium phosphate (DCP), salt, DL-methionine, B-complex, vitamin and trace mineral premix. The birds were given water ad libitum and provided with constant light 14 hrs/day.

Semen collection and Physical analysis

Semen samples from the experimental birds were collected by abdominal massage method (Burrows and Quinn, 1937). During the study period precautions were taken to avoid contamination of semen with fecal matter, urates and transparent fluid which deteriorate the semen quality. A tuberculin syringe of 1.0 ml capacity (graduated to measure 0.01ml sample) was used to quantify the semen volume. Immediately after collection, a drop of semen (4-5 µl) was taken with the help of Pasteur pipette on a dry, clean glass slide and was spread uniformly using a cover slip. On the basis of the activity of swirls, semen was graded and thus per cent motility of spermatozoa was scored for individual samples. The sperm concentration (million per ml) was determined from the standard calibration curve established between absorbance and sperm concentration in a double beam UV-VIS spectrophotometer at 550 nm (Brillard and McDaniel, 1985). The percentage of live and dead spermatozoa was examined as per the method described by Lake and Stewart (1978). For the estimation of abnormal spermatozoa, in the smears as prepared for sperm viability estimation, a total of 100 spermatozoa were counted under the oil-immersion objective of the microscope and classified as either normal or abnormal and the total % of abnormal spermatozoa was calculated.

Biochemical analysis

Preparation of seminal plasma was done by centrifuging the semen samples at 4°C in a cooling centrifuge at 5000 rpm for 10 minutes. After centrifugation supernatant was collected as seminal plasma and stored in the freezer (-20°C) till further use. The evaluation of various biochemical parameters of seminal plasma such as total protein by biuret method, triglyceride by glycerol-3-phosphate

oxidase/phenol+aminophenazone method, cholesterol by cholesterol oxidase/ phenol+aminophenazone method, transaminase enzymes (GPT- Glutamic pyruvate transaminase and GOT- Glutamic oxaloacetic transaminase) by Reitman and Frankel's method , alkaline phosphatase (ALP) by modified Kind and King's method , calcium by o-cresolphthalein method and uric acid by uricase/ phenol+aminophenazone method were done as per the protocol of commercial kits procured from Coral Clinical Systems, Goa. The neat semen pH was determined by digital pH meter (Thermo Fisher Scientific, USA) fitted with a microelectrode.

Statistical analysis

The data were subjected to analysis of variance using statistical software package SPSS (version 20) and the significant differences among means were determined by Duncan's multiple range tests (Duncan, 1955).

Results and Discussion

The physical characteristics of semen of both the indigenous breeds of chicken are shown in Table 1. The semen volume per ejaculate was recorded significantly ($p<0.05$) higher in the Nicobari breed followed by Ankleshwar breed. The variations in the ejaculate volume are ascribed to breed differences, intermix of transparent fluid during massage method and hormonal fluctuations (Kundu and Panda, 1990). No significant differences were found between the breeds in terms of sperm motility, sperm concentration, viability, though ankleshwar chicken showed slightly higher values than nicobari chicken. The percentage of morphologically abnormal sperms were found to be significantly ($p<0.05$) higher in Nicobari breed than ankleshwar. Abnormal sperm have been found to be negatively correlated with fertility in various studies (Mohan *et al.,* 2011).

The biochemical characteristics of semen of both the indigenous breeds of chicken are shown in Table 2. The total protein concentration showed no found significant difference between the native breeds but their presence in seminal plasma reflects the seminal quality. It has been demonstrated in mammalian semen that the basic proteins in seminal plasma would bind to the sperm membranes thereby increasing its permeability (Moore and Hibbit, 1976). Thurston *et al.,* (1982) stated that the presence of proteins in the seminal plasma was poorly correlated with blood proteins.The triglyceride and cholesterol concentration were observed more in ankleshwar breed than the nicobari breed. The results on cholesterol level in chicken seminal plasma were comparable with Biswas (2007). Studies proved that the seminal plasma lipids influence the fertilization capacity and that the oxidation of triacylglycerols helps to meet the energy demand of spermatozoa (Beer-Ljubic *et al*., 2009). Douard *et al.,* (2000) observed significant amounts of cholesterol esters and triglycerides in seminal plasma in contrast to spermatozoa.

The transaminase (GPT and GOT) enzyme activity were found to be higher in the seminal plasma of nicobari birds, however GPT did not show significance. The higher ($p<0.05$) activity of GOT in nicobari chicken may be due to sperm cell membrane instability and in turn reflect semen quality as these enzymes leak from spermatozoa and other structures of male reproductive tract into the seminal plasma under stress conditions. Hence the increase in seminal plasma transaminase may serve the index for poor quality sperms (Al-Daraji *et al.,* 2001). Manoharan (2000) reported breed differences for difference in enzyme activity among

different breeds. The enzyme activities of GOT in our study is 15 - 20 times greater than GPT values. Hammond *et al.,* (1965) and Datta *et al.,* (1980) reported 43-63 times higher activity of GOT and Mohan *et al.,* (2011) observed 39-55 times higher activity of GOT than GPT. Hence, the concentration of glutamic acid is remarkably high in the seminal plasma of cocks in all observations. According to Lake and McIndoe (1959), this amino acid constitutes as much as 90% of the total amino acids in the seminal fluid and might have a special role in maintaining its osmotic balance and pH. The metabolic origin of glutamic acid in seminal plasma is however, uncertain. It might be envisaged that the high activity of GOT is primarily directed towards the formation of this important amino acid. Though alkaline phophatase activity showed no breed differences among native chicken, their presence in seminal plasma is essential in the membrane transport, carbohydrate hydrolysis and energy provision to spermatozoa (Mohan *et al.,* 2011).

Table.1 Physical characteristics of semen in indigenous Nicobari and Ankleshwar breeds of chicken (Mean ± SE, n=6)

Parameter	Nicobari	Ankleshwar
Semen volume (ml)	0.55 ± 0.03^{a}	0.43 ± 0.01^{b}
Semen motility (%)	78.89±0.97	79.13±1.20
Sperm concentration (x 10^{9}cells/ml)	4.93±0.15	5.02±0.10
Live spermatozoa (%)	88.63±0.69	89.37±0.53
Dead spermatozoa (%)	11.36±0.68	10.75±0.56
Morphological abnormal spermatozoa (%)	4.30 ± 0.25^{a}	3.43 ± 0.17^{b}

Means bearing different superscripts in a row differ significantly ($p<0.05$)

Table.2 Biochemical characteristics of seminal plasma in indigenous Nicobari and Ankleshwar breeds of chicken (Mean ± SE, n=6)

Parameter	Nicobari	Ankleshwar
Total protein (g/dl)	2.27±0.02	2.24±0.02
Tryglyceride (mg/dl)	$77.97^{b}\pm1.85$	$86.47^{a}\pm2.80$
Cholesterol (mg/dl)	$33.24^{b}\pm1.64$	$38.50^{a}\pm1.16$
GPT (IU/L)	14.67±1.23	12.76±1.17
GOT (IU/L)	$188.09^{a}\pm4.38$	$176.08^{b}\pm2.85$
ALP (KA Units)	5.37±0.38	5.54±0.37
Calcium (mg/dl)	5.61±0.16	5.68±0.14
Phosphorus (mg/dl)	4.12±0.08	4.30±0.10
Uric acid (mg/dl)	$4.95^{b}\pm0.20$	$5.55^{a}\pm0.20$
Semen pH	7.29±0.03	7.28±0.02

Means bearing different superscripts in a row differ significantly ($p<0.05$)

The mineral concentrations (Calcium and Phosphorus) showed no significant differences between breeds. The uric acid concentration of ankleshwar breed was significantly ($p<0.05$) higher than nicobari breed. The concentration of uric acid in seminal plasma was positively correlated with sperms of normal morphology in mammals, which is comparable with our findings (Zhang *et al*., 2007). Also uric acid is reported to increase the antioxidant strategy of seminal plasma by scavenging the free radicals (Aitken *et al*., 2012). The hydrogen ion concentration (pH) showed no significance between the native breeds in our study.

The various semen characteristics observed in the present study showed significant variations between the indigenous breeds. This exhibits the difference in the reproduction potential among the breeds. Ankleshwar birds donated comparatively good quality semen than the Nicobari breed in our study.

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