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Semen quality and reproductive ability of *in ovo* thermal manipulated roosters at different ages

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ABSTRACT

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The aim of the present study was to evaluate higher temperature exposure during incubation on rooster semen quality at different ages. Dahlem Red breeder male chicks were obtained by hatching under normal incubation temperature (37.5°C and 65% relative humidity) and elevated incubation temperature (39.5°C and 65% relative humidity) applied for three hours during 16-18th days of incubation. The two groups of chicks were reared till 72 weeks of age. Semen collected at 29, 39, 59 and 69 weeks of age was evaluated for different physical parameters. An artificial insemination trial was carried at 30 weeks of age. Environmental variables during the weeks of semen evaluation were used for calculation of temperature humidity index (THI). Results indicated that roosters hatched from higher incubation temperature did not produce semen of better quality compared to control roosters.

Keywords: Thermal manipulation, Semen, Rooster, Temperature humidity index, Fertility

INTRODUCTION

High ambient temperature has deleterious effect on the semen quality of roosters. Thus, during summer or on exposure to high ambient temperature the semen parameters are adversely affected (Joshi *et al.,* 1980; McDaniel *et al.,* 1996). Different strategies have been adopted to reduce the adverse effects of ambient temperature on rooster semen quality like supplementation of minerals etc. In poultry, thermotolerance is improved by the mechanism of thermal manipulation during embryogenesis. This is based on the theory that thermal manipulation during critical periods causes epigenetic adaptation mechanisms and adapts the birds for the expected post natal environmental conditions (Tzschentke and Halle, 2009). The epigenetic adaptation is brought through changed neuronal hypothalamic thermo-sensitivity. Beneficial effects of thermal manipulation during broiler chick embryogenesis on thermo tolerance acquisition (Yahav, 2009) and post natal body weight (Tzschentke and Halle, 2009) has been reported. The present study aimed to evaluate the effect of thermal manipulation during incubation on semen quality at different intervals up to 72 weeks of age.

MATERIALS AND METHODS

The experiment was carried out at the experimental poultry farm of ICAR-Directorate of Poultry Research, Hyderabad, India. Four hundred and sixty eggs obtained from Dahlem Red breeder line were randomly divided into two groups (Control- 228; Heat exposed-232). The Control (C) group eggs were incubated at standard incubation conditions (37.5°C and 65% relative humidity). Another set of eggs, heat exposed (HE) group, were incubated at higher temperature 39.5°C from 16-18th days of incubation for 3 hours each day and relative humidity was maintained at 65% (Yahav *et al.,* 2004). A total of 367 chicks (190 C and 177 HE) were obtained after hatching of eggs. Initially the chicks were reared in battery brooders. From 18 weeks of age the roosters and hens were reared in individual breeder cages in an open-sided house under natural photoperiod and climatic conditions. The birds were provided with *ad libitum* feed and water. The roosters were trained for semen collection from 21 weeks of age and semen was periodically collected from the birds. Semen collected at ages 29, 39, 59 and 69 weeks from roosters of each group were evaluated for different gross semen parameters. The mean ambient temperature (Ta) in Celsius and percent relative humidity (RH) in the farm area during the weeks of semen collection was used to calculate the THI, according to the formula: THI= (0.8 × Ta) + [(RH / 100) × Ta – 14.3)] + 46.4) (Mader *et al.,* 2010). The weekly average THI during the experimental duration has been provided in table 1. The experiment was carried out according to the guidelines and approval of the Institutional Animal Ethics Committee.

The roosters were trained for semen collection by abdominal massage (Burrows and Quinn, 1937). The semen collected was diluted four times using high temperature (HT) diluent (Chaudhuri and Lake, 1988) and used for further evaluation. The volume of semen was calculated using 1 ml syringe having an accuracy of 0.02 ml. The raw semen appearance was visually scored 1 to 5 (McDaniel and Craig, 1959). Sperm motility was subjectively assessed as percentage of progressively

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motile sperm by placing a drop of the diluted semen on a Makler chamber and examined under 20× magnification. The sperm concentration was calculated as described by Taneja and Gowe (1961) using a colorimeter at 540 nm wavelength. Tetrazolium dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction test was carried out and absorbance measured at 570 nm (Hazary *et al.,* 2001). The percent live sperm was calculated by using eosin–nigrosin differential staining technique (Campbell *et al.,* 1953). Using the same slide, the percent abnormal sperm were estimated on the basis of observable abnormalities.

At 30 weeks of age an insemination trial was carried to evaluate the *in ovo* heat exposure on fertility and hatchability. Semen from C and HE roosters were pooled group wise and inseminated in corresponding group hens (30 hens/group) once in a week for five consecutive weeks. A fixed dose of sperm (100 million sperm/0.1 ml semen) was used for insemination of a hen. Eggs collected were incubated at standard incubation conditions and chicks were hatched in three hatches. The percent fertility and hatchability data were recorded.

Data were analysed by Students *t* test using SAS 9.2 software and $P < 0.05$ was considered significant. Percent values were arcsine transformed and used for analysis of data.

RESULTS AND DISCUSSION

The HE birds had significantly higher sperm concentration only at 39 weeks of age and lower MTT dye reduction test values at 29 weeks of age. There was no significant difference between the C and HE group roosters in other semen parameters studied (Table 1). The average fertility of C and HE group was 81.21% and 79.78% respectively and the average hatchability on total egg set of C and HE group was 72.12 % and 70.19% respectively. Analysed data did not reveal any significant difference in fertility traits due to *in ovo* heat exposure.

Prenatal temperature exposure influences post natal neuronal hypothalamic thermosensitivity due to epigenetic temperature adaptation (Tzschentke and Basta, 2002). This lifelong imprinting occurrence is more particularly at the end of incubation (Tzschentke and Plagemann, 2006). Thus, thermal manipulation during 'critical period' will result in long lasting warm adaptation during post hatch development.

In the present study consistent benefits of in ovo heat exposure on rooster's semen quality could not be observed. A threshold THI of 70 has been reported for laying hens (Karaman, 2007). The THI during the study period was higher when the birds were at of advanced age. The HE roosters during this higher THI period did not show any better semen quality characteristics compared to the C roosters. Similarly, the fertility trial also did not produce higher fertility parameters by the HE roosters. The incubation protocol followed in the present study has been reported to improve thermotolerance acquisition in broiler chicks (Yahav *et al.,* 2004). However, in the present study of semen parameters during high Ta the incubation protocol did not produce any beneficial effects. In an earlier report (Shanmugam et al., 2016) a detailed semen evaluation during high ambient temperature indicated no difference in semen quality due to *in ovo* thermal manipulation in the Dahlem Red roosters.

In conclusion an increased incubation temperature of 2°C between 16-18th day of incubation of chicken eggs did not impart any advantage in rooster semen quality during higher environmental temperature conditions. Thermal manipulation of different duration

Table 1: Mean±SE semen quality parameters of control and heat exposed roosters during different ages and the respective week average THI

	29 weeks		39 weeks		59 weeks		69 weeks	
Semen parameters	**C(N=19)**	**HE(N=15)**	**C(N=13)**	**HE(N=19)**	**C(N=11)**	**HE(N=9)**	**C(N=7)**	**HE(N=7)**
Semen volume (ml)	0.43±0.04	0.40±0.06	0.60±0.05	0.54±0.05	0.46±0.05	0.37±0.04	0.53±0.07	0.45±0.05
Appearance	3.32±0.13	3.27±0.12	3.15±0.22	3.27±0.12	3.63±0.20^{a}	3.11±0.11^{b}	3.29±0.13	3.14±0.14
Progressive motile sperm (%)	50.53±2.22	47.67±2.67	46.15±4.57	51.33±2.69	37.73±5.45	41.11±3.88	46.43±2.34	50.00±2.43
Sperm concentration (million/ µl)	5.37±0.32	5.65±0.34	4.32±0.43^{b}	5.91±0.42^{a}	5.78±0.75	5.18±0.44	5.93±0.73	5.51±0.43
MTT dye reduction test (nm of MTT Formazan /min/ million sperm)	20.21±0.59^{a}	18.36±0.70^{b}	15.96±1.39	17.67±0.52	15.64±1.03	14.80±1.33	17.29±1.05	15.63±0.89
Live Sperm (%)	93.04±1.14	92.45±0.91	91.40±2.12	93.58±1.33	87.48±1.97	87.47±2.23	96.00±0.63	91.53±2.81
Abnormal Sperm (%)	4.52±1.63	2.29±0.50	5.61±1.07	3.85±0.67	3.54±1.15	2.39±1.67	5.70±3.03	3.43±0.60
THI	76.14		68.26		80.19		78.98	

Means with different superscripts within an age in a row differ significantly ($P < 0.05$)

and stages of embryonic development during incubation should be explored for obtaining better semen quality during heat stress in rooster.

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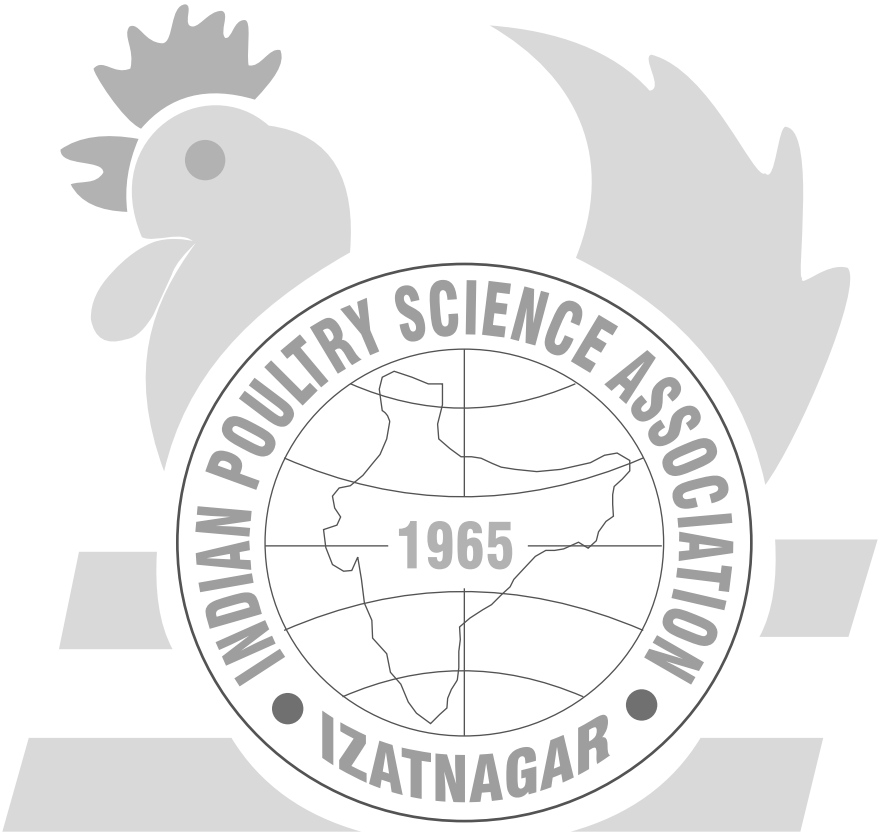
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