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Thermal manipulation during embryogenesis improves certain semen parameters in layer breeder chicken during hot climatic conditions

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ABSTRACT

Thermal manipulation during incubation has been shown to improve post hatch performance in poultry. The aim of the present experiment was to evaluate thermal manipulation on semen quality of roosters during hot climatic conditions. Eggs obtained after artificial insemination from Dahlem Red layer breeders were randomly divided into two groups control (C) and heat exposed (HE). C group eggs were incubated at 37.5 °C throughout the incubation period while the HE group eggs were exposed to higher temperature 40.5 °C from 15th to 17th day of incubation for 3 h each day. The relative humidity was maintained at 65% in both the groups throughout incubation. The chicks hatched were reared separately under standard husbandry conditions. During high ambient temperature semen from roosters (45 weeks of age) was collected and evaluated for different gross parameters, sperm chromatin integrity and sperm *HSP27* and *HSP70* gene expression by real-time PCR. The seminal plasma was evaluated for lipid peroxidation, ferric ion reducing antioxidant power (FRAP), triiodothyronine (T_3) and matrix metalloproteinase-2 (MMP-2) activity. The shed average Temperature Humidity Index (THI) during the experiment period was 78.55. The percent live sperm and FRAP level were significantly ($P < 0.05$) higher and sperm gene expressions were significantly ($P < 0.05$) lower in the HE group. No differences in other parameters were observed between the groups. Thus from the results it could be concluded that thermal manipulation during incubation improves certain semen parameters of roosters at high ambient temperature.

1. Introduction

The environmental temperature is an important factor affecting the semen quality and fertility of rooster in tropical countries. The semen quality deteriorates during summer or on exposure to high ambient temperature (Boone and Huston, 1963; Joshi et al., 1980). Even if the sperm motility was not affected by heat treatment of broiler males, the fertility and sperm egg penetration declined (McDaniel et al., 1995). It was suggested that heat exposure might have resulted in nuclear abnormality resulting in declined fertility. Furthermore, it has been shown that heat stress depresses the number of sperm penetrating the perivitelline membrane (McDaniel et al., 1996). Heat stress will lead to oxidative stress in semen (Rao et al., 2015) due to high levels of free radicals, more particularly reactive oxygen species (ROS). This higher free radical level will lead to damage of different sperm components. Chicken sperm are rich in polyunsaturated fatty

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acids and are readily susceptible to oxidation by free radicals. There exists an enzymatic and non-enzymatic defence mechanism to counter the damage due to oxidative stress. The non-enzymatic defence consists of dietary components and metabolites such as vitamin E, vitamin C, uric acid, bilirubin etc. These non-enzymatic compound activities in biological fluids can be measured and expressed as ferric ion reducing antioxidant power (FRAP) (Benzie and Strain, 1996). Mild or transient scrotal heat stress for shorter duration of 30 min in lab animals has been shown to cause DNA damage in the developing sperm (Banks et al., 2005; Paul et al., 2008). Furthermore, determination of the sperm chromatin integrity offers valuable information on the male fertility potential (Agarwal and Said, 2003). Sperm Chromatin Dispersion (SCD) testis a simple and inexpensive method for the analysis of sperm DNA fragmentation (Fernández et al., 2003) and the test was optimized for chicken sperm in our lab (Shanmugam et al., 2014). This test is based on the principle that sperm with DNA fragmentation fail to produce halo of dispersed DNA loops when mixed with agarose followed by acid denaturation and nuclear protein removal.

Heat Shock Proteins (HSPs) are a set of highly conserved proteins that act under physiological conditions as molecular chaperones and are also induced by cytotoxic stressors including temperature (Neuer et al., 2000). HSPs are classified based on their molecular weight and among them HSP70 and HSP27 were seem to be closely associated with heat tolerance (Samali et al., 2001; King et al., 2002). In response to heat stress differential expressions of HSP70 and HSP27 gene or protein in different tissues of chicken has been reported (Yahav et al., 1997; Wang and Edens, 1998).

The matrix metalloproteinase-2 (MMP-2) is a zinc dependant endopeptidase that hydrolyses a variety of extracellular matrix and non-extra cellular matrix proteins. MMP-2 has been detected in seminal plasma and other reproductive tissue fluids of human, dog, ram, boar and stallion (Metayer et al., 2002; Tentes et al., 2007; Saengsoi et al., 2011; Warinrak et al., 2015). MMP-2 has been shown to be correlated with sperm concentration and motility (Baumgart et al., 2002; Saengsoi et al., 2011). The presence or activity of MMP-2 in chicken seminal plasma is not known. Thyroid hormones play role in the regulation of metabolic rate. Chronic heat stress has been shown to reduce plasma T_3 concentration in layer hens (Decuypere and Kuhn, 1988; Melesse et al., 2011).

The procedure of thermal manipulation during embryogenesis has been shown to improve thermotolerance in poultry (Yahav et al., 1997; Piestun et al., 2008; Al-Zghoul et al., 2013). The epigenetic thermal adaptation described as changes that occur in a short critical developmental period during pre- or early postnatal ontogeny affects physiological control systems for a lifelong adaptation to an expected post-natal environmental condition (Tzschentke and Plagemann, 2006). Thermal manipulation during broiler embryo development has been shown to improve post-hatch performance under hot conditions (Piestun et al., 2011). The aim of the present study was to investigate the effect of thermal manipulation during embryonic development on rooster sperm characteristics associated with sperm function during hot climatic conditions.

2. Materials and methods

2.1. Experimental birds and husbandry

The experiment was carried out at the experimental poultry farm of the institute following the approval of the Institutional Animal Ethics Committee. Eggs obtained after artificial insemination from Dahlem Red layer breeder were randomly divided into two groups control (C) and heat exposed (HE). C group eggs were incubated at 37.5 °C and 65% relative humidity throughout the incubation period whereas the HE group eggs were exposed to higher temperature of 40.5 °C during 15–17th days of incubation for 3 h each day with relative humidity maintained at 65%. The hatchability on fertile eggs set in C and HE group were 87% and 83% respectively. The chicks hatched were wing banded and reared group wise in battery brooders and starting from 18 weeks of age the roosters were housed in individual breeder cages in an open-sided house under natural photoperiod and climatic conditions. The birds had free access to feed and water. The roosters were trained for semen collection from 22 weeks of age and semen collected and discarded periodically unless used.

2.2. Temperature data

The semen collection and evaluation was carried out during middle of summer when the maximum environmental temperature will reach up to 45 °C. Mean ambient temperature (T_a) in Celsius and percent relative humidity (RH) in the shed during the week of the experiment was used for calculation of the Temperature Humidity Index (THI), according to the formula: $THI = (0.8 \times T_a) + [(RH/100) \times T_a - 14.3] + 46.4$ (Mader et al., 2010). The shed average THI, temperature and relative humidity during the week of semen evaluation were 78.55, 31.08 and 43.4 respectively.

2.3. Semen collection and evaluation

All chemicals used in the experiment were purchased from Sigma–Aldrich (USA) unless otherwise specified. Semen sampling in nineteen randomly selected roosters (45 weeks of age) from each group was done twice during the week of experiment in an interval of five days. The initial semen collected was used for analyzing gross semen parameters, biochemical parameters and SCD test and the second sampled semen was used for gene expression in sperm and MMP activity determination in seminal plasma. Semen samples collected by abdominal massage (Burrows and Quinn, 1937) after assessing for ejaculate volume and appearance, were diluted four times with high temperature (HT) diluent (NaCl 0.8 g; TES 1.374 g; 1 M NaOH 2.75 ml; glucose 0.6 g, dissolved in 100 ml of double distilled water, pH 7.4) (Chaudhuri and Lake, 1988). This fourfold diluted semen samples were used for laboratory evaluation of seminal parameters.