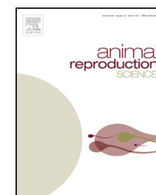
Animal Reproduction Science 145 (2014) 81–85

Contents lists available at ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci



Evaluation of semen quality in roosters of different age during hot climatic condition

M. Shanmugam*, A. Vinoth, K.S. Rajaravindra, U. Rajkumar

Directorate of Poultry Research, Rajendranagar, Hyderabad 500 030, India

ARTICLE INFO

Article history:
Received 2 August 2013
Received in revised form
18 December 2013
Accepted 21 December 2013
Available online 3 January 2014

Keywords:
Age
Chicken
Semen
Sperm chromatin dispersion test

ABSTRACT

The present experiment was conducted to evaluate the semen quality of roosters of different ages during hot climatic condition. Semen from roosters ($n = 8$/age group) of 23, 42 and 65 weeks of age was collected and evaluated for different physical parameters. The sperm membrane integrity was evaluated by hypo-osmotic swelling test, whereas sperm DNA fragmentation was assessed by Sperm Chromatin Dispersion (SCD) test. The seminal plasma cortisol level was assessed by EIA. The shed average Temperature Humidity Index (THI) during the experiment period was 79.32. Semen volume and sperm DNA fragmentation were significantly different ($P \leq 0.05$) between the age groups tested. Roosters of 42 weeks age had higher semen volume and lower sperm DNA fragmentation during study period. None of the other parameters were influenced by the age of the birds. The results indicated that semen quality was affected by the age of the birds. The extreme heat condition also appears to exert a negative influence on the sperm chromatin in roosters.

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

The semen quality of the rooster is affected by different factors like breed, age, feed and environmental stressors like temperature and humidity (Zhang et al., 1999; Karaca et al., 2002; Shanmugam et al., 2012). The semen volume in broiler breeder males increased from 24 to 48 weeks of age (Shanmugam et al., 2012), decreased in White Leghorn roosters with advancing age (46 weeks and above) (Clark and Sarakoon, 1967) or fluctuated between the weeks of collection (Kelso et al., 1997). Similarly the sperm concentration decreased with age of the bird (Sexton et al., 1989; Hocking and Bernard, 1997; Zhang et al., 1999) or remained constant up to 53 weeks of age and afterwards started declining (Wilson et al., 1971, 1987; Cerolini et al., 1997). The sperm activity assessed through 3-[4,5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide (MTT) dye reduction test and number of live sperms was shown to be increased from early age to mid age in broiler breeder males (Shanmugam et al., 2012).

The environmental temperature is an important factor influencing the semen quality and fertility of rooster in tropical countries where the birds are mostly raised in open sided poultry house. It has long been reported that semen quality is decreased during summer or on exposure to high ambient temperature (Boone and Huston, 1963; Joshi et al., 1980). Though the sperm motility was not affected by heat treatment of broiler males, fertility and sperm egg penetration declined, however, the underlying mechanism is not known (McDaniel et al., 1995). The authors had suggested that heat exposure might have resulted in nuclear abnormality leading to declined fertility. It is well documented in lab animals that mild or transient scrotal heat stress for shorter duration of 30 min causes DNA damage in the developing sperm (Banks et al., 2005; Paul et al., 2008). There is no report that examines the effect of high ambient temperature on sperm nuclear status in chicken. Determination of the sperm chromatin integrity

* Corresponding author. Tel.: +91 4024015651;
fax: +91 4024015651/7000
E-mail addresses: dr_shan@rediffmail.com, physioshan@gmail.com
(M. Shanmugam).

0378-4320/$ – see front matter © 2014 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.anireprosci.2013.12.015

provides valuable information on the male fertility potential (Agarwal and Said, 2003). Different tests are available for detection of sperm chromatin/DNA damage like COMET assay (Gliozzi et al., 2011), TUNEL assay (Martins et al., 2007), SCSA (Partyka et al., 2010) and Sperm Chromatin Dispersion (SCD) test (Fernández et al., 2003). Among these assays the SCD test is a simple and inexpensive method for the analysis of sperm DNA fragmentation. This method 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 (Fernández et al., 2003). The halos can be visualised using bright-field microscopy after staining with Wright's stain (Fernández et al., 2005).

The aim of the present study was to determine the semen quality of different age roosters during hot summer conditions. Further in the present study the status of sperm chromatin damage of the different age birds was assessed using SCD test.

2. Materials and methods

2.1. Experimental birds and husbandry

The experiment was carried out at the experimental poultry farm of Directorate of Poultry Research located at Hyderabad, India. Dahlem Red, a brown tinted layer pure line chicken maintained at the institute was used in the experiment. Roosters ($n = 8$/age group) of three different ages 23, 42 and 65 weeks were housed in individual breeder cages from 18 weeks of age in an open-sided elevated house under natural photoperiod and climatic conditions. The birds had free access to both feed and water. They were trained for semen collection from 20 weeks of age and semen collected and discarded weekly unless used. The trial was conducted following the approval of the Institute Animal Ethics Committee.

2.2. Temperature data

Mean ambient temperature (T_a) in Celsius and percent relative humidity (RH) in the shed during the week of the experiment was used to calculate the temperature humidity index (THI), according to the formula: $\text{THI} = (0.8 \times T_a) + [(\text{RH}/100) \times T_a - 14.3)] + 46.4)$ (Mader et al., 2010). The average THI during the week of semen evaluation was 79.32.

2.3. Semen collection and evaluation

Semen was collected in a glass funnel by abdominal massage method (Burrows and Quinn, 1937). The semen collected once during the week of the experiment was used for analysis. The samples were immediately diluted four times using 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). The four fold diluted semen samples were brought to the lab and used for laboratory evaluation of seminal parameters.

The ejaculate volume was assessed by drawing the sample in to 1 ml syringe with an accuracy of 0.02 ml. Sperm motility was assessed as percentage of progressively motile sperms and a drop of the diluted semen was kept on a clean glass slide and cover slip was applied to examine under high power magnification (40×) and was subjectively assessed. The concentration of sperm was estimated by the method described by Taneja and Gowe (1961) using a colorimeter at 540 nm of wavelength. Tetrazolium dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction test was carried out in duplicate tubes and absorbance was measured with a colorimeter (CL 157, Elico Ltd, India) at 570 nm according to Hazary et al. (2001).

The live and dead sperm percent was calculated by differential staining technique using eosin–nigrosin (Campbell et al., 1953). The slides were used for estimating the percent abnormal sperms on the basis of observable abnormalities. A minimum of 200 sperms were counted in each slide for calculating live, dead and abnormal sperm per sample.

2.4. Hypo-osmotic swelling test

The plasma membrane integrity was determined through hypo-osmotic swelling test as described for chicken spermatozoa (Santiago-Moreno et al., 2009). In a glass tube 25 μl of diluted semen was taken and 500 μl of hypo-osmotic solution (100 mOsm/kg; 1 g of sodium citrate in 100 ml distilled water) was added and incubated for 30 min at 37 °C. The samples fixed in 25 μl of 2% glutaraldehyde were examined at 400× under phase-contrast microscope by counting 200 sperm. The percentage of spermatozoa having coiled mid-pieces and tails were calculated.

2.5. SCD test

The test was performed according to the procedure of Fernández et al. (2003), with minor modifications. Equal volumes of diluted semen sample and 1% low-melting agarose were mixed at 37 °C. An aliquot of 30 μl of the mixture was pipetted onto glass slides precoated with 0.65% normal melting agarose, covered with cover slip and kept on ice pack for 4 min to solidify. Immediately after careful removal of cover slip the slides were immersed horizontally in a tray containing acid denaturation solution (0.08 N HCl) for 3 min at 22 °C in the dark to generate restricted single-stranded DNA (ssDNA) motifs from DNA breaks. Then the denaturation was stopped and proteins were removed by transferring the slides to a tray with neutralising and lysis solution (0.4 M Tris, 0.8 m DTT, 1% SDS, 50 mM EDTA, pH 7.5) for 5 min at room temperature. The slide was then washed in Tris-borate-EDTA buffer (0.09 M Tris-borate and 0.002 M EDTA, pH 7.5) for 2 min, dehydrated in sequential 70%, 90% and 100% ethanol (2 min each) and air dried. The prepared slides may be stored in tightly closed box in dark if not immediately stained. For bright-field microscopy slides were horizontally covered with a mix of Wright's stain and buffer solution (380 mg $Na_2 PO_4$, 547 mg $KH_2 PO_4$ in 100 ml distilled water) for

Download English Version:

https://daneshyari.com/en/article/2072888

Download Persian Version:

https://daneshyari.com/article/2072888

Daneshyari.com