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Comparison of semen variables, sperm DNA damage and sperm membrane proteins in two male layer breeder lines

M. Shanmugam*, T.R. Kannaki, A. Vinoth

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

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ABSTRACT

Semen variables are affected by the breed and strain of chicken. The present study was undertaken to compare the semen quality in two lines of adult chickens with particular reference to sperm chromatin condensation, sperm DNA damage and sperm membrane proteins. Semen from a PD3 and White Leghorn control line was collected at 46 and 47 weeks and 55 weeks of age. The semen was evaluated for gross variables and sperm chromatin condensation by aniline blue staining. Sperm DNA damage was assessed by using the comet assay at 47 weeks of age and sperm membrane proteins were assessed at 55 weeks of age. The duration of fertility was studied by inseminating 100 million sperm once into the hens of the same line as well as another line. The eggs were collected after insemination for 15 days and incubated. The eggs were candled on 18th day of incubation for observing embryonic development. The White Leghorn control line had a greater sperm concentration and lesser percentage of morphologically abnormal sperm at the different ages where assessments occurred. There was no difference in sperm chromatin condensation, DNA damage and membrane proteins between the lines. Only low molecular weight protein bands of less than 95 kDa were observed in samples of both lines. The line from which semen was used had no effect on the duration over which fertility was sustained after insemination either when used in the same line or another line. Thus, from the results of the present study it may be concluded that there was a difference in gross semen variables between the lines that were studied, however, the sperm chromatin condensation, DNA damage, membrane proteins and duration over which fertility was sustained after insemination did not differ between the lines.

1. Introduction

The rooster reproductive potential is known by assessing the semen quality. The semen quality and quantity are affected by the breed and strain of chicken (Omeje and Marire, 1990; 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). The White Leghorn lines selected for higher egg production had poorer semen quality – less semen volume, a lesser sperm concentration and greater seminal plasma lipid peroxidation in comparison with that of the control line. Apoptosis, also referred to as programmed cell death, occurs through a series of cellular, morphological and biochemical alterations without eliciting an inflammatory response. The comet assay is a simple method to study apoptosis-like DNA fragmentation in the sperm samples, and this method was used to study the effects of chicken sperm cryopreservation (Gliozzi et al., 2011). The comets

* Corresponding author.
E-mail addresses: physioshan@gmail.com, dr_shan@rediffmail.com (S. M.).

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formed after electrophoresis is usually stained with fluorescent dye and visualized using a fluorescent microscope (Madeddu et al., 2010; Gliozzi et al., 2011). Alternatively, the comets can be silver stained and visualized under a light microscope (Nadin et al., 2001) that can be visually scored or analyzed using computer software (García et al., 2007).

During the course of transport of sperm in the ductus deferens the sperm adsorb proteins from the luminal fluid (Esponda and Bedford, 1985). The sperm surface proteins in chicken sperm were studied and shown to be important in the sperm traversing the vagina to reach sperm storage tubules (Steele and Wishart, 1996).The sperm membrane proteins and role in fertility have been studied in humans (Rajeev and Reddy, 2004) and cattle (Roncoletta et al., 2006). In the literature, no articles reporting sperm membrane proteins in chickens have been found.

There are adverse effects of genetic selection for higher egg production on rooster semen variables such as sperm concentration and sperm viability, however, subcellular changes may occur in sperm as a result of selection for egg production in chickens. The aim of the present study was to assess the rooster semen quality, sperm DNA damage and sperm membrane proteins of chicken lines selected for egg production.

2. Materials and methods

The study was conducted at the experimental poultry farm of the institute located in Hyderabad, India. The roosters of two parent layer breeder lines, the White Leghorn control line and PD3 line (derived from Dahlem Red breed), were used in the experiment. The White Leghorn control line is a random bred pedigreed population without any selection being practiced and the PD3 line is selected for part period egg mass up to 40 weeks of age. The White Leghorn control line produces white eggs and the PD3 line brown eggs. Roosters from the two lines of the same hatching event were randomly selected and reared in individual cages in an open-sided house. The birds were provided with a layer breeder diet of 2600 ME (kcal/kg), 16% crude protein, 1% Ca, and 0.4% available P, and all the birds had free access to feed and water. The semen from the birds was collected at 46, 47 and 55 weeks of age. The experiment was conducted according to the guidelines of the Institutional Animal Ethics Committee.

2.1. Semen collection and evaluation

Semen from the birds was collected by abdominal massage (Burrows and Quinn, 1937) and evaluated for different gross semen variables such as semen volume, sperm concentration, sperm motility, dye reduction assay, live and abnormal sperm. Soon after collection the neat semen was diluted four times (volume/volume) using a high temperature diluent (suitable for storing semen at 20 or 40 °C), with a composition of NaCl 0.8 g; TES 1.374 g; 1MNaOH 2.75 ml; glucose 0.6 g, dissolved in 100 ml of double-distilled water and pH adjusted to 7.4, osmotic pressure to 382 mOsmol/kg water; (Chaudhuri and Lake, 1988) and used for further analysis. The volume of the ejaculated semen was assessed by using a 1 ml syringe. The raw semen appearance was visually scored 1–5 (McDaniel and Craig, 1959). The percentage of progressively motile sperm was assessed subjectively by placing a drop of diluted semen on a Makler chamber and examining at 20 × magnifications. Sperm concentration was determined in a colorimeter at 540 nm (Taneja and Gowe, 1961). The Tetrazolium dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction test was conducted and absorbance was recorded using a colorimeter (CL 157, Elico Ltd, Hyderabad, India) at 570 nm (Hazary et al., 2001). The samples that had greater metabolically active sperm produce a deep purple colored end product. The MTT dye reduction test may be used as predictor of sperm fertilizing capacity. Percent live sperm was estimated by the differential staining technique using eosin–nigrosin stain (Campbell et al., 1953). The slides were used for estimating the percent abnormal morphological sperm on the basis of observable abnormalities. Sperm were assessed for chromatin condensation by use of aniline blue staining (Park et al., 2011). Briefly, a smear of semen was made on a glass slide and was fixed in 4% formalin for 5 min. The slide was then stained with 5% aniline blue solution in PBS, pH adjusted to 3.5 using 4% acetic acid for 5 min. The slide was rinsed, dried and examined under oil immersion. Immature sperm heads stained dark blue were considered as aniline blue positive while mature sperm with protamine protein stained light blue in color were considered as aniline blue negative. A total of 100 sperm per slide was counted and percent of light blue stained sperm was calculated.

2.2. Duration of fertility after insemination

At 55 weeks of age semen samples from the roosters were collected, pooled line wise and diluted to have 100 million sperm in 0.1 ml semen. This fixed sperm dose from each line was inseminated intra-vaginally at approximately a 2.5 cm depth once into the same and other line of hens (20 hens per line) at 1500 h. The eggs were collected from the second day after insemination for15 days to study the duration of fertility. The eggs were marked for line and day of collection, and stored under refrigeration until incubation. The eggs were candled on the 18th day of incubation for observing developing embryos and percent fertility was calculated. Infertile eggs were opened by breaking the shell and contents were assessed for confirmation of fertilization.

2.3. COMET assay

The status of sperm DNA damage was assessed by comet assay. Semen samples of seven birds from each breed collected at 47 weeks of age was analyzed for sperm DNA damage. The neutral comet assay for determining DNA fragmentation was performed as described by Gliozzi et al. (2011) for chicken sperm with a few modifications. Briefly, the semen samples were washed twice by centrifugation in PBS. The sperm (approximately 1×10^6/ml) were mixed with low melting point agarose gel solution (LMPA, 0.8% w/v in PBS) at 37 °C and pipetted on a glass slide coated with normal melting point agarose (NMPA, 1.5% w/v in PBS)