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Effect of semen parameters on duration of fertility in layer breeder chicken

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ABSTRACT

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The present study was conducted to evaluate the effect of semen parameters on duration of fertility. Eleven birds from White Leghorn and PD3 lines (29 weeks age) were randomly selected for semen collection by abdominal massage. Semen samples were evaluated for different gross semen parameters. Seminal plasma was separated and evaluated for lipid peroxidation and progesterone level. White Leghorn males had significantly (P<0.05) higher sperm concentration, MTT dye reduction activity and membrane integrity (hypo-osmotic swelling test). The semen volume and abnormal sperm were significantly (P<0.05) higher in PD3 line. There were no significant differences in seminal plasma lipid peroxidation and progesterone levels. At 30th week an artificial insemination (AI) trial was conducted using fixed dose of sperm (100 millions) in 0.1ml of semen for studying duration of fertility. Semen from each line was pooled and inseminated into 20 hens of respective lines. No difference in duration of fertility was observed between the two lines. The results of this study indicated that though there were differences in semen quality between the lines it did not influence the duration of fertility.
Keywords: Semen, Fertility, Breeder, Layer Chicken

INTRODUCTION

Semen quality is affected by many factors like heredity, feed nutrients and semen collection techniques. Semen evaluation is essential before artificial insemination and semen quality is an important factor affecting fertility. Among different physical semen characters motility, live sperm and morphology fairly reflects fertility outcome but predicting fertility only with these parameters is very difficult. For example, it has been shown that plasmalemma associated glycoproteins removed sperm had normal motility but failed to reach the sperm storage tubules (SST) (Wishart and Steele, 1990). In poultry sperm are stored in uterovaginal SST, periodically released and fertilization takes place. It is still unclear that how sperm are selected, stored and periodically released from the SST. Sperm having normal membrane integrity swell in a hypo-osmotic environment (Jeyendran *et al*., 1984). There is scanty information available on effect of certain semen parameters like metabolic activity, membrane integrity and sperm morphology on duration of fertility in chicken. Thus, present study was carried out to evaluate the effect of different semen parameters on duration of fertility in layer chicken.

MATERIALS AND METHODS

The experiment was conducted at the experimental poultry farm of the Institute located at Hyderabad, in accordance with the guidelines of the Institutional Animal Ethics Committee. Eleven roosters (29 weeks age) of PD3 and White Leghorn layer (IWA) lines of same hatch reared in individual cages in an open-sided house were randomly selected and used for the experiment. PD3 line was evolved through selection from Dahlem Red breed. Semen from the birds was collected by abdominal massage (Burrows and Quinn, 1937) and evaluated for different gross semen parameters. Soon after collection the neat semen was diluted four times using high temperature diluent (Chaudhuri and Lake, 1988) and used for further analysis. The volume of the ejaculated semen was assessed by using a 1 ml syringe. The appearance of raw semen was scored visually in a scale of 1 to 5 (McDaniel and Craig, 1959). The progressively motile sperm was assessed subjectively after placing a drop of diluted semen on a Makler chamber and examining at 20 × magnification. The concentration of sperm was determined in a colorimeter at 540 nm (Taneja and Gowe 1961). Tetrazolium dye 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) reduction test was carried out and absorbance was recorded using a colorimeter at 570 nm (Hazary *et al*., 2001). The live sperm was estimated by using eosin–nigrosin stain (Campbell *et al*., 1953). The slides were also used for calculating the percent abnormal sperm on the basis of observable abnormalities. The plasma membrane integrity was determined through hypo-osmotic swelling test as described for chicken spermatozoa (Santiago-Moreno *et al*., 2009). Seminal plasma was separated by centrifugation of raw semen samples at 1500 x g and assessed for level of lipid peroxidation (LP) (Hsieh *et al*., 2006) and progesterone (P4) concentration by EIA kit (Diametra, Italy).

At 30 weeks of age the semen samples collected from the roosters was pooled line wise and diluted to contain 100 million sperm in 0.1 ml semen. This fixed sperm dose was then inseminated once into respective

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line hens of same age (20 hens per line) that were never inseminated before. Eggs were collected from second day after insemination till twenty days for studying duration of fertility. The eggs were marked for line and day of collection, stored under refrigeration till incubation. The eggs were candled on eighteenth day of incubation for observing developing embryos and percent fertility calculated. Infertile eggs were broke opened for confirmation.

Statistical analysis

Statistical analysis to determine differences in semen parameters and duration of fertility between the lines was done by Student's t test (SAS 9.2). The treatment means were compared at P<0.05. Percent values were arcsine transformed before analysis of data.

RESULTS AND DISCUSSION

The semen quality was comparatively inferior in PD3 with low sperm concentration, MTT dye reduction activity, percent hypo osmotic sperm swelling and high abnormal sperm percent (Table 1). The duration of fertility was 17 and 15 days in PD3 and White Leghorn respectively (Fig. 1). There was no significant difference in percent overall fertility between the lines. Comparison of the duration of fertility as first seven days and rest of the period also did not give any significant difference between the lines.

Table 1: Gross semen and seminal plasma parameters (mean±SE) in layer breeder lines

Semen parameters	PD3 (n=11)	White Leghorn (n=11)
Volume (ml)	0.49±0.05[a]	0.30±0.04[b]
Appearance	3.45±0.21	3.90±0.28
Sperm motility (%)	49.09±1.62	53.64 ± 2.34
Sperm concentration (million/ µl)	4.61±0.32[b]	5.96±0.30[a]
MTT dye reduction test (nM of MTT Formazan/ min/million sperm)	17.91±1.16[b]	21.01±1.29[a]
Live sperm (%)	91.53±1.65	94.13±0.72
Abnormal sperm (%)	2.55±0.53[a]	0.98±0.20[b]
Hypo-osmotic sperm swelling (%)	87.65±1.02[b]	92.97±1.03[a]
Seminal plasma lipid peroxidation (nmol MDA/mg protein)	1.77±0.36	1.69±0.27
Seminal plasma Progesterone (ng/ml)	0.15±0.01	0.14±0.02

Means with different superscripts in a row differ significantly (P< 0.05)

The sperm concentration was different between the lines and which may affect the fertility results; the insemination dose was fixed uniform. It is well accepted that for evaluating sperm fertilizing ability among different treatments the number of sperm used for insemination should be fixed. The MTT dye reduction test indicates the metabolic activity of the sperm and is highly correlated with sperm ATP content and fertilizing ability (Hazary *et al*., 2001). In the present study though there was difference between the lines in MTT dye reduction test it could not be observed to be translated in the fertility data. In the PD3 line different sperm abnormalities observed were knotted head, bent head, bending or

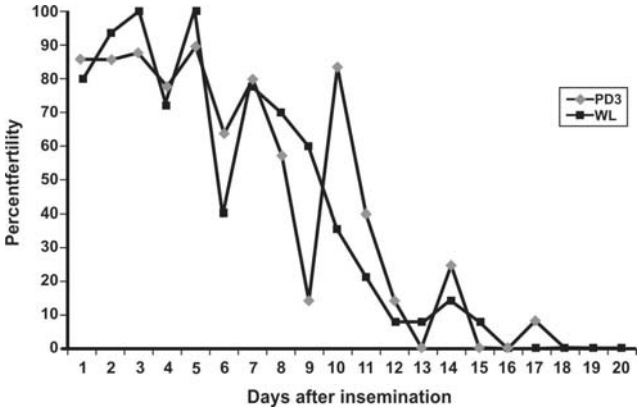

Fig. 1: Duration of fertility after artificial insemination in PD3 and White Leghorn lines

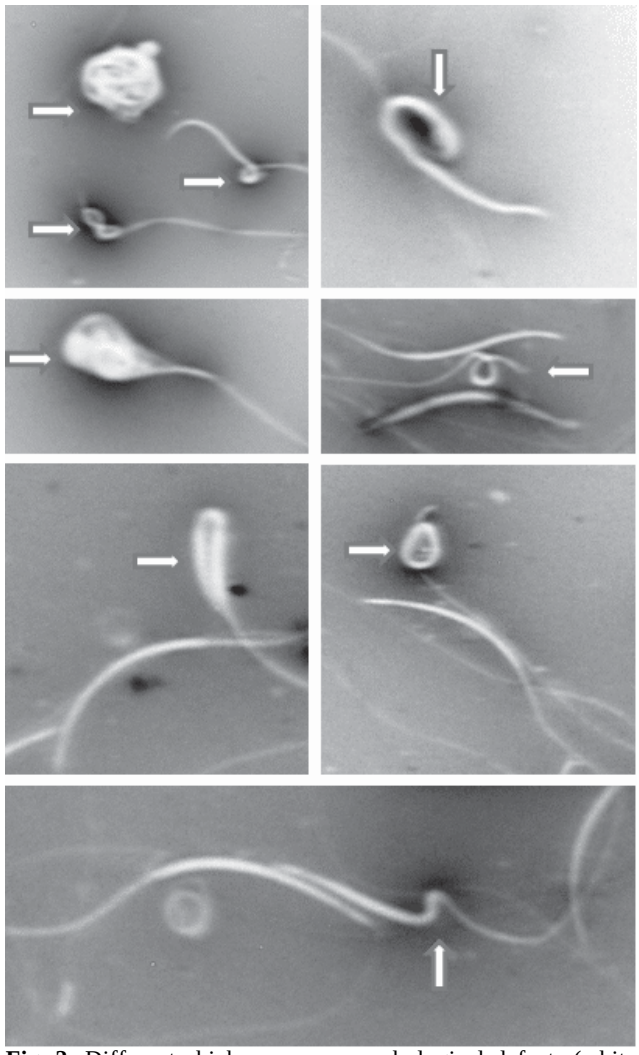

Fig. 2: Different chicken sperm morphological defects (white arrows)

knotting at head mid piece junction and curled tail (Fig. 2). The difference in percent abnormal sperm between the lines did not affect the duration of fertility. It may be due to the reason that during populating the SST the abnormal sperm are eliminated, however this needs confirmation. Significant higher seminal plasma P4 concentration than that in serum was reported earlier (Anderson and Navara, 2011). Furthermore, it was suggested that higher seminal plasma P4 might result in reduced sperm fertilizing ability. In the present study there was no difference in the seminal plasma P4 level between the two lines studied. The P4 levels in the present study was very low but could not be compared to the earlier report due to difference in assay procedure and processing of sample (extraction of P4). In the fertility results, apart from the quality of semen the contribution of females is equally important. Reports indicate that the age of hen and repeated inseminations have adverse effects on storage capacity of SST and finally fertility (Végi *et al*., 2013; Das *et al*., 2005). The hens utilized in this experiment were young and inseminated first time and the above said disadvantages were not present.

In the inseminated sperm about 1% only is retained in the SST (Brillard, 1993). Furthermore, a sperm dose of 25 million is sufficient to achieve more than eighty percent fertility (Wishart and Steele, 1985). Thus, in the above-mentioned processes, damaged or defective sperm are eliminated and normal sperm gets populated in the SST resulting in fertility duration comparable to that in the line having better sperm characteristics. Our results are similar to that reported by Kirby *et al*. (1998); the authors could not find any difference in duration of fertility in five broiler breeder lines though there was difference in sperm integrity.

From the results of this study it can be concluded that traditional semen parameters like sperm motility, mitochondrial activity and morphological defects may not provide accurate information on the fertility duration outcome in layer breeders.

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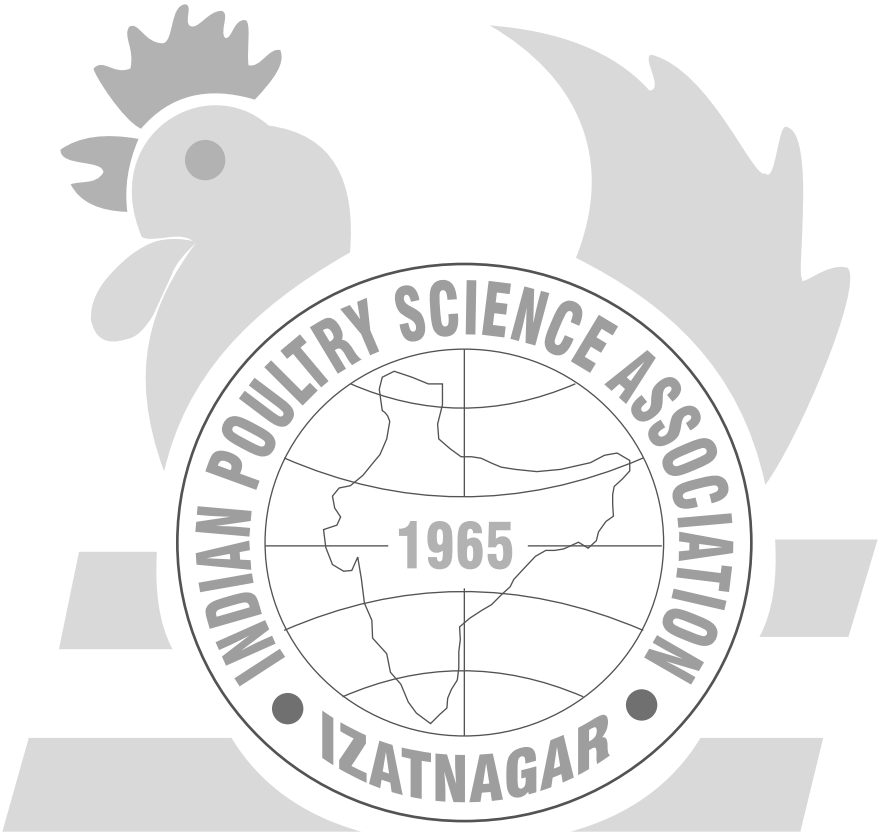
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