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Research Article

Effect of Diluents and Preservation on Time Native Cock Semen

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ABSTRACT

Evaluation, dilution and preservation are the essential part of quality determination of native cock semen. Total of 21 semen samples were collected by abdominal massage from 7 native cocks at 3 days interval. Fresh semen was examined macroscopically for physical parameters. Most of the samples were found in yellowish white in color. The mean±SD volume, motility, concentration, dead and morphologically abnormal spermatozoa of semen samples varied from 0.3±0.01 to 0.5±0.03 ml, 58.3±2.9 to 71.7±3.5%, 3733±153 to 6067±130 x 10^6/ml, 22.3±4.51 to 33.7±1.53% and 18.3±7.8 to 30.7±1.5%, respectively. Cock # 1 showed the highest body weight (1.3 kg) and produced significantly ($P<0.05$) highest ejaculate volume and motility among the cocks. However, significantly ($P<0.05$) higher concentration of spermatozoa was observed in cock # 5 compared with others. The most frequent sperm abnormalities recorded in the mid-piece (8.5 to 13.0 %), followed by the head (3.5 to 6.0%) and tail (1.5 to 6.0%) of spermatozoa. After diluted in Lake A and Lake B (antibiotic added) diluents and preserved at 4ºC for two days, semen samples were examined on Day 0 (15 min chilled), Day 1 and Day 2, respectively. With the advancement of preservation, sperm motility, live spermatozoa and sperm morphology deteriorated progressively in both the diluents. However, significantly ($P<0.05$) higher percentage of motility was observed on Day 0 (60.6±2.4%) compared with Day 2 (39.2±2.0%) in Lake A diluent only. it was observed that Lake B diluent showed significantly ($P<0.05$) higher number of motile spermatozoa than Lake A on Day 1 and 2. Similar with the motility, significantly ($P<0.05$) higher proportion of live spermatozoa was present in Lake A on Day 0 compared with Day 2. However, proportion of live spermatozoa was significantly higher in Lake B than Lake A on Day2 only. Significantly higher ($P<0.05$) number of tail abnormality was present on Day 2 compared with Day 0 and 1 in both diluents. The overall abnormalities were found higher when diluted in Lake A than Lake B. The semen quality can be affected by the absence of antibiotic in the diluents and various preservation times.

Key words: Cock, Semen, Diluents, Preservation

INTRODUCTION

Artificial insemination (AI) is the important technology for genetic improvement through male line. Evaluation of semen quality for breeding soundness is of great importance for the feasibility of AI. For good results through AI of chickens, the quality of semen should be ensured (Alkan *et al.,* 2001). The importance of semen evaluation in poultry breeding for selecting breeding males or for routinely monitoring their reproductive performance are well recognized in elsewhere (Cheng *et al.,* 2002). The increasing importance of AI in poultry reproduction has caused investigators to become interested in developing the proper conditions for liquid (short-term) and frozen (long-term) semen storage (Van Wambeke, 1967, Lake and Ravie, 1981, Lukaszewicz *et al.,* 2004). The cock ejaculate is generally small in volume, but highly concentrated so there is a potentiality of extending it with relevant diluents, at specific rates, prior to AI and storage (Blesbois, 2007). There are many unique characteristics of cock spermatozoa that limits its viability for AI, either fresh or post freeze/thawing. The sperm motility and fertilizing ability of cock spermatozoa generally deteriorates within one hour after collection, if stored in vitro (Dumpala *et al.,* 2006).

Several studies on AI and cock semen viz: artificial insemination and its application to poultry industry, improvement of reproductive efficiency through prediction of fertilizing ability and high temperature semen extender (Chaudhuri, 1996), effect of supplementary feeding on

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production performance (Rahman *et al.,*1998), effects of feeding on semen production in native cock (Das, 2002a), AI in native chicken with special reference to the role of immunocopetent cells in the sperm host glands to spermatozoa (Das, 2002b) and AI by raw semen: its advantages and disadvantages in deshi chicken (*Gallus domesticus*) (Das *et al.,* 2004) have been conducted in Bangladesh. However, no comprehensive study on effects of diluents and storage time on the quality of cock semen have been performed. Therefore, it seems to be inclination to evaluate the native cock semen immediately after collection, determine the proper diluents and storage time and morphological evaluation of cock semen for betterment of artificial insemination in poultry industry throughout the country.

MATERIALS AND METHODS

The study was conducted in the Department of Surgery and Obstetrics, Bangladesh Agricultural University, Mymensingh during the period from July 2010 to November 2010.

Selection of birds

Apparently healthy seven (n= 7) mature local cocks were purchased from the various local markets of the Mymensingh district devoid of any external anatomical deformities. The average age and body weight of cocks were 34±1.15 weeks and 1.14±0.13 kg respectively.

Housing and management

Each bird was kept in individual large cages. They were dewormed against digestive and gastrointestinal round worm by using Avinex® powder (Renata Limited, Bangladesh) and vaccinated against Ranikhet diseases using intramascular injection of RDV (LRI, Mohakhali, Bangladesh). Each bird was fed with 120gm commercial Layer Layer 1 (Quality Feeds Limited, Bangladesh) mash diet daily, containing 2900 kcal/kg ME and 19% CP. Total feeds were given in two splits per day. Water was provided ad-libitum. During the four weeks of pre-experimental period, cocks were trained twice daily for semen collection by the massage technique.

Semen collection

Each cock was ejaculated three times at three days interval between 9.00 to 10.00 AM throughout the experimental period. The cocks were taken gently from the cage and manipulated immediately. Semen was collected by abdominal massage technique (Lake, 1957) followed by manipulation of cloaca as described by Tabatabaei *et al.* (2009). The ejaculated volume was collected by 1ml disposable syringe. To minimize the stress and maximize the quality of semen, collection was always performed by the same operators, at the same time, and under the same conditions. Special care was taken to avoid contamination of semen with urine and transparent fluid, which could degrade the semen quality.

Dilution of semen

Within 30 min. of collection, semen samples were divided into 3 parts. One part was kept for fresh semen evaluation viz; mass activity, motility, concentration, viability and morphology. Other two parts were diluted in to 1:4 ratio (1part of semen: 4 part of extender) at room temperature with 2 different extenders viz: Lake A and Lake B (Table 1) and was placed into 10ml glass tubes and stored at 4°C in a refrigerator for further evaluation. The evaluation was done for the effects of two different semen extenders on the motility, viability and sperm morphology on immediately after addition of diluents and Day 1 and Day 2 hrs of preservation.

Preparation of Nigrosin-Eosin stain

The stain was prepared according to Evans and Maxwell (1990), by dissolving 0.85 gm of Eosin (*E. Merck*, Germany), 5 gm of Nigrosin (H_2O soluble, British Drug House Ltd. England) and 1.45 gm of sodium citrate (H_2O soluble; *E. Merck*, India) in 50 ml of distilled water.

Semen evaluation

Several parameters were evaluated to characterize each cock's fresh semen quality and effects of diluents and preservation periods. These were semen volume, color, mass activity, motility, concentration, viability and morphology.

Volume and color: The ejaculate volume and color of the fresh semen was recorded directly from the semen collection syringe immediately after collection by necked eyes. The color was scored into 4 scales: 1=opalescent, 2=milky white, 3=yellowish white and 4=creamy white.

Mass activity: To evaluate mass activity, a drop (25µl) of semen was placed on a pre-wormed (+37ºC) glass slide without cover slip and examined under light microscope at low magnification (10X). Mass activity was considered into 4 scales, 1=no mass activity, 2=slow wave motion without forming any waves, 3=rapid wave motion with formation of eddies at the end of waves, 4=very rapid wave motion with distinct eddies.

Motility: For evaluation of motility, one drop of the diluted semen was placed on the slide and covered with glass cover. The sperm motility was estimated by microscopic observation (40X). Motility was expressed as the percentage of motile spermatozoa with moderate to rapid forward progressive movement. At least 10 microscopic fields were examined for each sample.

Concentration: The concentration of spermatozoa was determined by using hemocytometer technique as described by Bane (1952). A drop of (10µl) diluted semen (1:100) was placed on the edge of cover slip and spermatozoa were allowed to settle for five minutes on the hemocytometer before placing on the stage of microscope. The spermatozoa were then counted in five large squares; four at corners and one at the centre of 25 large squares. The numbers of spermatozoa in five large squares were counted and average number was recorded. The concentration of the spermatozoa per ml of semen was calculated by multiplying the average number of spermatozoa in large five squares with dilution factor and expressed as 10^6 million per ml.

Table 1: Composition of diluents used for preserving cock semen for 24 and 48 hours respectively at 4°C

Components[1]	Diluents[2]	
	A	B
Sodium glutamate	1.35	1.35
Potassium citrate	0.128	0.128
Sodium acetate	0.51	0.51
Glucose	0.80	0.80
Streptomycin sulphate	-	0.20
Penicillin G sodium	-	0.04

[1]Values are given in grams per 100ml distilled water; [2]A= Diluent described by Lake (1960), B= Modification of Lake A

Sperm viability: The proportion of live spermatozoa was evaluated in Nigrosin-Eosin smears under a light microscope. In every slide, 200 cells were counted and classified as live (unstained cells) and dead (every cell stained red with Eosin against the dark Nigrosin background).

Sperm morphology: Sperm morphology was also evaluated in Nigrosin-Eosin smears under a light microscope With a Nigrosin-Eosin staining; it is possible not only to demonstrate the viability of spermatozoa, but also to identify within a live sperm population the fraction of spermatozoa with abnormalities in morphology. This method is well tested and has been widely used to assess semen quality (Lake *et al.,* 1958, Blesbois *et al.,* 1999). In every slide, 200 cells were counted. The morphological defects of head, mid-piece, tail and their proportions were evaluated. Spindle-shaped head with well-marked acrosome and visible tail within the fraction of live cells were classified as morphologically normal spermatozoa. On the other hand with a swollen head, bent neck, defective midpiece, or other deformity (coiled tail, lack of tail, bent tail, knotted tail etc.) were classified as morphologically abnormal. The morphological evaluation of spermatozoa and proportion of live and dead in a sample was assessed on the basis of 200 spermatozoa.

Statistical analyses

The mean and standard deviation (SD) of ejaculate volume, color, mass activity, motility, concentration, dead sperm and sperm with abnormal head, mid-piece and tail were measured by using descriptive statistics in Microsoft Office Excel work sheet 2007. The percentage of motility, live sperm, morphologically abnormal spermatozoa and the proportion of different (Head, Mid-piece and Tail) abnormalities were calculated as pooled data. The data recorded were compiled, tabulated and analyzed in accordance with the objective of the study by one way analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) using MSTATC® software. The difference between values was considered significant when the P value was less than 0.05.

RESULTS

Evaluation of fresh semen

Body weight of cock and volume of ejaculate: The body weights of cocks # 1, 2, 3, 4, 5, 6 and 7, used in this experiment were 1.1±0.13 kg. The mean volume of semen from different cocks were 0.5±0.03, 0.4±0.02, 0.4±0.01, 0.3±0.05, 0.3±0.03, 0.3±0.01 and 0.3±0.02 ml, respectively (Table 2).Cock # 1 showed the highest body weight (1.3 kg) and produced significantly (P<0.05) highest ejaculate volume (0.5 ml) than other cocks (Table 2).

Color: Most of the cock ejaculated yellowish white to creamy white color semen.

Mass activity: The mean±SD value of mass activity of fresh semen of seven different cocks # 1, 2, 3, 4, 5, 6 and 7 were 3.0±0.0, 3.7±0.58, 3.7±0.58, 3.0±1.0, 3.3±0.58, 3.0±0.0 and 3.7±0.58, respectively (Table 2). Mass activity was considered into 4 scales.

Motility: The mean±(SD) value of motility of spermatozoa (%) immediately after collection from cocks # 1, 2, 3, 4, 5, 6 and 7 were 71.7±3.51, 58.3±2.88, 64.7±5.85, 65.7±4.93, 65.3±5.03, 62.3±5.13 and 61.3±3.51%, respectively (Table 2). The sperm motility varied from 55 to 75% and were significantly (P<0.05) high in cocks # 1 compared to other cocks.

Sperm concentration: The mean±SD value of sperm concentration (X 10^6/ml) of fresh semen of different cocks # 1, 2, 3, 4, 5, 6 and 7 were 4093±122, 4640±151, 3733±153, 4967±321, 6067±130, 5367±252 and 5267±153, respectively (Table 2). The sperm concentration varied from 3600-6200 x10^6 /ml. The differences in sperm concentration among the cocks # 3, 4 and 5 were significantly (P<0.05) different. The sperm concentration was significantly high in cock # 5 compared to rest of the cocks.

Dead spermatozoa: The mean±SD value of dead spermatozoa (%) of fresh semen of different cocks # 1, 2, 3, 4, 5, 6 and 7 were 22.3±4.51, 25.3±3.51, 27.7±3.21, 23.3±3.21, 25.7±4.73, 33.7±1.53 and 31.7±3.06%, respectively (Table 2). The number of dead sperm was varied from 22 to 34%, whereas cock # 6 showed highest number (33.7±1.53%) of dead sperm. Significantly (P<0.05) higher number of dead sperm was present in cock # 6 compared with cock # 1, 2, 4 and 5. Similar difference was present in cock # 4 compared with cock # 7 (Table 2).

Sperm morphology of fresh cock semen

The mean±SD values of sperm morphology of fresh cock semen are presented in the Table 3.

Sperm head abnormalities: The normal head shape of cock spermatozoa is cylindrical, not wide in diameter and containing less cytoplasm. The sperm head abnormalities (%) were recorded as acrosomal defects (acrosomal detachment and acrosomal swelling), bent head, head detachment, knotted head, smaller and larger head, respectively. The mean proportion (%) of sperm with abnormal head of seven cocks # 1, 2, 3, 4, 5, 6 and 7 were 3.5±0.20, 4.3±0.20, 4.6±0.02, 5.6±0.30, 6.0±0.20, 4.5±0.20 and 4.3±0.01%, respectively (Table 3). The number of sperm with abnormal head in the fresh semen varied from 3.5 to 6.0%. Significantly higher (P<0.05) abnormal head was observed in cock # 5 compared with others. Similar difference was observed between 2 and 3 compared with 4 and 5.

Table 2: Mean evaluated characteristics of fresh cock semen

Cock no	Body weight (kg)	Volume (ml)	Color (1 to 4 scales)	Mass activity (1 to 4 scales)	Motility (%)	Concentra-tion (x 10^6/ml)	Dead sperm (%)
1	1.3	0.5±0.03^{a}	Yellowish white	3.0±0.00^{a}	71.7±3.51^{a}	4093±122^{bc}	22.3±4.51^{c}
2	1.2	0.4±0.02^{b}	Creamy white	3.7±0.58^{a}	58.3±2.88^{c}	4640±151^{bc}	25.3±3.51^{bc}
3	1.1	0.4±0.01^{b}	Yellowish white	3.7±0.58^{a}	64.7±5.85^{ab}	3733±153^{c}	27.7±3.21^{abc}
4	0.9	0.3±0.05^{c}	Yellowish white	3.0±1.00^{a}	65.7±4.93^{b}	4967±321^{b}	23.3±3.21^{c}
5	1.1	0.3±0.03^{c}	Creamy white	3.3±0.58^{a}	65.3±5.03^{b}	6067±130^{a}	25.7±4.73^{bc}
6	1.2	0.4±0.01^{b}	Creamy white	3.0±0.00^{a}	62.3±5.13^{b}	5367±252^{b}	33.7±1.53^{a}
7	1.2	0.3±0.02^{c}	Yellowish white	3.7±0.58^{a}	61.3±3.51^{bc}	5267±153^{b}	31.7±3.06^{ab}

a, b, c = The values with superscript within the same column differed significantly from each other (P<0.05).

Table 3: Abnormalities of cock spermatozoa

Cock no	Head (%)	Mid-piece (%)	Tail (%)	Total abnormalities (%)
1	3.5±0.20^{d}	8.5±0.20^{f}	6.0±0.10^{a}	18.3±7.8^{c}
2	4.3±0.20^{c}	9.0±0.20^{e}	6.0±0.02^{a}	24.7±1.5^{abc}
3	4.4±0.02^{c}	10.3±0.05^{cd}	4.1±0.02^{b}	18.7±2.5^{c}
4	5.6±0.30^{b}	12.1±0.02^{b}	6.0±0.20^{a}	22.3±1.5^{bc}
5	6.0±0.20^{a}	10.5±0.01^{c}	2.5±0.10^{c}	21.3±2.1^{bc}
6	4.5±0.20^{c}	13.0±0.20^{a}	1.5±0.20^{d}	30.7±1.5^{a}
7	4.3±0.01^{c}	10.0±0.50^{d}	6.0±0.02^{a}	27.7±2.1^{ab}

a, b, c, d, e, f = The values with superscript within the same column differed significantly from each other (P<0.05).

Table 4: Effects of diluents and storage times on the motility (%) of spermatozoa

Diluents	Preservation Period		
	Day 0	Day 1	Day 2
Lake A	^{x}60.6±2.4^{a}	^{x}52.9±2.3^{b}	^{y}39.2±2.0^{b}
Lake B	^{x}61.7±3.1^{a}	^{x}58.1±3.3^{a}	^{x}52.2±2.4^{a}

Number of observations in each cell = 21, a, b= the values with superscript within the same column and x, y = within the same row differed significantly from each other (P<0.05).

Table 5: Effects of diluents and preservation periods on the live spermatozoa (%)

Diluents	Preservation Period		
	Day 0	Day 1	Day 2
Lake A	^{x}69.0±4.5^{a}	^{x}62.6±4.4^{a}	^{y}45.1±3.8^{b}
Lake B	^{x}70.2±4.3^{a}	^{x}64.9±4.6^{a}	^{x}58.0±4.2^{a}

Number of observations in each cell = 21, a, b= the values with superscript within the same column and x, y = within the same row differed significantly from each other (P<0.05).

Table 6: Effects of diluents and preservation periods on the proportion of abnormal spermatozoa with respect to head

Diluents	Head abnormalities (%)		
	Day 0	Day 1	Day 2
Lake A	^{x}6.0±1.5^{a}	^{x}7.3±1.6^{a}	^{x}9.7±1.3^{a}
Lake B	^{x}5.7±5.0^{a}	^{x}6.7±1.4^{a}	^{x}8.4±1.7^{a}

Body weight may be a good indicator of semen volume in native cock; Number of observations in each cell = 21, a, b= the values with superscript within the same column and x, y = within the same row differed significantly from each other (P<0.05).

Sperm mid-piece abnormalities: The sperm mid-piece abnormalities were recorded as mid-piece knotting, mid-piece detachment, mid-piece swelling and mid-piece bent. The mean number of sperm with abnormal mid-piece (%) of seven cocks # 1, 2, 3, 4, 5, 6 and 7 were 8.5±0.20, 9.0±0.20, 10.3±0.05, 12.1±0.02, 10.5±0.01, 13.0±0.20 and 10.0±0.50%, respectively. The number of sperm with abnormal mid-piece in the fresh semen varied from 8.5 to 13.0%. Significantly (P<0.05) higher mid-piece abnormalities was observed in cock # 6 compared with others. Similar difference was also observed among the other cocks (Table 3).

Sperm tail abnormalities: The sperm tail abnormalities were recorded as bent tail, tail knotting and tail detachment. The mean number of sperm with abnormal tail (%) of seven cocks # 1, 2, 3, 4, 5, 6 and 7 were 6.0±0.10, 6.0±0.02, 4.1±0.02, 6.0±0.20, 2.5±0.10, 1.5±0.20 and 6.0±0.02%, respectively (Table 5). The number of sperm with abnormal tail in the fresh semen varied from 1.5 to 6.0%. Significant (P<0.05) variation was observed with abnormal tail among the cocks # 3, 5, 6 compared with other (Table 3).

Comparison of diluted semen

Effects of diluents and preservation times on the sperm motility (%) (Mean±SD)

The effects of diluents and preservation times on the sperm motility of pooled semen (n= 21) samples are presented in Table 4. Sperm motility varied from 39 to 62% and decreased gradually with the advancement of preservation time irrespective of the diluents used. Fifty percent sperm motility was maintained upto Day 2 in Lake B diluents only. Significantly higher (P<0.05) number of motility was present on Day 0 compared with Day 2 in Lake A diluent only. Between two diluents, significantly (P<0.05) higher number of motility were observed on Day 1 and Day 2 in Lake B than Lake A, respectively. However, no significant difference was existed between the two diluents on the day of collection.

Effects of diluents and storage times on the live sperm (%) (Mean±SD)

The effects of diluents and preservation times on the live sperm of semen samples are presented in Table 5. Live sperm varied from 45 to 70% depending on the storage times and diluents used. In both the diluents live sperm decreased gradually with the advance of preservation time. Fifty percent live sperm was maintained up to Day 2 in Lake B diluent and Day 1 in Lake A diluent. Regarding live sperm, both Lake A and B diluents showed the similar results like that of sperm motility. Significantly higher live sperm was present in Lake A on Day 0 compared with Day 2. Similar with motility significantly higher live spermatozoa was present in Lake B compared with Lake A diluents on Day 2 (P>0.05).

Effects of diluents and preservation periods on the proportion of abnormal spermatozoa with respect to head (Mean±SD)

Depending on the preservation periods and diluents used the mean proportion of abnormal spermatozoa with respect to head varied from 5.7 to 9.7% (Table 6). The abnormalities of head increased gradually with the

advancement of preservation period irrespective of the diluents used. There was no difference among different preservation time on head abnormality within each diluent. Between two diluents, head abnormality was found slightly higher in Lake A diluent than B. However, there was no significant (P>0.05) variation between them (Table 6).

DISCUSSION

The characteristics and morphology of fresh semen and the effects of Lake Diluents (A and B) on different preservation on sperm motility (%), live sperm (%) and morphology of semen of native cock was observed. The effects of body weight on ejaculate volume were also determined.

Body weight may be a good indicator of semen volume in native cock. Generally, poultry breeds with heavier body weights larger testes and produce more spermatozoa during spermatogenesis and thus yield bigger semen volume (Adeyemo *et al.,* 2007). Similarly in the present study, cock with a heavier body weight (1.3 kg) resulted in highest semen volume. On the other hand, the ejaculate volume in the present study was lower (Tuncer *et al.,* 2008) or higher (Bah *et al.,* 2001, Galal, 2007, Peters *et al.,* 2008) than other studies. There may be many reasons contributing to these lower semen volumes, e.g. breed, age, individual differences, body weight, stimulation, season and environmental and management factors.

The motility of cock spermatozoa was found 58.3 to 71.7 % in native cock during this study. Sperm motility was found significantly higher in cock which had a heavier body weight. However, the lighter body weight cock did not show much lower value of sperm motility. So that, it can be said that cock with lighter body weight could be used as breeding purpose. Mosenene (2009) has reported the percentage of motile sperm varied between 67.9±0.5% and 70.1±0.6% for fresh semen samples of cocks, which is near to the findings of this study. Some researchers (Chalah *et al.,* 1999, Bah *et al.,* 2001) have also recorded the sperm motility in fresh semen samples varied from 73.9±0.2 to 83.2±0.6%. These results are higher than the results obtained in this study. This may also be due to the time taken before the semen was processed and evaluated and the season of semen collection.

In this study, the sperm concentration varied from $3600x10^6$ to $6200x10^6$ per ml. These results are in agreement with Hafez and Hafez (2000), they showed that the sperm concentration in the domestic cock ranged from 3000 to 7000 million per ml. Chalov (1970), McDaniel *et al.* (2004) also reported the value of sperm concentrations of 3800×10^6 and $7500\pm0.21 \times 10^6$ per ml, respectively. These results were also in agreement with the present study. But Keskin *et al.* (1997) and Ezekwe *et al.* (2003) reported a lower value of sperm concentration (2000×10^6 per ml), which is contrary to this study. The differences in the semen concentration among ejaculates may be attributed to several factors such as age, season, individual performance and frequency of semen collection.

The proportion of dead spermatozoa in fresh semen samples varied from 22.3 to 33.7% in this study, which is considered high. The present findings are more or less consistent with the 18 to 28% recorded by Siudzinska and Lukaszewicz (2008b). However, Tabatabaei *et al*. (2009) recorded much lower percentage (11 to 18%) of dead spermatozoa in fresh semen sample which is contrary to current findings. The higher number of dead sperm recorded in the study may be attributed to various factors as mentioned above for the motility.

The present results show that the morphologically abnormal spermatozoa in fresh semen samples varied from 18.3% to 30.7% among the cocks studied. Tselutin *et al.* (1999) reported the number of morphologically abnormal spermatozoa of cock semen varied from 6 to 9%, which is lower than that of the present study. However, Siudzinska and Lukaszewicz (2008a) recorded 20 to 30% morphologically abnormal sperm, which is again more consistent with the results obtained in this study. The most frequent sperm abnormality recorded in this study was in the sperm mid-piece (8.5 to 13.0 %), followed by that in sperm head (3.5 to 6.0%) and tail (1.5 to 6.0%). Alkan *et al.* (2001) recorded that, head, mid-piece and tail abnormalities were 6.8, 26.9 and 6.0%, respectively. These variations in the proportion of morphological abnormalities of spermatozoa in the study may be attributed to the techniques used for collection and evaluations.

For the extender added to dilute semen, motility is an indicative of the viability of spermatozoa and the quality of diluted semen. The motility was found higher in fresh semen than semen diluted with Lake A and B diluents. During different preservation times (Day 0= 15 minutes after addition of diluents, Day 1 and Day 2 kept at 4ºC) the sperm motility decreased with the advancement time of preservation. However, the difference did exist only in Lake A diluent. The higher motility was found in Lake B diluent compared with Lake A, indicating the good effect of using Lake B diluent for preservation of cock semen. Most of the components used of these two diluents were same; except antibiotic was added in Lake B diluent. The sperm motility may be affected by preservation periods, storage temperature and ingredients used in the diluents.

In this study, Lake A diluent has shown an adverse effect on motility according to the increasing preservation periods. These observations are in agreement with the (Keskin *et al*., 1997 and Mosenene, 2009). Antibiotics can also increase motility and fertility of spermatozoa when used as a diluent in semen (Sexton, 1980, Bearden *et al*., 2004). There are several factors affecting sperm motility following semen dilution. Parker and McDaniel, (2006) reported avian sperm motility to be dependent on the amount of oxygen and Ca++ ions present and advise to evaluate sperm motility before and after preservation. The present results concluded as diluent with antibiotics has beneficial effects of cock's semen for preservation.

In the present study, significantly lower number of live spermatozoa (45.1±3.8%) was found in the Lake A diluent on Day 2 with respect to Day 0 and Day 1. In Lake B diluent there was no significant variation found among the preservation periods. As mentioned above the addition of antibiotic might result this differences between two diluents. The number of live spermatozoa was reduced according to the increased preservation periods. These observations are also in accordance with the other researchers (Keskin *et al*., 1997, Mosenene, 2009).

During the present study, the proportion of morpho-logically abnormal sperm did not differ significantly

according to the diluents used. Significantly higher number of abnormal spermatozoa was found at Day 2 in Lake A diluent in respect to Day 1 and Day 0. Although no difference was found between two diluents, however, decreased percentages of morphological abnormal spermatozoa in Lake B indicate the better use of this agent in diluent for preservation of cock semen. This concluded that, antibiotic has no effect on sperm morphology. Sperm head morphology is determined during spermatogenesis and does not get altered afterwards. The commercial diluent also contain antibiotic in their ingredients.

The current trial showed that the simple inexpensive method of eosin-nigrosin staining could be used to evaluate cock semen which was used by various researchers (Lake and Stewart, 1978, Lukaszewicz *et al.,* 2008, Mosenene, 2009). The quantity and quality of fresh semen still stays largely dependent on the individual male (Lukaszewicz and Kruszynski, 2003). The present study found that, during the time of storage, a decrease percentage in live, morphologically normal spermatozoa and an increase in dead spermatozoa, which was in agreement with studies performed by other investigators (Blesbois *et al.,* 1999, Lukaszewicz *et al*., 2004). Regardless of the semen extender used, the number of live and normal sperm declined due to the semen storage time, as temperature changes affect sperm viability, hence increased sperm abnormalities (Lukaszewicz and Kruszynski, 2003). In this study, the Lake A diluent seemed to have the less beneficial effect on stored semen than Lake B diluent. This conclusion confirmed the results of an earlier study, which demonstrated that, compared with two other media, only in the Lake A diluent the number of live spermatozoa in cock semen stored at 4ºC declines significantly according to increase preservation time (Howarth, 1979).

It was also observed that the sperm mid-piece and tail defects were higher than the head abnormalities in preserved semen. Similar results have been reported by Alkan *et al.* (2001) and attributed to the cock sperm cell having a relatively long mid-piece. A number of researchers have reported that the acrosome and the mid-piece are the most sensitive regions in the cock's sperm, with the mid-piece being quicker to deteriorate than the other regions. It has also been found that the connecting area between the sperm head and mid-piece of poultry is more sensitive to external factors. The most frequent type of sperm tail defects recorded in the cock was bending and folding and the technique of making the stain could contribute to these defects (Alkan *et al.,* 2001).

Conclusions

On the basis of experiment concluded that the quality of native cock semen depends on various factor body weight, nutrition and housing; Cock having heavier body weight produced higher ejaculate volume and diluents with antibiotic was preferable as dilution for keeping at 4ºC for two days.

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