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Research Article

Effect of Storage on the Physico-Biochemical Characteristics and Fertility of Guinea Fowl Semen

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ABSTRACT

Pooled semen samples from 30 adult and healthy guinea fowl (pearl variety) were collected and examined for their physico-biochemical characteristics and fertility at 0 hr (freshly ejaculated) vs 24 hr stored semen (3-5 ^{0}C). For both the storage conditions, semen samples were diluted in CARI diluent (1:1). Semen volume was found 0.048±0.002 ml. A significant (P<0.05) reduction in sperm motility was investigated after 24 hrs of storage of semen. A decline (P<0.05) in per cent live counts, linked with enhanced dead and morphologically abnormal spermatozoa were noticed in preserved semen. Per cent dead and abnormal sperm have been found to be negatively correlated with fertility. Sperm concentration was found 3.27 ± 0.14 × 10^9 cells per ml. Biochemical characteristics of guinea fowl seminal plasma revealed a significant increase (P<0.05) in GOT activity and MBRT in stored (24 hrs) seminal plasma at low temperature (3-5 ^{0}C) when compared to fresh seminal plasma/semen. Profile of GPT activity was nearly similar in both the fresh and stored semen. No significant difference was noticed in ALP activity between fresh and preserved seminal plasma of guinea fowl. The cholesterol content was found 235±12.11 mg/dl and remained unchanged during preservation in the semen of guinea fowl. The content of glucose could not be detected in the seminal plasma of the guinea fowl. No variation was noticed in protein content in fresh vs stored seminal plasma. The pH of the fresh seminal plasma was found 7.15±0.01 and increased during storage condition. This study suggests that during liquid storage, quality of semen was deteriorated as evidenced by physico-biochemical characteristics and fertility of guinea fowl semen. Therefore, a guinea fowl species-specific semen diluent is to be developed to maintain good fertility during liquid storage of semen.

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INTRODUCTION

Artificial Insemination (AI) was first practiced in America during the 1920 and became widely used in Australia with the introduction of laying cages in the late 1950s. It has been a critical component of reproduction in turkeys since the 1960s and is used almost exclusively for commercial flock production. The differences in the size of the toms (large white strains approximately 33 kg) and hens (approximately 9 kg at the onset of lay) resulting into unsuccessful mating and consequent low fertility of the heavy broad-breasted strains after natural mating has forced the adoption of AI in commercial poultry production (Gee et al., 2004). The technique of AI is also getting momentum in other poultry species. For example, fertility in the broiler breeds continues to decline as males are selected for growth, AI may become cost effective in broiler breeder management (Reddy, 1995). AI in poultry expresses better fertility than natural mating (Saeki and Nagomi, 1964). Even when under natural mating 80–85% of eggs are fertile, fertility can be increased by another 5–10% simply by adding AI to the propagation program (Gee et al., 2004). At present, AI in other domestic alternate poultry species is not used extensively. But this scenario may change in future. In guinea fowl only few reports are available on AI aspects. For the improvement of this species, this necessitates the research work on this technology.

To achieve better fertility by AI, fresh semen should be inseminated within 15-30 min after collection (Lake and Stewart, 1978).Within this short period it is difficult to inseminate the large number of birds. These enforce the researcher to enhance the storage period of avian semen under *in vitro* condition. Therefore, in the present study attempts have been made to examine if freshly ejaculated diluted semen could be stored at 4-6 ^{0}C for 24 hr without much deterioration in its characteristics by evaluating its physical and biochemical characteristics.

MATERIALS AND METHODS

Semen samples for physico-biochemical characterization of guinea fowl were collected by abdominal massage method (Burrows and Quinn, 1937) from 30 healthy adult birds (pearl variety). To determine the fertility in guinea fowls, same numbers of female birds (pearl variety) of the same hatch were maintained in individual cages under the uniform husbandry conditions. They were given a normal breeder/layer ration and water *ad libitum* with 14 hr light per day. Various physical and biochemical parameters of guinea fowl semen were assessed in fresh (0 hr) and 24 hrs stored (3-5 ^{0}C) semen diluted (1:1) in CARI semen diluent (Mohan *et al*., 2000) with certain modifications. Semen samples for 24 hrs storage were kept in 5

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ml capacity round bottom glass tube (length=7cm, diameter=1 cm) covered with aluminium foil.

Semen volume was measured by a pipette and sperm concentrations were determined with a Neubauer haemocytometer (Lake, 1960). The percent of live-dead and abnormal spermatozoa were counted after preparing smears and staining them with eosin and nigrosin according to the methods described by Lake and Stewart (1978). Stained spermatozoa were counted as dead. The percentage of abnormal spermatozoa was evaluated in the same sample by examining the morphology of a total count of 100 spermatozoa. The sperm motility was assessed by examination of a drop of semen (4-5 µl) under the microscope at 10 x magnification as described by Wheeler and Andrews, (1943). To determine the fertility, hens were inseminated with the diluted semen (1:1). Fertility of birds was assessed by incubating the eggs layed by hens 2 to 6 days after intravaginal insemination. The percent fertility was determined by the ratio of numbers of fertile eggs to the number of total egg set in the incubator. Biochemical parameters were examined in the seminal plasma using the standard methods. Seminal plasma was obtained after centrifugation of semen at 750 g for 15 minute. Alkaline phosphatase (ALP) was estimated by Kind and King's Method using Qualigens diagnostics manufactured by Sigma Diagnostics, Baroda, India. Glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) enzyme were estimated by Reitman and Frankel methods (1957) using the commercial kits from the same manufactures. Total cholesterol in semen was analyzed by using the Span Diagnostics, Surat, India. Protein in samples was determined by using follin and ceocalten's phenol reagent (Lowry et al., 1951). Methylene blue reduction time (MBRT) test in semen was done using the method of Beck and Salisbury (1943). Glucose was estimated in seminal plasma using the Nelson and Somogyi (1944) method. The pH of diluted semen was determined by pH meter, 320213 (Cyberscan 2100) fitted with microelectrode. Mean values were compared using Student's t-test (Snedecor and Cochran, 1994).

Result and Discussion

Proper evaluation of semen characteristics prior to AI or storage is very important to avoid the losses of fertility (Reddy and Sadjadi, 1990; Hammerstedt and Graham, 1992). Evaluation of physical and biochemical characteristics of poultry semen reflects the reproductive potential of cock (Zahraddeen et al., 2005). Hence, physical and biochemical evaluation of semen is carried out in this study to predict the fertility potential of guinea fowl semen under *in vitro* storage condition using CARI semen extender as this diluent developed by this Institute expressed good fertility than others. Mean values of physical characteristics of semen are given in Table 1. Semen volume was found 0.048±0.002 ml. Volume of semen of guinea fowl obtained in this study is higher than as reported by Nwakalor et al. (1988) and lower (0.075 ml) than as observed by Lake and Stewart (1978). A significant ($P<0.05$) reduction in sperm motility was investigated after 24 hrs of storage of semen which reduced the quality of semen. Johari et al. (1986) observed a negative correlation between the sperm motility and MBRT which is in agreement with the present study. A decline ($P<0.05$) in per cent live counts, linked with enhanced dead and morphologically abnormal spermatozoa were noticed in preserved semen. We have also observed a drastic reduction in fertility after 24 hr of storage of semen at low temperature. Similar results were observed by Siudzinska and Lukaszewicz (2008) in different breeds of chicken in which a decrease in the number of live spermatozoa with an increase in dead and morphological abnormal spermatozoa with bent necks were observed during storage at 3-5^{0}C. Per cent dead and abnormal sperm have been found to be negatively correlated with fertility. Wilson et al. (1969) found that when the number of dead sperm increases more than 10%, fertility was lowest. A significant decrease in fertility was noticed when only 1 % dead sperm were found. Sperm concentration was found 3.27 ± 0.14 × 10^9 cells per ml that was comparable to the earlier studies on guinea fowl (Pal et al., 1999; Fujihara et al., 1986). However, Lake and Stewart (1978) reported higher sperm concentration in guinea fowl semen.

Table 1: Physical characteristics (fresh Vs preserved) of semen in guinea Fowl (Pearl).
Semen was diluted (1:1) with CARI diluents (mean±, N=6).

Parameter	Fresh (0 Hrs)	Preserved (24 Hrs)
Semen volume (ml)	0.048±0.002	No change
Sperm motility (%)	85.00 ± 5.76	71.12* ± 7.73
Livespermatozoa (%)	87.22 ± 3.17	74.36* ± 4.31
Dead spermatozoa (%)	7.67 ± 0.10	15.11* ± 0.66
Morphologically abnormal spermatozoa (%)	5.11 ± 0.14	10.59 ± 0.27
Concentration of spermatozoa (x10^9 cells/ml)	3.27 ± 0.14	No change
Fertility (%)	82.00± 6.46	45.00*±8.67

*Mean values of preserved semen compare with corresponding fresh semen ($P<0.05$) using Student s t – test.

Mean values on biochemical characteristics of seminal plasma of guinea fowl are presented in Table 2. Biochemical characteristics of guinea fowl seminal plasma revealed a significant increase ($P<0.05$) in GOT activity and MBRT in preserved (24 hrs) seminal plasma at low temperature (3-5 ^{0}C) when compared to fresh seminal plasma/semen. Increase of the mean values of MBRT in preserved semen may be due to the more number of dead sperm in stored semen. Various workers have reported that per cent dead sperm significantly related to the MBRT (Marini and Goodman, 1969; Mc Daniel and Craig, 1962). Profile of GPT activity was nearly similar in both the fresh and preserved semen. The enzymatic profile of GOT in our study was noticed nearly 47-54 times more than GPT. We have noticed nearly similar trend as observed by Hammond et al. (1965) and Datta et al. (1980) in chicken seminal plasma. They have reported 43-63 time higher activity of GOT than GPT. This reflects the physiological significance of GOT over GPT enzymes in seminal plasma of guinea fowl. In this context, it is of interest to note that the concentration of glutamic acid in the seminal plasma of cocks is exceptionally high and according to Lake and Mclndoe (1959) this amino acid constitutes as much as 90% of the total amino acids in the fluid, might have a special role in maintaining its osmotic balance and pH. Significant release of GOT enzyme in preserved semen of guinea fowl may be due to the stress on sperm cells (Dhami and Kodagali, 1990). Dilution and storage of semen at low temperature is a kind of stress on spermatozoa. No significant difference was noticed in ALP activity between fresh and preserved seminal plasma of guinea fowl. Our results in this regard are in good agreement with the work carried out by Mahapatra et al. (1994) in broiler chicken in which ALP activity remained constant in fresh vs preserved semen. The cholesterol content was found 235 mg/dl and remained unchanged during preservation in the semen of guinea fowl.

The cholesterol may be correlated positively with sperm concentration as it is found in the sperm membrane. Cholesterol and phospholipids ratio regulate the sperm

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membrane fluidity. Therefore, variation in cholesterol content in spermatozoa of different breeds may be associated with the difference in the membrane fluidity. This may be the reason that freezability of semen vary from species to species. Sperm membrane fluidity can be used as an indicator of sperm freezability (Blesbois et al., 2005).The content of glucose could not be detected in the seminal plasma of the guinea fowl. This is in agreement of our earlier observations in chicken (Mohan et al., 2007). No variation was noticed in protein content in fresh vs stored seminal plasma. As the protein concentration of seminal plasma remained unchanged during the 24 hr of storage, hence it cannot be taken as an indicator of semen evaluation. The pH of the fresh seminal plasma was found 7.15 and increased during storage condition in guinea fowl (Table 2). Similar pattern was recorded by Mahapatra et al. (1994) in broiler semen.

It was concluded that during liquid storage, quality of semen was deteriorated as indicated by physico-biochemical characteristics and fertility of guinea fowl semen. This research work necessitates developing a suitable semen diluent for short-term liquid storage (24 h) of guinea fowl semen that maintains good quality of semen without reduction in the fertility.

Table 2: Biochemical characteristics (fresh Vs preserved) of seminal plasma in Guinea Fowl (pearl). Semen was diluted (1:1 with CARI diluents (mean ± SEM, n=6).

Parameter	Fresh (0 Hrs)	Preserved (24 Hrs)
GOT (Unit/ml)	180 ± 5.05	212* ± 6.65
GPT (Unit/ml)	3.83 ± 0.15	3.95 ± 0.10
ALP (KA unit)	4.57 ± 0.46	4.05 ± 0.18
Protein (mg/ml)	7.80 ± 0.17	7.67 ± 0.25
**cholesterol (mg/dl)	235 ± 12.11	240* ± 15.27
Glucose (mg/dl)	ND	ND
MBRT (sec)	125 ± 5.35	152* ± 6.70
pH	7.15 ± 0.01	7.25 ± 0.01

*Mean values of preserved semen compare with corresponding fresh semen ($P<0.05$) using Student s t – test
ND- Not Detected
**** Cholesterol was determined in semen.**

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