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Selection of Diluent for Short Term Preservation of Guinea Fowl Semen

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ABSTRACT

This study was conducted to identify the suitable diluent for short-term preservation (0, 24 and 48 h) of Guinea fowl (pearl variety) semen. Semen samples were collected and diluted with normal saline, CARI, BPSE and Lake's semen diluents and kept at 6°C for storage. No significant difference was found in sperm motility among the different diluents at 0 h (freshly ejaculated) storage period. A significant ($p<0.05$) reduction was found in sperm motility among all the diluents at 24 h storage period. Subsequently, there was no sperm motility was recorded in normal saline diluted semen samples at 48 h storage period. While a reduction ($p<0.05$) in sperm motility was recorded in rest of the three semen diluents. The mean values for fertility with freshly ejaculated semen during 2-12 days of fertile period were recorded as 66.27±4.08, 78.90±3.08, 64.90±4.87 and 67.99±6.36% whereas, after 24 h storage of semen, fertility reduced drastically such as: 0, 41.97±7.27, 36.09±8.72 and 30.90±7.12% in normal saline, CARI, BPSE and Lake's semen diluent, respectively. No fertility was noticed at 48 h storage in any semen diluent. Irrespective of the storage period and diluents, CARI semen diluent expressed better fertility than others in Guinea fowl.

Key words: Semen diluent, Guinea fowl, motility, fertility

INTRODUCTION

In some parts of the world Guinea fowls are an important source of high quality protein in terms of eggs and meat but the supply of these products are not regular because the birds are seasonal breeders. In addition, the behaviour of male Guinea fowl is monogamous which drastically limits the reproductive efficiency of the bird especially when the male bird dies or culled. Under these conditions female is not readily copulate naturally with a new male (Aire *et al*., 1983; Biswas, 1983). Hence, to supply the high quality protein to the growing human population and to fight against the malnutrition, reproductive efficiency of guinea fowl must be improved by breaking the seasonality and adopting the Artificial Insemination (AI) in this bird.

Ivanov (1907) reported first AI in bird. Since then, AI has gained momentum as a valuable breeding tool. Superiority of AI to natural mating was noticed by Saeki and Nagomi (1964) from analysis of four years of comparative data collected from domestic fowl in Japan. Further success of this technique needs suitable semen diluent. Diluents are essential for semen in order to increase number of birds inseminated by per unit volume of semen and it prolongs the sperm survival under

both short and long term preservation of semen *in vitro*. Considerable information is available on the semen diluent of chicken and turkey (Lake, 1960; Van Wambeke, 1967; Sexton, 1977; Lake and Ravie, 1981; Mohan *et al*., 2011) and frequently used wherever the technique of AI is employed. Studies indicated that most of the semen diluents were targeted for chicken and turkey and there is no proper diluent for guinea fowl semen. Hence, the information is scanty for the specific diluent of Guinea fowl semen. As to improve the reproductive efficiency of the bird, this study was carried out to select the suitable diluent for Guinea fowl from available poultry semen diluents such as: CARI semen diluent (Mohan *et al*., 2000), Beltsville Poultry Semen Extender (BPSE) (Sexton, 1977) and Lake's semen diluents (Lake, 1960). Normal saline was also taken as a diluent in this investigation because it is routinely employed for diluting the avian semen (Shinde *et al*., 2013; Ogbu *et al.,* 2014).

MATERIALS AND METHODS

Sixty healthy and adult female Guinea fowl (pearl variety) from the same hatch were taken randomly and maintained in individual cages under uniform husbandry conditions. For the fertility study, thirty healthy and adult males from the same hatch of the same variety were taken and maintained in the similar manner. They were given normal breeder ration and water *ad libitum* with a constant light of 14 h day^{-1}. Semen samples were collected every alternate day during study period by abdominal massage method (Burrows and Quinn, 1935). Pooled semen of Guinea fowl was diluted in various semen dilutor such as normal saline (0.89% (w/v) NaCl), CARI diluent (Mohan *et al*., 2000) with modifications (under process of patent), Beltsville Poultry Semen Extender (BPSE) (Sexton, 1977) and Lake's diluent (Lake, 1960). One part (1 mL) of good quality semen was taken in 5 mL round bottom glass tube (length = 7 cm, diameter = 1 cm) and mixed with equal volume of the respective diluent. Artificial insemination was carried out in birds at 3 different time intervals (0, 24 and 48 h) after semen collection. Diluted semen samples targeted for AI were stored at 6°C for 24 and 48 h.

Sixty hens of guinea fowl were equally divided in to 4 groups with 15 birds in each. Group 1, 2, 3 and 4 of hens were inseminated with the semen diluted (1:2) with normal saline, CARI, BPSE and Lake's diluent respectively. Before AI diluted semen of each group was examined for sperm motility at different storage period (0, 24 and 48 h). It was determined under low magnification (10X) of light microscope with cover slip and scored as described by Wheeler and Andrews (1943). For artificial insemination, vaginal eversion was made by gentle pressure on left side of the abdomen through the cloaca (Quinn and Burrows, 1936). Subsequently, with the help of tuberculin syringe which contain the doses (60-100 million spermatozoa) of good quality diluted semen, insemination was done (0.1 mL) into the well everted oviduct (to the depth 3-4 cm or as close as possible to the sperm host gland) and concurrently with the release of pressure on the abdomen so that oviduct revert to its normal position.

Fertility of birds was assessed by incubating the eggs (99.5°F temperature and 55-60% relative humidity) laid by hens from 2-12 days after single intra vaginal insemination. To determine the fertility, eggs were examined by candling at 9th day of incubation. Some eggs were broken for fertility assessment in which fertility was not clear by candling. The percent fertility was calculated as a ratio of number of fertile eggs to the number of total egg set in the incubator. Statistical analysis was done using statistical software package (SPSS-16) for ANOVA (Snedecor and Cochran, 1994) and Duncan's multiple range tests (Duncan, 1955) by comparing means for significant differences.

RESULTS AND DISCUSSION

The mean values of sperm motility in guinea fowl are presented in Table 1. All the semen diluents expressed the sperm motility between the ranges of 82.00±1.79-87.46±2.80% at 0 h of storage. There was no significant difference among the different diluents at this storage period. Shinde *et al*. (2013) conducted the study on chicken semen storage using various diluents and found no significant difference in sperm motility among the different diluents. At 24 h of storage, a significant ($p<0.05$) reduction was found in sperm motility among all the diluents. Similar results were observed by Mohan *et al*. (2013) using CARI diluent after 24 h storage of guinea fowl semen. Subsequently, no sperm motility was recorded in normal saline diluted group at 48 h of storage at low temperature. This may be due to the fact that normal saline is lack of energy source (sugar) and constituents like glutamic acid responsible for maintaining osmotic pressure of avian semen (Lake and McIndoe, 1959; Sexton and Fewlass, 1978). A significant reduction in sperm motility during 24/48 h storage was also recorded in rest of the three semen diluents (Table 1). Similar results were obtained in chicken by other workers (Shinde *et al*., 2013). It is known that sperm motility and the fertilizing ability of undiluted neat fowl semen stored *in vitro* usually decreases within 1 h of collection (Carter *et al*., 1957). Therefore, to store Guinea fowl semen, the type of diluent and storage temperature is very important to avoid a reduction in sperm quality.

Irrespective of the diluents, sperm motility (Table 1) and its fertilizing ability (Table 2 and 3) showed a declining trend as storage time progresses from 0-48 h. The mean values for fertility with freshly ejaculated semen during 2-12 days of fertile period were recorded as 66.27±4.08, 78.90±3.08, 64.90±4.87 and 67.99±6.36% by employing normal saline, CARI, BPSE and Lake's semen diluent respectively (Table 2). During the same fertile period after 24 h storage of semen, fertility was reduced markedly such as: 0, 41.97±7.27, 36.09±8.72 and 30.90±7.12% in normal saline, CARI, BPSE and Lake's semen diluent, respectively (Table 3). This suggested that fertilizing ability of Guinea fowl spermatozoa reduced drastically during low temperature storage. Overall, during short term storage (24 h) of Guinea fowl semen CARI diluents exhibited superior pattern of fertility (Fig. 1) than others (Table 2 and 3). These results are in agreement with the work of

Table 1: Effect of different semen diluents on sperm motility of Guinea fowl spermatozoa

Storage period (h)	Normal saline	CARI	BPSE	Lake's
0	$85.06±1.94^{c1}$	$87.46±2.80^{c1}$	$85.70±1.75^{c1}$	$82.00±1.79^{c1}$
24	$37.16±2.60^{b1}$	$71.16±2.94^{b2}$	$68.00±1.15^{b2}$	$67.83±1.54^{b2}$
48	000^{a1}	$52.50±1.23^{a2}$	$45.33±1.85^{a2}$	$44.50±1.64^{a2}$

BPSE: Beltsville poultry semen extender, Means bearing different superscript (a,b,c) within columns differ significantly ($p<0.05$), Means bearing different superscript (1,2) within rows differ significantly ($p<0.05$), (Mean±SEM, n = 6)

Table 2: Effect of different semen diluents on fertilizing ability of 0 h stored Guinea fowl spermatozoa

Days (fertile period)	Normal saline	CARI	BPSE	Lake's
2-6	$82.40±3.41^{b1}$	$88.00±3.06^{b1}$	$76.20±6.44^{b1}$	$78.40±6.00^{b1}$
7-12	$50.83±6.94^{a1}$	$71.33±1.83^{a2}$	$55.50±4.51^{a1}$	$57.66±5.72^{a1}$
2-12	$66.27±4.08^{ab1}$	$78.90±3.08^{ab2}$	$64.90±4.87^{ab1}$	$67.99±6.36^{ab1}$

BPSE: Beltsville poultry semen extender, Means bearing different superscript (a,b) within columns differ significantly ($p<0.05$), Means bearing different superscript (1,2) within rows differ significantly ($p<0.05$), (Mean±SEM, n = 5)

Table 3: Effect of different semen diluents on fertilizing ability of 24 h stored Guinea fowl spermatozoa

Days (fertile period)	Normal saline	CARI	BPSE	Lake's
2-6	0	$58.80±9.36^{b}$	$51.60±7.17^{b}$	$48.00±6.54^{b}$
7-12	0	$25.50±7.21^{a}$	$20.50±6.33^{a}$	$16.66±9.35^{a}$
2-12	0	$41.97±7.27^{ab}$	$36.09±8.72^{ab}$	$30.90±7.12^{ab}$

BPSE: Beltsville poultry semen extender, Mean values bearing different superscripts (a,b) in column differ significantly ($p<0.05$), (Mean±SEM, n = 5)

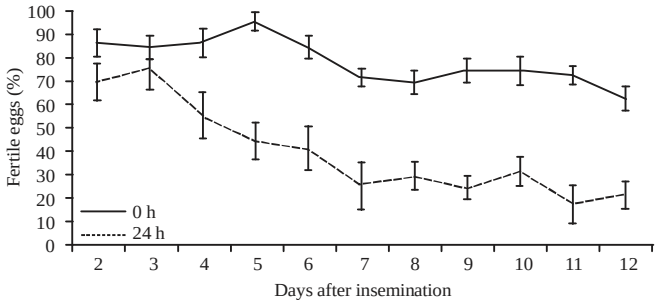

Fig. 1: Fertilizing ability of Guinea fowl spermatozoa after single insemination using CARI semen diluent (Mean±SEM)

Shinde *et al*. (2013) carried out in chicken using various semen diluents. Kammeree *et al*. (1972) found that the number of progressively motile sperm per ejaculate was the most consistent and reliable trait correlated with fertility. Positive correlations between motility and fertility have been reported by several investigators (Kamar, 1960; McDaniel and Craig, 1962; Kammeree *et al*., 1972; Monsi *et al*., 1975). Hence, reduction of motility (Table 1) with the advancement of storage period of the spermatozoa either *in vivo* (sperm storage tubules) or in vitro (at 6°C) may be the reason for decline in fertility (Table 2 and 3) in Guinea fowl. Absolutely no fertility was noticed when 24 h stored normal saline diluted semen was inseminated to the hens. As discussed earlier that normal saline is fluid which is deficient of energy and buffering capacity. Further, it is unable to maintain osmotic pressure of spermatozoa during short term storage at low temperature.

From the present investigation, it is concluded that to store Guinea fowl semen, the type of diluent and storage temperature plays a crucial role in storage of Guinea fowl semen as indicated by the sperm quality. Further, it is interpreted that at all the storage period (0 and 24 h), CARI semen diluents expressed higher fertility than others. This suggests that the ingredients of CARI diluent are comparatively more suitable to the survival of spermatozoa of Guinea fowl.

REFERENCES

Aire, T.A., J.S.O. Ayeni and M.O. Olowo-Okorun, 1983. Some Aspects of the Reproductive Biology of the Guinea Fowl (*Numida meleagris galeata pallas*). In: The Helmet Guinea Fowl (*Numida meleagris galeata pallas*) in Nigeria, Ayeni, J.S.O. (Ed.). Kainji Lake Research Institute, New Bussa, Nigeria, pp: 189-193.

Biswas, E.R.I., 1983. Management of Guinea Fowl for Breeding. In: The Helmet Guinea Fowl (*Numida meleagris galeata pallas*) in Nigeria, Ayeni, J.S.O. (Ed.). Kainji Lake Research Institute, New Bussa, Nigeria, pp: 196-197.

Burrows, W.H. and J.P. Quinn, 1935. Artificial insemination of chicken and turkeys. Poult. Sci., 14: 251-254.

Carter, R.D, M.G. Mccartney, V.D. Chamberlin and J.W. Wyne, 1957. The effect of storage time and temperature on fertilizing capacity of turkey semen. Poult. Sci., 36: 618-621.

Duncan, D.B., 1955. Multiple range and multiple *F* tests. Biometrics, 11: 1-42.

Ivanov, E.I., 1907. De la fecondation artificielle chez les mammiferes. Arch. Sci. Biol., 12: 377-511.

Kamar, G.A.R., 1960. The influence of semen characteristics on hatching results of chicken eggs. Poult. Sci., 39: 188-193.

Kammeree, D.M., R.E. Moreng, H.D. Muller and H.W. Hobbs, 1972. Turkey semen evaluation for fertility prediction. Poult. Sci., 51: 77-82.

Lake, P.E. and O. Ravie, 1981. An attempt to improve the fertility of stored fowl semen with certain additives in a basic diluent. Reprod. Nut. Dev., 21: 1077-1084.

Lake, P.E. and W.M. McIndoe, 1959. The glutamic acid and creatine content of cock seminal plasma. Biochem. J., 71: 303-306.

Lake, P.E., 1960. Studies on the dilution and storage of fowl semen. J. Reprod. Fertil., 1: 30-35.

McDaniel, G.R. and J.V. Craig, 1962. Predicting male fertilizing capacity in high and low fertility strains of chickens. Poult. Sci., 41: 866-869.

Mohan, J., J.M. Khanday, R.P. Singh and J.S. Tyagi, 2013. Effect of storage on the physico-biochemical characteristics and fertility of Guinea Fowl semen. Adv. Anim. Vet. Sci., 1: 65-68.

Mohan, J., R.P. Moudgal and R.V. Singh, 2000. A process forpreparation of a new and simple diluents for chickensemen. Patent No. 191190, Washington, DC., USA.

Mohan, J., R.P. Singh, K.V.H. Sastry, R.P. Moudgal, A. Biswas and N. Shit, 2011. Influence of chicken native breeds on some physical and biochemical characteristics and short-term storage of semen. Br. Poult. Sci., 52: 395-400.

Monsi, A., H.L. Enos, R.E. Moreng and B.W. Pickett, 1975. Low sperm concentration as a method of evaluating fertility among toms. Poult. Sci., 54: 1797-1798.

Ogbu, C.C., S.O.C. Ugwu and C.V. Ezebili, 2014. Use of coconut milk as an extender for cock semen. Res. Opin. Anim. Vet. Sci., 4: 571-577.

Quinn, J.P. and W.H. Burrows, 1936. Artificial insemination of fowls. J. Hered., 27: 31-38.

Saeki, Y. and Y. Nagomi, 1964. Seasonal differences in fertility and hatchability of eggs produced by the natural mating and artificial insemination methods of chickens. Jpn. J. Anim. Reprod., 10: 37-43.

Sexton, T.J. and T.A. Fewlass, 1978. A new poultry semen extender 2. Effect of the diluent components on the fertilizing capacity of chicken semen stored at 5°C. Poult. Sci., 57: 277-284.

Sexton, T.J., 1977. A new poultry semen extender 1. Effect of extension on the fertility of chicken semen. Poult. Sci., 56: 1443-1446.

Shinde, A.S., J. Mohan, K.V.H. Sastry, R.P. Singh, L. Chouhan and J.S. Tyagi, 2013. CARI diluent for short-term preservation of Kadaknath chicken semen. Indian J. Poult. Sci., 48: 240-243.

Snedecor, G.W. and W.G. Cochran, 1994. Statistical Methods. 8th Edn., Oxford and IBH Publishing Co., New Delhi, India.

Van Wambeke, F., 1967. Storage of fowl semen: 1. Preliminary results with new extenders. J. Reprod. Fertil., 13: 571-575.

Wheeler, N.C. and F.N. Andrews, 1943. The influence of season on semen production in the domestic fowl. Poult. Sci., 22: 361-367.