EFFECT OF SINGLE AND DOUBLE WASHING ON THE LIVEABILITY OF BUFFALO BULL SPERMATOZOA AT 37°C

M. Ahmad, S. Rehman, A. Khan[1] and K.M. Ahmad
Department of Animal Reproduction, [1]Department of Veterinary Pathology, University of Agriculture, Faisalabad-38040, Pakistan

ABSTRACT

This study was conducted to investigate the effect of washing on motility, liveability (hours) and absolute index of liveability of buffalo bull spermatozoa at 37°C. Five pooled (Ist and 2nd ejaculate) samples from each of the two buffalo bulls were collected. Each pooled ejaculate was divided into five aliquots (A, to E). Of these, B to E were centrifuged at 3000 rpm for 15 minutes. The seminal plasma was removed from aliquots C, D and E while aliquot B was kept as such having intact seminal plasma. Aliquot D was given single washing while aliquot E was subjected to double washing with sodium citrate solution. The remaining fifth aliquot A served as control. Treated and control aliqouts were diluted at 1:10 with extender (milk-egg yolk-glycerol) and incubated at 37°C. Statistical analysis of the data revealed that removal of seminal plasma and washing with sodium citrate solution have a beneficial effect on liveability of buffalo bull spermatozoa. However, double washing of spermatozoa yielded better results than other treatments as well as control.

INTRODUCTION

Seminal plasma, the fluid part of semen, is normally an isotonic and almost neutral fluid. Spermatozoa are nourished by it and acts as a vehicle for sperm transport to the female genital tract (Salisbury and Van Demark, 1961).

Extraneous energy yielding materials are also utilized by the spermatozoa (Singh *et al.*, 1977). However, the glucose uptake has apparently been reduced in the presence of seminal plasma (Flipse, 1954). During *in vitro* storage of semen early death of spermatozoa has been evidenced (Sahni and Mohan, 1990). Seminal plasma may contain some factors which affect the sperm physiology (Baas *et al.*, 1983). A heat labile toxic protein is reported to be present in the bovine seminal plasma (Shannon, 1965). Decapacitation factors obtained from the seminal plasma of various species inhibit corona penetrating enzyme necessary for fertilization process (McRorie and Williams, 1974). Moreover, phospholipase-A (Cowper's gland secretion) affects the preservation of goat semen by hydrolysing lecithin of egg yolk to fatty acids and isolecithin which are harmful for spermatozoa (Iritani and Nishkawa, 1961). Therefore, the seminal plasma appears to be unnecessary (Rodger, 1975).

Removal of seminal plasma have shown to improve survivability of buffalo spermatozoa (Ahmad *et al.*, 1996). Significant beneficial effect of removal of seminal plasma through washing on motility and acrosome morphology of frozen thawed goat spermatozoa have also been reported (Memon *et al.* 1985).

The present research work was conducted to study the effect of removal of seminal plasma by washing the semen on motility percentage, liveability (hours) and absolute index of liveability of buffalo bull spermatozoa at 37°C.

MATERIALS AND METHODS

Collection of semen

Two Nili-Ravi buffalo bulls (B1 and B10) maintained at the Department of Animal Reproduction, University of Agriculture, Faisalabad were used for collection of semen. Five pooled semen samples, each consisting of two ejaculates, were collected from each bull twice a week.

Evaluation of semen samples

The semen was evaluated on the basis of physical characteristics described by Salisbury and Van Demark (1978). The ejaculates having at least 60% motility, 6.5 to 7.2 pH and creamy white colour were selected for further processing.

Centifugation and extension of semen

Each pooled semen sample from each bull was divided into five aliquots (A to E). Four aliquots (B to E) were centrifuged at 3000 rpm for 15 minutes. The

seminal plasma was removed from three aliquots (C, D and E) whereas B was kept with intact seminal plasma. After removal of seminal plasma from samples D and E, sediment was resuspended in sodium citrate (2.9%) solution. They were again centrifuged at 3000 rpm for 15 minutes. One of these aliquots (D) was given single washing by removing the supernatant, whereas the other (E) was again centrifuged after removing the supernatant and adding the sodium citrate solution to give two washings. All the treated samples C, D and E were added with the milk-egg yolk-glycerol extender (Table 1) to make volume equal to that before centrifugation. The fifth aliquot (A) was kept as control. Treated and control samples were diluted with extender separately to make the ratio of 1:10. All samples were incubated at 37°C.

Table 1: Composition of experimental extender

Ingredients	Quantity
Milk (ml)	75
Egg yolk (ml)	20
Glycerol (ml)	5
Penicillin (IU/ml)	1000
Streptomycin (mg/ml)	1

Motility, liveability and absolute index of liveability

Immediately after dilution, motility (%) of each sample was recorded and then at one hour interval till the death of all spermatozoa. The time for which spermatozoa remained motile was taken as liveability (hours). The absolute index of liveability was calculated from the observed data by the application of Melovenof's equation (1962).

Statistical Analysis

The data were subjected to analysis of variance and Duncan's multiple range (DMR) test using the statistical Micro-computer programme "Minitab".

RESULTS AND DISCUSSION

The liveability (hours) of spermatozoa and absolute index of liveability in control and treated samples obtained from bull B1 and B10 determined at hourly interval till the death of all spermatozoa are presented in Table 2. Data revealed that the liveability (hours) of spermatozoa was significantly ($P<0.001$) higher in the samples washed twice with sodium citrate solution than rest of the treated as well as control samples. Single washing with sodium citrate solution and removal of seminal plasma also yielded significantly ($P<0.001$) higher liveability of spermatozoa as compared with control. The samples in which seminal plasma was kept intact showed significantly ($P<0.001$) low liveability of spermatozoa when compared with control as well as other treated samples (Table 2).

The results of current study are comparable with those of Ritar and Salamon (1982) who noted the higher surviving percentage after single and double washing of fresh and frozen-thawed spermatozoa than the samples which were given no washing. The double washing yielding maximum percentage than single washing as well as without washing. The removal of toxic proteins and certain deletarious factors after single or double washing might be the major factor which are responsible for the enhancement of liveability of spermatozoa. A toxic protein in the bovine seminal plasma has been reported (Shanon, 1965) which might depress the motility of spermatozoa thus ultimately resulting in reduced liveability. Similar results had also been reported by Corteel and Baril (1975) who studied the effect of washing in the preservation of goat semen. They reported that there was no loss of motility of spermatozoa in washed than unwashed spermatozoa.

Shah (1993) indicated that the removal of seminal plasma had highly better results than control in respect of liveability of buffalo bulls spermatozoa. The results are also in agreement with those of Ahmad *et al.* (1996) who reported a higher survivability of spermatozoa in the samples for which seminal plasma was removed (15.7 ± 1.2 hours) than samples with seminal plasma (12.9 ± 1.3 hours).

Improvement in the surviability of buck spermatozoa during preservation at 5°C without seminal plasma has been reported by Deka and Rao (1986). Similarly, Sahni and Mohan (1990) achieved highly beneficial effect of removal of seminal plasma during preservation of buffalo bull semen at 5°C. The viability of buffalo bull spermatozoa was as high on day 9 as on day 1 or 3. They noted that buffalo spermatozoa were highly resistant to cold shock and the plasma which contains unidentified spermiostatic factor proved damaging to liveability of spermatozoa.

In the study under report the samples in which seminal plasma was kept intact the liveability of spermatozoa was significantly reduced than the samples from which seminal plasma was removed as well as control. The significant reduced liveability in samples with intact seminal plasma might be due to release of intracellular proteins, enzymes and ions into the

Table 2: Means ± SE of liveability (hours) and absolute index of liveability of spermatozoa at 37°C with control and treated samples of buffalo bull semen

Treatments	Liveability (hours)	Absolute index of liveability
A (Control)	11.90 ± 0.57a	433.50 ± 28.57a
B (Intact Seminal Plasma)	9.40 ± 0.52b	326.85 ± 17.94b
C (Seminal Plasma Removed)	14.00 ± 0.70c	535.55 ± 31.17c
D (Seminal Plasma Removed Single Washing)	14.00 ± 0.42c	539.55 ± 25.97c
E (Seminal Plasma Removed Double Washing)	16.10 ± 0.67d	589.50 ± 31.39c

Values having differed letters in a column differ significantly ($P<0.001$).

surrounding medium which are responsible for reduced motility and liveability of spermatozoa resuspended into supernatant. After centrifugation these substances are released due to mechanical injury to sperm cell by centrifugation which leads to alteration in permeability of sperm cell membrane (Mann, 1951).

The inhibitory factor in the seminal plasma for motility is at a high level in buffalo semen than in cattle semen (Ganguli, 1978) and adverse effect is more marked in buffalo semen as compared to cattle semen (Sahni, 1990).

In the present study absolute index of liveability was significantly higher in the samples from which seminal plasma was removed than the samples with intact seminal plasma as well as control samples. The samples in which seminal plasma was kept as intact showed the least absolute index of liveability (Table 2). These rsults were in agreement with those by Ala-ud-Din *et al.* (1996) who reported that removal of seminal plasma is beneficial for absolute index of liveability in buffalo bulls.

It may be concluded from this study that removal of seminal plasma from semen and double washing of spermatozoa increases the liveability of buffalo bull spermatozoa.

REFERENCES

Ahmad, M., A. Khan, Z.A. Shah and K.M. Ahmad, 1996. Effect of removal of seminal plasma on the survival rate of spermatozoa. Anim. Reprod. Sci., 41: 193-199.

Ala-ud-Din, Z.I. Qureshi, L.A. Lodhi and M. Ashraf, 1996. Effect of removal of seminal plasma on the fertility of Nili Ravi buffalo bull spermatozoa. Pakistan Vet. J., 16: 62-64.

Baas, J., P.C. Molan and P. Shamon, 1983. Factors in seminal plasma of bulls that affect the viability and motility of spermatozoa. J. Reprod. and Fert., 68: 275-280.

Corteel, J.M. and G. Baril, 1975. Effect of washing on the preservation of goat semen at low temperatures. Elevate et Insem., 146: 20-36.

Deka, B.C and A.R. Rao, 1986. Motility of buck spermatozoa during preservation at 5°C with and without seminal plasma. Indian Vet. J., 63: 169-170.

Flipse, R.J., 1954. Metabolism of bovine semen, uptake of glucose by bovine spermatozoa. J. Dairy Sci., 37: 425-430.

Ganguli, N.C., 1978. Biochemistry of semen processing. In: Buffalo reproduction and artificial insemination. Proceedings of the seminar sponsered by the FAO/SIDA/Govt. of India.

Iritani, A. and Y. Nishikawa, 1961. Studies on the egg yolk coagulating factor in goat semen. II. Properties of coagulating factor and influential conditions for coagulation. Proc. Silver Jubilee Lah Anim. Hush., Kyoto Univ., pp: 97-104.

Mann, T., 1951. Mechanical separation of sperm from seminal plasma; release of intracellular proteins from damaged spermatozoa. Biochem. J., 48: 386.

Martinus, R.D., P.C. Molan and P. Shannon, 1991. Deleterious effects of seminal plasma in the cryopreservation of bovine spermatozoa. New Zealand J. Res., 34: 281-286.

McRorie, R.A and W.I. Williams, 1974. Biochemistry of mammalian fertilization. Ann. Rev. Biochem, 43: 777-803.

Memon, M.A., K.N. Bretzlaff and R.S. Ott, 1985. Effect of washing on motility and acrosome morphology of frozen thawed goat spermatozoa. Am. J. Vet. Res., 46: 473-475.

Melovenof, U.K., 1962. Biology of reproduction. Published for Agriculture literature, Journals and pamphlets, Moscow, USSR, pp: 451-452.

Ritar, A.J and S. Salamon, 1982. Effect of seminal plasma and its removal and of egg yolk in the diluent on the survival of fresh and frozen-thawed spermatozoa of the Angora goat. Australian J. Biol. Sci., 35: 305-312.

Rodger, J.C, 1975. Seminal plasma, an unnecessary evil? Theriogenology, 3: 237-239.

Sahni, K.L and G. Mohan, 1990. Effect of removal of plasma on preservation of bovine semen. Indian J. Anim. Sci., 60: 783-785.

Sahni, K.L., 1990. Inhibitory effect of seminal plasma on motility of bovine semen. Indian J. Anim. Sci., 60: 786-788.

Salisbury, G.W and N.L. Van Demark, 1961. Physiology of reproduction and artificial insemination of cattle. Ist ed. W.H. Freeman & Company, San Farancisco, U.S.A. pp: 259-267.

Salisbury, G.W. and N.L. Van Demark, 1978. Physiology of Reproduction and Artificial Insemination of Cattle, 2nd ed. W.M. Freeman & Company, San Francisco, U.S.A. pp: 402-403, 442-446,498.

Shah, Z.A., 1993. Effect of removal of seminal plasma from buffalo-bull semen at 37°C. M.Sc. Thesis, Deptt. Anim. Reprod., Univ. Agri., Faisalabad.

Shannon, P., 1965. Presence of a heat labile toxic protein in bovine seminal plasma. J. Dairy Sci., 48: 1362-1365.

Singh, J., 1977. Revivability of buffalo spermatozoa after deep freezing using various extenders. M.Sc. Thesis, Haryana Agri. Univ., India.