WASHING OF BUFFALO BULL SEMEN AFFECTS CONCEPTION RATE UNDER FIELD CONDITIONS

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ABSTRACT

Twenty pooled good quality buffalo bull semen samples were used in this study. Each sample was divided into five aliquots (A to E). Aliquot A was used to serve as control. Seminal plasma was removed by centrifugation from aliquot C, D and E. whereas it was not removed after centrifugation from aliquot B. Aliquot D was washed simply with 2.9% sodium citrate solution and aliquot E twice. All treated and control aliquots were extended in milk-egg-yolk glycerol extender and were used for insemination to note effect on conception rate (CR). CR was checked 45 days after insemination. The poorest 38.27% CR was observed with semen sample B. The CRs of samples A (53.85%), C (58.90%) and D (64.20) differed non significantly ($P<0.05$) from each other. But CR of E (69.30%) differed significantly ($P<0.05$) from sample A. However CR of sample E differed non significantly with the CR of C and D. It may be concluded that removal of seminal plasma and washing (single/double) improves conception rate in buffaloes.

Keywords: Buffalo bull semen, washing, conception rate, field conditions

INTRODUCTION

Seminal plasma has been found to have damaging effects on sperm, as it inhibits sperm motility and causes membrane damage (Shannon, 1965; Bass *et al.*, 1983). A motility inhibiting protein has been isolated from seminal plasma in the rabbit, ram, human, boar and bull (De Lamirande *et al.*, 1984). Separation of seminal plasma and its favourable effects on sperm survival in fluid and frozen state preservation of semen have been reported by many research workers (Shah, 1993; Ahmad *et al.*, 1997; Khan *et al.*, 1998). The literature about beneficial effects of seminal plasma removal on conception rate in buffalo is scanty. The objective of the present study was to investigate the effect of washing of buffalo semen on its fertility under field conditions.

MATERIALS AND METHODS

Semen samples collected from two adult Nili-Ravi buffalo bulls of approximately same age and kept under similar managerial conditions were used in this study. On every collection day two ejaculates were collected from each bull with the artificial vagina. Immediately after collection, first and second ejaculates were pooled and evaluated following Salisbury *et al.* (1961). In all twenty pooled (ten from each bull) good quality semen samples were used. Each sample was divided into five equal aliquots (A, B, C, D and E). Aliquot A was used as untreated control. Four aliquots (B, C, D and E) were centrifuged at 3000 rpm for 10 minutes (Iqbal *et al.*, 1987). From aliquots C, D and E seminal plasma was removed, however, aliquot B was kept with intact seminal plasma. Sediments of aliquots D and E were resuspended in 2.9% sodium citrate solution and were again centrifuged at 3000 rpm for 10 minutes. The supernatant was removed. Sediment of aliquot E was again added with sodium citrate solution and was centrifuged third time to give double washing. After centrifugation sediment of E was separated from supernatant.

All samples (control and treated) were extended (1:10) using milk-egg yolk-glycerol extender (Milk 75 ml, Egg yolk 20 ml, glycerol 5 ml, penicillin 1000 I.U/ml and streptomycin 1 mg/ml). For the rate of extension of treated samples, the volume before treatment was considered. Immediately after extension, motility (%) of each aliquot was recorded and they were stored at 4°C in the refrigerator for two days. Semen was used for A.I. of public animals visiting the clinic of Animal Reproduction Department, University of Agriculture, Faisalabad. Buffaloes were randomly allotted to various aliquots and after confirmation of heat by rectal palpation were inseminated. More than one hundred females were inseminated with each aliquot. Rectal palpation was done to note the pregnancy rate at least 45 days after A.I. Data

were subjected to 2 X 2 relative frequency Chi square test (Samuels, 1991).

RESULTS AND DISCUSSION

The results are presented in the Table 1.

Table 1: Conception rate of buffaloes inseminated with unwashed and washed spermatozoa.

Sample	Number of buffaloes inseminated	Examined for pregnancy	Pregnant	Conception rate (%)
A	119	91	49	53.85b
B	125	81	31	38.27a
C	112	90	53	58.90bc
D	125	81	52	64.20bc
E	155	101	70	69.30c
Total	636	444	255	57.43%

Values with different subscripts differ significantly (P<0.05).

The data presented in Table 1 indicate that the poorest conception rate (38.27%) was observed with the semen sample B which was centrifuged but the seminal plasma was not removed as compared to control and other treated samples. Poor liveability at 37°C of semen samples from which seminal plasma was not removed following centrifugation has been reported by Ahmad *et al.* (1998). Centrifugation results in the release of intracellular enzymes, proteins and ions (Mann, 1951). Ahmad *et al.* (1998) observed that alanine transaminase (ALT) and aspartate transaminase (AST) levels were high in bufalo semen samples from which seminal plasma was not removed after centrifugation. ALT and AST leakage has toxic effect on spermatozoa. The conception rates of sample A (53.85%), C (58.90%) and D (64.20%) differed non significantly from each other but sample E (69.30%) differed significantly (P<0.05) from sample A (53.85%). However C.R. of sample E (69.30%) differed non significantly (P<0.05) with the C.R. of C (58.90%) and D. (64.20%).

The data reveal improvement of conception rate with the removal of seminal plasma and washing (single or double) of spermatozoa. The present results are supported by the findings of various research workers (Shannon, 1965; Shah, 1993; Ahmad *et al.*, 1997) who observed improvement in survivability of spermatozoa after seminal plasma removal and washing (single/double). Ala-ud-Din *et al.* (1996) reported 60% conception rate with semen from which seminal plasma was removed as compared to 50% in control which supports the findings of present study. However, these results differ from the findings of Avenell (1982) who reported decreased fertility rate in cattle if extended semen was inseminated without seminal plasma. This may be due to the fact that the buffalo seminal plasma is more depressive for sperm than cattle (Ibrahim *et al.*, 1981).

Centrifugation and washing removes the toxic substance, protein in nature normally present in the seminal plasma (Shannon, 1965) which more adversely affects the buffalo spermatozoa as compared to cattle (Sahni, 1990). ALT and AST levels in the seminal plasma are reported to have negative correlation with fertility (Pace and Graham, 1970; Dhami and Shani, 1993).

It may be concluded from the present study that removal of seminal plasma and washing (single/double) improve the conception rates in buffaloes. Moreover, it is necessary to remove seminal plasma after centrifugation otherwise leaked ALT and AST enzymes can adversely affect sperm survival and fertility.

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