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Effect of live sperm count per inseminate on pregnancy rate in buffaloes

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ABSTRACT

Semen from 10 Murrah buffalo bulls was split-frozen in 0.5 ml French straws to get mean concentrations of 22.6, 17.3, 11.0 and 8.5 x 106 (C1, C2, C3 & C4, respectively) spermatozoa per dose. A total of 1941 buffaloes were inseminated at 20 AI centres and pregnancy rates were 59.4, 58.1, 66.7 and 52.4 per cent for C1, C2, C3 and C4 respectively. Pregnancy rates differed significantly among bulls and among technicians. After correcting for the variations due to bulls as well as technicians, the pregnancy rates were not affected by the sperm concentration employed. It is concluded that the reduced sperm concentration up to 11 x 106 spermatozoa or even lower concentration (8.5 million) per inseminate does not affect the overall fertility of buffalo bull semen, though individual differences exist between bulls regarding maintenance of fertility at lower sperm concentration per inseminate.

Key words : Buffalo bull, Number of spermatozoa, Pregnancy rate.

Currently most frozen semen stations in India are freezing between 25-30 million bovine spermatozoa per dose. Lowering the sperm number per dose, without compromising fertility, would reduce semen production costs and ensure more efficient use of elite bulls. Several reports are available on influence of sperm numbers per straw on non-return rates in cattle (Jondet, 1968, Willmington, 1980; Pace *et al.*, 1981; Gerard & Humblot, 1991). However, we are not aware of similar studies in the buffalo. Since buffalo is a major milk producing animal in our country, the objective of this investigation was to study the fertility of buffalo spermatozoa at varying sperm concentrations per insemination dose.

MATERIALS AND METHODS

The trial included 10 young Murrah buffalo bulls of unknown fertility in the age group of 3-6 years, maintained at Sabarmati Ashram Gaushala, Bidaj (Gujarat). The semen was collected by using artificial vagina and was evaluated as per standard laboratory procedure. Only the ejaculates which met laboratory standards i.e. more than 70% progressively motile sperm and total sperm number above 3 x 109 were processed. The ejaculates of similar quality were pooled and split into fractions for further dilution. The dilution was done in one stage using Tris-Citric acid-Yolk-Fructose diluter with 6% glycerol. The sperm concentration of neat semen samples was determined by using a calibrated spectrophotometer (CECIL make). The semen dilution rate was calculated so as to achieve 25, 20, 15 and 10 million sperm per straw (0.5 ml French straw). The sperm concentration in 0.5 ml straws was determined by counting of spermatozoa using haemocytometer. Duplicate samples were pooled and four counts using haemocytometer were made to arrive at average sperm concentration in the straw.

The mean sperm concentrations finally obtained in 0.5 ml straws were 22.0 (C-1), 17.3 (C-2), 11.0 (C-3) and 8.5 (C-4) million sperms per dose.

The straws (0.5 ml) were printed with the bull number (01 to 10) and the sperm concentration was mentioned with serial numbers as 1, 2, 3 & 4 succeeding to the bull number (i.e 011 to 014 for bull number 1 & 101

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to 104 for bull number 10). The serial number of 1, 2, 3 & 4 of the sperm concentration denotes 25, 20, 15 & 10 million spermatozoa per straw respectively. The straws were frozen in a LNR 330 wide mouth LN container in which the liquid nitrogen level was adjusted to four centimeters from straws after completion of equilibration period of 4-5 hours.

These straws with varying known sperm counts were distributed for a field trial in the Panchmahal District of Gujarat State. The frozen semen doses were supplied to 20 dairy cooperative AI centres where inseminations were carried out by trained technicians. All the buffaloes covered under the trial were ear tagged after insemination. Pregnancy diagnosis was done by rectal palpation after 70 days of insemination.

RESULTS AND DISCUSSION

During the trial, a total of 1941 buffaloes were inseminated. Out of these 1541 could be followed up for pregnancy and 907 (58.8%) were found pregnant. Pregnancy rates at different sperm concentrations were found to be 59.47, 58.19, 66.76 and 52.42 for average sperm concentrations of 22.6, 17.3, 11.0 and 8.5 million spermatozoa per dose respectively (Table 1). The results indicated that the decrease in sperm concentration upto 11 million sperm per dose did not reduce pregnancy rates, though non significant decline was recorded with 8.5 million sperm/dose (C4).

Since the observed variations in conception rates can be due to bull, sperm concentration, technician and interaction between bull and concentration. hence to find out the effects due to a particular parameter, the data was subjected to ANOVA. Conception rates across different sperm concentrations were not significantly different. Also, the interaction of bulls and treatments was not significant. At the same time, variations among the technicians were highly significant. This indicates that the skill of a technician is crucial in the pregnancy rates achieved under the field conditions. The results also indicated that increase in sperm concentration did not improve pregnancy rates.

Gerard and Humblot (1991) indicated that in cattle, conception rate was significantly reduced when total sperm concentration was reduced to 8 or 12 million from 16 million. While, Foulkes (1977), could not find significant differences in non return rates between sperm counts of 20 million and 12 million. The present study shows that the pregnancy rate in buffaloes is not significantly affected even when sperm concentration per insemination dose is reduced to 8.48 million. These results are in agreement with Jondet (1968) who reported acceptable conception rates in cattle with live sperm concentration of only three million/dose. Willmington (1980) also reported that 6.6 million live sperms per dose gave a satisfactory NR rate in cattle. However, Almquist (1975) reported that optimal fertility was obtained with a live sperm dose of 12.4 million in cattle. In the present study, maximum conception rates were obtained when the total sperm concentration was 11.98 million per inseminate.

Analysis of data without including treatment effect revealed significant differences in pregnancy rates among bulls. Similar phenomenon has also been reported earlier in cattle [Foulkes, 1977; Pace et al., 1981; Nanke den Daas, 1990 (personal communication)]. It indicates that the threshold number of sperm concentration per dose of semen for optimal fertility varies among bulls. Thus, in order to make extensive use of a superior bull, it would be ideal to

Table 1. Effect of varying sperm concentration per inseminate on pregnancy rate in the buffalo

Mean sperm conc./dose	No. of AI done	No. followed	No. of animals pregnant	Percent-pregnant
22.6 (C1)	482	380	226	59.5
17.3 (C2)	533	409	238	58.2
11.0 (C3)	476	340	227	66.8
8.5 (C4)	450	412	216	52.4
Total	1941	1541	907	58.8

determine the optimum sperm concentration for each bull and process the semen accordingly.

In conclusion, this trial indicated use of less number of spermatozoa per dose for AI in buffalo without affecting fertility. However, for determining the minimum number of sperm to achieve optimum pregnancy, fertility of each bull need to be ascertained individually.

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