



## Effect of Reducing Sperm Concentration per Insemination Dose in Cryopreserved Semen on Conception Rate

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### Abstract

The objective of this study was to evaluate the effect of reducing sperm concentration per semen dose in cryopreserved bull semen on the conception rate in artificial insemination technology in dairy animals. For this purpose, semen was collected from six bulls (2 each from HF100%, HF75%, and Murrah buffalo). The ejaculates were split-sampled and packed in 20 million, 15 million, and 10 million spermatozoa per straw. After dilution, the semen was cooled to 4°C, equilibrated for 4 hours, and packed in French Mini straws. A total of 8,418 inseminations were performed over a period from June 2012 to April 2014. The overall mean conception rate in HF100%, HF75%, and Murrah buffalo was recorded as  $57.79 \pm 0.83$ ,  $58.09 \pm 0.94$ , and  $53.63 \pm 1.06$  percent respectively. Sperm concentration-wise conception rate was recorded as  $56.75 \pm 0.77$ ,  $56.57 \pm 1.14$  and  $57.08 \pm 1.01$  percent, for 20, 15, and 10 million sperm concentration per straw respectively, non-significant difference in conception rate was noticed on account of different sperm concentrations per straw. In conclusion, the reduction of sperm concentration from existing 20 million to 15 million or 10 million per straw did not affect the fertility of cryopreserved cattle or buffalo bull semen, however, it was felt that more studies are needed to confirm the results before a policy decision is taken in this respect.

**Keywords:** Sperm Concentration; Cryopreserved Semen; Conception Rate; Artificial Insemination; Hf100%, Hf75%; Murrah Buffalo Bulls

### Introduction

Artificial Insemination (A.I.) is the first-generation assisted reproductive technology that has made a profound contribution to the genetic improvement in dairy cattle. The development of frozen semen technology has greatly enhanced the A.I. program. The fertilizing ability of spermatozoa depends not only on the initial quality of semen but also on the subsequent laboratory processes like semen dilution, freezing, storage, transportation, and thawing for insemination that ends up with deposition of semen in the genital tract of a cow. Numerous efforts have been made to find the optimal sperm concentration per insemination dose without compromising bull fertility [1]. The influence of a number of spermatozoa per insemination dose of cryopreserved semen revealed that  $5$  to  $15 \times 10^6$  progressively motile spermatozoa are necessary

to achieve an acceptable level of fertility [3,10]. Earlier, 30 million sperms used to be packed per straw which presently is reduced to 20 million as per Minimum Standard Protocol (MSP) guidelines of the Government of India and there is thinking going on to still reduce the sperm concentration without affecting the existing level of field animals' fertility. The present study has been planned to study the effect of reducing sperm concentration per insemination dose on the conception rate of cryopreserved bull semen.

### Materials and Methods

Six bulls (2 each from HF100%, HF75%, and Murrah buffalo) were selected for frozen semen production. Housing, feeding, and semen collection as well as its evaluation and processing were undertaken as per the laid down Standard Operating Procedure

(SOP) of MSP formulated by the Government of India. After final dilution, semen was splitted into three equal parts and packed in French Mini (0.25 ml) straws with IMV IS4, filling-sealing system in 3 different types of concentrations, viz. regular 20 million (Bull name/tag No), 15 million (Bull name/Tag No. 'A') and 10 million (Bull name/Tag No. 'B'). Prepared straws underwent 4 hours of equilibration (4 to 5°C) a cold handling cabinet before freezing in Bio-freezer. Post-thaw motility of each ejaculate was examined after 24 hours and semen showing post-thaw motility above 50 percent was preserved and kept for 30 days quarantine and then dispatched to the semen bank. Such frozen semen of different concentrations was issued for utilization on farmer-owned animals through BAIF's two Cattle Development Centers (CDC) in Pune districts (Uruli Kanchan & Ashtapur). Month-wise semen distribution schedule was prepared and twenty semen doses from each breed X bull X concentrations (6 bulls x 3 types x 20 semen doses) amounting to 360 semen doses were supplied to each CDC per month till the project semen availability. Semen utilization guidelines written in the local language were provided to CDC in-charges explaining the random use of an equal number of semen doses on each day and ensuring proper entry of bull name with code on A.I. register. Total 8,418 inseminations were performed during the period from June 2012 to April 2014 on field animals. The cows and buffaloes not repeated within 60 and 90 days post insemination, respectively, were confirmed for pregnancy by rectal palpation. The conception rate was calculated using the following formula suggested by [6].

$$\text{Conception Rate \%} = \frac{\text{Number of animals detected pregnant}}{\text{Number of animals tested for pregnancy}} \times 100$$

The data was analyzed using statistical methods suggested by [9] and significance within subgroups was tested by Duncans Multiple Range Test as modified by [4].

**Results and Discussion**

The CDC's selected for the study purposes are functioning for more than 25 years and in-charges working as A.I. technicians have more than 22 years' experience of A.I. and they are working on these CDC's since more than 18 years at the same locations. Both the in-charges were performing more than 3800 A.I.'s annually, in which cattle and buffalo A.I. ratio is approx. 80:20. The overall mean conception rate for HF 100%, HF75% and Murrah buffalo was recorded 57.79 ± 0.83, 58.09 ± 0.94 and 53.63 ± 1.06 percent respectively and sperm concentration-wise conception rate was recorded as 56.75 ± 0.77, 56.57 ± 1.14 and 57.08 ± 1.01 percent, for 20, 15 and 10 million sperm concentration per straw, respectively (Table 1).

**Table 1:** Breed-wise mean conception rate for different sperm concentrations per straw.

Bull Breed	Sperm concentration/straw			Total
	20 million	15 million	10 million	
HF100%	59.33 ± 1.10 (1994)	55.08 ± 1.98 (630)	56.28 ± 1.66 (892)	57.79 ± 0.83 (3516)
HF75%	57.20 ± 1.42 (1201)	58.42 ± 1.92 (659)	59.09 ± 1.63(853)	58.09 ± 0.94 (2713)
Murrah	50.84 ± 1.61 (958)	56.10 ± 2.04 (590)	55.54 ± 1.96 (941)	53.63 ± 1.06 (2189)
Overall	56.75 ± 0.77 (4153)	56.57 ± 1.14 (1879)	57.08 ± 1.01 (2386)	56.81 ± 0.54 (8418)

Figures in parenthesis indicate the number of observations

The results were noticed to be non-significant on account of different sperm concentrations per straw as well as A.I. year and season, however significant (p < 0.05) between CDC's (Table 2).

**Table 2:** Breed-wise analysis of variance of conception rate.

Source of variance	HF100%		HF75%		Murrah buffalo	
	Degree of freedom	MSS	Degree of freedom	MSS	Degrees of freedom	MSS
CDC	1	11120.26*	1	3777.36 NS	1	5159.93 NS
A.I. year	2	884.78 NS	2	413.06 NS	2	2896.91 NS
A.I. Season	2	2043.34 NS	2	7102.44 NS	2	2316.26 NS
Concentration	2	2715.86 NS	2	597.21 NS	2	6916.81 NS
Residual	3508	2435.46	2705	2434.09	2181	2481.26

(\*p < 0.05), NS: Non-Significant

Authors did not come across any reported work in the literature comparing three sperm concentrations for freezing and fertility of semen in cattle or buffaloes, however, the findings in Pakistan noticed a non-significant effect of 15 vs 30 million sperm concentration on conception rate in buffalo [2].

As per existing procedures followed in the BAIF semen freezing laboratory, 20 million spermatozoa with 80 percent initial forward motility are packed routinely in each straw as per the MSP protocol of

the Government of India. Following this procedure, an effective dose of 8 to 10 million motile sperms are available at the time of A.I. The scientist [7] opined that 6 to 10 million living sperm per dose would secure normal breeding results. Other scientists [5] in their review article quoted the reference of [8,11] and stated that 15 million total frozen spermatozoa in a French mini straw have been found to achieve acceptable conception rates provided that post-thaw sperm survival is equal to or greater than fifty percent. According to the research workers [12] besides semen quality, the conception rate is also affected by a number of other factors including female reproductive status, animal genetics, management of animals at farmer's herd, nutrition, inseminator skill, farmer awareness etc.

### Conclusions

Based on the results obtained, it can be concluded that reduction of sperm concentration from existing 20 million to 15 million or 10 million spermatozoa per insemination dose does not affect the fertility of cryopreserved bull semen investigated through pregnancy rate under field conditions, however, more study from other parts may help further for additional reliable results before taking any further decision.

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