ADDITION OF CHLOROQUINE DIPHOSPHATE OR ASCORBIC ACID IN JERSEY BULL SEMEN AND SUBSEQUENT EVALUATION WITH RESPECT TO POST-THAW INCUBATION TIME AND CONCEPTION RATE

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ABSTRACT

Total 36 ejaculates (6 from each Jersey bull) were used in the study and aliquots were added with Chloroquine diphosphate or Ascorbic acid or served as control. Semen straws from all the groups were analyzed at 0, 1 and 2 h post-thaw incubation at 37°C for percent viability, progressive motility, reaction to hypo-osmotic solution and acrosomal integrity. Following post-thaw incubation, an improvement ($p<0.05$) in all the evaluated semen parameters was recored in groups with semen additives compared to control. The conception rate was better ($p<0.05$) using semen fortified with ascorbic acid.

Keywords: Acrosomal integrity, Ascorbic acid, Chloroquine diphosphate, Conception rate, HOST

INTRODUCTION

Mammalian spermatozoa are extremely sensitive to oxidative damage, *in-vivo* as well as *in-vitro*. The process of peroxidation induces structural alterations, particularly in the acrosomal region, a fast and irreversible loss of motility, a deep change in metabolism and a high rate of intracellular components release (Cecil and Baskt, 1993). Membrane stabilizers and antioxidants have beneficial effect on the membrane integrity and biofunctional activity of spermatozoa. Ascorbic acid, a biologically active reducing agent, restores fertility possibly by the reduction of anti-agglutination factor on sperm membrane from inactive form to active form (Lindahl, 1966). Chloroquine diphosphate as membrane stabilizer was used earlier in preservation of buffalo semen (Kumar, 1992). The success of cryopreservation depends largely on the specific susceptibility of sperm cells to low temperature. The overall impact is seen in fertility in terms of lowered conception than with the fresh semen.

The present study investigated the impact of semen additives (Chloroquine diphosphate and Ascorbic acid) on routine semen evaluation parameters with respect to post-incubation time and conception rate.

MATERIALS AND METHODS

The study was conducted on 36 ejaculates (6 from each bull) collected twice a week from each of six apparently healthy purebred Jersey breeding bulls (age, 2.5-8.0 yr), maintained at Sperm Station, Palampur, Himachal Pradesh, India (32.6^{O}N, 76.3^{O}E, altitude 1290.8 m). After initial evaluation, the neat semen extended in TRIS-based extender was divided into three aliquots (10 ml diluted semen) viz. control (G_1), Chloroquine diphosphate @ 10^{-5}M concentration (G_2) or Ascorbic acid @ 0.02% concentration (G_3). All the semen dilution, extension and modified extension procedures were carried out at 37^{o}C with 80M spermatozoa/ml of diluted semen. Tested semen samples were filled in 0.25 ml French mini plastic straws and these were frozen as per the standard procedures. Semen straws from all the groups were thawed at 37^{o}C for 30 second and were evaluated at 0, 1 and 2 h post-thaw for percent viability, progressive motility, reaction to 150 mOsmol hypo-osmotic solution, and acrosomal integrity. For fertility trials, 55 cows were inseminated

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Table 1: Relationship between post-thaw incubation time with semen evaluation parameters following the addition of semen additives. G1, Control; G2, Chloroquine Diphosphate; G3, Ascorbic acid

Group	Correlation Coefficient	Regression Estimate	Regression Equation
Live Sperms, %			
G_1	-0.72**	-6.34±0.81	y = 53.85±1.04–6.34x±0.81
G_2	-0.73**	-6.67±0.81	y = 57.28±1.11–6.67x±0.81
G_3	-0.75**	-6.62±0.17	y = 57.98±0.93–6.62x±0.17
Progressive Motility, %			
G_1	-0.80**	-9.65±0.71	y = 47.84±0.91–9.65x±0.71
G_2	-0.76**	-9.06±0.72	y = 50.10±0.93–9.06x±0.72
G_3	-0.83**	-9.31±0.61	y = 51.24±0.80-9.31x±0.61
HOST, %			
G_1	-0.78**	-7.71±0.59	y =40.74±0.77–7.71x±0.59
G_2	-0.81**	-7.38±0.53	y =46.18±0.63–7.38x±0.53
G_3	-0.75**	-7.53±0.65	y =45.53±0.84–7.53x±0.65
Acrosomal Integrity, %			
G_1	-0.78**	-7.82±0.61	y =67.78±0.79–7.82x±0.61
G_2	-0.69**	-5.96±0.59	y =68.25±0.76–5.96x ±0.59
G_3	-0.79**	-6.63±0.49	y =70.01±0.64–6.63x±0.49

**p<0.01

with straws of each group The data were analyzed by SPSS® 20 level version for windows.

RESULTS AND DISCUSSION

All the semen quality assessment traits viz. live sperms, progressive motility, HOST reactive spermatozoa and acrosomal integrity were negatively and significantly (p<0.05) correlated with the post-thaw incubation time (Table 1), as observed earlier (Sharma *et al.*, 2012; Rastegarnia *et al.*, 2013). Furthermore, the post-thaw deterioration of semen quality was comparatively less in semen with additives in comparison to unfortified one (Table 1). Out of 165 cows inseminated (n=55 in each group), the conception rate was 45.5, 56.4 and 65.5% in cows of control, Chloroquine diphosphate and Ascorbic acid group, respectively. Thus, ascorbic acid as an anti-oxidant improved (p<0.05) the post-thaw quality of frozen semen of Jersey bulls. Higher conception rates with no statistical difference were also observed in the semen preserved with Chloroquine diphosphate in buffaloes (Singh *et al*., 2000). The decline in progressive motility observed in the present study after storage may be associated with decrease in live-dead sperm ratio and gradual exhaustion of some vital endogenous reserves in a highly differentiated cell which has lost its capacity for protein synthesis (Kumar, 2007).

In brief, it can be concluded that semen parameters had negative correlation with post-thaw incubation time, however, post-thaw deterioration of the semen quality was comparatively less in semen with additives and the conception rate was better following the use of fortified semen.

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