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Research Article

Studies on effect of antioxidants on cattle and buffalo semen during storage at refrigeration temperature

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ABSTRACT

Effects of antioxidants, viz. butylated hydroxy anisole, Vitamin E, Vitamin C and n-Propyl gallate on physico-morphological characters of semen of Jersey, Holstein Friesian (HF) and Murrah bulls during storage at refrigeration temperature were studied. It was observed that semen of Jersey, HF and Murrah bulls had better preservability after addition of antioxidants in the diluents. It was further revealed that progressive sperm motility and live sperm percentage were maximum in n-Propyl gallate fortified diluents as compared to other antioxidants' fortified diluents. Butylated hydroxy anisole did not exhibit positive role for the improvement of progressive sperm motility and live sperm percentage, whereas, it had beneficial effects on acrosomal integrity. Addition of optimum concentration of antioxidants in diluents could be effective to improve semen preservability by preventing lipid peroxidation in sperm cell membrane and thus, its fertility.

Key words: Cattle, buffalo, antioxidant, semen

Artificial Insemination (AI) in bovine has been recognized the world over as the fastest and most suitable means of dissemination of superior germplasm material for increasing milk yield. The effectiveness of AI technique has been enhanced with invention of frozen semen technology. An important reason for decreased fertility during storage of liquid semen is mainly the formation of lipid peroxides in the presence of reactive oxygen species or oxygen radicals due to cold shock.

The sperm plasma membrane contains high amount of polyunsaturated fatty acids and is therefore, particularly susceptible to peroxide damage with subsequent loss of membrane integrity, impaired cell functions and decreases motility of spermatozoa (Alvarez *et al.*, 1987; Griveau *et al.*, 1995). The process of peroxidation induces structural alteration, particularly in acrosomal region of sperm, changes in metabolism and enhances the rate of intracellular component release (Jones and Mann, 1977). Superoxide dismutase, catalase and glutathione peroxidase are to provide first defence against reactive oxygen species (Witting, 1980). However, bovine spermatozoa have little concentration of catalase enzyme (Holland, *et al.*, 1982), thus, bovine spermatozoa have lack of protection against lipid peroxidation. Various antioxidants like n-Propyl gallate (Bains *et al.*, 1996; Masaki *et al.*, 1994) and Butylated hydroxyl anisole helps to prevent the lipid peroxidation in sperm cells and hence protect them from the reactive oxygen species liberated in the process. Hence, development of an improved cryopreservation technique by the addition of suitable antioxidants can be of great help in improving the quality of frozen semen used for Artificial Insemination for genetic improvement of buffalo. Keeping in view the above facts, the present study was carried out with the objective of studying the effect of antioxidants (Butylated hydroxy anisole, Vitamin E, Vitamin C and n-Propyl gallate) on cattle and buffalo semen diluted in EYC and stored at refrigeration temperature.

MATERIALS AND METHODS

One each of Jersey, HF and Murrah breeding bulls of known fertility and in good health were taken as

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experimental animals in the present study. Animals were maintained under uniform husbandry and managemental conditions. The semen was collected twice weekly for ten successive weeks using artificial vagina. A total of 12 ejaculates were collected from each bull. Efforts were made to find correlation coefficients among various physico-morphological characters in neat semen of the three bulls. The preservation of semen of all the bulls was carried out in EYC, EYC + ethanol, EYC + butylated hydroxy anisole, EYC + Vitamin E, EYC + Vitamin C and EYC + n-Propyl gallate employing an extension rate of 1:10. Different concentrations of antioxidants were used to study their effects on quality of semen during its preservation. For convenience, codes for various concentrations were given as under:

EYC - C_1, EYC + ethanol – C_2, EYC + BHA (25 M) – B_1, EYC + BHA (12.5 M) – B_2, EYC + Vitamin E (15 M) – E_1, EYC + Vitamin (10 M) – E_2, EYC + Vitamin C (45 M – VC_1, EYC + Vitamin C (30 M) – VC_2, EYC + n-Propyl gallate (25 M) – nPg – 1 and EYC + n-Propyl gallate (15 M) – nPg – 2.

Undiluted and processed semen was evaluated for progressive sperm motility, live sperm percentage, abnormalities of sperm head, mid-piece, tail and acrosome, R – value and methylene blue reduction test at 15 minutes, 24 hrs and 48 hrs of storage of semen.

RESULTS AND DISCUSSION

The mean values for volume, sperm mass motility, progressive sperm motility, sperm concentration, live sperm percentage, sperm head, mid-piece, tail and total abnormalities, acrosome abnormalities, resistance to 1% Sodium chloride and methylene blue reduction test in neat semen of Jersey, HF and Murrah bulls were 4.17±0.19 ml, 3.66±0.86 grade, 71.25±1.01%, 986.33±38.54 x 10^6/ml, 86±1.34%, 7.10±0.79%, 5.17±0.27%, 5.5±0.31%, 17.50±1.08%, 6.0±0.34%, 6641.67±226.13 and 6.38±0.28 min (Jersey); 5.05±0.86 ml, 3.58±0.64 grade, 80.58±1.29%, 997.08±99.41 x 10^6/ml, 87.33±1.29%, 2.83±0.22%, 4.0±0.27%, 2.67±0.22%, 9.58±0.28%, 9.83±0.40%, 5666.67±309.77 and 7.75±0.52 min (HF) and 3.78±0.12 ml, 3.52±0.12 grade, 80.00±1.50, 1093.29±111.94 x 10^6/ml, 88.75±1.44%, 3.0±0.27%, 4.67±0.28%, 2.58±0.14%, 10.25±0.59%, 10.92±0.78%, 5083.33±280.91 and 6.17±0.37 min (Murrah), respectively. The semen volume, live sperm percentage, sperm concentration, sperm abnormalities, R-value and metabolic rate varied significantly ($P<0.05$) between the bulls. It indicated that these characters were subjected to great variation due to genotypic effects of bulls. However, none of the characters was affected due to variation between the replicates indicating conformity in reproductive behaviour of bulls, semen collection and processing, etc. throughout the experimental period. The results of the present and earlier findings indicated that there existed a minor difference between the investigators, which might be possible because of genetic make-up, age, season and variation in the function of different accessory glands (Bhoserekar, 1980; Dhami and Sahni, 1994; Dhami *et al.*, 1998; Jainudeen *et al.*, 1982; Nadroo, 1983).

Study of correlation coefficients (Table 1, 2 and 3) among various semen characteristics revealed that semen volume was significantly ($P<0.05$) correlated with live sperm percentage (0.989) in Jersey bull. Mass sperm motility had highly significant ($P<0.01$) correlationship with live sperm percentage in Jersey (0.858) and in Murrah bulls (0.593). The correlation was significantly positive between mass sperm motility and progressive sperm motility in Murrah bull. Mass sperm abnormalities was also significantly ($P<0.01$) but negatively correlated with acrosomal abnormalities (-0.566) and MBRT value (-0.748) in Jersey bull only. The progressive sperm motility had significantly positive ($P<0.05$) correlation (0.657) with sperm concentration in Jersey bull, whereas, its correlationship was significantly negative with MBRT value (-0.660) in Jersey and acrosomal abnormalities (-0.674) in HF bull. Live sperm percentage showed significant ($P<0.05$) correlation (0.561) with sperm head abnormalities and MBRT value (-0.809) in Jersey bull, a significant ($P<0.01$) positive correlation (0.634) with sperm concentration and negative correlation (-0.674) with acrosomal abnormalities in HF bulls. However, in Murrah bull, the live sperm percentage had significantly ($P<0.05$) positive correlation with sperm head abnormalities (0.612). Sperm concentration was significantly ($P<0.05$) and positively correlated (0.657)

Table 1: Correlation coefficient among various characters in neat semen of Jersey bull (n = 12)

Parameter	2	3	4	5	6	7	8	9	10	11	12
Volume (ml)	-0.017	-0.297	0.989*	-0.102	0.450	-0.234	0.146	0.349	0.096	-0.480	-0.127
Mass sperm motility (0-5)		0.452	0.858**	0.210	0.370	-0.038	0.196	0.345	-0.566**	0.547	-0.748*
Progressive sperm motility (%)			0.539**	0.657*	-0.261	0.124	-0.059	-0.196	-0.107	0.249	-0.660*
Live sperm (%)				0.189	0.561*	-0.125	0.126	0.451	-0.421	0.445	-0.809**
Sperm conc. (x 10^6)					-0.241	0.686*	-0.113	-0.07	0.017	-0.133	-0.542
Sperm head abnormality (%)						-0.042	0.376	0.908**	-0.169	-0.053	-0.386
Sperm mid piece abnormality (%)							0.089	0.266	0.080	-0.293	-0.169
Sperm tail abnormality (%)								0.639*	0.069	-0.050	-0.146
Total sperm abnormality (%)									-0.007	-0.144	-0.402
Acrosome abnormality (%)										-0.588*	0.526
R value											-0.210
MBRT (min)											

* Significant at 5% level

**Significant at 1% level

Table 2: Correlation coefficient among various characters in neat semen of Holstein Friesian (n = 12)

Parameter	2	3	4	5	6	7	8	9	10	11	12
Volume (ml)	0.081	-0.204	0.374	0.114	0.497	-0.098	0.200	0.229	-0.513	0.512	-0.185
Mass sperm motility (0-5)		0.584*	0.539	-0.476	0.107	-0.219	0.195	0.127	0.127	0.252	0.103
Progressive sperm motility (%)			0.290	-0.157	0.118	-0.449	-0.096	0.660*	0.285	0.090	0.316
Live sperm (%)				-0.601	0.612*	-0.017	-0.311	0.067	-0.674*	-0.050	0.426
Sperm conc. (x 106)					0.080	0.217	0.657*	-0.030	-0.315	0.346	-0.532
Sperm head abnormality (%)						-0.678*	0.383	0.204	-0.089	0.417	-0.012
Sperm mid piece abnormality (%)							0.039	0.855**	-0.363	-0.256	-0.151
Sperm tail abnormality (%)								0.624*	-0.269	0.612	-0.530
Total sperm abnormality (%)									-0.342	0.142	0.146
Acrosome abnormality (%)										0.548*	-0.152
R value											-0.426
MBRT (min)											

* Significant at 5% level

**Significant at 1% level

Table 3: Correlation coefficient among various

Table 3: Correlation coefficient among various characters in neat semen of Murrah bull (n = 12)

Parameter	2	3	4	5	6	7	8	9	10	11	12
Volume (ml)	-0.027	-0.097	0.089	-0.142	0.350	-0.434	0.600	0.142	0.317	-0.380	-0.147
Mass sperm motility (0-5)		0.858	0.593*	0.312	0.470	-0.138	0.298	0.445	-0.356	0.447	-0.748*
Progressive sperm motility (%)			0.503	0.518	-0.561	0.521	-0.447	-0.096	-0.407	0.349	-0.360
Live sperm (%)				0.612*	0.361	-0.225	0.126	0.352	-0.521	0.246	-0.409
Sperm conc. (x 106)					-0.678**	0.765**	-0.113	-0.147	0.007	-0.233	-0.342
Sperm head abnormality (%)						-0.427	0.612*	0.408	-0.269	-0.254	-0.488
Sperm mid piece abnormality (%)							0.029	0.855	0.295	-0.221	-0.169
Sperm tail abnormality (%)								0.624*	0.022	0.287	0.252
Total sperm abnormality (%)									-0.184	-0.148	-0.241
Acrosome abnormality (%)										0.451	0.491
R value											-0.466
MBRT (min)											

* Significant at 5% level
**Significant at 1% level

* Significant at 5% level
**Significant at 1% level

with sperm tail abnormalities in HF bull, whereas, sperm concentration of buffalo bull had significant (P<0.01) correlation with sperm head abnormalities (-0.678) and mid-piece abnormalities (0.765).

Sperm head abnormalities were significantly (P<0.05) positively correlated with the sperm mid-piece abnormalities in HF (0.678) and sperm tail abnormalities in Murrah bull (0.612), while a significant (P<0.01) correlation was observed with total sperm abnormalities in Jersey bull (0.908).

The sperm tail abnormalities were significantly (P<0.01) positively correlated (0.855) with total sperm abnormalities. Sperm tail abnormalities had significant (P<0.05) positive correlation (0.639, 0.624 and 0.624) with total sperm abnormalities in Jersey, HF and Murrah bulls, respectively. The results of correlation study among various characters of semen were fairly agreed with the findings of Gopal Krishna and Rao, 1979; Hazarika *et al.*, 1990; Saxena and Tripathi, 1980. The minor differences in the values may be due to different breeds of bulls, age, agro-climatic condition and hereditary functions.

Results of preservation of semen with various antioxidants revealed that most of the antioxidants used in the present study had beneficial effects on maintenance of progressive sperm motility, live sperm percentage and acrosomal integrity. Butylated hydroxyl anisole (B-1 and B-2) had no beneficial effect on progressive sperm motility and lives sperm percentage, while beneficial effect was observed on acrosome integrity. Acrosome abnormalities were low in butylated hydroxyl anisole treated groups than controls. No beneficial effects of antioxidants, except that of n-Pg-1, were found on the sperm morphology in the semen of Jersey, HF and Murrah bulls. Findings of the present investigation were fairly in agreement with the findings of Anderson *et al.*, 1994; Arora *et al.*, 1996; Beconi *et al.*, 1993; Lindemann *et al.*, 1988; Singh *et al.*, 1996; Singh *et al.*, 198; Wang *et al.*, 1997.

It may be concluded that the semen of Jersey, HF and Murrah bulls had better preservability after addition of antioxidants in the diluents. It was further

observed that percentage of progressive sperm motility and live sperm percentage was maximum in n-Propyl gallate fortified diluent (nPg-1, nPg-2) after storage as compared to other antioxidant's fortified diluents. Beside this, the acrosomal abnormalities were also lowest in nPg-1 and nPg-2 during storage of semen. In this study, butylated hydroxyl anisole did not exhibit positive role for the maintenance of progressive sperm motility and live sperm percentage, whereas, it had beneficial effect on maintenance of acrosomal integrity. Findings indicate that lower concentration of BHA (B-2) had some beneficial effects on progressive motility and live percentage, but these values were low as compared to control. It may be further inferred that the environmental stress in the exotic and high yielding indigenous bulls of the tropics could influence their semen quality, quantitatively and morphologically. Addition of optimum concentration of antioxidants in dilutors could be effected to improve semen preservability by preventing lipid-peroxidation in sperm cell membrane and thus its fertility.

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