Effect of Antioxidant Fortification on Preservability of Buffalo Semen

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ABSTRACT : During the process of freezing, spermatozoa suffer cold shock which increases their susceptibility to lipid peroxidation which plays an important role in ageing of spermatozoa, shortening their life span and affecting the preservation of semen. An experiment was therefore conducted to study the effect of addition of natural antioxidants into semen diluents on the preservability of buffalo semen. Split semen samples were extended in milk egg yolk diluents fortified with vitamin E (MYE), vitamin C (MYC) and control group (MYO); Tris-egg yolk diluents fortified with vitamin E (TYE), vitamin C (TYC) and control group (TYO) and evaluated for their preservabilities at 4-7°C and 37°C. Overall least squares mean of percent motility observed after 0, 24, 48, 72 and 96 h of preservation at 4-7°C were 66.70, 54.00, 36.80, 21.90 and 12.50, respectively while the estimates for semen extended in MYE, MYC, MYO, TYE, TYC and TYO were 44.80, 42.70, 38.70, 36.00, 35.20 and 33.00 percent, respectively. The results showed that motility was significantly ($p<0.01$) affected by extender (extender-antioxidant combination) and preservation interval. Overall least squares mean percent motility observed after 0, 4, 8, 12 and 24 h of preservation at 37°C were 68.50, 58.90, 45.00, 38.10 and 18.10 percent, respectively, while the estimates for semen extended in MYE, MYC, MYO, TYE, TYC and TYO were 48.20, 49.30, 46.80, 45.30, 42.30 and 42.50 percent, respectively. Extender and storage interval were found to be significantly ($p<0.01$) affecting spermatozoa motility on room temperature preservation. The results indicated that the incorporation of antioxidants, especially vitamin E, had beneficial effect on preservability of buffalo semen. ***(Asian-Aust. J. Anim. Sci. 2002. Vol 15, No. 1 : 16-18)***

Key Words : Antioxidant, Buffalo Semen, Murrah, Semen Preservability

INTRODUCTION

To meet the objective of augmenting milk production, special attention is required to make available sufficient number of semen doses from superior buffalo bulls. The present scenario of semen production is beset with a number of problems such as low sperm harvest, seasonal variation in semen quality, freezability and inconsistencies in frozen semen production technologies. During the process of freezing, spermatozoa are exposed to cold shock, which increases their susceptibility to lipid peroxidation. Lipid peroxidation induces ageing of spermatozoa, reducing their life span and affecting the preservation of semen for AI (Alvarez and Storey, 1982). Slow cooling of semen from 30 to 5°C has been observed to improve fertility performance of cattle. However, slow cooling may lead to increase in respiration, which is subsequently detrimental to sperm viability. Addition of antioxidant might be useful in preventing the damage under such condition.

With an overall objective of improving upon the existing methodologies of cryopreservation of buffalo semen and developing a suitable package, the present experiment was conducted to study the effect of addition of natural antioxidants on preservability of buffalo semen.

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MATERIALS AND METHODS

Semen ejaculates from Murrah buffalo bulls maintained at Artificial Breeding Complex, National Dairy Research Institute, Karnal, were used for the present study. Split semen samples extended in milk egg yolk diluents fortified with vitamin E (MYE), vitamin C (MYC) and control group (MYO); Tris-egg yolk diluents fortified with vitamin E (TYE), vitamin C (TYC) and control group (TYO) were evaluated for their preservabilities at refrigerator temperature (4-7°C) and room temperature (37°C). Antioxidant fortification was done by addition of antioxidant to the diluent at the rate of 1 mg/ml of vitamin E (alpha- tocopherol acetate, Loba Chemie, India) for MYE and TYE and 5 mM of Vitamin C (Sodium ascorbate, Fluka, Biochemica) for MYC & TYC extenders. Percent motility (%) was recorded after 0, 24, 48, 72 and 96 h of preservation at 4-7°C, and after 0, 4, 8, 12 and 24 h of preservation at 37°C. Observations on percent motility were made independently by three persons and the average of the observations was taken as percent motility. To study the effect of antioxidant (extender-antioxidant combination) and stage/interval of preservation on preservability of semen, the data were analysed by least squares technique (Harvey, 1975) using the following model:

$$Y_{ijk}=\mu+E_i+I_j+(E\ I)_{ij}+e_{ijk}$$

Where,

Y_{ijk}=kth observation of j^{th} stage of/interval of preservation.

Table 1. Percent motility estimate (%) of buffalo spermatozoa preserved at 4-7°C in antioxidant fortified extenders

Hours\Extender	MYE	MYC	MYO	TYE	TYC	TYO	Least squares mean
0	67.50±2.81	66.67±2.47	65.83±2.01	67.50±2.81	65.83±1.54	66.67±2.47	66.70±0.20
24	59.17±2.81	56.67±1.67	52.50±3.35	50.83±3.74	54.17±3.27	50.83±3.74	54.00±0.20
48	43.33±3.80	42.50±2.14	40.00±2.89	31.67±2.47	34.17±4.36	29.17±2.39	36.80±0.20
72	32.50±3.10	29.17±2.71	23.33±3.58	18.33±1.67	15.00±1.83	13.33±1.67	21.90±0.20
96	21.67±3.07	18.33±1.67	11.67±1.05	11.67±1.05	6.67±1.05	5.00±0.00	12.50±0.20
Least squares mean	44.80±0.22	42.70±0.22	38.70±0.22	36.0±0.22	35.20±0.22	33.00±0.22	

Table 2. Percent motility (%) of buffalo spermatozoa preserved in antioxidant fortified extenders at room temperature (37°C)

Hours\Extender	MYE	MYC	MYO	TYE	TYC	TYO	Least squares mean
0	68.33±2.47	69.17±2.71	67.50±2.81	68.33±2.47	68.33±2.47	69.17±2.71	68.50±0.18
4	65.00±3.65	65.00±2.89	59.17±3.52	59.17±2.39	54.17±2.39	50.83±2.01	58.90±0.18
8	47.50±1.71	51.67±2.79	46.67±2.79	44.17±2.39	40.00±2.24	40.00±1.83	45.00±0.18
12	39.17±1.54	41.67±3.33	38.33±1.67	38.33±2.11	35.00±2.24	35.83±2.39	38.10±0.18
24	20.83±2.01	19.17±2.39	22.50±1.12	15.00±1.83	14.17±1.54	16.67±1.05	18.10±0.18
Least squares mean	48.20±0.20	49.30±0.20	46.80±0.20	45.30±0.20	42.30±0.20	42.50±0.20	

Table 3. Least squares analysis of variance of percent motility (%) of buffalo spermatozoa preserved in antioxidant fortified extenders

Source of variation	Preservation at 4-7 °C		Preservation at 37 °C	
	df	M.S.	df	M.S.
Extender	5	314.6**	5	96.4**
Interval	4	7213.0**	4	5106.6**
Extender -interval interaction	20	39.0	20	16.0
Residual	150	16.2	150	13.3
Total	179	187.9	179	129.7

** Significant at 1% level of significance.
Df : Degree of freedom ; M.S. : Mean squares.

μ=Overall mean
E_i=the effect of i^{th} antioxidant (Extender-antioxidant combination)
I_j=the effect of j^{th} stage / interval of preservation
$(E\ I)_{ij}$=the effect of $(ij)^{th}$ antioxidant (Extender-antioxidant combination) - stage/interval of preservation interaction
e_{ijk}=Random error associated with $(ijk)^{th}$ observation, NID(0, $\sigma^2 e$)

RESULTS AND DISCUSSION

The results of percent motility (%) of semen preserved at 4-7°C are depicted in table 1. Overall least squares mean of percent motility (%) observed after 0, 24, 48, 72 and 96 h of preservation were 66.70, 54.00, 36.80, 21.90 and 12.50, respectively. The least squares mean of percent motility for semen extended with MYE, MYC, MYO, TYE, TYC and TYO were 44.80, 42.70, 38.70, 36.00, 35.20 and 33.00 percent, respectively. The results showed that percent motility was significantly ($p<0.01$) affected by extender (extender-antioxidant combination) and preservation interval (table 3). However, percent motility was not significantly affected by extender-interval interaction. With the increase in storage interval, there was deterioration in semen quality. Incorporation of antioxidants improved the performance of extenders. At all the stages of preservation, semen diluted in Milk-egg yolk extender fortified with vitamin E showed maximum percent motility. From the results it could be concluded that Milk-egg yolk extender fortified with vitamin E was most suitable for preserving motility of buffalo bull spermatozoa upon storage at 4-7°C.

Overall least squares mean percent motility after 0, 4, 8, 12 and 24 h of room temperature (37°C) preservation were 68.50, 58.90, 45.00, 38.10 and 18.10 percent, respectively (table 2). The overall mean percent motility with MYE, MYC, MYO, TYE, TYC and TYO were 48.20, 49.30, 46.80, 45.30, 42.30 and 42.50 percent, respectively. Extender and stage of preservation were found to be significantly ($p<0.01$) affecting spermatozoa motility on room temperature preservation (table 3). Whereas the effect of interaction was not statistically significant.

The overall results indicated that the incorporation of antioxidants, especially vitamin E, had beneficial effect on preservation of semen. In conformity with the present

findings, several investigations using antioxidants for preservation of semen in various species such as ram (Srivastava et al., 1987; Nauk and Boronchuk, 1992), boar (Nishimura and Morri, 1992), bull (AI-Khanak and AI-Hanak, 1989; Beconi et al., 1993) indicated that natural antioxidants exert a protective effect on the plasma membrane, preserving both metabolic activity and cellular viability. The present results were in agreement with other reports on different species, such as bull, boar and ram (Stolbov and Rimanova, 1983; Golyshev, 1985; Beconi et al., 1993; Salmon and Maxwell, 1995).

Positive results in favour of milk-egg yolk extender fortified with antioxidant, especially vitamin E suggested that the addition of antioxidant may lead to achieving better preservability of buffalo semen, thus increasing the number of semen doses available from a buffalo bull.

REFERENCES

AI-Khanak, K. and H. AI-Hanak. 1989. The protective action of tocopherol during cryopreservation of bull semen. Zhivotnov'dni Nauki. 26:70-74. Anim. Breed. Abstr. 59:3193.

Alvarez, J. G. and B. T. Storey. 1982. Spontaneous lipid peroxidation in rabbit epididymal spermatozoa: its effect on sperm motility. Biol. Reprod. 27:1102-1108.

Beconi, M. T., C. R. Francia, N. G. Mora and M. A. Affranchino. 1993. Effect of natural antioxidant of frozen bovine semen preservation. Theriogenology 40:841-851.

Golyshev, N. A. 1985. Improving the technology of freezing boar semen. Zhivotnovodstvo 7:49-51.

Harvey, W. R. 1975. Least squares analysis of data with unequal sub-class numbers. ARS H-4, USDA, Washington, DC.

Nauk, V. A. and G. V. Boronchuk. 1992. Effectiveness of antioxidants in freezing of ram semen. Cited in Anim. Breed. Abstr. 60:6419.

Nishimura, K. and H. Morri. 1992. Effect of alpha-tocopherol on motility of frozen-thawed boar spermatozoa. J. Reprod. Develop. 38:55-59.

Salmon, S. and W. M. C. Maxwell. 1995. Frozen storage of ram semen. 1. Processing, freezing, thawing and fertility after cervical insemination. Anim. Reprod. Sci. 37:185-249.

Srivastava, R. S., A. K. Mathur and D. B. Kalra. 1987. Effect of alpha tocopherol on preservability of ram semen. Indian J. Anim. Sci. 57:553-554.

Stolbov, V. M. and L. D. Rimanova. 1983. The effect of vitamins in the diluent on the quality of thawed bull semen. Kriokonservatsiya gameti embryonov sel'.khoz.zhivotnykh: 54-57.