

CAFFEINE AS A SEMEN ADDITIVE TO IMPROVE MURRAH BUFFALO (*Bubalus bubalis*) SEMEN CRYOPRESERVATION

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ABSTRACT

Caffeine was incorporated in egg yolk tris glycerol extender (EYTG) at the time of dilution of Murrah semen at a concentration of 1.0, 3.0 or 5.0 mM. The samples were subsequently frozen. The post thaw progressive motility and live sperm per cent improved significantly ($P < 0.05$) with addition of 1.0 (70.0 ± 0.99 and 88.80 ± 0.84) and 3.0 mM (65.83 ± 0.88 and $86.16 \pm 0.96\%$) caffeine to the extender before semen dilution and cryopreservation as compared to the control (61.94 ± 1.15 and $79.05 \pm 1.02\%$), when evaluated at 0 h post freeze. All the three concentrations of caffeine significantly improved the hypoosmotic swelling (HOS) % and protected the sperm from becoming abnormal as observed at 0 and 48 h after freezing. Thus incorporation of caffeine (1.0 or 3.0 mM) in EYTG extender, prior to freezing may be useful in improving the quality of cryopreserved bubaline semen.

Keywords: Murrah semen, stimulant, caffeine, cryopreservation

INTRODUCTION

During cryopreservation and thawing, solution effects inflict considerable damage to the spermatozoa (Mazur, 1984). The conception rate to AI with frozen thawed semen in buffalo is only about 33% (Chohan *et al.*, 1992; Bhosrekar *et al.*, 2001) which may be due to decline in motility, damaged acrosome and altered plasma membrane integrity (Rasul *et al.*, 2001) due to cryodamage inflicted during the process of freezing.

Caffeine exerts a beneficial effect by stimulating the kinetic activity and respiration in cattle spermatozoa (Drevius, 1973) by causing the inhibition of enzymes like nucleotide phosphodiesterase involved in sperm glycolysis (Hardman *et al.*, 1971) resulting in increased intracellular cAMP and/ or cGMP concentration (Hoskins *et al.*, 1975) which is essential for the maintenance of dyein generated sperm motility (Lindermann *et al.*, 1983). The cAMP also acts by binding to sperm membrane causing conformational changes leading to increased transport of substrates (Garbers *et al.*, 1973) accompanying metabolic activation (Hicks *et al.*, 1972).

Keeping in view the importance of buffalo as an important dairy animal for Indian livestock

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economy and the need for scientific intervention into cryopreservation process of buffalo semen, present study was conducted to study the effect of caffeine on Murrah semen cryopreservation and to standardize suitable concentration of Caffeine required for optimum protection from cryoinjuries.

MATERIALS AND METHOD

Semen samples were collected from three Murrah buffalo bulls, having known good spermogram over past many years. The bulls were maintained in an intensive housing system and subjected to identical conditions of management and feeding. A total of 18 ejaculates, collected in a sterilized goat AV, were used to evaluate the effect of stimulant caffeine on semen cryopreservation. Prepuce of the bulls was washed 12 h and 30 minutes before the semen collection with 0.01% acriflavin solution. After collection, the semen samples were immediately transferred to a water bath maintained at 35°C and kept under laminar airflow to avoid exposure to direct sunlight and bacterial contamination.

Semen samples were evaluated for progressive sperm motility (Tomar, 1997), hypoosmotic swelling test (Jeyendran *et al.*, 1984), per cent live sperm (Campbell *et al.*, 1960) and sperm abnormalities (Roberts, 1982) immediately after collection.

Soon after the neat semen evaluation, sperm concentration was evaluated by haemocytometer method. Each ejaculate was diluted (to ensure 60 million progressively motile sperm/ml) in egg yolk tris glycerol (EYTG) extender. The ejaculates after dilution were split into four parts and treated as follows:

1. Part 1: Diluted in EYTG (Control)
2. Part 2 : Diluted in EYTG + 1.0 mM caffeine (C1)
3. Part 3 : Diluted in EYTG + 3.0 mM caffeine (C2)
4. Part 4 : Diluted in EYTG + 5.0 mM caffeine (C3)

Diluted semen was put in French medium straws and sealed with PVA powder. These straws were kept for equilibration at 4°C for 4 h. Subsequently the semen samples were frozen using the horizontal liquid nitrogen vapour freezing technique (Verma *et al.*, 1975). Frozen semen samples were thawed at 37°C for 1 minute, immediately (0 h) and 48 h after freezing and examined for progressive sperm motility, live (%), sperm morphology and HOS %.

All the chemicals used in the experiment were from Sigma- Aldrich, USA.

The results were subjected to one way analysis of variance and the paired t-test (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

The results of the trial conducted to evaluate the effect of 1.0 mM (C1), 3.0 mM (C2) and 5.0 mM (C3) concentrations of caffeine are summarized in Table 1.

As evident from the table, significant ($P < 0.01$) beneficial effect of 1.0 mM and 3.0 mM caffeine on the progressive sperm motility, live sperm, total sperm abnormalities and HOS % recorded in the present study is in conformity with the reports of the beneficial effect of 2.0 mM caffeine reported by others (Fattouh *et al.*, 1985; Fattouh and Abdou, 1991). Significantly

higher post-thaw progressive sperm motility, live sperm per cent and reduced sperm abnormalities in buffalo semen fortified with 4.0 mM caffeine (Singh and Raina, 2000) prior to freezing has also been reported .

The difference in the effect of 1.0 and 3.0 mM caffeine was also significant (P<0.01). Supporting reports of better effect of 2.0 mM caffeine on cryopreservation of buffalo (Fattouh *et al.*, 1985; Fattouh and Abdou, 1991) and bovine (Garbers *et al.*, 1971) semen as compared to 4.0 or 6.0mM has also been recorded in the past. There was enhanced fertilizing ability when semen was diluted with 2.0 mM caffeine as compared to that diluted with 5.0 or 10.0 mM caffeine (Aitken *et al.*, 1983).

These reports and the significant beneficial effect of 1 mM caffeine as compared to the control and other three concentrations, recorded in the present study, suggests that even lower concentrations of caffeine may be effective in protecting sperm from cryoinjuries.

In the frozen thawed buffalo semen, some spermatozoa are alive but non motile because of their low metabolic level (Makler *et al.*, 1980; Gehlaut and Srivastava, 1987). The improvement in the progressive motility after freezing of semen with 1.0 or 3.0 mM caffeine, recorded in the present study, may also be attributed to the stimulatory effect of caffeine on progressive motility, respiration and fructolysis of epididymal (Garbers *et al.*, 1973) and

Table 1. Effect of caffeine on progressive motility, livability and abnormalities of sperm during cryopreservation Murrah semen.

Attributes Control/ Experimental Groups		Progressive sperm motility (%)	Live sperm (%)	Sperm abnormalities (%)	HOS (%)
Neat semen		78.42±0.70 ^a	90.55±0.74 ^a	10.00±0.38 ^a	69.53±0.40 ^a
Pre-freeze	C	76.67±0.90 ^a			
	C1	78.05±0.82 ^a			
	C2	77.78±0.72 ^a			
	C3	77.50±0.83 ^a			
Post-freeze (0 h)	C₀	61.94±1.15 ^b	79.05±1.02 ^b	14.50±0.32 ^b	61.14±0.80 ^b
	C1	70.00±0.99 ^c	88.80±0.84 ^a	11.78±0.16 ^c	67.00±0.69 ^{cc}
	C2	65.83±0.88 ^d	86.16±0.96 ^c	12.05±0.33 ^{cc}	65.17±0.59 ^{cd}
	C3	63.05±0.67 ^{bd}	82.33±1.03 ^d	13.22±0.29 ^d	63.19±0.90 ^d
Post-freeze (48 h)	C₄₈	61.39±0.88 ^b	78.30±1.07 ^b	14.61±0.29 ^b	60.39±0.86 ^b
	C1	70.28±1.05 ^c	89.11±0.25 ^a	12.17±0.28 ^{cc}	67.58±0.79 ^{ac}
	C2	66.11±0.89 ^d	86.50±0.94 ^c	12.89±0.31 ^{dc}	63.72±0.58 ^d
	C3	62.78±1.02 ^b	82.50±0.85 ^d	13.67±0.32 ^{bd}	62.78±0.80 ^d

Different superscripts within a column indicates significant difference (P<0.05).

C: control at pre freeze stage; C₀: control at 0 h post freezing; C₄₈: control at 48 h post freezing; C1, C2 and C3 represent 1.0, 3.0 and 5.0 mM Caffeine.

ejaculated spermatozoa (Fink *et al.*, 1985; Garbers *et al.*, 1971).

Cryopreservation damages sperm plasma membrane (Mann and Mann, 1981) resulting in disturbed regulation of Ca level in the cells (Aitken *et al.*, 1983), which may be detrimental to sperm motility (Drevius, 1973) possibly due to an increased intracellular Ca level. The cAMP associated outwardly directed Ca pump helps in regulating the Ca level inside the cell (Peterson *et al.*, 1979). Increased cAMP concentration due to inhibition of nucleotide phosphodiesterase might be responsible for efflux of calcium through the cAMP associated outwardly directed Ca pump and the mechanism seems to be more relevant in semen diluted with EYTG extender, which does not contain Ca, as the Ca efflux might be facilitated by a steep concentration gradient.

Interestingly the 1.0 mM caffeine in the present study was found to be better than 3.0 or 5.0 mM Caffeine. It appears that 1.0 mM or lesser concentrations of caffeine are sufficient to induce a beneficial effect on cryopreservation of buffalo semen and there is a need to explore the effects of caffeine at concentrations less than 1.0 mM .

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