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Role of EDTA and cysteine hydrocloride as additivies in EYC and TEFC extenders for preservations of beetal bulk's spermatozoa

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ABSTRACT

The semen sample of Beetal bucks extended in six extenders (EYC, EYC + EDTA, EYC + Cysteine Hcl, TEYFC, TEYFC + EDTA and TEYFC + Cysteine Hcl) and preserved at refrigerator temperature were evaluated for motility, livability and abnormality up to 96 hours at 24 hours interval.The effects of extender and additive on seminal attributes were significant ($P \leq 0.01$). The post preservation seminal attributes were better in TEYFC extender incorporated with Cysteine Hcl as compared to other extenders , indicating that TEYFC is an ideal semen diluent as compared to EYC and that inclusion of 0.01% Cysteine Hcl in both extenders showed beneficial effect towards improving the keeping quality of liquid semen of Beetal bucks.

Key words: EDTA, cysteine Hcl, buck, spermatozoa

Relative efficacy of EDTA and Cysteine Hcl in improving seminal quality in terms of either sperm motility, morphology or preservability of bull, ram and buffalo semen has been reported by several workers (Singh *et al.*, 1989; Dhami and Sahni, 1994 and Kumar *et al.*, 1996). Cysteine Hcl restrict aerobic metabolism and stimulate anaerobic metabolism (Sengupta *et al.*, 1969). Whereas EDTA is a powerful chelating agent and forms stable complexes with heavy metals (Abdou and El Guindi, 1977).

MATERIALS AND METHODS

This experiment was conducted on semen samples of 6 Beetal bucks maintained at Ranchi Veterinary College, Ranchi (Jharkhand) under uniform feeding and managemental conditions. A schedule of twice weekly semen collection was followed with the help of Artificial Vagina. The ejaculates having + 4 and above initial motility were pooled and centrifuged at 3000 rpm for 15 mts to remove the seminal plasma. A total of six extenders in 1:10 ratio with EYC (Salisbury *et al.*, 1941) and TEYFC (Davis *et al.*, 1963) containing each additive mixed at the rate of 1 mg/ml of extender. The extended semen was preserved at refrigerator temperature and evaluated for progressive motility, live sperm and sperm abnormility at 24 hours interval up to 96 hours of preservation. Data were subjected to statistical analysis by F test and means were compared by critical difference test (Snedecor and Cochran, 1967).

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RESULTS AND DISCUSSION

Mean values of seminal attributes at different hours of preservation in various extenders have been presented in Table 1. Higher percentage of progressive motility was observed in TEYFC + Cysteine Hcl extender at all hours of preservation, whereas lowest in EYC extender without additive (Table 1). At 0 hour of preservation , motility percentage did not differ significantly when either EDTA or Cysteine Hcl was added with TEYFC extender. The motility % was significantly higher in TEYFC extender having Cysteine Hcl as additive as compared to EDTA additive up to 96 hours of preservation. A decline trend in motility was observed in all the extenders with the increase in duration of preservation.

The increase in hours of preservation resulted decline in livability (Table 1). Percentage of live spermatozoa was significantly more in TEYFC extender

Table 1. Mean values for seminal attributes of Beetal bucks at different hours of preservation in different extenders

Hours	Extenders					
	EYC (12)	EYC+EDTA (12)	EYC+Cyst. Hcl (12)	TEYFC (12)	TEYFC+EDTA (12)	TEYFC+ Cyst. Hcl (12)
Motility (%)						
0	77.96± 0.216^{d}	78.57 ±0.168^{c}	78.97± 0.166^{c}	80.78±0.205^{b}	81.57± 0.253 a	82.04±0.212^{a}
24	74.08±0.197^{e}	75.17±0.172^{d}	76.20±0.197^{c}	76.67±0.238^{c}	77.45±0.198 b	78.77±0.202^{a}
48	60.98±0.171^{e}	62.90±0.125^{d}	65.58±0.114^{c}	65.88±0.124^{c}	67.85±0.109 b	70.84±0.118^{a}
72	46.40±0.174^{f}	50.12±0.171^{e}	55.21±0.131^{c}	53.35±0.144^{d}	58.35±0.533 b	62.42±0.139^{a}
96	33.23±0.153^{d}	38.99±0.136^{c}	46.27±0.144^{b}	38.58±0.219^{e}	46.73±0.272 b	52.61±0.211^{a}
Live sperm (%)						
0	86.91±0.202^{f}	87.72±0.160^{e}	88.51±0.166^{d}	90.45±0.183^{c}	91.29± 0.100b	91.90±0.188^{a}
24	82.38±0.166^{e}	83.56±0.153^{d}	84.91±0.253^{c}	84.79±0.209^{c}	86.29± 0.300b	87.72±0.185^{a}
48	67.23±0.165^{f}	69.91±0.131^{e}	74.12±0.153^{c}	72.47±0.142^{d}	75.37± 0.130b	78.48±0.190^{a}
72	54.31±0.154^{f}	59.23±0.144^{e}	63.42±0.146^{c}	60.98±0.107^{d}	65.82± 0.148b	70.21±0.133^{a}
96	39.35±0.147^{e}	47.61±0.231^{c}	55.16±0.191^{b}	45.42±0.247^{d}	55.50± 0.190 b	61.27±0.213^{a}
Acrosomal abnormality (%)						
0	0.84±0.002^{a}	0.84±0.002^{ab}	0.83± 0.002^{bc}	0.82±0.003^{cd}	0.82± 0.003 cd	0.82±0.003^{d}
24	1.81±0.011^{a}	1.60±0.004^{b}	1.55± 0.016^{c}	1.50±0.014^{d}	1.39± 0.014 e	1.30±0.010^{f}
48	3.14±0.023^{a}	2.85±0.010^{b}	2.55± 0.013^{d}	2.72±0.015^{c}	2.51± 0.023 d	2.33±0.012^{c}
72	4.41±0.025^{a}	4.09±0.014^{b}	3.79± 0.013^{c}	4.13±0.025^{b}	3.81± 0.025 c	3.55±0.015^{d}
96	5.63±0.026^{a}	5.33±0.020^{b}	5.14± 0.026^{c}	5.36±0.029^{b}	4.91± 0.017 d	4.58±0.018^{e}
Head abnormality (%)						
0	0.07±0.049	0.07±0.049	0.07±0.048	0.07±0.048	0.07± 0.047	0.07±0.046
24	0.08±0.052	0.08±0.052	0.08±0.051	0.08±0.051	0.07± 0.049	0.07±0.048
48	0.08±0.055	0.08±0.053	0.08±0.057	0.08±0.053	0.08± 0.052	0.08±0.052
72	0.12±0.061	0.11±0.058	0.11±0.057	0.11±0.058	0.11± 0.056	0.10±0.054
96	0.16±0.069	0.15±0.066	0.15±0.063	0.15±0.065	0.15± 0.063	0.14±0.059
Tail abnormality (%)						
0	2.73±0.016^{a}	2.64±0.016^{b}	2.61±0.017^{bc}	2.64±0.016^{b}	2.62±0.015^{c}	2.59±0.017^{c}
24	4.51±0.020^{a}	3.62±0.016^{b}	3.55±0.014^{c}	3.61±0.025^{bc}	3.15±0.023^{d}	3.03±0.021^{e}
48	6.37±0.060^{a}	5.81±0.033^{b}	5.26±0.030^{c}	5.83±0.033^{b}	5.29±0.029^{c}	4.76±0.024^{d}
72	8.28±0.039^{a}	7.63±0.041^{b}	6.66±0.048^{d}	7.68±0.041^{b}	7.09±0.051^{c}	6.28±0.105^{e}
96	10.47±0.089^{a}	9.92±0.075^{b}	8.95±0.060^{d}	9.96±0.077^{b}	9.39±0.072^{c}	8.39±0.057^{e}

Values bearing same superscripts in a row for each trait separately did not differ significantly. Figures in parentheses are number of samples.

having Cysteine Hcl additives as compared to EYC extenders having either EDTA or cysteine Hcl additives and TEYFC extender with EDTA., which supports the reports of Saxena and Tripathi (1984) in rams; Nadroo *et al.*(1988): Singh *et al.*(1989); Dhami and Sahni (1994) and Kumar *et al.* (1996) in bovines. In this study extenders influenced live sperm percentage significantly, which corroborates with the observations of Singh *et al.*(1995) and Singh and Purbey(1996). However, Singh *et al.* (1982) could not observe significant difference in the live sperm percentage due to extender.

The percentage of acrosomal abnormality varied significantly due to extender at different hours of preservation (Table 1). The acrosomal abnormality values were significantly lower in TEYFC extenders having Cysteine Hcl. The values reported for acrosomal damage by Mishra *et al.* (1993) are higher than the values observed. In this study extender influenced significantly the acrosomal abnormality which supports the findings of Singh *et al.* (1995) and Mishra *et al.* (1993). Head abnormality (Table 1) did not vary significantly due to extender at different hours of preservation. The present results pertaining to head abnormality are in agreement with the findings of Singh *et al.* (1995). The variation in tail abnormality due to extender was significant ($P < 0.01$) at different hours of preservation. It was significantly lower in TEYFC with Cysteine Hcl extender as compared to other extenders.

It can be inferred that incorporation of EDTA and Cysteine Hcl improved post-preservation seminal quality than control. The detoriation in seminal quality on preservation were less in extenders having either Cysteine Hcl or EDTA. However, more beneficial effect was observed by the addition of Cysteine Hcl than EDTA.

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