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Preservation of dog semen at refrigeration temperature

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ABSTRACT

A total number of 36 semen ejaculates, six ejaculates from each dog was collected by digital manipulation from 6 Mongrel dogs at weekly interval. Immediately after semen collection physical and morphological characters were evaluated and good quality semen was extended in three extenders viz., egg yolk tris (EYT), egg yolk citrate glycine glucose (EYCGG) and goat milk (GM) extenders by split sample technique. Extended semen samples were preserved at refrigeration temperature and sperm motility, percentage of live sperm, abnormal spermatozoa and acrosomal integrity were evaluated at 24 hours interval for five days with the ultimate objective of recommending suitable extender for preservation of dog semen at refrigeration temperature. There was significantly higher percentage of sperm motility and live sperm, lower percentage of abnormal sperms and acrosomal damage in EYT and EYCGG than in GM. Eventhough, the values are not statistically significant among EYT and EYCGG. EYT was found to have higher percentage of sperm motility and live sperms, lower percentage of abnormal sperms and acrosomal damage when compared to EYCGG. Besides EYT was also found to have better clarity for microscopical examination when compared to EYCGG. hence, it could be inferred that egg yolk tris is superior to egg yolk citrate glycine glucose and goat milk for preservation of dog semen at 4°C.

Key words : Preservation, dog semen refrigeration temperature

The technique of artificial insemination has gained momentum among dog breeders resulting in increased interaction and exchange of dog semen nationally and internationally. These turn of events necessitated a detailed investigation on semen preservation and A.I. In India, because of commercialization of dog breeding, AI has gained importance in recent time. Although, excellent media for preservation of bovine semen in both chilled and frozen state have been developed, only very few attempts have been made on the techniques required to prolong the viability of canine sperm. Few studies reported from other countries to evolve a suitable extender for preservation of dog semen, show diversity of opinion about the best extender (Harrop, 1956; Foote and Leonard, 1964; Roychoudhary and Dubay, 1974; Bouchard *et al.*, 1990; Rota *et al.*, 1995). Moreover, most of these studies were concentrated only on evaluation of diluents based on motility. Therefore, the study was undertaken to compare the efficacy of three extenders on the basis of motility, live and dead sperm, abnormal sperm and acrosomal integrity after preservation of 4°C with the ultimate object of recommending a suitable diluent for preservation of dog semen at refrigeration temperature.

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MATERIALS AND METHODS

A total of 36 ejaculates, six ejaculates from each dog were collected at weekly interval by digital manipulation from six adult Mongrel dogs. The three fractions of semen were collected separately in three graduated collection vials, attached with glass funnels. Immediately after semen collection physical and morphological characteristics of dog semen were assessed as per standard methods. The good quality sperm rich fraction was extended in three different extenders viz., egg yolk tris (EYT) (Rota *et al.*, 1995). Egg yolk citrate glycine glucose (EYCGG) (Foote and Leonard, 1964) and goatmilk (Harrop, 1956) at the rate of 1.4 by split sample technique. Diluted semen was filled in 1 ml screw caped serum vials, kept in the water bath at 38°C and gradually cooled at 4°C and stored at that temperature for preservation. The sperm motility, live sperm count, abnormal sperm morphology and acrosomal integrity were evaluated at 24 hour interval for five days. The acrosomal changes were studied by using Giemsa staining technique (Watson, 1975) and acrosomal morphology was classified as swollen, vesiculated, lost and other abnormalities (Rota *et al.*, 1995).

RESULTS AND DISCUSSION

The mean sperm motility in three extenders at different time intervals on preservation at 4°C are presented in Table 1.

Table 1. Sperm characteristics in EYT, EYCGG and GM extenders at 4°C

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Sperm	Extender	Days					
		0	1	2	3	4	5
Motility (%)	EYT	86.38±1.04[a]	79.86±1.32[a]	73.47±1.72[a]	66.80±1.99[a]	58.47±2.41[a]	49.86±2.74[a]
	EYCGG	80.83±1.20[a]	75.41±1.59[a]	70.13±1.85[a]	64.44±1.96[a]	55.41±2.49[a]	48.33±2.62[a]
	GM	76.25±1.26[b]	61.94±1.76[b]	41.66±1.92[b]	26.11±2.04[b]	7.50±1.61[b]	0[b]
Viability (%)	EYT	89.54±0.7[a]	86.44±0.84[a]	80.75±0.90[a]	74.65±0.99[a]	68.87±1.13[a]	60.87±1.58[a]
	EYCGG	87.87±0.87[a]	84.78±0.91[a]	78.85±0.98[a]	72.54±1.32[a]	66.24±1.64[a]	57.67±2.24[a]
	GM	85.28±0.88[b]	76.48±1.07[b]	56.75±1.54[b]	41.78±1.97[b]	28.87±2.23[b]	18.56±2.87[b]
Acrosomal damage (%)	EYT	8.88±0.58[a]	12.96±1.04[a]	17.19±1.48[a]	21.29±1.76[a]	26.19±2.21[a]	31.54±2.73[a]
	EYCGG	10.23±0.71[a]	14.69±1.10[a]	19.48±1.70[a]	24.78±2.42[a]	29.58±2.80[a]	34.56±3.10[a]
	GM	15.02±1.07[b]	25.36±1.91[b]	38.13±2.93[b]	51.66±3.49[b]	62.93±3.66[b]	69.45±4.20[b]
Abnormality (%)	EYT	8.82±0.49[a]	11.65±0.64[a]	14.17±0.85[a]	16.67±0.85[a]	19.35±0.99[a]	22.28±1.17[a]
	EYCGG	9.88±0.53[a]	12.73±0.67[a]	15.39±0.79[a]	18.13±0.92[a]	21.07±1.04[a]	24.99±1.44[a]
	GM	12.51±0.67[b]	18.74±1.12[b]	24.43±1.55[b]	30.70±1.82[b]	37.03±1.85[b]	44.75±2.20[b]

Figures with different superscript in a column within a character of spermatozoa differ significantly ($P < 0.05$)

Analysis revealed that there was no significant difference on sperm motility between EYT and EYCGG extender from day 0 to 5. But significantly ($P < 0.01$) lower sperm motility was recorded in GM in comparison with other two extenders. The sperm motility in EYT is in accordance with the findings of Rota *et al.* (1995) who reported the average percentage of sperm motility as 76.8±14.0, 73.6±15.5, 70.4±15.4, 60.9±18.9 and 53.6±20.1, respectively on day 0, 1, 2, 3 and 4. But slightly higher values (100, 98, 90, 87, 80 and 35) were recorded by Morton and Burce (1989) in tris-citrate glycerol-egg yolk extender on day 0, 1, 2, 3, 4 and 5. Slightly lower motility of 74.8 per cent at 0 hour and 59.2 per cent at 24 hours were reported by Ekrod (1989).

The sperm motility in EYCGG extender is in accordance with the finding of Foote and Leonard (1964). Sperm motility reduced immediately after dilution in EYCGG than that in EYT. But the rate of reduction of motility per day was slightly lower as compared to EYT. These observations are in agreement with Gabriel (1955) in bull semen. The average percentage of sperm motility in GM in this study was significantly lower, which is in agreement with that of Foote and Leonard (1964). They suggested that milk extender was less satisfactory.

EYT and EYCGG extenders were maintaining significantly ($P < 0.01$) higher motility than GM from day 0 to day 5. Though EYT and EYCGG did not differ significantly in sperm motility during preservation, a trend of higher values was observed in EYT extender. The mean percentage of live sperm in three extender after preservation at 4°C are presented in Table 1. Average percentage of live sperm on day 0 to day 5 did not vary significantly between EYT and EYCGG after storage at 4°C. But significantly ($P < 0.01$) lower number of live sperm was noted in GM in comparison with other extenders. The rate of decrease of live spermatozoa per day was significantly ($P < 0.01$) higher in GM.

Average percentage of abnormal sperm in three extenders during storage at 4°C from day 0 are presented in Table 1. The mean percentage of abnormal spermatozoa on day 0 to day 5 did not significantly ($P < 0.01$) differ between EYT and EYCGG after storage at 4°C. But significantly ($P < 0.01$) higher number of abnormal sperm was obtained in GM in comparison with other extenders. The rate of development of sperm abnormalities per day was significantly ($P < 0.01$) higher in GM when compared to other extenders.

The mean percentage of acrosome damage in three extender after preservation at 4°C from day 0 to day 5 are presented in Table 1. From this study average percentage of acrosomal damage on day 0 to day 5 did not differ significantly in EYT and EYCGG extender after preservation at 4°C. But

significantly ($P < 0.01$) higher damage of acrosome occurred during storage at 4°C in GM in comparison with EYT and EYCGG. The rate of acrosomal damage per day was significantly ($P < 0.01$) higher in GM than in other two extenders.

Though, acrosome damage did not differ significantly in EYT and EYCGG extender, a trend of less damage was recorded in EYT. Rota *et al.* (1995), reported that egg yolk tris maintained the integrity of acrosome better than egg-yolk milk and egg yolk cream extender.

Though, acrosome damage did not differ significantly in EYT and EYCGG extender, a trend of less damage was recorded in EYT. Rota *et al.* (1995), reported that egg yolk tris maintained the integrity of acrosome better than egg-yolk milk and egg yolk cream extender. This may be due to the ability to maintain the spermatozoa alive and motile longer, thereby preventing the acrosome reaction that occur in association with sperm death or irreversible damage (Aalseth and Sacke, 1985).

The above finding clearly shows that EYT and EYCGG maintains higher percentage of sperm motility and live sperm, lower percentage of abnormal sperms and acrosomal damage than in GM. Eventhough, the values are not statistically significant among EYT and EYCGG, EYT was found to have higher percentage of sperm motility and live sperms, lower percentage of abnormal sperms and acrosomal damage when compared to EYCGG. Besides EYT was also found to have better clarity for microscopical examination when compared to EYCGG. Hence, it could be inferred that egg yolk tris is superior to egg yolk citrate glycine glucose and goat milk for preservation of dog semen at 4°C.

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