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Short Communication

REFRIGERATORY PRESERVATION OF CAMEL SEMEN

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ABSTRACT

A total of 28 semen samples were collected from 6 males, each ejaculate was split into two and extended with Tris and Biociphos dilutors, respectively. The semen samples were extended at room temperature and were slowly cooled to 4°C. The individual sperm motility was recorded at 0, 24, 48, 72, 96 and 120 hours or till the motility ceased. None of the 28 samples extended in Biociphos could retain motility at 24 hours after collection while 11/28 (39.28%), 10/28 (35.71%), 5/28 (17.85%) and 3/28 (10.71%) semen samples extended in Tris could retain motility for 24, 48, 72 and 96 hours, respectively. The study was then continued with Tris buffer and 66 more semen samples (totaling 94) were extended and evaluated for preservability of sperm motility. The results indicated that 76/94 (80.85%), 35/94 (37.23%), 25/94 (26.59%), 9/94 (9.57%), 5/94 (5.32%) and 1/94 (1.06%) samples extended in Tris could retain motility for 0, 24, 48, 72, 96 and 120 hours, respectively. It is concluded that Biociphos is not a suitable extender for preservation of camel semen and preservability is also low in Tris extender. The composition of Tris buffer need to be modified for improving preservability of camel semen.

Key words : Camel, semen preservation, Tris and Biociphos extenders

Artificial insemination (AI) is an essential pre requisite for any breeding strategy for genetic improvement in livestock species. Extension and preservation of semen for prolonged period is a key step to develop AI. Extenders like Glucose-EDTA, Lactose, Tris, sodium citrate, skimmed milk and commercial diluents like Laiciphos, Androhep, Dimitropolous - 11 and Green Buffer etc. have been tried by various workers. It is believed that it is possible to preserve camel semen for 36 hours at 4°C. Best results concerning motility and morphology of sperm have been reported with Laiciphos, Androhep, sodium citrate extender, 11% lactose and Green buffer (Sieme *et al*, 1990). But, the conception rates have been reported to decrease dramatically on preservation (Naslaji, 2003). It is interesting to note that no pregnancies were achieved as yet after inseminating dromedary camels with frozen semen (Skidmore, 2003). Experimental inseminations of female camels at NRCC, Bikaner have indicated that pregnancies could be successfully achieved by deposition of undiluted whole camel semen only. No pregnancies could be possible with diluted chilled semen (Aminu Deen *et al*, 2003). These results indicated that more research is required to develop AI in this species, particularly on *in vitro* preservation of spermatozoa.

This study on refrigeratory preservation of camel semen was conducted with Tris and another commercial diluent Biociphos (IMV, France) and results obtained are presented and discussed.

Materials and Methods

Collection of semen

A 30 cm long bovine artificial vagina (AV) was used. The inner chamber was filled with approximately 750 ml water of 45 to 50°C temperature depending upon weather conditions in order to maintain an internal AV temperature of 41 to 42°C. Air was infiltrated to maintain adequate pressure. Director's funnel, collection of glass tube and insulation bag were applied as used in bovine. Application of lubricant was avoided as camel secretes lot of pre ejaculate prior to intromission of penis into artificial vagina to lubricate it adequately.

A female camel restrained in sternal recumbency was used as a dummy. The male was allowed to mount her. An operator approached the mounted male from the left side as males usually fall on to the right side after copulation. The AV was held firmly in the left hand of operator and the erect penis was directed into AV with the right hand on the sheath of the male camel throughout the course

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of copulation to prevent extrusion of the penis from the AV. During copulation, at least two assistants were required to control the male camel to prevent accidental falling of the male on to the operator.

Semen collected in this way was used for refrigeratory preservation using Tris and Biociphos dilutors.

Initially 28 semen samples from 6 male camels were split into two to be extended with Biociphos (IMV, France) and Tris extenders, respectively. The semen samples were extended (1:3) at room temperature and cooled slowly to refrigeratory temperature over a period of 2 hours. Another 66 samples from same 6 male camels were extended and cooled in Tris extender only. The extenders used were prepared as follows:

Tris (S. D. Fine Chem. Ltd., Mumbai)	: 30.28 gm
Fructose (S. D. Fine Chem. Ltd., Mumbai)	: 12.50 gm
Citric acid [E. Merck (India) Ltd., Mumbai]	: 16.70 gm
Caffeine (Hi Media Laboratories Ltd., Mumbai)	: 0.039 gm

Distilled water add to 1000 ml

The buffer was autoclaved at 1.1 kg/cm^2 pressure for 30 minues. Final semen extender was prepared as follows :

Buffer	: 80 ml
Egg yolk	: 20 ml
Benzyl Penicillin	: 1000 i.u./ml
Streptomycin	: 1000 µg/ml

Biociphos extender was prepared as follows :

Stock solution	: 1 part
Distilled water	: 4 part

Individual sperm motility were recorded under 40 x objective at 0, 24, 48, 72, 96 and 120 hours or till the motility ceased, whichever is earlier. Motility > 30% were considered as successful preservation.

Results

Results in regards to preservability of camel semen at refrigeratory temperature in Tris and Biociphos extenders are presented in table 1 and 2. These tables show average motility % at different time interval along with number of samples which had motility > 30% at that particular time. None of the 28 semen samples extended in Biociphos could maintain motility for 24 hrs, while 94 semen samples extended in Tris could maintain sperm motility > 30% in 35 (37.23%), 25 (26.59%), 9 (9.57%), 5 (5.32%) and 1 (1.06%) for 24, 48, 72, 96 and 120 hours, respectively.

Discussion

Mixing spermatozoa with extender allows the addition of many ingredients that sustain and protect the spermatozoa thereby preserving fertility until they are used for insemination. The major functions of semen extenders are to preserve the fertility of sperm cells and to increase the total volume so that

Table 1. Preservability of semen in Tris and Biociphos semen extenders.

Sl. No.	No. of observations	Extender	Average motility (%)				
			0 hour	24 hour	48 hours	72 hours	96 hours
			(No. of motile samples with motility> 30%)				
1.	4	Tris	55.00 ± 17.95	18.75 ± 12.99	10.00 ± 7.81	8.00 ± 8.48	8.00 ± 8.48
		Biociphos	27.50 ± 10.92	1.25 ± 1.44	0.00	0.00	0.00
2.	4	Tris	52.50 ± 15.89	26.25 ± 13.61	13.75 ± 7.94	11.25 ± 7.59	11.25±7.59
		Biociphos	50.00 ± 8.16	0.00	0.00	0.00	0.00
3.	5	Tris	46.00 ± 10.36	16.00 ± 9.74	9.00 ± 8.73	6.00 ± 6.70	2.00 ± 2.23
		Biociphos	34.00 ± 5.70	0.00	0.00	0.00	0.00
4.	7	Tris	60.00 ± 8.19	26.00 ± 9.49	18.85±9.18	8.85 ± .80	5.00 ± 4.58
		Biociphos	31 .42 ± 11.93	1.42 ± 0.99	0.714 ± 0.74	0.00	0.00
5.	5	Tris	44.00 ± 9.08	29.00 ± 10.06	23.00 ± 10.24	12.00±6.51	5.00 ± 2.50
		Biociphos	32.00 ± 5.47	0.80 ± 0.54	0.00	0.00	0.00
6.	3	Tris	63.33 ± 8.16	15.00 ± 9.35	13.33 ± 10.20	9.00 ± 6.82	3.33 ± 4.08
		Biociphos	40.00 ± 21.2	0.66 ± 0.81	0.00	0.00	0.00
Total	28	Tris	(24) (85.71%)	(11) (39.28%)	(10) (35.71%)	(5) (17.86%)	(3) (10.71%)
		Biociphos	(20)	(0)	(0)	(0)	(0)

Table 2. Preservation of sperm motility in camel semen with Tris buffer.

Sl. No.	No. of samples in which motility observed after refrigeration	Average motility (%)					
		0 hour	24 hour	48 hours	72 hours	96 hours	120 hours
		(No. of motile samples with motility> 30%)					
1.	12	56.66 ± 6.85	13.08 ± 5.67	7.08 ± 3.37	3.92 ± 2.65	2.66 ± 2.60	1.66 ± 1.74
2.	19	48.94 ± 7.15	26.05 ± 6.18	14.58 ± 3.70	10.00 ± 3.14	8.05 ± 2.95	3.42 ± 1.57
3.	13	54.61 ± 5.22	25.54 ± 6.58	14.61 ± 4.47	7.69 ± 2.86	3.61 ± 1.79	2.69 ± 1.73
4.	21	44.28 ± 5.40	18.66 ± 4.66	11.38 ± 3.94	5.14 ± 2.36	2.14 ± 1.52	1.90 ± 1.52
5.	15	48.33 ± 4.45	18.80 ± 5.15	11.93 ± 4.49	5.80 ± 2.60	2.46 ± 1.10	1.60 ± 0.78
6.	14	49.28 ± 5.68	20.07 ± 5.61	11.64 ± 4.12	7.42 ± 3.60	4.28 ± 2.60	0.36 ± 0.37
Total	94	76 (80.85%)	35 (37.23%)	25 (26.59%)	9 (9.57%)	5 (5.32%)	1 (1.06%)

the proper dose of cells for insemination can be conveniently packaged and used. Semen extenders used for cattle and buffalo semen have been found to preserve sperm motility and fertility for a period of 3 -7 days at 4°C temperature. Preservability of camel semen as revealed in present study using Tris and Biociphos extenders were observed to be low. None of the 28 samples extended in Biociphos could sustain motility for 24 hours. Performance of Tris in terms of sperm motility was though superior to Biociphos, but the results were not comparable with those of cattle and buffalo. Only 37.23, 26.59, 9.57, 5.32 and 1.06% samples were observed to maintain greater than 30% sperm motility in Tris extender at 24, 48, 72, 96 and 120 hours. Previous workers have reported that best results concerning motility and morphology of sperm have been observed with commercial diluents like Laiciphos, Androhep and Green buffer (Sieme *et al*, 1990) and non commercial extenders such as 11% lactose and sodium citrate extenders but exact quantification as reported in present study in terms of average percentage motility at different time intervals and % of samples maintaining > 30% motility at different periods were not given. It is generally opined that camel semen could be preserved for 36 hours at 4°C. Based on this study, we are of the opinion that preservation of semen in camel was not possible with Biociphos extender and preservability in Tris buffer was also graded to be low, 26.59% for 36 hours.

It is concluded that Biociphos is not a suitable extender for preservation of camel semen and preservability is also low in Tris extender. The composition of Tris buffer need to be modified for improving preservability of camel semen.

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Robot-Jockeys for Camel Races in Qatar

The president of the organising committee of camel races in this Gulf state said that three phases in the production of the robot have been successfully completed and waiting a visit by the engineers handling the project to start the fourth and probably the last, phase. According to the Sheikh Hamad bin Jaseem bin Faisal al-Thani the robots are being developed by a swiss company and are expected to be ready by 2005. Officials in Qatar's organising committee of camel races have been proudly circulating sketches of the robots. These suggest that the final product will be a much more advanced version of the one used on a trial basis earlier this year. One of them shows a human shaped robot in the saddle. Another features a remote control device to command the 'jockey' to make hand movements to direct the camel. According to Sheikh Hamad the Swiss company was paid around $ 1.37 millions to produce the robots. They will cost under $ 5,500 a piece. The committee will buy 100 robots and rent them out at prices subsidised by the government.

(**Source:** *The Hindu, 21st October 2004)*

A Camel Cart Journey to Promote the Casteless Society

A camel cart journey was being undertaken from Jaipur (Capital of Rajasthan State) to Delhi (Capital of India) to promote the concept of casteless society. The journey, Bharat Kalyan Unt Gadi Yatra was led by Selam Velu Gandhi, a Gandhian from Tamil Nadu. The journey was flagged off on September 9 to create awareness among the public on the need for a casteless society and to end communal discord.

(**Source:** The Hindu, 8th September, 2004)

Caravan of Light

Jim Wiltens and Denial Wright stated an adventurous journey on the back of camels from Bikaner on 21st September 2004. These tourists were given a traditional send off from Bikaner. They will travel 1400 miles from Bikaner to Kathmandu (Nepal). They will spread a message of education in the villages enroute and will donate solar lamps in the villages without electricity. They will also study the wild-life enroute and shall regularly update their web site made for this purpose. They bought four camels from adjoining villages of Bikaner and learnt the feeding and management of camels for two weeks.

Cameleers return back to home

A low intensity cyclonic storm threatened to hit the coastal areas of Kutch region of Gujarat region of India in last week of September 2004, led to migration of many families and majority of them were tribal cameleers. They returned back to their homes on the back of camels on 3rd October 2004 when the cyclone threat was over. Note the camels loaded with house hold items on the back.

(Source: *The Hindu, 4th October 2004)*