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Short Communication

Resistance of frozen-thawed epididymal and ejaculated spermatozoa of buffalo bulls*

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ABSTRACT

Storage and thermal resistance (per cent motile sperm at 5°C and 37°C, respectively) of frozen-thawed buffalo epididymal spermatozoa were studied in comparison to ejaculated ones. Storage thermal resistance were significantly (P<0.01) higher in epididymal semen than in the ejaculated one at all periods of storage/incubation.

Key words : Epididymis spermatozon, thermal resistance, buffalo bull, , freezing, thawing spermatoza

Frozen-thawed ejaculated spermatozoa have been evaluated using various tests including incubation at different temperatures and evaluation to record sperm motility and morphology (Dhami *et al.*, 1991; Onkarappa *et al.*, 1999). However, such an information is not available for frozen-thawed epididymal spermatozoa. Present work was, therefore, undertaken to study the storage and thermal resistance of frozen-thawed epididymal spermatozoa of buffalo bulls in comparison to ejaculated ones.

The experimental material comprised of 120 cauda epididymes of buffalo bulls collected after their slaughter. Caudal semen was collected by micro-puncture technique, pooled from same animal (N = 60), diluted in tris extender, evaluated and categorized (Gupta *et al.*, 1998) into caudal semen I (poor quality samples showing sperm motility less than 50%) and II (good quality samples showing sperm motility 50% and above). Both subtypes of caudal semen were frozen in medium French straw (Gupta *et al.*, 2001). Simultaneously 36 ejaculated buffalo semen samples were also processed. All the semen samples were thawed at 40°C for 30 seconds. Split samples were stored/incubated for 72 hrs at 5°C and for 3 hrs at 37°C respectively. The stored/incubated samples were evaluated 24 and 1 hourly by recording sperm motility i.e. storage resistance at 5°C and thermal resistance at 37°C respectively. The data were analyzed using Harvey's least square technique (Steel and Torrie, 1981).

RESULTS AND DISCUSSION

The results of present investigation have been summarised in Table 1 and 2. It will be worth comparing caudal semen II and ejaculated semen because the quality of caudal semen II is same at 0 hr i.e. post-thaw (PT) stage.

The storage and thermal resistance (per cent motile sperm at 5°C and 37°C, respectively) were significantly (P<0.01) affected by semen types, storage/incubation periods and their interaction. They declined significantly (P<0.01) from 0 hr to final storage/incubation period. These findings are in agreement with earlier reports (Chinnaiya and Balkrishnan, 1988; Onkarappa *et al.*, 1999). However, the decline was less in caudal semen II giving maximum recovery rate of 64% motile sperm (storage resistance) as against 15% in the ejaculated semen and 47% motile sperm (thermal resistance) as against 9% in the ejaculated semen after final storage/incubation period,

The storage and thermal resistance of caudal semen II was significantly (P<0.01) higher than that of ejaculated semen at all storage/incubation periods except at 0 hr (Tables 1 and 2). As such the higher resistance of caudal semen II observed in the present study is

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Table 1. Mean ±SE for storage resistance (% motile sperm) of post-thaw epididymal and ejaculated semen of buffalo at 5°C

Storage period (hr)	Motile sperm (%)			
	Caudal semen I (18)	Caudal semen II (42)	Ejaculated semen (36)	Overall (96)
0	15.00±1.85**	43.81±1.56	41.94±1.86	33.59±1.20
24	14.44±1.66**	37.14±1.61**	25.00±2.31	25.67±1.20
48	11.11±1.59	33.57±1.70**	14.72±1.92	19.70±1.20
72	6.67±1.62	27.86±1.72**	6.11±1.51	13.55±1.20
Overall	11.81±1.04**	35.60±0.68**	21.94±0.73	23.13±0.48

Figures in parenthesis indicate number of samples studied.
** (P<0.01) indicate significant difference between epididymis and the ejaculate.

Table 2. Mean ±SE for thermal resistance (% motile sperm) of post-thaw epididymal and ejaculated semen of buffalo at 37°C

Incubation period (hr)	Motile sperm (%)			
	Caudal semen I (18)	Caudal semen II (42)	Ejaculated semen (36)	Overall (96)
0	15.00±1.85**	43.81±1.56	41.94±1.86	33.59±1.20
1	14.44±1.66**	38.81±1.81**	22.64±2.68	25.30±1.20
2	10.00±1.98	30.48±2.26**	10.97±1.90	17.15±1.20
3	5.00±1.46	20.48±1.96**	3.75±0.78	9.74±1.20
Overall	11.11±1.11**	33.40±0.73**	19.83±0.79	21.45±0.52

Figures in parenthesis indicate number of samples studied.
**(P<0.01) indicate significant difference between epididymis and the ejaculate.

conceivable because of higher cold shock and thermal resistance of neat epididymal spermatozoa in comparison to ejaculated ones (Gupta *et al.*, 1998; Hafez and Hafez, 2000). It may be concluded that the higher thermal and cold shock resistance of neat epididymal buffalo spermatozoa than ejaculated ones (Gupta *et al.*, 1998) might have rendered them less susceptible to injury during freezing and thawing resulting into higher post-thaw resistance. The higher resistance of frozen-thawed epididymal spermatozoa might prove an asset in semen freezing provided fertility of such semen is worked out because the post-thaw resistance has a direct bearing on fertility of frozen semen (Ellitt, 1978).

Therefore, it was concluded that the storage and thermal resistance (per cent motile sperm at 5°C and 37°C, respectively) of frozen-thawed epididymal spermatozoa of buffalo bulls were significantly (P<0.01) higher than those of frozen-thawed ejaculated spermatozoa at all periods of storage/incubation.

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