COMPARISON OF THREE PROGRAMMABLE FREEZING PROTOCOLS FOR THE CRYOPRESERVATION OF BUFFALO BULL SEMEN

J. DALAL[1], A. KUMAR[2]*, M. HONPARKHE[3], S. SINGHAL[4] AND N. SINGH[5]

Department of Veterinary Gynaecology and Obstetrics
Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana - 141 004

Received: 01.05.2016 Accepted: 18.06.2016

ABSTRACT

Twenty-one ejaculates from three Murrah buffalo bulls (7 ejaculates per bull) were frozen according to three different freezing protocols using a bio-freezer. Post thaw semen samples were evaluated and the average percentage of individual motility, progressive motility, viability, sperm membrane integrity and total sperm abnormalities was similar (p>0.05) in three protocols.

Keywords: Buffalo bull, Cryopreservation, Freezing rate, Programmable freezing, Semen

Almost 40-50% sperms loose motility and viability during the freezing and thawing process (Watson, 2000). Now a days, a controlled cooling method (programmable freezing) is available and is found superior to vapour freezing method for retaining sperm functional capabilities (Santymire *et al*., 2007). There is no established programmable protocol available for the freezing of buffalo bull semen. Our aim was to compare the efficiency of atleast three programmable freezing protocols for the freezing of buffalo bull semen.

Three Murrah buffalo bulls (age ~4 year) being maintained under similar conditions of nutrition and management in a progeny testing program were used for semen collection by artificial vagina method. Twenty-one ejaculates from three bulls (7 ejaculates per bull) were collected. Semen was evaluated for mass motility, individual motility and sperm concentration. Semen was extended gradually in Tris egg yolk extender at 37°C till final sperm concentration reached 80 million/ml. The extended semen having individual motility >80% was used for further processing. The packaging of semen was done in 0.25 ml straws using automatic filling, sealing and printing machine. The straws were kept in cold handling cabinet and allowed to equilibrate at 4°C for 4 h. Fifteen straws from each ejaculate were selected and out of these, five straws each were earmarked for three freezing protocols (Table 1) constructed in the monitor unit of programmable freezer. The freezing of straws was carried out in programmable biofreezer, Mini Digitcool (ZH 400, IMV technologies, France) to reach -140°C. At this stage, straws were plunged and stored into liquid nitrogen at -196°C. Thereafter, semen was thawed at 37^0C for 30 seconds in a water bath and percent individual and progressive motilities

Table 1: Programmable freezing protocols for the cryopreservation of buffalo bull semen

Freezing protocol	**Temperature**, °C	**Freezing rate**, °C/min
Protocol A	+04 to -10	-05
	-10 to -100	-40
	-100 to -140	-20
Protocol B	+04 to -10	-05
	-10 to -40	-60
	-40 to -100	-40
	-100 to -140	-20
Protocol C	+04 to -12	-04
	-12 to -60	-40
	-60 to -140	-50

[1]Ph.D. Scholar, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar-125 004; [2]Assistant Professor, [3]Assistant Gynaecologist, [4]Assistant Gynaecologist; [5]Assistant Reproduction Physiologist, Department of Livestock Farms Complex; *ajeetvet@yahoo.com

Table 2: Post-thaw semen quality following three programmed freezing protocols (A, B and C). IM, Individual motility; PM, progressive motility; SA, Sperm abnormalities

	IM, %	**PM**, %	**Viability**, %	**HOST**, %	**SA**, %
A	30.71±1.96	15.61±1.23	42.62±3.08	15.47±2.42	12.67±2.64
B	31.43±1.66	15.98±1.96	41.81±2.35	15.21±2.52	13.14±1.44
C	32.14±1.85	16.04±1.76	43.81±2.39	15.73±2.31	11.90±1.77

$p<0.05$, Data non-significant

were assessed as well as the live sperm count (Eosin-Nigrosin stain), hypo-osmotic swelling test and sperm morphology (Rose-Bengal stain) was performed. The data analysis was performed using SPSS (V.20) software and Games Howell *post hoc* test and ANOVA were applied.

The results indiacted that individual motility, progressive motility, viability, membrane integrity (HOST) and total sperm abnormalities were similar ($p>0.05$) in all three freezing protocols (Table 2). These protocols are widely used for the freezing of cattle bull semen, however, our study revealed that unlike cattle bull semen, these protocols are not yielding optimum post-thaw semen quality in buffalo bulls. The suboptimal post-thaw recovery of buffalo bull semen may be due to differences in phospholipids of buffalo bull sperm membrane as phosphatidylcholine and phosphatidylethanolamine constitute 66% and 23% (Cheshmedjieva and Dimov, 1994), against only 50% and 10%, respectively in cattle bull (Parks *et al.,* 1987). Another reason for poor post-thaw recovery of buffalo bull semen may be the critical temperature range, during which relatively greater chances of cryodamage to the sperm occur such as -5 to -15 °C (Drobnis *et al.,* 1993), **-** 5 to **-** 50°C (Kumar *et al.,* 2003). In all three protocols, freezing rates were similar in the critical range. It might be the reason for similar outcome of post-thaw parameters. Thus, the similar effectiveness of all these protocols for freezing of buffalo bull semen warrants more extensive study to compare freezing rates from slow to moderately high in critical temperature range.

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