COMPARISON OF INITIAL PARAMETERS AND FUNCTIONAL ATTRIBUTES AT DIFFERENT STAGES OF PROCESSING IN FREEZABLE AND NON-FREEZABLE SEMEN EJACULATES OF MURRAH BUFFALO BULLS

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ABSTRACT

Six Murrah buffalo breeding bulls with the history of donating semen of good freezability and fertility parameters, and another six Murrah bulls frequently donating either initial poor quality semen or higher degree of damage during processing were selected to study their functional parameters viz. motility, live and dead count, reaction to hypo-osmotic solution (HOS) and acrosomal integrity at four stages viz. immediately post-dilution at 37°C, post-equilibration at 4°C and at 0 h and 1 h post-thaw. In brief, it was concluded that a higher (P<0.05-0.01) livability, progressive motility, HOS activity and percent intact acrosomes was present in freezable ejaculates at all the four stages of processing, in comparison to samples with poor freezability.

Keywords: Buffalo, Correlation, Freezable, Non-freezable, Semen

INTRODUCTION

The application of AI with frozen-thawed semen is limited in buffalo due to poor freezability of buffalo bull spermatozoa compared to cattle (Kumaresan *et al*., 2005). The use of best bulls is often restricted by the limited number of semen doses produced as there are several inherent and functional constraints in realizing the breeding goals through AI. Apart from the fact that buffalo bulls are known for poor libido, there are also some anatomical and physical limitations to production of quality germplasm. The relatively smaller testicular size, lower daily sperm production rate and epididymal sperm reserve in buffalo bulls compared to cattle are some of the natural inbuilt constraints of this species (Singh *et al.,* 2003). The objective of the present study was to compare the initial and functional seminal attributes of freezable and non-freezable ejaculates.

MATERIALS AND METHODS

The study was conducted on 12 Murrah buffalo breeding bulls, aged between 5-7 years, maintained at the frozen semen Bank RCDF Ltd., Bassi, Jaipur, India (26.92ºN, 75.82ºE and 431m AMSL). The selected bulls were divided into 2 groups, each comprising of 6 bulls according to their known ejaculate quality (donating good or poor quality semen and subsequently freezable or non-freezable, respectively) to compare their functional attributes. Group-1 comprised those bulls, which were donating semen of excellent quality with good freezability and fertility parameters, whereas group-2 included those which were frequently donating either initial poor quality semen or higher degree of damage during processing (during equilibration or cryopreservation), but were otherwise healthy.

A total of 96 ejaculate (8 ejaculates each from of 12 buffalo bull) were collected using an artificial vagina on biweekly schedule. Immediately after collection, each ejaculate was placed in a water bath at 37°C and examined using various standard laboratory tests. The semen samples selected for processing after initial examination were evaluated for progressive motility, live and dead count, reaction to hypo-osmotic solution and acrosomal integrity (Sharma *et al.,* 2012) at four

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Table 1: Comparative initial parameters (Mean±SE) **of freezable versus non-freezable semen of Murrah buffalo bulls** (6 bulls with 48 ejaculates in each group)**.**

Semen Quality	Volume (ml)	Sperm concentration ($x10^6$)	Mass activity (0-5 Scale)	pH
Freezable	3.92±0.08[a] (3.0-5.0)	1593.88±24.22[a] (1257-1947)	4.30±0.08[a] (3.0-5.0)	6.90±0.003[a] (6.84-6.93)
Non- freezable	3.38±0.13[b] (1.5-5.5)	1503.10±24.60[b] (1242-1887)	1.66±0.083[b] (0.0-3.0)	7.35±0.027[b] (6.95-7.82)

Figures within parenthesis indicate range; Figures with different superscripts within a column differ significantly (P<0.01)

stages viz, immediately post-dilution at 37°C, post-equilibration at 4°C and at 0 h and 1 h post-thaw. The data obtained were analysed using SAS statistical package version 9.2.

RESULTS AND DISCUSSION

In the present study, the freezable or good quality ejaculate had higher ejaculate volume, sperm concentration and mass activity with low pH (<7.0) in comparison to non-freezable or poor quality ejaculate (P<0.01, Table 1). In a given breed, a number of factors such as sexual development and maturity of the bull, nutrition, reproductive health, size of testes, breed, age of the bull and climate affect the quality of an ejaculate (Javed *et al.,* 2000). Moreover, there is impact of individual variation, season, management and collection procedure (Sharma *et al.,*1991). Similarly, the higher pH in poor quality ejaculate can be attributed to a larger amount of fluid from urethral and accessory glands (Salisbury and Van Denmark,1962).

The processing of semen lead to progressive decline (P<0.01) in mean livability (Table 2). However, at all stages of evaluation, the mean livability was higher (P<0.01) in good quality freezable ejaculates in comparison to poor quality non-freezable ejaculates (Table 2). Cryopreservation of spermatozoa, though results in the extension of their longevity, causes severe stress for spermatozoa, resulting in cryo-injuries which include the destruction of structural and functional integrity of membranes ultimately resulting in low sperm survival rates (Fickel *et al*., 2007). A positive (P<0.05) correlation of live-sperm percentage (livability) was observed with spermatozoa motility, HOS reactivity and intact acrosome percentage in freshly diluted (Table 3), as well as frozen thawed (Table 4) freezable and non-freezable semen. Similar positive correlation was reported earlier (Kirk *et al.*, 2005).

A decline (P<0.01) was observed in mean progressive motility at different stages of processing in both freezable and non-freezable ejaculates, however, the mean progressive motility remained higher (P<0.01) at all four stages of semen evaluation in freezable ejaculates (Table 2). Spermatozoal motility is known to be dependent on mitochondrial function. The ATP generated by oxidative phosphorylation in the inner mitochondrial membrane is transferred to microtubules to drive motility. Hence, reduced sperm motility induced by cryopreservation is believed to be mainly associated with mitochondrial damage (Januskauskas and Zillinskas, 2002). A positive (P<0.05) correlation of progressively motile spermatozoa percentage was observed between with HOS reactivity and intact acrosome percentage in freshly diluted (Table 3) as well as frozen thawed (Table 4) freezable and non-freezable semen. Similar correlations were reported earlier (Gillian *et al*., 2008).

The evaluation of HOS reactivity at various stages of processing showed that the HOS reactive spermatozoa percentage declined (P<0.01) from post-dilution to post-equilibration and then at post-thaw stages in both types of ejaculates (Table 2). However, at all stages of semen processing, a higher (P<0.01)

Table 2: Comparative functional parameters (Mean±SE) **of freezable (F) versus non-freezable (NF) semen during various stages of processing in Murrah buffalo bulls** (6 bulls with 48 ejaculates in each group)**.**

Sperm Parameter (n=48)	Quality	Stage of semen processing			
		Fresh diluted	Equilibration	0 h Post-thaw	1 h Post-thaw
Live (%)	F	90.88±0.26^{aA} (86-94)	86.44±0.25^{aB} (82-90)	78.29±0.31^{aC} (73-83)	74.00±0.36^{aD} (69-80)
	NF	64.42±0.91^{bA} (49-79)	48.77±1.11^{bB} (32-63)	29.42±1.10^{bC} (15-44)	10.75±0.78^{bD} (02-25)
Progressive Motile (%)	F	86.15±0.34^{aA} (79-90)	73.17±0.38^{aB} (68-80)	63.50±0.29^{aC} (60-68)	40.46±0.50^{aD} (32-49)
	NF	44.48±0.75^{bA} (30-54)	31.25±0.69^{bB} (20-43)	18.15±0.69^{bC} (08-29)	5.42±0.58^{bD} (00-13)
HOS reactive (%)	F	89.19±0.26^{aA} (84-92)	85.27±0.23^{aB} (81-89)	77.05±0.31^{aC} (72-82)	72.90±0.39^{aD} (68-79)
	NF	63.17±0.93^{bA} (47-77)	47.58±1.09^{bB} (31-61)	28.44±1.09^{bC} (14-43)	9.94±0.79^{bD} (00-24)
Intact acrosome (%)	F	91.21±0.30^{aA} (87-95)	85.48±0.37^{aB} (80-90)	78.77±0.35^{aC} (74-83)	70.79±0.32^{aD} (67-76)
	NF	65.33±0.88^{bA} (46-80)	49.65±1.04^{bB} (34-61)	32.06±0.81^{bC} (19-43)	15.88±0.64^{bD} (05-24)

Figures with different superscripts within a column (a,b) and within a row (A,B) differ significantly (P<0.01)

HOS reactivity was observed in freezable ejaculates in comparison to non-freezable ejaculates (Table 2). An intact and functionally active membrane is a prerequisite for metabolism, capacitation, acrosome reaction, attachment and penetration of oocyte (Jeyendran *et al*., 1984). The deterioration of spermatozoa function due to change in structural components occurs during the process of semen processing, freezing, cryo-storage and thawing (Centola *et al*., 1992). This accounted for the gradual fall in the percentage of sperms responsive to HOS test post-thaw. A positive (P<0.05) correlation was observed between HOS reactive spermatozoa percentage and intact acrosome percentage in freshly diluted (Table 3) as well as frozen thawed (Table 4) freezable and non-freezable semen. These observations were in accordance with earlier studies (Lodhi *et al*., 2008).

Intact acrosome percentage was higher (P<0.01) in freezable ejaculates as compared to non-freezable ejaculates at all stages of semen evaluation (Table 2). The decline (P<0.01) in percent intact acrosome spermatozoa during successive stages of cryopreservation was observed in freezable and non-freezable ejaculates (Table 2). The stresses of freezing and thawing usually lead to acrosomal damage (Gilbert and Almquist,1978).

In conclusion, higher livability, progressive motility, HOS activity and percent intact acrosomes were observed in freezable ejaculates at all stages of processing in comparison to ejaculates with poor freezability. A positive correlation was observed between functional attributes of spermatozoa in freshly diluted as well as frozen thawed freezable and non-freezable semen.

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Table 3: Inter-relationship between some functional parameters in freshly diluted freezable (F) versus non-freezable (NF) semen of Murrah buffalo bulls (6 bulls with 48 ejaculates in each group)**.**

Relationship between Parameters		Quality	Correlation Coefficient	Regression Estimate	Regression Equation
Livability	**Motility**	F	0.90315**	0.58±0.04	y=40.76+0.58x
		NF	0.63213**	0.76±0.14	y= 30.42+0.76x
	HOST	F	0.93348**	0.94±0.05	y=6.66 +0.94 x
		NF	0.99209**	0.97±0.018	y= 3.00+0.97x
	Acrosome	F	0.57791**	0.54±0.11	y=41.16 +0.54x
		NF	0.90992**	0.94±0.06	y= 2.81+0.94x
Motility	**HOST**	F	0.89201**	1.02±0.12	y=-4.39+1.02x
		NF	0.61303**	0.50±0.09	y=13.10+0.50x
	Acrosome	F	0.50559**	0.74±0.18	y=18.53+0.74x
		NF	0.59814**	0.51±0.10	y=10.99+0.51x
HOST	**Acrosome**	F	0.48501**	0.45±0.19	y= 47.90+0.45x
		NF	0.92161**	0.97±0.06	y=-0.50+0.97x

*P<0.05, **P<0.01, NS - not significant

Table 4: Inter-relationship between some functional parameters in post-thawed freezable (F) versus non-freezable (NF) semen of Murrah buffalo bulls (6 bulls with 48 ejaculates in each group)**.**

Relationship between Parameters		Quality	Correlation Coefficient	Regression Estimate	Regression Equation
Livability	**Motility**	F	0.53047**	0.57±0.14	y=41.97+0.57x
		NF	0.21542 NS	0.34±0.23	y=23.16+0.34x
	HOST	F	0.85688**	0.86±0.77	y= 11.98+0.86x
		NF	0.99507**	1.00±0.01	y=0.82+1.00x
	Acrosome	F	0.43047**	0.38±0.12	y=48.37+0.38x
		NF	0.87913**	1.19±0.09	y=-8.75+1.19x
Motility	**HOST**	F	0.47647**	0.44±0.12	y= 29.33+0.44x
		NF	0.21470NS	0.14±0.09	y=14.29+0.14x
	Acrosome	F	0.30898*	0.25±0.12	y=43.57+0.25x
		NF	0.33172*	0.28±0.12	y=9.14+0.28x
HOST	**Acrosome**	F	0.31709*	0.28±0.12	y=55.10+0.28x
		NF	0.88104**	1.18±0.09	y=-9.40+1.18x

*P<0.05, **P<0.01, NS - not significant

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