

Effect of Season on Physico-Morphological Attributes of Cryopreserved Haryana Bull Spermatozoa

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Abstract

The present study was undertaken to evaluate the effect of seasonal variations on various seminal attributes and freezability of Haryana bull semen. The study was conducted over a period of four months and divided into two peak seasons as winter (December-January) and summer (May-June). Twenty-four ejaculates were collected from 4 bulls in each season. The physico-morphological characteristics of semen, viz., volume (ml), mass activity (0-5 scale), pH, sperm concentration (million/ml), progressive motility (%), sperm livability (%), HOST (%), total morphological sperm abnormality (%) and acrosomal integrity (%) were estimated at different stages of cryopreservation in different seasons. During summer season the ejaculate volume and seminal pH were found non-significantly higher than in winter, whereas the percentage of morphologically abnormal spermatozoa was significantly ($p < 0.05$) higher in summer. Post-thaw percentage of progressively motile spermatozoa was significantly ($p < 0.05$) higher in winter than summer season. Live sperm per cent were significantly ($p < 0.05$) higher in winter than summer season both at fresh and pre-freeze stage. HOST positive sperm and acrosomal integrity were significantly ($p < 0.01$) higher in winter season than summer at all three stages. The study generated basic information that the summer season adversely affects the various physico-morphological characteristics of Haryana bull semen altering its freezability.

Introduction

Haryana is one of the most prominent dual purpose cattle breed of Indo Gangetic plain and is well adapted to the tropical environments, mainly because of the high degree of heat tolerance and resistance to many tropical diseases. The indigenous breeds, which traditionally form an integral part of agriculture, are decreasing in number in last couple of decades due to mechanization of agriculture. For conservation of this valuable germplasm, the knowledge of semen quality and its freezability is much imperative. Semen evaluation, a very

important tool for breeding purposes, has always been of great significance to assess the fertility status of the bull. Conventional parameters used for evaluation of semen have limited application because they only help to assess the structural integrity of the cell (Neild *et al.*, 1999).

Season is one of the important factors that influence the semen quality and fertility. Among different seasons hot-dry and hot-humid season is reported to be unfavorable for production as well as reproduction (Bhakat *et al.*, 2011). Season affects the animal directly through macro and micro climatic factors, like temperature, humidity,

rainfall and photoperiod and indirectly it acts by affecting the vegetation, forage quality and soil-plant-animal interaction. The magnitude of variations differs from breeds, location, prevailing climatic conditions, feeding and general management (Mandal *et al.*, 2000).

During cryopreservation and thawing, the major challenges to spermatozoa like cold and warming shocks, formation and dissolution of ice which causes changes in osmolarity and formation of ice crystals (Watson, 1995^a) are responsible for damage to spermatozoa. Osmotic (Watson, 2000) and oxidative stress is an important cause of sperm damage which elicited the greater interest in recent years (Aitken and Krausz, 2001). The knowledge of trend of seasonal influence on semen characteristics would help to know the requirement of bulls to meet the demand of frozen semen and to provide any suitable additional managerial requirements time to time. Hence, the present study was undertaken to investigate the effect of seasons on various characteristics of semen production and its freezability in Hariana bulls with the specific consideration of climatic components.

Materials and Methods

Four healthy Hariana bulls (nearly 5.5 to 6.5 years of age and body weight of 450 to 550 kg) maintained at semen biology lab, Department of Veterinary Gynaecology and Obstetrics, DUVASU, Mathura were utilized for the collection of semen for the present study. The institute is located at an altitude of 287 meters above the mean sea level and at latitude of 27.17° N and a longitude of 77.41° E. The bulls were maintained under identical and optimum conditions of feeding and management during entire period of study. The study was conducted during two seasons: peak winter (December- January) and peak summer (May-June). A total 48 ejaculates (24 in each season; 12 each from 4 bulls) were collected in the morning twice a week by using sterilized bovine artificial vagina.

Soon after collection each ejaculate was placed in a water bath at 34°C and various standard laboratory tests for semen were recorded. The ejaculates having mass activity $\geq +3.5$ and progressive motility $\geq 70\%$ were used for further processing. Fresh semen samples

were evaluated for volume, mass activity, pH, concentration (haemocytometer), initial progressive motility, livability, total morphological abnormality, HOST (Jayendran *et al.*, 1984) and acrosomal integrity (Watson, 1975^b).

The semen extended with Tris-egg yolk, glycerol (TYFG) dilutor @ 100 million sperm/ml, was packaged in 0.25 ml French mini straws and subjected to a combined cooling with equilibration period of 4 h at 4-5°C in the cold handling cabinet. The straws were kept in automatic programmable bio-freezer (IMV technology, France) till temperature of straws reached -140°C. Then straws were plunged into liquid nitrogen (-196°C) for storage. Semen samples were evaluated at pre-freeze and post-thaw stage for individual progressive motility, livability, morphological abnormality, HOST and acrosomal integrity. Frozen straws were thawed at 37°C for 30 sec after 24 h of freezing for post-thaw evaluation. The data were analyzed statistically using SPSS version 16.00.

Results and Discussion

Physico-morphological attribute of fresh semen:

Least square means of various seminal attributes of Hariana bulls observed during winter and summer seasons are presented in Table 1. There was no effect of season on ejaculate volume. Khan *et al.* (2007) also reported that the season did not significantly influence the volume of semen in zebu bulls. However, higher ejaculate volume during summer followed by winter season was reported in Tharparkar bulls by Rajoriya *et al.* (2015), while Mandal *et al.* (2005) reported higher ejaculate volume in winter than summer season in Tharparkar and Sahiwal bulls.

Mass activity of semen in present study did not reveal any significant difference between winter and summer season, as documented earlier by Tomar and Gupta (1984) and Mandal *et al.* (2005) in Hariana and Sahiwal bulls. The sperm concentration did not show any significant difference between winter and summer season. It was in agreement with Rajoriya *et al.* (2015) in Tharparkar bull semen. Tiwari *et al.* (2012) reported significantly higher sperm concentration during winter in Red Sindhi bulls. Tomar and Gupta (1984) and Bhakat *et al.* (2014)) also

Table 1: Physico morphological attributes of Haryana bull semen during winter and summer season

Seminal attributes	Season		
	Winter	Summer	Level of significance
Ejaculate volume (ml)	5.96±0.22	6.11±0.40	NS
Mass activity(0-5 scale)	3.92±0.07	3.82±0.08	NS
Concentration(million/ml)	1269.75±81.97	1324.17±63.25	NS
Seminal pH	6.71±0.02	6.76±0.01	NS
Initial progressive motility (%)	83.92±1.08	82.92±0.73	NS
Sperm liability (%)	89.83±0.60 ^b	88.00±0.58 ^a	p<0.05
Acrosomal integrity (%)	88.33±1.20 ^b	85.00±0.66 ^a	p<0.01
HOST reactive sperm (%)	80.38±0.66 ^b	74.08±0.84 ^a	p<0.01
Morphological abnormality (%)	2.71±0.19 ^a	4.00±0.33 ^b	p<0.01

Means bearing different superscripts in a row differ significantly.

reported significantly higher sperm concentration in winter than summer season in Haryana bulls.

Seminal pH was insignificantly higher in summer than winter season. This was in agreement with Bhakat *et al.* (2014, 2015) in Karan Fries and Murrah buffalo bull semen. High rise in the seminal pH in summer season might be due to more cholesterol efflux in summer season. The initial progressive motility of semen did not reveal any significant difference in winter and summer season. This observation was in agreement with Rajoriya *et al.* (2015) in Tharparkar bulls. On the contrary Tomar and Gupta (1984) and Mandal *et al.* (2005) have reported that the season significantly affect the initial motility of the semen in different zebu bulls semen.

Significant (p<0.05) seasonal difference was observed in live sperm percentage between two seasons in fresh semen, being higher in winter than summer season. Similar effect of season on live sperms has been reported earlier (Bhakat *et al.*, 2014). Seasonal variations with lower live sperm percentage during humid hot season have been reported in bulls. On the contrary, no significant effect of seasonal variation in relation to sperm livability was noted in Tharparkar bulls (Rajoriya *et al.*, 2015). The occurrence of lowest percentage of live spermatozoa synchronizing with the part of year characterized by the highest mean ambient temperature suggests that the summer environment becomes instrumental in causing death of sperm.

The HOST reactive spermatozoa and intact acrosome per cent varied significantly (p<0.01) between two seasons in fresh semen, being maximum during winter than summer season. Similar were the observations particularly for HOST in Karan Fries (Bhakat *et al.*, 2014) and in Tharparkar bulls (Rajoriya *et al.*, 2015). The highest HOST positive sperms indicate higher percent of membrane integrity of the spermatozoa. HOST assesses the functional and physiological aspect of the membrane permeability.

A highly significant (p<0.01) difference was observed for morphologically abnormal spermatozoa per cent in fresh semen, being higher in summer than winter season. This was in agreement with the reports of Singh *et al.* (2000) and Bhakat *et al.* (2014) in half breeds and in Karan Fries bulls. However, others (Tomar *et al.*, 1985; Rajoriya *et al.*, 2013) did not observe such significant difference in sperm morphology between seasons.

Physico-morphological attributes of pre-freeze and post-thaw semen:

The physico-morphological characteristics of Haryana bull semen at pre-freeze and post-thaw stage are presented in Table 2. The mean pre-freeze motility did not differ significantly between seasons, while post-thaw motility was significantly (p<0.01) higher in winter than summer season. This observation of post thaw motility was in agreement with Mandal *et al.* (2005), who also reported significantly higher post thaw motility

during winter and lowest during rainy seasons in Sahiwal bulls.

The mean live sperm percentage at pre-freeze stage was significantly ($p < 0.05$) higher in winter than summer season, but no such difference was noted at post-thaw stage between seasons. Similar seasonal variation with lower live sperm percentage during humid hot season has been reported in bulls (Bhakat *et al.*, 2014). While in another study there was no significant effect of season in relation to sperm livability in Tharparkar bull at pre-freeze and post-thaw stage (Rajoriya *et al.*, 2015).

In present study, highly significant ($p < 0.01$) differences for mean HOST reactive sperm per cent as well as intact acrosome per cent were found between two seasons, both at pre-freeze and post-thaw stages, being higher in winter than summer season. Similar finding on intact acrosome in Murrah buffalo bull semen for winter and summer season has been reported earlier

by Mandal *et al.* (2000). However, Rajoriya *et al.* (2015) did not find such difference in per cent intact acrosome at pre-freeze and post-thaw stage in different seasons. The significant ($p < 0.01$) variation in HOS reactive spermatozoa per cent between two season during pre-freeze and post-thaw stage, being higher in winter than summer season, was in agreement with finding in Karan Fries bulls (Bhakat *et al.*, 2014), while Rajoriya *et al.* (2015) did not find such difference in pre-freeze and post-thaw HOS response between seasons in Tharparkar bulls.

The mean morphologically abnormal sperm per cent was significantly ($p < 0.01$) higher in summer than winter season both at pre-freeze and post-thaw stages. It concurred with observations of Singh *et al.* (2000) and Bhakat *et al.* (2014) in half breeds and Karan Fries bulls, while Rajoriya *et al.* (2013) reported the per cent abnormal spermatozoa had no significant correlation between summer and winter season in pre-freeze and post-thaw stages.

Table 2: Physico-morphological attributes of Haryana bull spermatozoa during winter and summer season at pre-freeze and post-thaw stage

Seminal attributes		Season		
		Winter	Summer	Level of significance
Individual progressive sperm motility (%)	Pre-freeze	77.29±1.20	77.92±0.96	NS
	Post-thaw	58.13±0.89 ^b	52.83±0.94 ^a	$p < 0.01$
Sperm livability (%)	Pre-freeze	86.00±0.60 ^b	84.04±0.55 ^a	$p < 0.05$
	Post-thaw	71.54±0.93	71.29±1.20	NS
Acrosomal integrity (%)	Pre-freeze	83.83±1.22 ^b	81.13±0.59 ^a	$p < 0.01$
	Post-thaw	80.50±0.85 ^b	70.71±0.64 ^a	$p < 0.01$
HOST reactive sperm (%)	Pre-freeze	73.42±0.73 ^b	70.13±0.91 ^a	$p < 0.01$
	Post-thaw	62.96±0.85 ^b	56.13±1.02 ^a	$p < 0.01$
Morphological sperm abnormality (%)	Pre-freeze	4.42±0.24 ^a	6.04±0.37 ^b	$p < 0.01$
	Post-thaw	8.25±0.34 ^a	11.96±0.54 ^b	$p < 0.01$

Conclusions

It is concluded that the summer season adversely affects the various physico-morphological characteristics of Haryana bull semen. Winter is the most favorable season for good quality semen production. Therefore it is suggested that during summer, breeding bulls should be kept cool and comfortable, protected

from heat stress from direct wind, fed during cool hours and have a free access to cool and clean drinking water for better performance.

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Competing Interest:

Authors have no competing interest.

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