

*Original Research***Impact of Seasons and Extenders on Quality and Freezability of Surti Buffalo Bull Semen under Middle Gujarat Climate****D. V. Chaudhari, K. K. Hadiya, J. A. Patel and A. J. Dhami***

Department of Animal Reproduction Gynaecology and Obstetrics, College of Veterinary Science & Animal Husbandry, Anand Agricultural University, Anand-388 001, Gujarat, INDIA

***Corresponding author:** ajdhami@aaau.in

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Abstract

A study was conducted to evaluate the seasonal influence (peak winter and summer) and the efficacy of three extenders (egg yolk based TFYG extender and egg yolk free soya bean based commercial extenders Optixcell and Andromed) on quality and freezability of Surti buffalo bull semen in middle Gujarat. Nutritionally the bulls were managed identically throughout the year. Semen ejaculates (6/bull/season, total 36) revealed mean ejaculate volume 3.23 ± 0.18 ml, sperm concentration 963.06 ± 64.74 million/ml, progressive motility 79.94 ± 0.83 %, live sperm 83.67 ± 0.62 %, abnormal sperm 7.69 ± 0.41 %, and sperm with intact plasma membrane 83.19 ± 0.75 % and intact acrosome 94.92 ± 0.24 %. Only the progressive sperm motility (80.56 ± 0.98 vs. 73.33 ± 0.57 %) and HOST (85.33 ± 0.81 vs. 81.06 ± 1.07 %) were significantly ($p < 0.05$) higher with lesser sperm abnormality (6.17 ± 0.53 vs. 9.22 ± 0.37 %) during winter than in summer. Semen samples split diluted with TFYG, Optixcell and Andromed extenders revealed the overall mean values (%) of progressive sperm motility, livability, abnormality, plasma membrane integrity and acrosomal integrity during winter season as 75.09 ± 0.49 , 76.41 ± 0.51 , 5.52 ± 0.21 , 77.22 ± 0.38 and 92.61 ± 0.28 at pre-freeze stage and 42.13 ± 1.25 , 54.81 ± 0.88 , 8.46 ± 0.29 , 33.26 ± 0.96 and 90.78 ± 0.28 at post-thaw stage, respectively. The respective values (%) in summer season were 65.37 ± 0.53 , 71.56 ± 0.50 , 9.81 ± 0.28 , 68.17 ± 0.96 and 92.81 ± 0.28 at pre-freeze stage, and 33.43 ± 0.80 , 50.78 ± 0.76 , 14.46 ± 0.29 , 28.04 ± 0.78 and 90.43 ± 0.29 at post-thaw stage. The overall mean sperm post-thaw motility/longevity at 0, 30, 60 and 120 min of incubation at 37°C was 43.13 ± 1.34 , 36.72 ± 1.41 , 29.47 ± 1.42 and 19.83 ± 1.34 % during winter season and 34.73 ± 0.81 , 29.31 ± 0.83 , 21.86 ± 0.69 and 14.34 ± 0.82 % in summer season, respectively. The initial quality as well as freezability of semen in terms of motile, live, normal and HOS reactive sperm including post thaw longevity were significantly ($p < 0.01$) better in winter season than in summer season. Further, the values of all the five semen quality parameters studied were comparatively better in Optixcell than TFYG and Andromed extenders with significant differences mainly in sperm progressive motility, viability and post-thaw longevity in both the seasons. However, the season $\times$ extender interaction was not significant for any of the sperm quality parameters studied. Winter was the best season for harvesting optimum quality and freezability of buffalo semen in Optioxcell extender.

Key words: Buffalo Bull, Freezability, Gujarat Climate, Post Thaw Longevity, Season, Semen Quality

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Introduction

Semen cryopreservation and AI offer many advantages to the livestock industry. Bull fertility and semen quality are of vital importance to the bovine industry as sub-fertile bulls can cost producers a significant amount of loss (Kastelic and Thundathil, 2008). Among various climatic elements, high ambient temperature is the most important one affecting semen quality and animal fertility (Koivisto *et al.*, 2009), probably through change in quality of feed and its digestibility. Further, the semen extender may aggravate the situation. Egg yolk has been used as a basic component of extenders for bovine ejaculate since 1939 (Amirat *et al.*, 2004) and has still remained popular because of their excellent protection of sperm cells (Celeghini *et al.*, 2008). Nevertheless, the use of egg yolk as a cryoprotectant has recently been restricted in some countries for reasons of immunologic and hygiene risks (Thun *et al.*, 2002). An alternative to replace the components of animal origin in semen extenders is the soy lecithin. Hardly few studies aiming to evaluate the efficiency of soy lecithin as a primary source of lipoproteins in semen extenders are available on buffalo semen (Akhter *et al.*, 2010, Chaudhari *et al.*, 2015). We postulate that buffalo sperm motility is negatively affected by the hot season and poor extender and that season/extender induced alterations might affect the sperm's motility, viability and fertilizing ability. Therefore, the aim of this study was to evaluate the effect of season on semen quality and extenders with and without egg yolk on its freezability based on pre-freeze and post-thaw sperm quality parameters in Surti buffalo bulls under native climate.

Materials and Methods

The study was conducted during peak winter (Nov-Dec) and peak summer (May-Jun) on semen of three mature healthy Surti buffalo bulls (*Bubalus bubalis*), 6-8 years old (Av. 6.66 yr), managed identically with similar feeds & fodders throughout the year at the Sperm Station of the College in Anand, Gujarat. The bulls were under regular twice a week semen collection schedule using artificial vagina in the early morning between 7.30 and 8.30 hrs. The ejaculates (6 per bull/season, total 36) after collection were immediately transferred in to a water bath at 34°C and evaluated for gross quality, motility and sperm concentration (by Accucell photometer). Only the ejaculates with >70% initial motility were used for further processing.

Preparation of Extenders

The standard Tris-citrate-fructose-egg yolk-glycerol (TFYG) extender was prepared fresh daily and antibiotics benzyl penicillin 1000 IU/ml and streptomycin sulphate 1000 µg/ml were added as recommended by FAO (1979). The commercial Optixcell® (IMV, France) and Andromed® (Minitube, Japan) were prepared fresh for use by diluting 4 and 2 times, respectively, with Milli-Q water according to manufacturer's instructions.

Semen Processing

Qualifying ejaculates from each bull were split into three equal aliquots and diluted in single step at 34°C with each of three extenders @ 100×10^6 spermatozoa ml⁻¹ and were evaluated for progressive sperm motility. They were soon filled and sealed in French mini straws using IS4 System of IMV, France. The straws were then cooled to 4-5 °C within 60-90 minutes and further equilibrated for 4 hrs in cold handling cabinet. Freezing of straws was carried out in LN₂ vapour using a programmable bio-freezer (Digitcool 5300 CE ZH 350, IMV). The straws were then plunged in liquid nitrogen (-196 °C) for overnight storage. Semen straws were thawed next day in a water bath at 37 °C for 30 seconds.

Assessment of Sperm Quality

The samples were evaluated for various sperm quality parameters, viz., motility, viability, morphology, acrosome integrity and HOS test at initial (on dilution), pre-freeze (after equilibration) and post-thaw stage using standard procedures and phase contrast microscope. The sperm progressive motility was determined at 37°C temperature under high power magnification (40 X) and viability with eosin-nigrosin stained semen smears under oil emulsion lens of a phase contrast microscope. Simultaneously, sperms were also examined for various types of abnormalities. The percentages of spermatozoa with intact acrosome were assessed using Geimsa stain (Watson *et al.*, 1975) and plasma membrane integrity was assessed using a hypo-osmotic swelling (HOS) test employing 150 mOs/L solutions with 30, 60 and 120 minutes of incubation at 37°C (Jayendran *et al.*, 1984).

Statistical Analysis

The data were analyzed statistically using ANOVA and Duncan's Multiple Range Test by employing IBM SPSS Statistics version 20.00 to know the effect of season, extender and their interaction on various sperm quality traits during cryopreservation.

Results and Discussion

The pooled mean ejaculate volume, mass activity, sperm concentration, individual motility, viability, morphology and plasma membrane as well as acrosomal integrity of Surti buffalo semen averaged 3.23 ± 0.18 ml, 3.42 ± 0.05 , 963.06 ± 64.74 million/ml, $79.94 \pm 0.83\%$, $83.67 \pm 0.62\%$, $7.69 \pm 0.41\%$,

83.19±0.75 % and 94.92±0.24 %, respectively. The semen quality was apparently better in winter season than summer season, with significant ($p<0.05$) differences in sperm progressive motility (76.39±0.97 vs. 71.94±1.00 %), HOS reactive sperms (85.33±0.81 vs. 81.06±1.07 %) and abnormal sperm (6.17±0.37 vs. 8.56±0.30 %). The observations soon after dilution and at pre-freeze and post-thaw stage are presented in Table 1.

Table 1: Seasonal variation in semen quality parameters (%) of buffalo bulls on dilution, at pre-freeze and at post-thaw stage in different semen extenders

Stage	Season	Extender	Progressive motility	Viability	Plasma memb. integrity	Sperm Abnormality	Acrosomal Integrity
On dilution	Winter	TFYG	80.56±0.98	81.61±0.83	82.83±0.75	5.11±0.41	94.22±0.44
		Optixcell	81.94±0.72	80.94±0.94	83.06±0.72	5.11±0.35	94.11±0.50
		Andromed	79.72±0.75	80.50±0.96	81.17±0.70	4.50±0.32	93.83±0.45
		Overall	80.74±0.48 _p	81.02±0.52 _p	82.35±0.43 _p	4.91±0.21 _p	94.06±0.26
	Summer	TFYG	73.33±0.57 ^a	77.72±0.48 ^a	76.11±1.54	7.11±0.41	94.22±0.44
		Optixcell	75.72±0.81 ^b	80.06±0.65 ^b	77.06±1.50	7.06±0.41	94.28±0.50
		Andromed	73.89±0.65 ^a	78.50±0.60 ^{ab}	76.50±1.49	7.39±0.44	93.78±0.46
		Overall	74.31±0.41 _q	78.76±0.35 _q	76.56±0.85 _q	7.19±0.25 _q	94.09±0.27
Pre-freeze	Winter	TFYG	75.00±0.99 ^{ab}	77.61±0.63	77.61±0.62	5.06±0.36	92.78±0.48
		Optixcell	76.39±0.79 ^b	76.22±1.01	77.78±0.78	5.61±0.35	92.89±0.48
		Andromed	73.89±0.65 ^a	75.39±0.90	76.28±0.56	5.89±0.35	92.17±0.50
		Overall	75.09±0.49 _p	76.41±0.51 _p	77.22±0.38 _p	5.52±0.21 _p	92.61±0.28
	Summer	TFYG	64.72±0.85 ^a	71.22±0.80 ^{ab}	67.61±1.83	9.39±0.41	92.94±0.47
		Optixcell	67.22±0.92 ^b	73.28±0.88 ^a	70.56±1.48	9.28±0.30	93.17±0.49
		Andromed	64.17±0.83 ^a	70.17±0.82 ^b	66.33±1.57	10.17±0.44	92.33±0.50
		Overall	65.37±0.53 _q	71.56±0.50 _q	68.17±0.96 _q	9.81±0.28 _q	92.81±0.28
Post-thaw	Winter	TFYG	41.39±2.21	54.61±1.62	31.28±1.38	8.94±0.50	90.67±0.46
		Optixcell	44.44±2.17	56.06±1.48	35.28±1.74	7.22±0.45	91.28±0.48
		Andromed	40.56±2.13	53.78±1.49	33.22±1.80	9.22±0.55	90.39±0.53
		Overall	42.13±1.25 _p	54.81±0.88 _p	33.26±0.96 _p	8.46±0.29 _p	90.78±0.28
	Summer	TFYG	31.94±1.15 ^a	50.94±1.23	28.22±1.55	13.83±0.27	90.33±0.47
		Optixcell	36.11±1.25 ^b	52.61±1.32	29.56±1.44	13.48±0.51	90.94±0.49
		Andromed	32.22±1.58 ^a	48.78±1.34	26.33±0.92	15.75±0.34	90.00±0.52
		Overall	33.43±0.80 _q	50.78±0.76 _q	28.04±0.78 _q	14.46±0.29 _q	90.43±0.29

Means with different superscripts (a, b) differ significantly ($p<0.05$) within the column between extenders for a season/stage and overall means with subscripts p,q differ significantly ($p<0.05$) between seasons for a stage.

The sperm progressive motility, viability, morphology and plasma membrane integrity were found significantly ($p<0.05$) better in winter season than summer season on dilution and at pre-freeze and post-thaw stage, but no such variation was noted in acrosomal integrity. Among the diluters used for semen extension, Optixcell followed by TFGY gave better protection against cold shock than Andromed as characterized by significantly ($p<0.05$) better sperm progressive motility and viability, particularly during

summer, but other sperm parameters showed no significant differences among three extenders in any of the seasons at initial or pre-freeze and post-thaw stage (Table 1).

Further, the overall mean sperm post-thaw longevity/motility was significantly ($p<0.05$) better during winter season than summer season at all incubation (37°C) intervals with pooled values of 43.13 ± 1.34 vs. 34.73 ± 0.81 , 29.47 ± 1.42 vs. 21.86 ± 0.69 and 19.83 ± 1.34 vs. 14.34 ± 0.82 % at 0, 1 and 2 hrs, respectively. At each stage of post-thaw incubation, Optixcell diluent proved better than other two dilutors, and the values at all post-thaw incubation intervals were significantly better during winter than summer season in all three diluents, with non-significant effect of season x extender interaction (Table 2).

Table 2: Post-thaw longevity (% sperm progressive motility) in buffalo bulls in various semen diluters in different season

Season	Dilutor	Post-thaw longevity (% sperm progressive motility)			
		0 min	30 min	60 min	120 min
Winter	TFYG	42.39 ± 2.21	36.11 ± 2.16	28.61 ± 2.09	17.67 ± 2.06
	Optixcell	45.28 ± 2.04	40.00 ± 2.02	33.33 ± 2.06	24.72 ± 2.16
	Andromed	41.39 ± 1.97	35.56 ± 1.80	27.22 ± 1.91	16.11 ± 2.20
	Overall	43.13 ± 1.34	36.72 ± 1.41	29.47 ± 1.42	19.83 ± 1.34
Summer	TFYG	33.67 ± 1.07	28.67 ± 1.07	21.11 ± 0.95	13.61 ± 1.20
	Optixcell	37.28 ± 1.18	31.44 ± 0.89	23.61 ± 1.13	16.94 ± 1.00
	Andromed	32.83 ± 1.09	27.28 ± 1.03	19.89 ± 0.86	11.91 ± 0.82
	Overall	$34.73\pm0.81^{**}$	$29.31\pm0.83^{**}$	$21.86\pm0.69^{**}$	$14.34\pm0.82^{**}$

** Significant ($p<0.01$) between seasons within the column for overall values.

In the present study, initial semen evaluation revealed better sperm progressive motility and lesser sperm abnormalities during winter season as compared to summer season. At pre-freeze and post-thaw stages also, the semen collected during summer had reduced progressive motility, viability, and a tendency toward a higher proportion of abnormal sperm with damaged plasma membrane, relative to semen collected in the winter. The photoperiod is an important factor that influences the seminal characteristics of buffaloes (Sansone *et al.*, 2000) and the effect of the hot environment on the reproduction and sexual activity of buffalo bulls has previously been reported, not only affecting the libido but also the quality and freezability of semen (Vale, 2007); and the effect is probably aggravated by changed feed/fodder quality and its ruminal digestibility. However, in the present study care was taken to provide uniform feed and fodders throughout the year to the bulls under investigation. Thus, the results of present study substantiate the observations of these researchers.

We found seasonal variations in freezability of buffalo semen. Freezability was found to be best when semen was cryopreserved in winter compared with summer, and concurred with the findings of Bahga and Khokar (1991), Tiwari *et al.* (2011), Alam *et al.* (2015) and many others. Castro *et al.* (2017) suggested that the physical and morphological characteristics of the ejaculates showed a significant

statistical difference in wave motion, motility, vigor, major defects, minor defects, total defects and plasma membrane integrity between the hot and cool periods ($p < 0.05$). In tropical region, the ideal period for using buffalo ejaculates, are the months of the cooler season. Alam *et al.* (2015) based on their findings also concluded that sperm quality of Egyptian water buffalo is affected by the elevated temperature during summer season that might be related to the increased production of reactive oxygen species (ROS).

Tiwari *et al.* (2011) reported significantly ($p < 0.05$) lower initial and post-thaw sperm progressive motility in summer than winter in Murrah buffalo bull semen, although semen volume and sperm output per ejaculate were not affected by season. Higher values in winter, followed by summer and monsoon seasons have also been reported by Sagdeo *et al.* (1991) in Surti buffalo bulls. Koivisto *et al.* (2009) observed significant seasonal influence on gross-motility, progressive sperm motility, vigor and morphological sperm defects. All these reports collaborate to our present findings. Orgal *et al.* (2012) also recorded lower post-thaw velocity, motility and progressive motility in summer than winter samples. The reduction in sperm motility in frozen semen could be attributed to the reduction of total protein concentration and absence of specific protein from seminal plasma due to seasonal effect (Smith *et al.* 1999). Plasma membrane integrity is crucial to sperm survival inside the female reproductive tract, and it plays an important role in several events taking place during fertilization, e.g. capacitation, acrosome reaction, sperm fusion with the oocyte. In our study, hypo-osmotic swelling test (HOST) showed a marked decrease in plasma membrane integrity in samples collected during summer season compared to winter at initial as well as at post-thaw stage. These results were in agreement with those reported by Mandal *et al.* (2003) and Koonjaenak *et al.* (2007).

The present findings on sperm quality parameters in terms of live and abnormal sperm observed in semen at different stages of cryopreservation with egg yolk and soybean based semen extenders coincided well with many previous reports (Akhter *et al.*, 2010, Meena *et al.*, 2010, Chaudhari *et al.*, 2015, Kumar *et al.*, 2015) either for one or more of the above quality parameters in buffalo semen. The findings regarding the performance of TFYG and Andromed extenders are supported by Kumar *et al.* (2015), who found better post-thaw motility, viability and post-thaw longevity in semen extended with egg yolk based extender than Andromed extender. Our findings showed some significantly better sperm quality parameters in semen frozen with Optixcell and TFYG than Andromed. In the present study, Optixcell gave relatively improved performance than the TFYG, but the differences at most instances, except sperm motility, were not significant, as has been reported earlier by many workers in India and abroad (Beran *et al.*, 2012, Orgal *et al.*, 2012, Chaudhari *et al.*, 2015). There might be incorporation of some antioxidant and unknown additives in Optixcell extender, which favoured the performance of this extender apart from its

visual transparency in absence of egg yolk. However, the season x extender interaction was not significant for sperm quality parameters studied.

Conclusion

The results discussed above with a hot tropical climate suggest a special management needs to be implemented for buffalo bulls to control THI (temperature humidity index) such as application of foggers, air circulating fans, access to daily baths/wallowing in ponds, providing good quality green fodder, feeding during cooler hours, etc especially during warmer times, so that they can dissipate body heat from metabolism and that caused by the environment. It is very important to protect buffaloes against thermal stress, since their thermoregulatory system is less efficient when compared to cattle. In general, the Optixcell and TFYG extenders were found superior over Andromed extender in both summer and winter seasons, and heat stress depressed the initial semen quality and thereby freezability during peak summer. Therefore, suitable managemental intervention for amelioration of seasonal stress on buffalo bulls especially heat stress should be implemented.

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