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Influence of Seasons and Extenders on Quality and Freezability of Gir Bull Semen under Middle Gujarat Climate

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Abstract

The 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 Gir bull semen in Middle Gujarat. Semen ejaculates (6/bull/season, total 36) revealed mean ejaculate volume 6.49±0.30 ml, sperm concentration 1212.36±58.10 million/ml, progressive motility 74.17±0.78 %, live sperm 81.39±0.80 %, abnormal sperm 7.36±0.31 %, and sperm with intact plasma membrane 81.31±0.98 % and intact acrosome 94.81±0.24 %. Only the progressive sperm motility was significantly ($P<0.05$) higher (76.39±0.97 % vs. 71.94±1.00 %) with lesser sperm abnormality (6.17±0.37 % vs. 8.56±0.30 %) during winter than in summer. Semen samples split diluted with TFYG, Optixcell and Andromed extenders recorded the overall mean values of progressive sperm motility, livability, abnormality, plasma membrane integrity and acrosomal integrity during winter season as 77.87±0.51, 77.50±0.45, 5.56±0.20, 76.02±0.81 and 94.35±0.29 on dilution; 72.41±0.51, 70.50±0.64, 5.96±0.26, 71.20±0.79 and 93.09±0.32 at pre-freeze stage; 41.30 ±0.94, 50.28±1.03, 9.15±0.31, 29.89±0.40 and 90.65±0.40 at post-thaw stage, respectively. The respective values in summer season were 72.13±0.60, 75.50±0.60, 7.48±0.25, 75.61 ±0.55 and 94.09±0.30 on dilution; 65.46±0.66, 69.41±1.05, 8.89±0.28, 69.70±0.66 and 92.63 ±0.33 at pre-freeze stage; 31.48±0.52, 45.09±0.85, 13.48±0.33, 26.85±0.71 and 91.26±0.38 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 41.20±1.51, 35.19±1.47, 28.80±1.75 and 17.50±1.47 % during winter season and 31.57±0.89, 26.20±0.77, 20.37±0.83 and 13.80±0.77% 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 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 only in sperm progressive motility in both the seasons. The season x extender interaction was not significant for any of the sperm quality parameters studied.

Key Words: Gir bull, Season, Semen quality, Freezability, Post thaw longevity, Gujarat climate.

Introduction

Bull fertility and semen quality are of vital importance to the bovine industry as sub-fertile bulls can cost producers a significant amount of money. Among all climatic elements, temperature is the most important parameter affecting animal fertility (Kunavongkrit *et al*., 2005). However, the effect of heat stress and semen extender on semen quality may also contribute to the phenomenon. The high ambient temperature increases the scrotal temperature in males and consequently a decline in semen quality. Exposure to elevated ambient temperature increases the proportion of morphologically abnormal sperm and attenuates sperm motility in cattle (Yaeram *et al*., 2006). We hypothesize that bovine spermatozoon motility is negatively affected by the hot season and poor extender, and postulate that season/extender induced alterations might affect the sperm's motility, viability and fertilizing ability. Perhaps semen collected during the summer and morphologically classified as being of good quality is, in fact, less competent to survive freezing and to fertilize ovum. Therefore, the aim of this study was to examine the association between season, extender, semen quality and its freezability in Gir bulls under native climate.

Materials and Methods

The study was conducted during peak summer and peak winter on semen of three sexually mature healthy Gir bulls (*Bos indicus*), 4-6 years old, managed identically 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 34ºC and evaluated for gross quality, motility and sperm concentration (by Accucell photometer). Only the ejaculates with >70% initial motility were used for further processing and freezing.

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 (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 $\times 10^6$ spermatozoa ml^{-1} and were evaluated for progressive sperm motility. They were soon filled and sealed in French mini straws (at least 10 from each aliquot) using IS4 System of IMV Technologies, France. The straws were then cooled to 4-5 °C within 60-90 minutes and further equilibrated at the same temperature for 4 hrs in cold handling cabinet. Freezing of straws was carried out in LN_2 vapour using a programmable bio-freezer (Digitcool 5300 CE ZH 350, IMV Technologies). 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 (Olympus BX20, Tokyo, Japan). 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 solution of sodium citrate and fructose with 30 minutes of incubation at 37°C (Jayendran *et al.,* 1984). Nearly 200 spermatozoa were assessed from different fields for each trait and the spermatozoa with morphological defects, intact acrosome and intact plasma membrane (swollen coiled tail) were counted and expressed as per cent.

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 extenders, processing steps and their interaction on various sperm quality traits (Snedecor and Cochran, 1994).

Results and Discussion

Among the parameters evaluated for fresh semen, the ejaculate volume, mass activity, sperm concentration, individual motility, viability, morphology and plasma membrane as well as acrosomal integrity did not differ significantly between seasons, and the values averaged 6.49±0.30 ml, 3.42±0.05, 1212.36±58.10 million/ml, 74.17±0.78%, 81.39±0.80 %, 7.36±0.31%, 81.31±0.98 % and 94.81±0.24 %, respectively. However, the quality parameters were better in winter season than summer season, and significantly ($p<0.05$) better sperm progressive motility (76.39±0.97 vs. 71.94±1.00 %) with lesser sperm abnormality (6.17±0.37 vs. 8.56±0.30 %) was found in winter season than summer season in freshly ejaculated semen. The observations soon after dilution are presented in Table 1.

At pre-freeze evaluation, the overall sperm progressive motility was found significantly ($p>0.05$) better in winter season than summer season on dilution (77.87±0.51 vs 72.13±0.60 %) and after equilibration (72.41±0.51 % vs. 65.46±0.66 %). However, the sperm viability, plasma membrane integrity and acrosomal integrity (70.50±0.64, 71.20±0.79 and 93.09±0.32 % in winter, and 69.41±1.05, 69.70±0.66 and 92.63±0.33 % in summer season) did not vary significantly between two seasons, and were little better in winter season. Among the diluters used for semen extension, Optixcell followed by TFYG gave better protection against cold shock than Andromed as characterized by significantly ($p<0.05$) better sperm progressive motility, but other sperm parameters showed no significant differences among three semen extenders in any of the seasons at initial or pre-freeze stage (Table 1).

At post-thaw stage, significantly ($p<0.05$) higher overall sperm progressive motility, more viability and lesser sperm abnormalities were recorded in winter season (41.30±0.94, 50.28±1.03 and 9.15±0.31 %) than those of summer season (31.48±0.52, 45.09±0.85 and 13.48±0.33 %, respectively). Although there were no significant differences in plasma membrane and acrosomal integrity between two seasons (29.89±0.40 and 91.26±0.38 % in winter vs. 26.85±0.71 and 90.65±0.40 % in summer), the values were comparatively higher in winter season. In both the seasons Optixcell and TFYG diluents gave better freezability than Andromed as depicted by significantly ($p<0.05$) better sperm progressive motility and plasma membrane integrity (Table 1).

Further, the overall mean sperm post-thaw longevity was significantly ($p<0.05$) better during winter season than summer season at 0, 30, 60 and 120 min of incubation, viz. 41.20±1.51, 35.19±1.47, 28.80±1.75 and 17.50±1.47 % vs. 31.57±0.89, 26.20±0.77, 20.37±0.83 and 13.80±0.77 %, respectively. At each stage of post-thaw incubation, Optixcell diluent proved better than other two (Table 2).

Although the deleterious effects of thermal stress on the female reproductive tract have long been known, the effect of environmental thermal stress on semen quality and its association with low fertility during the hot season are unclear. In the present study, the semen collected during summer had reduced progressive motility, had more dead spermatozoa and a tendency towards a higher proportion of abnormal sperms with damaged plasma membrane, relative to semen collected in the winter at both pre-freeze and post-thaw stages. Similar findings were also observed earlier by Fiaz *et al.* (2010) and Bhakat *et al.* (2014). The individual motility, acrosomal integrity and HOST are considered as good parameters for semen quality and are used for prediction of fertility potential of bulls. Their percentages in semen were decreased ($p<0.05$) during hot humid season as compared to spring season in crossbred bulls (Mishra *et al.,* 2013; Bhakat *et al.,* 2014). Heat stress enhances the production of ROS (reactive oxygen species) which may disrupt the sperm membrane, as the membrane is highly rich in phospholipids, sterols and polyunsaturated fatty acids, therefore, the sperm membrane is always prone to free radical attack. Shukla *et al.* (2010) and Bhakat *et*

Table 1: Seasonal variation in semen quality parameters (%) of Gir bull 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
Initial on dilution	Winter	TFYG	76.39±0.97^{a}	77.22±0.71	76.78±1.39	5.50±0.32	94.44±0.49
		Optixcell	79.44±0.69^{b}	78.17±0.77	76.72±1.43	5.28±0.39	94.56±0.53
		Andromed	77.78±0.83^{ab}	77.11±0.86	74.56±1.39	5.89±0.32	94.06±0.53
		Overall	77.87±0.51*	77.50±0.45	76.02±0.81	5.56±0.20	94.35±0.29
	Summer	TFYG	71.67±0.99^{ab}	75.72±0.66	75.83±0.97	7.17±0.41	94.22±0.50
		Optixcell	73.89±0.76^{a}	76.50±0.49	76.33±0.89	6.58±0.45	94.28±0.55
		Andromed	70.83±1.23^{b}	74.28±1.52	74.67±1.03	8.11±0.40	93.78±0.52
		Overall	72.13±0.60**	75.50±0.60	75.61±0.55	7.48±0.25	94.09±0.30
Pre-freeze	Winter	TFYG	70.83±0.93^{a}	72.22±0.99	72.22±1.29	6.00±0.50	93.22±0.53
		Optixcell	74.17±0.61^{a}	70.67±1.07	71.50±1.39	5.78±0.42	93.33±0.55
		Andromed	72.22±0.92^{ab}	68.61±1.25	69.89±1.45	6.11±0.45	92.72±0.59
		Overall	72.41±0.51*	70.50±0.64	71.20±0.79	5.96±0.26	93.09±0.32
	Summer	TFYG	65.00±1.07^{ab}	70.00±1.54	69.72±1.22	8.61±0.42^{a}	92.78±0.56
		Optixcell	67.78±1.01^{a}	70.33±1.74	70.83±1.15	7.94±0.43^{a}	93.00±0.57
		Andromed	63.61±1.20^{b}	67.89±2.11	68.56±1.05	10.11±0.47^{b}	92.11±0.62
		Overall	65.46±0.66**	69.41±1.05	69.70±0.66	8.89±0.28	92.63±0.33
Post-thaw	Winter	TFYG	42.22±1.58^{a}	50.11±1.79	29.39±0.65^{a}	8.61±0.51	91.39±0.67
		Optixcell	43.89±1.69^{a}	52.17±1.67	31.83±0.53^{b}	8.83±0.51	91.50±0.62
		Andromed	37.78±1.29^{b}	48.56±1.85	28.44±0.68^{a}	10.03±0.56	90.89±0.69
		Overall	41.30±0.94*	50.28±1.03*	29.89±0.40	9.15±0.31*	91.26±0.38
	Summer	TFYG	31.39±0.89^{ab}	43.72±1.55	27.89±1.42	13.33±0.57^{a}	90.72±0.71
		Optixcell	33.33±0.81^{b}	46.78±1.34	27.39±1.08	12.17±0.41^{a}	91.00±0.67
		Andromed	29.72±0.85^{a}	44.78±1.49	25.28±1.13	14.94±0.57^{b}	90.22±0.73
		Overall	31.48±0.52**	45.09±0.85**	26.85±0.71	13.48±0.33**	90.65±0.40

Means with different superscripts (a, b) within the column between extenders for a season/stage differ significantly ($p<0.05$). */** significant ($p<0.05/0.01$) between seasons for a stage.

al. (2014) noticed higher ($p< 0.05$) percentage of major sperm abnormalities during hot-humid season in crossbred bulls. Better spermatozoal morphology was reported in winter and spring seasons in bulls (Vilakazi and Webb, 2004). The higher ambient temperature and humidity might have an adverse effect on spermatogenesis and negative effect on LH secretion. This might be one of the reasons for poor semen quality in bulls under tropical climate, like ours.

Shrivatava *et al.* (2013) reported highest frequency of discarded ejaculates due to poor sperm concentration followed by poor initial motility, poor post thaw motility and lower volume during

Table 2: Post-thaw longevity (% sperm progressive motility) in Gir bull in various semen dilutors in different season

Season	Dilutor	Post-thaw longevity (% sperm progressive motility)			
		0 min	30 min	60 min	120 min
Winter	TFYG	41.67±1.51	35.83±1.58	29.72±1.96	18.06±1.47
	Optixcell	43.06±1.57	36.94±1.47	31.11±1.69	21.11±1.18
	Andromed	38.89±1.43	32.78±1.35	25.56±1.61	13.33±1.76
	Overall	41.20±1.51*	35.19±1.47*	28.80±1.75*	17.50±1.47*
Summer	TFYG	31.39±0.89	26.11±0.76	20.83±0.73	13.61±0.68
	Optixcell	33.33±0.81	28.33±0.81	22.78±0.83	15.83±0.93
	Andromed	30.00±0.99	24.17±0.73	17.50±0.93	11.94±0.72
	Overall	31.57±0.89**	26.20±0.77**	20.37±0.83**	13.80±0.77**

*/** significant ($p<0.05/0.01$) between seasons within the column.

summer season in HF crossbred bulls. Exposing males to an elevated temperature can subsequently compromise spermatozoon competence (Yaeram *et al.,* 2006). In the current study although, better sperm progressive motility and lesser sperm abnormalities were observed in winter season at initial and at pre-freeze stage, the total viable sperm and sperm with intact plasma membrane and acrosome, were not significantly affected by season. However, further examination following the freeze-thaw process revealed that semen collected during the summer is highly sensitive to cryopreservation, as expressed by reduced progressive motility, viability and plasma membrane integrity. Orgal *et al.* (2012) also observed reduced motility, and impaired progressive motility after thawing in semen collected during summer season as compared to winter season. In light of these findings, it is suggested that the characterization of semen quality would be more accurate if examinations were performed post freeze-thaw rather than at collection in fresh semen. Season affects the normal reproduction process multi-dimensionally, like by reducing feed intake, and by inhibiting the release or response to important reproduction hormones like GnRH, FSH and LH. LH is inhibited by increasing level of plasma corticosteroids due to heat stress. Due to extreme heat stress bulls get physically exhausted and have an ultimate effect on production of sperms (Levine, 1999).

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 cattle and buffalo semen. The findings regarding the performance of TFYG extender and Andromed extender were supported by Beran *et al.* (2012) and 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 TFYG and Optixcell than Andromed extender. In the present study, Optixcell gave relatively improved performance than the TFYG, but the differences at most instances, except sperm motility, were not significant. There might be incorporation of some antioxidant and unknown additives in Optixcell extender, which favoured the performance of this extender. But we cannot underestimate TFYG extender, as it gave good or satisfactory results in the present study and has also been reported by many workers in India and abroad (Beran *et al.,* 2012; Orgal *et al.,* 2012; Chaudhari

et al. (2015).

The study in general suggests a seasonal & extender effect on semen quality: ejaculates collected during the summer and those extended in Andromed were more sensitive to cryopreservation than those collected in the winter & extended in Optixcell or TFYG, as reflected by reduced progressive motility in pre-freeze and post-thawed semen. Therefore, suitable managemental intervention for amelioration of seasonal stress especially heat stress and selection of ideal extender must be exercised.

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Conflict of Interest: Authors declare no conflict of interest for this research work.

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