Journal of Animal Research: v.6 n.2, p. 121-125. April 2016

DOI: 10.5958/2277-940X.2015.00179.5

Influence of Season on Semen Quality in Karan Fries (Tharparkar × Holstein Friesian) Bulls

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Received: 02 February, 2016 **Accepted**: 18 February, 2016

ABSTRACT

Availability of good quality semen is essential throughout the year to achieve the sustainable productive growth of dairy cattle. Therefore, the present study was designed from five (*n*=5) adult healthy Karan Fries bulls from Animal Breeding Research Centre (ABRC) of National Dairy Research Institute (NDRI), Karnal (India) with emphasis to observe the semen quality during different seasons (winter, spring, hot-dry and hot- humid). Weekly ejaculates were collected using artificial vagina (42-45 °C). Immediately after collection, the ejaculates were placed in a water bath (37 °C) for assessing semen evaluation parameters. Ejaculate volume (3.53 ± 0.08 VS 3.96 ± 0.12 mL), individual motility (48.00 ± 1.12 VS 55.16 ± 1.32 %), concentration (1487.10 ± 26.55 VS 1590.77 ± 25.90 in 10^6/mL), non-eosinophilic sperm count (62.42 ± 0.75 VS 68.05 ± 0.77 %), Hypo-osmotic swelling test (59.75 ± 0.57 VS 64.97 ± 0.84 %) and acrosomal integrity (74.22 ± 0.62 VS 83.40 ± 0.48 %) decreased ($p<0.05$) during hot-humid season as compared to spring season. The major abnormalities (10.74 ± 0.18 %) increased ($p<0.01$) in hot-humid as compared to spring season. It can be concluded that the semen quality of Karan Fries bulls deteriorate during hot humid season as compared to other seasons of the year under tropical climatic conditions.

Keywords: Season, semen quality, Karan Fries bulls

The testicular temperature of most mammals are maintained at about 2-6°C below their body temperature for the production of morphologically normal and fertile spermatozoa (Waites, 1970). Higher ambient temperature and humidity may have negative effects on spermatogenesis, resulted poor semen quality and fertility (Bhuiyan and Shamsuddin, 1998; Mathevon *et al.* 1998). Good quality semen is very crucial for successful artificial insemination (AI) throughout the year. The embryonic death during summer may also contributed by spermatozoa (Setchell *et al.* 1988). Valeanu *et al.* (2015) observed lowest ($p<0.05$) percentage of live spermatozoa during summer compared to spring season and DNA fragmentation index percentage (DFI %) was higher ($p<0.05$) during summer compared to spring season. Several studies have reported significant effect of season on semen quality (Mathevon *et al.* 1998; Nichi *et al.* 2006; Koonjanek *et al.* 2007; Fiaz *et al.* 2010). Summer season (heat stress) in tropical climate is one of the major hindrances of exotic and crossbred animal's performance. The impact of climate change on livestock production is highly concerned for food security and sustainable livestock growth. There is need of availability of good quality fresh and frozen semen throughout the year for improvement of Indigenous and crossbred cattle in India. Therefore, the present study was conducted to evaluate the semen quality during different seasons in Karan Fries bulls under tropical climatic conditions.

MATERIALS AND METHODS

Selection of animals and details of environmental variables

Study was conducted on five (*n*=5) Karan Fries bulls (4-6 years of age and average body weight 655.2 ± 52.03 kg) from December to August maintained at Animal Breeding Research Centre (ABRC), Indian Council of Agricultural Research-National Dairy Research Institute (ICAR-

NDRI), Karnal, Haryana (India). Weekly ejaculates were collected during winter (December to mid February), spring (mid February to April), hot dry (May to June) and hot humid (July to August) seasons. The environmental variables during experimental period are presented in table 1. All the experimental animals were maintained under uniform feeding and managemental regime practiced at ABRC-NDRI. Water was made available for the animals throughout the day. Bulls were subjected to physical exercise in bull exerciser in the morning.

Table 1. Environmental variables during the experimental period (Average)

Season	Max. Temp (°C)	Min. Temp (°C)	T_{db} (°C)	T_{wb} (°C)	RH %	THI
Winter	19.36	7.4	13.86	15.02	77.66	61.39
Spring	26.36	12.13	20.38	15.84	63.83	66.67
Hot dry	39.25	23.95	26.75	23.2	48.25	80.68
Hot humid	34.5	25.5	30.4	26.65	75.25	81.64

Maximum temperature (Max.Temp), Minimum temperature (Min. Temp), dry bulb temperature (T_{db}), wet bulb temperature (T_{wb}), relative humidity (RH) and temperature humidity index (THI)

Semen analysis

Semen samples were collected using artificial vagina (42-45°C) at early in the morning and immediately, placed in a water bath (37°C) to carry out semen evaluation parameters viz: volume, mass motility, individual motility, hypo-osmotic swelling test (HOST), non-eosinophilic sperm count, acrosomal integrity, sperm concentration and abnormalities. Ejaculate volume was recorded just after semen collection from graduated glass centrifuge tube. A drop of fresh semen sample was placed on preheated (37ºC) glass slide; gently cover slip was placed upon the drop and observed under phase contrast microscope (Nikon eclipse E600, Tokyo, Japan) in low magnification (10X). The semen samples were graded on the basis of wave movement as given in table 2.

Individual motility was assessed by diluting the neat semen with egg yolk medium (1:100). One drop of sample was taken in a pre heated glass slide (37°C), cover slip was placed gently and observed under light microscope (40X, Labomad).

Table 2. Grading of semen on the basis of mass activity

Observation	Grade	Descriptive value
Waves not present, sperm cells immotile	0	Very poor
Waves not present, sperm cells motile	+	Poor
Barely distinguishable waves in motion	++	Fair
Waves apparent, moderate motion	+++	Good
Dark distinct waves in rapid motion	++++	Very good

The HOST was carried out as per the method of Jeyendran *et al.* (1984). Briefly, 100 µL of neat semen was added in 900 µL of hypo-osmotic solution (150 mosm/ L osmolality) in 2 mL Eppendorf tube and incubated at 37 °C for 60 minutes in a CO_2 incubator. After incubation, a small drop of well mixed sperm suspension was taken on a clean, dry and grease free glass slide, covered with cover slip and observed under light microscope (40X). Non-eosinophilic spermatozoa were assessed by eosin-nigrosin (EN) stain as suggested by Blom (1950). EN stain was also used for counting sperm abnormalities [(Major abnormalities: proximal cytoplasmic droplet, pyriform heads, folded/ coiled tails and middle piece defects) (Minor abnormalities: distal cytoplasmic droplets, tailless normal heads, simple bend, terminally coiled tail, narrow and small heads)]. EN stain was prepared by proper mixing of 1:5 ratio of eosin and nigrosin in 10 mL of 2.9 % (pH 6.8) sodium citrate buffer with the help of a magnetic stirrer at 70-80°C for 40-60 minutes. The content was filtered through filter paper and was kept at 4°C. Then 2.5 µL of neat semen and 20 µL of EN stain were placed on clean pre warm glass slide, mixed properly and 5-8 µL of mixture was drawn in a clean pre-warm glass slide, a very thin smear was made and air dried. The dead sperm stained pink (eosin) and partially stained sperm were also considered as dead while live sperm remained unstained. Two hundred spermatozoa were counted under oil immersion per slide from different fields. Acrosomal integrity was assessed by the method of Hancock (1951). Neat semen (3 µL) was placed on a clean glass slide and thin smear was prepared, the slides were air dried and subjected to staining in 5% Giemsa for overnight. The working solution of 5% Giemsa was

Table 3. Seminal parameters during different seasons in Karan Fries (Tharparkar X Holstein Friesian) bulls.

Sl. no.	Parameters	Winter (Dec to Mid Feb)	Spring (Mid Feb to April)	Hot-dry (May to June)	Hot-humid (July to August)
1	Ejaculates (mL)	3.83 ± 0.09^{ab}	3.96 ± 0.12^{a}	3.72 ± 0.12^{ab}	3.53 ± 0.08^{b}
2	Mass Motility (0-5scale)	2.6 ± 0.09^{a}	2.7 ± 0.09^{a}	2.53 ± 0.09^{a}	2.4 ± 0.08^{a}
3	Individual Motility (%)	52.50 ± 1.33^{a}	55.16 ± 1.32^{a}	51.00 ± 0.88^{ab}	48.00 ± 1.12^{b}
4	Concentration (10^6/mL)	1579.65 ± 22.31^{ab}	1590.77 ± 25.90^{a}	1523.00 ± 28.14^{ab}	1487.10 ± 26.55^{b}
5	Non-eosinophilic count (%)	66.23 ± 0.88^{a}	68.05 ± 0.77^{a}	65.86 ± 0.77^{a}	62.42 ± 0.75^{b}
6	HOST (%)	63.34 ± 0.73^{ab}	64.97 ± 0.84^{a}	61.48 ± 0.89^{bc}	59.75 ± 0.57^{c}
7	Acrosomal Integrity (%)	83.09 ± 0.60^{a}	83.40 ± 0.48^{a}	78.70 ± 0.60^{b}	74.22 ± 0.62^{c}
8	Major Abnormalities (%)	7.79 ± 0.24^{bc}	7.61 ± 0.28^{c}	8.66 ± 0.22^{b}	10.74 ± 0.18^{a}
9	Minor Abnormalities (%)	8.13 ± 0.28^{a}	7.96 ± 0.18^{a}	8.29 ± 0.25^{a}	8.36 ± 0.20^{a}
10	Total Sperm Output (million)	6036.03 ± 143.40^{a}	6276.80 ± 201.15^{a}	5660.83 ± 210.85^{ab}	5229.05 ± 133.13^{b}
11	Total Live Sperm (million)	3996.60 ± 107.14^{ab}	4265.62 ± 140.87^{a}	3706.63 ± 212.36^{b}	3264.43 ± 93.14^{c}

prepared by mixing 5 mL of Giemsa stock solution (Qualigens, India) along with 7.5 mL of stock solution A (0.06M Na_2HPO_4=1.0673 g in 100 mL of double distilled water) and stock solution B (0.06 M KH_2PO_4 = 0.8165 g in 100 mL of double distilled water). The total volume was adjusted to 100 mL by adding 80 mL of double distilled water. The pH of solution A and B was maintained at ≈ 6.8 and stored in clean glass bottles at room temperature. 200 spermatozoa were counted per slide under oil immersion from different fields. Sperm concentration was determined by haemocytometer method (Neubauer improved, Marienfeld).

Statistical analysis

The data analysis was carried out by SAS software, Version (9.1) of the SAS system for Window, Copyright© (2011) SAS Institute Inc., Cary, NC, USA. One way ANOVA was used to observe the seasonal affect on semen quality in Karan Fries bulls and pair-wise comparison of mean was done by Tukey's multiple comparison test.

RESULTS

Semen evaluation parameters showed a decreasing trend from spring to hot humid seasons (Table 3). The ejaculate volumes (3.53 ± 0.08 vs. 3.96 ± 0.12 mL), individual motility (48.00 ± 1.12 vs. 55.16 ± 1.32 %), sperm concentration (1487.10 ± 26.55 vs. 1590.77 ± 25.90 million/mL), Hypo-osmotic swelling test (59.75 ± 0.57 vs. 64.97 ± 0.84 %), acrosomal integrity (74.22 ± 0.62 vs. 83.40 ± 0.48 %), total sperm output (5229.05 ± 133.13 vs. 6276.80 ± 201.15 million) and total live sperm output (3264.43 ± 93.14 vs. 4265.62 ± 140.87 million) were significantly ($p<0.05$) lowered during hot humid season as compared to spring season whereas the major abnormalities were significantly ($p< 0.05$) higher during hot humid (10.74 ± 0.18 %) followed by hot dry season (8.66 ± 0.22) (Table 3).

DISCUSSION

Cattle living in the un-favourable environment who are not adapted to that climatic condition are also one of the causes of sub fertility (Barros *et al.* 2011). When the seminiferous tubules expose to high temperature, a degenerative process occurs in the testes (Blanchard *et al.* 1996; Rahman *et al.* 2011) that may lower the production of physiologically normal spermatozoa (Vogler *et al.* 1993; Wildeus and Entwistle, 1986). Shrivatava *et al.* (2013) reported highest frequency of discarded semen due to poor sperm concentration followed by poor initial motility,

poor post thaw motility and lower volume during summer season in Holstein Friesian (HF) crossbred bulls. Higher quantity of ejaculate volume with higher concentration of normal spermatozoa increases the frozen semen doses for artificial insemination.

Present study recorded lower ($p<0.05$) ejaculate volume during hot humid season as compared to spring season. Sperm concentration, total sperm output and total non-eosinophilic sperm output were lowered ($P<0.05$) during hot humid compared to spring season. Similar findings were also observed by Goswami *et al.* (1991), Fiaz *et al.* (2010) and Bhakat *et al.* (2014) during summer season. Good quality of semen was observed during spring season where the environment temperature was approximately 25 °C. The individual motility and HOST are considered as good parameters for semen quality and use for prediction of fertility potential of bulls. Their percentage decreased ($p<0.05$) during hot humid season as compared to spring season. Acrosomal integrity is also considered one of the good indicators of sperm quality (Correa and Zavos, 1994) which was also lowered ($p< 0.05$) during hot-humid as compare to spring season. This finding is in agreement with Mishra *et al.* (2013) and Bhakat *et al.* (2014) in crossbred bulls. 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. An overloaded oxidative stress has negative effect on spermatogenesis which may lead to damage of sperm membrane and DNA. ROS activity is positively correlated with higher serum Inhibin B in human and it can directly suppress spermatogenesis (Richthoff *et al.* 2003). Shukla *et al.* (2010) and Bhakat *et al.* (2014) noticed higher ($p< 0.05$) percentage of major abnormalities during hot-humid season in crossbred bulls. Better spermatozoal morphology was reported in winter and spring seasons in bulls (Salah *et al.* 1992; Vilakazi and Webb, 2004). The higher ambient temperature and humidity might have an adverse effect on spermatogenesis and negative effect on LH secretion (Gilad *et al.* 1993). This might be one of the reasons for poor semen quality in crossbred bulls raised under tropical climatic conditions. The findings of the present study also indicate poor adaptability of Karan Fries bulls under tropical climatic conditions; they may be under heat stress during hot dry and hot humid seasons.

CONCLUSION

The availability of good quality semen is must throughout the year for successful artificial insemination. From the present study it can be concluded that the hot humid season is stressful to Karan Fries bulls under tropical climatic conditions. Therefore, special measures should be taken to Karan Fries bulls under tropical climatic conditions for sustainable semen production throughout the year.

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