Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
Accessible at: www.pvj.com.pk

RESEARCH ARTICLE

# Seasonal Variations in Certain Physical and Biochemical Attributes of Semen from Cholistani Bulls

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ARTICLE HISTORY

Received: December 31, 2012
Revised: June 19, 2013
Accepted: August 07, 2013

**Key words:**
Cholistani bulls
Season
Seminal attributes
Stress-free
Stressful

**ABSTRACT**

In the present study, effects of stress-free and stressful seasons on certain physical and biochemical attributes of semen of Cholistani AI bulls were investigated. A total of 382 ejaculates from six bulls were collected at weekly intervals during four seasons viz. stress-free autumn (October-November), stressful winter (December-January), stressful dry summer (May-June) and stressful wet summer (July-August). These ejaculates were evaluated for various physical and biochemical attributes. Overall mean values (±SEM) for ejaculatory volume, number of ejaculates per bull, mass motility, individual sperm motility, sperm concentration, number of doses of semen frozen per bull, percentages of live, morphologically normal sperm and those with intact acrosome were 4.92±0.14 mL, 1.89±0.02, 2.26±0.06, 63.51±1.03%, 989.73±27.4 million/mL, 208.26±8.42, 86.74±0.55%, 86.34±0.51% and 86.64±0.48%, respectively. In general, all the parameters revealed significantly ($P<0.05$) better results in stressful seasons as compared to stress-free season. Overall mean values for $Na^+$, $K^+$, cholesterol and glucose in the seminal plasma were 102.79±3.95 & 36.65±0.99 mEq/L, 152.02±3.17 & 127.22±3.65 mg/dL, respectively. Amongst these, $Na^+$ was higher ($P<0.05$) during dry summer, whereas $K^+$ was higher in dry summer and winter. Cholesterol and glucose were elevated ($P<0.05$) during dry summer season. Correlation analysis revealed significant positive correlations between mass and individual sperm motility, morphologically normal and live sperm and sperm acrosome integrity and live sperm ($P<0.01$). Among biochemical attributes of seminal plasma, $Na^+$ and $K^+$, cholesterol and $K^+$ and glucose and $Na^+$ were correlated ($P<0.01$). Considering seminal plasma biochemical constituents and physical attributes, only seminal plasma cholesterol was positively correlated with ejaculatory volume ($P<0.01$). In conclusion, various physical and biochemical attributes of Cholistani bull semen were better in stressful seasons as compared to stress free season.



**To Cite This Article:** Farooq U, A Ijaz, N Ahmad, H Rehman and H Zaneb, 2013. Seasonal variations in certain physical and biochemical attributes of semen from Cholistani bulls. Pak Vet J, 33(4): 510-514.

## INTRODUCTION

Variation in semen quality of an animal depends upon many intercalated factors such as macro- and micro-climatic conditions (temperature, humidity, rainfall and photoperiod), feeding and management (Mandal *et al*., 2005), breed (Mukhopadhyay *et al*., 2010), age, health status (Mandal *et al*., 2005), genetic make up (Koivisto *et al*., 2009), and the sexual activity of the animal at the time of semen collection (Bhakat *et al*., 2011). Season seems to influence the seminal attributes directly through its macro- and micro-climatic factors and indirectly through affecting vegetation, forage quality and soil-plant interaction (Chacón *et al*., 2002; Bhakat *et al*., 2009).

Fifteen well recognized indigenous cattle breeds of Pakistan constitute about 43% of the total cattle population in the country and belong to zebu (humped; *Bos indicus)* cattle. Although their ability of thermo-tolerance and tick-resistance has widely been accepted (Farooq *et al*., 2010), yet a precise knowledge of their reproductive potentials, particularly in terms of bull fertility under their local climatic conditions, is still a far

fetched milestone. The work published from Pakistan and certain other tropical countries for Zebu bulls is mostly on Sahiwal breed (Ahmad *et al*., 2003). Similarly, the buffalo bulls have also been studied extensively, revealing their previously hidden potentials. However, the reproductive potentials of many other indigenous cattle breeds still remain unearthed. Cholistani breed of cattle is a zebu *(Bos indicus)* or humped breed, being reared mostly by the nomadic pastoralists of Cholistan Desert of Pakistan. Animals of this breed are the major source of socio-economic uplift in the area. However, there is relatively little information available in the literature on various seminal attributes of this breed.

In a recent study (Farooq *et al*., 2013), we published preliminary data on semen quality and freezability of six Cholistani bulls collected over a period of three months. In the present study, an attempt was made to assess seasonal variations in certain physical and biochemical attributes of fresh semen and seminal plasma based on the data collected from the same six Cholistani bulls during stress-free and stressful seasons. Moreover, relationships among various seminal attributes were also investigated.

## MATERIALS AND METHODS

**Experimental area:** The present study was conducted at the Semen Production Unit (SPU), Karaniwala, Bahawalpur, located in the Cholistan Desert of Pakistan. Geographical location of this area has been described earlier (Farooq *et al*., 2012). The climate of this area is arid tropical, May and June (dry summer) are the hottest months with the maximum temperature exceeding 45ºC. July and August (wet summer) bring in Monsoon winds with negligible rains. Thus, the area is least influenced by rains and remains water scarce and drought stricken. October and November have temperature of 24-27ºC, whereas December and January are the coldest months with temperature of 17-20ºC.

The study was conducted in the months of October, November and December, 2011 and January, May, June, July and August 2012. Keeping in view the prevailing climatic conditions of the study area, 4 seasons of 2 months duration each were defined viz. i) stress free autumn (October-November), ii) stressful winter (December-January), stressful dry summer (May-June) and iv) stressful wet summer (July-August). Mean values of temperature, relative humidity and rainfall for four seasons in the study area are presented in Table 1.

**Semen collection and evaluation:** Six adult Cholistani AI bulls with clinically normal reproductive tract and donating semen of acceptable quality were selected. The details regarding feeding and management of these animals have been described elsewhere (Farooq *et al*., 2013). Semen from each experimental bull was collected at weekly intervals, using an artificial vagina (AV). The final temperature of AV before semen collection was in the range of 41-44°C (Sharma *et al*., 1957). Two ejaculates were collected from each bull on each collection. However, occasionally some bulls refused to give the second ejaculate. Thus, a total of 382 ejaculates were available for analysis.

All ejaculates were evaluated for physical characteristics including ejaculatory volume, mass motility, individual sperm motility, sperm concentration, percentages of live and morphologically normal sperm and those with intact acrosome. The number of doses to be frozen (0.5 mL straws) was also obtained from the photometer with a final dilution of 40 million spermatozoa/0.5 mL straw. The detail of semen collection and evaluation has been given earlier (Farooq *et al*., 2013).

**Seminal plasma collection and analysis:** Seminal plasma extraction was carried out fortnightly and 96 samples from six bulls (04 samples per bull per season) were available. Seminal plasma was extracted as described earlier (Pesch *et al*., 2006) and stored at -20ºC till analyzed for sodium ($Na^+$), potassium ($K^+$), cholesterol and glucose with a chemistry analyzer (Metrolab 1600 DR, Diaton Group, Budapest, Hungary), using commercially available kits.

**Statistical analysis:** Mean values (±SEM) for various physical and biochemical attributes were calculated. Magnitude of variation in these attributes was ascertained through ANOVA, using completely randomized design. Differences between mean values were compared through Duncan's multiple range test. Pearson's correlation coefficients were also calculated between various spermiogram attributes, between seminal plasma constitutes, and between spermiogram attributes and seminal plasma constituents.

## RESULTS

**Spermiogram attributes:** Overall mean values (±SEM) for ejaculatory volume, number of ejaculates per bull, mass motility, individual sperm motility, sperm concentration and total number of doses of semen frozen for the six Cholistani AI bulls were 4.92±0.14 mL, 1.89±0.02, 2.26±0.06, 63.51±1.03%, 989.73±27.4 million/mL and 208.26±8.42, respectively (Table 2).

Results of various spermiogram parameters of Cholistani AI bulls as affected during stress free and stressful seasons are also presented in Table 2. In general, all the parameters revealed significantly ($P<0.05$) better results in stressful seasons as compared to stress free season, with the exception of number of ejaculates per bull which was not affected significantly by seasons. Ejaculatory volume was significantly higher ($P<0.05$) in dry and wet summer than stress free autumn. However, the difference in ejaculatory volume between autumn and winter was non-significant. Same was true between winter and dry summer.

Mass motility was significantly higher ($P<0.05$) during dry summer as compared to autumn and winter, the difference between the latter two seasons was non-significant. In wet summer, mass motility was significantly higher ($P<0.05$) than that in autumn or winter, while it differed non-significantly from that in dry summer. The individual sperm motility was higher in dry summer ($P<0.05$) as compared to autumn and wet summer, the difference between the latter two seasons was non-significant. Sperm concentration was lower ($P<0.05$)

**Table 1:** Average values for the climatic data of the study area during four seasons

| Parameters | Stress Free Autumn (Oct/Nov) | Stressful Seasons | | |
|---|---|---|---|---|
| | | Winter (Dec/Jan) | Dry Summer (May/June) | Wet Summer (July/Aug) |
| Temperature (°C) | 25.1 | 14.2 | 35.2 | 34.0 |
| Relative Humidity (%) | 61.0 | 58.0 | 40.4 | 63.0 |
| Rainfall (mm) | 0 | 2.5 | 3.0 | 72.2 |

Source: Regional Meteorological Centre, Lahore, Pakistan.

**Table 2**: Mean values (±SEM) of spermiogram attributes of Cholistani AI bulls as affected by different seasons

| Parameters | Stress Free Autumn (Oct/Nov) | Stressful Seasons | | | Overall mean |
|---|---|---|---|---|---|
| | | Winter (Dec/Jan) | Dry Summer (May/June) | Wet Summer (July/Aug) | |
| Ejaculatory volume (mL) | 4.35±0.26a | 4.57±0.28ab | 5.26±0.27bc | 5.42±0.30c | 4.92±0.14 |
| No. of ejaculates/bull | 1.96±0.03 | 1.86±0.045 | 1.85±0.050 | 1.90±0.041 | 1.89±0.02 |
| Mass motility (Score 1-5) | 2.07±0.12ab | 1.93±08a | 2.64±0.13c | 2.40±0.14bc | 2.26±0.06 |
| Individual sperm motility (%) | 62.66±2.19a | 63.22±1.66ab | 68.75±1.37b | 59.33±2.66a | 63.51±1.03 |
| Sperm concentration (million/mL) | 1047.61±44.77b | 825.03±42.61a | 1094.57±56.59b | 1003.95±62.22b | 989.73±27.40 |
| No. of doses frozen per bull | 191.40±14.22b | 143.92±11.25a | 245.37±17.84c | 250.37±17.75c | 208.26±8.42 |
| Live sperm (%) | 80.72±1.74a | 90.31±0.71c | 89.38±0.48c | 85.52±0.81b | 86.74±0.55 |
| Morphologically normal sperm (%) | 80.70±1.40a | 90.61±0.71c | 88.94±0.51c | 84.11±0.73b | 86.34±0.51 |
| Acrosome integrity (%) | 80.70±1.43a | 89.28±0.71c | 89.08±0.53c | 86.46±0.62b | 86.64±0.48 |

Values with different letters within a row are significantly different from one another (P<0.05).

**Table 3:** Mean values (± SEM) of seminal plasma constituents of Cholistani AI bulls as affected by different seasons

| Parameters | Stress Free Autumn (Oct/Nov) | Stressful Seasons | | | Overall mean |
|---|---|---|---|---|---|
| | | Winter (Dec/Jan) | Dry Summer (May/June) | Wet Summer (July/August) | |
| Sodium (mEq/L) | 96.96±3.04a | 100.72±3.70a | 117.54±2.89b | 95.80±2.33a | 102.79±3.95 |
| Potassium (mEq/L) | 32.31±0.98a | 39.69±1.02b | 43.36±0.50b | 31.26±0.24a | 36.65±0.99 |
| Cholesterol (mg/dL) | 144.55±4.92ab | 157.90±4.53b | 173.70±4.24c | 131.95±7.33a | 152.02±3.17 |
| Glucose (mg/dL) | 110.00±3.95a | 129.75±3.82ab | 147.95±7.74b | 121.20±9.52a | 127.22±3.65 |
| Na:K ratio | 3.00±0.82ab | 2.54±0.76a | 2.71±0.80a | 3.06±0.80b | 2.80±0.66 |

Values with different letters within a row are significantly different from one another (P<0.05).

**Table 4:** Significantly positive correlation coefficients between various spermiogram attributes and seminal plasma constituents of Cholistani AI bulls

| Parameters | r value |
|---|---|
| Individual motility × mass motility | 0.619** |
| Normal morphology × viability | 0.848** |
| Acrosome integrity × viability | 0.790** |
| $Na^+ \times K^+$ | 0.341** |
| Cholesterol × $K^+$ | 0.390** |
| Glucose × $Na^+$ | 0.232* |
| Cholesterol × volume | 0.222** |

* Correlation is significant at the 0.05 level (2-tailed);
** Correlation is significant at the 0.01 level (2-tailed)

during winter as compared to other three seasons, the latter differed non-significantly from one another.

In terms of semen production, a total of 39778 doses of 0.5 mL straws were frozen during the study period. Significantly higher number of doses per bull (P<0.05) was frozen during dry and wet summer as compared to autumn and winter; the difference between the latter two seasons was also significant, while it was non-significant between the former two seasons.

The subjective analysis of spermatozoa revealed that percentages of live, morphologically normal sperm and those with intact acrosome were 86.74±0.55, 86.34±0.51 and 86.64±0.48, respectively. These values were higher (P<0.05) during stressful winter and dry summer as compared to wet summer and autumn, the difference between the former two seasons was, however, non significant. Moreover, mean values for these attributes were significantly higher during wet summer as compared to autumn.

**Seminal plasma constituents:** The overall mean values (±SEM) for $Na^+$, $K^+$, cholesterol and glucose in the seminal plasma of Cholistani AI bulls included in the present study were 102.79±3.95 & 36.65±0.99 mEq/L, 152.02±3.17 & 127.22±3.65 mg/dL, respectively (Table 3). Amongst these, $Na^+$ was significantly higher (P<0.05) during dry summer, whereas K+ was higher in dry summer and winter as compared to other seasons. Cholesterol and glucose were found to be significantly elevated (P<0.05) during dry summer season. Na:K ratio was higher during wet summer as compared to winter and dry summer (Table 3).

**The correlations:** The results of correlation analysis (Table 4) revealed significantly positive correlations between individual and mass motility, morphologically normal and live sperm, and sperm acrosome integrity and live sperm (P<0.01). Among biochemical attributes of seminal plasma, $Na^+$ and $K^+$, cholesterol and $K^+$, and glucose and $Na^+$ were correlated. Correlating the seminal plasma constituents with the spermiogram attributes, only seminal plasma cholesterol was found to be positively correlated with ejaculatory volume (P<0.01).

## DISCUSSION

**Spermiogram attributes:** The overall mean ejaculatory volume of Cholistani AI bulls recorded in the present study is relatively higher than the studies published for bulls of various *Bos indicus* breeds. A preliminary data of three months on the same breed revealed a lower mean ejaculatory volume of 4.45±0.76 mL (Farooq *et al*., 2013). A mean ejaculatory volume of 4.38±0.09 mL for adult (5-years old) Sahiwal bulls has been reported (Ahmad *et al*., 2003). Similarly, a lower mean value of 3.4±1.3 mL was recorded for indigenous non-descript zebu bulls in India (Siddiqui *et al*., 2008). Another study from Brazil

recorded mean semen volume ranging from 4.8 to 5.4 mL for *Bos indicus* bulls (Koivisto *et al*., 2009). These differences can be attributed to breed characteristics, as well as the meteorological and nutritional variations (Bhakat *et al*., 2011).

The results on seasonal variation revealed significantly higher ejaculatory volume in stressful dry and wet summer seasons than the stress free autumn season. Previously, some workers have negated the effect of season on semen volume (Mathevon *et al*., 1998). However, others have reported a lower ejaculatory volume in dry or humid summer owing to high temperatures and poor grazing quality (Ahmad *et al*., 2003; Koivisto *et al*., 2009). The effect of season on ejaculatory volume can be minimal if bulls are fed adequately and well adopted to high temperature (Mathevon *et al*., 1998). Higher ejaculatory volume recorded in the present study during stressful wet and dry summer suggests an adequate plain of nutrition and management of the animals under farm conditions.

The overall mean mass motility recorded in the present study is congruent with earlier work reported for Sahiwal bulls (Ahmad *et al*., 2003). Results regarding seasonal variation revealed highest mass motility in dry summer, followed by wet summer which is in line with earlier published results for *Bos indicus* bulls (Koivisto *et al*., 2009). The results of the present study are suggestive of the fact that *Bos indicus* bulls are thermo-tolerant and well adapted to stressful conditions of dry and wet summer.

The overall mean individual motility in the present study is in line with a previous report (Bhakat *et al*., 2011). Regarding the seasonal influence, individual sperm motility was highest in dry summer and lowest in wet summer. Similar results have previously been reported (Ahmad *et al*., 2003). However, Mostari *et al*. (2005) reported deteriorated sperm motility during summer season in Holstein-Friesian and Jersey bulls maintained in Pakistan. Better results in the present study during dry summer can be attributed to an innate ability of these bulls to be well adapted to the harsh, hot desert climate. Furthermore, it has been established that the sperm motility depends more on mating frequency than on seasonal climatic changes (Chacón *et al*., 2002; Koivisto *et al*., 2009).

The overall mean sperm concentration in the present study is lower than 2541.9±41.2 and 1471±37.0 million/mL reported for crossbred and Sahiwal bulls, respectively (Sarder, 2003; Siddiqui *et al*., 2008). Seasonal influence revealed a significant decrease in sperm concentration during winter as compared to other seasons, which is in accordance with previous reports (Koivisto *et al*., 2009; Bhakat *et al*., 2011). The plausible justification for significant decrease in sperm concentration during winter could be the displacement of testes towards abdomen during winter due to severe cold, consequently raising the testicular temperature and hence, hampering its functionality in terms of sperm production (Zafar *et al*., 1988).

In terms of production of semen, the overall mean number of ejaculates per bull was higher than that reported for Sahiwal bulls (Ahmad *et al*., 2003). The seasonal trend revealed highest number of doses frozen in dry and wet summer seasons, the period when ejaculatory volume and sperm concentration were also higher. Thus, higher semen production during dry and wet summer can be attributed to better semen quality in terms of ejaculatory volume and sperm concentration.

Mean values for spermatological parameters (live and morphologically normal sperm and those with intact acrosome) were higher in winter and dry summer than autumn and wet summer. However, a report on Brahman bulls revealed non significant effect of season on viability and acrosome integrity of spermatozoa (Chacón *et al*., 2002).

**Seminal plasma constituents:** The present study revealed that the overall $Na^+$ level in seminal plasma was higher than 106.46±11.92 mg/dL (46.29±2.13 mEq/L) and 90.40±25.10 mEq/L reported for Murrah buffalo and Holstein Friesian bulls by Shukla *et al*. (2009) and Petrunkina *et al*. (2001), respectively. Conversely, the overall mean seminal plasma $K^+$ levels of the present study were lower than 98.18±11.67 mg/dL (42.69±2.07 mEq/L) and 66.50±7.5 mEq/L reported for Murrah buffalo and Holstein Friesian bulls by the above referred workers, respectively. These variations may be indicative of adaptation of Cholistani bulls to the hot arid environment or a genetic predisposition. The effect of variation in method of estimating these cations can not be ruled out. It is noteworthy that neither $Na^+$ nor $K^+$ showed any correlation with any of the spermiogram attributes in the present study.

Regarding the seasonal influence, seminal plasma concentration of $Na^+$ was higher in dry summer, whereas $K^+$ was higher during winter and dry summer than other seasons. To the best of our knowledge, none of the previous studies has addressed the seasonal influence on the concentrations of these cations in the seminal plasma of AI bulls. Whether these variations depict an adaptation to the environment or are purely genetic in origin, is yet not clear.

The concentrations of cholesterol and glucose in the seminal plasma were highest during dry summer in Cholistani AI bulls under study. A rise in the level of cholesterol during summer season has been reported earlier (Okab, 2007). Conversely, a study on rams has reported its level to be at a nadir point in dry summer (Zamiri *et al*., 2010). Higher levels of seminal cholesterol during summer seem to be due to increased thyroid activity and hepatic mechanisms that remove cholesterol from circulation (Shukla *et al*., 2009). Furthermore, cholesterol is known to be a precursor of androgenic hormones and its rise in summer season indicates its availability for conversion into sex hormones and hence higher sex drive (Shukla *et al*., 2009). In addition to this, cholesterol has been known for its potential as a protective agent against environmental stress (Jacyno *et al*., 2009), hence, its increase in summer season in the present study may also be an adaptive mechanism to minimize thermal stress. Similarly, high level of glucose in seminal plasma during dry summer seems to be necessary for meeting the higher sugar demand due to increased thyroid activity during stressful summer season (Taha *et al*., 2000).

**Correlations:** Significant positive correlations were recorded between individual sperm motility and mass

motility, normal morphology and viability, and acrosome integrity and viability. A significant positive correlation between $Na^+$ and $K^+$ was recorded in the present study. This positive correlation seems to be necessary for maintaining osmolarity and metabolic activity of spermatozoa. However, none of the cations was found to be correlated with any of the seminal attributes which is in contrast to earlier reports which have revealed a positive correlation of these cations with mass motility and individual motility in the buffalo (Shukla *et al*., 2009).

A positive correlation between cholesterol and ejaculatory volume was noticed in the present study. Seminal plasma of young boars from Poland (Jacyno *et al*., 2009) also presented same correlation along with its additional close correlations with other seminal attributes such as initial motility and sperm count. A positive correlation between cholesterol and sperm count has been reported for bulls being bred in Turkey (Cevik *et al*., 2007).

Glucose showed no correlation with any of the spermiogram attributes, although it was significantly correlated with seminal plasma $Na^+$ contents. However, a previous report (Cevik *et al*., 2007) has demonstrated a close correlation of glucose with various semen attributes and ultimately to fertility due to its importance in spermatozoa energy production. In the present study, only six bulls were included. Further studies with a larger sample size are suggested for better understanding regarding seasonal effects on these seminal attributes.

**Conclusion:** Various physical and biochemical attributes of Cholistani bull semen were better in stressful seasons as compared to stress free season. The acceptable quality of semen of Cholistani bulls recorded during wet/dry summer in the present study is an indicative of the fact that this breed has an innate ability of being well adapted to the harsh, hot desert climate. This adaptability helps them maintain their reproductive abilities at optimum levels even in stressful seasons.

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