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Semen Quality of Bulls as Influenced by Breed, Body Condition Score and Ascorbic Acid under Heat Stress

Muamber Shah[1], Muhammad Subhan Qureshi[1], Rifat Ullah Khan[1], Muhammad Mobashar[1], Mubashar Ali Khalique[2], Irfan Khattak[3], Ambrina Tariq[4], Ijaz Ahmad[1] and Shabana Naz[5,*]

[1]Faculty of Animal Husbandry and Veterinary Sciences, The University of Agriculture, Peshawar
[2]Faculty of Veterinary and Animal Sciences, University of Poonch, Rawlakot, Azad Kashmir
[3]College of Veterinary Science and Animal Husbandry, Abdul Wali Khan University, Mardan
[4]Civil Veterinary Hospital, Dera Ismail Khan, Directorate of Livestock and Dairy Development, Peshawar
[5]Department of Zoology, Govt. College University, Faisalabad

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ABSTRACT

This study evaluated the effect of breed, body condition score and ascorbic acid supplementation on the semen quality of breeding bulls. A total of 8 adult (≥2 year) bulls (2 each from Jersey, Friesian, Sahiwal and cross bred) were selected with body condition score (BSC) at a scale of (0-5). The results showed that mean semen volume was significantly (P<0.05) high in Friesian bull. Sperm concentration and motility were significantly (P<0.05) high in Jersey bull while live sperm percentage was significantly (P<0.05) high in Jersey bulls and sperm abnormality was significantly (P<0.05) high in Sahiwal and Friesian-Sahiwal cross. In addition, semen quality was improved in all the breeds after supplementation of vitamin C. There was no significant effect of BCS on the sperm quality of bulls. The results give important information regarding the semen quality of different breeds of bulls under heat stress condition.

INTRODUCTION

When the animal is exposed to high environmental temperature a state of heat stress could occur which affects the quality of semen (Marai *et al*., 2010). Heat stress may directly affect reproductive performance in the form of impaired spermatogenesis and lower testosterone level (Murugaiyah, 1992). In Pakistan, during summer, the atmospheric temperature ranges from 30-45°C. For exotic breeds, high ambient temperature has adverse effect on the reproductive performance (Ihsanullah *et al*., 2017). Ascorbic acid works as a natural antioxidant and is found in elevated level in seminal fluid (Thiele *et al*., 1995). Vitamin C also plays an important role in reproduction and showed significant results (Luck *et al*., 1995; Khan *et al*., 2012). Its role in the physiological reproduction had been undefined. Ascorbic acid prevents sperm oxidative damage by keeping genetic integrity of sperm cells. Considering the merits and future potential and benefits of using ascorbic acid, its effects on the semen parameters are needed to be explored. Therefore, the present study was designed to investigate the effects of different levels of ascorbic acid and body condition score (BCS) on semen quality and semen traits of bulls. The present study assessed the effect of heat stress on the semen quality under different BCS and the ameliorative effect of ascorbic acid supplementation on semen traits of different breeds of bulls.

MATERIALS AND METHODS

A total of 8 adult (≥2 year) bulls (2 each from Jersey, Friesian, Sahiwal and cross bred) were selected. Body condition score was recorded at a scale of (0-5) points as described by Ihsanullah *et al*. (2016). Further 12 animals of different breeds were selected and divided into four treatment groups. Ascorbic acid was supplemented in feed at the rate of 0, 50, 100, and 200 mg/day of feed. The experiment was continued for 3 weeks.

Semen evaluation

Semen was collected from the experimental bulls early in the morning using artificial vagina (AV). Soon

* Corresponding author: drshabananaz@gcuf.edu.pk
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after collection, semen was transferred to water bath with a temperature of 35°C for further investigation. The collection was done twice a week i.e., Monday and Thursday. The semen samples were subjected to determination of semen volume, sperm concentration, percentage of motile spermatozoa, live dead and sperm morphology as described by Majid *et al*. (2015).

Temperature and humidity index (THI)

The THI was calculated using the formula of Ihsanullah *et al*. (2017) as follow:

$THI = (0.8 \times T_{mix}) + [(H/100) \times (T_{mix} - 14.4)] + 46.4$

Where, T_{mix} is maximum temperature in centigrade and H is percent relative humidity.

Different levels of THI in dairy breeds: THI=< 72 (no stress), THI = 72-79 (mild stress), THI = 80-89 (moderate to high stress) and THI = > 96 (very danger and death may occur).

Statistical analyses

Data was statistically analyzed with the help of statistical software, Statistix (version 8.1). Means of control and treated groups were compared by using one way analysis of variance (Steel *et al*., 1997).

RESULTS

Among the different breeds, maximum semen volume was produced by Holstein Friesian (HF) bulls followed by Jersey, Friesian Sahiwal Crossed (FSC) and Sahiwal as given in Table I. The overall mean volume of semen was also significantly higher (P<0.05) in HF breed as compared to other breeds in this study even in condition of high temperature and humidity. The results also presented that the volume of the semen in all breeds was high during first week and then reduced with an increase in temperature and humidity index. The concentration of semen was also significantly different (P<0.05) in different breeds of bulls in this study. Significantly, the highest semen concentration was found in Jersey bulls followed by HF, FSC and Sahiwal even at in high temperature and humidity. The overall mean of high concentration was also significantly high in sperm of Jersey bulls as shown in Table I. The result presented in Table I describes sperm percent motility of different breeds of bulls in different weeks at high temperature and humidity. The sperm percent motility was significantly (P<0.05) increased in Jersey bulls followed by the HF, FSC and Sahiwal breeds of bulls. The overall mean of sperm percent motility was also significantly high in Jersey breed of bulls compare to others. Results on live percentage of sperm among different breeds during various weeks at high temperature and humidity are shown in Table II. Live percentage of sperm was significantly (P<0.05) higher in Jersey breed of bulls followed by the HF, FSC and Sahiwal breed. The overall live percentage was also significantly high in Jersey bulls. Sperm primary abnormality of different breeds of bulls at different weeks in different temperature and humidity are shown in Table II. However, FSC bull breed showed significantly (P<0.05) greater sperm primary and secondary abnormalities at high temperature and humidity as compared to other bull breeds.

Table I.- Mean values of semen traits of different bull breeds at different temperature and humidity index (n=64).

Breed	Parameters	Week 1	Week 2	Week 3	Week 4	Overall mean
Friesian	Volume (ml)	6.26±0.14^{a}	6.20±0.13^{a}	5.75±0.15^{a}	5.50±0.28^{a}	5.68±0.03^{a}
	Concentration (106/ml)	1375.0±14.43^{b}	1375.0±14.43^{b}	1300.0±28.86^{b}	1275.0±14.43^{b}	1331.3±1.04^{b}
	% Motility	89.00±0.28^{b}	88.50±1.15^{b}	88.50±0.28^{b}	87.50±0.28^{b}	88.40±0.05^{b}
Jersey	Volume (ml)	5.35±0.14^{b}	5.30±0.12^{b}	5.25±0.10^{b}	5.25±0.14^{a}	5.50±0.00a^{b}
	Concentration (106/ml)	1725.0±14.43^{a}	1675.0±14.43^{a}	1600.0±28.86^{a}	1600.0±28.86^{a}	1650.00±7.21^{a}
	% Motility	92.00±0.28^{a}	91.50±0.86^{a}	91.50±0.57^{a}	91.50±0.86^{a}	91.63±0.20^{a}
Sahiwal	Volume (ml)	5.22±0.14^{b}	5.16±0.10^{b}	5.00±0.09^{c}	4.75±0.28^{b}	5.06±0.10^{c}
	Concentration (106/ml)	900.00±14.43^{d}	875.00±14.43^{d}	875.00±28.86^{d}	825.00±14.43^{d}	868.75±10.8^{d}
	% Motility	85.50±0.57^{d}	83.00±0.86^{b}	83.00±1.15^{c}	82.50±0.28^{d}	83.50±0.28^{d}
FSC**	Volume (ml)	5.50±0.14^{b}	5.50±0.12a^{b}	5.25±0.28b^{c}	5.00±0.14^{a}	5.38±0.14^{c}
	Concentration (106/ml)	1200.0±28.86^{c}	1200.0±57.73^{c}	1150.0±28.86^{c}	1125.0±14.43^{c}	1168.8±18.1^{c}
	% Motility	90.00±0.28^{c}	86.50±1.15^{a}	85.00±0.28^{c}	84.50±0.57^{c}	86.53±0.43^{c}
THI		83.68	84.75	85.22	86.16	84.95

^{a,b,c,d}, means with different superscripts within the same column for the same parameter differ significantly (P<0.05) for different breeds. THI, Temperature and humidity index; FSC, Fresian Shiwal cross; THI, temperature and humidity index.

The results present in Table II describes the sperm secondary abnormality of different breeds of bulls at different weeks at high temperature and humidity showing that sperm secondary abnormality was significantly (P<0.05) high in FSC breed of bulls followed by Sahiwal, Friesian and Jersey breeds. The overall sperm secondary abnormality was also significantly (P<0.05) high in cross breed bulls as compare to other breeds in this study.

The ascorbic acid supplementation significantly (P<0.05) increased the volume of semen and sperm concentration in bulls. The highest volume of semen and sperm concentration was produced by the dose rate of 200 mg as compared to non-supplemented or low supplemented group as shown in Table III. The overall mean volume was also significantly high in the same group.

The result of the sperm concentration of different group of bulls at different weeks is given in Table III. The sperm concentration was significantly high (P<0.05) in all four weeks in group (C200) bulls as compare to all other groups in the study. The overall mean of sperm concentration was also significantly high in group (C 200) of bulls. However, Ascorbic acid supplementation didn't influence live percent motility of sperm among different bull breeds (Table IV).

Table II.- Mean values of live percent and abnormalities percentage of sperm of different bull breeds at different temperature and humidity index (n=64).

Breed	Parameters	Week 1	Week 2	Week 3	Week 4	Overall mean
Friesian	Live %	93.00±0.57^{a}	91.50±0.28^{b}	90.50±0.28^{b}	90.50±0.28^{b}	91.37±0.07^{b}
	ABN % (P)	5.50±0.28^{c}	6.00±0.57^{c}	6.00±0.57^{b}	7.00±0.57^{bc}	6.12±0.36^{c}
	ABN % (S)	13.50±0.28 ^{c}	13.50±0.86^{b}	14.00±1.15^{a}	15.00±0.57^{b}	14.00±0.72^{b}
Jersey	Live %	93.50±0.28^{b}	92.50±0.28^{a}	92.00±0.28^{a}	90.50±0.57^{a}	92.12±0.07^{a}
	ABN % (P)	3.00±00^{d}	3.50±0.28^{d}	5.50±0.28^{d}	5.50±0.28^{d}	4.37±0.07^{d}
	ABN % (S)	13.50±0.28^{c}	13.50±0.57^{b}	13.50±0.86^{a}	13.00±0.28^{b}	13.37±0.36^{b}
Sahiwal	Live %	89.50±0.57^{c}	89.50±0.28^{c}	89.00±0.86^{b}	89.00±0.57^{c}	89.25±0.14^{d}
	ABN % (P)	7.50±0.28^{b}	7.50±00^{b}	9.00±0.57^{a}	10.00±0.28^{b}	8.50±0.14^{b}
	ABN % (S)	15.00±0.28^{b}	15.50±0.57^{a}	16.50±0.57^{a}	17.00±0.86^{ab}	16.00±0.14^{a}
FSC	Live %	91.50±0.28^{b}	90.50±0.75^{c}	90.50±0.28^{b}	89.00±0.28^{b}	90.37±0.21^{bc}
	ABN % (P)	8.50±0.28^{a}	10.50±0.28^{a}	10.50±0.57^{a}	11.00±0.86^{a}	10.12±0.21^{a}
	ABN % (S)	15.50±0.28^{a}	16.50±0.28^{a}	17.50±0.28^{a}	17.50±0.86^{a}	16.75±0.28^{a}
THI		83.68	84.75	85.22	86.16	84.95

^{a,b,c,d}, means with different superscripts within the same column for the same parameter differ Significantly (P<0.05) for different breeds. ABN (P), primary abnormality; ABN (S), secondary abnormality; FSC, Fresian Sahiwal cross.

Table III.- Effect of different supplementations of ascorbic acid on mean values of semen traits of different bull breeds at different weeks (n=96).

Group	Parameters	Week 1	Week 2	Week 3	Week 4	Overall mean
C 00	Volume (ml)	4.66±0.16^{b}	4.83±0.16^{b}	4.83±0.16^{b}	5.06±0.06^{b}	4.83±0.04^{b}
	Concentration (106/ml)	1266 .7±28.86^{b}	1276.7±44.09^{b}	1300.0±14.53^{b}	1316.7±16.66^{b}	1290.3±16.4^{b}
	% Motility	89.00±0.57^{a}	89.66±1.20^{a}	88.00±0.57^{a}	88.66±0.33^{a}	88.83±0.33^{a}
C 50	Volume (ml)	5.50±0.28^{a}	5.50±0.28^{a}	5.50±0.28^{a}	5.50±0.28^{a}	5.50±0.14^{a}
	Concentration (106/ml)	1300.0±33.33^{ab}	1300.0±28.86^{ab}	1316.7±28.86^{b}	1333.3±16.66^{b}	1312.7±21.6^{b}
	% Motility	86.00±0.57^{a}	88.00±0.57^{a}	88.33±0.88^{a}	89.33±0.66^{a}	87.91±0.58^{a}
C 100	Volume (ml)	5.50±0.28a^{b}	5.50±0.28^{b}	5.33±0.33^{ab}	5.66±0.33^{b}	5.50±0.14^{ab}
	Concentration (106/ml)	1300.0±28.86^{b}	1300.3±16.66^{ab}	1333.0±28.86^{b}	1333.3±16.66^{b}	1316.7±8.33^{b}
	% Motility	87.66±2.02^{a}	87.66±0.33^{a}	88.66±0.88^{a}	89.33±0.66^{a}	88.33±0.79^{a}
C 200	Volume (ml)	5.66±0.28^{a}	6.00±0.66^{a}	6.00±0.57^{a}	6.36±0.44^{a}	6.01±0.49^{a}
	Concentration (106/ml)	1383.3±44.09^{a}	1383.3±18.86^{a}	1400.0±16.66^{a}	1433.3±44.09^{a}	1400.3±3.94^{a}
	% Motility	89.00±0.66^{a}	88.66±0.66^{a}	88.33±0.57^{a}	88.33±0.33^{a}	88.58±0.44^{a}

^{a,b}, means with different superscripts within the same column for the same parameter differ significantly (P<0.05)for different supplementation level. C 00, C50, C100 and C200 denote different vitamin C supplementation at rate of 0, 50, 100 and 200 mg/day of feed.

Table IV.- Effect of different levels of ascorbic acid on sperm live parentage, sperm primary and secondary abnormalities of different bull breeds (n=96) during different weeks.

Group	Parameters	Week 1	Week 2	Week 3	Week 4	Overall mean
C 00	Live %	92.00±0.57	91.33±1.20	91.00±0.57^{a}	90.66±0.33^{a}	91.08±0.36^{a}
	ABN % (P)	9.66±0.88	8.00±0.66	7.33±0.33^{a}	6.66±0.57^{a}	7.33±0.36^{a}
	ABN % (S)	14.00±0.57	15.33±0.88	12.00±0.57^{a}	11.33±0.88^{a}	13.16±0.46^{a}
C 50	Live %	91.33±0.88	93.33±1.33	94.00±1.15^{a}	90.33±0.33^{a}	91.41±0.36^{a}
	ABN % (P)	8.66±0.88	7.00±0.57	8.33±0.66^{a}	5.33±0.33^{b}	7.20±0.16^{ab}
	ABN % (S)	13.66±1.76	13.00±0.57	12.00±o.57^{a}	11.33±0.66^{a}	12.50±0.76^{a}
C 100	Live %	93.00±0.57	90.33±0.33	92.00±1.15^{a}	90.33±0.33^{a}	91.16±0.30^{a}
	ABN % (P)	8.33±0.33	6.33±0.33	8.33±0.33^{a}	5.33±0.33^{b}	7.18±0.22^{ab}
	ABN % (S)	13.66±0.88	13.33±0.88	13.66±0.88^{a}	12.00±0.57^{a}	13.16±0.08^{a}
C 200	Live %	93.00±1.00	91.00±0.57	92.00±0.57^{a}	91.33±0.33^{a}	91.75±0.14^{a}
	ABN % (P)	9.33±0.88	7.66±0.33	6.33±0.33^{b}	6.00±0.57^{b}	7.16±0.22^{b}
	ABN % (S)	14.00±1.15	14.00±0.57	12.00±0.57^{a}	11.33±0.33^{a}	12.83±o.16^{a}

Values with different superscripts within the same column differ significantly (P<0.05) for different supplementation levels. C00, C50, C100 and C200 represent 0, 5, 100 and 200 mg/feed day. ABN (P), primary abnormality; ABN (S), secondary abnormality.

Table V.- Mean values of different semen parameters of different bull breeds under variable body condition score (n=64).

Breed	BCS	Volume (ml)	Concent. (10^{6}/ml)	% Motility	Live %	% ABN (P)	% ABN (S)**
Friesian	3.25	6.05±0.21	1350±20.41	87.75±1.10	91.00±0.40	7.00±0.40	15.25±0.47
	3.50	5.77±0.27	1325±32.27	88.25±1.31	92.00±0.81	6.50±0.28	15.50±0.64
Jersey	3.25	6.05±0.21	1687.5±23.93	89.25±1.43	91.25±0.62	4.25±0.25	12.00±0.40
	3.75	5.65±0.11	1650.0±35.35	88.75±1.03	89.00±0.40	4.25±0.62	12.00±0.70
Sahiwal	3.00	5.30±0.28	837.50±23.93	82.75±0.47	88.75±0.47	5.75±0.47	12.50±0.50
	3.25	5.00±0.14	837.50±12.50	84.50±0.50	87.50±0.28	6.50±0.86	13.50±0.00
FSC	3.00	5.60±0.07	1237.5±12.50	85.25±1.49	87.50±1.32	9.75±0.47	17.25±0.47
	3.5	5.57±0.13	1237.5±37.50	83.50±2.21	89.00±0.90	8.50±0.28	17.75±0.47

Mean values within the same column for the same parameters are not significantly different (P>0.05). BCS, body score condition; ABN (P), primary abnormality; ABN (S), secondary abnormality; FSC, Friesian Sahiwal cross.

The results revealed in Table IV that there was non-significant (P>0.05) difference in live percentage of sperm in different groups of bulls with different levels of ascorbic acid supplementation. Furthermore, primary abnormality in sperm among different bull breeds during various weeks was significantly higher (P<0.05) in control group while secondary abnormalities remained unaffected among treatments.

The different semen parameters like semen volume and concentration and percent motility and live percentage, primary abnormality and secondary abnormality of sperm of different bull breeds at various weeks were not significantly affected by variable body condition score (Table V).

DISCUSSION

High environmental temperature has negative effects on fertility of bulls. In a previous study, it was reported that Holstein Friesian bulls are mostly affected by heat stress as compared to other breeds (Garacia *et al.*, 2005). In the present study, sperm concentration was significantly higher in Jersey bulls compared to other breeds. This is in agreement with findings of Ahmad *et al*. (1993) and Shaha *et al*. (2008) who also found higher concentration of sperm in Jersey bulls. Similarly, in the present study, Jersey bulls also had lesser primary and secondary abnormalities in comparison with other bull breeds. While FSC bulls had significantly higher primary and secondary sperm abnormalities, which might be linked with its genetic disturbance and imbalance. It was also observed that live percentage and percent motility of sperm was significantly high in Jersey bull breed and high dead sperm ratio was found in Sahiwal breed. The high concentration of sperm, less dead percentage of sperm, high motility and lesser primary and secondary abnormality in sperm of

Jersey breed showed that Jersey breed is lighter and very favorable for the subtropical environment. According to the breeding policy in Pakistan, the semen of Jersey breed is suitable for local cattle breeds.

In the current study, the semen volume was significantly increased and the sperm primary abnormalities were decreased in the ascorbic acid supplemented groups of bulls. Yousef *et al*. (2003) also observed similar pattern of semen volume with ascorbic acid treatment in rabbits. Similarly, higher sperm concentration was found in bulls at ascorbic acid level of C200. Dawson *et al*. (1987) also found significantly higher sperm concentration in human with higher level of ascorbic acid. Interestingly, sperm motility, live percentage of sperm and sperm abnormalities were not influenced by ascorbic acid treatment.

Animals with high BCS have highest sperm reserve count as well as sperm count (Salisbury *et al.,* 1978). In the present study, all the semen parameters mentioned above at different weeks under variable BCS of different breeds' bulls were not significantly different. Our results are in contrast with the results of Salisbary *et al*. (1978). The reason could be that we have collected data from semen processing unit, where all high quality semen producing animals were kept and the difference among the body condition score were not high. All the animals kept in SPU were healthy and there were no weak or obese animals which could not make any statistically difference in the semen traits.

CONCLUSION

Ascorbic acid should be supplemented to all bulls used for artificial insemination to improve the semen quantitative and qualitative characteristics under heat stress. The body condition score within range of (3-3.75) had no significance effect on semen traits. Jersey breed of cattle is the best adapted summer stressed breed.

Statement of conflict of interest

The authors declare no conflict of interest.

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