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Effect of Season on Bacterial Load in Semen of Different Breeds of Cattle

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ABSTRACT

Fresh semen ejaculates collected from 56 cow bulls of different breed during three different seasons (summer, rainy and winter of the year) were subjected to bacteriological examination. Total viable bacterial count of fresh semen was determined using standard plate count method and result expressed as mean (±SEM) CFU/ml of semen. Significantly ($P<0.05$) higher bacterial load (33571 ± 3842 CFU/ml) in bovine semen was reported during rainy season as compared to summer and winter season. During rainy season, Gir semen showed significantly ($P<0.05$) higher bacterial count (37500 ± 7500 CFU/ml). During summer season, bacterial count was significantly ($P<0.001$) higher in semen of exotic bulls (37143 ± 3595 CFU/ml) followed by crossbred (32000 ± 5333 CFU/ml) and indigenous bulls (19359 ± 1875 CFU/ml), whereas non significant variation were noticed during rainy and winter season. Among breeds, significantly ($P<0.005$) higher count was reported in Jersey and HF cross during summer; and in Jersey and Gir during rainy season, however; Tharparkar and Red Sindhi bulls yielded significantly ($P<0.005$) lower bacterial load in their semen as compared to other breeds during whole seasons of the year. It could be concluded from the results of the present study that season had significant effect on bacterial load in semen of different breeds of cow bulls.

Keywords: Bacterial load, Semen, Cow bull, Breed, Season

Artificial insemination (AI) technique is routinely used for improving the production potential and genetic merit of nondescript indigenous cows using superior germplasms. The success of this programme mainly depends on the quality of semen used for AI. The bacterial contaminants adversely affect the semen quality and hence the subsequent fertility (Grieveu *et al.,* 1995) and the same can be affected by change in season (Azawi and Ismaeel, 2012; Sannat *et al.,* 2015b).

As the season of our country varies markedly, special attention should be given to the fertility of bulls of different breed category, all the year round. The bulls used for AI in cattle included indigenous, exotic or crossbred bull; which however varied among region. The adaptability of exotic and cross-bred bulls in hot and humid climate as prevailing in India is limited. Though cattle are not normally considered a seasonal species, seasonal variations have already been recorded in some parameters of cow bull semen (El-Tayeb *et al.,* 2007; Sarder, 2007; Fiaz *et al.,* 2009).

A better knowledge of the influence of seasons of the year during semen collection on semen microbes will help the AI industry to adapt a standard management of bulls to improve semen output. The present study was therefore designed to investigate the effect of seasons on bacterial load in semen of indigenous, crossbred and exotic bulls used for AI in Chhattisgarh and surrounding state in India.

MATERIALS AND METHODS

Location

Present study was conducted at Central semen station, Anjora, District-Durg in Chhattisgarh state of India. Durg is situated between 17-23.70 N Latitude and 80.43-83.380 E Longitudes in central western part of Chhattisgarh

and semen station, Anjora is located on the bank of river Shivnath.

Distribution of season

Durg generally has a dry tropical and sub-humid type climate which is moderate; however summer is a little bit hotter. Climate of Durg district (durg.gov.in/District profile_General.html) was broadly categorized into three main seasons i.e. summer (March to Mid June), winter (November to January) and rainy season (Mid June to September). Details of climatic condition during study period were:

Summer

Rise of temperature begins from the month of March to May. The peak temperatures were observed in May and maximum temperature recorded was high as 48°C. The atmospheric humidity was very low as 15-20% during peak summer months.

Rainy

The onset of monsoon was usually from June and the season extended up to September, with monsoon peaking during July and August. The atmospheric humidity was very high (>90%) during monsoon months.

Winter

A very short winter season was reported starting form last of November to mid of January with minimum temperature of 15°C.

Cow bulls

56 cow bulls belonging to indigenous breeds namely Sahiwal (14), Gir (8), Red Sindhi (8) and Tharparkar (9); crossbred bull namely HF cross i.e. Holstein Friesien X Sahiwal (7) and Jersey cross i.e. Jersey X Red Sindhi (3) ; exotic breed namely Jersey (7) were undertaken in present study.

Feeding and management of bulls

Feeding and managemental system as recommended in minimum standard for production of bovine semen, National Dairy Development Board (NDDB, 2012) was practiced in semen collection center. All the bulls were kept under identical conditions of management, feeding (seasonal fodder) and watering. The bulls were housed individually in pens with sufficient cross ventilation and protection against summer heat and in an open space for sunbathing in winter. However, the bulls of the present study were reared under more or less stable environmental conditions.

Samples

Samples included fresh semen ejaculates of cow bulls. Strict aseptic measures were practiced during collection and handling of semen samples. Ejaculates were collected by means of sterile artificial vagina using routine collection technique (Shukla, 2008) and were processed immediately for bacteriological examination within one hour after collection. Overall nine ejaculates (three ejaculates in each season) were taken from each bull during the whole year. Samples were being collected from a bull keeping an interval of at least 10 days between two samples.

Determination of Bacterial load

Standard plate count (SPC) method by serial plate dilution was used to determine bacterial load in the semen samples (Shukla *et al.*, 2011). Culture media and reagents of HiMedia were used throughout the study. Tenfold serial dilution of the semen sample (1:10, 1:100, 1:1000 and 1:10000) was made in sterile nutrient broth. Four test tubes, each containing 0.9 ml nutrient broth were taken. As a procedure, 0.1 ml of freshly collected semen was added and mixed thoroughly into first test tube to obtain 1:10 dilution. Then, 0.1 ml (1:10 dilution) volume from first test tube was added into second test tube and mixed thoroughly to get 1:100 dilution and similarly step wise serial dilution was done to obtain 1:1000 and 1:10000 dilution of sample. Inoculums size of 0.5 ml from each dilution was spread on to separate SPC agar plate. Separate plates were used with each dilution and two SPC agar plates were taken for a single batch of semen. Sample was allowed to diffuse and then incubated at 37°C for 72 h. Colonies per plate were read and counted with the help of colony counter. Highest dilution showing visible colonies were taken into consideration for calculation of colony forming unit (CFU) per ml of sample using following formula.

$$\text{CFU/ml of sample} = \text{No. of CFU's} \times \text{dilution} \times 2$$

If plate with dilution 1:10000 shows two number of colony, then CFU/ml of sample = 2 ×10000 X2 = 40000. Average count of two plates with similar dilution was taken as final value.

Data recording and statistical analysis

Data were expressed as means (±SEM) colony forming unit (CFU) /ml of semen and analyzed by applying GLM (General Linear Model) for factorial experiments using SPSS computer software package (Version 16.0.0.247 ©2007). DMRT was done to make specific treatment comparisons for values that were found significant by ANOVA according to procedure outlined by Steel and Torrie (1980). Pearson's correlation between seasons was calculated using bivariate ($r_{12)}$ analysis.

RESULTS AND DISCUSSION

Comparative variations of bacterial load in semen from different breeds of cattle due to seasonal influences are shown in Table 1 and 2. Significantly (P<0.05) lower bacterial load in bovine semen was observed during summer and winter season as compared to rainy season, which is supported by findings of Azawi and Ismaeel (2012) who also reported lower bacterial count in ram semen during late summer. As there is negative correlation between bacterial count in semen and sperm motility (Shukla, 2005), poor sperm motility may be correlated with increased bacterial count. So, present report is also supported by Sarder (2007) who observed increased sperm motility during summer season in cow bull semen. Reason behind lower bacterial count during summer season needs to be explored. Seasonal effects are caused by several factors such as cleanliness, ambient temperature, relative humidity and photoperiod and it was reported that sunlight of summer significantly causes bacterial inhibition, however winter light reduces bacterial growth markedly when exposed for long time (El-Tayeb *et al.*, 2007). Sun light (red light) is associated with stimulation of reactive oxygen species and thus lethal to catalase negative bacteria. In another aspect, higher bacterial contamination was recorded in semen during rainy season as it is more difficult to keep bull's bedding and alleys clean, with consequent increasing amount of dirt on legs, flanks and preputial orifice during rainy environment. Effect of temperature and relative humidity also played important role in growth of microorganisms. The ambient temperatures of 35-40°C with a relative humidity of 35 to 45% reduced sperm quality significantly (Skinner and Louw, 1966) as bacterial population in semen grow best at temperature of 20-40°C. During course of present study, poor availability of sunlight at the time of semen collection during rainy season; higher temperature of about 40-48°C during summer and lower temperature (15-30°C) during winter season, could account for increased bacterial growth during rainy season while bacterial inhibition during summer and winter season, respectively.

Table 1: Seasonal variation of bacterial Load in fresh semen of indigenous, exotic and crossbred bull

	Bacterial load (mean±SEM) CFU/ml of semen		
Breed category of bulls	**Summer Season**	**Rainy Season**	**Winter Season**
Indigenous	19359±1875$^{a***(A\dagger)}$	31795±4072$^{(B\dagger)}$	24564±3764$^{(AB)}$
(N=39)	(n=117)	(n=117)	(n=117)
Exotic	37143±3595^{b***}	50000±19272	22857±15386
(N=7)	(n=28)	(n=28)	(n=28)
Crossbred	32000±5333^{b**}	29000±5859	19000±7371
(N=10)	(n=40)	(n=40)	(n=40)
Total	**23839±1890$^{(A\dagger)}$**	**33571±3842$^{(B\dagger)}$**	**23357±3422$^{(A\dagger)}$**

Note: *Values with different superscript (a,b) with in a column differ significantly at 0.01** and 0.001*** level.*

Values with different superscript (A,B) with in a row differ significantly at 0.05† level.

'N' values denotes number of bulls and 'n' values represent number of ejaculates tested. SEM- Standard error of mean

Table 2: Seasonal effect on bacterial load in fresh semen from different breeds of cattle

	Bacterial load (mean±SEM) CFU/ml of semen		
Breed of bulls	**Summer Season**	**Rainy Season**	**Winter Season**
Sahiwal	21428±2537^{a**}	35714±7964	30000±6874
(n=14)	(n=42)	(n=42)	(n=42)
Gir	23750±3750$^{ab*(AB\dagger)}$	37500±7500$^{(B\dagger)}$	21000±3525$^{(A\dagger)}$
(n=8)	(n=24)	(n=24)	(n=24)
Tharparkar	16667±5773^{a***}	21111±8571^{a*}	17777±5720
(n=9)	(n=27)	(n=27)	(n=27)
Red sindhi	14375±2577^{a***}	31250±7425	26250±12238
(n=8)	(n=24)	(n=24)	(n=24)
Jersey	37142±3595^{b***}	50000±19272^{b*}	22857±15386
(n=7)	(n=28)	(n=28)	(n=28)
Jersey Cross	20000±10000^{a*}	23333±3333	10000±5773
(n=3)	(n=12)	(n=12)	(n=12)
HF cross	37142±5654^{b***}	31428±8289	22857±10169
(n=7)	(n=28)	(n=28)	(n=28)
Total	**23839±1890$^{(A\dagger)}$**	**33571±3842$^{(B\dagger)}$**	**23357±3422$^{(A\dagger)}$**

Note: *Values with different superscript (a,b) with in a column differ significantly at 0.05*, 0.01** and 0.005*** level.*

Values with different superscript (A,B) with in a row differ significantly at 0.05† level.

'N' values denotes number of bulls and 'n' values represent number of ejaculates tested. SEM- Standard error of mean

Bacterial load in semen of different breed category was also affected significantly (P<0.05) by seasonal change (Table 1). Bacterial load in indigenous breeds are significantly (P<0.05) influenced by season and thus higher viable count was observed during rainy season as compared to summer and winter season, where as seasonal variations were not noticed in crossbred and exotic bulls. Within season and between breed categories; variations were although significant (P<0.001) during summer season; no significant (P>0.05) finding was recorded during rainy and winter season. During summer season, significantly (P<0.001) higher bacterial load was reported in semen of exotic bull followed by crossbred and indigenous cattle which is well supported by the findings of Fiaz *et al.* (2009) who observed deteriorated sperm motility in Jersey during summer season. On contrary, non-significant effect of season on bacterial load and sperm motility of semen was observed by Brito *et al.* (2002). Exotic bulls are more susceptible than indigenous and crossbred bulls to high ambient temperatures and crossbred cattle are well adapted to the local hot and humid climatic conditions in India (Anjum *et al.,* 2009). Durg, being a tropical and sub-humid region of Chhattisgarh has a long summer period that extends from March to June, with average ambient temperature ranging from 30 to 48°C. These environmental conditions are not suitable for exotic breeds from temperate regions and therefore can adversely affect reproductive efficiency of HF and Jersey bulls and thus a continuous evaluation of their semen quality is required to achieve higher non return rates and also to keep the crossbreeding programme economically viable.

Among breeds, seasonal variation on bacterial load in semen was observed only in case of Gir bulls which showed significant difference (P<0.05) between rainy and winter season which is well supported by earlier findings (Sannat *et al.,* 2015a). Gir bulls are being predisposed to prolapse of preputial sheath thus exposed to environment, which might accounts for higher bacterial count in their semen (Gaur *et al.,* 2003).

Table 3: Pearson's correlations between seasons of bacterial load in semen of different breeds of cattle

Season	Name of Breed	Fresh semen			Frozen semen		
		Summer	Rainy	Winter	Summer	Rainy	Winter
Summer	Sahiwal	1	0.105	0.063	1	0.18	0.506
	Gir	1	-0.206	-0.418	1	-0.169	0.118
	Tharparkar	1	**0.683***	0.098	1	0.508	0.204
	Red sindhi	1	-0.32	0.556	1	0.602	-0.324
	Jersey	1	**0.893****	0.455	1	0.627	0.367
	Jersey Cross	1	**-1****	-0.866	1	_	0.866
	HF cross	1	-0.697	-0.059	1	-0.53	0.482
Rainy	Sahiwal	0.105	1	0.25	0.18	1	0.122
	Gir	-0.206	1	0.257	-0.169	1	0.621
	Tharparkar	**0.683***	1	0.176	0.508	1	0.57
	Red sindhi	-0.32	1	0.361	0.602	1	-0.333
	Jersey	**0.893****	1	0.385	0.627	1	0.482
	Jersey Cross	-1**	1	0.866	_	1	_
	HF cross	-0.697	1	-0.036	-0.53	1	0.161
Winter	Sahiwal	0.063	0.25	1	0.506	0.122	1
	Gir	-0.418	0.257	1	0.118	0.621	1
	Tharparkar	0.098	0.176	1	0.204	0.57	1
	Red sindhi	0.556	0.361	1	-0.324	-0.333	1
	Jersey	0.455	0.385	1	0.367	0.482	1
	Jersey Cross	-0.866	0.866	1	0.866	_	1
	HF cross	-0.059	-0.036	1	0.482	0.161	1

Note: **Pearson correlation is significant at the 0.05 level (2-tailed)*

***Pearson correlation is significant at the 0.01 level (2-tailed)*

In another part, within season and between breeds variations were also recorded (Table 2). During summer season, significantly (P<0.005) higher bacterial count was observed in semen of Jersey and HF cross followed by Gir, Sahiwal, Jersey cross, Tharparkar and significantly (P<0.005) lower in Red Sindhi. During rainy season, comparatively higher bacterial load was noted in semen of all breeds with highest count in Jersey bull followed by Gir, Sahiwal, HF cross, Red Sindhi, Jersey cross and Tharparkar. Bacterial load in semen of Jersey differed significantly (P<0.05) with Tharparkar bull during rainy season. Increased bacterial count in semen of Jersey during present study was due to increased count in two individual bulls. There was non significant (P>0.05) variation of bacterial count in semen between different breeds during winter season.

Bulls of Red Sindhi and Tharparkar showed significantly decreased bacterial contamination as compared to bulls of other breeds, which might be attributed to their well adaptability in Chhattisgarh region and their sturdy and heat resistant characteristics.

Correlation of bacterial load between seasons is shown in Table 3. Bacterial load in Jersey and Tharparkar bull semen during summer season was significantly (P<0.01 for Jersey and P<0.05 for Tharparkar) and positively correlated with the same in rainy season, whereas significant (P<0.01) negative correlation was reported between the summer and rainy season in case of Jersey cross. Bacterial load in

semen of all bulls except Tharparkar and Jersey showed non-significant negative correlation between summer and rainy season, which indicated uniformity of bacterial load in semen during summer as well rainy season by Jersey and Tharparkar bulls. Likewise, non significant negative correlation was found between summer and winter seasons in Gir, Jersey cross and HF cross; between rainy and winter seasons in all bulls except HF cross. So, non significant but positive correlation of bacterial load was observed between summer and winter; and between rainy and winter season.

It can be concluded from present study that changing season in Durg district of Chhattisgarh (INDIA) had significant effect on bacterial load in semen of different breeds of cattle as significantly ($P<0.05$) lower bacterial load was reported in bovine semen during summer and winter as compared to rainy season.

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