SEMEN CHARACTERISTICS OF CROSSBRED (FRIESIAN X SAHIWAL) BULLS AT LIVESTOCK RESEARCH STATION, NATIONAL AGRICULTURAL RESEARCH CENTRE, ISLAMABAD

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ABSTRACT

The aim of the present study was to investigate the semen characteristics (volume, motility, concentration and morphology) of crossbred (Friesian x Sahiwal; F x S) bulls maintained at Livestock Research Station of National Agricultural Research Centre, Islamabad. Moreover, sperm head, mid-piece and tail dimensions in crossbred (F x S) and pure-bred (Friesian and Sahiwal) bulls were also studied. Semen was routinely collected twice a week during the months of June 1997 to May 1998 from seven crossbred (F x S; No. 36, 37, 38, 39, 40, 41 and 42) bulls by means of prewarmed (42 ^{0}C) artificial vagina. Crossbred bulls No. 36 and 37 had the highest volume of semen (4.8 ml), whereas, bull No. 40 yielded the lowest volume of semen (3.9 ml). Semen volume was high during August, September, October, November and December which averaged 4.96 ml and low in the remaining part of the year (4.06 ml). Mean semen volume was higher ($P<0.05$) in the first ejaculate than in the second ejaculate in all the crossbred bulls. Crossbred bull No. 36 showed highest ($P<0.05$) sperm motility (61%), whereas bull No. 40 and 42 expressed the lowest sperm motility (34 %). Mean sperm motility was high from October to December which averaged 55% and low in rest of the year (44 %). Mean sperm motility was higher ($P<0.05$) in the first ejaculate than in the second ejaculate of all the crossbred bulls. Crossbred bulls bearing No. 36, 37 and 39 had the highest concentration of sperm in ejaculate (0.81 x 10^{9}/ml), while bulls having tag No. 38, 40, 41 and 42 yielded the least sperm concentration (0.63 x 10^{9}/ml). Sperm concentration was high during most of the months (0.77 x 10^{9}/ml), while it was low in April, May and August (0.52 x 10^{9}/ml). Mean total sperm abnormalities were higher ($P<0.05$) in bull No. 40 and 42 (39.15%) as compared with other bulls (25.46%). Total sperm abnormalities were high ($P<0.05$) during June and July (60%) than other months (December, January, February, March, April and May) which averaged 19.6%. Sperm head, mid-piece and tail length was shorter ($P<0.05$) in crossbred (F x S) than pure-bred (Friesian and Sahiwal) bulls. The results of present study demonstrate that changes in semen characteristics of crossbred (F x S) bulls were observed to be occasioned by bull, month of semen collection or ejaculate number. There was a relative difference in sperm head, mid-piece and tail length of crossbred (F x S) and pure-bred (Friesian and Sahiwal) bulls spermatozoa. Therefore, it can be concluded that genetics and seasons (months) clearly contribute to semen characteristics of crossbred (F x S) bulls.

Keywords: Crossbred bull, semen volume, sperm motility, concentration, morphology.

INTRODUCTION

Crossbreeding of local cattle breeds (*Bos indicus*) with exotic breeds (*Bos taurus*) in Pakistan was initiated during early 1970's to combine the superior dairy potential of *Bos taurus* with better resistance of *Bos indicus* to heat stress and local diseases in the offsprings (Ishaq *et al.*, 1981; Shah *et al.*, 1982). Investigations revealed that productive and reproductive traits were best only in crossbred cows that have 50% exotic inheritance level as compared with cows having other levels of inheritance (Chaudhry *et al.*, 1990). To exploit and maintain half of the heterosis effect and half of the additive difference between the indigenous *Bos indicus* and exotic *Bos taurus* breeds, availability of frozen or fresh semen from the same halfbred bulls is required (Cunningham and Syrstad, 1987; Usmani *et al.*, 1993). Quality of semen plays a vital role in success of a breeding plan for improving dairy cattle through Artificial Insemination (A.I).

It is well established that characteristics of bull semen vary widely, not only between bulls, but also between ejaculates within bulls and from time to time or season to season (Nadaraja, 1967). Semen volume, concentration of spermatozoa, proportion of dead and abnormal spermatozoa, and motility of spermatozoa are

recognized as important indices of semen quality (Saacke, 1984; Bajwa *et al.*, 1986). Semen producing ability and quality of crossbred bulls are essential to ensure the supply of superior quality germplasm for maintaining the production performance of future crossbred progeny in the country (Usmani *et al.*, 1993). The objective of this study was to record observations on semen quality in term of volume, motility, concentration and morphology of crossbred (Friesian x Sahiwal; F x S) bulls used at Livestock Research Station for semen cryopreservation technology at different months of the year. Moreover, sperm head, mid-piece and tail dimensions in crossbred (F x S) and pure-bred Friesian and Sahiwal bulls were also determined.

MATERIALS AND METHODS

Source and collection of semen

Seven crossbred (Friesien x Sahiwal; No. 36, 37, 38, 39, 40, 41 and 42; age averaged 4 years) bulls maintained under uniform conditions of feeding and management at Livestock Research Station, National Agricultural Research Centre, Islamabad ($33^0.3'$ N latitude and $73^0.0'$ E longitude), were used in this study. Semen was routinely collected twice a week during the months of June 1997 to May 1998. On the day of semen collection, each bull was sexually stimulated by permitting 10 minutes restraint in semen collection yard and one false mount over a male teaser. After sexual stimulation, ejaculates were collected from each bull by means of prewarmed (42 ^{0}C) artificial vagina (AV) and brought to semen evaluation room within one minute of collection.

Evaluation of semen

Each semen ejaculate was examined for it's volume, sperm motility, concentration and morphology. Volume of the semen was recorded directly from graduated collection tube fitted with AV. For sperm motility, a drop of semen was placed on the pre-warmed (37 ^{0}C) glass slide, covered with micro cover glass and viewed under phase contrast microscope (Laborlux-D, Leitz, Germany) using X 400 magnification. Visual motility was examined by observing 4 to 6 fields per slide. Sperm motility was expressed in percentage.

Sperm concentration in semen ejaculate was determined by using a digital-photometer (Dr. Lange, LP300 SDM, Germany) at 546 nm wave length. Semen samples (25 µl) were diluted in 4975 µl normal saline in a 10 ml round bottom test tube. After mixing well by inverting the tube twenty times, the mixture was transferred to rectangular plastic cuvettes and placed in the cuvette holder of the photometer. The bull number, volume and motility of semen collected was entered to the instrument to get the result of sperm concentration in billion/ml of ejaculate.

Morphology of Spermatozoa

On first collection of each month, a drop of semen was added to small cuvettes containing one ml formal saline solution. A drop of solution was placed on clean glass slide, covered with a micro cover glass and examined under the phase contrast microscope (Laborlux-D, Leitz, Germany) at X 1000 magnification. One hundred spermatozoa of each sample were studied for head, mid-piece and tail abnormalities.

Biometry of Spermatozoa

Seven crossbred (F x S; No. 36, 37, 38, 39, 40, 41 and 42), one Friesian (pure-bred; No. 1) and two Sahiwal (pure-bred; No. 2 and 4) bulls were used for biometry of spermatozoa. A drop of semen sample was placed on a clean glass slide and gently mixed with Nigrosin Eosin stain (Eosin 1 gm, Nigrosin 2 gm, Sodium Citrate 3.5 gm and distilled water upto 100 ml). Smear was prepared, air dried and 15 straight spermatozoa per bull per replicate were examined under the phase contrast microscope (Olympus BH-2, Japan; X 1000 magnification). Head, mid-piece and tail dimensions were measured by using a calibrated ocular micrometer at X 1000 magnification.

Statistical analyses

Data on semen volume, sperm motility, concentration and morphology were subjected to analysis of variance using two factors (bull and month/ejaculate) completely randomized design. Tukey's test paired comparison was used to establish statistical differences between means, where necessary. Data on size of sperm head, mid-piece and tail were subjected to analysis of variance using single factor (breed) completely randomized design. All analyses were conducted using statistical software SYSTAT (SPSS Inc., 1996, Chicago, IL).

RESULTS AND DISCUSSION

Semen productivity and quality of crossbred (F x S) bulls during different months of the year are presented in Tables 1 to 5. In the current study changes in semen characteristics of crossbred (F x S) bulls were observed to be occasioned by bull, month of semen collection or number of the ejaculate.

Bull No. 36 and 37 had the highest volume of semen (4.8 ml), whereas, bull No. 40 yielded the least volume of semen (3.9 ml) as compared with other bulls (Table 1). Month wise mean values of semen volume in crossbred bulls are presented in Table 2. Semen volume was high ($P<0.05$) during August, September, October, November and December which averaged 4.96 ml and low ($P<0.05$) in the remaining part of the year (4.06 ml). Mean semen volume was higher ($P<0.05$) in the first ejaculate (4.7 ml) than in the second ejaculate (4.2 ml) in all the crossbred bulls (Table 3).

In this study volume of ejaculate in crossbred bulls was significantly affected by bull, month and number of the ejaculate. Volume of semen has been reported to be affected by season (Wildevs and Entwistle, 1984; Usmani *et al.*, 1993) and frequency of semen collection (Sattar and Mirza, 2002) in different crossbred and pure-bred cow bulls. However, Raja and Rao (1983)

Table 1: Mean values (± SE) of semen volume, sperm motility and concentration in crossbred (Friesian x Sahiwal) bulls

Bull No.	Volume (ml)	Motility (%)	Concentration (10^9/ml)
F x S 36	4.8 ± 1.7^{a}	61.0 ± 18.3^{a}	0.933 ± 0.67^{a}
F x S 37	4.8 ± 2.1^{a}	52.2 ± 20.01^{bc}	0.759 ± 0.55^{ac}
F x S 38	4.01 ± 1.2^{bc}	46.0 ± 15.7^{b}	0.664 ± 0.425^{bc}
F x S 39	4.6 ± 1.7^{acd}	49.0 ± 19.2^{b}	0.756 ± 0.549^{ac}
F x S 40	3.9 ± 1.9^{bc}	35.0 ± 19.73^{bd}	0.632 ± 0.501^{bc}
F x S 41	$4.0 \pm 1.7b^{df}$	51.8 ± 20.0^{b}	0.689 ± 0.513^{bc}
F x S 42	4.61 ± 2.5^{acef}	33.1 ± 22.0^{bd}	0.568 ± 0.459^{bc}

Values with different superscripts in a column differ significantly ($P<0.05$).

Table 2: Mean values (± SE) of semen volume, sperm motility and concentration in crossbred (Friesian x Sahiwal) bulls (n=7) during different months

Month	Volume (ml)	Motility (%)	Concentration (10^9/ml)
January	3.8 ± 1.2^{a}	37.5 ± 22.0^{a}	0.892 ± 0.531^{a}
February	4.18 ± 1.33^{ac}	42.1 ± 23.6^{ac}	0.88 ± 0.742^{a}
March	3.8 ± 2.2^{a}	43.6 ± 24.2^{ad}	0.712 ± 0.618^{ac}
April	3.8 ± 2.2^{a}	44.9 ± 27.2^{ae}	0.58 ± 0.410^{ad}
May	3.7 ± 2.7^{a}	41.4 ± 27.0^{af}	0.546 ± 0.494^{bcde}
June	4.5 ± 1.5^{ad}	47.2 ± 7.5^{ag}	0.782 ± 0.521^{aef}
July	4.4 ± 1.5^{ae}	45.4 ± 15.6^{ah}	0.606 ± 0.501^{aeh}
August	4.7 ± 1.6^{ae}	49.0 ± 18.3^{bcdefgh}	$0.446 \pm 0.269^{bedeghi}$
September	5.1 ± 1.5^{bcde}	48.2 ± 14.2^{ah}	0.668 ± 0.315^{aehjk}
October	4.6 ± 1.7^{ae}	55.7 ± 14.8^{bgh}	0.816 ± 0.453^{aehjk}
November	5.2 ± 2.1^{bcde}	54.0 ± 20.9^{bdegh}	0.792 ± 0.519^{aehk}
December	5.2 ± 1.69^{bcde}	55.2 ± 17.2^{bgh}	0.840 ± 0.496^{ahk}

Values with different superscripts in a column differ significantly ($P<0.05$)

Table 3: Effect of ejaculate number on semen volume, sperm concentration and motility of crossbred (Friesian x Sahiwal) bulls (n=7)

Ejaculate No.	Volume (ml)	Motility (%)	Concentration (10^9/ml)
1	4.7 ± 1.8^{a}	49.0 ± 19.4^{a}	0.750 ± 0.529
2	4.2 ± 2.0^{b}	45.1 ± 23.0^{b}	0.680 ± 0.545

Means ± SE with different superscripts in a column differ significantly ($P<0.05$).

found no seasonal effect on ejaculate volume of crossbred bulls maintained under tropical environments of India. In an another study it was noted that average ejaculate volume of crossbred bulls was comparatively more than of pure-bred bulls (Nazir *et al.*, 1987). Similar studies in *Bos indicus* bulls revealed that little seasonal variation was present in their semen producing ability (Tomar and Gupta, 1984; Usmani et *al.*, 1985). Findings of the present study as compared with other studies, therefore, indicate that crossbred (F x S) bulls are less resistant particularly to summer months in terms of semen producing ability than indigenous bulls.

Table 4: Mean morphological sperm abnormalities (± SE) in individual crossbred (Friesian x Sahiwal) bulls

Bull No.	Head (%)	Mid-piece (%)	Tail (%)	Total abnormalities (%)
F x S 36	5.91 ± 8.096^{a}	8.83 ± 9.495^{a}	6.833 ± 4.91^{a}	21.5 ± 19.1^{a}
F x S 37	10.00 ± 7.909^{ac}	9.18 ± 8.451^{a}	8.250 ± 7.263^{ac}	27.3 ± 19.0^{a}
F x S 38	7.83 ± 7.837^{ad}	11.75 ± 7.545^{ac}	9.417 ± 6.788^{ad}	28.3 ± 17.5^{a}
F x S 39	5.25 ± 5.57^{a}	12.58 ± 9.13^{ad}	7.08 ± 0.46^{ae}	24.8 ± 15.9^{a}
F x S 40	10.83 ± 5.37^{ae}	18.0 0± 12.8^{bcde}	11.6 ± 5.33^{bcdef}	58.7 ± 17.9^{b}
F x S 41	10.60 ± 8.47^{af}	10.66 ± 10.13^{ade}	7.167 ± 6.43^{afg}	25.4 ± 17.7^{a}
F x S 42	13.33 ± 6.282^{bcdef}	19.50 ± 9.349^{bcde}	8.91 ± 9.13^{afg}	39.6 ± 16.8^{b}

Values with different superscripts in a column differ significantly (P<0.05).

Table 5: Mean morphological sperm abnormalities (± SE) in semen of crossbred (Friesian x Sahiwal) bulls (n=7) in different months

Month	Head (%)	Mid-piece (%)	Tail (%)	Total abnormalities (%)
January	4.0 ± 3.9^{a}	6.29 ± 1.86^{a}	12.14 ± 9.22^{a}	21.50 ± 19.1^{a}
February	4.0 ± 2.7^{a}	3.14 ± 3.5^{a}	9.50 ± 5.96^{bae}	16.70 ± 7.6^{a}
March	9.1 ± 4.7^{ac}	5.7 ± 3.9^{ad}	4.50 ± 4.8^{a}	18.70 ± 8.2^{a}
April	8.0 ± 6.6^{a}	5.28 ± 2.9^{ae}	6.57 ± 3.7^{a}	19.80 ± 8.2^{a}
May	6.2 ± 2.4^{a}	8.00 ± 8.4^{af}	5.14 ± 2.47^{a}	19.40 ± 10.0^{a}
June	21.4 ± 6.8^{bd}	17.20 ± 4.5^{acdfe}	22.50 ± 4.6^{be}	61.20 ± 4.3^{bc}
July	17.0 ± 5.7	27.40 ± 5.2^{bef}	15.40 ± 1.71^{be}	60.00 ± 5.1^{bc}
August	9.57± 4.2^{aef}	15.80 ± 7.4^{acdefg}	13.40 ± 4.9^{be}	39.00 ± 7.8^{be}
September	2.14± 1.95^{afg}	23.10 ± 15.0^{befgh}	4.70 ± 4.3^{a}	29.70 ± 14.9^{bdf}
October	5.71± 3.1^{afi}	23.80 ± 13.3^{befghi}	6.10 ± 3.5^{a}	35.60 ± 11.5^{bdf}
November	11.42± 6.4^{aefhi}	11.86 ± 3.9^{aefghij}	7.86 ± 4.811^{a}	31.12 ± 7.0^{bdf}
December	10.71± 11.0^{aefhi}	7.42 ± 6.1^{aezgj}	3.57 ± 2.37^{a}	21.71 ± 6.96^{a}

Values with different superscripts in a column differ significantly (P<0.05).

Table 6: Mean sperm head, mid-piece and tail dimensions (± SE) of crossbred (Friesian x Sahiwal; n=7), pure-bred Friesian (n=1) and Sahiwal (n=2) bull spermatozoa

Breed	Head (μm)	Mid-piece (μm)	Tail (μm)
Crossbred (F x S)	8.433 ± 0.526^{a}	13.347 ± 0.555^{a}	43.131 ± 5.455^{a}
Friesian	9.148 ± 0.193^{c}	13.22 ± 0.568^{c}	45.354 ± 6.856^{b}
Sahiwal	8.851 ± 0.889^{b}	13.719 ± 0.507^{b}	45.061 ± 3.689^{b}

Values with different superscripts in a column differs significantly (P < 0.05).

Mean sperm motility of each crossbred bull is presented in Table 1. Bull No. 36 showed highest ($P<0.05$) sperm motility (61%), whereas bull No. 40 and 42 expressed the least sperm motility (34%). Month wise mean sperm motility values of crossbred bulls (F x S) are presented in Table 2. Mean sperm motility was high from October to December which averaged 55% and low in rest of the year (44%). Mean sperm motility was higher ($P<0.05$) in the first ejaculate (49%) than in the second ejaculate (45.1%) of all the crossbred bulls (Table 3). In the present study sperm motility in semen of crossbred (F x S) bulls was significantly affected by bull, month and the ejaculate number. These observations are consistent with early studies in different crossbred and pure-bred cow bulls (Nazir *et al.*, 1987; Usmani *et al.*, 1993; Sattar and Mirza, 2002), and buffalo bulls (Bhosrekar *et al.*, 1980; Anzar *et al.*, 1988; Zafar *et al.*, 1988). Regardless of season, the sperm motility of crossbred bull semen was found lower than that of the pure-bred bull semen (Bhatt and Chauhan, 1982; Raju and Rao, 1982; Usmani *et al.*, 1993).

Sperm concentration of individual crossbred bulls (F x S) is presented in Table 1. Crossbred bulls bearing No. 36, 37 and 39 had the highest concentration of sperm which averaged 0.81 x 10^9/ml of semen, while bulls having tag No. 38, 40, 41 and 42 yielded the least sperm concentration that averaged 0.63 x 10^9/ml of semen. Month wise mean values of sperm concentration in semen of crossbred bulls are presented in Table 2. Sperm concentration was high during most of the months which averaged 0.77 x 10^9/ml of semen, while it was low in April, May and August (0.52 x 10^9/ml of semen). The results of the present study demonstrated that the concentration of sperm in semen of crossbred (F x S) bulls was significantly affected by bull and month. Similar studies in crossbred (Wildevs and Entwistle, 1984) and pure-bred bulls (Kumi-Diaka *et al.*, 1981; Raja and Rao, 1983; Mathevon *et al.*, 1998) indicate that the sperm concentration is under the influence of season and cannot withstand a wide range of temperature during different months. These observations are contrary to the findings of Brito *et al.* (2002), who found that neither ambient temperature and humidity nor season affect sperm concentration in *Bos indicus* and *Bos taurus* bulls in tropics. This difference could be due to genetic, managemental or nutritional factors.

Occurrence of sperm head, mid-piece and tail abnormalities in crossbred bull (F x S) semen was significantly affected by bull (Table 4) and month of semen collection (Table 5). Mean total sperm abnormalities were higher ($P<0.05$) in bull No. 40 and 42 (39.15%) as compared with other bulls (25.46%). Month wise mean values of sperm abnormalities in crossbred bulls are presented in Table 5. Total sperm abnormalities were high ($P<0.05$) during June and July (60%) as compared with other months (December, January, February, March, April and May) which averaged 19.6%.

In the current study sperm head, mid-piece and tail abnormalities in crossbred (F x S) bull semen were observed to be significantly modulated with bull and month. Head, mid-piece and tail abnormalities were high during June and July. Same findings have been reported by Nazir *et al.* (1987) and Prasad *et al.* (1990) regarding frequency of various sperm abnormalities in crossbred bulls having different levels of exotic inheritance. Similarly Gilbert and Shook (1987) have reported that when ambient temperature increases bovine semen samples exhibit high frequency of abnormal spermatozoa. These findings are also congruent with those of Kumi-Diaka *et al.* (1981), who found that exotic bulls showed higher number of sperm abnormalities during the summer as compared with the *Bos indicus* bulls, suggesting that thermal stress in the tropics exhibits adverse effects on semen quality of non-indigenous breeds. Contrary to these findings, Chacon *et al.* (2002) found in Brahaman bulls *(Bos indicus)* reared under tropical environments that there was no relationship between climatic variables and sperm morphology, suggesting that temperature is not a major factor influencing spermiogramme, but nutrition may be a main factor affecting seasonal variations in semen quality parameters. In an another study neither ambient temperature and humidity nor month affected sperm morphology in *Bos indicus* and *Bos taurus* bulls maintained under tropical environmental conditions of Brazil. While Chacon (2001) found in zebu bulls managed under dry tropics of Costa Rica that frequency of sperm abnormalities was affected by some other factors like scrotal length and testicular consistency.

Sperm cell dimensions in crossbred (F x S) and pure-bred (Friesian and Sahiwal) bulls are presented in Table 6. Sperm head, mid-piece and tail length in crossbred (F x S) bulls spermatozoa was shorter ($P<0.05$) than that of pure-bred (Friesian and Sahiwal) bulls. In the present study, the biometry of sperm head, mid-piece and tail of crossbred (F x S) and pure-bred (Friesian and Sahiwal) bulls were also conducted. Significant variation in sperm size of cross and pure-bred bulls was noticed. Sperm head, mid-piece and tail size were shorter in crossbred bull spermatozoa than in pure-bred bulls. Mukherjee and Dott (1971) observed a highly significant correlation between fertility and

sperm head length in Hariana bulls. Short size of crossbred (F x S) bull spermatozoa may, therefore, be related to poor sperm profiles and ultimately with reduced fertility.

The results of the present study demonstrate that changes in semen characteristics (volume, motility, concentration and morphology) of crossbred (F x S) bulls were occasioned by bull, month of semen collection or ejaculate number. There was a relative difference in sperm head, mid-piece and tail length of crossbred (F x S) and pure-bred (Friesian and Sahiwal) bulls spermatozoa. Therefore, it can be concluded that genetics and seasons (months) clearly contribute to semen characteristics of crossbred (F x S) bulls.

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