

Effect of age and season on semen quality parameters in Sahiwal bulls

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Abstract The objective of the study was to determine the effect of season, period, age, bull, and ejaculate on semen quality in Sahiwal bulls. Semen production records from 1996 to 2006 of 5,483 ejaculates from 46 Sahiwal bulls maintained at Artificial Breeding Complex, NDRI, Karnal, India were analyzed using least square analysis of variance by LSML software package. The overall least squares means of ejaculate volume (VOL), total volume per day (VOLD), mass activity (MA), initial motility (IM), sperm concentration per ml (SPC), and sperm concentration per ejaculate (SPCE) were 3.79 ± 0.02 ml, 5.81 ± 0.06 ml, 2.32 ± 0.01 , $55.47 \pm 0.001\%$, $766.69 \pm 5.50 \times 10^6/\text{ml}$ and $3023.25 \pm 30.15 \times 10^6$, respectively. All semen traits (VOL, VOLD,

MA, IM and SPCE) were significantly ($P < 0.01$) affected by age groups, season and period, whereas season had significant effect on VOL at 5% level. During hot-humid season, highest value of VOL, VOLD, MA, IM, SPC, and SPCE were observed followed by summer and cold season. Highest value of VOL, VOLD, IM, and SPCE were observed during period-3 (2004–2006), whereas highest value of MA and SPC were observed during period-1 (1996–1999). However, lowest magnitude of MA, IM, SPC, and SPCE during period-2 (2000–2003) was observed. Ejaculate characteristics like VOL, VOLD, and SPCE increased with the increasing age of bull up to 5 years and then decreased. Significant ($P < 0.01$) bull to bull variation was found in VOL, VOLD, MA, IM, SPC, and SPCE traits. First ejaculate had significantly ($P < 0.01$) higher MA, IM, SPC, and SPCE. Hence, it could be concluded that during rainy season and period-1 and period-3 the quality of semen is quantitatively and qualitatively good. Better quality semen was obtained up to 5 years of age in Sahiwal bulls.

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Introduction

India possesses first rank in milk production, which is mostly contributed by about 80% of non-descript low milk-producing livestock. Among them, Sahiwal is the most excellent native milch breed of India and contribute considerable amount of milk to the country's milk production (National Cattle Breeding Policy 1999). Sahiwal is adaptable to a high degree of heat tolerance, resistant to certain diseases and ability to survive on low feed and fodder resources. Sahiwal semen can be used for grading up

in India to improve milk production (National Cattle Breeding Policy 1999). However, their population in India is gradually declining due to negligence in systematic breeding plans (Mandal et al. 2005). To mitigate the above problem, special attention is required to make available sufficient number of frozen doses from superior bulls. In a breeding farm, extent of variations of semen quality over a long period may depend on existing macro- and micro-climatic conditions, feeding and general management, besides the sexual activity of the animal influenced by surrounding environment during semen collection (Mandal et al. 2000). Semen quality is affected by breed (Mukhopadhyay et al. 2010); reproductive health status of bulls, technical skills, age (Mandal et al. 2010; Ahmad et al. 2011), and genetic constitution of bull (Koivisto et al. 2009) over a long period may not be consistent as it is not under a single managerial hand.

Information regarding the effect of season, period and age on semen characteristics is available in different breeds and species (Mandal et al. 2000; Mathur et al. 2002; Bhat et al. 2004; Ghosh 2004; Bhoite et al. 2005; Walzl et al. 2006; Chauhan 2007; Helbig et al. 2007; Koonjaenak et al. 2007; Mandal et al. 2010; Ahmad et al. 2011). Further, reports are also available regarding effect of bull and ejaculate on semen characteristics (Ravimurgan et al. 2003; Walzl et al. 2006). But scanty information is available on Sahiwal bulls as this breed is confined to the limited areas of India. Therefore, the present study was undertaken to investigate effect of season, period, age, bull and ejaculate influencing quality semen production of Sahiwal bulls.

Materials and methods

Semen quality data records were recorded from 1996 to 2006 of 46 Sahiwal bulls maintained at the semen production unit, Artificial Breeding Complex, National Dairy Research Institute, Karnal, Haryana, India. The farm is located at an altitude of 250 m above the mean sea level on 29.43°N latitude and 72.2°E longitude. Records had information on each ejaculate included date of collection, ejaculate number for the day, original volume of the ejaculate, concentration of sperm per milliliter, and percentage of motile sperm. Edition of data set included those bulls that refused to mount or who did not donate any semen and breeding bulls with less than 40 ejaculates or less than 6 months in production were also excluded. The bulls were maintained on identical conditions of feeding and management during the entire period. All the bulls were vaccinated against foot and mouth disease, hemorrhagic septicemia, and black quarter annually. The year was subdivided into three seasons: hot dry or summer (April to June); hot humid or rainy (July to October), and cold or winter (November to March)

and the factor year of collection was grouped in three (period-1=1996–1999, period-2=2000–2003, and period-3=2004–2006). Age of the bull has been proved to affect semen quality, such that all bulls were classified into four age groups (A1, <3 years; A2, ≥3 to <4 years; A3, ≥4 to <5 years; A4 ≥5 years).

Semen was collected in the morning once a week from the bulls using sterilized bovine artificial vagina (IMV model-005417; maintained between 42–45°C) over a male dummy bull. Immediately after collection, semen was evaluated for volume and mass activity as per standard procedure (Tomar 1984). After dilution in egg-yolk-tris-dilutor, the semen was kept in water-bath at 30°C for 10–15 min for evaluation of initial motility percentage using differential interference contrast phase contrast microscope (Nikon Eclipse E600, Tokyo, Japan) with Tokoiheat thermal stage. Sperm concentration was determined by using the hemocytometer (Neubauer's chamber) method (Tomar 1984). Sperm concentration per ejaculate was calculated by multiplying volume with sperm concentration.

Statistical analyses

The semen production records were subjected to statistical analysis using least square analysis of variance by LSML software package (Harvey 1975). To study the effect of non genetic factors on the semen quality parameters [ejaculate volume (VOL), total volume per day (VOLD), mass activity (MA), sperm concentration (SPC), sperm concentration per ejaculate (SPCE), and motility(IM)] the following model has been used. Prior to the analysis, proportionality data (motility) was transformed using the arcsine transformation [$\text{asin}(\sqrt{\text{percent}/100})$] (Snedecor and Cochran 1994) with adjustment to allow for zero values.

$$Y_{ijkl} = \mu + S_i + P_j + A_k + e_{ijkl}$$

Where,

- Y_{ijkl} l th record of seminal parameter collected in i th season and j th period on a bull belonging to k th age group
- S_i Effect of i th season of collection ($i = 1, 2, \text{ and } 3$)
- P_j Effect of j th period of collection ($j = 1, 2, \text{ and } 3$)
- A_k Effect of k th age group ($k = 1, 2, 3, \text{ and } 4$)
- e_{ijkl} Random error associated with Y_{ijkl} which is assumed to be normally and independently distributed with mean zero and constant variance

To study the effect of bull and ejaculate on the semen quality parameters, the data was adjusted against the

season, period, and age with respect to significance and then the adjusted data were subjected to the following least square model.

$$Y_{ijk} = \mu + B_i + E_j + e_{ijk}$$

Where,

- Y_{ijk} k th record of seminal parameter in i th bull and j th ejaculate
- B_i Effect of i th bull ($i = 1, 2, 3, \dots, 46$)
- E_j Effect of j th ejaculate ($j = 1, 2$)
- e_{ijk} Random error associated with Y_{ijk} which is assumed to be normally and independently distributed with mean zero and constant variance

Results

Seminal attributes of Sahiwal bulls

The overall least squares means of various seminal attributes like ejaculate VOL, VOLD, MA, IM, SPC, and SPCE were 3.79±0.02 ml, 5.81±0.06 ml, 2.32±0.01, 55.47±0.001%, 766.69±5.50×10⁶/ml, and 3023.25±30.15×10⁶, respectively (Table 1). The common colors of Sahiwal semen are watery, milky, lemon, and creamy with thin to thick consistency. Most of the semen samples were milky (47.00%) followed by creamy (29.65%), watery (13.76%) and lemon (9.59%).

Effect of season

The present study demonstrates that season had significant effect ($P < 0.05$) on VOL, VOLD and SPCE (VOL at 5% level, VOLD and SPCE at 1% level) of Sahiwal bulls (Table 2). Semen volume and total volume per day was highest during rainy (3.87±0.04 and 6.09±0.09 respectively) and the lowest on winter (3.72±0.04 and 5.63±0.08, respectively) season. Significant differences ($P < 0.05$) in volume between rainy and winter were observed. Total volume per day in summer (5.72±0.10) and winter (5.63±0.08) seasons significantly differ ($P < 0.01$) with rainy season (6.09±0.09). Mass activity, IM, and SPC were highest during rainy (2.35±0.02, 55.97±0.003, and 779.77±8.41) and lowest during winter (2.30±0.02, 54.88±0.002, and 754.58±7.97) seasons but non-significant ($P < 0.05$). During the rainy season average SPCE was significantly higher (3,141.01±46.05) as compared to winter (2,919.64±43.68) season, respectively.

Effect of period

Period had highly significant effect ($P < 0.01$) on all the seminal attributes of Sahiwal bulls under study (Table 2).

Table 1 Least squares means ± S.E. for effect of period on semen quality parameters of Sahiwal bulls

Parameters	S1	S2	S3	P1	P2	P3	A1	A2	A3	A4	Overall
Ejaculate volume (ml)	3.78±0.05 ^{bc} (N=1286)	3.87±0.04 ^a (N=1979)	3.72±0.04 ^{bc} (N=2218)	3.38±0.05 ^A (N=1301)	3.83±0.03 ^B (N=2567)	4.17±0.04 ^C (N=1615)	3.34±0.05 ^A (N=1037)	3.86±0.04 ^{BD} (N=2124)	4.14±0.05 ^C (N=1293)	3.83±0.05 ^D (N=1029)	3.79±0.02 (N=5483)
Ejaculate volume per day (ml)	5.72±0.10 ^A (N=841)	6.09±0.09 ^B (N=1255)	5.63±0.08 ^A (N=1457)	4.90±0.11 ^A (N=894)	6.04±0.07 ^B (N=1625)	6.49±0.10 ^C (N=1034)	5.09±0.12 ^A (N=666)	5.93±0.08 ^{BD} (N=1381)	6.31±0.10 ^C (N=850)	5.92±0.12 ^D (N=656)	5.81±0.06 (N=3553)
Mass activity (0–5 scale)	2.32±0.03 (N=1239)	2.35±0.02 (N=1923)	2.30±0.02 (N=2124)	2.39±0.03 ^A (N=1233)	2.21±0.02 ^B (N=2454)	2.36±0.02 ^A (N=1599)	2.30±0.03 ^A (N=996)	2.39±0.02 ^B (N=2040)	2.30±0.03 ^A (N=1243)	2.31±0.03 ^A (N=1007)	2.32±0.01 (N=5286)
Initial motility (%)	55.56±0.004 (N=1286)	55.97±0.003 (N=1979)	54.88±0.002 (N=2218)	56.11±0.004 ^A (N=1301)	52.96±0.002 ^B (N=2567)	57.34±0.003 ^A (N=1615)	53.99±0.005 ^A (N=1037)	56.16±0.002 ^B (N=2124)	54.88±0.004 ^{AB} (N=1293)	56.85±0.005 ^B (N=1029)	55.47±0.001 (N=5483)
Sperm concentration (million/ml)	765.71±10.30 (N=1286)	779.77±8.41 (N=1979)	754.58±7.97 (N=2218)	794.72±10.65 ^A (N=1301)	723.26±7.17 ^B (N=2567)	782.07±9.35 ^A (N=1615)	747.45±11.69 (N=1037)	784.47±7.88 (N=2124)	762.49±10.32 (N=1293)	772.34±11.58 (N=1029)	766.69±5.50 (N=5483)
Sperm concentration per ejaculate (million)	3009.11±56.41 ^{AB} (N=1286)	3141.01±46.05 ^A (N=1979)	2919.64±43.68 ^B (N=2218)	2861.15±58.35 ^A (N=1301)	2804.10±39.26 ^A (N=2567)	3404.49±51.24 ^{BD} (N=1615)	2617.31±64.03 ^A (N=1037)	3133.61±43.18 ^{BD} (N=2124)	3278.33±56.55 ^B (N=1293)	3063.75±63.46 ^{CD} (N=1029)	3023.25±30.15 (N=5483)

Least squares means bearing different superscripts within same row differ significantly (^{ab} $P < 0.05$, ^{AB} $P < 0.01$). S1 = hot dry (summer), S2 = hot humid (rainy), S3 = cold humid (winter), P1 = 1996 to 1999, P2 = 2000 to 2003, P3 = 2004 to 2006; A1 = <3 years, A2 = ≥3 to <4 years, A3 = ≥4 to <5 years, A4 = ≥5 years

Table 2 ANOVA showing effect of season, period and age on semen quality parameters for Sahiwal bulls

Parameters	Source of variation	Degree of freedom (<i>df</i>)	Mean sum of square (M.S.S.)
Ejaculate volume (ml)	Season	2	11.74 ^a
	Period	2	213.49 ^b
	Age		118.07 ^b
	Error	5,475	2.55
Ejaculate volume per day (ml)	Season	2	75.72 ^b
	Period	2	604.32 ^b
	Age		182.52 ^b
	Error	3,545	8.62
Mass activity (0–5 scale)	Season	2	1.21
	Period	2	17.37 ^b
	Age		3.23 ^b
	Error	5,278	0.82
Initial motility (%)	Season	2	214.14
	Period	2	3295.63 ^b
	Age		622.89 ^b
	Error	5,475	163.01
Sperm concentration (million/ml)	Season	2	331,237.34
	Period	2	2,704,214.2 ^b
	Age		333,038.59
	Error	5,475	128,791.29
Sperm concentration per ejaculate (million)	Season	2	25,674,376 ^b
	Period	2	186,401,440 ^b
	Age		86,981,424 ^b
	Error	5,475	3,864,808

^a Significant at 5%^b Significant at 1% level

The data in Table 1 shows that the highest value (4.17 ± 0.04) for semen volume was recorded during period-3 and lowest (3.38 ± 0.05) during period-1 and revealed significant ($P < 0.01$) differences in ejaculate volume and volume per day between all the periods. The average values for volume per day were 4.90 ± 0.11 ; 6.04 ± 0.07 , and 6.49 ± 0.10 , in periods-1, -2, and -3, respectively. The average value for mass activity was minimum during period-2 (2.21 ± 0.02) and maximum during period-1 (2.39 ± 0.03). During period-3, it had intermediate values (2.36 ± 0.02). Initial motility was highest during period-3 (57.34 ± 0.003) and lowest during period-2 (52.96 ± 0.002). The concentration of spermatozoa averaged 794.72 ± 10.65 , 723.26 ± 7.17 , and 782.07 ± 9.35 million per ml during periods-1, -2, and -3, respectively. Significant ($P < 0.01$) variation was noticed during period-1 and period-3 with period-2 for mass activity, initial motility, and SPC. The sperm concentration per ejaculate was maximum ($3,404.49 \pm 51.24$) during period-3 and minimum ($2,804.10 \pm 39.26$) during period-2,

whereas period-1 had intermediate values ($2,861.15 \pm 58.35$). Highly significant ($P < 0.01$) differences of periods-1 and -2 with period-3 were observed in the present investigation.

Effect of age

All semen traits were significantly ($P < 0.01$) affected by age, except sperm concentration (Table 2). Ejaculate volume significantly ($P < 0.01$) enhanced with the increasing age of bulls (3.34 ± 0.05 , A1; 3.86 ± 0.04 , A2; and 4.14 ± 0.05 , A3) but decreased again for older age group (3.83 ± 0.05 , A4) bulls. A similar trend was found in case of total volume per day (A1, 5.09 ± 0.12 ; A2, 5.93 ± 0.08 ; A3, 6.31 ± 0.10 ; and A4, 5.92 ± 0.12), regarding effect of age on the said semen quality parameters as it was observed in case of ejaculate volume. Mass activity of semen significantly ($P < 0.01$) increased as the bull matured but decreased again for older age group (A3 and A4). Mass activity was highest in A2 age group bulls and lowest in A1 and A3 age group bulls. Percentage of progressive motile sperm were 53.99 ± 0.005 , 56.16 ± 0.002 , 54.88 ± 0.004 , and 56.85 ± 0.005 for the four age groups, respectively. Highest and lowest sperm concentration were found for the bulls in A2 age (784.47 ± 7.88) group and A1 age group (747.45 ± 11.69), respectively. Sperm concentration per ejaculate was higher ($3,278.33 \pm 56.55$) in A3 age group bulls than the other age groups (A1, 2617.31 ± 64.03 ; A2, 3133.61 ± 43.18 ; and A4, 3063.75 ± 63.46), respectively.

Effect of bull and ejaculate

The least squares means of various seminal attributes of Sahiwal bulls and analysis of variance for bull and ejaculate differences has been presented in Tables 3 and 4, respectively. *F* statistics show significant ($P < 0.01$) effect of bull on all the semen quality parameters studied in Sahiwal bulls. The overall least squares means of various seminal attributes like VOL, MA, IM, SPC and SPCE were 3.79 ± 0.02 ml, 2.30 ± 0.01 , $55.28 \pm 0.001\%$, $764.80 \pm 5.41 \times 10^6$ /ml, and $3061.28 \pm 29.46 \times 10^6$, respectively. There were 3,408 to 3,537 first ejaculates and 1,878 to 1,946 second ejaculates in a once-a-week two-ejaculates schedule. The variation in the number of ejaculates may be due to adjustment of data against season, period, and age. After data adjustment, the zero values were automatically deleted from the data set during analysis. On analysis of data, semen volume, mass activity, motility, sperm concentration and sperm concentration per ejaculate showed a decreasing trend in successive ejaculates. Table 4 represents the analysis of variance for seminal characteristic and *F* statistics showing the significant ($P < 0.01$) differences between first and second ejaculates for all semen quality parameters except volume.

Table 3 Least squares means \pm S.E. for effect of ejaculate on semen quality parameters of Sahiwal bulls

Parameters	Ejaculate 1	Ejaculate 2	Overall
Ejaculate volume (ml)	3.81 \pm 0.03 (<i>N</i> =3,533)	3.77 \pm 0.03 (<i>N</i> =1,943)	3.79 \pm 0.02 (<i>N</i> =5,476)
Mass activity (0–5 scale)	2.36 \pm 0.02 (<i>N</i> =3,408)	2.24 \pm 0.02 (<i>N</i> =1,878)	2.30 \pm 0.01 (<i>N</i> =5,286)
Initial motility (%)	56.18 \pm 0.001 (<i>N</i> =3,537)	54.36 \pm 0.002 (<i>N</i> =1,946)	55.28 \pm 0.001 (<i>N</i> =5,483)
Sperm concentration (million/ml)	792.36 \pm 6.09 (<i>N</i> =3,537)	737.24 \pm 7.99 (<i>N</i> =1,946)	764.80 \pm 5.41 (<i>N</i> =5,483)
Sperm concentration per ejaculate (million)	3175.38 \pm 33.18 (<i>N</i> =3,488)	2947.17 \pm 43.45 (<i>N</i> =1,918)	3061.28 \pm 29.46 (<i>N</i> =5,406)

Discussion

Value of initial motility is similar with the findings of Mandal et al. (2005) (52.71 \pm 1.31%) but higher values was reported by Singh et al. (2000) in the same breed. However, Shanmugavel and Singh (2002) and Mandal et al. (2005) reported lower semen volume (3.34 \pm 0.13 and 3.15 \pm 0.13 ml), higher mass motility (2.63 \pm 0.08 and 2.66 \pm 0.07), and sperm concentration (1,227.5 \pm 44.18 and 1335.25 \pm 51.00 $\times 10^6$ /ml) in semen of Sahiwal bulls as compared to the present values. Haq et al. (2003) reported lower semen volume (3.64 \pm 0.09) and mass activity (1.36 \pm 0.04) in Sahiwal bulls. Haq et al. (2003) reported higher mass motility in Sahiwal bulls. The result of the present

study are not in agreement with the findings of Mathur et al. (2002) as they reported higher semen volume (4.06 \pm 0.05 ml), mass motility (2.94 \pm 0.02), sperm concentration (1342.27 \pm 19.04 $\times 10^6$ /ml), and lower motility (52.48 \pm 0.78%) in Sahiwal crossbred bulls. The variation in result may be due to short-term study period and less number of observations to other authors.

Our results indicated that in Sahiwal bulls, semen quality was best during rainy season, followed by summer, whereas during winter season it was poor, which is in contrast to the findings of Mandal et al. (2005). Koivisto et al. (2009) reported ejaculate volume, sperm concentration, gross-motility and progressive sperm motility were significantly influenced by season and genotype ($P < 0.05$). Mostari et al. (2005) found that season had significant ($P < 0.05$) effect on semen volume and initial sperm motility. Mandal et al. (2005) reported non-significant ($P < 0.05$) effect of season on MA and SPC. This may be due to less fodder availability during the first 2 months of winter. The results are similar during the early summer period due to feeding practices (silage fed to bulls), which are common over years. Seasonal effect on semen quality parameters is not only affected by ambient temperature but also availability of fodder in scarce period. In the current study, it seemed that both the extreme cold and extreme hot–dry conditions depressed the semen quality parameters. The possible reason for the poor semen production during cold season, in spite of higher thyroid activity, may be due to the displacement of testis from the scrotal pouch to the abdomen and due to severe cold ambient temperatures which leads to stress for the animals (Zafar et al. 1988). Information regarding the effect of seasons on semen characteristics has been reported to be of diverse in nature due to variability of climatic conditions, ambient temperature, humidity, feeding, housing and day length (Mathevon et al. 1998a, b) which can naturally lead to variations in findings of various authors.

Our results regarding significant effect of period on various semen characteristics are in consonance with

Table 4 ANOVA showing effect of bull and ejaculate on semen quality parameters for Sahiwal bulls

Parameters	Source of variation	Degrees of freedom (<i>df</i>)	Mean sum of square (M.S.S.)
Ejaculate volume (ml)	Bull	45	73.09 ^a
	Ejaculate	1	2.05
	Error	5429	1.95
Ejaculate volume per day (ml)	Bull	45	115.97 ^a
	Error	3491	3.43
Mass activity (0–5 scale)	Bull	45	15.81 ^a
	Ejaculate	1	19.61 ^a
	Error	5239	0.69
Initial motility (%)	Bull	45	4010.85 ^a
	Ejaculate	1	1363.29 ^a
	Error	5436	130.97
Sperm concentration (million/ml)	Bull	45	2,885,176.5 ^a
	Ejaculate	1	3,782,222.2 ^a
	Error	5436	105,497.42
Sperm concentration per ejaculate (million)	Bull	45	85,607,072 ^a
	Ejaculate	1	63,913,548 ^a
	Error	5359	3,073,412.5

^a Significant at 1% level

reports of some researchers (Kapoor 1979) who reported significant effect of period on volume. Similarly, Bhoite et al. (2005) and Chauhan (2007) reported that the influence of period of semen collection on semen volume and mass activity was significant. An overview of the results indicated that the performance of Sahiwal bulls in terms of quality semen production was better in periods-1 and -3, which could be due to uniform management practices in both the periods. In the first period, the bulls were raised and trained for semen donation in the main herd. Later on, bulls born were raised in the main herd, shifted at later age to the newly created Artificial Breeding Complex, and trained for semen production. For the third period, males born in the main herd were kept for 6 months with the female stock and shifted at younger age for training for semen production at Artificial Breeding Complex. During this period, better managerial conditions were provided and bulls were managed individually in protected bullpens, which have resulted in better semen quality due to better growth and subsequently better production. Therefore, it can be emphasized that bull management in the younger age also had significant effect in quality semen productivity.

The present findings regarding semen volume with age groups are in agreement with the findings of Nordin et al. (1990); Younis (1996) and Javed et al. (2000). These indicate that Sahiwal bulls produce a maximum volume of semen around 4 to 5 years of age and thereafter it begins to decline, probably due to onset of senile changes. In a report, Ahmad et al. (2003) emphasized that that semen collected from mature bulls (adult bulls) is of better quality than the semen of young and old bulls. Ahmad et al. (2005) also reported that adult bulls showed better libido than young and old bulls. Ahmad et al. (2011) reported that ejaculate volume and sperm concentration increased ($P < 0.05$) in mature bulls compared to younger ones whereas motility remained insignificant due to age. The increase of ejaculate volume with age of bull is in agreement with previous studies (Bhosrekar et al. 1992a, b; Murugan and Raman 2003, Ahmad et al. 2011). On the contrary, Amstalden et al. (1994) and Ghosh (2004) reported that age of bull had no significant effect on ejaculate volume and sperm motility at younger age. These findings regarding mass motility were comparable to the findings of Younis (1996) and Javed et al. (2000) of higher mass activity in adult than young and old bulls. On the contrary, Nordin et al. (1990) observed higher mass activity in adult bulls. However, Bhosrekar et al. (1992a, b) reported that there was significant increase in mass activity as the bulls grew beyond 36 and 30 months of age. But Ghosh (2004) reported that age of bull had no significant effect on mass activity during maturation stage.

The highest percentage of progressive motility was found in bulls older than 5 years of age. However, Bhosrekar et al.

(1992a, b) reported increased motility with age of bull. Whereas in other studies, Amstalden et al. (1994) and Ghosh (2004), age of bulls showed no effect on sperm concentration, similar with our findings. Increase in semen volume and total number of sperm per ejaculate with age were also reported in various studies (Garner et al. 1996; Mathevon et al. 1998a, b).

Volume and number of cells per ejaculate tended to increase with age of the bull up to 4 to 5 years of age. This observation could be explained that the main factor determining the total number of sperms produced is the size of the testes (Almquist 1978), which increases until at least 5 years after puberty (Amann and Almquist 1976). The higher mass activity in the age group of 3 to 4 years was probably due to higher sperm concentration and low sperm abnormalities, which is also stated by Dhami and Kodagali (1988). Similarly, Koonjaenak et al. (2007) reported that age had significant effect on total sperm number per ejaculate and initial sperm motility. It should be noted that the effect of age of bull is also influenced by managerial conditions. Artificial insemination (AI) centers are highly interested in producing a maximum number of straws of elite bulls while at the same time younger test bulls are of less interest. Additionally, less demanded bulls might not reach a higher age leaving a selected group of bulls in higher age classes. Time of sexual preparation was reported to have significant effects on ejaculate volume and number of doses per ejaculate (Kommissrud and Berg 1996). As the older bulls have an economic impact on the AI industry, their sexual preparation might be done more carefully than that of the younger testing bulls. Older bulls are already sexually mature; their semen production probably was more stabilized than the semen production of growing bulls. The effect of age of the bull is important because of physiological changes that occur as bulls grow to sexual maturity. However, it has been found that some young bulls raised in all-male groups may show temporary deficiencies in libido and or mating ability (Chenoweth 1981), which affect the semen production. Semen quality deterioration after certain ages may be due to onset of senile changes.

The superiority of first ejaculates with regards to higher volume, sperm concentrations, and total number of spermatozoa per ejaculate is in accordance with many other reports (Taylor et al. 1985; Walzl et al. 2006). Walzl et al. (2006) also reported higher progressive motility in second ejaculate contrary to our findings.

Conclusions

Sahiwal bulls produced best semen during rainy season followed by summer and winter, probably due to variation

in quality of feeding, ambient temperature, and humidity. Periods-1 and -3 were better in terms of quality semen production of Sahiwal bulls which reflects uniform management practices during the periods. The bulls of 4–5 years of age group were able to produce better quality semen which might be due to better testicular size and preferential managemental conditions. Bull to bull variation in semen production performance is also evident. Semen quality of first ejaculates was superior as compared to second ejaculate.

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