EFFECT OF SEASON ON SEMINAL CHARACTERISTICS OF
HOLSTEIN BULL UNDER SEMI-ARID ENVIRONMENT
I. BIOPHYSICAL CHARACTERISTICS

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Summary

Eight healthy Holstein bulls, 4-6 years old were used to study the effect of season of the year on the biophysical characteristics of semen. Semen was collected twice a week by AV (artificial vagina) over one-year period. The analyses revealed that all the basic seminal traits studied were differed significantly due to season, except the ejaculate volume and consistency and the percentage of swollen spermatozoa in a hypo-osmotic fructose-citrate solution. Ejaculates collected during hot summer season had significantly lower sperm motility, concentration and total counts, and higher percentage of dead spermatozoa than those collected during winter time. Warm spring had moderate semen quality. The temperature-humidity index was calculated and it was associated (p < 0.01) negatively with the ejaculate pH, sperm concentration and total counts, and positively with the % of dead sperms. Ejaculate volume, percentage of swollen spermatozoa, individual motilities did not correlate significantly with the change in temperature-humidity index values. The total live, motile spermatozoa per ejaculate during both the winter and spring seasons showed significant increase of about 37% and 32%, respectively over the summer season. Also, rectal temperatures of the bulls were elevated during the hot summer season, while the values of blood hemoglobin and packed-cell volume were decreased.

(Key Words: Season, Semen, Holstein Bulls, Semi-Arid)

Introduction

The effect of weather conditions on semen quality for different species has been studied by many authors. Contradictory results were often seen due to poorly defined environmental conditions, type of animal or breed. Under tropical conditions, the exotic breeds showed significantly seasonal fluctuations in semen quality with lower rates during hotter periods (Fields et al., 1979; Bhosrekar et al., 1980; Kumi-Diaka et al., 1981; Rekwot et al., 1987). In their report, Kumi-Diaka et al. (1981) concluded that semen quality in exotic (Bos taurus) was affected by season but not in indigenous (Bos indicus). But this was at variance with Rekwot et al. (1987) who reported seasonal variations in both exotic and indigenous breeds. In respect to certain semen characteristics, the controversy is holding. Working with Zebu and Friesian bulls and their crosses in Nigeria, Rekwot et al. (1987) revealed significant differences between seasons in respect of ejaculate volume, sperm concentration and total sperm counts per ejaculate, while Saxena and Tripathi (1984), working with Jersey bulls in India, were not able to discern any such seasonal effects. However, both authors reported significant influence of season on percentage live and abnormal spermatozoa and non-significant influence on sperm motility.

In Saudi Arabia (semi-arid climates), the importation of high performance dairy cattle, the exotic temperate evolved Holstein cows, have willingly increased throughout the last decade. These animals and their generations have faced, and still, the stressors of the new environment, considerably the unusual prolonged summer heat stress (over 35°C for up to 8 months). Both male and female were affected by that environment. Milk production is reduced (Salah et al., 1988) and conception rate is diminished (Salah and Mogawer, 1990). The role of the bull did not tested seriously in many dairy farms where natural service is dominated. To overcome some effects
of this extreme environment, the A I technique was applied in most of the dairy farms utilizing proven semen from abroad. Due to the expense of the imported semen and the slaughtering of many good young male males, the government established a newly A I center at the Kharj region in order to select the high genetic merit locally-born bulls and utilize them in A I program across the country. Their semen would be tested routinely before any use. As preliminary work at this center, selected bulls have to be subjected to semen evaluation and to visualize the changes occurred in the quality of semen throughout the different seasons of the year, especially during the summer. These were the ultimate goals of this study.

**Materials and Methods**

Eight healthy dairy Holstein bulls aged 4 to 6 years belonging to the Germ Plasm Bank and Artificial Insemination Center of Al-Kharj Agriculture Project, Al-Hassa Irrigation and Drainage Authority, Ministry of Agriculture and Water, Saudi Arabia were used in this study. The experiment was conducted at the above center during the period from June 1, 1989 to May 30, 1990. The May in 1989 was a pre-experimental period to accustom the animals to the manipulations. Bulls were maintained individually in a 10 x 12 m separate pen with a 4 x 4 m shelter at the center. They were fed freely on fresh alfalfa and hay supplemented with sufficient concentrate mixture (16% C.P.) to maintain body weight.

Semen samples were collected twice a week from each bull at early morning (05:00-06:00 hr) by the method of AV using a teaser male. Each bull was allowed to serve the AV one time per collection within 5 minutes after a false mount. Immediately after collection, the volume of the whole ejaculate from a given bull was measured to the nearest 0.1 ml. Colour and consistency were assessed by direct visual examination. The consistency was graded (4-0) as thick creamy, thin creamy, milky, cloudy and clear. The pH was measured using Whatman Indicator papers (ranges 5-9 with 0.5 unit intervals). Then the semen tube was placed in a water bath at 37°C. Motility of the spermatozoa was estimated within 10-15 min from the time of semen collection. Mass motility was carried out by placing a small drop of undiluted semen on a slide glass warmed on an electric stage heater (34°C), mounted with cover slip and then examined under the lower power of the light microscope. A score of 0-5 was given according to the intensity of the swirling bands (Moule, 1965). Progressive (individual) motility was examined in semen diluted with one or two drops of citrate buffer (pH 6.9), on a slide glass covered with slip, using high power magnification. The percentage of individual spermatozoa displaying progressive motility across the field was estimated and recorded (Herman and Maddren, 1972).

The concentration of spermatozoa per μl of semen was determined in a 1.200 diluted semen drawn into standard red cell dilution pipette by direct cell count on improved Neubauer haemocytometer. The dilution fluid consists of a weak eosin solution made up of 50 ml distilled water, 1 ml of 2% eosin and 1 ml of 3% NaCl solution.

The proportion of dead spermatozoa in each ejaculate was recorded by using a freshly prepared nigrosin-eosin vital stain (Dott and Foster, 1972).

The swelling of sperm tail in the presence of a hypotonic solution was tested, as a measure for the functional integrity of the sperm membrane, using the technique of Jeyendran et al. (1984). The percentage of spermatozoa that showed typical tail abnormality indicative of swelling was calculated (HOS%).

Observations of rectal temperature (RT) were made just before semen collection time. Blood samples were taken, from the external Jugular vein in 10 ml vacutainer tubes containing ethylene diamine-tetra-acetate acid (EDTA) as anticoagulant, once a week from each bull about an hour before obtaining one of the two weekly ejaculates. The haemoglobin (Hb, gm%) and packed cell volume (PCV, %) were measured. Also, minimum, maximum and average daily temperatures and humidities were obtained from a local weather bureau adjacent to the project for the purpose of seasonal comparisons. The period of study was categorized into three seasons according to the average mean monthly temperatures and relative humidities as follows: Summer (Hot-dry, June 1-October 31); Winter (Cold-humid, November 1-February 28); Spring (Warm-humid, March 1-May 31). The recorded dry (db) and wet bulb (wb) temperatures (°C) were used in calculating
the temperature-humidity index (THI), in evaluation of animal response to the environmental conditions, from the following equation:

\[ \text{THI} = 0.72 \left( C_{\text{aw}} + C_{\text{wb}} \right) + 40.6. \]

After adjustments for the effect of bull’s age, the obtained data were statistically analyzed for the effect of season using the Statistical Analysis System (SAS USER’S GUIDE, 1986). The GLM, and LSMEANS procedures were used. Also the procedure CORR was applied to assess the interrelationships among semen characteristics, the physiological and meteorological measurements.

Results

Table 1 shows the meteorological data recorded during different seasons of the one-year experimental period and the average corresponding changes in RT, Hb and PCV in the Holstein bulls. The average maximum air temperatures were 50.6, 26.8 and 36.5°C during the hot-dry (summer), the cold humid (winter) and the warm-humid (spring) seasons, respectively. The corresponding values of the minimum air temperatures were 23.2, 10.8 and 17.5°C. Average maximum relative humidity for the three seasons were 24, 68 and 47 percent respectively, while the corresponding minimum values were 10, 25 and 17%. The calculated Temperature-Humidity Index, THI, was 79.4 during summer, 65.3 during winter and 70.2 during spring.

Bulls in hot-dry season had significantly \((p < 0.01)\) lower Hb values than during the cold-humid and the warm-humid seasons. Bulls did not significantly differ in their Hb values between the cold and warm seasons. The PCV values were differed significantly \((p < 0.01)\) between the three seasons. It was the highest during the cold season, and the least during the hot season. Season of the year affected significantly the rectal temperature of the individual bulls. It was higher \((p < 0.01)\) during the months of summer and spring than during the winter months.

The ejaculate characteristics of the Holstein bulls in relation to season of the year are summarized in table 2. The analyses revealed that all the semen traits studied did differ significantly \((p < 0.01)\) due to season, except the mean ejaculate consistency and volume and the percentage of spermatozoa showed swollen tail in hyposmotic fructose citrate solution. The appearance of the ejaculates varied between the light milky and the creamy, with the majority being milky-white. The ejaculate volume averaged 5.9 ± 0.1 ml, with a minimum of 3 ml and a maximum of 12 ml. The value of semen pH averaged 6.5 ± 0.01 with one-tenth point decrease \((p < 0.01)\) from cold to warm season, and from warm to hot season.

The mass movement of the spermatozoa was vigorous and swirl formation was between rapid (very good) and moderate (good). The best

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Hot-Dry</th>
<th>Cold-Humid</th>
<th>Warm-Humid</th>
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<tbody>
<tr>
<td>Air temperature (°C)</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Maximum</td>
<td>50.6 ± 0.5a</td>
<td>26.8 ± 0.7b</td>
<td>36.5 ± 0.6c</td>
</tr>
<tr>
<td>Minimum</td>
<td>23.2 ± 0.4b</td>
<td>10.8 ± 0.6b</td>
<td>17.5 ± 0.4c</td>
</tr>
<tr>
<td>Average</td>
<td>33.8 ± 0.4b</td>
<td>18.9 ± 0.6b</td>
<td>25.3 ± 0.5c</td>
</tr>
<tr>
<td>Relative humidity (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum</td>
<td>24.4 ± 0.4a</td>
<td>68.3 ± 2.6b</td>
<td>47.4 ± 1.8c</td>
</tr>
<tr>
<td>Minimum</td>
<td>10.0 ± 0.4a</td>
<td>25.2 ± 1.5b</td>
<td>17.1 ± 0.7c</td>
</tr>
<tr>
<td>Average</td>
<td>17.2 ± 0.3a</td>
<td>46.3 ± 1.3b</td>
<td>32.3 ± 0.9e</td>
</tr>
<tr>
<td>Temperature-Humidity Index</td>
<td>79.4 ± 0.2a</td>
<td>60.3 ± 0.4b</td>
<td>71.2 ± 0.6e</td>
</tr>
<tr>
<td>Haemoglobin concentration</td>
<td>11.5 ± 0.1a</td>
<td>12.0 ± 0.1b</td>
<td>11.9 ± 0.1b</td>
</tr>
<tr>
<td>Packed cell volume (%)</td>
<td>32.4 ± 0.2a</td>
<td>34.0 ± 0.2b</td>
<td>33.2 ± 0.2c</td>
</tr>
<tr>
<td>Rectal temperature (°C)</td>
<td>38.4 ± 0.2a</td>
<td>38.1 ± 0.2b</td>
<td>38.4 ± 0.3a</td>
</tr>
</tbody>
</table>

* Different small letters indicate significant differences among means \((p < 0.01)\).
TABLE 2. LEAST SQUARES MEANS AND STANDARD ERRORS FOR VARIOUS BIOPHYSICAL SEMEN CHARACTERISTICS OF HOLSTEIN BULLS DURING DIFFERENT SEASONS AND SEASONS POOLED TOGETHER UNDER SEMI-ARID CONDITIONS

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Season</th>
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<tbody>
<tr>
<td></td>
<td>Hot-Dry</td>
<td>Cold-Humid</td>
<td>Warm-Humid</td>
<td>Pool</td>
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<tr>
<td><strong>Ejaculate:</strong></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Colour (Score 1-4)</td>
<td>1.91 ± 0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.20 ± 0.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.84 ± 0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.73 ± 0.05**</td>
</tr>
<tr>
<td>Consistency (Score 0-4)</td>
<td>2.42 ± 0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.47 ± 0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.46 ± 0.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.44 ± 0.05N.S</td>
</tr>
<tr>
<td>Volume (ml)</td>
<td>5.70 ± 0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.85 ± 0.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.87 ± 0.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.94 ± 0.11N.S</td>
</tr>
<tr>
<td>pH</td>
<td>6.44 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.59 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.54 ± 0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.48 ± 0.01**</td>
</tr>
<tr>
<td><strong>Spermatozoa:</strong></td>
<td></td>
<td></td>
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<tr>
<td>Mass motility (Score 0-5)</td>
<td>3.63 ± 0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.23 ± 0.13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.67 ± 0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.54 ± 0.07**</td>
</tr>
<tr>
<td>Individual motility (%)</td>
<td>76.49 ± 0.81&lt;sup&gt;a&lt;/sup&gt;</td>
<td>80.87 ± 1.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>78.02 ± 1.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>76.73 ± 0.55**</td>
</tr>
<tr>
<td>Dead (%)</td>
<td>18.04 ± 0.70&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.83 ± 0.87&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.52 ± 0.94&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.31 ± 0.47**</td>
</tr>
<tr>
<td>Concentration × 10&lt;sup&gt;9&lt;/sup&gt;/ml</td>
<td>1.35 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.62 ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.62 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.52 ± 0.03**</td>
</tr>
<tr>
<td>Total count × 10&lt;sup&gt;9&lt;/sup&gt;/ejaculate</td>
<td>7.63 ± 0.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.61 ± 0.37&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.45 ± 0.89&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.23 ± 0.21**</td>
</tr>
<tr>
<td>HOS&lt;sup&gt;2&lt;/sup&gt; (%)</td>
<td>58.93 ± 0.72&lt;sup&gt;a&lt;/sup&gt;</td>
<td>59.33 ± 1.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>58.63 ± 1.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>58.94 ± 0.46N.S</td>
</tr>
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</table>

** Significant at p < 0.01; N.S = Non-significant.
<sup>1</sup> Different small superscript letters indicated significant different means (p < 0.05).
<sup>2</sup> Spermatozoa swollen in the citrate-fructose hypo-osmotic solution.

movement was shown during the cold season (p < 0.05) compared to the warm and hot seasons, respectively. The individual sperm motion in average was between rapid rectilinear (very good motion) during the months of November and December, and moderate (fair motion) during the month of September. It was better during the cold season (81%) than during the warm (p < 0.05; 78%) and the hot (p < 0.01; 76%) seasons. Both mass and individual motilities did not differ significantly between the latter two seasons.

The average sperm concentration/ml of ejaculate was 1.62 ± 0.03 billion during both the cold and warm seasons. The dry season had lower (p < 0.01) sperm concentration (1.35 ± 0.03 billion). The highest mean concentration (1.81 ± 0.05 billion sperm) for individual bulls was recorded during the month of February and the lowest (1.08 ± 0.04 billion) was recorded during the month of September. The total number of spermatozoa per ejaculate was 9.6 ± 0.4, 9.5 ± 0.9 and 7.6 ± 0.3 billion for the cold, warm and hot seasons, respectively. The corresponding percentages for the dead spermatozoa were 10.8 ± 0.9, 12.5 ± 0.9 and 18.0 ± 0.7. The overall means for these two traits were 9.2 ± 0.2 billion and 15.3 ± 0.5%. The hypo-osmotic swelling test showed that about 59% of the spermatozoa in the ejaculate are expressing high fertilizability.

The association between the temperature-humidity index, THI, and pH of the ejaculate, sperm concentration and total count, was negative and significant (p < 0.01), and with dead and abnormal percentages of spermatozoa was positive and significant (p < 0.01), whereas it was non-significant with ejaculate volume, HOS percentage, mass and individual motilities values of the spermatozoa.

**Discussion**

Rectal temperature of the bulls varied significantly between seasons being the highest during the hot summer season and the lowest during the cold season mainly due to the elevated ambient temperature during summer time. The average maximum air temperature during the hot-dry season was higher by about 14°C and 24°C than that during the warm-humid and cold-humid seasons, respectively. Owing to the dry nature of the atmosphere in the central region of Saudi Arabia, humidity seems to have less effect on semen quality than temperature did. The combined effect of temperature and humidity was elevated by calculating the Temperature-Humidity Index (THI). The upper critical THI
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for high producing temperate-evolved Holstein cows was reported to be 72 (Johnson, 1987). It is logic to assume that bulls of the same breed might come close in THI average to that in cows. THI during the summer season exceeded that critical limit by more than 7 points and was slightly lower during the warm season, while it decreased during the cooler season to be lower than the upper critical one. This might be a reason for the seasonal variations obtained in both the physiological and the seminal traits in the present study.

The rise in RT with elevated air temperature agrees with other reports on dairy cattle (Lee et al., 1976; Holmes et al., 1980; Ludri, 1985; El-Nouty et al., 1990b). In dairy bulls, Quinlan and Riemerchmidt (1941) found that elevation of atmospheric temperatures from 15.2 to 37.8°C increased body temperature from 38.6 to 39.1°C. The decline in Hb and PCV observed in the present study due to the elevation in ambient temperature during summer was also observed in dairy cattle by various authors (Roussel et al., 1972; Tripathi et al., 1976; Shaffer et al., 1981; El-Nouty et al., 1986; El-Nouty et al., 1991a). This effect may be attributed to the haemodilution by drinking water transported in the circulation for evaporative cooling (Misra and Mukherjee, 1980; Shaffer et al., 1981) and/or to a reduction in cellular oxygen requirements in order to reduce metabolic heat load and, consequently, to compensate for elevated environmental heat load (Lee et al., 1976). Heat induces an increase in blood flow to the periphery, including the testes in order to meet their tissue requirements for semen production, causing depression in blood Hb and PCV. There was an equal relationship (r = 0.39, p < 0.01) between total sperm count per ejaculate and both Hb and PCV values. In the other hand, the high blood Hb and PCV observed in bulls during cooler seasons may be ascribed to their fast metabolic process necessary for sustained sperm production. Total live, motile spermatozoa output per ejaculate during the cold and warm seasons were higher compared with the summer season by about 32 and 37%, respectively. The variations among bulls in RT, Hb and PCV values may reflect different degrees of acclimatization and quality of semen.

Despite the significant variations in semen characteristics, semen quality of the Holstein bulls under the local prevailing condition, semi-arid environment, is within the limit that permit a good fertilizing rates as indicated by Hafez, 1987. The obtained semen characteristics stand very close to those reported in the temperate breeds under temperate condition (Zemjanis, 1970; Ball et al., 1974; Parkinson, 1987) as well as in the tropical condition (Bhatt and Chauhan, 1982; Bujabarauah et al., 1982; Saxena and Tripathi, 1984; Rekwot et al., 1987).

The average volume of the ejaculate obtained in the present study was within the range reported for dairy bulls by other authors. The non-significant influence of season of the year on the ejaculate volume is in agreement with the findings of many authors (Ahquist and Cunningham, 1967; Igboeli and Rakha, 1971; Rao and Rao, 1978; Fields et al., 1979; Saxena and Tripathi, 1984), and is in disagreement with the findings of others (Rutlie et al., 1975; Djimde and Wengen, 1986; Rekwot et al., 1987) who reported significant dependency of volume of bull ejaculate on season of the year. These variations may be due to the effect of frequency of semen collections and the handling of the bulls before collection. Also the breed should have its effect, since under the tropical condition of India, Bhosker et al. (1980) found seasonal (p < 0.05) differences in ejaculate volume of the Holstein-Friesian bulls but not in the Jersey bulls; may be as an effect of body size (Herman and Swanson, 1941).

Good quality semen tends to be slightly acidic (6.5 to 6.9). Bulls known to have semen of pH ranging between 6.5 and 8.0. In the present study, the average pH for the Holstein bull semen was at the lower limit of that range with lower value during the hot summer season. Rollinson (1962) reported pH value of 6.5, while Salisbury et al. (1978) reported mean value of 6.75. The significant seasonal variations in semen pH of the present study was observed also by other authors (Djimde and Wengen, 1986). Others (Igboeli and Rakha, 1971) were not able to discern any such seasonal effects on pH. The variation in the pH values might depend on the proportion of the constituent from the epididymis and that of accessory glands as the contents of the former are more acidic (Milovouev, 1962). The effect of elevated air temperature during summer time could be directed on the epididymis function
adding more acidic secretion to the seminal plasma, lowering the pH of the ejaculate. The relationship of pH and THI was negative \( r = -0.48 \) and significant \( (p < 0.01) \).

The significant effects of season on mass and individual motility of spermatozoa were observed by Rao and Rao (1975) and Rao and Rao (1978). However, Saxena and Tripathi (1984), Djinmede and Weniger (1986) and Rekwot et al. (1987) reported non-significant dependency of mass motility of the sperm on the seasonal influences. In general, the activity during the hotter months was less than during the cooler ones (Donaldson, 1963; Rao and Rao, 1975; Rao and Rao, 1978). Mass activity is the product of density and livability of spermatozoa (Bishop, 1962). The density is indicated by the total number of sperm (live and dead). In the present study, mass activity was found to be associated with the percentage of dead spermatozoa in all seasons. Therefore, the higher \( (p < 0.01) \) incidence of dead spermatozoa detected during the hot summer could be the reason for the observed reduction in mass activity during summer compared to winter. The THI had non-significant relationship with sperm motility but it was significantly related \( (p < 0.01) \) to the percentage of dead spermatozoa. The individual sperm motility followed the trend of the mass motility. The correlation coefficient between the two traits was positive and significant \( (p < 0.01) \). Its magnitudes for the hot, cold and warm seasons were 0.78, 0.72 and 0.84, respectively.

The percentage of the live spermatozoa in the present study was over 80\%, within the acceptable level needed for good fertility (Hancock, 1959; Saacke, 1970) even during the hot season; Good samples of semen showed a maximum average of 25 percent dead sperms (Arthur et al., 1982). In the present study, bulls that showed high incidence of live spermatozoa had semen of significantly \( (p < 0.01) \) good sperm motility \( (r = 0.44) \). The appreciable increase in the percentage of dead spermatozoa observed during the hot-dry season agrees with the previous reports (Rao and Rao, 1975; Rao and Rao, 1978; Saxena and Tripathi, 1984; Rekwot et al., 1987).

The present investigation revealed significant \( (p < 0.01) \) lower concentration of spermatozoa in semen collected during the hot season than in semen collected during both winter and spring seasons. The relationship between air temperature and sperm concentration was negative \( (p < 0.01) \); agreed with that reported by Nishiyama et al. (1968). Reduced sperm concentration during summer has been noted in Hereford bulls by Almquist and Cunningham (1967) and in Zebu bulls by Igboeli and Rakha (1971). In pure-bred bulls, under tropical conditions, Rao and Rao (1975) and Rao and Rao (1978) reported significant seasonal variations in sperm concentration, while Djinmede and Weniger (1986) and Rekwot et al. (1987) showed no seasonal dependency in sperm concentration in different exotic and indigenous breeds and their crosses. Under the same condition, Bhosrekar et al. (1980) reported significant seasonal variations in sperm concentration in Jersey bulls but not in Holstein-Friesian bulls. It is apparent that this discrepancy could be related either to breed differences or to the magnitude of the seasonal variations in which bulls are subjected.

Although there was no seasonal effect on ejaculate volume in bulls under the present study, the total sperms counted per ejaculate showed significant seasonal variations. This indicated that the effect of season on testicular function is greater than that on the accessory glands function. The compensating effect of volume and concentration on sperm output suggested by Everett et al. (1978) did not apply here. The higher load of heat stress on bulls during the summer period could cause discomfort to the bulls, reflected in lower rate of sperm production in addition to slow libido. Other authors (Annan, 1976; Everett et al., 1978; Bhosrekar et al., 1980; Rekwot et al., 1987) reported also seasonal effect on total sperm output of bulls with lower values during the hotter seasons. Parkinson (1985) found positive correlation between plasma testosterone and sperm output. Testosterone production was found to be depressed by prolonged (60 minutes) temperature of 39.5°C (Eik-Nes, 1965).

The bovine spermatozoa have the ability to swell in a hypo-osmotic medium (Drevius and Eriksson, 1966; Brederman and Foohe, 1969; Drevius, 1972). The present study confirmed this ability with an average swelling close to that reported for human spermatozoa using the same hypo-osmotic solution (Jeyendran et al., 1984). This ability is a sign of normal water transport across the membrane, i.e., a sign of membrane
integrity and normal functional activity. This is not only essential for the maintenance of sperm motility, but also for the induction of the acrosome reaction and possibly other events related to fertilization. Bulls were not differed significantly in their spermatozoal ability to swell in a hypo-osmotic solution. Also, the variations between seasons were not significant. This may be an indication of an intrinsic-dependent effect not related to seasonal changes in weather condition, but may be related to the breed of the bull. The relationship of THI with the HOS % of spermatozoa was not significant. HOS % was related significantly ($p < 0.01$) and positively ($r = 0.27$) to the sperm motility. The percentages of dead spermatozoa did not influence the HOS % neither in each season or in pooled seasons together. In man, the percentage of swollen spermatozoa in the same hypo-osmotic solution used in the present study showed higher and significant relationship with both percentages of the sperm motility and the dead spermatozoa (Jeyendran et al., 1984).

In the light of the present results, it can be concluded that season of the year affected significantly the quality of the Holstein bull semen, as well as the interrelationships between the various characteristics of the semen. Environmental conditions prevailing during winter and spring seasons were better suited for Holstein bulls to maintain normal spermatogenesis and hence good quality semen, which adversely affected during summer season due to the high ambient temperature. Good managerial manipulations for bulls might improve their semen quality during the hot summer season.

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