Seasonal variation in the characteristics of the Azarbaijani buffalo (*Bubalus bubalis*) semen

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Summary

To study the seasonal variation in the characteristics of the Azarbaijani buffalo semen, three 2-4-year-old bulls of Azarbaijani water buffaloes, kept in the Buffalo Breeding Center, in Urmia, northwest of Iran, were selected. Semen samples were collected once a week for a period of one year using artificial vagina and a buffalo cow as a dummy. Semen volume, colour, pH and motility and spermatozoa motility, viability, morphology and concentration were examined. During one calendar year, 129 semen samples were examined. The mean values were plotted and a curve was drawn for the annual variations in each parameter. The comparison of the mean values in each sampling and in different seasons and the correlation between these variations and ecological factors, such as temperature, rainfall and day length were also studied. Semen was characterized by a mean (±SEM) ejaculation volume of 4 ± 0.14 ml, semen colour density score of 3.75 ± 0.07, pH of 6.97 ± 0.03 and motility score of 2.89 ± 0.05. The sperm motility of 75.85% ± 1.59% and sperm viability of 73.2% ± 1.56% were observed; 11.55% ± 0.42% of spermatozoa had abnoraml morphology. The mean (±SEM) sperm concentration was 1239.9 ± 34.11×10^6 cells/ml. All of the studied parameters showed fluctuations throughout the year but these variations were statistically significant only in some occasions and were mostly correlated with length of the day. Semen of better quality was collected in summer and autumn.

Key words: Buffalo, Semen, Seasonal variations

Introduction

The population of the world buffalo is estimated to be more than 140 million. Despite its importance for the production of milk, meat and leather, buffaloes have not received sufficient attention regarding improvement in their breeding practices (Sansone *et al*., 2000). Buffaloes play an important role in the rural livestock production, particularly in Asia. Factors affecting their productivity are of paramount importance to agricultural economics in this region. Reproductive efficiency is the primary factor affecting productivity and is hampered by a number of factors including distinct seasonal reproductive patterns (Singh *et al*., 2000).

Buffaloes in Iran are bred mostly in two provinces of West Azarbaijan (northwest) and Khouzestan (southwest). Azarbaijani buffaloes are mostly accommodated around the Urmia Lake, which has a temperate rainy climate.

The quality of semen is of great importance in the fertility and hence productivity of many species including buffaloes. It has been reported (Sansone *et al*., 2000) that season has an effect on the libido and also on the quality of buffalo semen. These effects need to be studied in all areas where buffaloes are bred, due to the considerable environmental differences.

The present work was carried out to investigate the seasonal variations in the semen quality of buffalo bulls in West Azarbaijan, Iran.

Materials and Methods

Three Azarbaijani water buffalo bulls at the age of 2-4 years, in the Buffalo Breeding Center, in Urmia, northwest of Iran (37º 33´ N, 45º 4´ E) were selected and kept at the same ambient conditions and plane of nutrition. Semen samples were collected every 7 to 10 days during one year with a bovine artificial vagina and using a buffalo

cow as a dummy. Sampling was started at March 21 (day 1) and continued until the same date of the following year.

Semen evaluation was carried out within 15 min of collection. The following parameters were estimated twice in each sample and then the mean value was recorded.

Semen volume (ml) was determined by reading the volume of the ejaculate in a pre-warmed graduated collecting tube. Semen colour was assessed by visual examination and scored as 0 to 5, according to the appearance and the density of its colour (clear, watery samples scored 0 and thick, milky white ones scored 5). Semen pH was determined by using a Bachman portable digital pH meter (electrodes directly inserted into the sample).

Motility

Mass motility and progressive individually motility were assessed using the pre-warmed thick and thin smears, respectively. This was carried out according to the procedures recommended by Ax *et al*., (2000) by using a Zeiss (467085) light microscope and the magnifications of ×100 and ×250, respectively. At least 5 different microscope fields were examined for each recording.

Sperm viability

The percentage of live spermatozoa was determined in a smear of semen stained by eosin-aniline blue staining technique (1% yellowish eosin dye and 4% aniline blue solution, both in 3% sodium citrate solution). The smears were prepared according to the method recommended by Barth (1997) and examined under a light microscope with a magnification of ×400. At least 200 spermatozoa were counted for each examination.

Sperm morphology

This parameter was estimated in smears prepared for the spermatozoa viability determination. All the spermatozoa with abnormal morphology were classified according to the procedure recommended by Ax *et al*., (2000). At least 200 spermatozoa were examined and the proportion of abnormality was recorded.

Sperm concentration

This parameter was determined by preparing a 1:100 dilution of the sample in normal saline and counting the total number of sperm cells on a hemocytometer. The loading/scoring interval for this preparation was 5 min.

Statistical methods

The mean values (±SEM) were plotted to form a curve for seasonal variation in each parameter.

The correlation coefficients among the parameters and ecological factors such as minimum and maximum temperature, day length and rainfall rate were also determined. Pearson correlation coefficient (r) was used (SPSS, version 10). One-way analysis of variance with Duncan's test were used to compare the mean values between groups. The results of sperm viability examination, percentages of progressive sperms and the proportion of sperms with abnormal morphology were transformed by root square method as recommended by Armitage and Berry (1987) before analyses. General linear model was used to test the between subjects effects using SPSS, 10 software.

Results

During the period of the study, a total number of 129 semen samples were examined. Table 1 shows the climatological data for the year of experiment.

Semen volume

The overall mean (±SEM) of the semen volume was 4.0 ± 0.14 ml (Fig. 1). The mean values for the volume of the ejaculates were variable throughout the year but the variations were not statistically significant. This parameter had a significant ($P = 0.012$) correlation ($r = 0.38$) with the proportion of abnormal sperm morphology. Semen volume had no significant variations in different seasons (Table 2).

Semen colour density

The overall mean (±SEM) for colour

Table 1: Climatological data for the experimental year as reported by Urmia Meteorological Center

Season	Air temperature (°C)		Rainfall (mm)	Day length
	Maximum	Minimum	Total (Mean ± SEM)	(h)
Spring	20.05 ± 0.56* (7 to 32.4)°	7.56 ± 0.51 (-5 to 15)	195.3 (5.92 ± 1.12) (0.2 to 26)	13.96 ± 0.072 (12.4 to 14.91)
Summer	28.51 ± 0.294 (21 to 33.6)	14.55 ± 0.28 (8.3 to 19)	28.2 (5.64 ± 3.89) (0.2 to 22.8)	13.67 ± 0.28 (12.51 to 14.90)
Autumn	13.96 ± 0.787 (-3.8 to 25.6)	2.02 ± 0.06 (-12.8 to 14)	215.4 (7.43 ± 1.68) (0.4 to 40)	11.1 ± 0.06 (10.06 to 12.47)
Winter	7.33 ± 0.403 (-2.2 to 14.4)	-2.58 ± 0.065 (-9 to -3.8)	146.2 (7.31 ± 2.16) (0.2 to 40)	10.98 ± 0.065 (10.00 to 12.80)

*Mean ± Standard error of the mean; °Range

Table 2: Comparison of the results obtained in different seasons

Parameters	Spring (Mean ± SEM) n = 36	Summer (Mean ± SEM) n = 27	Autumn (Mean ± SEM) n = 30	Winter (Mean ± SEM) n = 36
Volume (ml)	3.68 ± 0.22	4.14 ± 0.18	3.59 ± 0.27	4.37 ± 0.247
Colour (scores)	3.43 ± 0.16	3.73 ± 0.10	3.94 ± 0.03*	3.83 ± 0.15*
pH	6.99 ± 0.03	6.63 ± 0.04*	6.96 ± 0.06*	7.1 ± 0.05*
Semen motility (scores)	3.02 ± 0.06	3.02 ± 0.09	2.78 ± 0.14*	2.75 ± 0.135*
Progressive sperm motility % (t%)	55.2 (7.43 ± 0.19)*	83.9 (9.16 ± 0.16)	82.26 (9.07 ± 0.23)	64.3 (8.02 ± 0.21)*
Viability % (t%)	75.1 (8.67 ± 0.2)	84.1 (9.17 ± 0.08)	70.3 (8.39 ± 0.23)*	60.4 (7.77 ± 0.14)*
Abnormal morphology % (t%)	10.4 (3.23 ± 0.01)	9.7 (3.12 ± 0.13)	8.12 (2.85 ± 0.15)	15.8 (3.98 ± 0.15)*
Concentration ($\times 10^6$ cells/ml)	1137.2 ± 54.37*	1280.8 ± 66.1	1297.0 ± 49.43	1281.4 ± 60.5

*P<0.05; (t%): Transformed percentages

density score was 3.72 ± 0.07. Although there were some fluctuations in the mean values for semen colour scores, these variations were not statistically significant. The colour of the ejaculate was correlated positively with the sperm concentration (r = 0.35; P = 0.02) and negatively with the day lentgh (r = -0.30; P = 0.04). The mean values in autumn and winter were significantly higher than spring and summer (Table 2).

Semen pH

The overall mean (±SEM) value for the semen pH was 6.97 ± 0.03. The pH of the semen was significantly lower in summer and higher during winter (Table 2). This parameter was correlated negatively with semen motility (r = -0.38; P = 0.01), sperm motility (r = -0.47; P = 0.001), sperm viability (r = -0.71; P<0.001), ambient temperature (minimum: r = -0.75; P<0.001, maximum: r = -0.73; P<0.001) and day length (r = -0.58; P<0.001) and positively with the proportion of sperms with abnormal morphology (r = 0.39; P = 0.01) and rainfall (r = 0.35; P = 0.02).

Motility

The overall mean (±SEM) value for semen (gross) motility scores (Fig. 2) was 2.89 ± 0.05. Semen motility scores in thick smears of samples showed a significant increase on days 105 and 349. It was negatively correlated with semen pH (r = -0.38; P = 0.01) and the rainfall (r = -0.33; P = 0.03) and positively with sperm viability (r = 0.41; P = 0.007), ambient temperature (minimum: r = 0.41; P = 0.007, maximum: r = 0.40, P = 0.007) and day length (r = 0.39; P = 0.01).

Fig. 1: Changes in the semen volume (Mean ± SEM)

Fig. 2: Changes in the semen motility scores on thick smears (Mean ± SEM)

Fig. 3: Changes in the sperm concentrations (Mean ± SEM); ($\times 10^6$) /ml

In thin smears of the semen, the overall mean (±SEM) value for the sperm motility was 75.85 ± 1.59% (transformed value: 8.63 ± 0.01%). The percentage of forward moving (progressive) spermatozoa had a significant increase on days 70 and 105 and a reduction on days 251 and 348. It was significantly higher in summer and autumn (Table 2). Sperm motility was negatively correlated with the semen pH (r = -0.47; P = 0.001), abnormal morphology (r = -0.66; P<0.001) and had a positive correlation with sperm viability (r = 0.62; P<0.001) and ambient temperature (minimum: r = 0.42; P = 0.005, maximum: r = 0.51; P<0.001).

Sperm viability

The overall mean (±SEM) value for the sperm viability was 73.20 ± 1.56% (8.46 ± 0.01% as transformed). There was a significant variation in the mean values obtained for the percentage of sperm viability only on day 338 that showed an increase in summer and decreased significantly during autumn and winter (Table 2). It was correlated negatively with semen pH (r = -0.70; P<0.001), proportion of sperms with abnormal morphology (r = -0.62; P<0.001) and rainfall (r = -0.39; P = 0.01). It had a positive correlation with semen (gross) motility (r = 0.4; P = 0.007), sperm motility (r = 0.62; P<0.001), ambient temperature (minimum: r = 0.78; P<0.001, maximum: r = 0.76; P<0.001) and day length (r = 0.67; P<0.001).

Proportion of sperms with abnormal morphology

The overall mean (±SEM) value for the percentage of abnormal morphology was 11.55 ± 0.42% (3.33 ± 0.06%). The mean values of this parameter had a reduction on days 15, 156, 286 and 338 and a significant increase on days 166, 293 and 348. The mean values in winter were significantly higher than other seasons (Table 2). It was positively correlated with semen volume (r = 0.38; P = 0.012), pH (r = 0.39; P = 0.010) and had a negative correlation with sperm motility (r = -0.66; P<0.001), sperm viability (r = -0.62, P<0.001) and ambient tem-perature (minimum: r = -0.39; P = 0.008, maximum: r = -0.42; P = 0.004).

Sperm concentration

The overall mean (±SEM) value for sperm concentration was 1239 ± 34.11 × 10^6 cells/ml (Fig. 3). The mean sperm concentration showed fluctuations throughout the year. The variations, however, were not statistically significant in most occasions. The mean values in spring were significantly lower than those in other seasons (Table 2). It was correlated with semen colour (r = 0.35; P = 0.02).

Discussion

Although female buffaloes are polyestrous, they exhibit a distinct seasonal variation in sexual activity. The breeding frequency in buffaloes is reported to be the highest during winter; decreases in autumn

and spring and is the lowest in summer (Singh *et al*., 2000). This requires the reproductive efficiency of buffalo bulls at these times. Photoperiod and other environmental factors are influencing the sexual activity of buffalo bulls. So far, several researchers reported the seasonal variability in libido and quality of buffalo semen (Vale, 1997; Sansone *et al*., 2000).

In the temperate regions of the world, it has been found that the semen is of better quality during winter and spring than in summer and autumn (Mohan and Sahni, 1990; Galli *et al*., 1993). In tropical regions, the quality of semen was observed to be satisfactory during the rain season. In warm and humid tropical Amazon region, the best time to obtain semen is between January and June (Vale, 1994b). Buffaloes are very sensitive to heat stress, thus a decline in the quality of semen is a common finding during the hot season of the year. No information is available on Azarbaijani buffaloes. Therefore, this work may provide useful information, though the number of samples was small.

Semen volume (Fig. 1) was normally fluctuating between 2 to 7 ml in our study, but values as low as 1 ml and as high as 12 ml were also observed. The lowest values were recorded in spring and autumn (Table 2). These values, with an overall mean (±SEM) value of 4 ± 0.14 ml are accord with values reported by Vale (1994a) (1–3 ml and 6 ml), Jainudeen and Hafez (2000) (5 ml), and Ahmad (2001) (1–6 ml).

The colour density of the semen in autumn had an increase and during winter had a significant decrease (Table 2). This variation which was accompanied by an increase in the sperm concentration and a decrease in the proportion of abnormal sperm morphology, led to the observation of a better quality semen in autumn.

Semen pH in this study (6.63–7.1 and an overall mean (±SEM) value of 6.97 ± 0.03) is in keeping with the values (6.4–7.0) reported by Raton (1990), Aguiar *et al*., (1994) and Vale (1997).

The assessment of semen (gross) motility in thick smear (Fig. 2), by putting a drop of semen on a warm glass slide and its microscopic examination by low magnification, helps in a quick evaluation of the samples. Samples with low scores are usually discarded, since they are not worth further processing. Mass motion depends on three factors: concentration, percentage of progressively motile cells and the speed of progression of spermatozoa. When any of these factors is depressed, the expected rapid swirling will be severely depressed or eliminated (Barth, 1997). In this study lower semen motility scores were observed in autumn and winter.

Estimation of sperm motility in a thin smear represents the motility of individual spermatozoa. In this study, low values of sperm forward motility (55.2% and 64.3%) observed during spring and winter, respectively. These values and their overall mean (±SEM) of 75.85 ± 1.59 % agree with the values (40 to 82%) previously reported by Galli *et al*., (1993) for buffalo bulls in Italy.

The percentage of spermatozoa viability determines the quality of the ejaculate. In this study, during the warm months of the year, percentage of live sperms fluctuated between 70 and 95% (mean = 84.1%), but in the cold months, it was between 35 and 80% (mean = 60.4%). This indicates the effect of the cold on survival of spermatozoa (r = 0.78).

Abnormal spermatozoa are detected by staining methods and are usually classified as head, middle-piece and tail abnormalities (Kumar *et al*., 1993). The percentages of sperms with abnormal morphology showed fluctuations in association with both an increase and a decrease in ambient temperature. The lowest values of 8.1% observed in autumn and the highest value (15.8%) was during winter.

The mean sperm concentration showed fluctuations throughout the year (Fig. 3) but the lowest values were observed in spring. The mean sperm concentration, which was measured by using a hemocytometer was from 1100 ± 54 to 1297 ± 49 × 10^6 cells/ml (range: 320-2280 and overall mean (±SEM) of 1239.9 ± 34.11 × 10^6 cells/ml). These figures have a wider range of values (690.6 ± 187.9 to 1290.7 ± 100 × 10^6 cells/ml) that reported by Galli (1993) for buffaloes bred in Italy and 300 to 1500 × 10^6 cells/ml reported by Jainudeen and Hafez (2000) and are higher than 1166.3 ± 17.5 × 10^6 cells/ml

reported by Aguiar *et al*., (1994) for buffalo bulls in Brazil, 524.1 ± 20.7 to 1031.4 ± 28.7 × 10^6 cells/ml reported by Kumar *et al*., (1993) for Murrah buffalo bulls bred in India and agree with those (1000–4000 × 10^6 cells/ml) reported by Ahmad (2001). The difference in the sperm concentration and some other semen parameters of individual bulls in each sampling made the variation of these parameters so great. The rhythmic fluctuation in the sperm concentration observed in each bull ejaculate at successive samplings may be a reflection of the cyclic pattern of the sperm production.

Day length influences all the parameters significantly except for the sperm viability. The high and low environmental temperatures have a negative influence on the proportion of sperms with abnormal morphology. The low ambient temperature negatively influences the semen colour and pH and the sperm concentration.

In conclusion, all parameters examined in this study to evaluate the quality of semen in buffalo bulls are affected by environmental factors such as day length, temperature and humidity. The quality of semen in summer and autumn is better than other seasons. Cold environment, by reducing semen motility, sperm viability and concentration and by increasing the percentage of abnormal sperms reduces the semen quality.

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