Scientific Papers. Series D. Animal Science. Vol. LXI, Number 1, 2018
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

QUALITY AND QUANTITY PARAMETERS IN BUFFALO SEMEN PRODUCTION

Dana TAPALOAGA[1], Makki Khalaf Hussein AL DULAIMI [2], Paul-Rodian TAPALOAGA[1]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania
[2]Al-Furat Al-Awasat Technical University, 51009 Babylon, Iraq

Corresponding author email: rodiantapaloaga@yahoo.com

Abstract

Semen production depends upon different factors as age, breed, collecting and environmental conditions. The present research has in view to establish the influence of age upon the quantitative and qualitative parameters of semen from few of the Romanian buffalo reproducers. There were analyzed 12 native buffalo males, from different age category. At every semen collecting there were recorded the following morphologic sperm parameters: sperm volume, sperm concentration(x 10^9spz/ml), colour, mass activity, pH and motility. The influence of the age was made along two years, studying the dynamics of the sperm parameters. Statistical analysis was performed as per standard statistical methods. The results reveal the fact that the best performances were recorded in bulls up than three years old. The study clearly demonstrates that there is a variation in reproductive parameters in the buffalo bulls, which could be studied at the molecular level to unveil any genomic markers associated with low fertility and/or infertility. The males may be used to obtain semen by subjecting the young ones to training at an early age, thereby decreasing the initial age of semen donation.

Key words*: sperm collecting, qualitative and quantitative indices, buffalo.*

INTRODUCTION

Artificial insemination is practiced very little in Europe and East Asian countries like Iran and Egypt (Borghese, 2014). Also, buffalo livestock and strategies are reported for all the countries in Europe, where buffalo specie is reared and used for food production, as Italy, Romania, Bulgaria, Germany, Macedonia, United Kingdome, Greece, Serbia, Albania, Ukraine and Hungary. It was reported that the percent of buffaloes covered by AI programs is only 5% in Italy, 3.7% in Azerbaijan, 0.3% in Egypt and 0.1% in Romania. In Bulgaria, in the large cooperative state farms, it is used on 80% of the buffaloes. It was also stated that the diffusion of AI in buffaloes is difficult.

The buffalo rearing in Romania is still in an uncertain phase, which could be considered a developing phase (Tapaloaga, 2015). Despite of remarkable contribution of buffaloes breeders in the European context in the past, and a sinuously importance during the last 30 years that leads to no more than sixty thousand animals in the whole country, there is always shortage of scientific Information on this animal especially in the field of reproduction.

Although artificial insemination (AI) is being widely used in nearly all cattle / buffalo breeding countries, but the number of breeding bulls has greatly reduced and consequently the quality of the bulls has become a matter of vital importance for this species. The present study was planned with the following objective: to investigate the relationship between semen quality and quantity parameters in some Romanian buffalo bulls.

Artificial insemination facilitated the choice of using the best possible males of proven quality in improving the genetic base of the bovine population, thus conveying to the primary goal for breeding, the increasing of the productivity and the profitability of the commercial herds, by increasing the number of the offspring produced by selected genetically superior males. Due to the fact that buffalo is one of the main dairy animal in many countries of the world, but not in the European countries, excepting Italy or Bulgaria and taking into consideration that Romanian buffalo livestock decreased so much, meanwhile the consumers trend in buffalo milk products is increasing, it is of major importance for Romanian scientists

and animal breeders to focus on this species advantages (Koonjaenak, 2007; Al Dulaimi, 2015; Tapaloaga, 2015). The objective of this study was to investigate the influence of buffalo age on some quantity and quality traits and to relate them to the animal fertility, thus contributing to the success of buffalo artificial insemination. The buffalo bull age factor has been investigated concerning its effect on morphologic and morph metric sperm features by many authors (Biswajit, 2014). Moreover, sperm analyze has been also used in fertility evaluation of male and it is recommended as part of the domestic animal sperm files (Al Dulaimi, 2015; Al Dulaimi, 2014).

MATERIALS AND METHODS

The present study was conducted in the Department of Animal Reproduction, Faculty of Animal Science, University of Agronomic+ Sciences and Veterinary Medicine, Bucharest, Romania, on 12 Romanian buffalo bulls (*Bubalus bubalis*) divided into three age groups, with four bulls in each group, the first group consisted in less than 4 years aged bulls, the second one consisted in 5-6 years aged bulls and more than 7 years aged bulls. All bulls were kept under identical conditions of management, feeding and watering throughout the study period.

They were selected according to the normal sperm quality in routine tests. They were individually penned and fed, including the population households. All buffalo bulls were sexually active and under a weekly semen collection regime throughout the study period. The recorded data were analysed according to the statistical procedures. All data were nearly normal distributed. Hypothesis testing was performed by parametric tests which included analysis of variance (ANOVA). Semen from all the experimental bulls was collected every two days with the aid of an artificial vagina (AV). The semen collected was brought to the laboratory immediately and it was placed in a water bath at 37°C. Two collected ejaculates were pooled and evaluated for total volume, colour, mass activity, motility, pH and sperm concentration.

Semen volume was recorded directly in the collector glass. The colour of the semen was recorded as creamy, milky or watery, depending on the thickness of the semen and was assigned a numerical weight from zero to two for statistical analysis. A numerical weight of 2 was assigned to creamy, 1 to milky and 0 to watery samples. The pH of semen was recorded with a pH meter. The mass activity of spermatozoa was observed by placing an undiluted semen drop on glass slide under warm stage of Phase Contrast Microscope (10x) and the grading was recorded as: 0 = no mass motility; + = less than 20 percent of sperms showing progressive motion; ++ = 40 to 60 percent showing progressive motion with slow wave; +++ = 60 to 80 percent showing progressive movement with wave more intense and ++++ = 80 to 100 percent showing progressive movement with rapid wave waking eddies (+++ or more are recommended for A.I. purpose). Motility, as a percentage of individually motile spermatozoa, was estimated by examining a drop of diluted fresh semen (with 2.9% sodium citrate solution) under a microscope at 400×. Motility was scored on the basis of the percentage of spermatozoa with normal forward progressive movement, while those showing circling movements or those oscillating at one place were regarded as immotile. Sperm concentration was measured using a photo colorimeter. The means were compared, and correlation coefficients among different parameters were also worked out. The recorded data were analysed according to the statistical procedures. All data were nearly normal distributed. Hypothesis testing was performed by parametric tests which included analysis of variance (ANOVA).

RESULTS AND DISCUSSIONS

In the present study the colour of all semen samples was white, creamy with normal appearance. Normal colour of buffalo semen is white to creamy white (Johnson, 1997; Tapaloaga, 2003). The current study findings are also in agreement to Brohi (1993) in Kundhi buffalo bulls and Kumar (1993) in Indian buffalo bull semen and Tapaloaga et al. (2015) in Romanian buffaloes.

The mean (+SEM) of ejaculate volume of the semen was found to be 4.07 (±0.02) ml ranging from 3.85-4.33 ml (Table 1). Analysis of

variance showed no significant (P>0.05) difference between the bulls for ejaculate volume. The overall mean value of ejaculatory volume was 4.07 ml (SD = 0.16; CV = 3.05%). The intra-assay CV ranged between 2.89% and 3.21%. Ejaculatory volume varied between 3.72 ml and 4.33 ml among semen ejaculates and 3.42 ml and 4.69 ml among bulls. Mature bulls had higher mean values of ejaculatory volume (4.33±0.01) than those of the young (3.85±0.03) bulls (Table 1). No significant interactions were found between bulls, age group and ejaculate on ejaculatory volume.

The volume in the current study falls in the range reported by Sansone (2000) and Tapaloaga (2016). In current study all the bulls were of the age and young therefore variation in the ejaculate was not expected. The variation might be due to the difference in group age.

Table 1. Mean ejaculatory volume in Romanian buffalo bulls (ml)

Age group	Group 1 (n=4) N_1=4 N_2=32	Group 2 (n=4) N_1=4 N_2=36	Group 3 (n=4) N_1=4 N_2=32	Overall N_1=4 N_2=100
Mean	3.85	4.33	4.21	4.07
SD	0.18	0.14	0.18	0.16
SEM	0.03	0.01	0.02	0.02
CV (%)	2.89	3.21	3.08	3.05

The mean (+ SEM) pH value of fresh semen was 5.81(± 0.06) with the range of 5.72 - 6.01 (Table 2). A significant (P<0.05) difference was observed between the bulls for pH values. The mean pH value (5.72 ±0.06) found in the current study is slightly lower than the mean (6.16) reported by Brohi (1993) in Kundhi buffalo and Younis (1996) in the Nili Ravi buffalo (6.04-6.93). This might have been due to concentration of semen, season and hygienic conditions (Alvi-Shoushtari and Babazadeha-Hebashi, 2006).

However none of the pH level recorded in the present study falls in the lethal level for sperm cells (Mann and Mann, 1988). Time interval after collection and individual aliquot also influence the level of pH in fresh semen as was the case in present study.

Table 2. Mean sperm pH in Romanian buffalo bulls

Age group	Group 1 (n=4) N_1=4 N_2=32	Group 2 (n=4) N_1=4 N_2=36	Group 3 (n=4) N_1=4 N_2=32	Overall N_1=4 N_2=100
Mean	5.72	5.79	6.01	5.81
SD	0.46	0.64	0.49	0.55
SEM	0.03	0.03	0.02	0.06
CV (%)	4.07	3.86	4.87	4.51

For assessing the mass activity, swirling movement was observed. All the sample appeared to have score of +++ to ++++. For statistical interpretation these were given numerical values. The mean (+ SEM) numerical value was found to be 3.32 (± 0.05), which ranged from 3 to 4 (Table 3). Analysis of variance showed no significant (P>0.05) difference between the bulls for mass activity. Mass activity found (3.32 ±0.05) in the current study was higher than the reported values (2.65 ±1.14) in Nili Ravi bulls (Javed et al., 2000; Heuer et al., 1982) and in Indian bulls (2.54) (Vyawanare et al., 1989). The desirability can be attributed to the effect of climatic conditions, in which sperm might be more rigors due to preservation temperature.

Table 3. Mean sperm mass activity in Romanian buffalo bulls

Age group	Group 1 (n=4) N_1=4 N_2=32	Group 2 (n=4) N_1=4 N_2=36	Group 3 (n=4) N_1=4 N_2=32	Overall N_1=4 N_2=100
Mean	3.12	3.76	3.72	3.32
SD	0.46	0.64	0.33	0.55
SEM	0.03	0.03	0.02	0.05
CV (%)	4.87	3.23	4.32	4.36

The mean (+SEM) motility percentage of Romanian buffalo bull semen was 71.5 (±0.03), which ranged between 69.2-72.3% (Table 4). The motility percentage (60-75%) found in the current study in different bulls were higher beside the ones reported (63%) by Brohi et al. (1993) in Kundhi buffalo bulls and Jainuddin et al. (1982) in swamp buffalo. Higher values than the current figures have been reported in Murrah buffalo bull by Jainuddin et al. (1982). However assessment of mean percentage of motility using simple method is readily available and cost efficient and provides rapid means of semen evaluation in field conditions but objective type of semen assessments are still needed to be applied for precise analysis of semen sample (Molinia et al., 1994).

Table 4. Mean sperm motility in Romanian buffalo bulls

Age group	Group 1 (n=4) N_1=4 N_2=32	Group 2 (n=4) N_1=4 N_2=36	Group 3 (n=4) N_1=4 N_2=32	Overall N_1=4 N_2=100
Mean	69.2	72.3	71.7	71.5
SD	0.36	0.44	0.31	0.55
SEM	0.02	0.01	0.02	0.03
CV (%)	5.09	3,86	4.36	4.51

The mean (+SEM) sperm concentration of semen was found to be $1.65x10^9$ per ml, which ranged from 0.89-1.83 billion/ml (Table 5). Analysis of variance showed no significant (P>0.05) difference for semen concentration between the bulls.

The sperm concentration of the ejaculates found in the current study in Romanian buffalo falls in the range (1-4 x 10^9/ ml) reported in other breeds of buffalo (Arther, 2003; Rehman, 2012, Aguiar et al., 1994) excepting the younger bulls, in the first category, which recorded values slightly under 1 billion cells/ml. This indicates that the number of cells required for maintaining fertility level of the semen from Romanian bulls was acceptable to be used for AI programme, but taking into consideration further researches due to the fact that bulls before 3 years old have a lower sperm concentration.

Table 5. Mean sperm concentration in Romanian buffalo bulls ($x10^9$)

Age group	Group 1 (n=4) N_1=4 N_2=32	Group 2 (n=4) N_1=4 N_2=36	Group 3 (n=4) N_1=4 N_2=32	Overall N_1=4 N_2=100
Mean	0.89	1.83	1.74	1.65
SD	0.23	0.42	0.46	0.55
SEM	0.03	0.03	0.02	0.03
CV (%)	3.46	3.21	4.71	4.51

The overall mean value of sperm concentration was 1.65 x 10^9 (SD = 0.55; CV = 4.51%). The intra-assay CV ranged between 3.21% and 4.71%. Sperm concentration varied between 0.68 x 10^9 and 1.96 x 10^9 among semen ejaculates and 0.85 x 10^9 and 1.92 x 10^9 among

bulls. Older bulls had lower mean values of sperm concentration (1.74 x 10^9±0.03) than those of the young (0.89 x 10^9±0.03) bulls (Table 4). No significant interactions were found between bulls, age group and ejaculate on sperm concentration.

It is well known that spermatozoa differ in shape and dimensions among species and also between individuals (Thurston et al., 2001). Abnormal bull sperm morphology has always been correlated with reduced fertility. However, a number of studies have shown no correlations between sperm morphology and fertility (Linford, 1976) with clear associations between normal bull sperm morphology and fertility continuing to remain elusive (Johnson, 1997). Our results have brought a modest contribution to describing the morphologic traits in native buffaloes, with values close to the ones reported by other researchers from the Asian continent on crossbred and native Murrah buffalo bulls (Biswajit Roy).

CONCLUSIONS

On the basis of current study it was concluded that Romanian buffalo bulls' qualitative and quantitative semen parameters in correlation with other objective characters can be useful tools for developing a fertility index. The study clearly demonstrates that there is a variation in reproductive parameters in the bovine bulls, which could be studied at the molecular level to unveil any genomic markers associated with low fertility and/or infertility. The males may be utilized to obtain semen by subjecting the young ones to training at an early age, thereby decreasing the initial age of semen donation.

REFERENCES

Aghangari Y.T., 1992. Cryopreservation of ram semen for A.I. Ph.D. Thesis, Univ. Wales, Bangor.

Al Dulaimi M.K.H., Tapaloaga P.R., Tapaloaga D., 2015. Age related morphometric features of spermatozoa in ram, Journal of Biotechnology, 208, Supplement, p S37, ISSN 0168-1656.

Al Dulaimi M.K.H., Tapaloaga P.R., Tapaloaga D., 2014. Artificial insemination sperm fertility assessment by CASMA procedures in ram, Journal of Biotechnology, 185, Supplement, September, p S41, ISSN 0168-1656.

Al Dulaimi M.K.H., Tapaloaga P.R., Tapaloaga D., 2015. Results regarding some morphometric features of spermatozoa in ram, Agriculture and Agricultural Science Procedia, 6, 232-235, ISSN 2210-7843.

Alvi S.S.M., Babazadeha-Hebashi B., 2006. Seasonal variations in the characteristics of the Azerbaijani buffalo. Iran. J. of Vet. Res., 7, 1-14.

Aguiar P.H.P., Andrade V.J., Abreu J.J., Gomez N.B.N., 1994. Physical and morphological semen characteristics of buffalo aged from four to eight year old. Proc. 4th Int. Buffalo Congr.,Sao Paulo, Brazil., 3, 488-488.

Arther, 2003. Artificial Insemination. Arther's Veterinary Reproduction and Obstetrics 8th edition London, W.B. Sunder, Co., 751-758.

Biswajit Roy, 2014. A comparative study on sperm morfometry of crossbred and Murrah buffalo bulls, International Journal of Agricultural Sciences and Veterinary Medicine, 2(3), 149-155.

Borghese A, 2014. A Scientific Bulletin of Escorena, vol 10.

Brohi N.A., 1993. Sexual behaviour and semen characteristics of Kundhi buffalo bulls. M.Sc. Thesis. Department of Anim. Repro. Sindh Agriculture University Tandojam.

Heuer C.H., Bader M.A., Bajwa, 1982. Sperm morphology of the Nili- Ravi buffaloes. Pak.Vet. J., 2, 155-160.

Javed M.T., Khan A., Kausar R., 2000. Effect of age and season on some semen parameters of Nili-Ravi buffalo (*Bubalus bubalis*) bulls. Vet.Arhiv., 70, 83.

Jainuddin M.R., Bangso T.A., Dass S., 1982. Semen characteristics of the swamp buffalo (*Bubalus bubalis*). Anim. Repro. Sci., 4, 213-217.

Johnson W.H., 1997. The significance of bull fertility of morphologically abnormal sperm. Bull fertility, Veterinary Clinics of North America: Food Anim. Practice, 13, 255-270.

Linford E. et al., 1976. The relationship between semen evaluation methods and fertility in bull. Journal of reproduction and fertility, 47, 283-291.

Mann T., Mann L.W., 1988. Male reproductive function and semen. Themes and trends in physiology. Biochemistry and investigative Andrology. Spring-Velog, Berlin, New York.

Molinia F.C., Evans G., Casares P.I., Maxwell W.M.C., 1994. Effect of monosaccharides and disaccharides in Tris-based diluents on motility, acrosome integrity and fertility of pellet frozen ram spermatozoa. Anim Reprod Sci., 36, 113– 122.

Rehman Z.U., Samo M.U., Qureshi T.A., Khan S., Qureshi M.S., Khan F.A., Bahadadr S., 2012. Studies on the freezability of Kundhi Buffalo bull semen. The Journal of Animal and Plant Sciences, 22(2), 18-23, ISSN 1018-7081.

Thurston I. et al., 2001. Morphologically distinct sperm subpopulations defined by Fourier Shape Descriptors in fresh ejaculates correlated with variation in boar semen quality following cryopreservation. J. Androl., 22, 382-394.

Sansone G., Nastri M.J.F., Fabbricini A., 2000. Storage of buffalo (*Bubalus bubalis*) semen. Anim. Repro. Sci., 55-76.

Tapaloagă P, 2003. Handbook of animal reproduction, Allprint Publishing House, Bucharest.

Tapaloaga D, Tapaloaga P.R., 2015. Study Regarding Age-Related Morphometric Features of Buffalo

Sperm, Agriculture and Agricultural Science Procedia, 6, 272-276.
Vyawanare R., Chauhan R.A.S., Nema S.P., Poswal M.L., 1989. Studies on seminal attributes, enzyme leakage and preservability of buffalo semen, Ind. Vet. J., 66, 1128-1132.
Younis M., 1996. Studies on semen quality, freezability and fertility of buffalo bulls during low and peak breeding seasons. Ph.D. Dissertatiot of Anim.Repro. University of Agriculture, Faisalabad, Pakistan.
Seri Koonjaenak, Vichai Chanatinart, Suneerat Aiumlamai, Tanu Pinyopumimintr, Heriberto Rodriguez-Martinez, 2007. Seasonal variation in semen quality of swamp buffalo bulls (*Bubalus bubalis*) in Thailand. Asian J Androl., 9(1), 92–101.

TECHNOLOGIES OF ANIMAL HUSBANDRY