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Study Regarding Age-Related Morphometric Features of Buffalo Sperm

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Abstract

Semen production quality from reproduction males depends upon different factors such as:age, breed, collection method, environmental conditions. The present research was conducted to establish the influence of age upon the quantitative and qualitative indices of the native buffalo semen. There were analyzed 20 native buffalo males, from different age categories. At every semen collecting there were recorded the following morphologic and morphometric sperm indices: sperm volume, sperm concentration (x 10^6spz/ml), total number of sperm cells/ejaculate(x10^9), head length, head width, tail length, head area and shape factor.The influence of the age was made along two years, studying the dynamics of the sperm indices through the computer image analysis. Descripted statistics were performed on the recorded data to determine normality. Statistical analysis was performed as per standard statistical methods.The results revealed the fact that the best performances were recorded in bulls older than three years old. 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.

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1. Introduction

Artificial insemination, the manual deposition of spermatozoa in the reproductive tract of a sexually receptive female by the use of artificial means, is considered the first reproductive biotechnology. It was created with the major intention of controlling the dissemination of venereal diseases and it still remains the most important technology for safe gene dispersion in the breeding of an animal livestock.

The biotechnology of artificial insemination has also provided the future developing other assisted reproductive biotechnologies, such as semen cryopreservation and sexing, as well as alternative methods for semen deposition, oestrous cycle regulation and control or embryo transfer.

Artificial insemination facilitated the choice of using the best possible males of proven quality in improving the genetic makeup 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.

The objective of this study was to investigate the influence of buffalo age on some morphometric quality traits and to relate them to the animal fertility, thus contributing to the success of buffalo artificial insemination.

Many authors suggest a correlation between the morphometric characteristics and males fertility in the ejaculates of dogs, stallions, boars, rabbits and bulls.

2. Research Methods

In animal breeding, artificial insemination procedure requires evaluation of sperm characteristics, having as aim to ensure its quality before service. Age of males is generally linked to cellular changes affecting sperm quality and of course its fertilizing ability. The buffalo bull age factor has been investigated concerning its effect on morphologic and morphometric sperm features by many authors (Boersma, 2001; Biswajit, 2014).

Moreover, sperm morphometry has been also used in fertility evaluation of male and it is recommended as part of the domestic animal sperm files (Padrik and Jaakma, 2002).

The present study was conducted over two years on animals in the centre and north-western part of Romania and involved 20 males. 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. As experimental design, the buffaloes were allocated in two groups. Each one consisted of 8-12 buffaloes, aged 3-4 years (young, n = 8) and 5-9 years (mature, n = 12). Semen samples were collected (one ejaculate/male/week) by the artificial vagina technique.

The recorded data were analysed according to the statistical procedures. All data were nearly normal distributed. The inter- and intra-assay coefficients of variation (CV) were estimated for sperm head dimensions. Hypothesis testing was performed by parametric tests which included analysis of variance (ANOVA).

3. Results and Discussions

The overall mean value of sperm concentration was 0.91 x 10^6µl (SD = 0.55; CV = 4.51%). The intra-assay CV ranged between 4.25% and 4.77% (Table 1).

Sperm concentration varied between 0.95 x 10^6µl and 0.87 x 10^6µl among semen ejaculates and 0.93 x 10^6µl and 0.89 x 10^6µl among bulls. Mature bulls had lower mean values of sperm concentration (0.84 x 10^6µl±0,03) than those of the young (0.98 x 10^6µl±0,03) bulls (Table 1). No significant interactions were found between bulls, age group and ejaculate on sperm concentration.

The overall mean value of ejaculatory volume was 4,07 ml (SD = 0.16; CV = 3.05%). The intra-assay CV ranged between 2.97% and 3.13%. Ejaculatory volume varied between 3.76 ml and 4.38 ml among semen ejaculates and 3.56 ml and 4.58 ml among bulls. Mature bulls had lower mean values of ejaculatory volume (4.21 ml±0.03) than

those of the young (3.94 ml±0.01) bulls (Table 2). No significant interactions were found between bulls, age group and ejaculate on ejaculatory volume.

Table 1. Sperm Concentration in Buffaloes Depending on Age

Age group	Young bulls (n=8) N_1=4 N_2=32	Mature bulls (n=12) N_1=3 N_2=36	Overall N_1=3-4 N_2=68
Mean	0.98 x 10^6µl	0.84 x 10^6µl	0.91 x 10^6µl
SD	0.46	0.64	0.55
SEM	0.03	0.03	0.03
CV (%)	5.09	3.86	4.51

Table 2. Ejaculatory Volume in Buffaloes Depending on Age

Age group	Young bulls (n=8) N_1=4 N_2=32	Mature bulls (n=12) N_1=3 N_2=36	Overall N_1=3-4 N_2=68
Mean	3.94 ml	4.21 ml	4.07 ml
SD	0.18	0.14	0.16
SEM	0.03	0.01	0.02
CV (%)	2.89	3.21	3.05

The overall mean value of sperm head length was 7.42 µm (SD = 0.35; CV = 3.83%). The intra-assay CV ranged between 4.13% and 3.53%. Sperm head length varied between 6.95 µm and 7.89 ml among semen ejaculates and 6.99 ml and 7.92 ml among bulls. Mature bulls had lower mean values of ejaculatory volume (7.61 µm ± 0.04) than those of the young (7.20 µm ± 0.04) bulls (Table 3). No significant interactions were found between bulls, age group and ejaculate on ejaculatory volume.

Table 3. Sperm Head Length in Buffaloes Depending on Age

Age group	Young bulls (n=8) N_1=4 N_2=32	Mature bulls (n=12) N_1=3 N_2=36	Overall N_1=3-4 N_2=68
Mean	7.20 µm	7.61µm	7.42 µm
SD	0.28	0.42	0.35
SEM	0.02	0.04	0.03
CV (%)	4.25	3.21	3.83

The overall mean value of sperm head width was 4.97 µm (SD = 0.26; CV = 4.14%). The intra-assay CV ranged between 3.12% and 5.16%. Sperm head width fluctuated between 4 and 5 µm among semen ejaculates and between 4.80±0.027 and 5.14±0.25 among bulls. The effect of bull and age on head width was significant. As shown in table 4, mature bulls had lower values of sperm head width than young bulls (4.93±0.01 than 5.02±0.01). Nevertheless, sperm head width of young bulls did not vary significantly from those of the mature bulls. Furthermore, bull and ejaculate by age interactions on head width was significant.

Table 4. Sperm Head Width in Buffaloes Depending on Age

Age group	Young bulls (n=8) N_1=4 N_2=32	Mature bulls (n=12) N_1=3 N_2=36	Overall N_1=3-4 N_2=68
Mean	5.02 µm	4.93µm	4.97 µm
SD	0.27	0.25	0.26
SEM	0.01	0.01	0.01
CV (%)	3.21	5.07	4.14

The tail length mean values are shown in Table 5. Tail length fluctuated between 56.24 and 56.96 µm in mature bulls versus young bulls. The intra-assay CV ranged between 5.45% and 4.85%, with an average value of 5.15%. Mature bulls had lower mean values of tail length (56.24 ±0.02) than those of the young (56.96 ±0.01) bulls (Table 5). No significant interactions were found between bulls, age group and ejaculate on tail length.

Table 5. Sperm Tail Length in Buffaloes Depending on Age

Age group	Young bulls (n=8) N_1=4 N_2=32	Mature bulls (n=12) N_1=3 N_2=36	Overall N_1=3-4 N_2=68
Mean	56.96 µm	56.24 µm	56.60 µm
SD	0.25	0.24	0.24
SEM	0.01	0.02	0.01
CV (%)	5.45	4.85	5.15

The overall mean value of sperm head shape was 0.66 (SD = 0.11; CV = 3.82%). The intra-assay CV ranged between 3.41% and 4.23%. Mature bulls had lower mean values of sperm head shape (0.64±0.01) than those of the young (0.69±0.02) bulls (Table 6). No significant interactions were found between bulls, age group and ejaculate on sperm head shape.

Table 6. Sperm Head Shape in Buffaloes Depending on Age

Age group	Young bulls (n=8) N_1=4 N_2=32	Mature bulls (n=12) N_1=3 N_2=36	Overall N_1=3-4 N_2=68
Mean	0.69	0.64	0.66
SD	0.11	0.12	0.11
SEM	0.02	0.01	0.01
CV (%)	3.41	4.23	3.82

The overall mean value of head area, expressed in μm^2 was 24.35 μm^2 (SD = 0.56; CV = 4.96%), and shown in Table 7. The intra-assay CV ranged between 4.81% and 5.12%. There were non significant fluctuations between young and mature bulls. The mean value of variability coefficient presented and demonstrated a low variability character in the studied population.

Table 7. Sperm Head Area in Buffaloes Depending on Age

Age group	Young bulls (n=8) N_1=4 N_2=32	Mature bulls (n=12) N_1=3 N_2=36	Overall N_1=3-4 N_2=68
Mean	24.3 μm^2	24.41 μm^2	24.35 μm^2
SD	0.62	0.51	0.56
SEM	0.04	0.06	0.03
CV (%)	4.81	5.12	4.96

Spermatozoa differ in shape and dimensions among species and also between individuals (Thurston et al., 2001). Abnormal bull sperm morphology has 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). In the bull, metric criteria for normal sperm head measurement have not been readily applied to fertility assessment. Our results have brought a modest contribution to describing the morphologic and morphometric 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 (Biwajit, 2014).

4. Conclusions and Recommendations

Computer- aided sperm head morphometry appears to be a precise method of assaying sperm head dimensions in buffalo bulls. Sperm morphology and morphometry in combination 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.

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