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Particularities of the conditioning of buffalo bulls in semen collection with conventional artificial vagina for use in favorable and unfavorable breeding seasons

[*Particularidades do condicionamento de touros bubalinos em coleta de sêmen com vagina artificial convencional para uso em estações reprodutivas favorável e desfavorável*]

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ABSTRACT

This study aimed to evaluate the sexual behavior and accommodation characteristics of buffalo recipients patented for semen collection with an artificial vagina. Thirty bulls were sampled during favorable (March to May) and unfavorable (November to December) breeding seasons in a property located in the state of Minas Gerais, Brazil (Latitude 20°41'45" South and Longitude 44°49'37" West). Data were analyzed descriptively according to whether ejaculation was successful, using a range of behavioral parameters. A multilevel logistic regression analysis was performed using the responses and explanatory variables mentioned above. The results show that when more than three parameters are manifested, the chance of obtaining ejaculation increases by 9.0%, a value that rises to 28.2% when seven to nine parameters manifested by the breeders are observed. These observations allow us to conclude that it is possible to condition sexually mature bulls for semen collection through the artificial vagina for use in artificial insemination and fixed-time artificial insemination programs.

Keywords: sexual behavior, semen collection, reproductive management

RESUMO

Este estudo teve como objetivo avaliar o comportamento sexual e as características de condicionamento de reprodutores bubalinos submetidos à coleta de sêmen com vagina artificial. Trinta touros foram avaliados durante as estações reprodutivas favorável (março a maio) e desfavorável (novembro e dezembro), em propriedade localizada no estado de Minas Gerias, Brasil (latitude 20°41'45'' Sul e longitude 44º49'37'' Oeste). Os dados foram analisados descritivamente de acordo com o sucesso ou não da ejaculação, usando-se uma série de parâmetros comportamentais. Uma análise de regressão logística multinível foi realizada utilizando-se as respostas e as variáveis explicativas mencionadas acima. Os resultados mostraram que, quando mais de três parâmetros se manifestam, a chance de se obter ejaculação aumenta em 9,0%, valor que sobe para 28,2% quando se observam sete a nove parâmetros manifestados pelos reprodutores. Essas observações permitem concluir que é possível condicionar touros sexualmente maduros para coleta de sêmen por meio da vagina artificial para uso em programas de inseminação artificial e inseminação artificial em tempo fixo.

Palavras-chave: comportamento sexual, coleta de sêmen, manejo reprodutivo

INTRODUCTION

In reproduction, the role of the bull is unquestionable since the bull can mate with several females. This importance is multiplied with the use of conventional artificial insemination or fixed-time artificial insemination (FTAI), when for success good resistance to cryopreservation is added to desirable characteristics of seminal quality. In both forms of fertilization, good reproductive libido is required to increase field coverage potential, particularly after estrus synchronization programs, or to allow semen collection for artificial insemination.

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In addition, there is the reproductive seasonality, a physiological characteristic of the bubaline species, and the difficulty of finding semen on the market, because of the reports of bubaline bulls that even conditioned to the collection with conventional artificial vaginas in central became resistant to artificial service over time (Prabhu and Bhattacharya, 1954; Bernardes, 2017 - personal communication), making it difficult and economically unfeasible to maintain them in semen freezing plants. This is mainly due to two reasons: 1) during semen collection, animals are sexually stimulated in such a way that premature activities (courtship and recognition of the female in heat) can be ignored to produce the desired number of ejaculations, in the shortest possible time (Mader and Price, 1984), because males of this species do not maintain regularity of quality ejaculates, which allow freezing for several weeks in a row, with several alternating collections of semen with and without quality for freezing and even interruption in ejaculations for no apparent reason.

Another problem is the lack of behavioral knowledge of the buffalo bulls involved in the process of handling, collecting and freezing semen. It is neglected the factor that the buffalo is more sensitive and fearful when compared to bovine bulls, requiring more time to get used to the change of environment, personnel involved in management and treatment, in addition to needing a longer time to acquire confidence in the person involved in the collection and respond appropriately to the methodologies used.

In the field, it is difficult to use electro-ejaculation in buffalo, due to their greater sensitivity to electrical stimuli (Ohashi, 2008), which can cause accidents, especially in adult animals. However, in younger animals, there has been some success (Ohashi *et al*., 2011).

Faced with so many difficulties, the search for knowledge and improvement in the techniques of conditioning and collection of semen in buffaloes are factors that make the activity even more challenging, but at the same time motivate and encourage technicians and professionals, who work with this species, to improve and seek new strategies to solve this problem.

A vast literature has emerged about the buffalo, but adequate scientific studies of its social behavior are still scarce, particularly concerning the conditioning for semen collection with an artificial vagina. This study aims to aggregate knowledge in this field and compare it with historical literature. For this, an evaluation of animal conditioning and the application of management techniques and their probable effects on the efficiency of semen collection in buffaloes was carried out during the favorable and unfavorable reproductive seasons for breeders raised on private property, as a tool to optimize the use of cooled and frozen semen, in artificial insemination (AI) and fixed-time artificial insemination (FTAI) programs.

MATERIAL AND METHODS

The experiment was carried out at Fazenda Bom Destino, in the municipality of Oliveira/MG, Brazil (Latitude 20°41'45" South and Longitude 44°49’37" West). The study was carried out after approval of all procedures by the Ethics Committee on the Use of Animals (CEUA) of the Veterinary School of the Federal University of Minas Gerais (EV/UFMG), according to protocol No. 368/2015.

The bulls were evaluated in semen collection, during the favorable reproductive periods “PRF” (autumn-winter; May to July) and unfavorable reproductive “PRD” (spring-summer; December to February) (Zicarelli, 2010). Thirty bulls from a private property were used during the years 2014 to 2017, with 10 bulls being used in 2014 during the PRD (November to December), and for the PRF (March to May) in 2015, 2016 and 2017 (9, 5 and 6 bulls, respectively). However, 6 of the bulls were present in more than one valuation period. The bulls had the following characteristics: ages between 3 and 11 years; weight from 620 to 1200 kg; ECC from 3 to 5 (1-5); Murrah (n = 21), Mediterranean (n = 4) and Mestizo (n = 5) breeds; bred in an extensive system and with sexual experience with females. These bulls were previously submitted to clinical and andrological evaluation according to the manual for andrological examination and evaluation of animal semen (Manual…, 2013), later presented to a female in natural heat or induced with estrogen and/or prostaglandin, contained in a specific trunk for collection, in the morning and afternoon.

A minimum time of 15 minutes was stipulated for the animal to perform the copulation (Vale, 2011). A table was also created, which recorded the types and numbers of signs manifested by the breeders (smelling urine in the soil, smelling the vulva, licking the vulva, Flehmen reflex, scratching the female's head, head-butting the female, supporting the chin on the female's rump, intermittent penile contraction before mounting, exposure of the penis and incomplete mounting without exposure of the penis), presented by the animal during the stipulated evaluation period.

Collections were performed with an artificial vagina with an internal temperature between 42-45 °C (Sansone, 2000; Ohashi, 2008; Vale, 2011; Almeida and Resende, 2021; Almeida *et al*., 2023). Coupled to the vagina collector cups (plastic tubes 15 mL) were used, graduated and sterile, previously heated and protected with an isothermal shirt, to avoid contact with ultraviolet (UV) rays and sudden temperature changes that could affect semen quality. For each collection, a false sample was induced in the first attempt (without collecting semen, to increase the sperm concentration of the ejaculate), and the ejaculate of the second sample was collected and directed to the laboratory, placed in a water bath at 37 °C, for the analyzes.

For statistical analysis, the response variable (dependent ejaculated or not) was created from whether at the time of observation the bull managed to ejaculate, either in the ground or ejaculate in the artificial vagina. The explanatory variable (independent 9 factors) was created from the number of events manifested by the bulls in the observations during semen collections.

The explanatory variable was defined in three categories, the first is whether the bull performed between zero and three parameters, this category being the reference category; the second category if the bull performed between four and six parameters and the third is if the bull performed seven to nine of the parameters.

In this context, a multilevel logistic regression analysis was performed using the answers and explanatory variables described above, which were controlled by the bull, the variance of the bull was controlled as a random variable and tested according to the Wald test. The bulls (n = 30) had 12 follow-up moments, and a model with all observations was performed (Dohoo *et al*., 2010).

RESULTS

The average frequencies of events related to the sexual behavior of 30 buffalo bulls, observed during the favorable reproductive period and unfavorable reproductive period, were studied in a particular property (Table 1) for four years (2014-2017). As well as the physical and morphological characteristics of the natural ejaculation of each bull and correlations between the parameters of sexual behavior are presented in Tables 2 and 3.

In table 4 it is possible to observe the variables between the parameters that are associated with the probability of ejaculation.

In table 5 it is possible to verify the overall sexual performance of 30 bulls during the evaluation years.

DISCUSSION

The results described in Tables 1, 2 and 3 come from an unfavorable reproductive period (2014) and three favorable reproductive periods (2015 to 2017), totaling twelve collection sessions for each bull with an artificial vagina, during a time of up to 15 minutes of tolerance per animal. This time was determined due to the buffalo male being considered of lower libido than the bulls of the bovine species (Vale *et al*., 2008), requiring more time to perform the jumps and consequently the copulations. This fact is attributed to a lower circulation of androgens (Nikan *et al*., 2005). Therefore, the reaction time of the buffalo male may be slightly longer than that observed in cattle, around 10 to 15 minutes (Sansone, 2000). However, bulls already conditioned to the collection with artificial vaginas, need only 1.9 minutes to perform the jump and have their semen collected (Henry *et al*., 2017a). It was also verified that the reaction time of the bulls seems not to be influenced by the seasons (Dexit *et al*., 1984; Sing *et al*., 2001; Samo *et al.*, 2005; Quezada-Casasola *et al*., 2016).

Table 1. Testicular and weight biometrics, and physical and morphological characteristics of buffalo semen (30 animals) collected during conditioning with artificial vagina for up to 15 minutes (12 sessions/animal), values referring to the best ejaculate obtained from each bull

Bulls	Parameters												
	Race	Age (years)	ECC (1-5)	Weight (kg)	PE (cm)	Aspect	Volume (mL)	Tourbillon (0-5)	Motility (%)	Vigor (0-5)	Concentration (x10^6/mL)	DM (%)	N (%)
1	Mur	9	4	900	40	milky	2.9	3	90	4	780	9	80
2	Mur	8	4	920	38	creamy	3.5	3	85	3	1960	10	80
3	Mur	6	3	740	37	creamy	3.2	4	95	5	1440	7	85
4	Mur	6	4	650	32.5	milky	4.0	3	80	4	660	11	83
5	Mest.	6	4	870	37.5	milky	3.5	4	90	5	1220	4	90
6	Med	11	3	750	40	aque.	3.0	3	90	5	1195	7	85
7	Mest.	9	3	890	39	aque.	2.1	2	60	2	315	17	65
8	Mur	9	4	840	38	aque.	1.1	2	50	1	125	21	65
9	Mur	4	5	1200	34	milky	2.1	3	70	2	360	10	80
10	Mur	3	3	700	33	aque.	1.4	1	60	2	165	12	75
11	Mest.	4	3	650	35	aque.	-	-	-	-	-	-	-
12	Mur	4	4	720	34	aque.	2.3	2	60	1	135	16	70
13	Mur	6	4	790	35	aque.	1.1	3	70	2	315	11	85
14	Mur	4	4	680	34	milky	0.8	2	60	1	280	19	75
15	Mur	4	3	700	35	aque.	1.1	1	70	2	330	10	80
16	Mur	11	3	820	36	milky	2.2	3	70	2	470	9	80
17	Med	3	3	580	32	aque.	1.4	1	60	1	115	13	75
18	Med	3	4	620	33	aque.	0.5	0	50	1	85	13	70
19	Mest.	5	4	650	35	milky	1.2	1	50	2	225	16	65
20	Mur	4	3	715	34	-	-	-	-	-	-	-	-
21	Mur	4	4	550	32	milky	1.5	2	70	2	500	10	75
22	Mur	4	3	600	31	aque.	2.0	3	70	2	490	11	70
23	Mur	5	4	750	30	milky	2.1	4	80	4	900	8	85
24	Mur	4	3	720	33	aque.	1.9	3	70	3	850	9	80
25	Med	3	3	630	32	milky	2.0	3	90	3	565	7	82
26	Mest.	4	4	660	33	-	-	-	-	-	-	-	-
27	Mur	5	4	715	34	aque.	3.3	3	85	3	490	9	81
28	Mur	4	5	835	36	aque.	1.5	3	75	2	398	10	79
29	Mur	5	4	735	35	milky	2.7	2	60	2	496	12	65
30	Mur	4	3	780	34	milky	3.6	4	70	3	668	9	79
X		**5.4**	**3.6**	**745.3**	**34.7**		**2.1±**	**2.5**	**71.5**	**2.6**	**575.3**	**11.1**	**77.2**
±DV	**-**	**±2.3**	**±0.6**	**±129.4**	**±2.3**	**-**	**1.0**	**±1.1**	**±13.4**	**±1.3**	**±449.0**	**±3.9**	**±7.1**

Aque. = aqueous; ECC: body condition score; PE: scrotal perimeter; DM: major defects; N: normal; Mur: Murrah; MED: Mediterranean; Mest.: Mestizo; X: average and DV: Standard deviation.

Table 2. Average frequencies of events related to the sexual behavior of buffalo bulls (n=30), during conditioning for semen collections with artificial vagina for up to 15 minutes (12 sessions/animal), in favorable (autumn and winter) and unfavorable (spring and summer) reproductive seasons from 2014 to 2017

Parameters	Number of attempts (%/n)									Accumulated (%/N)			
	1	2	3	4	5	6	7	8	9	1-3	1-6	1-9	X/DV
Smelling urine in the soil	49.2 /63	58.0 /100	34.4 /125	16,7 /18	-	-	-	-	-	43.1 /360	44.1 /360	-	1.3±0.9
Smell vulva	100.0 /5	69.1 /55	47.8 /138	25.0 /92	20.0 /15	100.0 /1	-	-	-	35.6 /360	44.1 /360	-	1.3±0.9
Lick vulva	100.0 /4	92.0 /25	61.1 /72	39.6 /91	29.9 /77	13.8 /29	12.5 /8	-	-	23.2 /360	43.8 /360	44.1 /360	2.2±0.9
Flehmen reflex	100.0 /11	56.6 /113	37.1 /140	18.4 /38	33.3 /3	100.0 /1	-	-	-	41.5 /360	44.1 /360	-	2.2 ±0.8
Scratch your head on ♀	68.6 /67	40.2 /102	41.6 /89	24.4 /41	20.0 /5	0.0 /1	0.0 /1	-	-	40.5 /360	44.1 /360	-	1.4±0.9
Headbutt the ♀	53.9 /117	42.9 /105	33.3 /72	30.0 /10	0.0 /1	0.0 /1		-	-	43.1 /360	44.1 /360	-	0.9±0.8
Support the chin on the ♀	46.3 /149	50.5 /49	25.0 /52	40.0 /5	100.0 /1	-	-	-	-	43.1 /360	44.1 /360	-	0.7±0.9
Penile contraction	83.3 /10	100.0 /3	92.3 /13	91.2 /34	72.6 /51	46.8 /47	31.6 /38	14.7 /34	8.0/20	8.2 /360	37.6 /360	44.1 /360	0.8±0.9
Exposure of the penis	59.1 /132	41.8 /110	19.6 /56	0.0 /7	0.0 /1	-	-			44.1 /360	-	-	0.7±1.8
M. inc. w/o penis exp.	57.7 /156	36.5 /96	20.0 /50	0.0 /3	0.0 /1	-	-	-		44.1 /360	-	-	1.3±0.9

% = percentage of attempts with ejaculation; n = number of attempts; ♀ = female; M.inc. s/exp. of the penis = incomplete mount without exposure of the penis and($p<0.05$).

Table 3. Coefficients of phenotypic linear correlations between sexual behavior characteristics in adult buffalo bulls (n=30) submitted to semen collection with artificial vagina during favorable reproductive seasons (autumn and winter) and unfavorable reproductive seasons (spring and summer) between 2014 and 2017

Parameters	Smell urine in soil	Smell vulva	Lick vulva	Reflection of Flehmen	Scratch the head on ♀	Headbutt on♀	Support the chin on ♀	Contraction penile	Exposure of the penis
Smell vulva	0.3291839^{ns}								
Lick vulva	0.9706328***	0.4980601^{ns}							
Flehmen reflex	0.3445921^{ns}	0.9640989**	0.4067813^{ns}						
Scratch your head on ♀	0,7629877^{ns}	0.215457^{ns}	0.9427476**	0.1425599^{ns}					
Headbutt the ♀	0.7725079^{ns}	0.263288^{ns}	0.9434002**	0.0991088^{ns}	0.8444553*				
Support the chin on the ♀	0.1594234^{ns}	-0.5929301^{ns}	0.315879^{ns}	-0.4454725^{ns}	-0.4506901^{ns}	-0.7391352^{ns}			
Penile contraction	0.7699267*	0.5358988^{ns}	0.8460538**	0.471928^{ns}	0.8037585**	0.8037663**	0.6634858*		
Exposure of the penis	0.8847054^{ns}	0.5508588^{ns}	0.9516336***	0.465555^{ns}	0.931495*	0.9724389*	-0.3291481^{ns}	0.5859942*	
Mounts incompl./ penis exp.	0.844991^{ns}	0.5545297^{ns}	0.9439896***	0.4747785^{ns}	0.9569032**	0.9753304*	-0.3461315^{ns}	0.5790225^{ns}	0.9888312**

^{ns} = Not significant; * = low correlation (p<0.05); ** = moderate correlation (p<0.01); *** = high correlation (p<0.001) and ♀ = female.

Table 4. Mixed final logistics for variables associated with semen collection from 30 buffalo bulls in 12 sessions (n = 360) between 2014 and 2017

Variable	Odds ratio	EP	P
0-2 parameters	1	Reference class	
3 to 6 parameters	9.0	1.8	0.07
> 6 parameters	28.2	2.7	0.00
Variance between bulls	1.2	-	-

Odds ratio = chance of occurring.

Table 5. General sexual performance of buffalo bulls (n=30) in 12 collection sessions (n=360) during the years 2014 to 2017

Bulls (n)	Ejaculation (%/n)		Jumps (%/n)	
	Successful attempts	Unsuccessful attempts	Attempts effected	Attempts refused
24	57.3(165)	42.7(123)	50.4(145)	49.7(143)

n = number; % = percentage; Bulls = 30 and Total collection sessions = 360.

Among the 30 bulls evaluated, the largest number of bulls is of the Murrah breed with 21 animals. All animals were over 3 years of age, considered sexually mature (Fischer and Bodhipaksha, 1992; Gordon, 1996; Henry *et al*., 2017b), with ECC above 3 and an average weight of 745.3 kg. The animals had good nutrition and weight development conditions. All the bulls had sexual experience in the field.

For PE, an average value of 34.7 cm was obtained, and all animals presented PE above the minimum value 30 cm for animals older than three years (Manual…, 2013; Henry 2017b), thus being able to be used in reproduction when using the PE parameter for the selection of breeders. However, although important, this parameter alone does not guarantee that the bull can be used as a breeder. Thus, the physical and morphological characteristics of semen are of paramount importance for the recommendation and release of a buffalo for reproduction.

In table 1 it is possible to observe that only the first six and the last two bulls of the ratio reached the minimum values of seminal quality (whirlwind, motility, and vigor) recommended by the manual for andrological examination and evaluation of animal semen (Manual…, 2013)

and that would allow its release for mounting in the field. However, it is necessary to make a caveat, that all these animals were in batches of cows and carrying coverings. This may justify a lower semen quality of some bulls, due to sexual exhaustion due to a high bull:cow ratio verified in some batches (reaching in some cases 1:100).

However, one must be alert, as animals with good phenotypic and weight characteristics sometimes do not have enough spermatic characteristics to be allocated together with the breeding females. In this particular property, gynecological examinations by rectal palpation in the years 2014 (November), 2015 (February), 2016 (March) and 2017 (April) diagnosed high percentages of non-pregnant buffaloes [45.6% (230/504), 41.7% (148/355), 47.8% (215/450) and 30.4% (157/517), respectively], after the females had spent all the reproductive seasons with bulls. Given this finding, it is important to perform andrological examinations on the breeders before the beginning of the reproductive season.

In the general average of the sperm parameters for the bulls evaluated (Table 1), it is possible to verify that the volume, motility, concentration and percentage of normal sperm are above the values recommended by the manual for andrological examination and evaluation of animal semen (Manual…, 2013) for the bubaline species, with mean values of 2.1mL, 71.5%, $575.3x10^6$ SPTZ/mL and 77.2%, respectively. Henry *et al*. (2017a) reported similar mean values for the same characteristics studied when evaluating 13 bubaline breeders for 4 years, totaling more than 1460 collections.

However, ejaculates with higher volumes (3-4mL) were found by (Koonjaenak and Rodriguez-Martinez, 2007; Sajjad *et al*., 2007) in Nili-Ravi ejaculates. In a previous study (Zorzetto *et al*., 2016), they reported having obtained a volume of 3.6 to 4.5 mL in Murrah bulls. These results are consistent with the results obtained in this study, in which bulls aged 3 to 11 years had mean volumes of 2.1 mL, as mentioned above. However, they are lower than the 4 to 8 mL that is usually obtained in fully mature adult bulls (Nikan *et al*., 2005; Ohashi, 2008; Vale, 2011).

For the parameters of tourbillon and vigor and major defects, the average values found (2.5, 2.6 and 11.1%, respectively) are below that recommended by the manual for andrological examination and evaluation of animal semen (Manual…, 2013). For Henry *et al*. (2017a) these low values only reflect the time of collection.

Probably several of these bulls, which did not reach the minimum parameters of seminal quality, could do so, if more time were offered to demonstrate (>15 minutes for interaction with the female), given that the animals were evaluated in 12 sessions of attempts at collections in at most 45 days, some animals exposed to this atypical environment and in the human presence, would probably require more time to acquire confidence and perform the jumps and ejaculations. Probably a change of feeding, a sexual rest, reduce the number of females per bull, since on the property was observed bull:cow ratio of up to 1:100 in some lots. But some animals would likely be taken out of breeding if a detailed andrological examination had been carried out before the start of the breeding season. As a result, the percentage of empty cows at the end of the reproductive season would probably be much lower than reported.

As for the larger defects, in this study, a predominance of strongly bent or curled tails was observed, followed by abnormal contour defects. The minor defects most found were a curled or curled tail and a curled tail in the terminal portion. These results contradict (Saeed *et al*., 1990), who reported in Murrah bulls a higher incidence of pathologies in the sperm head (5.8%), while the intermediate part abnormalities were less than 1%. These same authors reported that the appearance of cytoplasmic gout was less than 1%, a value like that of the present study. However, Zorzetto *et al*., 2016 reported that among the minor defects distal cytoplasmic gout was the second largest defect found. This may have occurred if the age, treatment, protocols, number of semen harvests and management used by the authors are different from the one used in the present study, influencing the different changes in buffalo spermatozoa. However, both studies present percentages of total sperm morphological defects within the acceptable range recommended by the manual for andrological examination and evaluation of

animal semen (Manual…, 2013), which is < 30%.

The creamy aspect presented the highest sperm concentration in 1960 and $1440x10^6$sptz/mL, followed by the milky aspect. However, half of the bulls that ejaculated 50% (11/22) had watery-looking ejaculates. The results for the highest concentration obtained from two bulls for the creamy aspect differ from those cited by (Hafez and Hafez, 2004), who reported that the highest concentration of spermatozoa was presented for the milky aspect.

However, the warning is that often, animals with good phenotypic and weight characteristics sometimes do not have enough spermatic characteristics to be allocated together with the breeding females. In this particular property, gynecological examinations by rectal palpation in the years 2014 (November), 2015 (February), 2016 (March) and 2017 (April) diagnosed high percentages of non-pregnant buffaloes [45.6% (230/504), 41.7% (148/355), 47.8% (215/450) and 30.4% (157/517), respectively], after the females had spent all the reproductive seasons with bulls. Given this finding, it is important to perform andrological examinations on the breeders before the beginning of the reproductive season.

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For the parameters of tourbillon and vigor and major defects, the average values found (2.5, 2.6 and 11.1%, respectively) are below that recommended by the manual for andrological examination and evaluation of animal semen (Manual…, 2013). According to (Henry *et al*., 2017a) these low values may reflect only the moment of collection.

Probably several of these bulls, which did not reach the minimum parameters of seminal quality, could do so, if more time were offered to demonstrate (>15 minutes for interaction with the female), given that the animals were evaluated in 12 sessions of attempts at collections in at most 45 days, some animals exposed to this atypical environment and in the human presence, would probably require more time to acquire confidence and perform the jumps and ejaculations. Probably a change of feeding, a sexual rest, reduce the number of females per bull, since on the property was observed bull:cow ratio of up to 1:100 in some lots. But some animals would likely be taken out of breeding if a detailed andrological examination had been carried out before the start of the breeding season. As a result, the percentage of empty cows at the end of the reproductive season would probably be much lower than reported.

As for the larger defects, in this study, a predominance of strongly bent or curled tails was observed, followed by abnormal contour defects. The minor defects most found were a curled or curled tail and a curled tail in the terminal portion. These results contradict (Saeed *et al*., 1990), who reported in Murrah bulls a higher incidence of pathologies in the sperm head (5.8%), while the intermediate part abnormalities were less than 1%. These same authors reported that the appearance of cytoplasmic gout was less than 1%, a value like that of the present study. However, Zorzetto *et al*., 2016 reported that among the minor defects distal cytoplasmic gout was the second largest defect found. This may have occurred if the age, treatment, protocols, number of semen harvests and management used by the authors are different from the one used in

the present study, influencing the different changes in buffalo spermatozoa. However, both studies present percentages of total sperm morphological defects within the acceptable range recommended by the manual for andrological examination and evaluation of animal semen (Manual…, 2013), which is < 30%.

The creamy aspect presented the highest sperm concentration 1960 and $1440x10^6$sptz/mL, followed by the milky aspect. However, half of the bulls that ejaculated 50% (11/22) had watery-looking ejaculates. The results for the highest concentration obtained from two bulls for the creamy aspect differ from those cited by (Hafez and Hafez, 2004), who reported that the highest concentration of spermatozoa was presented for the milky aspect.

The creamy aspect presented the highest sperm concentration in 1960 and $1440x10^6$sptz/mL, followed by the milky aspect. However, half of the bulls that ejaculated 50% (11/22) had watery-looking ejaculates. The results for the highest concentration obtained from two bulls for the creamy aspect differ from those cited by (Hafez and Hafez, 2004), who reported that the highest concentration of spermatozoa was presented for the milky aspect.

CONCLUSIONS

Buffalo bulls can be conditioned to collect semen with an artificial vagina on their farms of origin. Of all the events studied, penile contraction was the most important in the sexual behavior of buffalo bulls, demonstrated by the high proportions of manifestation in relation to all sexual behaviors expressed in the presence of a female in estrus. During the four years of evaluating the behavior and conditioning of the bulls for collection, it was found that the animals ejaculate both in the favorable reproductive period and in the unfavorable reproductive period.

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REFERENCES

ALMEIDA, J.; NEVES, B.P.; BRITO, M.F.; BECERRA, V.A.B. *et al*. Avaliação da longevidade espermática em sêmen refrigerado de Búfalos (*Bubalus bubalis*) à 5 ^oC. *Pubvet*, v.17, p.1-14, 2023.

ALMEIDA, J.; RESENDE, A.O. Condicionamento de reprodutores bubalinos para coleta e congelamento de sêmen na propriedade. In: REUNIÃO ANUAL DA ASSOCIAÇÃO BRASILEIRA DE ANDROLOGIA ANIMAL, 5., 2021, Campo Grande. *Anais*... Campo Grande: ABRAA, 2021. p.136-140.

DIXIT, N.K.; AGARWAL, S.P.; DWARAKNATH, P.K. Seasonal variations in serum levels of thryroid hormones and their relation with seminal quality and libido in buffalo bulls. *Theriogenology*, v.22, p.497-507, 1984.

DOHOO, I.R.; MARTIN, W.; STRYHN, H. *Veterinary epidemiologic research.* 2.ed. Charlottetown, Canadá: University of Prince Edward Island, 2010. 865p.

FISCHER, H.; BODHIPAKSHA, P. Reproduction in swamp buffaloes. In: TULLOH, N.M.; HOLMES, J.H.G. (Eds.). *Buffalo production*. Amesterdam, Netherland: Elsiever Science Publisher, 1992. 505p.

GORDON, I. *Controlled reproduction in cattle and buffaloes*. Willingford, UK: CAB International, 1996. p.432-466.

HAFEZ, B.; HAFEZ, E.S.E. *Reprodução animal*. 7.ed. Barueri: Manole, 2004. 513p.

HENRY, M.; BRITO, M.F.; NEVES, B.P.; AULER, P.A. *et al*. Peculiarities of the buffalo species for andrological evaluation - results of four years of study and weekly semen collection schedule. *Anim. Reprod*., v.14, p.1225-1233, 2017a.

HENRY, M.; BRITO, M.F.; NEVES, B.P.; AULER, P.A. *et al*. Exame andrológico de bubalinos. *Rev. Bras. Reprod. Anim*., v.41, p.188-194, 2017b.

KOONJAENAK, S.; RODRIGUEZ-MARTINEZ, M. Assessment of semen quality in Swamp Buffalo AI Bulls in Thailand. *Ital. J. Anim. Sci*., v.6, suppl.2, p.701-704, 2007.

MADER, D.R.; PRICE, E.O. Effect of sexual stimulation on the sexual performance of Hereford bulls. *J. Anim. Sci*., v.59, p.294-300, 1984.

MANUAL for andrological examination and evaluation of animal semen. 3.ed. Belo Horizonte: *Brazilian College of Animal Reproduction*, 2013. 104p.

NIKAN, S.R.; DESHMUKH, B.T.; VADNERE, N.B.; NAGVEKAR, A.S. *et al*. Comparative appraisal of seminal plasma lipid profile in buffalo and cow bulls. *J. Bombay Vet. Col*., v.13, p.46-49, 2005.

OHASHI, O.M. Inseminação artificial em bubalinos. In: GONÇALVES, P.B.D. *Biotécnicas aplicadas à reprodução animal*. 2.ed. São Paulo: Roca, 2008. p.97-110.

OHASHI, O.M.; SANTOS, S.S.D.; MIRANDA, M.S.; CORDEIRO, M.S. *et al*. Morfologia do sistema genital, distúrbio reprodutivo e manejo do macho bubalino (*Bubalus bubalis*). *Rev. Bras. Reprod. Anim*., v.35, p.88-94, 2011.

PRABHU, S.S.; BHATTACHARYA, P. Influence of factors affecting sex drive on semen production of buffaloes. I - Physiological state of the "teaser" cow. *Indian J. Vet. Sci. Anim*. Husband, v.24, p.35-50, 1954.

QUEZADA-CASASOLA, A.; MARTÍNEZ-ARMENDÁRIZ, K.E.; CARRERA-CHÁVEZ, J.M.; PÉREZ-EGUÍA, E. *et al*. Effect of season on scrotal circumference, semen characteristics and testosterone serum concentration in Mexican Corriente and other beef breed bulls. *Anim. Reprod*., v.13, p.787-794, 2016.

SAEED, A.; CHAUDHRY, R.; KHAN, I.H.; KHAN, N.U. Morphology of semen buffalo bulls of different age groups. In: ACHARYA, R.M. *Recent advances in buffalo research*. New Delhi: Indian Soc. Buffalo, 1990. p.17-19.

SAJJAD, S.; ALI, S.; ULLAH, N.; ANWAR, M. *et al*. Blood serum testosterone level and its relationship with scrotal circumference and semen charactristics in Nili-Ravi buffalo bulls. *Pak. Vet. J*., v.27, p.63-66, 2007.

SAMO, U.M.; BROHI, N.A.; KAKA, I.; QURESH, T.A. Study on sexual behaviour and seminal quality characteristics of Kundhi buffalo bulls. *Pak. J. Biol. Sci*., v.8, p.1628-1629, 2005.

SANSONE, G.; NASTRI, M.J.F.; FABBROCINI, A. Storage of buffalo (*Bubalis bubalus*) semen. *Anim. Reprod. Sci*., v.62, p.55-76, 2000.

SINGH, P.; SENGUPTA, B.P.; TRIPATHI, V.N. Effect of multiple showering and vitamin supplementation on sexual behavior, quality and freezability of buffalo bull semen. *Asian-Austral J. Anim. Sci.*, v.14, p.184-188, 2001.

VALE, W.G. Avances biotecnológicos em reproducción de búfalos. *Tecnol. Marcha*, Nesp., v.24, p.89-90, 2011.

VALE, W.G.; RIBEIRO, H.F.L.; SOUZA, J.; SILVA, A.O.A. *et al*. Seleção e avaliação andrológica do reprodutor bubalino. *Rev. Bras. Reprod. Anim*., v.32, p.141-155, 2008.

ZICARELLI, L. Enhancing reproductive performance in domestic dairy water buffalo (*Bubalus bubalis*). *Soc. Reprod. Fertil. Suppl*., v.67, p.443-445, 2010.

ZORZETTO, M.F.; SILVA, Y.F.R.S.; ZOCCA, S.; MONTEIRO, G.A. *et al*. Aspectos físicos e morfológicos do sêmen de búfalos da raça Murrah em maturidade sexual. *Vet. Zootec*., v.23, p.647-455, 2016.