Determination of Quality Characteristics of Kundhi Buffalo Bull Semen

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Abstract: The objective of present study was to investigate the quality characteristics of semen collected from kundhi bulls. Semen was collected by an artificial vagina and transported into laboratory immediately for evaluation. Total of 96 (Twenty four from each bull) semen the samples were evaluated and found creamy white. The mean (±SEM) were mass activity (+++), volume (2.25 ± 0.01 ml), pH (6.10 ± 0.007), progressive motility (69 ± 0.34%,) sperm concentration (1542 ± 9.20x10^6/ml), morphology (79 ± 1.37%) and sperm membrane (55.56 ± 1.37%) respectively. Non-significant (P>0.05) difference between the bulls for the characteristics except percentages morphology and sperm membrane where a significant (P<0.05) variation was found.

It was concluded that the quality characteristics of khundi buffalo bull maintained standard score considered for freezing and cryopreservation and A.I programme.

Keywords: Buffalo, bull, semen, pre freezing evaluation.

1. INTRODUCTION

The domestic water buffalo *(Bubbalis bubbalis)* is an essential animal in the agrarian economy of many countries mainly India and Pakistan [1]. The population of buffalo is about 158 million of which 153 million are found in the Asia [2]. Pakistan has Kundhi and Nilli Ravi buffalo breeds possess qualities of adaptation under stressful environment; they have achieved the reputation of being best multipurpose breeds in the tropical and subtropical world [1].

Pakistan shares 28.4 million buffaloes from total buffalo population of world. They are the main dairy animals in the our country and share around 71% of the milk and 49% of meat production. In addition to milk and meat, buffaloes also support in draught power and agricultural operations [3].

Artificial insemination (AI) is the only tool which is used for improvement of animal genetic. The productive efficiency of buffalo is being improved by superior sires produced through artificial insemination. Laboratory assessment of sperm quality before AI is important semen preservation technology, which includes sperm motility, morphology, and structural integrity.

Therefore, the current study was conducted to examine and establish basic norms of characteristics of the semen for Kundhi Buffalo bull.

2. MATERIALS AND METHODS

This study was carried out at the Department of Animal Reproduction, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University Tando jam. The sample and data were recorded for three months on evaluating the parameters such as volume, color, pH, concentration, mass activity, motility, morphology and sperm membrane.

2.1. Experimental Animals and their Management

Four bulls with an age range of 3 to 4 years were used in the study. Each bull was housed in individual bull pen. Seasonal green fodder wheat straw, concentrate (cotton seed cake) and clean water were provided. Seasonal vaccine and Drenching were done.

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2.1.1. Semen Collection

Samples were collected twice a week for 90 days through an artificial vagina (AV). Preparation of AV was done by the procedure described by [4] maintaining the inner temperature of AV at 42°C - 45°C. Two samples were collected on every collection and processed after pooled together.

2.1.2. Semen Evaluation

Semen samples were collected and brought into laboratory for evaluation. It was examined for the following using stated methods.

2.1.3. Colour

Semen colour was examined visually direct from collected tube and was classify as milky, creamy, creamy white and translucent as described by [5].

2.1.4. Volume

Volume was recorded through graduated collection tube.

2.1.5. pH

pH of the semen was obtained by using digital pH meter made up of Eutech company having serial number 67460.

2.1.6. Mass Activity and Progressive Motility

The mass activity was evaluated by placing a drop of semen on pre warmed sterilized glass slide according to method described by [18].

2.1.7. Sperm Concentration

Sperm concentration was determined using a hemocytometer as described by [18].

2.1.8. Assessment of Sperm Membrane (ORT)

Fresh semen was used for the Hypo Osmotic Swelling (HOS) test using the method developed in cattle [6].

2.1.9. Morphological Characteristics

These were determined by eosin–nigrosin staining procedure described by [8].

2.2. Statistical Analysis

Data was analyzed by ANOVA and Least significant difference (LSD). The data are presented as mean ± standard error of mean.

3. RESULTS

The study was conducted on four young Kundhi buffalo bulls for three months. Ninty six (Twenty four from each bull) semen samples were utilized for this purpose. The results are described below.

3.1. Macroscopic Characteristics of Fresh Semen of Kundhi Buffalo Bull

Volume and pH values of each bull is presented in Table **1**. And it was observed that there was no significant difference ($p>0.05$) among bulls. In the current study, all the four bulls produced creamy white semen. It was thick in consistency.

3.2. Microscopic Characteristics of Fresh Semen of Kundhi Buffalo Bull

The mean (± SEM) of Microscopic characteristics of fresh semen of Kundhi buffalo bull is presented in Table **2**. Motility and concentration were observed non significant. However, morphology and membrane integrity was observed significant.

The fresh sperm exhibit a typical swirling movement under microscopic field called mass activity. It was found to be +++ in all the Kundhi buffalo bulls. The sperms were making rapid swirling motion forming eddies at the end of each motion (Table **2**).

4. DISCUSSION

Decline in semen quality is common in hot seasons due to sensitivity of buffalo to heat stress. This study

Table 1: Macroscopic Characteristics of Fresh Semen of each Kundhi Buffalo Bull

Bull no	Volume (ml)		Colour	pH	
	Mean±SEM		Creamy White	Mean±SEM	
1	2.2	0.12	Creamy White	6.32	0.09
2	2.2	0.12	Creamy White	6.02	0.09
3	2.26	0.11	Creamy White	6.02	0.10
4	2.24	0.15	Creamy White	6.06	0.08
Mean	2.25	0.01	Creamy White	6.10	0.07

Table 2: Microscopic Characteristics of Fresh Semen of Kundhi Buffalo Bull

Bull NO	Motility %		Mass activity	Concentration m/ml		Morphology%	
	Mean±SEM			Mean±SEM		Mean±SEM	
1	68	1.30	+ + +	1548	19.85	77*	2.00
2	69.4	1.96	+ + +	1518	32.31	82.2**	1.02
3	69.4	1.75	+ + +	1540	12.25	81.2*	0.8
4	69.2	1.59	+ + +	1562	16.85	77*	2
Mean	69	0.34	+ + +	1542	9.20	79.35	1.37

LSD = morphology = 4.66.

was also performed during summer months of the year. The results are discussed under appropriate headings.

Table 3: Mean (± SEM) Sperm Cells having Intact Sperm Membrane of Fresh Semen

Bull number	ORT reacted cells	
	Mean	±SEM
1	60.4	1.63
2	49.6	1.50
3	56.6	1.66
4	56	1.39
Mean	55.56	2.24

LSD= sperm membrane = 4.64.

Volume of buffalo semen varies from 2 to7ml [9;19] depending on season, age, breed [10]. The mean (±SEM) volume (2.25 ± 0.015ml) found in this study was lower than those reported by [11] and [12] in Kundhi buffalo. The variation might be due to the difference in age of bulls. The bulls used in the present study were very young and were under training. The results reported by [9] in Azerbaijani buffalo and [14] and [10] in Murrah buffalo are similar to present study.

The pH of buffalo semen ranges 6.4 to 7.4 [10]. Slight variation has been reported due to the season [9]. The Mean (±SEM) pH observed (6.1 ±0.0722) agrees with the results reported by [11 and 12] in Kundhi buffalo bulls. The results reported by [9] in Azerbaijani buffalo bulls, and [10] in Murrah buffalo bulls also fall in the range of present study. In some cases the pH is reported as 5.1-6.53 [19]. However variation in pH values might be due to the activity of sperms and life of the semen producing more lactic acid making semen acidic, but it was not within the lethal level.

Buffalo semen usually varies from white to translucent [5] in colour depending on variation in concentration of sperm [10]. Colour recorded was creamy white semen. The same was also observed by [11] in Kundhi buffalo semen and others [9; 10; 14; 19; 20] in different breeds of buffalo.

The Motility of the spermatozoa used for freezing in most of the A.I organization is above 60 percent [15]. Mean (±SEM) motility percentage observed (69±.3367) in the current study was higher than that of [12] in Kundhi buffalo breed. Difference in motility supposed to due to age and season of bulls, as the sperm in warm climatic condition are more active utilizing more energy more sources. The results reported by [9;10; 15] in various buffalo breeds are similar.

Mass activity found (+++) was higher than those reported by [11] and [12] in Kundhi buffalo bulls. This difference in mass activity may be due to the age of bulls and the season during which semen was collected. The results reported by [13;15;16] in different buffalo breeds are similar to current study.

Sperm concentration varies per unit volume semen, different ejaculates and time of collection. Mean (±SEM) sperm concentration observed in the (1542±9.201 $x10^{6/}$ml) was lower than that observed by [12] in Kundhi buffalo bulls as the bulls of the current study were very young and still are under training. This difference may be due to varriation in age of bulls. [14] observed 1031 $x10^{6}$l in Murrah buffalo bred in India and [9] 1281 $x10^{6}$ ml in Azerbaijani buffalo, [10] 1200 $x10^{6}$ ml in Italian buffalo, (Aguir *et al.* 1994) 1166 $x10^{6}$ ml in brazil. This difference in sperm concentration might be due to breed difference.

Buffalo sperm has distinct morphological features. A typical buffalo bull spermatozoa is shoter than Boss taurus bulls and measures 62 micron. Mean (±SEM) normal morphology of sperm observed in current study (79.35±1.372%) corroborates with the results of [9] in Azerbaijani buffalo bulls, [14] in Murrah buffalo bred in

India and [17] in Brazilian buffalo reported results similar.

Plasma membrane is of prime significance during the freezing and fertilizing ability of sperm. Mean (±SEM) of plasma membrane observed (55.56±2.24%) was lower than that observed by [15] in Nilli Ravi buffalo bulls. The difference might be due to breed variation.

5. CONCLUSION

On the basis of present study, it has been concluded that the Kundhi buffalo semen could be cryopreserved. Objective methods developed for evaluation of cattle semen can be used buffalo bull semen.

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