Journal of Veterinary Science & Technology

ISSN: 2157-7579

Srinivas et al., J Vet Sci Technol 2016, 7:6
DOI: 10.4172/2157-7579.1000391

Research Article Open Access

Semen Characteristics of Normal and Low Grade Ejaculates in Ongole (*Bos indicus*) Bulls

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Abstract

The normal and low-grade ejaculates collected from the eight bulls under study were evaluated of various semen parameters. The overall means of HOS-G sperm was recorded as 56.50 ± 0.50 and 30.35 ± 0.66 percent for HOS positive and acrosome positive (HPAP), 17.30 ± 0.54 and 19.48 ± 0.66 percent for HOS positive and acrosome negative (HPAN), 18.73 ± 0.54 and 30.11 ± 0.89 percent for HOS negative and acrosome positive (HNAP) and 7.64 ± 0.47 and 19.63 ± 0.94 percent for HOS negative and acrosome negative (HNAN) in fresh semen of normal and low grade ejaculates, respectively. The overall mean sperm penetration distance was recorded as 19.75 ± 0.19 and 14.40 ± 0.19 mm/20 min in normal and low grade ejaculates (Table 1) with a highly significant difference between them (P<0.01). The sperm penetration distance (mm/20 min) differed only amongst bulls of low grade ejaculates and not among those of normal ejaculates. It was concluded from the present study that routine semen evaluation tests which are mainly subjective in nature suffered human bias and have limited ability to predict the fertilizing capacity of the sperm, hence still more sensitive tests like functional assessment of plasma membrane and acrosome membrane (HOS-G Test) and sperm penetration distance in synthetic medium are essential to predict optimum fertility.

Keywords: Semen characteristics; HOS-G; Sperm penetration distance; Normal ejaculates; Low-grade ejaculates; Ongole bulls

Introduction

Reproductive soundness evaluation facilitates the elimination of sub-fertile sires from breeding programmes. Andrological investigation of breeding bulls is important to evaluate them for their fertility which includes scrotal and testicular biometry, ultrasonography of male reproductive tract and testes and laboratory evaluation of semen quality with fertility [1]. It has been noticed that in indigenous as well as cross-bred bulls a huge percentage of ejaculates are discarded due to high proportion of abnormal sperms and their inability to withstand to freezing [2,3]. Earlier studies on semen from indigenous Gir and Jafrabadi bulls was observed to be consistently poor in its quality and freezability, in spite of proper inputs in health care, feeding and management. The quality of low-grade ejaculates could be improved by filtration methods, which removes the dead, non-motile and abnormal sperms. Thus studies on low grade ejaculates are of immense value in exploiting the breeding potential of genetically superior sires giving poor semen which was destined to be discarded [4]. Therefore, keeping in view of the above facts, the present study was attempted to evaluate the membrane integrity and sperm penetration distance of normal and low grade ejaculates in Ongole breeding bulls.

Materials and Methods

Semen was collected from eight bulls under study once in a week between 6.00 AM and 7.00 AM with the standard artificial vagina using an anoestrus cow as a dummy. Each time two ejaculates were collected at a gap of 20 to 30 minutes after allowing one or two false mounts. Soon after the collection, the semen collection tubes were numbered and transferred to a water bath at 37°C. During the period under report a total of 549 ejaculates were collected from the eight bulls under study they included normal ejaculates and low grade ejaculates discarded at various stages of semen processing. Ejaculates having initial motility between 40 and 50% were classified as low grade as per the method of Ahmed et al. [5]. Functional membrane integrity was assessed by hypo-osmotic swelling-Giemsa (HOS-G) test. 1 ml of hypo osmotic swelling solution (100 mOsmol/L) was mixed with 0.1 ml of semen and incubated at 37°C for 1 hour. Similarly, 0.1 ml of semen was incubated in isotonic solution (300 mOsmol/L). After incubation at 37°C for 30 minutes one drop of well-mixed sample was smeared on a warm glass slide and then stained with Giemsa. Minimum of 200 spermatozoa were observed in a phase-contrast microscope under oil immersion and expressed in percentage for different subpopulations. Sperm penetration distance was carried out as per the method described by Kumar [6]. Polyacrylamide gel a synthetic migration medium was prepared as per the method described by Lorton et al. [7] with slight modification.

Results

The overall means of HOS-G sperm was recorded as 56.50 ± 0.50 and 30.35 ± 0.66 percent for HOS positive and acrosome positive (HPAP), 17.30 ± 0.54 and 19.48 ± 0.66 percent for HOS positive and acrosome negative (HPAN), 18.73 ± 0.54 and 30.11 ± 0.89 percent for HOS negative and acrosome positive (HNAP) and 7.64 ± 0.47 and 19.63 ± 0.94 percent for HOS negative and acrosome negative (HNAN)

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Received February 29, 2016; **Accepted** October 07, 2016; **Published** October 12, 2016

Citation: Srinivas M, Sreenu M, Srilatha CH, Rao BK, Naidu K (2016) Semen Characteristics of Normal and Low Grade Ejaculates in Ongole (*Bos indicus*) Bulls. J Vet Sci Technol 7: 391. doi: 10.4172/2157-7579.1000391

in fresh semen of normal and low grade ejaculates, respectively (Table 1).

HPAP, HPAN, HNAP and HNAN percentages differed significantly between normal and low grade ejaculates as well as between the bulls in both grades of ejaculates (Table 2). HPAP sperm percent of normal fresh semen had a highly significant negative correlation on HNAN sperm percent of fresh semen ($P<0.01$) and a significant positive correlation with live sperm percent and HPAP sperm percent of post diluted semen ($P<0.05$), while the HPAP sperm percent of fresh low grade semen had a highly significant positive correlation with HPAP sperm percent post diluted low grade semen ($P<0.01$).

The overall mean sperm penetration distance was recorded as 19.75 ± 0.19 and 14.40 ± 0.19 mm/20 min in normal and low grade ejaculates (Table 1) with a highly significant difference between them ($P<0.01$). The SPD (mm/20 min) differed only amongst bulls of low grade ejaculates and not among those of normal ejaculates (Table 2). Sperm penetration distance of fresh semen had a significant negative correlation with abnormal sperm count ($P<0.05$), while the same for low grade semen revealed a significant positive correlation with post diluted HNAP sperm percent and post diluted sperm penetration distance ($P<0.05$). Sperm penetration distance of normal fresh semen had a significant positive correlation with fertility ($P<0.05$), while it had a non-significant negative correlation in low grade ejaculates.

Discussion

In the present study HOS positive and acrosome positive (HPAP) percent differed significantly between normal and low grade ejaculates ($P<0.05$) and had a highly significant difference between the bulls in both the grades of semen ($P<0.01$). HOS positive and acrosome negative (HPAN) percent differed non significantly between normal and low grade ejaculates and had a highly significant difference between the bulls in both the grades of semen ($P<0.01$) which was in agreement with the observations of Sushant and Kumar [8].

HPAP sperm percent of normal fresh semen had a non-significant positive correlation with fertility, while the HPAP sperm percent of low grade fresh semen had a non-significant negative correlation with fertility. Kumaresan et al. [9] opined that HOS test evaluates the biochemical intactness plasma membrane as normal, intact active sperm plasma membrane was essential for fertilization to occur.

The overall mean sperm penetration distance recorded in the present investigation was in corroboration with those of Kumar et al. [10] while Sushant and Kumar [8] reported higher sperm penetration distance values assessed for 60 minutes in Holstein Friesian bulls and their crosses and concluded that the values differed significantly between bulls and breeds as observed in the present study.

Sperm penetration distance of normal fresh semen had a significant negative correlation on abnormal sperm count of fresh semen ($P<0.05$) and had a significant positive correlation with fertility ($P<0.05$), while it had a non-significant negative correlation in low grade ejaculates as spermatozoal characteristics such as motility, morphology, concentration and acrosome integrity significantly affect the sperm penetration as opined by Anilkumar et al. [11].

It was concluded from the present study that routine semen evaluation tests which are mainly subjective in nature suffered human bias and have limited ability to predict the fertilizing capacity of the sperm, hence still more sensitive tests like functional assessment of plasma membrane and acrosome membrane (HOS-G Test) and sperm penetration distance in synthetic medium are essential to predict optimum fertility as reported by Kumaresan et al. [9]. These tests have been performed in the present investigation with ease and produced consistent results and hence could be adopted for routine semen evaluation as synthetic medium (2% polyacrylamide gel) could be stored in the refrigerator for prolonged use to evaluate sperm penetration distance as availability of bovine cervical mucus is restricted and could not be stored for prolonged usage.

Parameter	Ejaculate Quality	408	409	417	424	426	465	1011	1027	Over All Mean
HOS-G HPAP (%)	Normal	59.90 ± 0.55^{a}	56.70 ± 1.41^{ab}	58.70 ± 1.12^{ab}	55.60 ± 1.60^{b}	57.60 ± 1.26^{ab}	55.40 ± 1.30^{b}	57.90 ± 0.72^{ab}	50.20 ± 0.83^{c}	56.50 ± 0.50^{A}
	Low Grade	35.00 ± 1.89^{ab}	28.50 ± 0.81^{cd}	31.10 ± 2.00^{bc}	30.70 ± 1.43^{bc}	30.70 ± 1.16^{bc}	25.20 ± 1.37^{d}	36.70 ± 1.45^{a}	24.90 ± 1.17^{d}	30.35 ± 0.66^{B}
HOS-G HPAN (%)	Normal	13.50 ± 0.43^{c}	20.30 ± 1.57^{ab}	16.40 ± 1.62^{bc}	16.00 ± 0.92^{bc}	15.90 ± 1.41^{bc}	20.80 ± 1.37^{a}	16.10 ± 1.03^{bc}	19.40 ± 2.13^{ab}	17.30 ± 0.54^{B}
	Low Grade	18.80 ± 1.55^{abc}	14.70 ± 1.31^{c}	17.90 ± 1.92^{bc}	17.00 ± 1.92^{bc}	21.10 ± 1.53^{ab}	20.90 ± 1.39^{ab}	21.50 ± 1.38^{ab}	23.90 ± 2.48^{a}	19.48 ± 0.66^{A}
HOS-G HNAP (%)	Normal	22.50 ± 0.78^{a}	16.40 ± 1.19^{bc}	20.30 ± 0.73^{ab}	22.60 ± 1.19^{a}	18.70 ± 1.52^{abc}	14.70 ± 1.09^{c}	17.70 ± 1.10^{bc}	16.90 ± 2.34^{bc}	18.73 ± 0.54^{B}
	Low Grade	32.60 ± 1.38^{b}	42.90 ± 1.35^{a}	28.20 ± 1.25^{bc}	32.90 ± 1.92^{b}	24.40 ± 1.54^{cd}	27.90 ± 2.07^{bc}	29.40 ± 1.93^{bc}	22.60 ± 2.32^{d}	30.11 ± 0.89^{A}
HOS-G HNAN (%)	Normal	4.30 ± 0.45^{c}	6.60 ± 0.85^{bc}	4.60 ± 0.65^{c}	6.60 ± 1.24^{bc}	7.80 ± 1.10^{b}	9.10 ± 1.12^{b}	8.60 ± 0.99^{b}	13.50 ± 1.52^{a}	7.64 ± 0.47^{B}
	Low Grade	13.40 ± 2.35^{c}	12.10 ± 1.75^{c}	22.80 ± 2.35^{ab}	18.40 ± 2.04^{bc}	23.00 ± 1.59^{ab}	24.40 ± 2.78^{ab}	14.40 ± 1.85^{c}	28.60 ± 1.54^{a}	19.63 ± 0.94^{A}
SPD (mm/20 min)	Normal^{NS}	20.50 ± 0.45	19.10 ± 0.56	20.50 ± 0.43	20.00 ± 0.65	19.30 ± 0.30	20.30 ± 0.72	19.40 ± 0.48	18.90 ± 0.38	19.75 ± 0.19^{A}
	Low Grade	15.50 ± 0.69^{a}	14.50 ± 0.43^{abc}	15.20 ± 0.49^{ab}	14.30 ± 0.26^{abc}	13.30 ± 0.42^{c}	13.90 ± 0.55^{bc}	13.90 ± 0.31^{bc}	14.60 ± 0.60^{abc}	14.40 ± 0.19^{B}

[a] Means bearing different superscripts within a row differ significantly
[A] Overall means bearing different superscripts within a column within a parameter differ significantly

Table 1: HOS-G and sperm penetration distance of normal and low grade fresh semen (Means ± SE) in Ongole bulls.

Source of variation	df	Mean sum of squares with F value in parenthesis				
		HOS-G HPAP (%)	HOS-G HPAN (%)	HOS-G HNAP (%)	HOS-G HNAN (%)	SPD (mm/20 min)
Between groups	1	27352.900 (1000.824)**	189.225 (6.482)*	5187.006 (120.028)**	5760.000 (130.853)**	1144.900 (424.435)**
Error	158	27.330	29.195	43.215	44.019	2.697
Between groups-normal ejaculates	7	87.60 (6.62)**	66.714 (3.37)**	82.479 (4.66)**	85.684 (7.90)**	4.229 (1.61)NS
Error	72	13.233	19.483	17.675	10.843	2.631
Between groups-low grade ejaculates	7	174.857 (8.23)**	86.021 (2.88)**	393.727 (12.76)**	355.713 (8.30)**	5.171 (2.17)*
Error	72	21.225	29.831	30.860	42.840	2.375

* Significant ($P < 0.05$)
** Significant ($P < 0.01$)
NS Non significant

Table 2: Analysis of variance for HOS-G and sperm penetration distance between normal and low grade fresh semen in Ongole bulls.

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