International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2017; 5(4): 887-891

Received: 01-05-2017
Accepted: 02-06-2017

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Effect of age on fresh spermatozoa concentration and motion characteristics of frieswal bulls under computer assisted semen analyzer (CASA)

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Abstract
In this study, motion characteristics of spermatozoa was assessed by computer assisted semen analyser (CASA) for evaluating fertility potential of Frieswal bulls. Forty bulls were selected and grouped into threeon the basis of age (AG-I < 24 months old; AG-II 25-36 month and AG –III > 36 months old). The following CASA parameters i.e., velocity average path (VAP, µm/s), velocity straight line (VSL, µm/s), velocity curvilinear (VCL, µm/s), amplitude of lateral head displacement (ALH, µm) and straightness (STR) were recorded. Results of the study revealed that higher sperm concentration (1493.48±94.96 Million/ml), VAP (118.83±2.81 µm/s), ALH (11.25±1.86 µm), BCF (23.65±0.39 Hz), area of head (14.5±0.38 µm sq) and Elongation (40.51±0.1 %) in more than 36 month old bulls except VSL (µm/s), STR (67.16±0.03 %) and linearity (36.67±0.01 %) in 25 to 36 month old bulls. In most motion characteristics especially in motility and linearity of the motion, younger bulls better than older bulls. That indicated the possibility of selecting bulls at an early age on the basis of concentration of spermatozoa to save the money, space and time which otherwise spent on rearing such inferior bulls. This study also clearly indicated that CASA is a good supplementation to aid for selection of breeding bulls.

Keywords: frieswal bulls, age, spermatozoa concentration, motion characteristics, computer assisted semen analyzer (CASA)

Introduction
Semen analyses typically produce a wide variety and number of semen characteristics that are correlated, indicating that underlying measures of semen quality can be used to reduce the number of variables evaluated [1]. Frieswal is a cross bred strain being developed at Project Directorate of Cattle, Meerut, India by crossing Sahiwal with Holstein Friesian and stabilizing the exotic inheritance at 62.5 per cent. Like other cross bred bulls, the problem of poor quality semen production and preservation also exists for the strain. Since accurate morphological screening of the ejaculate allows elimination of bulls with a potential low fertility, prior to use bulls in the progeny testing programme and the preservation of semen. It is a well-known fact that sire is half the herd. The importance of genetically superior bull is to produce large number of normal fertile spermatozoa in AI programme. Again if a bull is to be used extensively for AI breeding, it becomes necessary to ensure that the bull is kept in optimum breeding condition. This implies that such methods of management have to be put in vague, which would aim at maintaining the proper physiological process of the bull and in retaining its reproductive potential over a longer period.

In this respect, the advent of Computer-assisted semen analysis (CASA) has brought a new dimension to semen evaluation. CASA is a recent laboratory tool for evaluating semen samples objectively and provides an opportunity to assess sperm kinetics more precisely, rapidly and accurately. The CASA technique yields repeatable and highly reliable results on kinematics of ejaculates based on measurements of individual sperm cells [11].

Adoption of CASA technique has been reported as the potential tool for improvements in evaluation of semen to enhance fertility [1]. Hence, it is of great interest to use a combination of semen motion characteristics which can predict bull of high fertility performance more accurately than a single test. Moore and Akhondi (1996) indicated that CASA provided significant information for determining sperm fertilizing capacity and will be a useful technique for reproductive toxicology.

Conventionally, sperm motility estimation is done by visual approximation of progressively moving spermatozoa using phase contrast microscope. The progressive motility estimation is only an assessment of 'quantity' of moving spermatozoa. However, the assessment of quality of motility in terms of velocity, swimming pattern and sperm head behaviour etc., may help in better understanding of the possible sperm function. The advent of computer assisted semen analysis (CASA) has brought a new dimension to semen evaluation. The CASA technique yields repeatable and highly reliable results on kinematics of ejaculates based on measurements of individual sperm cells [2]. Therefore, assessment of sperm motility coupled with kinetic measurements would help in better evaluation of semen quality.

2. Materials and methods

2.1 Description of the study site

The study was carried out at Central Institute of Research on Cattle (CIRC), Meerut with collbaration Sardar Vallbhbhai Patel University and Technology, Meerut, Uttar Pradesh, India. The farm is situated at an altitude of 228 m above the mean sea level on 28°58′48″ N latitude and 77°42′22″ E longitude. The range of atmospheric temperature varies from near (8°C) in winter months to about 24.7°C in summer months. The average annual rainfall is approximately 933 mm, which is received mostly during months of July to August. Relative humidity varies from as low as 25 per cent to as high as 71 per cent.

2.2 Animals and semen collection

Forty breeding Frieswal bulls (age 24 to 60 months and weight > 350 kg) were selected and were grouped into three on the basis of age groups (AGI < 24 months old; AG II 25 to 35 and more than 36 months old). Semen samples (120) were collected in the morning hours between 8:00 to 9:00 am with the help of artificial vagina (AV) using dummy bull. The bulls were thoroughly washed, cleaned and dried at least 15 to 30 min before collection. The temperature of A.V. was maintained at 42 ^{0}C by filling warm water of 45^{0}C. Prior to semen collection prepural orifice of the bulls was cleaned with soft paper napkin soaked in normal saline to avoid contamination of semen with dung or mud. Each bull was given one false mount followed by a restrain before actual collection. Semen was collected directly into a clean dry graduated centrifuge tube attached to the latex cone of the AV. Immediately after collection, tubes containing semen were marked and placed in the water bath at 32 ^{0}C.

2.3 Feeding and management of bulls

All experimental bulls were kept in individual pens made up of brick and cement with concrete floor and asbestos roof. The bull pens had the facilities of fanning and water sprinkling during stressful weather condition. The bulls fed balance ration as per standard military farm feeding schedule. They were being regularly vaccinated against important contagious and infectious diseases.

2.4 Feeding schedule of bulls

Each adult bull was offered feed viz., Burseen/Green maize/Jowar @ 30 to 40 per cent, Bhoosa: 5.0 to 5.5 kg**,** Concentrate: 3.5 to 4.0 kg and Service ration: 1 kg additional concentrate (Table 1).

2.5 Computer Assisted Semen Analysis (CASA)

CASA is a catch-all phrase for automatic or semi-automatic semen analysis techniques. Most systems are based on image analysis, but alternative methods exist such as tracking cell movement on a digitizing tablet. Computer-assisted techniques are most-often used for the assessment of sperm concentration and mobility characteristics, such as velocity and linear velocity. Now a days, there are CASA systems, based on image analysis and using new techniques, with near perfect results, and doing full analysis in a few seconds.

2.6 Sample preparation for CASA

0.1 ml of neat semen was added with tris buffer [3] according to the sample concentration (10 to 20 times). The diluted semen sample was loaded on the 4 well Leja slide. A range of 10 fields were acquired for motility analysis [4].

2.7 Setting of CASA

The Leja chamber temperature was maintained at 37^{0}C the time of semen sample analysis. A Hamilton Throne multi species sperm Analyzer (HTM-IVOS.V13.0C) was used for this study. The setting of instrument for the assessment of motility characteristics of bull spermatozoa were as fallow (Table 2).

2.8 Statistical method

The data obtained for various parameters were analysed with SPSS statistical software Version 13. Before analysis, the percentage values were converted to arcsin transformations and analyzed. After statistical analysis the results obtained were retransformed to obtain corresponding mean values. The between subject differences were estimated by multiple comparisons with LSD and Duncuns test.

3. Results and discussion

3.1 Effect of age concentration of spermatozoa

The effect of age on concentration of spermatozoa is given in Table 3. Research finding indicated that the highest sperm concentration (1493.48 ± 94.96 million/ml) found in those animal belong to more than 36 month age. While, lowest concentration of spermatozoa in Frieswal semen 1121.1±136.24 was found in the age group of upto 24 months of bulls with overall average 1385.97 ± 65.37million/ml in the age. It has been seen that the concentration of sperm (million/ml) increased with the increase of bulls age however, the difference between the age groups were non-significant.

3.2 Motion characteristic of spermatozoa head in Frieswal semen

The motion characteristic of spermatozoa is an important aspect for evaluation of semen quality. As all the motile sperm differ from another in the pattern of movement alone their axis, all of them may not give the same results subsequent to insemination. Wide array of motion patterns have been observed in the sperm and among them, the VSL, VAP, VCL, ALH and BCF are recorded by the CASA instrument. To record these measures, the CASA instrument recodes the sperm tracks in the form of series of dotes; wherein each dot represents the position of sperm head. The CASA software then connects these dots to construct the track of individual sperm.

3.2.1 Average path velocity (VAP)

This is defined as the average velocity over the smoothed cell path. This parameter is used to characterize the overall trajectory of the sperm cell. It is calculated by determining the length of the average path (μ) and divided it by the elapsed time in seconds. The overall VAP values in the present

investigation were 117.22±2.15 µm/s. The values were slightly higher in the bulls aged >36 months (118.83±2.81 µm/s) compared to 24 month (117.62±3.75 3 µm/s) and 25 to 36 months (115.17±3.67 µm/s) of age, however the differences were statistically non-significant. The results of present investigation are agreement with [5].

3.2.2 Straight line velocity (VSL)
VSL is the simplest velocity measure of sperm movement and is expressed as the distance traveled by a sperm head in straight line per unit of time and is commonly expressed in µm/s. It provides information about the rapidity of the sperm progress in a single direction and is thus a good indicator of sperm progressive motility. The overall VSL values in the Frieswal semen were 74.10±1.04 µm/s with a range of 71 to 75 µm/s. The effect of age of the bull on this motility parameter was non-significant. Higher values (98.8 µs) than reported in the present study have been observed in Belgian Blue (crossbred bull) semen by [5]. They have also reported that the value were even higher in pure HF (109.4 µm/s). Values as higher as 121.1 µm/s have also been reported by [6].

3.2.3 Curvilinear velocity (VCL)
VCL or curvilinear velocity is the velocity of sperm along its track and is expressed in µm/s. It is calculated by determining the sum of distance traveled by the sperm divided by the time. Since VCL is an average velocity along the sperm track, it is a good indicator of general vigor of sperm motion. However, it does not provide information about the direction of the motion or the progressiveness of the sperm. The VCL values are generally higher than the VAP values. The overall VCL values in Frieswal semen were 217.19µm/s. Although, higher value were recorded in young bulls (224.9 µm/s) than bulls aged >36 month of age, however, the differences were statistically non-significant. Comparatively lower values of VCL (193.9µm/s) and 89.2±15.67µm/s in fresh bovine semen have been reported by [6, 7].

3.2.4 Amplitude of lateral head displacement (ALH)
ALH means magnitude of lateral displacement of a sperm head about its spatial average trajectory. It can be expressed as a maximum or an average of such displacements. In the present study the overall mean values of 10.53±1.17 µm were recorded for ALH for Frieswal sperm. ANOVA showed non-significant differences between bulls. In Belgian Blue and HF bulls lower values then the present study were reported by [5]. Different CASA instruments compute ALH using different algorithms, so the values are not strictly comparable.

3.2.5 Beat/cross frequency beats/s (BCF)
BCF means the time-average rate at which the curvilinear sperm trajectory crosses its average path trajectory. In the present study the overall means of 22.84±0.4 Hz was recorded for Frieswal sperm with non- significant between bull differences. In Belgian Blue and HF bulls, lower values than the present study were reported by [5]. Values as higher as 36.2Hz have also been reported by [6]. Lower values of BCF (5.0 to 9.5 Hz) in different sub-population of Holstein bull semen have been reported by [8] whereas, comparable values to the present investigations (26.1 to 36.2 Hz) were reported in Brown Swiss bulls semen by [9] BCF gives an indirect indication about the progressive movement of the sperm. Spermatozoa with high BCF and low ALH moves faster in a unit time compared to those with less BCF and high ALH.

3.2.6 Area of head (µm sq)
The area or size of sperm head is important with respect to the sperm movement. Small deviations in head size can alter the overall velocity of any individual sperm. Therefore, the overall area of sperm head in a bull can affect the overall velocity of the sperm kinematics. In the present study the overall mean area of head was 13.75±0.29 sq µm (Table 4) which differed significantly between bulls.

3.2.7 Straightness (STR)
The results of straightness (STR), linearity and elongation of sperm head in motion are presented in Table 5. STR means linearity of the spatial average path ad is calculated as VSL/VAP. In the present study the overall mean of 65.66± 0.01% was observed and the differences were non-significant between bulls. Comparatively lower values of STR (86.9±0.4%) and 85.7±2.43 per cent in fresh bovine semen have been reported [6; 7].

3.2.8 Linearity (LIN)
LIN mean the linearity of the curvilinear trajectory, VSL/VCL. In the present study the overall LIN values of 36.22 ±0% were recorded for Frieswal bulls with non-significant differences between bulls. In fresh bovine semen higher values than the present study were reported by [7; 9]. Values as higher as 69.8 per cent have also been reported by [8].

3.2.9 Elongation (%)
Morphometrically differences in sperm head affect the velocity of sperm in a given direction. Small geometrical differences in sperm morphology can result in large differences in sperm hydrodynamics, and subsequently an impaired sperm velocity. In the present study the Elongation overall elongation of sperm head in Frieswal semen was 39.78 per cent. (Table 5) Minimum elongation values (37.82±0.01 %) were found up to 24 months while maximum elongation values (40.51±0.0%) were observed in bulls aged >36 months suggesting that with the advancement of age the spermatozoa improved in morphometrical parameters.

4. Conclusions
Based on the results, it is concluded that significantly high sperm kinetic characteristics of CASA, especially higher sperm linearity is recorded for bulls, which may indicate the possibility of including this sperm parameter in routine evaluation of bulls for better judgement of bulls for fertility and also the probability of culling bulls based lower concentration of spermatozoa, especially at an early age without spending money, space and time on rearing of such inferior bulls. Moreover, this study also clearly indicated that CASA is a good supplementation to aid genetic selection in breeding bulls. Nonetheless, further study which could encompass different age groups.

Table 1: Ingredient composition of concentrate mixture.

S. No.	Ingredient	Percentage
1.	Wheat bran	32
2.	Maize	18
3.	Barley	17
4.	GNC (decorticated)	30
5.	Mineral mixture	02
6.	Salt	01
7.	Total	100
8.	DCP	21.64
9.	TDN	76.97

Table 2: Analysis set-up of Hamilton Throne multi species sperm Analyzer (HTM-IVOS.V13.^{0}C) for evaluate bull spermatozoa.

Frames per sec	60Hz
No frames	30
Minimum contrast	35
Minimum cell size	5 pix
VAP cut off	20 µm/s
VCL cut off	30 µm/s
Chamber type	Leja 4
Chamber depth	20 µm
Stage position mm	(A) 3.7 (B) 13.7 (C) 20.0 (D) 28.9
Set stage temp.	37^{0}c
Cell intensity	110

Table 3: Effect of age on concentration of spermatozoa in Frieswal bulls

Age of months	Number of samples	Sperm concentration (million/ml)
upto 24	6	1121.01 ± 136.24
25 to 36	51	1284.35 ± 96.12
> 36	63	1493.48 ± 94.96
Overall	120	1385.97 ± 65.37

Table 4: Motion characteristics of spermatozoa head in Frieswal semen

Age	Sample	VAP (µm/s)	VSL(µm/s)	VCL(µm/s)	ALH(µm)	BCF(Hz)	Area of head (µm sq)
upto 24	6	117.62± 3.75	71.97 ± 2.69	224.92±6.07	8.53±0.32	22.08±0.67	13.0±0.42
25 to 36	51	115.17 ± 3.67	73.35±1.69	214.66±6.35	9.87±1.53	21.93±0.8	12.89±0.47
> 36	63	118.83 ± 2.81	74.91±1.41	219.87±4.83	11.25±1.86	23.65±.39	14.5±0.38
Overall	120	117.22 ± 2.15	74.1±1.04	217.91±3.71	10.53±1.17	22.84±0.4	13.75±0.29

Table 5: STR, linearity and elongation of sperm head in motion.

Age in month	STR (%)	Linearity (%)	Elongation (%)
upto 24	63.01 ± 0.01 (6)	34.15 ± 0.01 (6)	37.82 ± 0.01 (6)
25 to 36	67.16 ± 0.03 (51)	36.67 ± 0.01 (51)	39.12 ± 0 (51)
> 36	64.67 ± 0.01 (63)	36.06 ± 0 (63)	40.51 ± 0 (63)
Overall	65.66 ± 0.01 (120)	36.22 ± 0 (120)	39.78 ± 0 (120)

5. Acknowledgements

The authors acknowledge the Director, ICAR - Central Institute for Research on Cattle (CIRC), (Indian Council of Agricultural Research) Meerut Cantt.- 250 001 (U.P.), India with thanks for the facilities provided for the study and authors are also thankful to The Head, Dept. of Animal Husbandry, Sardar Vallbhabhai Patel University of Agriculture and Technology for proper guidance.

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