Volume 8, Issue 04, 2021, Pp. 293-299.
Available online at: www.indianfarmer.net
ISSN: 2394-1227 (Online)

ORIGINAL ARTICLE

OPEN ACCESS

CASA (Computer Assisted Semen Analysis): As a tool for analysis of bull fertility

Manisha Sethi[1]*, Nadeem Shah[2], Pratyush Kumar[2], Vinod Gupta[2], Anju Rohilla[1], Neeraj Bhatia[1], Nishant Kumar[3], Mukesh Bhakat[4] and Tushar K Mohanty[5]

[1]MVSc Scholar; [2]PhD Scholar; [3]Senior Scientist
(Animal Reproduction, Gynaecology and Obstetrics)
[4]Principal Scientist (Livestock Production Management)
[5]Principal Scientist (Animal Reproduction, Gynaecology and Obstetrics)
ICAR-National Dairy Research Institute (NDRI), Karnal-132001
**Corresponding Author: sethimanisha43@gmail.com*

***Article Received**: 10 April 2021* ***Published:** 11 April 2021*

Abstract

Male fertility is an important factor in bovine reproduction as a single bull is generally used to breed numerous cows especially after the introduction of AI technology. Therefore prediction of fertilization potential of sperm becomes the primary goal of semen analysis. Visual evaluation of sperm motility in semen samples using light microscopy is the most common assay used in most laboratories around the world. However, such a motility analysis does not reveal what factors distinguish fast, medium, and slow-moving sperm. While manual analysis of semen doesn't yield accurate results as they are subjective and prone to observer errors. Hence, there is a need to assess several seminal attributes at a time so that its fertilizing potential can be predicted to great extent. All this led to the discovery of computer-assisted semen analysis which can examine several semen attributes and also gives information about the velocities of the moving sperms.

Key words: CASA, Fertility, Sperm, Velocity

INTRODUCTION

What is CASA?

CASA refers to an automated system (hardware and software) to visualize and digitize successive images of sperm, process and analyze the information, and provide accurate, precise, and meaningful information on the kinematics of individual cells.

Kinematics is the branch of mechanics that deals with the study of the motion of objects without reference to the forces which cause the motion. Early systems required operator intervention, but preferred systems would require the operator only to ensure that the system is functioning properly, place the sample into the instrument, and examine/store output data. Computer-assisted semen analyzers (CASA) provide precise and accurate information on different sperm motion characteristics and are an objective assessment, independent of the interpretation of the technician and give detailed information on sperm movement.

Principle:

The basic principle behind most microscopy-based CASA systems is that a series of successive images of motile spermatozoa within a static field of view is acquired. CASA is equipped with software taking sequential images to identify individual spermatozoa (typically by sperm heads rather than tails) and trace their progression across the field of view. This involves recognizing the same cell in each image by its position and inferring its next position by estimating the likelihood that it will only have moved a certain maximum distance between frames. In practice, spermatozoa usually move out of the field of view within 3–5 s (depending on the microscope settings), but even with this limitation, it is routinely possible to generate tracks containing 150–250 sequential head positions. For the detailed study of individual sperm tracks, it is also possible to use a low power objective (×4) and record tracks with several thousand sequential head coordinates.

All the parameters through which analysis of a semen sample is to be made by CASA must be defined correctly in the CASA analysis setup. CASA identifies sperm by cell detection values already fed in the system and detection of both motile and static sperm cells is made. CASA uses these values to determine if objects in the field have the potential of being sperm cells. If the object meets all these criteria, the CASA then determines if the object is a motile cell or not. If the object is not motile, then CASA uses the static cell gates to identify the static cells versus debris. For tracking motile spermatozoa and motility parameters, CASA captures multiple "snapshots" extremely quickly (e.g., 30 images in 0.5 seconds). The computer then calculates percent motility and progressive motility, velocities, concentrations, motion parameters, etc. The camera in the system follows the individual sperm, captures the images, and then transfers them to the computer for it to analyze them and produce the results.

History of semen evaluation:

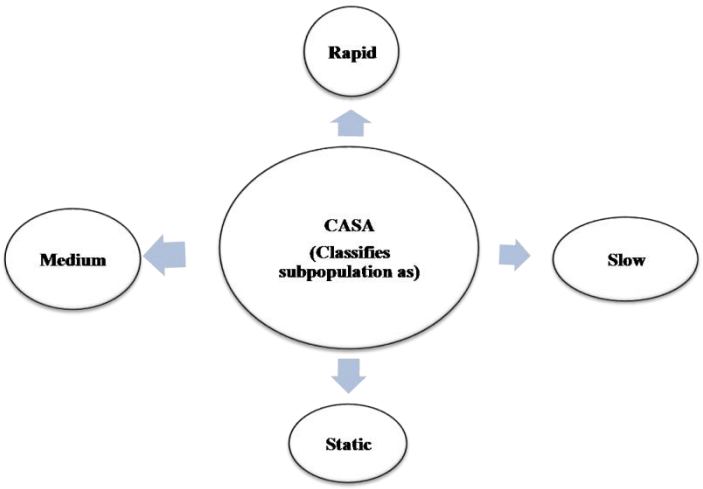
- Light microscopy-first used by Antony Von Leuwenhoek (1678) is the first generation device for visualization and analysis of sperm.
- Before World War II, European opticians developed phase-contrast optics. It is the second-generation device that was first used by pioneering andrology labs in the mid- 1950s, and it remains the primary instrument for observation of living sperm.
- CASA systems represent the third-generation devices for visualization and analysis of sperm motion.

- A method allowing the capture of successive images of spermatozoa and the analysis of their movement was first reported by Dott and Foster, (1979) and since then, the computer-assisted sperm analysis (CASA) has been presented and gradually developed as a method to objectively evaluate sperm motility parameters. The first commercial CASA system developed specifically for the evaluation of sperm motion was the Cell Softy system (CRYO Resources Ltd), in 1985. The second commercial system to develop an evaluation of sperm motion was the HTM-2000 (Hamilton-Thorn Research).

Sub Population of sperm cells in semen:

These computer-aided systems analyze the spermatozoa for various motility characteristics such as progressive motility, track speed, progressive velocity, path velocity, linearity, the amplitude of lateral head displacement, beat cross frequency (BCF).

It has been found that different subpopulations of spermatozoa coexist in most of the mammalian ejaculates including bovines. This may be attributed to the differences in the origin of individual spermatozoa and as well as the differential maturational status and age when they get mixed in the epididymis. These sub-populations may reflect discrete functional and possibly adaptive differences of spermatozoa during their genesis and subsequent storage in the epididymis. This heterogeneity of sperm population can be reflected by differences in morphology, motility, viability, and ultimately in fertilizing capacity of spermatozoa.



CASA classifies this different sub-population of spermatozoa based on path velocity into four categories: Rapid (VAP > MVV); Medium (LVV < VAP <MVV); Slow (VAP < LVV) and Static (fraction of cells that are not moving during the analysis). Also ejaculates with the highest sub-populations of rapid and progressive sperm were also the most resistant to cryopreservation CASA may help in identifying the motion characteristics of potentially fertile sub-population of spermatozoa in an ejaculate/inseminate which can reach the site of fertilization after surviving nature's rigorous selection process in the female genital tract.

Types and Parts of CASA

The basic parts of CASA are:

1. Microscope
2. Video camera.
3. Computer with software.

The video camera captures the image sends it to the computer which converts it into a digital image and produces the result. The software containing the program records images from a microscope and analyses sperm motility and kinematic motions.

Today there are more than 12 CASA systems marketed, somewhere in the world, for use with animal sperm, but CASA of Hamilton Throne is very popular. This company manufactures two types of CASA-

- IVOS Sperm Analyzer
- CEROS Sperm Analyzer

IVOS: Integrated Visual Optical System:

It integrated the optical system within the unit such that the external microscope is not needed. The IVOS hardware and software have been designed to provide users with a system that offers high-end performance while maintaining its ease of use. Since the settings for the integrated optics are made through the software, mechanical adjustments are rarely required. With a minimum investment of time, even users unfamiliar with computers will be performing fast, accurate, and reliable sperm analyses. As part of the integrated optical system, the unique computer-controlled specimen stage of the IVOS II provides precise control of temperature and position during analysis. The stage temperature, which may be set from ambient to 40°C, remains constant to within 0.5°C. Special slides are used in this called Leja slides. By adding an image capture rate of 60 frames per second, it offers the highest level of accuracy available today for measuring sperm velocities and motion parameters.

CEROS Sperm Analyzer:

This system offers the same level of accuracy and reliability for sperm analysis as IVOS but the internal optics of the IVOS analyzer are replaced with a microscope and camera. It uses an external negative phase microscope for image visualization and analysis. It is a cost-effective and space-saving alternative. Depending on the software that one can purchase, one can also perform morphology, viability, and DNA fragmentation analysis via CASA.

Sample preparation for CASA analysis:

Preparation of semen samples for analysis in CASA is critical from three aspects; sperm concentration, extender used to dilute semen, and loading volume used for analysis. Dilution of the semen sample is essential to adjust the concentration for successful analysis of individual sperm tracks. The concentration of sperms used for analysis is significant to obtain accurate kinetic measurements as higher concentrations can cause multiple individual collisions of sperm cells and falsify the motility results. The extender used to dilute the sample must not contain particles of a size similar to the sperm head. Various extenders such as Tris-egg yolk-glycerol, Tyrode's albumin-

lactate-pyruvate (TALP), Cornell University Extender, sodium citrate-casein, physiological saline have been used by different workers for evaluating semen in computer-aided systems. The velocity parameters (VAP, VSL, and VCL) and the percentage of motile, progressive, and rapid spermatozoa were higher for the semen samples diluted in HEPES-TALP or prostatic fluid in comparison with the other two diluents. Tris-egg yolk and physiological saline diluted semen samples showed similar measurements for most of the evaluated parameters except BCF, and the percentage of rapid and medium moving spermatozoa was significantly lower ($p < 0.05$) in tris-egg yolk extender. Further, velocity measures like VAP and VCL were the lowest in tris-egg yolk extender among the four diluents and this could be attributed to the higher viscosity of the diluents leading to slow down of motile spermatozoa. Therefore, it has to be mentioned that the effect of the diluent on motility characteristics of spermatozoa needs to be understood clearly before interpreting the CASA results.

Motion characteristics:

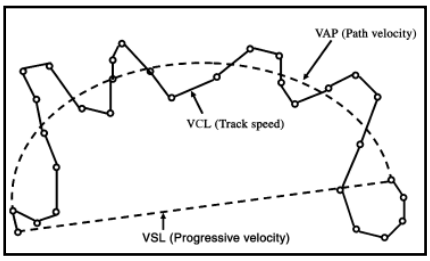


Fig. Sperm motion track showing different motion characteristics assessed by CASA (VCL: Curvilinear Velocity; VAP: Average Path Velocity; VSL: Straight Line Velocity)

The commonly reported CASA parameters include:

Total motility, Progressive motility, Curvilinear Velocity (VCL), Average Path velocity (VAP), Straight Line Velocity (VSL), Amplitude of lateral head displacement (ALH), Beat cross frequency (BCF), Straightness (STR), Linearity (LIN), etc.

Total Motility: is the ratio of motile cells to the total cell concentration expressed as a percentage.

Progressive Motility: Are the sperms moving in a forward direction.

VSL (Straight Line velocity): One of the simplest velocities, calculated as the distance traveled between the first and last point, divided by the time elapsed and expressed in microns per second.VSL by itself provides no direct information about the pattern of motion. For example, a sperm swimming slowly with a straight path might have the same VSL as a sperm swimming quickly but with a crooked or circular path.

VAP (Average Path Velocity): is defined as the total distance along the smoothened average path for each cell divided by the time elapsed.

VCL (Curvilinear Velocity): is the velocity of the sperm along its track expressed in microns/second. It is calculated by determining the sum of the distance traveled by the sperm (point-to-point) divided by the time. It is proportional to the frequency of sampling, i.e. to the video frame rate VCL does not, by definition, provide information

about the direction of motion or the progressiveness of the sperm, For example, a sperm with a circular path and one with a straight path might have the same VCL.

VAP is always lower than VCL and higher than VSL, and for sperm with relatively straight tracks and regular oscillations, VAP is more similar to VSL than VCL.

Straightness (STR): Straightness measures the departure of the cell path from a straight line. It is defined as VSL/VAP x 100

Linearity (LIN): measures the departure of the cell track from a straight line and is defined as VSL/VCL x 100.

The amplitude of lateral head displacement (ALH): is derived from the sperm track and average path. It approximates the diameter of the cylindrical sperm trajectory (Mortimer, 1997). CASA instruments determine ALH mathematically by calculating the maximum distance from the average path (as defined above) and the maximum excursion of the track from the path. This distance is then multiplied by two to capture the full amplitude of each wave in the track, expressed in microns.

Beat cross frequency (BCF): which is the number of times the sperm track crosses the average path per second. Thus, both ALH and BCF depend on VAP and therefore vary across CASA instruments that calculate VAP differently.

NOTE: VCL is always the highest of the three values while VSL is the lowest. With regular and linear trajectories of sperm movement, VAP is almost equal to VSL. When the sperm track is non-linear with a high degree of lateral head movement, then the VAP will be much higher than the VSL. In general, progressive sperm will have higher values for STR and LIN than sperm swimming in circular or irregular patterns. Therefore, these outcomes become useful for defining subpopulations of sperm based on their swimming patterns, such as hyper-activated sperm that are vigorous but non-progressive.

Tabular representation of definitions of sperm velocity descriptors measured by CASA

Descriptors	Abbreviation	Unit	Description
Curvilinear velocity	VCL	μms−1	Velocity of progression along the entire trajectory
Average path velocity	VAP	μms−1	Velocity of progression along the smoothed trajectory
Straight line velocity	VSL	μms−1	Velocity of progression from first to last coordinates
Beat cross frequency	BCF	Hz	Frequency that the sperm head crosses the smoothed trajectory
Amplitude of lateral head displacement	ALH	μm	Mean lateral sperm head displacement along the smoothed trajectory
Linearity of track	LIN	%	VSL/VCL×100
Straightness of track	STR	%	VSL/VAP×100

Significance of Different Sperm Motion Characteristics in Predicting Bull Fertility:

Many researchers have tried to correlate different motion characteristics of bull spermatozoa with oocyte penetration rate, in vitro fertility rate, and field fertility, and it was seen that among the different sperm motion characteristics assessed by CASA, progressive motility and velocity parameters like VCL, VSL and path velocity (VAP) can be of use in predicting the fertility of bull semen. Significant positive correlations

between different velocity parameters and fertilization percentage obtained in bull sperm are similar to the reports made in human spermatozoa. However, parameters like STR, LIN, BCF, and ALH were found to have almost no correlation with bull fertility in these studies, which is in contrast to the positive correlation between some of these parameters and in vitro fertilization rates of human spermatozoa.

Though CASA proves to be very helpful yet there are some limitations:

1. CASA systems are based on similar principles but their instrument components (e.g. optics and hardware characteristics, as well as algorithms for sperm identification and trajectory reconstruction) and settings (e.g. frame rate and frames per field) highly influence sperm motility parameters. There is a lack of homogeneous results between different commercial equipment.
2. The price of these systems is very high so cannot be applied at the field level.
3. In sperm sample preparation, the media used for dilution, sperm sample concentration, temperature and pH should be considered, which can also impact sperm motion characteristics evaluated by a CASA system.
4. CASA instruments are not 'ready-to-use robots, and the reliability of their results depends largely on the expertise and training of the user.
   Therefore, although a CASA system can provide an objective assessment of sperm motility parameters, a standardized protocol has now become necessary. With such a standardized protocol, sperm motion characteristics would be accurately reflected and would then permit comparison across systems and groups.

Conclusion

Sperm motility parameters are important semen characteristics correlated with fertility, which can be evaluated simultaneously, objectively, rapidly, and accurately with CASA. With traditional methods, only a few characteristics can be evaluated at a time and that too subjectively. Semen evaluation with CASA can provide a comprehensive objective assessment of motion, velocity, and morphology parameters of a semen sample to be used for prognosis of fertilizing potential of semen. Therefore, a semen sample quality score can be prepared and correlated with fertility across the species. The application of CASA technology is currently more restricted to research purposes and its use for routine laboratory semen evaluation could not become popular because of difficulties in achieving optimum setup procedures, operational complexity, and operational cost. If these things are taken care of by the CASA manufacturing companies, this technology can become routine in semen evaluation. Yet the technology provides a reliable assessment of semen characteristics for fine-tuning subjective assessment abilities of technicians engaged in frozen semen production for livestock species.