Portable systems extend computer-assisted semen analysis to insemination centres and reproductive facilities in the field – a review

Kenneth Matamoros[1], Francisco Sevilla[1,2], Ignacio Araya-Zúñiga[1,3], Arcesio Salamanca-Carreño[4], Ruth Ccalta[5], Alejandro Saborío-Montero[6], Anthony Valverde[1]

[1]Costa Rica Institute of Technology, School of Agronomy, Research and Development Centre for Sustainable Agriculture in the Humid Tropics, Animal Reproduction Laboratory, San Carlos Campus, Alajuela, Costa Rica
[2]Doctorado en Ciencias Naturales para el Desarrollo (DOCINADE), Instituto Tecnológico de Costa Rica, Universidad Nacional, Universidad Estatal a Distancia, Alajuela, Costa Rica
[3]Maestría en Ciencia y Tecnología para la Sostenibilidad, Instituto Tecnológico de Costa Rica, Alajuela, Costa Rica
[4]Cooperative University of Colombia, Faculty of Veterinary Medicine and Animal Husbandry, Villavicencio, Colombia
[5]National Institute of Agrarian Innovation, Andenes Cusco Experimental Station, Cusco, Perú
[6]University of Costa Rica, Faculty of Agri-Food Sciences, Zootechnics School and Alfredo Volio Mata Experimental Station, Cartago, Costa Rica

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Abstract

Semen quality assessment is essential to livestock production. Traditionally, such assessments have been performed using a subjective visual inspection often with unreliable accuracy. Over the last three decades, on the other hand, computer-assisted semen analysis (CASA) systems objectively measure semen quality, specifically sperm motility, concentration, kinematics, and morphology. This paper reviewed the current state of portable CASA systems on livestock farms. Breeders have used CASA technology to evaluate assisted reproductive techniques in animal breeding programs and fish spermatozoa. Despite their usefulness, benchtop CASA systems are expensive and large, which restricts their use to laboratories. New versions with portable devices, however, allow breeders to evaluate semen on-farm with various benefits thereof. Basic training is required to use the equipment and prepare samples whether *in situ* or *ex situ*. Currently, some portable systems have been calibrated for *in situ* use not only for livestock, but for domestic and wild animals including some endangered species. As these technologies are not yet widespread, their continued testing and training will only improve male reproductive selection and sample cryopreservation in livestock, wildlife, and domestic animals.

Breeding male selection, portable CASA, reproduction, spermatozoa, sperm kinematics

Semen quality assessment is valuable for selecting breeding males (Okere et al. 2005). New technologies for semen analysis have significantly improved evaluation accuracy and precision (Bompart et al. 2018; Gallagher et al. 2018; Soler et al. 2018). Standard analysis typically measures sperm concentration and total and progressive motility as well as kinematic variables. These indices vary between and within species and breeds (Valverde et al. 2018).

Evaluating semen quality sheds light on factors that affect male fertility (Santolaria et al. 2023). Functional and structural sperm indicators such as viability (Gomes et al. 2020), motility (Valverde et al. 2019a), kinematics (García-Molina et al. 2023), acrosome and DNA integrity (Sadeghi et al. 2016), mitochondrial function (Vahedi Raad et al. 2024), morphology (Pelzman and Sandlow 2024), and morphometry (Barquero et al. 2021a) are associated with fertility. Spermatozoan straight-line velocity and progressive motility, moreover, are widely recognized as reliable fertility predictors (Santolaria et al. 2015; Simonik et al. 2015).

Address for correspondence:
Anthony Valverde
Costa Rica Institute of Technology
Animal Reproduction Laboratory
San Carlos Campus, Alajuela, Costa Rica
E-mail: anvalverde@itcr.ac.cr

Several studies have indicated that sperm quality is a multifactorial trait (Valverde and Madrigal-Valverde 2018; Kaya et al. 2020; Garcia-Grau et al. 2022; Wang et al. 2022) that includes species, breed (Felton-Taylor et al. 2020), health (Pichardo-Matamoros et al. 2023), nutrition (Calderón-Calderón et al. 2022), sexual rest period (Araya-Zúñiga et al. 2023), season (Hensel et al. 2023), and age (Pascal et al. 2024; Araya-Zúñiga et al. 2025). The latter in turn affects various semen quality indicators (Sevilla et al. 2025a), for example, older bulls (4–5 years old) tend to have a larger scrotal circumference than younger bulls, which has been widely and positively associated with semen quality (Bhakat et al. 2011). Similarly, in young bulls, testicular size increases with age, leading to greater sperm production (Brito et al. 2002; Chandler et al. 1988). Older bulls (55–100 months) produce a larger volume of semen and higher sperm concentration, both mostly stable functional traits except for reduced live sperm and increased defects mid-age (Ahmad et al. 2011). Age also influences sperm morphoanomalies (Abah et al. 2023), with younger animals more likely to exhibit higher incidence of abnormal sperm morphology (Felton-Taylor et al. 2020). This combination of factors underscores the importance of evaluating breeding bulls of any production system, regardless of whether the system employs natural mating, controlled mating, or artificial insemination (Torres-Aburto et al. 2020).

CASA quantitatively measures sperm variables (Mortimer and De Jonge 2018; Valverde et al. 2019b, 2020) to predict fertility potential of breeding males (Maroto-Morales et al. 2015). These systems measure percentage of sperm motility and progressive motility, straight-line and curvilinear velocities, straightness indices, and oscillation-related parameters such as beat-cross frequency, based on sperm trajectories (Shi et al. 2006; Tomlinson et al. 2010; Buchelly Imbachí et al. 2018) at different frame rates (Bompart et al. 2019; Valverde et al. 2019c; Gacem et al. 2020). These tools are useful for evaluating breeding males during mating season in large-scale production systems (Viquez et al. 2021), as well as for assessing individual semen doses prior to use (Brito 2025). Artificial insemination centres with limited resources may be unable to use conventional benchtop CASA systems if they lack specialized microscopy and complex software algorithms (Hackerova et al. 2025). Simpler more affordable and portable systems therefore are easier to adopt for animal reproduction programs. The present work reviewed the current use of CASA portable devices in livestock production systems.

This study used articles indexed in Scopus and Web of Science. Specific keyword selection criteria were used; articles related to portable devices, CASA portable devices, CASA, or well-known trademarks were sought. No language restrictions limit article inclusion. Unpublished data were not considered and therefore were not a limitation.

Development of sperm motility testing

Traditional semen examination

Microscopic observation of sperm motility began in the 17th century (Sztein et al. 2018), remaining the most used method to evaluate semen quality by artificial insemination centres (Lenz et al. 2011).

Traditionally, seminal assessments have been made with subjective visual inspections. One example is mass motility, categorized visually using a five-point scale where 5 represents high motility and 1 represents no motility (Memon et al. 2007). A common way to compensate for the arbitrariness of this method was to increase the estimated number of sperm cells beyond what was necessary which resulted in significant loss of accuracy and reliability (Zhao et al. 2004; Soler et al. 2017). Using the eye to estimate total motile sperm percentage is highly subjective, limiting its utility as a reliable fertility marker (Walker et al. 1982). Several factors influence this subjectivity, including technician variation

(Verstegen et al. 2002; Rijsselaere et al. 2003; Gallego et al. 2018). Such weaknesses drove CASA technology development in the mid-1980s (Bompart et al. 2018; Yániz et al. 2018; Mortimer et al. 2015). Morphology assessments based on subjective observations, furthermore, suffer from low precision, poor repeatability, and limited accuracy (Hidalgo et al. 2006). In some species such as rams, the incidence of morphologically abnormal cells is particularly low (Sancho et al. 1998), which makes detection even more difficult. Additionally, most methods used for morphological evaluation are time-consuming (Soler et al. 2005). When evaluated, visual inspection focus is usually limited to identifying cytoplasmic droplets or clear cases of teratozoospermia (Zou and Yang 2000; Thundathil et al. 2001).

Benchtop CASA technology

CASA technology shifted the paradigm in semen analysis, first in humans and then in livestock (Mortimer 2000; Valverde and Madrigal-Valverde 2018). Engineers created a tool that could automatically and accurately measure semen characteristics, thereby eliminating the subjectivity and variability associated with visual assessments (Amann and Waberski 2014). The first CASA systems could not store large volumes of data.

CASA-evaluated semen quality has focused on total and progressive motility, as well as sperm concentration and kinematics (Rispoli and Roth 2023; Petričáková et al. 2024). Sperm motility indicates the energetic status of mammalian spermatozoa (Quintero-Moreno et al. 2004; Tourmente et al. 2019). Parameter measurements offer an objective assessment of the number of motile spermatozoa, and the total number of cells present (Amann and Waberski 2014; Valverde and Madrigal-Valverde 2018). Research centres and production systems use this information to estimate fertilization potential of breeding males (Gai et al. 2022), select ejaculates for cryopreservation, optimize semen doses (Brito 2025), select breeding bulls (Málková et al. 2024), and provide a more realistic view of sperm motility in the field (Ormachea et al. 2019).

These systems can also calculate kinematic parameters to identify categories based on sperm motility. Typically, spermatozoa are classified into categories such as rapid, rapid progressive, normal, and slow (Valverde and Madrigal-Valverde 2018). These velocity categories correlate with curvilinear velocity, while other variables such as average path velocity are associated with the sperm's ability to migrate through cervical mucus (Ormachea et al. 2019) (Plate II, Fig. 1).

CASA systems can now automatically evaluate sperm motility and kinematic properties (Amann and Waberski 2014; van der Horst et al. 2018; Alquézar-Baeta et al. 2019; Moraes et al. 2019). They assess fertility potential by tracking sperm trajectories with camera images rather than human eyes (Urbano et al. 2017; Gallagher et al. 2018; van der Horst 2020). This technology has been applied to fish spermatozoa (Caldeira et al. 2018), assisted reproduction in humans (García-Molina et al. 2023), and as a routine step prior to artificial insemination (Mellagi et al. 2023), and in animal breeding programs (Araya-Zúñiga et al. 2023; 2024; Solís et al. 2024; León et al. 2024). Several studies have reported significant correlations between CASA-evaluated sperm motility and fertility in livestock (Hirano et al. 2001; Broekhuijse et al. 2012; Rodríguez et al. 2013).

Non-portable CASA systems

Non-portable CASA systems measure more sperm parameters and variables with comprehensive analysis; however, they require a high initial investment and proper technical training (Rispoli and Roth 2023). A typical non-portable CASA benchtop setup comprises a microscope fitted with a heated stage and negative phase-contrast optics linked to a video camera; the camera's feed is routed to a desktop computer running specialized

software (Valverde et al. 2020). Proper semen sample handling and evaluation must be standardized (Ehlers et al. 2011; Palacín et al. 2013; Björndahl et al. 2022a) to ensure reproducibility across laboratories (Björndahl and Kirkman Brown 2022b).

Portable CASA technology

CASA portable devices evaluate semen *in situ* (Sevilla et al. 2023). These portable systems enjoy improved optical capacity, illumination, and instrumentation that differ from traditional benchtop CASA systems (Bulkeley et al. 2021). Similar to desktop computer-based CASA systems, portable devices also provide objective and automatic estimations of sperm motility patterns (Zhang et al. 2024) (Plate II, Fig. 2).

Advancements in computing technology nonetheless have significantly increased data storage, improved precision and accuracy of CASA algorithms, and standardized laboratory conditions and equipment (Finelli et al. 2021; Soler and Valverde 2023).

Portable CASA systems employ algorithms similar to those used in benchtop systems to process captured images, allowing them to estimate these same variables. Portable devices analyse semen directly on farms or at livestock facilities overcoming limitations of large benchtop CASA systems, which are expensive (Hackerova et al. 2025). Some mobile sperm analysis applications (hardware and software) are already commercially available such as iSperm® (Dini et al. 2019; Moraes et al. 2019), AndroScope® (Brito 2025), and Fertile-Eyez® (Kanakasabapathy et al. 2017; Thirumalaraju et al. 2019). These portable systems only require a smart phone or tablet, small device to analyse sperm, and the corresponding app to evaluate sperm motility (Matsuura et al. 2017). The process is simple: the semen sample must be diluted, an aliquot (volume varies between devices) placed onto a slide and sealed with a cover chip, forming an enclosed sperm counting chamber, and then inserted into the system (Plate III, Fig. 3).

Portable CASA technology has improved accessibility for small- and medium-scale producers due to its lower cost. Although portable systems also have limitations, for example some do not record sperm trajectories for all kinematic variables, they do represent an easy-to-use, accessible, and low-cost (10 × cheaper) option suitable for rural areas (Yu et al. 2018). This technology requires basic technical training in equipment handling, sample preparation, and interpretation of results (Domain et al. 2022). Therefore, its application is not limited to laboratory technicians; trained producers themselves can evaluate semen and breeding males with their own portable systems (Sevilla et al. 2023). These systems have analysed sperm from bulls (Silva et al. 2022), stallions (Brito 2025), boar (Suárez-Trujillo et al. 2022; Sevilla et al. 2023), and dogs (Domain et al. 2022). In addition, they have been used to evaluate endangered wildlife species such as rhinoceros (Rispoli and Roth 2023) and jaguar (Jorge-Neto et al. 2020).

In recent years, computational neural network-based models have also been developed for the automated identification of sperm morphology (Yüzkat et al. 2021; Keller et al. 2024). This technology could be integrated into new portable CASA system models to include novel field-testing panels, for example, sperm morphology analysis to detect morphological anomalies in the ejaculate based on standardized criteria (Brito et al. 2025). The information generated by these technologies has established *in situ* associations with reproductive parameters in females, such as sows (Barquero et al. 2021a, 2021b, 2021c). This opens new possibilities for improving fertility and other outcomes in animal production (Rocha et al. 2021).

Various portable semen analysis systems have been employed across multiple species including production animals, companion animals, wildlife, and even humans. Table 1 presents the mean values reported for total motility, progressive motility, curvilinear velocity, linearity index, and concentration obtained in studies using portable devices, categorized by species and commercial brand of each device.

Table 1. Results of semen analysis evaluated using portable CASA devices showing mean values and ranges of some sperm variables.

Species	TM	PM	VCL	LIN	SC	CASA system	Reference
Boar	75	30	-	-	167–224	iSperm®	Sevilla et al. 2023
Boar	90	-	-	-	-	iSperm®	Matsuura et al. 2017
Boar	80–82.57	-	-	-	262–304	Fertile-Eyez	Suárez-Trujillo et al. 2022
Stallion	58.2	48.7	157.7	-	-	Androscope®	Brito 2025
Bull	-	-	-	-	1380.57	iSperm®	Silva et al. 2022
Rhinoceros	0–94.3	-	-	-	9.0–69.3	iSperm®	Rispoli and Roth 2023
Dog	-	-	-	-	35.12	iSperm®	Domain et al. 2022
Stallion	76.7–90.3	62.4–64.8	-	-	-	Ongo	Buss et al. 2019
Human	58.3	-	-	-	43.9	Smartphone-based	Cheon et al. 2019
Stallion	20.2–37.4	6.7–20.2	60.2–84.8	46.1–66.2	-	iSperm®	Aitken et al. 2023
Bull*	75.92	42.37	53.79	-	88.01	SpermCell™	Başţan 2024
Human	49.59	-	-	-	-	OVIEW-M device®	Kim et al. 2022
Stallion	73.7	48.9	-	-	-	iSperm®	Medica et al. 2023
Ram	67.6	45.8	147.3	77.8	-	iSperm®	Málková et al. 2024
Rooster	73.60	16.40	78.80	61.90	-	iSperm Poultry 5®	Petričáková et al. 2024
Human	-	-	-	-	54.20	SpermCell™	Dincer et al. 2024
Jaguar	5	-	-	-	61.8	iSperm®	Jorge-Neto et al. 2020

*Research reporting average values of frozen-thawed semen. TM: total motility (%); PM: progressive motility (%); VCL: curvilinear velocity (μm/s); LIN: linearity index (%); SC: sperm concentration ($\times 10^6$ spermatozoa/ml).

This technology facilitates breeding animal selection in production systems (Matsuura et al. 2017). Because portable systems do not include all sperm functional properties to assess fertility (Suárez-Trujillo et al. 2022), analysis of different species requires field calibration to avoid inaccuracies or limitations. Laboratory studies have confirmed their precision with only minor differences compared to more sophisticated laboratory equipment (Silva et al. 2022). Moreover, comparisons of motile and kinematics with non-portable computerized systems have yielded promising results (Brito 2025). New artificial intelligence tools are going to be incorporated into portable CASA devices, just as they have been introduced into desktop systems, together with novel tools for semen sperm analysis that include motility, kinematics, morphology, among others (Sevilla et al. 2025b).

Conclusion

Portable CASA devices facilitate *in situ* semen analysis. By avoiding complex, large, and high-cost components, this technology has become more accessible to producers. Its growing field application has been important, since CASA provides valuable, objective information to expedite male reproductive selection and sample cryopreservation in livestock, wildlife, and companion animals. This emerging technology requires calibration and training for correct equipment handling. All of this is framed within the ongoing development of artificial intelligence and its potential integration into portable systems, allowing their connection to agricultural management databases in the pursuit of increasingly precise and efficient production systems.

Conflict of interest

The authors declare no conflict of interest.

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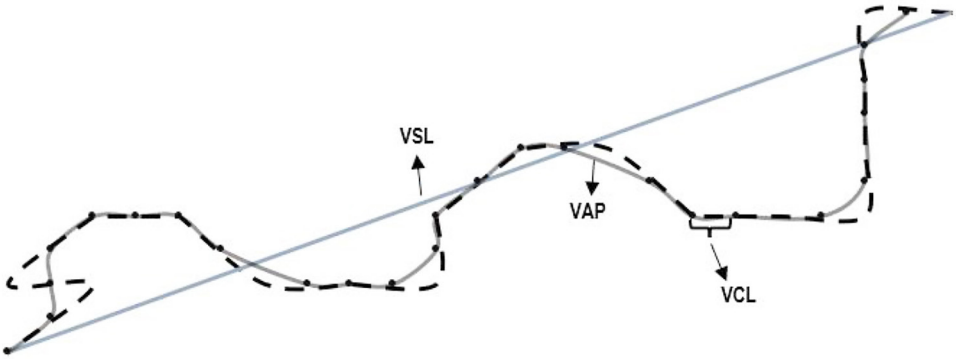

Fig. 1. Schematic representation of sperm movement patterns measured by a CASA system. Straight-line velocity (VSL): Ability and velocity of a sperm cell to move in a straight line. Curvilinear velocity (VCL): Velocity of a sperm cell along its actual path, not just in a straight line. Average path velocity (VAP): Estimated from the smoothed trajectory path.

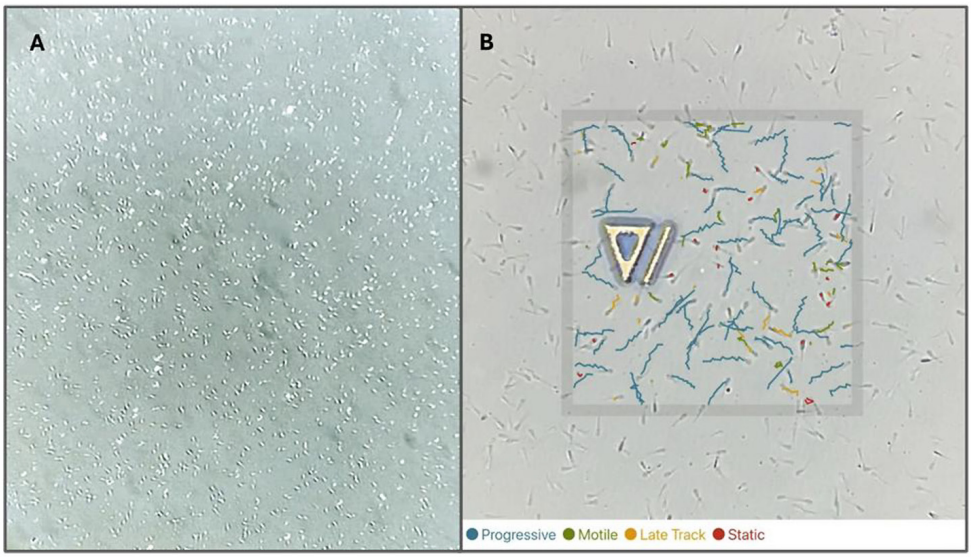

Fig. 2. Difference between A) a visual analysis using a benchtop phase-contrast microscope and B) an automatic analysis with a CASA portable device showing spermatozoa classified by progressive and total motility.

Plate III

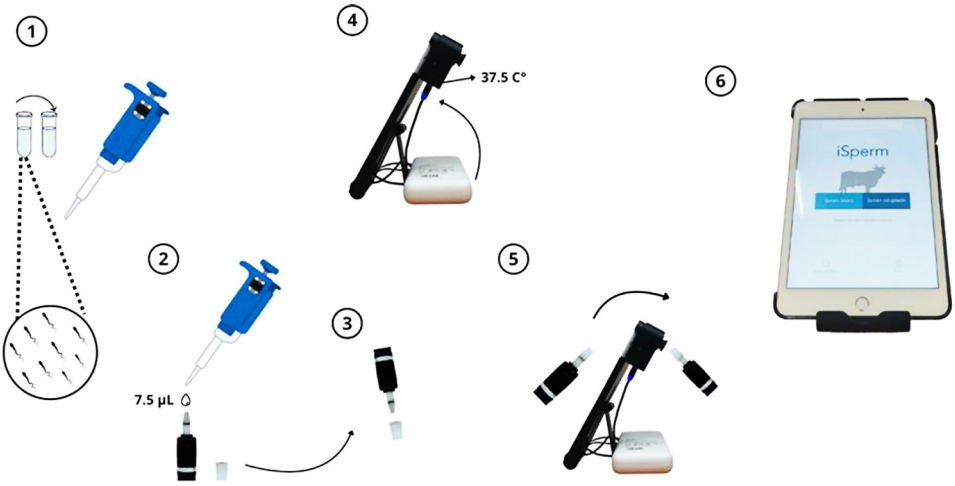

Fig. 3. General process for using a portable CASA system.

1) Dilution of the semen sample. 2) Application of an ejaculate aliquot into the sample collector and placement onto the base cap. 3) Placement of the base chip over the cover chip. 4) The system heater must first be connected to a power source (white box in figure). 5) Insertion of the sample collector into the portable device. 6) Analysis of sample with the app. The device clips onto a portable tablet and uses the portable tablet's camera and flash to film the spermatozoa in the sample.