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The application of computer-assisted semen analysis (CASA) technology to optimise semen evaluation. A review

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ABSTRACT. Currently, artificial insemination (AI) is the most applied technique for assisted reproduction in the livestock industry. The traditional analysis of seminal quality includes the evaluation of concentration and motility (total and progressive), the parameters on which the number of doses producible from an ejaculate is calculated. The introduction of automated sperm evaluation systems based on computer-assisted semen analysis (CASA) represented a revolution in the production of seminal doses and the knowledge in reproductive biology; however, this technology has been generally implemented without a critical analysis of its limitations and dependence on several methodological factors. In addition to the use of CASA technology, the estimation of kinematics and morphometry have been incorporated as novel parameters for sensitivity and reproducibility. AI has facilitated the rapid and universal dissemination of genetic material from a relatively small number of males. The CASA systems allow the analysis of a large number of sperm cells in a short time, thereby providing an array of quantitative data on kinematics and head sperm morphometry, thus making it possible to optimise the reliability of seminal analyses.

Introduction

Reduced sperm motility has been associated with lower fertility rates (Yániz et al., 2018). Motile and acrosome-reacted spermatozoa revealed a high percentage of the variation in fertility of the samples (Flowers, 2009). However, fertility is multifactorial and several factors including season may influence the outcome, number of sperms, time of copula before ovulation and seminal plasma profile (Vesseur et al., 1996), and it must be considered the female effect. Furthermore, functional and structural sperm parameters such as motility, kinematics, viability, acrosome and DNA integrity, mitochondrial function, morphology and morphometrics (Gosálvez et al., 2016) may be associated with the fertility process. Boars or bulls are not usually considered seasonal breeders; however, there may be seasonal variations in semen quality (Ibănescu et al., 2018). Variations in sperm parameters between summers and winters have been partially attributed to the changes in scrotal thermoregulation and heat dissipation mechanisms (Menegassi et al., 2015).

Computer-assisted semen analysis (CASA) technology allows the automated and accurate measurement of motile patterns of individual sperms in the ejaculate (Amann and Waberski, 2014). However, this standardisation has never been performed from an integrative point of view. There are three main aspects to consider when optimising

automated semen analyses using the CASA technology, namely the type and depth of the counting chamber (Bompart et al., 2019; Valverde and Madrigal-Valverde, 2019), the dilution media, and the frame rate of image acquisition (Valverde et al., 2019b). However, this is associated with the high frame rate values that introduce significant changes in the values of few sperm kinetic parameters. Therefore, this study intends to bridge the gap in the information regarding optimisation of semen analysis protocols (García-Molina et al., 2020). For human samples, there is a general agreement on the protocol for semen analysis as mentioned in different editions of the World Health Organization (WHO) manual. However, there are alternative protocols recognised by different international organisations. Therefore, the aim of this study was: 1) to review the main factors that could influence the output values of semen evaluation using CASA technology and 2) to examine the current sperm parameters for semen analysis using the CASA systems.

We reviewed the literature to elucidate the main factors that influence the accuracy and precision of the output values of semen evaluation using CASA technology. The structure of this review is organised according to the aims of the study. In the first half, the main assisted reproduction concepts, semen analysis, technical conditions, and limitations of CASA technology are reviewed. The second half of the paper focuses on the sperm variables for semen analysis using CASA systems, such as motility (total and progressive motility) and kinematics, including curvilinear velocity (VCL), straight line velocity (VSL), average path velocity (VAP), linearity (LIN), straightness (STR), wobble (WOB), amplitude of lateral head displacement (ALH), and beat-cross frequency (BCF). Additionally, sperm head morphometric parameters namely length, width, area, perimeter ellipsometry, rugosity, elongation, and regularity were also analysed.

Assisted reproduction in animal species

Reproduction within animal farms (i.e. dairy cattle and swine) is usually by artificial insemination (AI), which reflects the importance of this procedure that has increased and developed globally after its introduction in the first half of the 20th century (Amann and Waberski, 2014). For males, AI in dairy cattle involves the following steps: semen collection, assessment, dilution and freeze-thaw.

The collection can be performed using an artificial vagina (Barszcz et al., 2012), massage (Valverde et al., 2018) and electro-ejaculation (Palmer et al., 2004). Sperm motility (total and progressive) and concentration are the principal variables assessed at the AI centres or farms to calculate AI doses (Caldeira et al., 2019). For dilution of the sample species, specific extenders must be defined for both refrigeration and cryopreservation of the samples (Foote, 2002; Víquez et al., 2020). Cryopreservation-specific protocols for freeze-thaw, including vitrification, must be defined for each species (Yeste, 2016). Finally, insemination requires the development of specific catheters (Hernández-Caravaca et al., 2017).

AI facilitates the rapid dissemination of genetic material from a small number of genetically superior males to a large number of females in the population (Vishwanath and Shannon, 1997) and is the most important technique to facilitate the genetic improvement of animals (Howley et al., 2012).

Additionally, AI reduces the need for studies on farms and improves the accuracy of reproductive records (Funk, 2006). For instance, in pigs, the rate of genetic improvement can be increased with AI. Semen is collected from dominant males and artificially inseminated into 10–20 females with a single boar ejaculate (Johnson et al., 2000). The viability of semen doses may be compromised by the boar effect and extender used, thereby affecting the reproductive results of the sows. Therefore, the critical elements essential for AI must be retained (Knox, 2016).

Semen analysis

Microscopic sperm motility analysis began in the early 1600s (Sztein et al., 2018) and motility continues to be the most common parameter for semen quality evaluation in AI centres (Lenz et al., 2011). The evaluation of sperm motility provides relevant information on the energy status of mammalian sperms (Quintero-Moreno et al., 2004). Furthermore, motility plays an essential role in enabling the spermatozoa to reach the utero-tubal junction in mammals, which contains mucus (Jansen and Bajpai, 1982) and serves as a barrier to sperms with poor motility (Mortimer, 1997).

Motility evaluation presents a degree of subjectivity due to the visual estimation of the percentage of total motile spermatozoa, which reduces its potential as a fertility marker (Gaffney et al., 2011).

Subjective estimation of motility is affected by various factors, namely the technician effect (Gallego et al., 2018).

As mentioned previously, the typical approaches use a 5% approximation of motility and a speed of progression (SOP) score of 1 to 5 (slowest–to–fastest) to characterise the overall motility of a semen sample and to assess sperm quality. However, this approach lacks the precision required for accurate motility estimation (Zhao et al., 2004).

On the other hand, sperm morphology is also considered as an important part of semen analysis, which reflects the genetics of the spermatozoa (Murphy et al., 2013). Sperm cells with abnormal morphology have been associated with low fertility rates (Barth et al., 1992). However, subjective estimates of sperm morphology imply a lack of precision, repeatability and accuracy (Hidalgo et al., 2006; García-Herreros, 2016). In species such as rams, the number of significant morphologically abnormal cells is low (Sancho et al., 1998). In avian spermatozoa, morphology evaluation has been limited to subjective assessment despite the different morphological shapes in turkey, rooster and guinea fowl (Thurston and Hess, 1987). Moreover, most of the morphological observation techniques are time-consuming (Soler et al., 2005a). These limitations have been attributed to the fact that sperm morphology is not commonly evaluated during seminal dose production and is limited to the evaluation of cytoplasmic droplets or teratozoospermic samples (Zou and Yang, 2000).

All these limitations were the basis for the advent of the CASA technology in the beginning of the 80s of the last century (Gallagher et al., 2018).

Computer-assisted semen analysis (CASA) system

The CASA technology has been commercially available since the mid-1980s and provides an objective evaluation of sperm characteristics (Soler et al., 2018). The main components of a CASA system include a microscope equipped with a heated stage and a negative phase contrast optical device with an attached video camera. A computer, which contains specific software for different types of analyses, receives the camera signal.

The measurement of sperm motility and kinematics has been a target of semen research for over 25 years (see the special number of Reproduction Fertility and Development 30(6), 2018 for a thorough review of the state of the art). CASA-Mot systems offer an array of motility parameters in addition to the general motility evaluation. It has been shown that these parameters are sensitive to several hardware and software parameters as well as the variability of semen samples (Yeste et al., 2018).

In parallel to the development of CASA-Mot systems, CASA-Morph commercial systems were designed to reduce the subjectivity of sperm morphology assessment (Yániz et al., 2015). In addition to kinematics studies, morphometry has also been widely considered in the last few years (Yániz et al., 2015) (refer to the Asian Journal of Andrology 18(6), 2016 for a complete review of the state of the art). The predictive ability of the morphological aspects of sperm quality highly increased when morphometric, instead of morphological criteria, were used (Soler et al., 2005b).

In general, the CASA parameters enable the comparison of results, both within and among laboratories, thereby making it possible to detect quantitative differences in seminal parameters. These facilities apply to studies analysing different experimental or productive conditions (Palacín et al., 2013) and to find differences in the treatments and interactions between males participating in the same experiment (Verstegen et al., 2002).

Moreover, the capacity of CASA to generate large datasets comprising motility data from thousands of spermatozoa has been overlooked due to the summary statistics provided by the software, which does not display the intrinsic variability of the semen samples (Martínez-Pastor et al., 2011; Ibanescu et al., 2020). The first step in the evaluation of CASA datasets is a data outlier evaluation (Martínez-Pastor et al., 2011) described in the following sections.

Technical conditions and limitations of the CASA technology

Although CASA systems have been demonstrated to possess higher accuracy than the traditional methods (Didion, 2008), errors can occur in the automatic detection of sperm trajectory and silhouette identification (Simonik et al., 2015). However, the final results of the semen samples can be influenced by several factors, such as dilution of the semen samples, time elapsed since ejaculation until examination, sample temperature, counting chamber type (Del Gallego et al., 2017), location inside the camera (Nöthling and dos Santos, 2012), number of analysed fields (Broekhuijse et al., 2011), recording frame rate (Valverde et al., 2019b), staining technique for morphological evaluation (Soler et al.,

2005b; Valverde and Madrigal-Valverde, 2019) and type of CASA system (Boryshpolets et al., 2013).

Attention must be devoted to the counting chamber (Bompart et al., 2019). Different types of counting chambers can be used in the CASA-Mot systems that differ widely in volume inside the chamber, depth, shape and loading modality. Few studies have shown that the counting chamber used for semen analysis has a significant effect on sperm kinetics in cattle (Valverde et al., 2019a), goats (Del Gallego et al., 2017), humans (Peng et al., 2015), rams (Palacín et al., 2013), pigs (Valverde et al., 2019d) and stallions (Hoogewijs et al., 2012).

Another significant factor is related to the design of the CASA system. Although most of them are based on similar principles, they differ in optics, hardware and software characteristics, particularly in terms of the algorithms used for sperm segmentation, identification, trajectory reconstruction and silhouette identification (Gallagher et al., 2019). The CASA systems differ in their grayscale bit-depth, frame rate (Wilson-Leedy and Ingermann, 2007), number of consecutive frames analysed, grayscale thresholding method, image segmentation method to determine the pixel coordinates of the sperm, head versus midpiece tracking and strategies for handling collisions between spermatozoa (Shi et al., 2006) as well as the different morphological components of the cell (Yániz et al., 2016).

Furthermore, CASA systems can provide high accuracy and repeatability (Davis et al., 1992). Standardisation of equipment used in the process can further influence the results (Verstegen et al., 2002), based on the training level or expertise of the technician (Ehlers et al., 2011), the technical settings (Rijsselaere et al., 2003), the type of software (Tejerina et al., 2008) and the type of chamber used for analysis (Gloria et al., 2013).

In general, the effect of the different technical settings was found to show high relevance for kinematic (Contri et al., 2010) and morphometric (Yániz et al., 2015) evaluation. All these factors claim for well-defined inclusive protocols for the standardisation of results.

CASA parameters

Motility and kinematic variables

Most of the CASA-Mot systems plot the movement of a sperm head centroid, thereby creating a trajectory and analysing the kinematic parameters of the resulting track. Sperm kinematics include measurement of the distance between each head point for a given sperm during the acquisition period (Gallagher et al., 2019). Sperm motility parameters are mainly composed of three parameters on sperm motion velocity, three parameters on velocity ratio and two parameters reflecting sperm wobble characteristics. The main parameters are: VCL (μm/s), measured by summing the distance between the sperm head centroid positions frame by frame, divided by the elapsed time; VSL (μm/s), the distance between the first and last points of the sperm track, divided by the elapsed time; VAP (μm/s), the average path length, determined by smoothing the sperm head position in a running average (determination of the algorithm which changes among CASA-Mot brands), divided by the elapsed time; LIN (%), the level of linear progression calculated as the ratio VSL/VCL in percentage; STR (%), the ratio of VSL/VAP in percentage to measure track compactness; WOB (%), the oscillation of the actual path about the average path expressed as the ratio of VAP/VCL in percentage; ALH (μm), the amplitude of the approximate sinusoidal oscillation of the sperm head around the track (can be considered as the maximum or the mean value along the track); and BCF (Hz), the frequency with which the sperm head crosses the average path length during acquisition (Kay and Robertson, 1998; Soler et al., 2017) (Figure 1, Table 1).

As mentioned previously, few variables, such as sample concentration and dilution, frame rate (Valverde et al., 2019b), frequency of image acquisition (Acosta and Kruger, 1996), algorithm for the reconstruction of the trajectories (Morris et al., 1996), number of fields analysed and the counting chamber can affect motility results in semen evaluation even with the same CASA system (Bompart et al., 2019; Valverde and Madrigal-Valverde, 2019).

The frame rate might be the key factor as it was conditioned for a long time by the available video cameras. During the early ages of technology, a frame rate as low as 16 or 25 frames per second (fps) were used, but in recent times, an acquisition frequency rate of 50–60 Hz has been used, particularly for human samples (Morris et al., 1996). However, when cells display high speed and low linearity, for instance, a hyperactivated sperm, an increased frame rate is recommended (Valverde et al., 2019b).

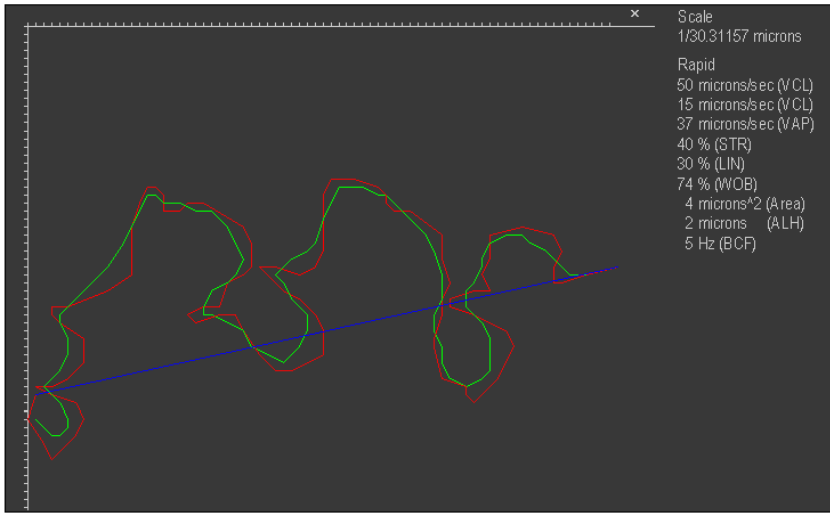

Figure 1. Kinematic parameters as presented by the ISAS®v1CASA system (Proiser R+D, Valencia, Spain). The red line shows curvilinear velocity (VCL), the blue line shows straight line velocity (VSL) and the green line shows average path velocity (VAP). STR – straightness (= VSL/VAP*100); LIN – linearity (= VSL/VCL × 100); WOB – wobble (= VAP/VCL × 100); ALH – amplitude of lateral head displacement; BCF – beat-cross frequency (Soler et al., 2018)

Table 1. Kinematics variables of spermatozoa (mean ± SEM) in different species obtained with the CASA-Mot system using 20 µm depth counting chamber

Species	VCL, µm/s	VSL, µm/s	VAP, µm/s	LIN, %	STR, %	WOB, %	ALH, µm	BCF, Hz	Reference
Bull$^{\alpha}$	207.3 ± 2.1	51.6 ± 0.9	173.5 ± 1.8	25.7 ± 0.4	31.7 ± 0.4	82.1 ± 0.3	1.1 ± 0.01	41.3 ± 0.4	Bompart et al. (2019)
Boar$^{\beta}$	106.33 ± 0.47	38.96 ± 0.15	56.34 ± 0.24	42.68 ± 0.19	71.34 ± 0.18	56.89 ± 0.15	1.45 ± 0.004	20.12 ± 0.10	Valverde et al. (2019d); Valverde and Madrigal-Valverde (2019)
Buck$^{\gamma}$	135.2 ± 0.7	98.3 ± 0.7	123.8 ± 0.7	75.3 ± 0.4					Del Gallego et al. (2017)
Ram$^{\delta}$	101.3 ± 2.1	72.6 ± 2.5	82.1 ± 2.6	67.0 ± 1.4	82.5 ± 0.8	78.4 ± 1.2	2.8 ± 0.1	8.5 ± 0.2	Palacín et al. (2013)
Stallion$^{\varepsilon}$	138.8 ± 2.2	68.8 ± 1.6	82.9 ± 1.5	49.7 ± 0.8	79.0 ± 0.7		5.2 ± 0.1	41.2 ± 0.3	Hoogewijs et al. (2012)
Rooster$^{\zeta}$	142.94 ± 6.63	37.65 ± 4.47	50.98 ± 5.27	24.92 ± 2.45	69.70 ± 2.38		2.01 ± 0.13	9.83 ± 0.23	Yan et al. (2017)
Caiman$^{\eta}$	54.16 ± 0.48	14.15 ± 0.21	23.64 ± 0.22	27.90 ± 0.35	57.29 ± 0.52	46.11 ± 0.30	2.58 ± 0.02	4.40 ± 0.05	Valverde et al. (2019c)
Eel$^{\theta}$	156.95 ± 6.34	81.76 ± 6.37	104.16 ± 6.03		77.83 ± 1.87			30.44 ± 0.75	Gallego et al. (2013); Caldeira et al. (2019)
Fox$^{\iota}$	114.52 ± 44.16	53.00 ± 31.62	70.87 ± 30.88	44.9 ± 20.39	70.76 ± 23.01	61.43 ± 13.85	4.35 ± 1.66	10.07 ± 4.12	Soler et al. (2014a)

VCL – curvilinear velocity; VSL – straight line velocity; VAP – average path velocity; LIN – linearity of forward progression; STR – straightness; WOB – wobble; ALH – amplitude of lateral head displacement; BCF – beat-cross frequency; SEM – standard error of the mean; $^{\alpha}$ – *Bos taurus*; $^{\beta}$ – *Sus scrofa*; $^{\gamma}$ – *Capra aegagrus hircus*; $^{\delta}$ – *Ovis aries*; $^{\varepsilon}$ – *Equus caballus*; $^{\zeta}$ – *Gallus gallus domesticus*; $^{\eta}$ – *Caiman crocodilus fuscus*; $^{\theta}$ – *Anguilla anguilla*; $^{\iota}$ – *Alopex lagopus*

Morphometry

Most published studies on sperm morphometry have focused mainly on the sperm head, although few others have also measured other parts of the sperm cell structure, such as the nucleus, acrosome, midpiece or whole flagellum (tail, including the midpiece) (Yániz et al., 2015). Different parameters have been used to describe the morphometry of the sperm head, but the most commonly accepted are: primary parameters that provide information on sperm head dimensions and usually include length (L, µm), width (W, µm), area (A, µm^2) and perimeter (P, µm), and derived parameters as approximations to head shape using a series of mathematical formulae, including ellipticity = L/W, rugosity (also known as roughness) = $4\pi A/P^2$, elongation (lack of roundness) = (L − W)/(L + W) and regularity = $\pi LW/4A$ (Figure 2). In few studies, ellipticity and elongation have provided redundant information as they describe the same phenomenon, i.e. the ratio between sperm head lengthening and widening (Sánchez et al., 2013), but, in general, the multivariate mathematical analysis considers both as significant (Vasquez et al., 2016).

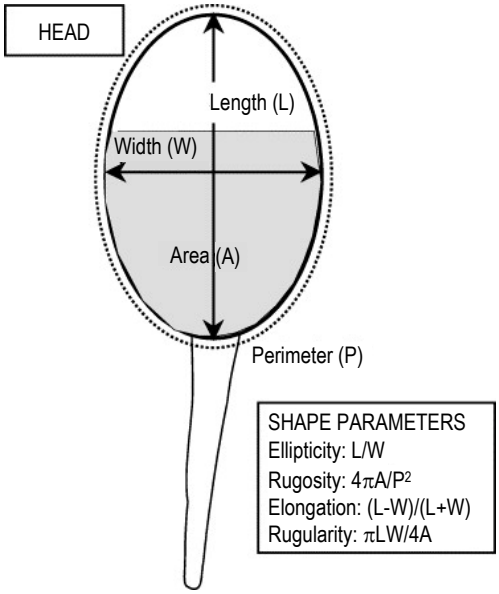

Figure 2. Morphometric sperm-head parameters. The length (L, along with the major axis), width (W, along with the shortest axis), area (A) and perimeter (P) of the head are self-evident. Shape parameters are mathematical combinations following the correspondent expressions (Soler et al., 2005a).

Morphometric results may be influenced by internal and external factors in males. Intrinsic factors include genetic or environmental factors, age, sexual maturity and sampling frequency (Valverde et al., 2016). Nevertheless, the most important factor is the introduction of artefacts as a consequence of the staining technique. In a way, this process implies the dehydration of the cells, which introduces a sensitive change in the final observed morphology (Yániz et al., 2015). However, fixation of the samples using chemical fixatives also introduces changes in the final cell morphometry (Sancho et al., 1998). To overcome this limitation, a new technique, named Trumorph®, was proposed (Soler et al., 2015; 2016), which involved the observation and analysis of sperm morphometry of cells in suspension without staining.

The morphology of live spermatozoa can be observed using the Trumorph® device that briefly increases the temperature of the sample to 45 °C to arrest motility and applies a light pressure of 6 kPa to spread the sample (2 µl) on a conventional slide under a 22 × 22-mm^2 coverslip. The depth between the slide and coverslip is ~6 µm, and the spermatozoa are restricted in movement to expose their flat upper surface parallel to the coverslip (Soler et al., 2015). Morphological observations were conducted with the UB203 microscope (Proiser R+D, Valencia, Spain) using negative phase contrast at 40× magnification.

Differences in sperm morphometry have been described between various related species (Soler et al., 2014b; Cucho et al., 2016; Valverde et al., 2019c), subspecies (Beletti et al., 2005) and breeds (Saravia et al., 2007; Valverde et al., 2016) (Table 2). Extrinsic factors may include those which influence the environment of the donor (Immler et al., 2010; Yániz et al., 2015).

Table 2. Morphometric traits of spermatozoa (mean ± SEM) in different species with the CASA-Morph system

Species	Area, µm^2	Perimeter, µm	Length, µm	Width, µm	Ellipticity	Elongation	Rugosity	Regularity	Reference
Bull$^{\alpha}$	32.11 ± 0.32	23.74 ± 0.12	8.99 ± 0.06	4.54 ± 0.03	1.99 ± 0.02	0.33 ± 0.00	0.72 ± 0.00	1.00 ± 0.00	Vicente-Fiel et al. (2013)
Boar$^{\beta}$	36.2 ± 1.71	26.6 ± 1.23	9.1 ± 0.34	4.6 ± 0.19	2.0 ± 0.11	0.3 ± 0.02	0.6 ± 0.05	0.9 ± 0.02	Saravia et al. (2007)
Buck$^{\gamma}$	29.02 ± 1.5	22.20 ± 0.60	8.47 ± 0.27	4.16 ± 0.18	2.08 ± 0.10	0.34 ± 0.02	0.74 ± 0.02	0.95 ± 0.03	Hidalgo et al. (2006)
Ram$^{\delta}$	27.3 ± 1.69	21.7 ± 2.11	8.1 ± 0.25	4.2 ± 0.17	1.9 ± 0.10	0.3 ± 0.07	0.7 ± 0.05		Martí et al. (2011)
Stallion$^{\varepsilon}$	14.72 ± 1.72	15.64 ± 0.92	5.87 ± 0.39	3.07 ± 0.27	1.92 ± 0.18	0.31 ± 0.04	0.75 ± 0.04	0.96 ± 0.03	Hidalgo et al. (2005)
Rabbit$^{\zeta}$	31.9 ± 1.7	23.9 ± 1.0	8.5 ± 0.2	4.7 ± 0.2					Lavara et al. (2013)
Caiman$^{\eta}$	6.03 ± 0.12	15.15 ± 0.19							Valverde et al. (2019c)
Mangabey$^{\theta}$	16.60 ± 1.58	15.22 ± 0.75	5.18 ± 0.26	3.80 ± 0.21					Gadea et al. (2019)
Falcon$^{\iota}$	10.0 ± 2.4	16.5 ± 2.8	7.0 ± 1.2	1.7 ± 0.3					Villaverde-Morcillo et al. (2017)
Deer$^{\kappa}$	28.38 ± 2.54	21.11 ± 1.08	7.67 ± 0.47	4.42 ± 0.25	1.74 ± 0.10	0.27 ± 0.03	0.80 ± 0.02	0.94 ± 0.03	Soler et al. (2005a)
Llama$^{\lambda}$	13.75 ± 2.82	14.80 ± 1.60	5.51 ± 0.69	3.38 ± 0.42	0.24 ± 0.06		0.87 ± 0.06	1.07 ± 0.06	Soler et al. (2014c)
Rooster$^{\mu}$	8.17 ± 4.24	15.68 ± 4.70	10.48 ± 4.13	1.39 ± 0.47	8.15 ± 4.14	0.74 ± 0.09	0.43 ± 0.16		García-Herreros (2016)
Guinea fowl$^{\nu}$	7.20 ± 4.10	14.68 ± 4.57	9.84 ± 3.88	1.29 ± 0.46	8.29 ± 3.96	0.75 ± 0.09	0.43 ± 0.15		García-Herreros (2016)

SEM – standard error of the mean; $^{\alpha}$ – *Bos taurus*; $^{\beta}$ – *Sus scrofa*; $^{\gamma}$ – *Capra aegagrus hircus*; $^{\delta}$ – *Ovis aries*; $^{\varepsilon}$ – *Equus caballus*; $^{\zeta}$ – *Oryctolagus cuniculus*; $^{\eta}$ – *Caiman crocodilus fuscus*; $^{\theta}$ – *Lophocebus aterrimus*; $^{\iota}$ – *Falco peregrinus brookei*; $^{\kappa}$ – *Cervus elaphus hispanicus*; $^{\lambda}$ – *Llama glama*; $^{\mu}$ – *Gallus domesticus*; $^{\nu}$ – *Numida meleagris*

Conclusions

In this study, we examined the main computer-assisted semen analysis (CASA) systems, technical conditions, and limitations of the CASA technology that affect semen evaluation. Furthermore, we reviewed the sperm variables used in semen analysis by CASA systems. To summarise, CASA technology provides an objective sperm characteristic evaluation and offers an array of motility and morphometric parameters, in addition to the general motility evaluation. However, the most important CASA-Mot parameters are the velocity indices, the velocity ratios and the sperm wobble characteristics, and the CASA-Morph sperm head dimensions may be influenced by intrinsic and extrinsic factors. The effect of different technical settings was found to be highly relevant for kinematics and morphometric evaluation. All these factors claim well-defined inclusive protocols for the standardisation of results.

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