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Original article

Influence of chamber type integrated with computer-assisted semen analysis (CASA) system on the results of boar semen evaluation

D. Gączarzewicz

Department of Animal Reproduction, Biotechnology and Environmental Hygiene,
Faculty of Biotechnology and Animal Breeding, West Pomeranian University of Technology,
Doktora Judyma 6, 71-466 Szczecin, Poland

Abstract

The objective of the study was to evaluate the effect of different types of chambers used in computer-assisted semen analysis (CASA) on boar sperm concentration and motility parameters. CASA measurements were performed on 45 ejaculates by comparing three commonly used chambers: Leja chamber (LJ), Makler chamber (MK) and microscopic slide-coverslip (SL). Concentration results obtained with CASA were verified by manual counting on a Bürker hemocytometer (BH). No significant differences were found between the concentrations determined with BH vs. LJ and SL, whereas higher ($p<0.01$) values of this parameter were obtained with MK. Compared to MK and SL, significantly higher values were recorded in LJ for velocity (VCL and VAP) as well as amplitude of the lateral head displacement (ALH) and beat cross frequency (BCF), which was associated with significantly higher percentages of motile, progressively motile and rapidly progressive motile spermatozoa. Higher values for the linearity (LIN) and straightness (STR) of sperm movement were obtained for the analysis performed in MK and SL. In both these chambers, the results of all the linearity and kinetic parameters of sperm were similar ($p>0.05$). The results obtained show that CASA assessment of boar semen should account for the effect of counting chamber on the results of sperm motility and concentration, which confirms the need for further study on standardizing the automatic analysis of boar semen.

Key words: boar semen, computer-assisted sperm analysis (CASA), motility, counting chamber

Introduction

The assessment of sperm motility is considered in terms of the functional test, which reflects the sperm's motor ability and energy status. This is because a significant percentage of the energy that is produced by mammalian sperm is directed towards maintaining motility, which determines the effective fertilization process (Quintero-Moreno et al. 2004, Schulze et al. 2013). Therefore, sperm motility and number, which

Correspondence to: D. Gączarzewicz, e-mail: dariusz.gaczarzewicz@zut.edu.pl

are still commonly considered as one of the most important parameters of semen quality, have been assessed in recent decades using new techniques that offer more accurate and reliable information, especially with regard to the subjective methods of light microscopy (Mortimer et al. 2000, Maes et al. 2010, Hoogewijs et al. 2012, Gloria et al. 2013). A promising alternative to conventional methods of visualization of sperm motility or concentration is the use of computer-assisted semen analysis (CASA) systems. Originally these systems were mainly used in spermatology research and in human andrology laboratories. Today they find increasing applications in semen processing in farm animal AI centers worldwide (Mortimer et al. 2000, Quintero-Moreno et al. 2004, Maes et al. 2010). The main reason is that CASA systems allow for rapid, accurate and simultaneous assessment of sperm motility and concentration, which provide a basis for estimating the number of insemination doses that can be obtained from one ejaculate, and this may translate into the economic aspect of production in AI centres (Maes et al. 2010, Broekhuijse et al. 2011).

CASA software enables each sperm to be individually detected, and, by determining successive positions of sperm heads on video frames, enables the trajectory of each sperm to be reconstructed (Rijsselaere et al. 2003, Contri et al. 2010). This allows for simultaneous calculation of the sperm kinetic parameters within a short space of time, which makes the computerized measuring devices highly accurate and repeatable (Rijsselaere et al. 2003, Contri et al. 2010, Broekhuijse et al. 2012, Palacín et al. 2013). Although CASA systems operate on similar principles, they differ in terms of optics and hardware characteristics, as well as algorithms for sperm identification and trajectory reconstruction (Contri et al. 2010, Hoogewijs et al. 2012). Therefore, for CASA-obtained data on motility to be reliable, this type of equipment must be present and the measurement procedure standardized (Mortimer et al. 2000, Rijsselaere et al. 2003, Contri et al. 2010, Gloria et al. 2013). Of great importance is proper definition of the criteria connected with sperm characteristics specific to a species, the technical settings of CASA systems (e.g., the frequency of frame acquisition, the number of fields analysed and time of analysis), and the preparation of semen for analysis (Verstegen et al. 2002, Contri et al. 2010, Broekhuijse et al. 2011). It has been recognized in recent years that CASA assessment of sperm motility or concentration can be significantly influenced by the type of chamber used for the analysis (Contri et al. 2010, Hoogewijs et al. 2012, Gloria et al. 2013, Palacín et al. 2013). The different types of chambers used on the CASA systems differ in terms of depth, size, shape and loading modality, which is often dependent on the volume of semen analysed (Hoogewijs et al. 2012, Gloria et al. 2013). The possibility of using different chambers may hinder the identification and quantification of factors potentially affecting CASA outcomes, and there is also a need to harmonize and standardize laboratory procedures used during CASA assessment for each species (Rijsselaere et al. 2003, Broekhuijse et al. 2011, Gloria et al. 2013). In a previous research, the effect of the type of chamber used has been studied both in man (Le Lannou et al. 1992) and in some animal species such as bulls (Contri et al. 2010, Lenz et al. 2011, Gloria et al. 2013), rams (Palacín et al. 2013), horses (Hoogewijs et al. 2012), rabbits (Massányi et al. 2008) and dogs (Iguer-Ouada and Verstegen 2001). However, little is known about the effect of different chambers designed for CASA analysis on the sperm concentration and especially on kinetic evaluation of boar spermatozoa. Thus, the aim of this study was to compare three different chambers commonly used for CASA assessment of boar sperm motility and concentration so as to find possible differences that could influence measured parameters.

Materials and Methods

Experimental animals and semen processing

Forty-five ejaculates, collected from healthy boars (Landrace, Large White, and Duroc × Pietrain) with proven fertility from the local AI center and with ages ranging from 1.5 to 2.5 years, were used in this study. Boars were housed in individual pens in an environmentally controlled building under uniform management practices. They were given *ad libitum* access to water and were fed a complete diet according to farm standards, in compliance with the nutritional requirements of adult boars.

Sperm-rich ejaculate fractions were collected by gloved hand technique into disposable filtering bags enclosed in insulated plastic thermos cups preheated to 37°C. Immediately after collection, the initial semen characteristics (sperm concentration, motility, morphology) were evaluated using routine AI laboratory procedures. Only those ejaculates with more than 200×10^6 sperm/ml and displaying a minimum of 70% progressive motility and 80% of morphologically normal spermatozoa were isothermically diluted in BIO'DIL® long-term commercial extender (Genes Diffusion, France) so that each AI dose contained approximately 2.7×10^9 spermatozoa. Dilution was based on sperm concentration, which had been estimated by optical density using a calibrated spectrophotometer (Accucell 60CI0394; IMV Technologies, France). The AI doses were then cooled to 16°C and transported to the laboratory for further analyses.

Procedures for assessing sperm motility and concentration

In this study, the objective assessments of motility and concentration were carried out with a CASA system – Sperm Class Analyzer® (SCA, Microptic, Barcelona, Spain) to compare the effect of different viewing chambers or slides on outcomes of semen analysis. CASA system was combined with Nikon Eclipse E-200 microscope (Nikon Corporation, Kanagawa, Japan) equipped with a negative phase-contrast 10× objective, a digital camera (A312FC/C, Basler, Germany), and with an attached heating stage set at 37°C (Semic Bioelektronika, Kraków, Poland).

The parameter settings for the SCA software were 25 frames with a spermatozoon present in at least 15 in order to be counted, time resolution 40 ms (25 Hz). The search radius was 11 µm, and the minimum and maximum areas of the detected objects were 10 µm^2 and 80 µm^2, respectively. The following motility variables recorded by CASA system were determined: total motile spermatozoa (TMS, %), progressively motile spermatozoa (PMS, %), rapidly progressive motile spermatozoa (RPMS, %), non-progressively motile spermatozoa (NPMS, %), non-motile spermatozoa (NMS, %), curvilinear velocity (VCL, µm/s), straight-line velocity (VSL, µm/s), average path velocity (VAP, µm/s), percentage of linearity (LIN, as the ratio between VSL and VCL, %), percentage of straightness (STR, as the ratio between VSL and VAP, %), wobble of the curvilinear trajectory (WOB, as the ratio between VAP and VCL, %), amplitude of the lateral head displacement (ALH, µm) and beat cross frequency (BCF, Hz). Spermatozoa with VAP ≥ 45 µm/s and STR > 45% were considered rapidly progressive and with VAP < 45 µm/s and STR > 45% progressive. Sperm cells with VAP less than 10 µm/s were recognized as immotile and those of the velocities between 10 and 25 µm/s were deemed as non-progressive motile.

The semen samples were analysed using three chambers: 8-cell chamber Leja slide (LJ; 20-µm depth; Leja Products B. V., Nieuw-Vennep, the Netherlands), Makler chamber (MK; 10-µm depth; Sefi-Medical Instrument, Haifa, Israel) and slide-coverslip (SL; 5 µl droplet of semen under a 22×22 mm coverslip corresponds to 10.3-µm depth (WHO 2010) (Heinz Herenz Medizinalbedarf GmbH, Hamburg, Germany). Prior to sperm assessment, AI doses were carefully mixed, and then 1.5 ml aliquots of each one were taken and re-warmed to 37°C for 20 min. All chambers were pre-warmed at 37°C and loaded in accordance with the manufacturer's recommendations; L8 was filled by capillarity flow of 2 µl incubated semen, MK and SL were loaded 5 µl of the incubated semen using the drop-filled cover slide technique. When air bubble was present, the slide was prepared again. After preparation, each slide was left to rest at 37°C for 30 s to avoid passive flow/move of liquids in the chamber. A random rotation was done with the used viewing chambers to prevent any effect of increased incubation time. A minimum of 500 spermatozoa per semen sample were evaluated in random microscopic fields in the central part of each chamber type at a magnification of 100×. Before the track sequence was to be analysed, the trajectories of identified and recorded objects were visually assessed to eliminate possible debris and to diminish the risk that unclear tracks were included in the analyses.

The sperm concentration was assessed by using a SCA system in different chambers: LJ, MK and SL. This concentration was verified by counting in Bürker hemocytometer (BH; Paul Marienfeld GmbH & Co. KG, Germany) that was performed following the procedure described by Bielański (1977).

Statistical analysis

Statistical analyses were performed using the Statistica software package (version 10.0; StatSoft Incorporation, Tulsa OK, USA). The mean ± standard error of the mean (SEM), median, minimum and maximum values, and 25 and 75 percentiles were calculated for the sperm concentration, percentages of sperm motility and kinematic parameters. The normality of the data was checked using the Shapiro-Wilk test, and since the data did not follow a normal distribution, they were subjected to a Kruskal-Wallis test for differences between the viewing chambers. Differences were considered significant if the probability of their occurring by chance was less than 5% ($p<0.05$).

Results

As shown in Table 1, the type of counting chamber had an effect ($p<0.001$) on the results of sperm concentration determined by CASA. Sperm concentration in the semen samples analysed in MK was higher ($p<0.01$) compared to the values determined hemocytometrically (in BH) as well as those estimated in LJ and SL. The average number of spermatozoa (44.95×10^6 in ml of semen) in MK was around $14\text{-}18 \times 10^6$ higher than in the other chamber types. No statistical differences were observed between the concentration of sperm determined in BH vs. LJ and SL.

Table 1. The concentration of the boar spermatozoa ($\times 10^6$/ml) determined by counting in Bürker hemocytometer (BH) or recorded by a CASA system (Sperm Class Analyzer®, Microptic, Barcelona, Spain) integrated with different chambers: 8-cell chamber Leja slide (LJ), Makler chamber (MK) and slide-coverslip (SL).

Parameters	BH (n = 45)	LJ (n = 45)	MK (n = 45)	SL (n = 45)
Mean ± SEM	29.64 ± 1.19	26.54 ± 1.07	44.95 ± 2.39	30.71 ± 1.41
Median	28.75^{A}	25.30^{A}	39.40^{B}	29.80^{A}
Min-Max	17.50-51.25	16.60-51.80	21.70-93.60	13.00-50.40
Percentiles*	23.75-33.75	21.30-29.60	35.10-53.90	23.30-36.40

* Percentiles: 25 and 75.
^{AB} Different letters indicate significant differences at $p<0.01$.

Table 2. The proportion of motile boar sperm determined by a CASA system (Sperm Class Analyzer®, Microptic, Barcelona, Spain) using different chambers: 8-cell chamber Leja slide (LJ), Makler chamber (MK) and slide-coverslip (SL).

Parameters*		LJ (n = 45)	MK (n = 45)	SL (n = 45)
TMS	Mean ± SEM	92.30 ± 1.61	90.05 ± 1.06	90.82 ± 0.81
	Median	94.50^{A}	92.30^{B}	92.80^{B}
	Min-Max	35.60-99.00	67.30-98.90	77.40-97.70
	Percentiles**	92.90-97.70	87.40-94.80	89.10-94.10
PMS	Mean ± SEM	78.14 ± 2.24	72.96 ± 1.86	71.69 ± 2.10
	Median	81.10^{a}	76.60^{b}	75.10^{b}
	Min-Max	26.50-94.90	32.70-93.70	40.30-94.00
	Percentiles	73.00-88.50	69.80-81.10	58.80-83.10
RPMS	Mean ± SEM	68.78 ± 2.60	42.30 ± 2.25	44.67 ± 3.22
	Median	71.70^{A}	45.30^{B}	43.60^{B}
	Min-Max	20.80-91.50	10.00-73.50	9.50-81.50
	Percentiles	64.30-80.30	32.80-52.60	28.00-63.00
NPMS	Mean ± SEM	14.16 ± 1.13	17.10 ± 1.16	19.13 ± 1.79
	Median	13.20	15.50	14.60
	Min-Max	2.80-36.60	5.10-35.30	3.50-44.10
	Percentiles	8.30-17.60	11.40-20.40	9.50-29.60
NMS	Mean ± SEM	7.70 ± 1.61	9.95 ± 1.06	9.18 ± 0.81
	Median	5.50^{A}	7.70^{B}	7.20^{B}
	Min-Max	1.00-64.40	1.10-32.70	2.30-22.60
	Percentiles	2.30-7.10	5.20-12.60	5.90-10.90

* TMS, total motile spermatozoa (%); PMS, progressively motile spermatozoa (%); RPMS, rapidly progressive motile spermatozoa (%); NPMS, non-progressively motile spermatozoa (%); NMS, non-motile spermatozoa (%).
** Percentiles: 25 and 75.
$^{ab, AB}$ Different letters indicate significant differences within rows, lowercase at $p<0.05$ and uppercase at $p<0.01$.

The effect of the counting chambers used on the proportion of motile sperm determined by CASA is presented in Table 2. The highest TMS, PMS and RPMS values of 92.30 ± 1.61%, 78.14 ± 2.24% and 68.78 ± 2.60%, respectively, were observed in LJ. These parameters were significantly higher than those determined in MK by slightly over 2% ($p<0.01$), 5% ($p<0.05$) and 26% ($p<0.01$), respectively, as well as higher than those obtained in SL by around 1.5% ($p<0.01$), 6.5% ($p<0.05$) and 24% ($p<0.01$), respectively. An inverse relation between the chambers occurred for the proportion of non-motile spermatozoa, with the NMS parameter in LJ being significantly ($p<0.01$) lower than that determined using MK and SL. No statistically significant differences were found in TMS, PMS, RPMS and NMS between MK and SL. The average percentage of NPMS, which ranged from 14.16 to 19.13%, was similar for all the chambers under study.

The results concerning different sperm velocities and parameters of sperm motility quality, determined in each chamber, are given in Table 3. The values of these parameters did not differ significantly between MK and SL. Compared to these two chambers, sperm analysed in LJ moved more rapidly; significant differences in VCL and VAP were found between LJ vs.

Table 3. Motility descriptors of the boar spermatozoa determined by a CASA system (Sperm Class Analyzer®, Microptic, Barcelona, Spain) using different chambers: 8-cell chamber Leja slide (LJ), Makler chamber (MK) and slide-coverslip (SL).

Kinematic parameters*		LJ (n = 45)	MK (n = 45)	SL (n = 45)
VCL	Mean ± SEM	71.48 ± 1.73	49.41 ± 1.47	50.84 ± 2.26
	Median	69.80^{A}	49.00^{B}	49.40^{B}
	Min-Max	38.30-96.00	29.20-75.90	28.50-80.50
	Percentiles**	65.50-77.10	43.30-55.50	39.20-61.20
VSL	Mean ± SEM	26.76 ± 1.33	21.25 ± 0.62	24.62 ± 1.25
	Median	23.70^{a}	21.10^{b}	23.50^{ab}
	Min-Max	13.40-50.00	12.60-31.40	10.70-47.80
	Percentiles	20.10-32.00	19.00-23.80	18.00-30.30
VAP	Mean ± SEM	48.87 ± 1.40	34.24 ± 0.88	36.70 ± 1.54
	Median	49.80^{A}	35.10^{B}	36.90^{B}
	Min-Max	26.80-62.00	19.30-44.80	16.90-62.70
	Percentiles	40.40-55.30	30.50-37.70	30.60-43.40
LIN	Mean ± SEM	38.30 ± 2.06	44.48 ± 1.69	50.91 ± 2.50
	Median	33.00^{A}	43.10^{AB}	51.40^{B}
	Min-Max	19.00-73.30	23.90-69.80	19.50-84.40
	Percentiles	26.10-50.30	34.40-52.50	41.00-63.00
STR	Mean ± SEM	54.72 ± 2.07	62.80 ± 1.57	67.67 ± 2.14
	Median	54.00^{Aa}	64.30^{ABb}	69.20^{Bb}
	Min-Max	32.20-80.90	43.80-82.60	37.20-91.80
	Percentiles	41.60-66.80	54.70-71.20	60.00-78.50
WOB	Mean ± SEM	68.62 ± 1.48	68.62 ± 1.75	72.61 ± 1.79
	Median	67.80	67.40	74.10
	Min-Max	46.60-90.60	10.10-85.70	38.70-92.00
	Percentiles	62.90-76.00	64.70-76.20	66.50-80.70
ALH	Mean ± SEM	2.89 ± 0.08	2.11 ± 0.05	2.04 ± 0.06
	Median	2.90^{A}	2.10^{B}	1.90^{B}
	Min-Max	1.70-3.60	1.40-3.10	1.40-3.00
	Percentiles	2.50-3.40	1.90-2.30	1.70-2.30
BCF	Mean ± SEM	9.17 ± 0.19	8.02 ± 0.13	7.92 ± 0.13
	Median	9.00^{A}	7.90^{B}	7.80^{B}
	Min-Max	6.60-11.90	6.60-10.50	6.60-9.50
	Percentiles	8.30-10.10	7.30-8.60	7.30-8.70

* VCL, curvilinear velocity (μm/s); VSL, straight-line velocity (μm/s); VAP, average path velocity (μm/s); LIN, percentage of linearity (the ratio between VSL and VCL, %); STR, percentage of straightness (the ratio between VSL and VAP, %); WOB, wobble of the curvilinear trajectory (the ratio between VAP and VCL, %); ALH, amplitude of the lateral head displacement (μm); BCF, beat cross frequency (Hz).
** Percentiles: 25 and 75.
$^{ab, AB}$ Different letters indicate significant differences within rows, lowercase at $p<0.05$ and uppercase at $p<0.01$.

MK and SL ($p<0.01$), and also in VSL between LJ and MK ($p<0.05$). In addition, higher ($p<0.01$) ALH and BCF values were noted in LJ than in the other two chamber types. Compared to LJ, semen analysed in SL was characterized by significantly higher ($p<0.01$) LIN and STR values. Between all the tested chambers, significant differences were not found only for WOB (Table 3).

Discussion

It is widely accepted that true fertility of many animal species is associated with normal motility of spermatozoa, which allows them to reach the fertilization site and to penetrate the zona pellucida (Gloria et al. 2013, Schulze et al. 2013). However, data from scientific literature are inconsistent on this point; some studies confirm that fertility and sperm motility characteristics are interrelated (Broekhuijse et al. 2012, Schulze et al. 2013), while others suggest that this relationship is small or non-existent (Quintero-Moreno et al. 2004, Gadea 2005, Didion 2008). These discrepancies are difficult to explain (Quintero-Moreno et al. 2004), but they may be due to the lack of thorough standardization procedures for CASA assessment of sperm motility, despite the fact that this method is considered more accurate, ob-

jective and repeatable than manual microscopic evaluation of motility (Rijsselaere et al. 2003, Contri et al. 2010, Broekhuijse et al. 2011, Hoogewijs et al. 2012, Gloria et al. 2013, Palacín et al. 2013). Sperm motility results obtained with CASA are dependent on many factors (see Amann and Waberski 2014) including some CASA system settings and preparation of semen samples for analysis (Rijsselaere et al. 2003, Contri et al. 2010, Broekhuijse et al. 2011). A considerable effect, which has received particular attention recently, can be exerted by the type of counting chamber, which was reported in humans (Le Lannou et al. 1992) and in some species of animals (Iguer-Ouada and Verstegen 2001, Massányi et al. 2008, Contri et al. 2010, Lenz et al. 2011, Hoogewijs et al. 2012, Gloria et al. 2013, Palacín et al. 2013). The present study demonstrates that chamber type influences sperm concentration results and sperm motility parameters obtained with CASA also in the case of boar semen.

In this study, sperm concentrations determined by CASA in three different chambers (LJ – capillary-loaded, 20-µm depth; MK – droplet-loaded, 10-µm depth; SL – droplet-loaded, 10.3-µm depth) were related to the concentration results obtained using standard hemocytometric method (BH) as a reference. The concentration estimated in MK was found to be significantly higher than sperm number determined in BH and in the other two chambers. Similar findings were reported by Hoogewijs et al. (2012). These authors found equine sperm concentration to be significantly higher in MK compared to the value determined with NucleoCounter SP-100 as the gold standard, from which the mean values estimated for Leja chambers of different depth (10 µm, 12 µm and 20 µm) did not differ. Some authors report that CASA systems are often used in conjunction with capillary-filled 20 µm chambers, which effectively keep the sperm cells within the focal plane of the microscope (Kuster 2005, Hoogewijs et al. 2012, Amann and Waberski 2014). During the loading of these chambers, the Segre-Silberberg effect (associated with Poiseuille flow which causes migration of suspended cells in a direction transverse to the flow) may lead to sperm assessment errors, which could be avoided after using a proper correction factor (see Douglas-Hamilton et al. 2005, Kuster 2005). In the present study we used LJ, which does not correct for Segre-Silberberg effect, but the results were comparable to the reference (BH). It is possible that the sample stabilization time (30s) applied prior to the analysis balanced the flow of fluid in the chamber and the uneven distribution of sperm directly after loading. Some studies indicate that the use of Leja chambers (of 20 µm depth) which correct for the Segre-Silberberg effect, produces similar results as those without the correction of this effect (Maes et al. 2010) or may overestimate the concentrations compared to the reference (Hoogewijs et al. 2012). In turn, it cannot be excluded that flattening of a semen droplet during the loading of MK causes a specific hydrodynamic flow of the fluid, which makes sperm unevenly distributed in the chamber, leading to high sperm concentration values obtained in MK. Some authors pointed out before that the assessment of concentration is less accurate with MK compared to hemocytometric chambers (Christensen et al. 2005), while others observed the most even distribution of cells in the chamber's central compared to edge fields using MK (Gloria et al. 2013).

Studies cited above as well as the present study suggest the need for a comprehensive determination and quantification of factors, which in a given type of chamber may significantly influence the assessed parameters prior to the analysis. This is all the more important because the concentration may significantly affect the kinetic parameters of sperm (Rijsselaere et al. 2003, Contri et al. 2010, Broekhuijse et al. 2011). It is also necessary to stress that the determination of sperm concentration by means of CASA is not universally accepted (Hoogewijs et al. 2012), which is due to discrepant results obtained in some species between different types of chambers compared in the same device, as well as between CASA results and those obtained using different concentration estimation techniques, such as BH measurements or results treated as the gold standard (Le Lannou et al. 1992, Rijsselaere et al. 2003, Kuster 2005, Maes et al. 2010, Hoogewijs et al. 2012).

Earlier studies suggest that different chamber types have different effects on CASA assessment of sperm motility and dynamics, which mostly depend in particular species on chamber loading procedure (Hoogewijs et al. 2012, Gloria et al. 2013) and chamber depth (Contri et al. 2010, Lenz et al. 2011, Hoogewijs et al. 2012, Gloria et al. 2013, Palacín et al. 2013). In general, compared to chambers of around 10 µm depth (MK and/or SL), 20 µm-LJ chambers (or those similar in depth and loading) exhibited lower values of some parameters of motility, such as TMS and PMS (Contri et al. 2010, Lenz et al. 2011, Gloria et al. 2013, Palacín et al. 2013) or velocity, such as VCL, VSL and VAP (Gloria et al. 2013, Palacín et al. 2013) or just VCL (Contri et al. 2010). Unlike the studies cited above, the present study found a significantly higher proportion of spermatozoa classified based on velocity (TMS, PMS and RPMS) in LJ compared to MK and SL, with a similar NPMS percentage in all the chambers. Also most of the kinetic parameters for LJ were different from those obtained for MK and SL (Table 3). In MK and SL, similar results

were obtained for sperm motility and kinetic parameters, which was probably due to the similar depth of both chambers. The lack of differences between these chambers in terms of some motility parameters had been reported before, even when the assessment in SL was performed in a semen layer of 20.6 µm thickness (Lenz et al. 2011, Gloria et al. 2013, Palacín et al. 2013).

Based on the data presented above, it is suggested that the greater depth of LJ allowed spermatozoa to move more freely, which contributed to the variable velocities and parameters of sperm movement quality. This was reflected in the higher VCL, VSL and VAP values, higher ALH and BCF values, and lower STR and LIN values compared to MK and SL. In such shallower chambers, it is more likely that the natural (three-dimensional) motion of sperm cells will be suppressed by limiting its tail whipping in one of the dimensions (Gloria et al. 2013, Amann and Waberski 2014). What is more, sperm velocity and movement patterns can also be affected by the proximity of surface, because spermatozoa tend to adhere to the surface, which may potentially depend on surface tension (Lenz et al. 2011, Amann and Waberski 2014). The present results demonstrate that sperm in the deeper chamber (LJ) had high energy (higher VCL, VAP, ALH and BCF) but were less progressive (lower STR and LIN) and moved in more irregular trajectories. In the two shallow chambers (MK and SL), sperm swam forward less energetically (lower VCL, VAP, ALH and BCF) along more rectilinear trajectories (higher STR and LIN), covering distances similar to those in LJ (VSL). In this context it can be assumed that chamber depth may play a significant role in the assessment of sperm motility parameters (such as ALH and BCF) with the activity pattern of the head and tail, with high degree of flagellar curvature motion. This suggestion needs to be validated in further research, but it seems important because much of the present research focuses on in-depth analysis of changes in the subpopulations of motile spermatozoa in different experimental conditions, also during hyperactivation (Schmidt and Kamp 2004, Flores et al. 2009). Indeed, in men, the assessment of hyperactive spermatozoa performed by means of CASA showed the need to use chambers at least 20 µm deep (Le Lannou et al. 1992), and, as indicated by Mortimer (2000), they should be at least 30 µm in depth. The advantage of deeper chambers is that they enable more accurate measurement of the parameters (such as ALH and BCF) that are crucial for determining hyperactive spermatozoa (Verstegen et al. 2002). However, where the chambers are too deep, the problem is to retain the sperm within the depth of field of the microscope, which may make their identification more difficult (Amann and Waberski 2014). It is conceivable that because of species specific properties of spermatozoa related to their size and motion dynamics, it may be necessary in CASA assessment to use chambers of different depth for different species. Compared to other mammals, boar spermatozoa are characterized by low mean values of motion parameters (Quintero-Moreno et al. 2004), which can be influenced by many factors during CASA assessment (Broekhuijse et al. 2011). In addition, the use of chambers differing in structure and depth in different studies with boar semen (Quintero-Moreno et al. 2004, Schmidt and Kamp 2004, Flores et al. 2009) may hinder the identification and analysis of these factors. With this in mind, and also considering the present results, it seems necessary to define the optimum chamber depth for measurement of boar sperm concentration and/or motility by means of CASA, taking into account the economic implications for artificial insemination practice associated with the cost of the chambers.

In summary, the present study shows that the type of chamber used for CASA assessment of boar semen may have a considerable influence on the measurements of sperm concentration and motility parameters. When assessing the concentration in MK, it should be considered that the number of spermatozoa in a sample may be overestimated. When analysing motility it is necessary to consider that chamber depth, by probably determining the way spermatozoa move in it, may contribute to differences between the motility results recorded by CASA for the same semen sample evaluated in layers of different depth (20 µm vs 10 µm). On the basis of the present study it seems that deeper chambers (such as LJ) enable a proper measurement of boar sperm concentration and an assessment in which the sperm motility is closer to the natural motility. Further research is necessary to determine, which type of chamber integrated with the CASA system will provide the most accurate information on the concentration and motility of boar spermatozoa in specific experimental or practical conditions.

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