Romanian Biotechnological Letters *Vol. 22, No. 6, 2016*

ORIGINAL PAPER

The effect of chamber type on liquid-stored boar semen examined with computerized instruments

Received for publication, March 1, 2015
Accepted for publication, August 7, 2015

IULIAN IBĂNESCU[1, 2,*], DAN DRUGOCIU[1], PETRU ROȘCA[1], IOANA SFARTZ[1], CLAUS LEIDING[2]
[1] University of Agricultural Sciences and Veterinary Medicine, Faculty of Veterinary Medicine, Aleea M. Sadoveanu 8, 700489, Iași, RomaniaȘ 2 Besamungsverein Neustadt an der Aisch, Germany
***Corresponding author:** iulian.ibanescu@yahoo.com; Tel.: +40756381861, +4915210344974.*

Abstract

The computer-assisted sperm analysis (CASA) is nowadays widely used for the examination of semen both in research laboratories and production units. Therefore, it is very important to know all the factors that can alter the CASA output. This study aimed to compare the results obtained with different disposable chambers used for the examination of boar sperm and to determine if they show significant differences. Samples of semen were obtained from five adult and healthy Pietrain boars and examined by means of SpermVision version 3.7 using the following viewing chambers: Leja (20µm), MofA (20µm) and Minitube (20 µm). The examination was performed at 0, 6 and 12 minutes after filling the chamber. The sperm concentration was also determined by SQA-Vp, Nucleocounter NC-100 and hemocytometer. The results showed a significant difference in terms of kinetic parameters between the MofA type and the other two chamber types, but also a significant difference between sperm concentration calculated by SpermVision (regardless of the chamber type) and that calculated by other methods ($p<0.05$).

Keywords: boar sperm, motility, concentration, computer-assisted analysis, chamber.

1. Introduction

The reproduction in swine breeding centers isperformednowadays almost exclusively by artificial insemination.The success of this procedure depends on a number of factors, including semen quality. When semen is inadequate in terms of volume, sperm number, sperm motility and/or morphology, the conception rate may be affected [1]. Many authors have demonstrated the correlation between some parameters of semen and its fertilizing capacity. For example, Flowers (1997) suggests that the use of semen with motility below 60% leads to lower values of fecundity and prolificacy [2], while other authors indicate some correlations between sperm parameters assessed bycomputer-assisted sperm analysis (CASA) and fertility indices [3-7]. The development of technology for semen evaluation (CASA systems) helped in increasing the accuracy and confidence in determination of seminal parameters. CASA provides more information than classical evaluation by conducting additional tests, such as dissociation between the total and progressive motilityor calculation of several special parameters, namely: the average velocity of spermatozoa, curvilinear velocity, straight line velocity, amplitude of lateral head displacement, etc. [8,9]. In addition, the availability of information recorded by computer facilitates the comparison of results and enablesthe detection of possible differences in motility according to different situations [10]. CASA systems are used increasinglyin centers that produce and examine boar semen, after several authors have proven that they are more objective than traditional methods [8, 9, 11-13], especially in terms of sperm motility. In these circumstances, it is essential to know all the factors that contribute to the achieving of results. The outputvalues may be influenced by the quality of semen itself, but also by some

external factors, including mistakes of the examiner or shortcomings of the equipment used [14]. Awareness of all extrinsic factors that can cause false results is essential in order to provide more precise and objective values. In general, CASA results may be influenced by software settings, image capture settings, the number of analyzed fields, concentration and dilution of the semen sample, adjustment of chamber, temperature of smear, the time from ejaculation to examination [9], standardization of equipment and examination protocol [15], the type of software used [16], the training level of the operator [17] and the type of analyzing chamber used [18-20]. Currently, there are various types of analyzing chambers availableon the market, with different shapes and sizes produced by various companies. In order to obtain more precise and accurate results forsemen examination, it is important to choose the type of chamber that offers a higher degree of confidence. This study aimed to determine whether the results of computerized analysis of liquid-stored boar semen can be significantly influenced by the type of disposable chamber used during examination.

2. Materials and methods

2.1. Semen

The semen samples originated from a commercial unit in Germany specialized in the production of doses for artificial insemination of sows. Boar semen was collected by the manual method [21] from five healthy Pietrain boars commonly used for reproduction. The dilution was carried out with the BTS® extender (Minitüb, Tiefenbach, Germany), and the evaluation was performed after two days of preservation in liquid state at 17°C in tubes of 100 ml (90 ml semen + 10 ml air).

2.2. CASA system

The assessment of seminal parameters was performed using SpermVision software version 3.7 (Minitube of America - MOFA®, Verona, WI, USA) connected to a Zeiss Axio Scope.A1 (Carl Zeiss MicroImaging GmbH, Göttingen, Germany) optical microscope equipped with a heating stage at 38°C. The following parameters were determined: sperm concentration (Conc), total sperm motility (TMot), progressive sperm motility (PMOT), straight line velocity (VSL), average path velocity (VAP), curvilinear velocity (VCL), linearity (LIN), straightness (STR), wobble (WOB), amplitude of lateral head displacement (ALH), beat cross frequency (BCF).Settings were adjusted as follows: for total motility were taken into account the sperm showing any movement, but for progressive motility have been considered only the sperm showing an average speed of minimum 25 µm/s and a coefficient of straightness of at least 0.3.

2.3. Disposable chambers

Within examinations, three types of disposable chambers have been used: i) Leja chamber (20 µm), elongated(LOT 391 415, Leja Products, Nieuw-Vennep, The Netherlands); ii) MofA chamber (20µm), circular(LOT 10114240, MOFA Global, Verona, WI, USA); iii) Minitube chamber (20 µm), elongated (MT14259 LOT-D, Minitüb GmbH, Tiefenbach, Germany).

2.4. The experimental design

Semensamples were warmed to 38°C and about 2 ml of each sample was deposited in Eppendorf tubespre-warmed at 38°C as well, in special devices (block thermostat HBT model 132-2, Haep Labor Consult, Bovenden, Germany). The samples were maintained at this temperature for about 10 minutes, in order to be certain that theyreached the optimal temperature entirely. The analyzing chambers were also preheated to 38°C, fitted into the microscope examination device and filled according to manufacturer's specifications (3µl for chambers Leja and Minitube, 2.5µl for chambers MofA). Each semen sample was analyzed with 3 slides (12 chambers) of each type, and the evaluation was performed in three time points: immediately after filling the chamber, at 6 minutes and at and 12 minutes, thus

summing a total of 540 examinations. The experiment was conducted by alternating the 3 types of chambers as follows: examination of chamber A of slide 1 for each type immediately after the filling, followed by the examination of chamber A of slide 1 for each type at 6 minutes after the filling, followed by the examination of chamber a of slide 1 for each type at 12 minutes after the filling, followed by the examination of chamber B of slide 1 immediately after the filling, and so on.

2.5. Additional methods for examination of sperm concentration

To check the accuracy of the results provided by the CASA system in terms of sperm concentration, this parameter was determined with some additional methods, widely used and considered to be very accurate: SQA-Vp device (A-Tech, Medical Electronic Systems Ltd, Los Angeles, CA, USA), Nucleocounter® device NC-100 ™ (Chemometec A / S, Allerod, Denmark) and by the hemocytometer method using the Thoma counting chamber of 0.1 mm height and 0.0025 mm^2 surface (Heinz HerenzMedizinalbedarf GmbH, Hamburg, Germany).

2.6. Statistical analysis

The data stored in the computer were processed using the software IBM SPSS® Statistics version 21 (IBM® Corporation, Chicago, IL, USA). Results are presented as mean values and standard deviation (SD). Values were considered statistically significant when $p<0.05$. To highlight where the differences among the 3 types of chambers were significant, the means were analyzed using One-Way ANOVA analysis, and to signal the presence of possible correlations between Leja, MofA and Minitube, the Pearson correlation (2-tailed)has been used.

3. Results and discussions

Over the time, many authors have compared the results obtained with different methods for determining sperm concentration [23-25] or sperm motility [26]in boar semen. However, to our knowledge, no study has been conducted to compare these three types of disposable chambers (Leja - 20µm, MofA – 20 µm and Minitube – 20 µm)to each other and/or to determine the accuracy of these chambers in calculating the sperm concentration.

3.1. The effect of the chamber on the kinetic parameters

The mean values, the standard deviation and the statistical significance of the differences among different types of chambers used for determination of sperm parameters are shown in table 1, table 2 and table 3.

Table 1. Mean values and standard deviation obtained during the assessment of boar sperm immediately after filling the chamber

Chamber type	Leja (mean value ± SD)	MofA (mean value ± SD)	Minitube (mean value ± SD)
Conc ($\times 10^6$)	13.00 ± 2.17^{a}	15.71 ± 3.18^{a}	11.42 ± 2.02^{a}
TMot (%)	76.47 ± 5.30^{a}	72.98 ± 6.51^{b}	75.69 ± 5.33^{a}
PMot (%)	69.86 ± 7.86^{a}	55.20 ± 9.71^{b}	68.53 ± 8.64^{a}
VAP (µm/sec)	58.31 ± 7.60^{a}	46.39 ± 4.16^{b}	58.19 ± 7.79^{a}
VCL (µm/sec)	95.55 ± 18.54^{a}	81.71 ± 13.29^{b}	93.12 ± 18.29^{a}
VSL (µm/sec)	51.17 ± 6.86^{a}	40.04 ± 4.84^{b}	51.82 ± 7.15^{a}
STR (VSL/VAP)	0.87 ± 0.04^{ab}	0.86 ± 0.05^{b}	0.89 ± 0.04^{ac}
LIN (VSL/VCL)	0.54 ± 0.09^{ab}	0.50 ± 0.11^{b}	0.57 ± 0.10^{ac}
WOB (VAP/VCL)	0.62 ± 0.08^{a}	0.58 ± 0.09^{b}	0.63 ± 0.09^{a}
ALH (µm)	2.51 ± 0.42^{a}	2.30 ± 0.44^{b}	2.36 ± 0.40^{ab}
BCF (Hz)	36.15 ± 1.99^{a}	34.26 ± 2.24^{b}	37.67 ± 2.49^{c}

Within the same row, different superscripts indicate significant difference at $p<0.05$

The differences in sperm concentration were not statistically significant ($p>0.05$). On the other hand, all kinetic parameters showed significant differences depending on the type of chamber used within the analysis ($p<0.05$). The best results were provided by the Leja chamber for TMot, PMot, VAP, VCL, VSL and ALH, while the Minitube chamber provided superior results for STR, LIN, WOB and BCF. All kinetic parameters showed lower values when MofA chamber was used.The values obtained for total and progressive motility were not significantly different betweenLeja and Minitube, which suggests that these two types tend to provide similar results in this regard. The standard deviation was roughly the same for all three chamber types suggesting that the degree of repeatability is approximately similar. Each producer uses his own technique and materials for the manufacture of a certain product, and this applies to semen analyzing chambers as well. The chemicals used for coating and the glue used to combine components may be toxic to spermatozoa.This would affect their ability of movement, being well known that decreased motility is one of the main signs of intoxication in sperm. In order to better observe this potential toxic effect, we examined the semen also at 6 and 12 minutes after filling the analyzing chamber. Table 2 shows the results obtained at 6 minutes after filling. motility, without significant differences ($p>0.05$) between them. Moreover, the results provided by the two above-mentioned types were very similar in terms of all kinetic parameters, with the exception of BCF.This suggests that they provide relatively similar conditions for sperm during the examination. For all three chamber types, the percentage of motile spermatozoa was lower at 6 minutes after loading the sample when compared to the values obtained immediately after filling. However, this decline wasmore severe forMofA type, not only insperm motility (21.95% for TMot and 29.97% for PMot) but also in the values of VAP, VCL and VSL. Thus, both the percentage of motile spermatozoa and the speed of their movementdecreased very much. Moreover, there was a significant increase in the values of the standard deviation, which indicates a decrease in uniformity of results and the repeatability of this chamber type. The decrease of kinetic parameters was even more visible at 12 minutes after loading the sample, for all the three chamber types but especially for MofA chamber where the value of progressive motility dropped to 5.42% (Table 3).

Table 2. Mean values and standard deviation obtained during the assessment of boar sperm, 6 minutes after filling the chamber

Chamber type	Leja (mean value ± SD)	MofA (mean value ± SD)	Minitube (mean value ± SD)
Conc ($\times 10^6$)	10.24 ± 1.95^{a}	10.62 ± 2.81^{a}	8.82 ± 2.63^{b}
TMot (%)	69.57 ± 9.25^{a}	51.03 ± 17.96^{b}	69.88 ± 12.18^{a}
PMot (%)	59.85 ± 12.77^{a}	25.23 ± 15.96^{b}	59.68 ± 15.85^{a}
VAP (μm/sec)	55.10 ± 9.20^{a}	32.57 ± 5.99^{b}	53.38 ± 7.88^{a}
VCL (μm/sec)	90.94 ± 17.86^{a}	64.32 ± 15.34^{b}	89.62 ± 14.40^{a}
VSL (μm/sec)	48.54 ± 8.73^{a}	26.74 ± 5.05^{b}	47.17 ± 7.99^{a}
STR (VSL/VAP)	0.88 ± 0.04^{a}	0.82 ± 0.04^{b}	0.88 ± 0.04^{a}
LIN (VSL/VCL)	0.54 ± 0.09^{a}	0.42 ± 0.08^{b}	0.53 ± 0.10^{a}
WOB (VAP/VCL)	0.61 ± 0.08^{a}	0.51 ± 0.08^{b}	0.60 ± 0.09^{a}
ALH (μm)	2.31 ± 0.46^{a}	2.07 ± 0.64^{b}	2.20 ± 0.38^{ab}
BCF (Hz)	36.72 ± 1.99^{a}	28.93 ± 3.90^{b}	38.22 ± 2.03^{c}

Within the same row, different superscripts indicate significant difference at $p<0.05$

Again, the Leja and Minitube chambers showed better and relatively similar results for sperm. The decrease of sperm motility over time is a normal phenomenon, and for this reason some authors recommend the analysis of the sample within 5 minutes from filling the analyzing chamber [27]. However, in our study we observed a much smaller decrease when

Leja and Minitube chambers were used. A possible cause for this fact could be the substances used in the production of MofA chamberwhich might have a toxic effect on boar sperm.

Table 3. Mean values and standard deviation obtained during the assessment of boar semen, 12 minutes after filling the chamber

Chamber type	Leja (mean value ± SD)	MofA (mean value ± SD)	Minitube (mean value ± SD)
Conc ($\times 10^6$)	9.29±1.46^{ab}	8.49±2.40^{bc}	6.89±2.04^{c}
TMot (%)	67.03±6.29^{a}	26.91±13.59^{b}	63.85±8.23^{a}
PMot (%)	57.76±7.91^{a}	5.42±5.46^{b}	55.77±9.58^{a}
VAP (µm/sec)	52.70±7.21^{a}	28.61±10.51^{b}	52.43±5.17^{a}
VCL (µm/sec)	85.39±16.98^{a}	47.44±20.76^{b}	85.37±14.55^{a}
VSL (µm/sec)	46.51±6.99^{a}	23.31±8.18^{b}	46.66±5.93^{a}
STR (VSL/VAP)	0.86±0.05^{a}	0.76±0.23^{a}	0.87±0.06^{a}
LIN (VSL/VCL)	0.54±0.09^{a}	0.47±0.17^{a}	0.55±0.12^{a}
WOB (VAP/VCL)	0.61±0.08^{a}	0.57±0.19^{a}	0.61±0.11^{a}
ALH (µm)	2.17±0.33^{a}	2.84±1.57^{a}	2.12±0.36^{a}
BCF (Hz)	36.24±2.39^{a}	18.03±8.68^{b}	37.65±1.60^{a}

Within the same row, different superscripts indicate significant difference at $p<0.05$

Again, no significant differences were observed between Leja and Minitube ($p>0.05$) in terms of all the kinetic parameters determined within this study. The effect of analyzing chamber on the percentage of motile spermatozoa is illustrated in Figures 1 and 2.

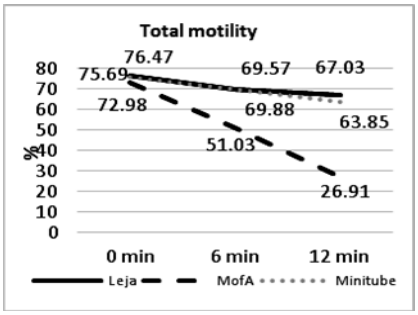

Figure 1 Total sperm motility according to the chamber type and time point

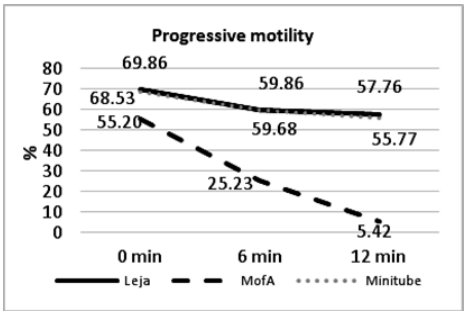

Figure 2 Progressive sperm motility according to the chamber type and time point

As mentioned above, the values of sperm motility (both total and the progressive) were lower, and the negative effect of time elapsed since filling the chamber was much higherfor MofA chamber. Mean value and standard deviation showed no significant differences between Leja and Minitube chambers, which means they offer an equal level of accuracy. Moreover, although we do not recommend a semen evaluation later than 5 minutes after filling the analyzing chamber, our results suggest that Leja and Minitube chambersmayenablean analysis of boar sperm with acceptable results even at 12 minutes after filling.

3.2. The effect of the chamber type on sperm concentration

Considering the possibility that the three chamber types could offer different results, we used additional methods for determining the sperm concentration, in order to decide which of the chambers is more accurate. Thus, this parameter was also determined by SQA-Vp, Nucleocounter® NC-100™ and Thoma hemocytometer. SQA-Vp device (Sperm quality analyzer version pig) converts the variations of optical density in electrical signals to estimate the number of sperm in the test sample and its efficiency has been demonstrated previously [25].

The Nucleocounter® device is equipped with a novel integrated fluorescence microscope, designed to detect signals from individual sperm cell nuclei by means of a nuclei staining dye, propidium iodide. Detected signals are correlated to direct sperm count. Nucleocounter tends to become a new reference method for determining sperm concentration. It shows a high degree of accuracy [23] and has some advantages over the hemocytometer method, for example much shorter analysis time and completion of a supplementary examination (calculation of viability). The cheapest and widely available method to determine sperm concentration in all species is the hemocytometer, a method often called the 'gold standard' for counting sperm [22, 28]. However, for hygienic and also practical reasons the current trend is to replace the classical hemocytometer with disposable chambers. In addition, the quality of disposable chambers is not affected by the damage from long term usage or multiple washes, thus reducing the risk of erroneous results. Table 4 shows the results obtained for sperm concentration, depending on the method and type of chamber used for its calculation.

Table 4. The values of sperm concentration, according to the method used for determination

Computer-assisted sperm analysis (CASA)			SQA-Vp ($\times 10^6$)	Nucleocounter ($\times 10^6$)	Thoma chamber ($\times 10^6$)
Leja (mean±SD) ($\times 10^6$)	MofA (mean±SD) ($\times 10^6$)	Minitube (mean ±SD) ($\times 10^6$)			
13.00±2.17[a]	15.71±3.18[b]	11.42±2.02[c]	24.18	25.52	25.50

-Within the same row, different superscripts indicate significant difference at $p<0.05$; For Leja, MofA and Minitube were taken into account the values obtained immediately after filling the chamber; The standard deviation was omitted in SQA-Vp, Nucleocounter and Thoma chamber because the number of determinations was much lower.

Significant differences were observed among the three types of disposable chambers, with MofA showing the highest mean value (and the highest standard deviation), Leja being the second and Minitube the last. However, none of these chambers presented results similar to those obtained using the additional methods of analysis. The Thoma counting chamber is often considered a standard method for counting sperm.In this study the SQA-Vp, the Nucleocounter and the Thoma counting chamber showed close values, which strengthens the hypothesis that their output is very close to the true value. Based on this, it seems that CASA may be deficient in calculating the sperm concentration in liquid-stored boar semen regardless of the chamber type used for analysis.Similar situations have been reported by other authors as well[23, 26]. This imprecision is most likely due to sperm agglutination within the test sample. Boar sperm are characterized by a high rate of agglutination during storage in liquid form at 17°C, some authors indicating the bacterial contamination as one of the causes [29, 30]. This induces the occurrence of sperm gathered in heaps and left undetectedby the CASA system because the software only identifies the sperm with loose heads. In this way, the total number of counted sperm is lower. On the other hand, SQA-Vp and Nucleocounter operate on principles that allow the counting of all sperm, including those that are agglutinated. The Pearson analysis indicatessignificant positive correlations ($p<0.01$) among the three types of disposable chambers in terms of the calculated sperm concentration (Table 5).

Table 5. Correlations among the three types of disposable chambers in terms of the calculated sperm concentration

		Leja	MofA	Minitube
Leja	Pearson Correlation	1	0.618**	0.594**
	Sig. (2-tailed)		0.000	0.000
MofA	Pearson Correlation	0.618**	1	0.693**
	Sig. (2-tailed)	0.000		0.000
Minitube	Pearson Correlation	0.594**	0.693**	1
	Sig. (2-tailed)	0.000	0.000	

**. Correlation is significant at p< 0.01 (2-tailed).

Thus, although all the chambers tested within this study (Leja, MofA and Minitube) offered results lower than the real value in terms of sperm concentration, it seems that their results are under the influence of a common factor. This strengthens the hypothesis of sperm agglutination as the cause of CASA imprecision in calculating sperm concentration of liquid-stored boar semen.

4. Conclusions

Our results suggest that the output of CASA is influenced by the chamber typewhen examining liquid-stored boar semen. In our study the Leja and Minitube chamber types showed similar values, while the MofA chamber offered significantly lower results. We think that further studies on MofA chamber are needed, in order to confirm or to refute the negative effect exerted on the kinetic sperm parameters and to determine whether this effect (if confirmed) is due to chemical or mechanical factors.We also recommend a study on boar raw semenand also onsemen of other species, the higher sensitivity of boar spermatozoa being well known. In addition, we found a significant difference between the concentration determined by CASA and that obtained using other methods (Nucleocounter, SQA-Vp, Thoma hemocytometer). Since all the other methods are widely recognized to be accurate and the differences among them were very small, we tend to think it was rather an underestimation by CASA system than an overestimation of the other devices. Based on this, we do not recommend the use of CASA for determining sperm concentration in liquid-stored boar semen, unless corrective measures are considered.

5. Acknowledgements

This study was conducted under the frame of European Social Fund, Human Resources Development Operational Programme 2007-2013, project no. POSDRU/159/1.5/S/132765. The authors want to thank Besamungsverein Neustadt an der Aisch, Germany, for their support during the study, by providing the semen samples and the technical instruments necessary for semen examination.

References

1. C. GLOSSOP, Infertility in the sow and gilt. *Arthur's Veterinary Reproduction and Obstetrics*, by D.E. NOAKES, T.J. PARKINSON and G.C.W. ENGLAND. W.B. Saunders. 2001, Pp. 621-638.
2. W.L. FLOWERS, - Management of boars for efficient semen production. *J Reprod Fertil* 52:67–78 (1997).
3. P.R. BUDWORTH, R.P. AMANN, P.L. CHAPMAN, Relationships between computerized measurements of motion of frozen-thawed bull spermatozoa and fertility. *J Androl.,*9:41–54 (1988).
4. Y. HIRANO, H. SHIBAHARA, T. SUZUKI, S. TAKAMIZAWA, C. YAMAGUCHI, H. TSUNODA, Relationships between sperm motility characteristics assessed by the computer-aided sperm analysis (CASA) and fertilization rates in vitro. *J Assist ReprodGenet.,* 18:213–8 (2001).
5. M. HIRAI, A. BOERSMA, A. HOEFLICH, E. WOLF, J. FOLL, T.R. AUMULLER. Objectively measured sperm motility and sperm head morphometry in boars (Susscrofa): relation to fertility and seminal plasma growth factors. *J Androl.* 22:104–110 (2001).

6. N. SUTKEVICIENE, M.A. ANDERSSON, H. ZILINSKAS, M. ANDERSON. Assessment of boar semen quality in relation to fertility with special reference to methanol stress. *Theriogenology*, 63:739–47 (2005).
7. F.J. McPHERSON, S.G. NIELSEN, P.J. CHENOWETH - Semen effects on insemination outcomes in sows. *Animal Reproduction Science* 151: 28–33 (2014).
8. B.A. DIDION. Computer-assisted semen analysis and its utility for profiling boar semen samples. *Theriogenology*, 70 (8): 1374–1376 (2008).
9. M.L.W.J. BROEKHUIJSE, E. ŠOŠTARIC, H. FEITSMAD, B.M. GADELLA. Additional value of computer assisted semen analysis (CASA) compared to conventional motility assessments in pig artificial insemination. *Theriogenology* 76:1473–1486 (2011).
10. I. PALACÍN, S. VICENTE-FIEL, P. SANTOLARIA, J.L. YÁNIZ, Standardization of CASA sperm motility assessment in the ram. *Small Ruminant Research,* 112:128– 135 (2013)
11. D.O. DAVIS, D.F. KATZ, Computer-aided sperm analysis: technology at a crossroads. *Fertil Steril,* 59:953–5 (1993).
12. W. KRAUSE, Computer-assisted semen analysis systems: comparison with routine evaluation and prognostic value in male fertility and assisted reproduction. *Hum Reprod*., 10:60–66(1995).
13. R.P. AMANN, D. WABERSKI, Computer-assisted sperm analysis (CASA): Capabilities and potential developments. *Theriogenology* 81:5–17 (2014).
14. I. IBĂNESCU, D. DRUGOCIU, P. ROŞCA, Ioana SFARTZ. Factors influencing sperm motility in boar. *Lucrări Ştiinţifice USAMV Iaşi – seria Medicină Veterinară*, 57(1-2):116-126. (2014)
15. J. VERSTEGEN, M. IGUER-OUADA, K. ONCLIN, Computer assisted semen analyzers in andrology and veterinary practice. *Theriogenology* 57:149-79 (2002)
16. F. TEJERINA, K. BURANAAMNUAY, F. SARAVIA, M. WALLGREN, H. RODRIGUEZ-MARTINEZ, Assessment of motility of ejaculated, liquid-stored boar spermatozoa using computerized instruments. *Theriogenology* 69:1129–1138 (2008).
17. J. EHLERS, M. BEHRB, H. BOLLWEIN, M. BEYERBACH, D. WABERSKI, Standardization of computer-assisted semen analysis using an e-learning application. *Theriogenology* 76:448–454 (2011).
18. M. KRAEMER, C. FILLION, B. MARTIN-PONT, J. AUGER, Factors influencing human sperm kinematic measurements by the Celltrak computer-assisted sperm analysis system. *Hum. Reprod.,* 13:611–619 (1998)
19. M. TOMLINSON, J. TURNER, G. POWELL, D. SAKKAS, One-step disposable chambers for sperm concentration and motility assessment: how do they compare with the World Health Organization's recommended methods? *Hum. Reprod*. 16 (1): 121-124. (2001).
20. A. GLORIA, A. CARLUCCIO, A. CONTRI, L. WEGHER, C. VALORZ, D. ROBBE. The effect of the chamber on kinetic results in cryopreserved bull spermatozoa. *Andrology* 1(6):879-85 (2013)
21. Ş.G. CIORNEI. *Materialul seminal de vier şi biotehnologii de reproducţie la suine*. Ed. "Ion Ionescu de la Brad", Iaşi, pp. 42-44.
22. N. PRATHALINGAM, W. HOLT, S.REVELL, S. JONES, P. WATSON - The Precision and Accuracy of Six Different Methods to Determine Sperm Concentration. *J Androl*., 27(2):257-62 (2006).
23. C. HANSEN, T. VERMEIDEN, J.P.W. Vermeiden, C. Simmet, B.C. Day, H. Feitsma - Comparison of FACSCount AF system, Improved Neubauer hemocytometer, Corning 254 photometer, SpermVision, UltiMate and NucleoCounter SP-100 for determination of sperm concentration of boar semen. *Theriogenology* 66:2188–2194 (2006).
24. A. CAMUS, S. CAMUGLI, C. LÉVÊQUE, E. SCHMITT, C. STAUB. Is photometry an accurate and reliable method to assess boar semen concentration? *Theriogenology* 75:577–583 (2011)
25. A. LÓPEZ RODRÍGUEZ, T. RIJSSELAERE, J. BIJTTEBIER, P. VYT, A. VAN SOOM, D. MAES - Effectiveness of the sperm quality analyzer (SQA-Vp) for porcine semen analysis. *Theriogenology* 75 : 972–977 (2011).
26. P. VYT, D. MAES, T. RIJSSELAERE, E. DEJONCKHEERE, F. CASTRYCK, A. VAN SOOM, Motility assessment of porcine spermatozoa: a comparison of methods. *ReprodDomest Anim.* 39(6):447-53 (2004).
27. A. CONTRI, C. VALORZ, M. FAUSTINI, L. WEGHER, A. CARLUCCIO, Effect of semen preparation on CASA motility results in cryopreserved bull spermatozoa. *Theriogenology* 74 (3): 424–435 (2010).
28. T. RIJSSELAERE, A. VAN SOOM, D. MAES, A. de KRUIF. Effect of technical settings on canine semen motility parameters measured by the Hamilton-Thorne analyzer. *Theriogenology* 60, 1553-1568 (2003).
29. J.L. UBEDA, R. AUSEJO, Y. DAHMANI, M.V. FALCETO, A. USAN, C. MALO, F.C. Perez-Martinez Adverse effects of members of the Enterobacteriaceae family on boar sperm quality. *Theriogenology*. 80(6):565-570 (2013)
30. N. PRIETO-MARTÍNEZ, E. BUSSALLEU, E. GARCIA-BONAVILA, S. BONET, M. YESTE. Effects of Enterobacter cloacae on boar sperm quality during liquid storage at 17°C. *Anim Reprod Sci*. 148 (1-2):72-82 (2014)