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# COMPARISON OF DIFFERENT EVALUATION CHAMBERS FOR ANALYSIS OF RABBIT SPERMATOZOA MOTILITY PARAMETERS USING CASA SYSTEM

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## ABSTRACT

In this study rabbit spermatozoa motility parameters, measured using different evaluation chambers, were compared. The measurement was done using CASA (Computer Assisted Semen Analysis) system; each sample was placed into four different chambers – microscopic slide, Zander Spermometer, Standard Count Analysis Chamber Leica 20 micron and Makler Counting Chamber. CASA showed that all measured parameters varied depending on chamber used as follows: an average spermatozoa concentration was 1.02 – 1.17 x $10^6$/ml, the percentage of motile spermatozoa was in range 59.85 – 77.78% and spermatozoa with progressive motility was ranged from 46.14 to 68.57%. Of other parameters, DAP was 19.23 – 24.44 µm, DCL 37.43 – 47.20 µm, DSL 14.27 – 18.92 µm, VAP 45.26 – 57.31 µm/s, VCL 87.45 – 110.37 µm/s, VSL was 33.77 – 44.31 µm/s, straightness 0.71 – 0.76, linearity 0.36 – 0.40, wobble 0.50 – 0.52, ALH 4.18 – 4.60 µm and BSF 23.58 – 28.16. Statistical analysis detected significant differences in almost all studied parameters in regards to evaluation chamber used. Particularly, highest values for concentration, percentage of motile and progressive motile spermatozoa were detected when microscopic slide with coverslip was used as a spermatozoa chamber. In parameters of the distance, velocity, linearity, straightness and BSF the highest values were obtained using Zander Spermometer, whilst the amplitude of lateral head displacement was the highest in the Makler chamber. These results clearly suggest that the type of evaluation chamber may influence a reliability of measurement of spermatozoa parameters.

**Keywords**: rabbit, spermatozoa, CASA, counting chamber

## INTRODUCTION

In recent years, a number of techniques for objective assessment of movement characteristics of human and animal spermatozoa have been introduced using computer–assisted (automated) semen analysis (CASA) systems. For the conventional analysis, a simple classification system, which provides the best possible assessment of sperm motility with no needs for complex equipment, is recommended (Massanyi et al., 2002; Chrenek et al., 2007, Okab 2007, Makarevich et al., 2008). The use of computer-assisted (automated) semen analyzer – CASA is a promising alternative to the traditional approach of microscopic visualization of spermatozoa motility and heamocytometric evaluation of spermatozoa concentration. The usefulness of the CASA system in clinical and experimental practice has been described in man (Farrell et al., 1996; Spiropoulos 2001; Chantler et al., 2004) as well as in various animals: bulls (Goffaux and Thibier 1986; Massanyi et al., 1995; 1996a,b; 1998a; 1999), rams (Massanyi et al., 1998b), stallion (Jasko et al., 1990; Massanyi et al., 1998c), fox (Massanyi et al., 1998d; 2002).

Semen analysis is a cornerstone of testing for male infertility problems. This test provides important information about the quality and quantity of the spermatozoa. Semen sample is analyzed for the volume, viscosity (thickness), pH and colour of the ejaculate,

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spermatozoa concentration, motility, morphology, and straight-forward progression of the spermatozoa. The sample is also examined for the presence of white or red blood cells which may indicate infection or inflammation. We perform both manual and computer assisted semen analyses (CASA). From this simple test, we can tell how many spermatozoa are present, how many appear normal and how many are moving (Mahony et al., 1988; Johnson et al., 1990).

The aim of this study was to compare four different chambers used for evaluation of spermatozoa motility by computer assisted semen analysis (CASA) to find possible differences that could influence measured parameters.

## MATERIAL AND METHODS

Rabbit semen was obtained according to a regular collection schedule and 16 samples (16 000 spermatozoa) from adult breeding rabbits (SARC, Nitra, Slovak republic) were used. Semen was collected from each animal and subsequently diluted in the semen diluent (Minitüb, Germany) by routine approach. After proceeding all samples were transported to the laboratory at room temperature. Analysis was done using a CASA system – SpermVision (Minitüb, Tiefenbach, Germany) combined with Olympus BX 51 microscope (Olympus, Japan).

Each sample was placed into four different chambers – microscopic slide with 20x20 mm coverslip (MS, with a depth of 18 µm), Zander Spermometer (ZS, a depth of 10 µm, Zander Medical, Germany), Standard Count Analysis Chamber Leica 20 micron (SC, Minitüb, Germany) and Makler Counting Chamber (MC, depth of 10 µm, Sefi–Medical Instruments, Germany). In each sample following parameters were evaluated: concentration ($10^6$ per ml); percentage of motile spermatozoa (motility > 5 µm/s), percentage of progressive motile spermatozoa (motility > 20 µm/s), DCL (distance curved line; µm), DAP (distance average path, µm), DSL (distance straight line, µm), VCL (velocity curved line, µm/s), VAP (velocity average path, µm/s), VSL (velocity straight line, µm/s), LIN (linearity – VSL:VCL), STR (straightness – VSL:VAP), WOB (wobble – VAP:VCL), ALH (amplitude of lateral head displacement, µm) and SCF (beat cross frequency, $H_2$).

Obtained data were statistically analyzed by Excel software and GraphPad Prism 3 using Dunn's multiple comparison tests.

## RESULTS

Computer assisted semen analysis showed that the average spermatozoa concentration was 1.02 – 1.17 x $10^6$ per ml. The percentage of motile spermatozoa ranged from 59.85 to 77.78% and percentage of spermatozoa with progressive motility was 46.14 – 68.57%. Analysis of distance parameters showed that DAP was 19.23 – 24.44 µm, DCL 37.43 – 47.20 µm and DSL 14.27 – 18.92 µm. In velocity parameter VAP was 45.26 – 57.31 µm/s, VCL 87.45 –110.37 µm/s and VSL was 33.77 – 44.31 µm/s. In other parameters these data were measured: straightness 0.71 – 0.76, linearity 0.36 – 0.40, wobble 0.50 – 0.52, ALH 4.18 – 4.60 µm and BSF 23.58 – 28.16 (Tables 1 - 4).

**Table 1: CASA results obtained using microscopic slide with coverslip (MS)**

| Parameter | $\bar{x}$ | SD | CV | minimum | maximum |
|---|---|---|---|---|---|
| concentration | 1.17 | 0.60 | 51.28 | 0.44 | 2.36 |
| % motile | 77.78 | 8.90 | 11.44 | 63.77 | 93.58 |
| % progressive | 68.57 | 12.89 | 18.80 | 43.71 | 89.42 |
| DAP | 19.23 | 3.47 | 18.04 | 12.60 | 24.23 |
| DCL | 37.43 | 6.92 | 18.49 | 22.53 | 46.89 |
| DSL | 14.27 | 3.16 | 22.14 | 9.52 | 20.11 |
| VAP | 45.26 | 7.94 | 17.54 | 30.22 | 56.84 |
| VCL | 87.45 | 16.03 | 18.33 | 53.10 | 106.67 |
| VSL | 33.77 | 7.21 | 21.35 | 21.93 | 47.93 |
| STR | 0.74 | 0.06 | 8.11 | 0.65 | 0.84 |
| LIN | 0.38 | 0.04 | 10.53 | 0.31 | 0.44 |
| WOB | 0.51 | 0.02 | 3.92 | 0.47 | 0.57 |
| ALH | 4.18 | 0.67 | 16.03 | 3.29 | 5.56 |
| BSF | 24.62 | 2.32 | 9.42 | 19.73 | 28.35 |

**Table 2: CASA results obtained using Zander Spermometer (ZS)**

| parameter | $\bar{x}$ | SD | CV | minimum | maximum |
|---|---|---|---|---|---|
| concentration | 1.05 | 0.73 | 69.52 | 0.13 | 2.40 |
| % motile | 72.46 | 18.26 | 25.20 | 41.87 | 94.86 |
| % progressive | 61.11 | 25.86 | 42.32 | 19.21 | 92.17 |
| DAP | 24.44 | 6.42 | 26.27 | 14.14 | 36.07 |
| DCL | 47.20 | 12.79 | 27.10 | 26.39 | 69.99 |
| DSL | 18.92 | 6.50 | 34.36 | 11.18 | 32.82 |
| VAP | 57.31 | 14.07 | 24.55 | 34.56 | 80.25 |
| VCL | 110.37 | 28.67 | 25.98 | 61.26 | 152.08 |
| VSL | 44.31 | 13.96 | 31.51 | 27.58 | 71.53 |
| STR | 0.76 | 0.10 | 13.16 | 0.62 | 0.91 |
| LIN | 0.40 | 0.07 | 17.50 | 0.30 | 0.49 |
| WOB | 0.52 | 0.03 | 5.77 | 0.49 | 0.60 |
| ALH | 4.27 | 1.09 | 25.53 | 3.10 | 6.35 |
| BSF | 28.16 | 6.13 | 21.77 | 21.42 | 40.81 |

**Table 3: CASA results obtained using Standard Count Analysis Chamber 20 micron (SC)**

| parameter | $\bar{x}$ | SD | CV | minimum | maximum |
|---|---|---|---|---|---|
| concentration | 1.02 | 0.61 | 59.80 | 0.15 | 1.62 |
| % motile | 71.53 | 10.71 | 14.97 | 53.33 | 89.27 |
| % progressive | 56.55 | 15.57 | 27.53 | 34.48 | 85.12 |
| DAP | 21.54 | 8.31 | 38.58 | 9.74 | 35.07 |
| DCL | 43.06 | 18.32 | 42.55 | 20.04 | 74.69 |
| DSL | 15.24 | 6.02 | 39.50 | 7.27 | 26.78 |
| VAP | 50.41 | 18.61 | 36.92 | 22.14 | 76.65 |
| VCL | 99.96 | 41.30 | 41.32 | 45.51 | 163.49 |
| VSL | 35.80 | 13.33 | 37.23 | 16.64 | 62.27 |
| STR | 0.71 | 0.06 | 8.45 | 0.61 | 0.81 |
| LIN | 0.36 | 0.04 | 11.11 | 0.29 | 0.42 |
| WOB | 0.51 | 0.04 | 7.84 | 0.45 | 0.57 |
| ALH | 4.48 | 1.14 | 25.45 | 2.58 | 6.15 |
| BSF | 23.85 | 4.81 | 20.17 | 18.66 | 35.24 |

**Table 4: CASA results obtained using Makler Counting Chamber (MC)**

| parameter | $\bar{x}$ | SD | CV | minimum | maximum |
|---|---|---|---|---|---|
| concentration | 1.08 | 0.53 | 49.07 | 0.26 | 1.94 |
| % motile | 59.85 | 26.54 | 44.34 | 14.55 | 92.06 |
| % progressive | 46.14 | 29.34 | 63.59 | 3.79 | 88.88 |
| DAP | 20.65 | 4.84 | 23.44 | 11.08 | 30.13 |
| DCL | 41.60 | 11.63 | 27.96 | 19.73 | 67.79 |
| DSL | 14.63 | 3.49 | 23.86 | 8.59 | 24.37 |
| VAP | 48.56 | 11.28 | 23.23 | 24.94 | 69.44 |
| VCL | 96.78 | 26.98 | 27.88 | 43.60 | 156.63 |
| VSL | 34.58 | 8.09 | 23.40 | 20.19 | 55.28 |
| STR | 0.72 | 0.08 | 11.11 | 0.62 | 0.89 |
| LIN | 0.36 | 0.05 | 13.89 | 0.29 | 0.50 |
| WOB | 0.50 | 0.03 | 6.00 | 0.44 | 0.57 |
| ALH | 4.60 | 1.02 | 22.17 | 2.52 | 6.28 |
| BSF | 23.58 | 4.44 | 18.83 | 16.76 | 32.70 |

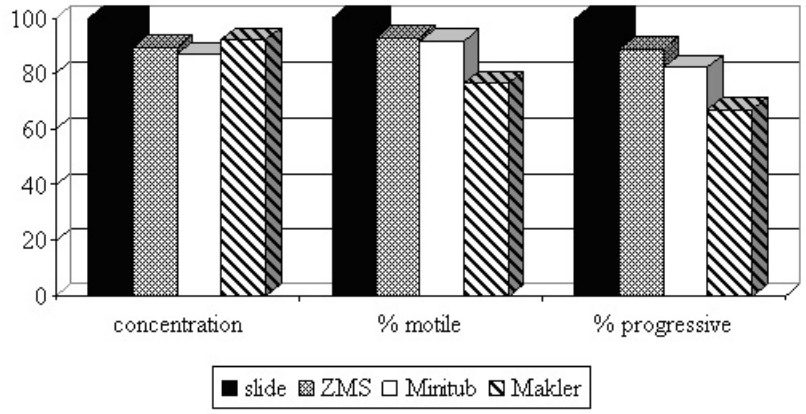


**Fig. 1: Differences in basic parameters (concentration, % motile, % progressive) in regards to counting chamber**

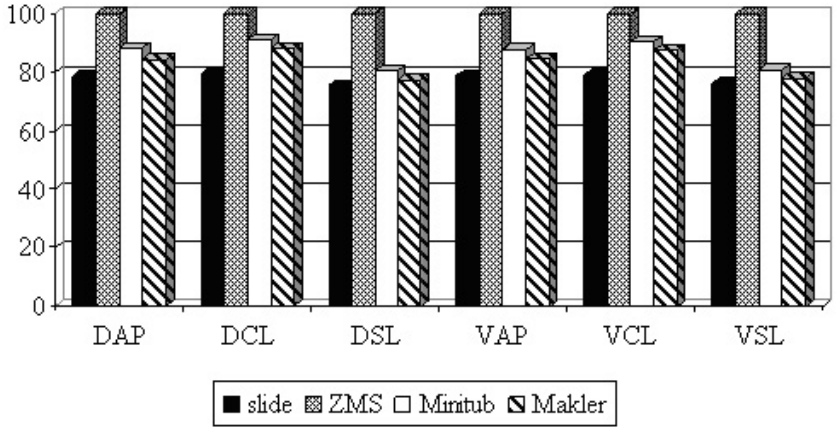


**Fig. 2: Differences in advanced paramters (DAP, DCL, DSL, VAP, VCL, VSL) in regards to counting chamber**

**Table 5: Significant difference between observed parameters in relation to various type of evaluation chamber (Dunn's multiple comparison test)**

| | MS - ZS | MS – SC | MS – MC | ZS – SC | ZS – MC | SC – MC |
|---|---|---|---|---|---|---|
| concentration | – | – | – | – | – | – |
| % motile | – | – | p<0.05 | – | – | – |
| % progressive | – | – | p<0.01 | – | p<0.01 | – |
| DAP | – | p<0.01 | – | – | p<0.05 | – |
| DCL | – | p<0.01 | – | – | – | – |
| DSL | p<0.001 | – | – | p<0.01 | p<0.01 | – |
| VAP | p<0.01 | – | – | – | – | – |
| VCL | p<0.01 | – | – | – | – | – |
| VSL | p<0.01 | – | – | p<0.01 | p<0.01 | – |
| STR | – | – | – | – | – | – |
| LIN | – | – | – | – | – | – |
| WOB | – | – | – | – | – | – |
| ALH | – | – | – | – | – | – |
| BSF | – | – | – | – | – | – |

MS – microscopic slide with coverslip; ZS – ZMS, Zander Spermometer; SC – Standard Count Analysis Chamber 20 micron; MC – Makler Counting Chamber; – - p>0.05

Statistical analysis detected no significant differences in concentration between all tested chambers. Differences were observed in percentage of motile spermatozoa (between MS and MC, p<0.05) and in percentage of progressive motile spermatozoa (between MS and MC, p<0.01 and between ZS and MC, p<0.01, Table 5, Figure 1).

In regards to type of spermatozoa movement, significant differences were observed in DAP (between MS and SC, p<0.01 and between ZS and MC, p<0.05), in DCL (between MS and SC, p<0.01), in DSL (between MS and ZS, p<0.001 and ZS and SC, p<0.01 and ZS and MC, p<0.01), in VAP (between MS and ZS, p<0.01), in VCL (between MS and ZS, p<0.01), in VSL (between MS and ZS, p<0.01, and ZS and SC, p<0.01, and ZS and MC, p<0.01) (Table 5, Figure 2).

No significant differences among chambers tested were found in STR, LIN, WOB, ALH and BSF parameters (Table 5).

## DISCUSSION

Compared to classic spermatozoa analysis using visual criteria for motility, a new system of computer assisted spermatozoa analysis (CASA) enables more objective and exact evaluation of spermatozoa quality including determination type of the movement. Despite this fact the correlation between multiple characteristics of semen quality measured by CASA and actual fertility in rabbits is 0.53 (Farrell et al., 1993). This correlation likely will be increased with refinement in instrumentation. We hypothesize that using CASA system the results of spermatozoa testing may be influenced by type of the evaluation chamber. Therefore in our study we aimed at verifying this assumption by comparison of four types of testing chambers of different cost.

Spermatozoa motility is one of major important factors of ejaculate characteristics. Evaluation of motility based on visual feelings of operator is rather subjective and needs some improvements. CASA is a high specific measuring system which allows defining different forms of spermatozoa motility, which is not possible to determine using classic method (for example using Burker Turk slide). Motility parameters, determined by this system, in combination with spermatozoa morphology analysis can provide additional information about the fertilizing capacity of rabbit spermatozoa (Lavara et al., 2005). Analysis of the motile spermatozoa revealed several types of trajectories (irregular, small circular, large circular and arcs, jagged and straight-line). Accuracy of classification varied from 70% to 96%, depending on the type of track (Perez–Sanchez et al., 1996).

In our study more differences in parameters of spermatozoa motility were determined between chambers MS and ZS (DSL, VAP, VCL, VSL) as well as between chambers ZS and MC (% progressive, DAP, DSL and VSL). On the other hand, no differences in all studied

spermatozoa motility parameters were found between SC and MC chambers.

There are several factors which can influence CASA results, particularly the collection site (Perez–Sanchez et al., 1996), dilution (Castellini et al., 2006), male age (Pizzi et al., 2005) and others. Basing on our data, the type of testing chamber for CASA measurement may be another factor, which can influence correctness of final results. In particular, differences detected in this study are most probably caused by the depth of the chamber (slide with coverslip has a 18 μm depth; Zander Spermometer - 10 μm; Analysis Chamber Leica - 20 μm and Makler Counting Chamber - 10 μm). Another possible factor may be adhesive characteristics of chamber surface. On the other hand, commercially available chambers are manufactured to reduce cell adhesion to glass surfaces in order to provide the highest motility and lowest lateral head displacement (Armant and Ellis, 1995).

Our results clearly suggest that the type of evaluation chamber may influence reliability of measurement of spermatozoa parameters. For evaluation of complex spermatozoa motility parameters only one unified type of chamber should be used to achieve reliable results.

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