

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

P. MASSÁNYI¹, P. CHRENEK²,³, N. LUKÁȹ, A.V. MAKAREVICH², A. OSTRO⁴, J. ŽIVČAK⁵, J. BULLA¹

<sup>1</sup>Slovak University of Agriculture, Nitra, Slovak Republic; <sup>2</sup>Slovak Agricultural Research Centre, Nitra, Slovak Republic; <sup>3</sup>Constantine the Philosopher University, Nitra, Slovak Republic; <sup>4</sup>Paul Joseph **Šafarik University, Košice**, Slovak Republic; <sup>5</sup>Technical University, Košice, Slovak Republic

#### **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  $1.02-1.17 \times 10^6$ /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,

Correspondence: E-mail: peter.massanyi@uniag.sk Received: February 2, 2008; Accepted: Marche 18, 2008

60 ISSN 1335-3686

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  $\mu$ m), Zander Spermometer (ZS, a depth of 10  $\mu$ m, Zander Medical, Germany), Standard Count Analysis Chamber Leica 20 micron

(SC, Minitüb, Germany) and Makler Counting Chamber (MC, depth of 10  $\mu m$ , Sefi–Medical Instruments, Germany). In each sample following parameters were evaluated: concentration ( $10^6$  per ml); percentage of motile spermatozoa (motility > 5  $\mu$ m/s), percentage of progressive motile spermatozoa (motility > 20  $\mu$ m/s), DCL (distance curved line;  $\mu$ m), DAP (distance average path,  $\mu$ m), DSL (distance straight line,  $\mu$ m), VCL (velocity curved line,  $\mu$ m/s), VAP (velocity average path,  $\mu$ m/s), VSL (velocity straight line,  $\mu$ m/s), LIN (linearity – VSL:VCL), STR (straightness – VSL:VAP), WOB (wobble – VAP:VCL), ALH (amplitude of lateral head displacement,  $\mu$ m) and SCF (beat cross frequency, H<sub>2</sub>).

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  $19.23-24.44\,\mu m$ , DCL  $37.43-47.20\,\mu m$  and DSL  $14.27-18.92\,\mu m$ . In velocity parameter VAP was  $45.26-57.31\,\mu m/s$ , VCL  $87.45-110.37\,\mu m/s$  and VSL was  $33.77-44.31\,\mu m/s$ . In other parameters these data were measured: straightness 0.71-0.76, linearity 0.36-0.40, wobble 0.50-0.52, ALH  $4.18-4.60\,\mu m$  and BSF 23.58-28.16 (Tables 1-4).

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

Parameter	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	$\frac{-}{x}$	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	_ 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. C	ASA results	obtained using	Makler Counting	g Chamber (MC)
Table 4. C.	ASA I CSUIIS	optamed using	IVIAKICI COUHUH	e Chambel (Mc)

parameter		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

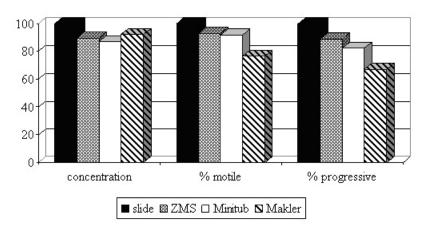


Fig. 1: Differences in basic parameters (concentration, % motile, % progressive) in regards to counting chamber

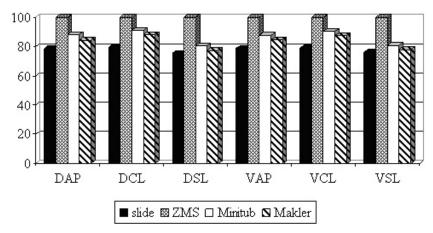


Fig. 2: Differences in advanced paramters (DAP, DCL, DSL, VAP, VCL, VSL) in regards to counting chamber

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 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.

# **ACKNOWLEDGEMENT**

This work was supported by grants APVV 0299–06, VEGA 1/0696/08 and the grant no. 1985/2007 of the Ministry of Education of Slovak Republic.

## REFERENCES

- AMBRIZ, D. ROSALES, A.M. SOTELO, R. MORA, J.A. ROSADO, A. GARCIA, A.R. 2002. Changes in the quality of rabbit semen in 14 consecutive ejaculates obtained every 15 minutes. In: *Arch. Androl.*, vol. 48, 2002, p. 389-395.
- ARMANT, D. R. ELLIS, M. A. 1995. Improved accuracy of sperm motility assessment using a modified Micro-Cell sperm counting chamber. In: *Fertil. Steril.*, vol. 63, 1995, p. 1128-1130.
- BOITI, C. CASTELINI, C. THEAU–CLEMENT, M. BESENFELDER, U. LIGUORI, L. RENIERI, T. PIZZI, F. 2005. Guidelines for the handling of rabbit bucks and semen. In: World Rabbit Sci., vol. 13, 2005, p. 71-91.
- CASTELLINI, C. PIZZI, F. THEAU–CLEMENT, M. 2006. Effect of different number of frozen spermatozoa inseminated on the reproductive performance of rabbit does. In: *Theriogenology*, vol. 66, 2006, p. 2182-2187.
- CHANTLER, E. ABRAHAM–PESKIR, J. ROBERTS, C. 2004. Consistent presence of two normally distributed sperm subpopulations within normozoospermic human semen: a kinematic study. In: *Intern. J. Androl.*, vol. 27, 2004, p. 350-359.

- CHRENEK, P. DRAGIN, S. MAKAREVICH, A.V. 2007. Reproductive characteristics of transgenic rabbit males with human protein C. In: *Slovak J. Anim. Sci.*, vol. 39, 2007, p. 131-134.
- FARRELL, P. B. FOOTE, R. H. SIMKIN, M. E. CLEGG, E. D. WALL, R. J. 1993. Relationship of semen quality, number of sperm inseminated, and fertility in rabbits. In: *J. Androl.*, vol. 14, 1993, p. 464-471.
- FARRELL, P. B. FOOTE, R. H. MCARDLE, M. M. TROURNTREND, V. K. TARDIF, A. L. 1996. Media and dilution procedures tested to minimize handling effects on human, rabbit, and bull sperm for computer–assisted sperm analysis (CASA). In: *J. Androl.*, vol. 17, 1996, p. 293-300.
- GOFFAUX, M. **THIBIER, M**. 1986. Evaluation of a semen analyzer for thawed bovine semen. In: *Elevate Insem.*, vol. 5, 1986, p. 215-224.
- JASKO, D. J. LEIN, D. H. FOOTE, R. H. 1990. A comparison of two computer–assisted semen analysis instruments for the evaluation of sperm motion characteristics in the stallion. In: *J. Androl.*, vol. 11, 1990, p. 453-459.
- JOHNSON, J.E. BOONE, W.R. SHARIPO, S.S. 1990. Determination of the precision of an automated semen analyzer. In: *Lab. Med.*, vol. 1, 1990, p. 33-38.
- LAVARA, R. MOCE, E. LAVARA, F. DE CASTRO, M.P.V – VINCENTE, J.S. 2005. Do parameters of seminal quality correlate with the results of on–farm inseminations in rabbits? In: *Theriogenology*, vol. 64, 2005, p. 1130-1141.
- MAHONY, M. C. ALEXANDER, N. J SWANSON, R. J. 1988. Evaluation of semen parameters by means of automated sperm motion analyzers. In: *Fertil. Steril.*, vol. 49, 1988, p. 876-880.
- MAKAREVICH, A. V. PARKANYI, V. ONDRUŠKA, Ľ. KUBOVIČOVÁ, E. FĽAK, P. SLEZÁKOVÁ, M. PIVKO, J. RAFAY, J. 2008. Evaluation of fertilizing capacity of rabbit sperm on the basis of annexin of Vlabelled membrane changes. In: *Slovak J. Anim. Sci.*, vol. 41, 2008, p.1-5.
- MASSÁNYI, P. MASSÁNYIOVÁ, K. PIZZI, F. TRANDŽÍK, J. 1995. Time dependent sperm motion in bulls evaluated by computerized semen analyzer. In: *Slov. Vet. J.*, vol. 20, 1995, p. 292-294.
- MASSÁNYI, P. LUKÁČ, N. TRANDŽÍK, J. 1996a. In vitro inhibition of the motility of bovine spermatozoa by cadmium chloride. In: *J. Environ. Sci. Health*, vol. A31, 1996, p. 1865-1879.
- MASSÁNYI, P. TRANDŽÍK, J. MASSÁNYIOVÁ, K. ČELECHOVSKÝ, M. 1996b. Determination of sperm motion by CellSoft automated semen analyzer. In: *Acta Zootech. Univ. Agric. Nitra*, vol. 51, 1996, p. 95-98.
- MASSÁNYI, P. HORNIAKOVÁ, E. LUKÁČ, N. HUSTÝ, S. 1998a. Evaluation of breeding bull ejaculate quality in relation to the level of nutrition. In: *Agriculture*, vol. 44, 1998, p. 589-599.
- MASSÁNYI P. **TRANDŽÍK**, J. **LUKÁČ**, N. **TOMAN**, R. 1998b. Time dependent motility of ram spermatozoa evaluated by a computer analyzer. In: *Slov. Vet. J.*, vol. 23, 1998, p. 107-109.
- MASSÁNYI P. **TRANDŽÍK**, J. **LUKÁČ**, N. **TOMAN**, R. 1998c. Time dependent motility of stallion spermatozoa evaluated by a computer analyzer. In: *Slov. Vet. J.*, vol. 23,

- 1998, p. 134-135.
- MASSÁNYI P. TRANDŽÍK, J. LUKÁČ, N. TOMAN, R. – SLAMEČKA, J. – KOVÁČIK, J. 1998d. Evaluation of spermatozoa motility in a male fox (Vulpes vulpes) by the computer technique. In: *Slov. Vet. J.*, vol. 23, 1998, p. 306-308.
- MASSÁNYI, P. MASSÁNYIOVÁ, K. PIZZI, F. TRANDŽÍK, J. – TOMAN, R. 1999. Time-dependent influence of low cadmium dose on bovine spermatozoa motion after refreezing. In: *Agriculture*, vol. 45, 1999, p. 287-295.
- MASSÁNYI, P. TRANDŽÍK, J. LUKÁČ, N. TOMAN, R. – HALO, M. – STRAPÁK, P. 2002. Evaluation of spermatozoa motility using computer technique. 1. st. Nitra : Slovak Agricultural University, 2002, 70 p. ISBN 80-8069-117-7
- OKAB, A. B. 2007. Semen characteristics and plasma testosterone of New Zealand male rabbits as affected by

- environmental temperatures. In: Slovak J. Anim. Sci. vol. 40, 2007, p.161-167.
- PEREZ-SANCHEZ, F. TABLADO, L. YEUNG, C. H. COOPER, T. G. SOLER, C. 1996. Changes in the motility patterns of spermatozoa from the rabbit epididymis as assessed by computer–aided sperm motion analysis. In: *Mol. Reprod. Develop.*, vol. 45, 1996, p. 364-371.
- PIZZI, F. GLIOZZI, T. M. ALETTI, B. 2005. Study of semen quality parameters in relation to genetic line and age of the boar. In: *Theriogenology*, vol. 63, 2005, p. 493-494.
- SPIROPOULOS, J. 2001. Computerized semen analysis (CASA): Effect of semen concentration and chamber depth on measurements. In: *Arch. Androl.*, vol. 46, 2001, p. 37-42.
- YANAGIDA, K. HOSHI, K. SATO, A. BURKMANN, L. 1990. Automated semen analysis shows an increase in sperm concentration and motility with time in Makler chambers having excess sample volume. In: *Hum. Reprod.*, vol. 5, 1990, p. 193-196.

Author's addresses: Peter Massányi, Norbert Lukáč, Jozef Bulla, Department of Animal Physiology, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic; Peter Chrenek, Alexander V. Makarevich, Slovak Agricultural Research Centre, Hlohovská 2, 949 92 Nitra, Slovak Republic; Peter Chrenek; Constantine the Philosopher University, Department of Zoology and Anthropology, Nábrežie mládeže 91, 949 74 Nitra, Slovak Republic; Alexander Ostro, II.Department of Reproduction and Obstetrics - Centre for Assisted Reproduction, Paul Joseph Šafarik University, Tr. SNP 1, 040 66 Košice, Slovak Republic; Jozef Živčák, Department of Biomedical Engineering, Automatization and Measurements, Technical University, Letná 9, 042 00 Košice, Slovak Republic