Journal of Microbiology, Biotechnology and Food Sciences

Błaszczyk et al. 2013 : 2 (Special issue 1) 1365-1376

REGULAR ARTICLE

SEMEN QUALITY ASSESSMENT OF NEW ZEALAND WHITE RABBIT BUCKS

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ABSTRACT

Rabbits have been extensively used as a model for large animals and humans. All the reproduction techniques employed with farm animals can be performed with the low-cost rabbit model, and certain placental membrane characteristics make them especially relevant for studies of human teratology. The purpose of this study was to assess semen quality of New Zealand White rabbits. The material represents semen samples collected from adult rabbits (n=30). The semen was obtained by means of artificial vagina. All samples were analyzed using CASA Sperm Vision™ system. To assessed spermatozoa morphology (the length and the width of head and tail; presence of abnormal spermatozoa) we used QuickPhoto Micro system. Received data were statistically analyzed. Our research showed decrease of semen parameters value after one hour storage in 37°C. Correlation analysis showed negative correlation between presence of spermatozoa with separated flagellum and CASA parameters value e.g. motility, progressive motility, DAP, DCL, DSL, VAP, VCL, VSL, ALH and BCF. From among 3000 analyzed spermatozoa 14.2% posed abnormal forms. We observed negative influence of semen storage on its quality. Also negative correlations between all types of tail defect and motility of spermatozoa were detected.

Keywords: CASA, pathological forms of spermatozoa, rabbit, spermatozoon

INTRODUCTION

Semen is a mixture of spermatozoa, produced by testicles, and seminal plasma secreted at different sites by accessories glands and by the epididymides. Seminal plasma also contains other particles of different size which affect the spermatozoa behavior during the transit along the female reproductive tract (**Castellini, 2008**). During the ejaculation, seminal plasma stimulates spermatozoa motility and guaranties protection against negative influence of environmental factors. Seminal plasma contains substances which are used as a source of energy for spermatozoa and substances which are responsible for transport along intra-uterine environment. Both components are completely different on account of structure and functions, so they must be examined separately during the quality assessment of semen (**Lukáč *et al.*, 2007**). This assessment must provide information about spermatozoa ability to inseminate.

The most relevant parameters correlated with the fertility rate are the number of spermatozoa inseminated and their motility, although the use of a single attribute is not sufficiently accurate to predict the fertilizing ability of semen (**Castellini, 2008**). When there are put 10-20 mL of spermatozoa in female rabbit reproductive tract, just 5000 reach oviduct, because of the big size of reproductive tract (**Nalbandov, 1966**).

Male sex cells undergo many modifications during the spermatogenesis process, which can be natural changes, but it also can be a reason to forming spermatozoon abnormalities. Primary changes took place when spermatozoa reached epididymis tail and it suggests that there are some functional disorders in urogenital tract. Secondary changes took place when spermatozoa are stored for a long time in epididymis tail (**Lukáč *et al.*, 2007**). Among all morphological anomalies of spermatozoon the most frequent are defects connected with head e.g. spermatozoon with two heads, spermatozoon with shapeless head, with elongated head or spermatozoon with changes in acrosomal region. The main structural defect of acrosome is partial or complete lack of it or immature acrosome. These pathological changes are connected with spermatozoa inability to conduct acrosomal reaction and to bounding with zona pellucida (**Lukáč *et al.*, 2007**). Another serious defect is the separation of the flagellum from the head. This “pin-head” defect is assumed to be genetically inherited. Instead of nucleus, a globular cytoplasmic mass surrounding the proximal segments of the decapitated flagellum may be misinterpreted as microcephalic spermatozoa under light microscopy (**Nikolettos, 1999**).

It should mention that rabbits have been extensively used as a model for large animals and humans. All the reproductive techniques employed with farm animals can be performed

with the low-cost rabbit model, and certain placental membrane characteristics make them especially relevant for studies of human teratology (**Foote, 2002**).

The aim of this study was to analyze quality of New Zealand White rabbit semen which included spermatozoa morphology examining with pointing percentage of abnormal forms and defining time influence on spermatozoa motility which was measured using CASA system. In our researches we didn't test seasonal changes in semen quality.

MATERIAL AND METHODS

Animals

Research was carried out at Slovak University of Agriculture in Nitra. The materials represented semen samples collected from adult New Zealand White rabbits (n=30) which came from husbandry situated in Lužianky (RIAP Nitra, Slovakia). Rabbits were selected on the basis of age normally associated with reproduction (12-14 months). They were in individual cages and fed with a commercial diet.

Semen collection

The semen was obtained by means of an artificial vagina filled with warm liquid. We collected semen twice a week. A doe was fitted with this device and presented to the buck (**Boiti, 2005**). After collecting semen samples were transported to the laboratory at room temperature (22°C). For the researches we used samples exhibiting a white color without presence on any urine and gel. Samples were diluted (1:5) with physiological solution (0.9% NaCl).

Spermatozoa motility

Each of samples were evaluated using a Computer Assisted Semen Analyzer (CASA) system Sperm Vision^{TM} (Minitüb,Tiefenbach,Germany). Spermatozoa were transferred by pipette to a Makler counting chamber with depth of 10 μm. The samples were placed in an Olympus BX51 microscope (Olympus, Japan) to assess the spermatozoa motility (**Roychoudhury *et al.*, 2010a,b; Lukáč *et al.*, 2009, 2011**). Motility was measure in fresh samples and after 1 hour in samples which was stored in thermostat (37°C). In each sample

the following parameters were evaluated – 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 BCF (beat cross frequency, Hz) (**Slivkova *et al*., 2010; Roychoudhury *et al*., 2009**).

Spermatozoa morphology

After 1 hour storage in temperature 37°C for morphology analysis semen samples were fixed with Hancock's solution and stained using Giemsa solution. In each sample at least 100 spermatozoa were analyzed (all slides were analyzed at magnification x50) and the percentage of pathological spermatozoa was recorded. These pathological changes were classified: flagellum ball, knob-twisted flagellum, super-twisted knob flagellum, broken flagellum, separated flagellum, retention of cytoplasmatic drop, acrosomal changes and different (spermatozoa with two heads or with two tails, separated head, separated flagellum) (**Slivkova *et al*., 2009**).

To assess spermatozoa morphology (the length and the width of head and tail) we used QuickPhoto Micro system (microscope Olympus CX41 connected with camera Olympus U-CMAD3). For each slides 100 spermatozoa photos were made.

Statistical analysis

Obtained data were statistically analyzed with the help of PC program Excel and a statistic package SAS 9.1 (SAS Institute Inc., USA) using Student's t-test and correlations like earlier made e.g. **Stawarz *et al*. (2009)** or **Roychoundhury *et al*. (2009).**

RESULTS AND DISCUSSION

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In the Tables 1 – 5 minimal and maximal value, mean and standard deviation for rabbit semen parameters measured using CASA system in time intervals 0 and 1 (60 minutes storage

in 37°C) are listed. In our research mainly 5 parameters: motility, progressive motility, VCL, ALH and BCF were analyzed.

Table 1 Spermatozoa motility (%) in time intervals 0 and 60 minutes (1)

MOTILITY (%)				
Time	**Min**	**Max**	**Mean**	**S.D.**
0	49.15	98.93	**81.17**	10.11
1	20.51	98.11	**78.82**	15.89

Table 2 Spermatozoa progressive motility (%) in time intervals 0 and 60 minutes (1)

PROGRESSIVE MOTILITY (%)				
Time	**Min**	**Max**	**Mean**	**S.D.**
0	22.54	95.00	**69.61**	15.70
1	4.54	98.11	**65.89**	21.92

Table 3 VCL (µm/s) in time intervals 0 and 60 minutes (1)

VCL (µm/s)				
Time	**Min**	**Max**	**Mean**	**S.D.**
0	58.80	188.33	**120.99**	24.29
1	42.98	204.93	**121.42**	31.50

Table 4 ALH (µm/s) in time intervals 0 and 60 minutes (1)

ALH (µm/s)				
Time	**Min**	**Max**	**Mean**	**S.D.**
0	1.56	6.73	**4.09**	0.85
1	1.64	6.93	**4.44**	1.04

Table 5 BCF (Hz) in time-intervals 0 and 60 minutes (1)

BCF (Hz)				
Time	**Min**	**Max**	**Mean**	**S.D.**
0	21.67	42.28	**33.41**	4.3
1	16.95	40.35	**30.55**	3.9

Min – minimum; Max – maximum; S.D. – standard deviation

As described in tables after 1 hour of storage the value of spermatozoa motility, progressive motility and BCF decreased. Interestingly, the value of VCL and ALH parameters slightly increased in the same time interval, but this change was statistically non-significant. Values of other parameters obtained by the CASA system also decreased with the exception of DCL. We observed increase from 52.57 μm/s to 52.96 μm/s, however this increase was also statistically non-significant. Detected CASA results are located in the referential values which were announced by the others scientists (**Boiti, 2005**). The results suggests that 1 hour storage indicated decrease of spermatozoa parameters value and what is connected with decrease of semen and spermatozoa quality.

Morphology

Spermatozoa morphology is an important parameter in the fertilization process *in vivo* and *in vitro,* as well as the progressive motility of spermatozoa. It can be used as a single and independent predictor for successful fertilization (**Nikolettos, 1999**). Analyses were made using QuickPhoto Micro system afforded following dimensions of spermatozoa morphology: 4.5 x 8.1 μm when for spermatozoa head and 42.4 x 1.1 μm for spermatozoa flagellum (tail).

Toxic and environmental factors cause reversible alterations in spermatozoa structure. Epidemiological studies on the influence of various work environments and contact with different toxic substances have shown important increases in spermatozoa defects in farmers and graziers (exposed to various pesticides) and men working in mechanical trades, chemical and petroleum workers (exposed to fuels, oils). Unusually large increases in the mean percentage of abnormal spermatozoa in smokers compared with non-smokers were reported. Various physical agents have deleterious influences on spermatozoa quality. Ionizing radiation effects on spermatozoa structure have been studied in humans exposed to high radiation doses after nuclear reactor accidents and in mice experimentally subjected to X-rays or radioisotopes. The main observations were nuclear and chromatin structural defects, decreased motility and sterility. Cryopreservation of human spermatozoa adversely affects spermatozoa morphology, motility, mitochondrial function and viability. Exposure to any factor that compromises the thermoregulatory function of the scrotum will adversely influence semen parameters. Lifestyles including posture and clothing, excessive use of sauna, high ambient temperatures and intensity of activity can induce higher scrotal temperatures and reversible spermatozoa abnormalities **(Chemes, 2003).** Our studies on spermatozoa abnormalities showed that the total number of pathological spermatozoa was 14.2%. From all

the pathological spermatozoa evaluated the highest number was tail abnormalities (12.31%). The most often abnormalities observed were flagellum ball (5.91%) and the most rarely abnormalities observed were separated flagellum (0.07%) and retention of cytoplasmatic drop (0.07%).

Correlation analysis detected positive correlation between the most of all CASA parameters, but more important was to detect correlation between CASA parameters and spermatozoon defects. This analysis showed negative correlation (medium strong) between presence on separated flagellum spermatozoa in semen and the value of CASA parameters such as motility (r=-0.52), progressive motility (r=-0.64), DAP (r=-0.57), DCL (r=-0.57), DSL (r=-0.51), VAP (r=-0.55), VCL (r=-0.44), VSL (r=-0.51), ALH (r=-0.42) and BCF (r=-0.62). Medium strong, negative correlation was also detected between LIN (r=-0.39) and WOB (r=-0.46) parameters and presence of knob-twisted flagellum spermatozoa in semen samples. Strong positive correlation (r=0.70) between ALH and different abnormalities of spermatozoa (2 heads or 2 tails) were found. Interestingly, we also observed positive correlation between presence on flagellum ball spermatozoa in semen and CASA parameters such as motility (r=0.28) and progressive motility (r=0.23). These dependences are listed in Table 6.

As mentioned earlier, in our research special attention to 5 CASA parameters (motility, progressive motility, VCL, BCF and ALH) was focused. In accordance to data in Table 1 the value of motility decreased from 81.17% to 78.82% after 1 hour storage. This result was similar to results which were obtained by **Lavara *et al.* (2008)** but simultaneously higher from result which was obtained by **Panella *et al.* (1994):** 57.4%. Probably this difference is connected with type of solvent – in our researches it was 0.9% NaCl and in **Panella *et al.* (1994)** it was phosphate buffer. Researches which were carried out by **Lavara *et al.* (2008)** detected significant positive correlation between width spermatozoa head and motility (r=0.42). They also observed dependence that spermatozoa with higher size of head are more motile and in consequence of this had higher fertility rate. In studies connected with spermatozoa motility very important is fact, that nickel deprivation significantly decrease spermatozoa motility **(Slivkova *et al.*, 2009).**

One hour storage also caused decrease of progressive motility from 69.61 to 65.89% and these results were similar to result which were obtained by other scientists e.g. **Farrell *et al.* (1993)** or **Oyeyemi and Okediran (2007).** Progressive motility in turn amount to 68% and 68.3%. **Farrell *et al.* (1993)** noticed positive correlation between the number of progressively motile spermatozoa per ejaculate and the percentage of fertilized oocytes (r=0.39). In the light

of this results, other experiment seems to be very important, because it proves that soymeal feeding has positive influence on progressive motility (because of high level of protein and fat in the soymeal) and male reproductive success (**Oyeyemi and Okediran, 2007**).

It is interesting that the value of VCL parameter increased after 1 hour storage, but here we should mention that researches which were carried out by **Garcia-Tomás *et al.* (2006)** proved that spermatozoa velocity, measured in μm/s of a linear trajectory, was not significantly correlated with fertility.

Increase of the value of ALH parameter after 1 hour storage in temperature 37°C was also observed. **Lavara *et al.* (2008)** detected that greater lateral displacement of the spermatozoa head indicates rapid spermatozoa motion, but this dependence in this research was not noticed.

In this study we observed decrease of BCF parameter value (Table 5). This disagree with **Farrell *et al.* (1993)** that the average value of this parameter should be located between 14 and 16 Hz. **Garcia-Tomás *et al.* (2006)** explained that differences obtained in values of CASA parameters come out of using different rabbit breed.

We have to mention, that correct results of semen assessment do not decide definitively about actual fertility of presented male, because this type of assessment does not detect e.g. spermatozoa DNA disorders.

Morphological results obtained in this study are comparable with analyses published by **Boiti (2005)**. **Banaszewska *et al.* (2007)** found that there is dependence between morphology of spermatozoon and the length of the day. They observed that the longer the day, the more spermatozoa with correct morphology in boar semen. Results described in this study undergo small fluctuation depending on season.

Abnormal sperm may reduce fertility in one of two ways: (1) failure to reach the fertilization site; or (2) inability to fertilize the ovum once they are at the fertilization site or to sustain development of the early embryo **(Chenoweth, 2005)**. As we mentioned earlier, total number of pathological spermatozoa was 14.2% and it was similar to those reported in previous studies **(Lukáč *et al.*, 2009)**. The annual average value of total deformation is significantly higher in the Angora bucks than in Pannon White and New Zealand White ones by 10% **(Bodnár *et al.*, 2000).** Differences can also have connection with the period of the year as studies describe that higher temperature cause decrease of semen quality **(Banaszewska *et al.*, 2007)** and increase of abnormalities frequency **(Bodnár *et al.*, 2000).**

Table 6 Correlations between CASA parameters and pathologies found in rabbit semen

	CON	MOT	PRO	DAP	DCL	DSL	VAP	VCL	VSL	STR	LIN	WOB	ALH	BCF	FB	KTF	BF	T	STKF	SF	RET	AKR	INNE
CON	1	0.21	0.18	-0.27	-0.35	-0.26	-0.24	-0.28	-0.16	0.03	0.12	0.25	0	-0.08	-0.08	-0.08	-0.18	0.11	-0.08	-0.21	-0.12	0.07	0.20
MOT	0.21	1	0.95	0.40	0.36	0.31	0.40	0.27	0.32	-0.14	0.11	0.36	0.61	0.20	0.28	0.06	-0.23	-0.12	0.36	-0.52	0.12	-0.11	0.05
PRO	0.18	0.95	1	0.58	0.53	0.50	0.57	0.40	0.50	-0.11	0.18	0.42	0.68	0.37	0.23	-0.06	-0.19	-0.05	0.31	-0.64	0.13	-0.10	0.13
DAP	-0.27	-0.40	0.58	1	0.95	0.94	0.99	0.79	0.91	-0.09	0.34	0.50	0.74	0.58	0.20	-0.19	-0.12	0.09	0.18	-0.57	-0.16	-0.04	0.14
DCL	-0.35	0.36	0.53	0.95	1	0.87	0.93	0.76	0.83	-0.21	0.12	0.24	0.67	0.57	0.27	-0.04	-0.09	0.06	0.16	-0.57	-0.09	-0.08	0.20
DSL	-0.26	0.31	0.50	0.94	0.87	1	0.94	0.72	0.98	0.22	0.56	0.52	0.63	0.68	0.14	-0.23	-0.06	0.15	0.19	-0.51	-0.16	-0.02	0.23
VAP	-0.24	0.40	0.57	0.99	0.93	0.94	1	0.78	0.93	-0.04	0.40	0.55	0.74	0.58	0.16	-0.24	-0.14	0.10	0.19	-0.55	-0.19	-0.05	0.14
VCL	-0.28	0.27	0.40	0.79	0.76	0.72	0.78	1	0.70	-0.17	0.19	0.33	0.63	0.31	0.20	-0.06	-0.10	-0.10	-0.03	-0.44	-0.07	-0.11	0.28
VSL	-0.16	0.32	0.50	0.91	0.83	0.98	0.93	0.70	1	0.27	0.62	0.56	0.62	0.68	0.12	-0.23	-0.11	0.14	0.14	-0.51	-0.18	-0.07	0.25
STR	0.03	-0.14	-0.11	-0.09	-0.21	0.22	-0.04	-0.17	0.27	1	0.72	0.21	-0.31	0.33	-0.25	-0.19	0.14	0.11	-0.06	0.12	-0.03	0.11	0.16
LIN	0.12	0.11	0.18	0.34	0.12	0.56	0.40	0.19	0.62	0.72	1	0.79	0.17	0.50	-0.18	-0.39	-0.07	0.18	0.17	-0.13	-0.22	0.06	0.06
WOB	0.25	0.36	0.42	0.50	0.24	0.52	0.55	0.33	0.56	0.21	0.79	1	0.52	0.32	-0.06	-0.46	-0.24	0.13	0.23	-0.27	-0.25	0.08	-0.11
ALH	0	0.61	0.68	0.74	0.67	0.63	0.74	0.63	0.62	-0.31	0.17	0.52	1	0.07	0.52	-0.21	-0.39	0.05	0.20	-0.42	-0.15	-0.10	0.07
BCF	-0.08	0.20	0.37	0.58	0.57	0.68	0.58	0.31	0.68	0.33	0.50	0.32	0.07	1	-0.25	-0.07	0.15	0.14	0.09	-0.62	-0.06	0.03	0.13
FB	-0.08	0.28	0.23	0.20	0.27	0.14	0.16	0.20	0.12	-0.25	-0.18	-0.06	0.52	-0.25	1	0.30	-0.22	-0.16	-0.01	-0.06	-0.26	-0.07	-0.08
KTF	-0.08	0.06	-0.06	-0.19	-0.04	-0.23	-0.24	-0.06	-0.23	-0.19	-0.39	-0.46	-0.21	-0.07	0.30	1	0.07	-0.23	-0.11	-0.13	0.20	-0.01	-0.16
BF	-0.18	-0.23	-0.19	-0.12	-0.09	-0.06	-0.14	-0.10	-0.11	0.14	-0.07	-0.24	-0.39	0.15	-0.22	0.07	1	0.03	0.15	0.13	-0.15	0.24	0.13
T	0.11	-0.12	-0.05	0.09	0.06	0.15	0.10	-0.10	0.14	0.11	0.18	0.13	0.05	0.14	-0.16	-0.23	0.03	1	0.09	-0.15	-0.11	0.54	-0.09
STKF	-0.08	0.36	0.31	0.18	0.16	0.19	0.19	-0.03	0.14	-0.06	0.17	0.23	0.20	0.09	-0.01	-0.11	0.15	0.09	1	0.05	0.08	-0.23	0.01
SF	-0.21	-0.52	-0.64	-0.57	-0.57	-0.51	-0.55	-0.44	-0.51	0.12	-0.13	-0.27	-0.42	-0.62	-0.06	-0.13	0.13	-0.15	0.05	1	-0.05	-0.07	-0.10
RET	-0.12	0.12	0.13	-0.16	-0.09	-0.16	-0.19	-0.07	-0.18	-0.03	-0.22	-0.25	-0.15	-0.06	-0.26	0.20	-0.15	-0.11	0.08	-0.05	1	-0.17	0.04
AKR	0.07	-0.11	-0.19	-0.04	-0.08	-0.02	-0.05	-0.11	-0.07	0.11	0.06	0.08	-0.10	0.03	-0.07	-0.01	0.24	0.54	-0.23	-0.07	-0.17	1	-0.25
INNE	0.20	0.05	0.13	0.14	0.20	0.23	0.14	0.28	0.25	0.16	0.06	-0.11	0.70	0.49	0.66	0.41	0.51	0.63	0.95	-0.10	0.04	-0.25	1

Legend: **CON** – concentration, **MOT** – motility, **PRO** – progressive motility, **FB** – flagellum ball, **KTF** – knob-twisted flagellum, **BF** – broken flagellum, **T** – torso, **STKF** – super-twisted knobbed flagellum, **SF** – separated flagellum, **RET** – retention of cytoplasmatic drop, **AKR** – acrosomal changes,

- strong correlation, - medium strong correlation

Previous study **(Kuzminsky *et al.*, 1996)** refer that the most often abnormalities are those connected with tail, also in the present study this dependence was found. **Kuzminsky *et al.* (1999)** explained that tail abnormalities are easily observed under the light microscope even at low magnification. Among identified categories flagellum ball represented the highest proportion as it was also reported by **Kuzminsky *et al.* (1996).** Retention of the cytoplasmatic drop represented the lowest proportion and it remains of variance with researches carried out by other scientist **(Roychounhury *et al.*, 2010; Slivkova *et al.*, 2009)**. Results of their research suggest that percentage of these abnormalities reached 1-3%. Observed opposites could be a result from differences in period, when the researches were made, because defect above are found more often in May and September, than in the other months of the year **(Banaszewska *et al.*, 2007).** In our studies we detected low percentage of spermatozoa with acrosomal changes, what is comparable to the results obtained by **Lukáč *et al.* (2009)** and at the same time contradictory to the results obtained by **Bodnár *et al.* (2000).**

CONCLUSION

The data showed that the value of parameters we obtained using CASA system as well as the number of abnormal spermatozoa has an influence on semen quality. The assessment of semen quality and quantity is very important and useful especially for breeders in diagnosing fertility problems. In the light of this fact, it seems to be necessary to perform a semen evaluation on an individual basis for every male from husbandry, to access its ability to fertilize female.

Acknowledgments: This study was supported by VEGA project 1/0532/11 and KEGA KEGA 013SPU-4/2012.

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