CORRELATION BETWEEN CASA AND ASMA PARAMETERS IN RABBIT SEMEN

Lavara R.*, Vicente J. S., Marco-Jiménez F., Baselga M.

Institute of Animal Techonolgy Science (ICTA), UPV, Camino de Vera, 46022 Valencia, Spain
*Corresponding author: rlavara@dca.upv.es

ABSTRACT

Computerized motility analysis (CASA) and quantitative, morphologic and morphometric analyses (ASMA) were performed in 187 semen samples from 23 adult males selected for daily weight gain between 28 and 63 days of age (Line R). The following traits were recorded: volume (VOL), concentration (Cn), normal apical ridge (NAR), abnormal sperm (ANR), length of sperm head (L), width of sperm head (W), area of sperm head (A), perimeter of sperm head (P), percentage of motile cells (MOT), average of curvilinear velocity (VCL), average straight-line velocity (VSL), average path velocity (VAP), linearity index (LIN), straightness coefficient (STR), wobble (WOB) and amplitude of lateral head displacement (ALH). Phenotypic correlations between sperm traits were estimated, and Bonferroni's correction was applied. Several significant correlations between volume, concentration, motility, morphological and morphometric parameters were also observed.
Positive correlations between sperm concentration and motility (r=0.22) and negative correlation with volume (r=-0.28) were evidenced. In this study we observed a significant correlation between some morphometric parameters like width and area of the sperm head and the percentage of motile cells (r=0.42, r=0.37, respectively) and abnormal sperm percentage (r=-0.25, r=-0.24, respectively) indicating the relationship between morphometric parameters and sperm quality in rabbit males. The quality of motion, measured by different indexes (LIN, STR, WOB), was positively correlated with width sperm and/or area head (r=0.31, r=0.26, r=0.24, respectively for LIN, STR,WOB, with W, and r=0.27, for LIN with A, respectively). Significant positive correlations were observed between different sperm velocity parameters and the amplitude of the lateral head displacement of sperm (r=0.50, r=0.78, r=0.60 for VSL, VCL, VAP), indicating that rapid sperm motion is associated with a greater lateral displacement of the sperm head. These results indicate that morphometric parameters of sperm head, in combination with sperm concentration, morphology analyses and motility parameters determined by CASA systems, can provide complementary information about the kinematic and morphometric characteristics of rabbit semen, and their inclusion in the spermiogram could improve the prediction of potential fertility.

Key words: CASA, Correlations, Rabbit sperm, Morphometric parameters.

INTRODUCTION

The most common semen analysis is mainly based on the assessment of the sperm concentration, the visual motility characteristics, and morphological classification of spermatozoa (Vestengerd, 2000), but these parameters are highly correlated with fertility when a poor value is obtained. Computer-assisted semen analysis methods (CASA) provide more objective motility parameters, and in recent years there has been an increase in the use of these systems to evaluate semen quality in rabbit (Farell *et al*., 1993; Brun *et al*., 2002; Lavara *et al*., 2005).The motion parameters typically derived using automated CASA systems provide information about the velocity, linearity and lateral displacement of sperm heads as spermatozoa progress along their trajectories.

Automated sperm morphometry analysis methods (ASMA) have been developed to reduce the technical variation in the sperm morphometry assay, in addition the possibility of numerous parameters evaluation in a single analysis and the analysis possibility is increased (Baker and Clarke,

1987). Automated sperm morphology analysis has been applied in rabbit semen by several authors (Gravance and Davis, 1995, Pérez-Sánchez *et al.*, 1998; Marco-Jiménez *et al*., 2005; Lavara *et al*., 2006).

In a previous work, Lavara *et al.* (2006) reported low variation coefficients in sperm head parameters but consistent differences inter-males. These results are in agreement with Morrow and Gage (2001) who reported in different populations that sperm head was male-specific and was repeatable between successive ejaculates. To date, there has been only one study describing the use of automated sperm morphometry analysis and fertility of individual male rabbits (Marco-Jiménez *et al*., 2005). In this trial the authors show the interest in the application of sperm head morphology for prediction and control of fertility in male rabbits. Hence, morphology and motility characteristics of sperm have both been implicated in sperm potential fertility.

In this study, carried out on male rabbits of a line selected for growth rate after weaning, we examined the relationship of the morphology of head spermatozoa to their motility parameters using automated systems for semen analysis (CASA-ASMA).

MATERIALS AND METHODS

Animals and breeding system

Mature males (15-20 months old) used (n= 23) were from a line of rabbits, selected for growth rate from weaning to slaughter (28–63 days). Selection methodologies were described by Estany *et al*. (1992). Males were housed at the experimental farm of the Departamento de Ciencia Animal, Universidad Politécnica de Valencia (Valencia, Spain), under a photoperiod of 16L:8D, in individual cages, fed with a commercial diet and provided water *ad libitum*. Each week, two ejaculates per male were collected on a single day using an artificial vagina, with a minimum of 30 min between ejaculate collections. At least 8 ejaculates per male were analyzed. Only ejaculates exhibiting a white colour were used in the experiment, and gel was removed if present. Then the volume was recorded.

Semen analyses

Semen samples of each ejaculate (10 µl) were diluted 1:20 in an extender Tris-citrate acid-glucose and BSA to prevent the spermatozoa from sticking to the glassware during motility analysis. Then, 10 µl of the sample was placed into a 10 µm deep Makler counting chamber (Sefi Medical Instruments, Haifa, Israel) for motility analysis using a computer-assisted sperm analysis (CASA) system (Sperm Class Analyzer, S.C.A., Microptic, Barcelona, Spain). Sperm motility was assessed at 37ºC at 10x using a phase contrast microscope. For each sample four microscopic fields were analyzed and a minimum of 100 sperm evaluated. The percentage of motile cells (MOT, %), average path velocity (VAP, µm/s; the average velocity of the smoothed cell path), curvilinear velocity (VCL, µm/s; the average velocity measured over the actual point to point track followed by the cell), straight-line velocity (VSL, µm/s; the average velocity measured in a straight line from the beginning to the end of the track), linearity index (LIN, %; the average value of the ratio VSL/VCL), amplitude of lateral head displacement (ALH, µm; the mean width of the head oscillation as the sperm cells swim), straightness coefficient (STR=(VAP/VCL)x100, %), and wobble (WOB = (VAP/VCL) x 100, %; a measure of the oscillation of the actual trajectory about its spatial average path) were recorded.

For the morphological analyses, an aliquot from each ejaculate (20 µl) was fixed with 180 µl of a solution of glutaraldehyde 2% in DPBS (Pursel and Johnson, 1974). A minimum of 100 sperm cells was evaluated at a magnification of 500x with a differential interference contrast microscope (Nomarski contrast). The status of the acrosome of the normal sperm (intact, AI, or damaged, AD), and the sperm abnormalities were evaluated (ANR). The percentage of sperm with a normal apical ridge (NAR) was calculated as the ratio: [AI/(AI + AD)] x 100. The percentage of abnormal sperm (ANR) was calculated as the ratio: [ANR/(AI + AD + ANR)] x 100. The sperm concentration was

determined using a Thoma-Zeiss counting cell chamber (Marienfield, Germany). For performing the morphometric analyses, a 10 µl drop of fixed sperm with glutaraldehyde was placed on a slide and covered with a coverslip (20 x 20 mm). The slides were placed under an Nikon Elipse-microscope, with a bright field x40 phase optic objective, on which was mounted a Sony CCD AVC-D7CE video camera (Sony Corporation; Tokyo, Japan) with a x3.3 photo-ocular connected to a morphometric module of a Sperm-Class Analyser (SCA) (Microptic, Barcelona, Spain). Sperm cells were displayed on the monitor at equivalent brightness, and all the cells that did not present any overlap with debris or other cells were considered for analysis. If after treatment of the images false correspondence between the original image and its mask were observed, the sperm were discarded. At least 100 normal sperm were measured for each ejaculate. Each sperm head was measured for four primary parameters (head area [A, μm^2], head perimeter [P, µm], head length [L, µm], head width [W, µm]).

Statistical analysis

Statistical analyses were performed using a commercially available statistics package (Statgraphics Plus, Version 5.1, STSC Inc., Rockville, MD, USA). Descriptive statistics were calculated for each seminal parameter. Pearson's correlation coefficients were calculated to determine the relationship between sperm parameters. Bonferroni's correction was applied and the statistical significance was indicated by a P value of less than 0.003.

RESULTS AND DISCUSSION

The summary statistics for the sperm parameters are shown in Table 1 and 2. Similar values for volume and motility have been obtained by different authors in ejaculates from adult rabbit males belonging to males selected for the same objective (Lavara *et al*., 2006; Quintero-Moreno *et al.*, 2007), and lines selected for different objectives (Brun *et al*., 2002). The percentage of normal apical ridge and percentage of abnormal spermatozoa were similar than results reported by García-Tomás *et al.* (2006) and Lavara *et al.* (2005). Morphometric parameters (Table 1) were similar than those observed in the literature (Gravance and Davis, 1995; Marco-Jiménez *et al.*, 2005; Lavara *et al.*, 2006).

Table 1: Summary statistics of quantitative, morphological and morphometric parameters

	VOL (ml)	Cn (10^6/ml)	NAR (%)	ANR (%)	L (µm)	W (µm)	A (μm^2)	P (µm)
N	187	185	178	178	187	187	187	186
Average	0.8	226.2	93.2	14.2	8.5	4.4	30.3	22.6
S.D.	0.4	136.4	7.1	11.5	0.3	0.2	2.1	0.7
Min	0.1	4.5	53.4	0.0	7.8	3.6	23.6	20.5
Max	2.00	791.7	100.0	54.8	9.3	4.9	35.0	24.5

N: number of observations; S.D: standard deviation; VOL: volume; Cn: concentration; NAR: normal apical ridge; ANR: abnormal sperm; L: length of sperm head; W: width of sperm head; A: area of sperm head; P: perimeter of sperm head

Table 2: Summary statistics of motility parameters

	MOT (%)	VCL (µm/s)	VSL (µm/s)	VAP (µm/s)	LIN (%)	STR (%)	WOB (%)	ALH (µm)
N	170	170	170	170	170	170	170	170
Average	77.8	73.9	38.9	47.8	54.8	72.7	67.1	2.2
S.D.	20.5	27.5	18.4	20.4	10.4	12.2	8.8	0.7
Min	15.8	20.0	4.7	8.9	27.5	21.3	42.2	0.8
Max	98.5	155.8	96.8	109.8	79.6	96.7	84.1	5.0

N: number of observations; S.D.: standard deviation; MOT: motility; VCL: curvilinear velocity; VSL: straight-line velocity; VAP: average path velocity; LIN: linearity index; STR: straightness coefficient; WOB: wobble; ALH: amplitude of lateral head displacement

Several significant correlations (Table 3; P<0.003) between volume, concentration, motility, morphological and morphometry parameters were also observed. Significant positive correlations between the percentage of motility and morphometry parameters like width and area (r=0.42; P<0.003,

and r=0.37; P<0.003, for width and area respectively) showed that spermatozoa with higher size of head are more motile. Marco-Jiménez *et al*. (2005) reported that male rabbits with high head showed high fertility rate.

A significant positive correlation was observed between width sperm head and some motility index like MOT, LIN, STR, and WOB (r=0.42, 0.31, 0.26 and 0.24, respectively, P<0.003), indicating that sperm head size is related with quality motion spermatozoa. In contrast, significant negative correlations between the percentage of abnormal sperm and width of sperm head (r=-0.25; P<0.003), and area of sperm head (r=-0.24; P<0.003) were observed. There is a wide variety of aberrations in the head shape of rabbit spermatozoa, one of this aberrations is the tapered head defect. The tapered head often appears to be smaller than normal spermatozoa and are very difficult to distinguish from normal cells (Barth and Oko, 1989).

Table 3: Correlations among seminal parameters

	VOL	Cn	NAR	ANR	MOT	VCL	VSL	VAP	LIN	STR	WOB	ALH	L	W	A
Cn	**-0.28***														
NAR	0.12	0.14													
ANR	0.01	-0.11	-0.02												
MOT	0.06	**0.22***	-0.04	-0.02											
VCL	-0.05	0.14	0.14	0.1	**0.43***										
VSL	0.00	0.14	0.06	-0.02	**0.60***	**0.81***									
VAP	-0.01	0.14	0.09	0.01	**0.58***	**0.90***	**0.98***								
LIN	0.13	-0.11	0.02	0.08	**0.37***	0.16	**0.56***	**0.46***							
STR	0.03	0.10	0.03	0.16	**0.66***	**0.40***	**0.56***	**0.53***	**0.59***						
WOB	0.13	-0.19	-0.02	0.14	**0.33***	0.23	**0.55***	**0.50***	**0.96***	**0.79***					
ALH	-0.01	0.15	0.14	0.04	0.22	**0.78***	**0.50***	**0.60***	-0.14	0.18	-0.12				
L	-0.04	-0.09	-0.06	0.14	-0.19	-0.12	-0.10	-0.11	0.02	-0.06	-0.01	-0.08			
W	-0.02	0.08	-0.06	**-0.25***	**0.42***	-0.03	**0.25***	0.17	**0.31***	**0.26***	**0.24***	-0.11	0.16		
A	-0.03	0.07	-0.13	**-0.24***	**0.37***	-0.09	0.20	0.11	**0.27***	0.22	0.18	-0.13	**0.42***	**0.94***	
P	-0.05	-0.10	0.04	0.13	-0.19	-0.15	-0.10	-0.11	0.10	-0.09	0.12	-0.12	**0.81***	**0.36***	**0.53***

VOL: volume (ml); Cn: concentration (10^6/ml); NAR: normal apical ridge (%); ANR: abnormal sperm (%); L: length of sperm head (µm); W: width of sperm head (µm) A: area of sperm head (µm^2); P: perimeter of sperm head (µm); MOT: motility (%); VCL: curvilinear velocity (µm/s); VSL: straight-line velocity (µm/s); VAP: average path velocity (µm/s); LIN: linearity index (%); STR: straightness coefficient (%); WOB: wobble (%); ALH: amplitude of lateral head displacement (µm)
*P<0.003

Moreover, there was a significant positive correlation between sperm concentration and motility, and a negative correlation with volume. These findings were in agreement with Lavara *et al*. (2005) and Brun *et al.* (2002) who observed a positive correlation coefficient (0.42; P<0.05) between sperm concentration and motility of purebred and crossbred rabbit bucks selected for litter size. Significant positive correlations were observed between different sperm velocity parameters and the amplitude of the lateral head displacement of sperm (r=0.50, r=0.78, r=0.60; for VSL, VCL, VAP; P<0.003), indicating that rapid sperm motion is associated with a greater lateral displacement of the sperm head. Similar results were reported by Lavara *et al*. (2005). Many correlations between motility, velocity sperm parameters (r=0.43, r=0.60, r=0.58 for VCL, VSL, VAP, P<0.003) and its derivates (r=0.37, r=0.66, r=0.33 for LIN, STR, WOB, P<0.003) were observed, indicating that great motility percentage is associated with high velocity parameters and its derivates.

CONCLUSIONS

The correlations observed between some morphometric parameters like width and area sperm head with motility percentage and abnormal sperm percentage indicated the relationship between morphometric parameters and sperm quality in rabbit males. The quality of motion measured by different indexes was positively correlated with the area and/or width sperm head. All the parameters included in the CASA-ASMA analysis provided information to define the kinematic and morphometric characteristics of rabbit semen, and their inclusion in the spermiogram could improve in the future the prediction potential of fertility.

ACKNOWLEDGEMENTS

This work has been supported by the Spanish Research Project (CICYT AGL2004-0271/GAN).

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