A COMPARISON BETWEEN COMPUTERISED SEMEN IMAGE ANALYSES AND VISUAL METHODS TO EVALUATE VARIOUS BIOLOGICAL PARAMETERS IN RABBIT SEMEN

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Abstract - In order to compare computerised semen image analyses (HTMA-IVOS Hamilton-Thorn) with standard evaluation methods of rabbit semen, 47 ejaculates were analysed. PH and visual parameters were measured immediately after collection and 1:5 dilution. After 1:40 subsequent dilution, each ejaculate was analysed with the HTMA, from 3 tubes, 3 drops and 4 fields (i.e. 36 analyses) or from 2 tubes, 2 drops and 2 fields (i.e 8 analyses). The play back option was used to count visually all cells incorrectly identified in order to know the exact total number of cells by field. The concentration estimated by HTMA correlates to a great extent with the exact total number of cells (r=0.999, P=0.0001) this proving its accuracy. Correlations were quite similar when calculated either from 36 or from 8 measurements. The percentage of HTMA estimated progressive cells and VAP correlated negatively with pH (r=-0.31, P=0.03 and r=-0.32, P=0.02 respectively). The percentage of HTMA estimated motile cells correlated positively with individual motility (r=0.52, P=0.0002). Despite a delay of about 2 hours, the percentage of HTMA estimated motile cells correlated highly with visual estimated ones (r=0.79, P=0.0001). HTMA concentrations correlated significantly with hemocytometer counts and optical density (r=0.68, P=0.001 and r=0.65, P=0.001 respectively). The authors conclude that HTMA accuracy is satisfactory for motility analyses. Its interest will be completely established when high correlations are obtained between its parameters and fertility.

INTRODUCTION

For the past decade, Computer Assisted Sperm Analyses (CASA) have been of practical use in human and animal reproduction, with the ultimate purpose of relating sperm motion parameters to the fertilising ability of semen doses. In rabbits, PIZZI *et al.* (1993) compared semen image analyser (Cellsoft system) with a standard method (counting chamber), for evaluation of semen concentration, but correlations did not exceed 0.57. More recently, FARREL *et al.* (1992) using a Hamilton-Thorn experimental system, could observe high correlations (r=0.99) between hemocytometer counts and semen analyser values for sperm concentration. Unfortunately, very little information was given in this work about experimental design and practical determination of set up adjustments. The performances of computer assisted semen analyses greatly depend on the correct setting of the system. These settings may differ greatly between species. Adequate set up must at first be determined before practical use of the equipment.

The aim of this work is to compare computerised semen image analyses (HTMA-IVOS Hamilton-Thorn Research, Beverly, MA/U.S.A.) with visual standard evaluation methods for rabbit semen.

MATERIAL AND METHODS

Semen from 47 A1029 and A1077 bucks were analysed. Experimental conditions are described in the previous publication (THEAU-CLEMENT *et al.*, 1996). For each ejaculate, a total of 3 tubes, were assessed with 3 drops per tube and four fields per drop, i.e. 36 analyses. Therefore, another approach to the statistical analyses was carried out with the first 2 tubes, the first 2 drops and fields 2 and 3 (i.e 8 analyses). Within each tube, concentrations were estimated using both a Thoma-Zeiss cell counter and a spectrophotometer (wave-length : 520nm) at a 1 : 200 final dilution. Visual measurement methods are described in BOUSSIT (1989).

Immediately after semen collection, pH and visual parameters were observed : general motility, individual motility, percentage of live spermatozoa, concentration (number of spermatozoa/ml) and optical density for

each tube. HTMA parameters were carried out between 30 minutes and 4 hours after collection. HTMA available for each field were : concentration, percent motile cells, percent progressive cells (if VAP>40μ/sec and straightness> 80%), path velocity (VAP : average velocity of the smoothed cell path in μ/sec), the amplitude of lateral head displacement (ALH, which corresponds to the average sperm track width) and linearity (LIN, measures the departure of the cell track from a straight line). Following each analysis, the playback option was used to count visually all cells incorrectly identified (uncounted or counted erroneously) by the HTMA, which allowed further quantification of the cell population present in each field (exact total number of cells).

Pearson correlations were applied to study the relationships between HTMA parameters, pH and visual data. Computation was made on HTMA data means by ejaculate. On Table 1, significant values ($P < 0.05$) are in bold characters.

Table 1 : Correlations between HTMA parameters and visual parameters

		HTMA parameters							
		Concentration 10^6/ml	Motile cells (%)	Progressive cells (%)	VAP	ALH	LIN	Uncounted cells	Counted by error
	Motile cells	0.49(1)							
	(%)	*0.42*(2)							
	Progressive cells	0.22	0.73						
	(%)	*0.06*	*0.70*						
	VAP	0.33	0.54	0.48					
		0.27	*0.42*	*0.31*					
HTMA	ALH	0.44	0.54	0.17	0.72				
parameters		*0.43*	*0.28*		*0.57*				
	LIN	0.01	0.44	0.88	0.36	-0.07			
		-0.16	*0.34*	*0.85*	*0.21*	-0.23			
	Uncounted cells	-0.21	-0.61	-0.56	-0.59	-0.32	-0.42		
		-0.12	*-0.54*	*-0.52*	*-0.43*	*0.12*	*-0.37*		
	Counted by error	0.30	0.194	-0.08	0.10	0.22	-0.14	-0.16	
		0.38	*0.22*	*-0.05*	*0.09*	*0.14*	*-0.16*	*-0.15*	
	pH	-0.39	-0.07	-0.33	-0.33	-0.11	-0.28	0.01	0.07
		-0.29	*-0.05*	*-0.31*	*-0.32*	*-0.22*	*-0.25*	*-0.04*	*0.16*
	General	0.40	0.45	0.17	0.06	0.09	0.03	-0.11	0.23
	motility	*0.36*	*0.46*	*0.20*	*0.07*	*0.06*	*-0.01*	*-0.17*	*0.24*
	Individual	0.17	0.73	0.51	0.35	0.47	0.29	-0.30	0.17
pH and	motility	*0.15*	*0.77*	*0.52*	*0.26*	*0.25*	*0.22*	*-0.25*	*0.16*
VISUAL	Live	0.28	0.78	0.54	0.49	0.55	0.34	-0.45	0.30
Parameters	spermatozoa (%)	*0.30*	*0.79*	*0.54*	*0.41*	*0.40*	*0.27*	*-0.40*	*0.32*
	Concentration	0.68	0.05	-0.31	-0.05	0.33	-0.46	0.25	0.30
	10^6/ml	*0.68*	*0.01*	*-0.35*	*-0.15*	*0.34*	*-0.48*	*0.28*	*0.18*
	Optical density	0.66	0.11	-0.25	-0.01	0.33	-0.34	0.09	0.40
		0.65	*0.09*	*-0.27*	*-0.10*	*0.31*	*-0.35*	*0.08*	*0.41*

(1) 36 analysis (3 tubes, 3 drops, 4 fields) ; (2) 8 analysis (2 tubes, 2 drops, fields 2 and 3)

RESULTS

Correlations between HTMA parameters

HTMA concentration correlated to a great extent ($r = 0.999$, $P < 0.001$) with the exact total number of cells (after addition of uncounted cells and subtraction of cells counted erroneously, Figure 1a).

First of all, we can notice that correlations were quite similar when calculated either from 36 or from 8 measurements (Table 1). Therefore, only the latter were considered in the following comments.

Concerning HTMA accuracy, it is interesting to notice that the higher the percentage of motile cells, the lower the uncounted cells ($r = -0.54$). The higher the concentration in working preparations is, the higher the risks of overestimations of this concentration ($r = 0.38$).

Correlations between HTMA and visual parameters

PH correlated negatively with HTMA estimates of the percentage of progressive cells and VAP ($r = -0.31$ and -0.32 respectively). The general motility visually estimated was positively, even poorly correlated with HTMA concentration ($r = 0.36$) and the percentage of motile cells ($r = 0.46$). Likewise, individual motility correlated to a great extent with HTMA percentage of motile cells ($r = 0.77$) and with the percentage of progressive cells ($r = 0.52$).

The value of correlations for the percentage motile cells between HTMA and visual estimations reached 0.79 (Figure 1b). Likewise, the correlation between HTMA concentrations and hemocytometer counts (Figure 1c) or optical density (Figure 1d) was slightly lower ($r = 0.68$, and 0.65 respectively).

DISCUSSION

The mean delay between collection and beginning of the HTMA analysis was 1h 30 mn (minimum = 30 mn, maximum = 4 h).

The high correlation between HTMA concentration and the exact total number of cells demonstrates the validity of the set up used and proves HTMA accuracy.

As found by DE YI LIU *et al.* (1991), BATAILLE *et al.* (1990) and PALMER and MAGISTRINI (1992) on equine frozen semen, sperm velocity was highly correlated with ALH ($r = 0.57$), as was the percentage of progressive cells with linearity ($r = 0.85$) and the percentage of motile cells with progressive cells ($r = 0.70$). The negative correlation between the percentage of motile cells and uncounted cells suggests the difficulty with HTMA of taking into consideration slow or dead spermatozoa. As observed by PANELLA and CASTELLINI (1990) and BENCHEIKH (1995), the negative correlation between pH and biological parameters partly reflects sperm metabolism, with higher concentrations of lactic acid and therefore lower pH, in samples with high motility.

Despite a delay of about 2 h (30 min-4h) between HTMA or visual estimations of motile percentage, correlations are quite high. By comparison, DE YI LIU *et al.* (1991) observed a correlation of $r = 0.58$ between these parameters with a 2030 HTMA. The correlations between HTMA concentration and hemocytometer counts is quite similar to PIZZI'S *et al.* observations ($r = 0.57$, Cellsoft System, 1993), but lower than data published by FARREL *et al.* ($r = 0.99$, 1992) and DE YI LIU *et al.* ($r = 0.88$, 1991). In our work HTMA concentrations were nearly twice as high as hemocytometer counts (1834 *vs* 684 10^6 spermatozoa/ml), while the mean correlation between corrected total number of cells / field and HTMA concentrations was 0.999 (Figure 1a). Therefore, it is our opinion that the "concentration program" proposed by the manufacturer may present a problem with the configuration used in the case of rabbit semen, as demonstrated by this observation.

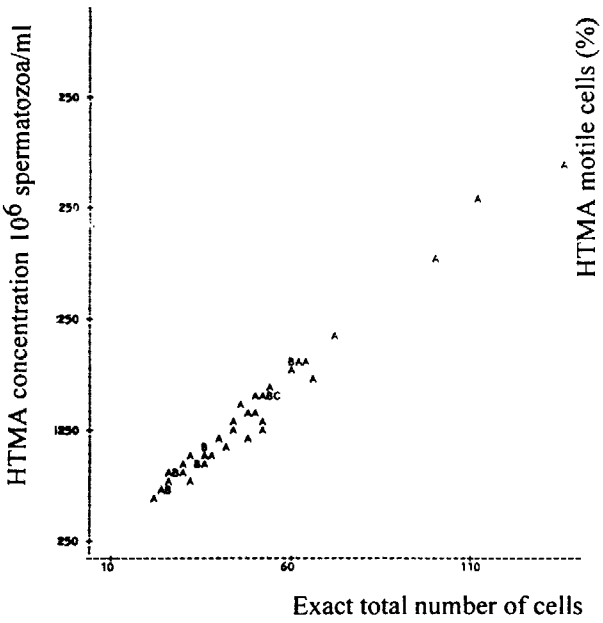

Figure 1a

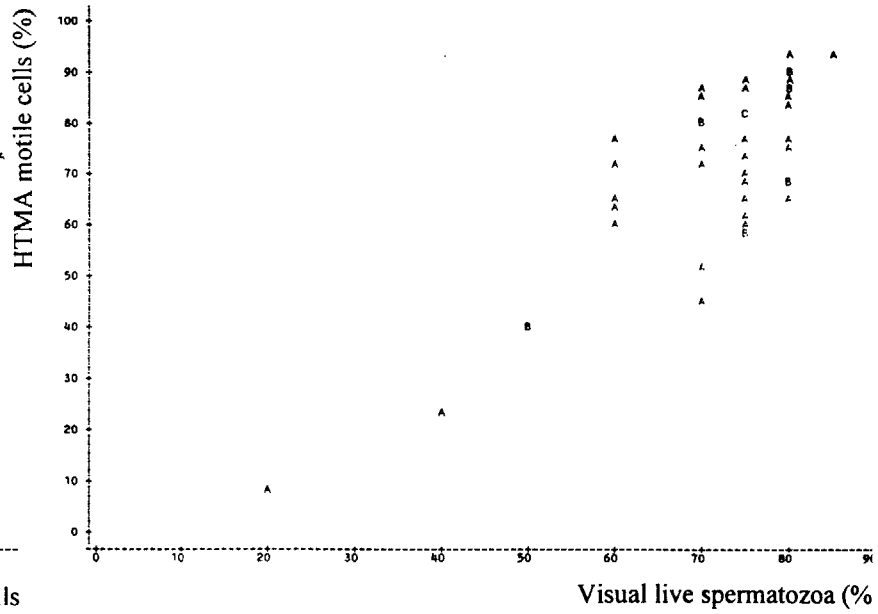

Figure 1b

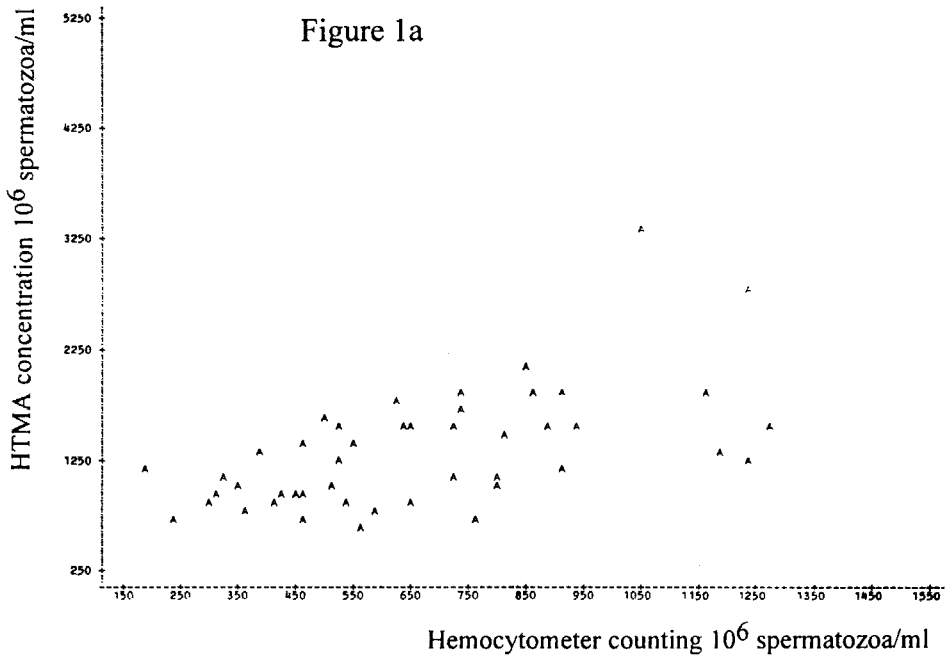

Figure 1c

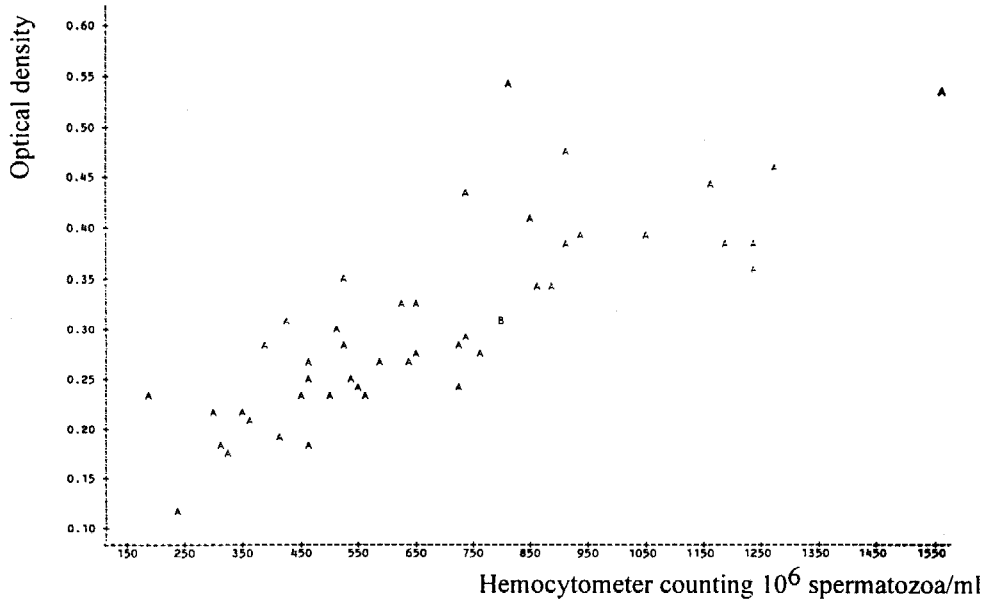

Figure 1d

Figure 1 Relationships between HTMA, pH and visual parameters

Legend : A = 1 observation, B = 2 observations ....

CONCLUSION

This work confirms HTMA accuracy for motility analyses. Correlations are maintained in spite of a lighter analysis design. Moreover, HTMA allows access to invisible parameters (the percentage of progressive cells, VAP) significantly correlated with pH or individual motility. The interest for HTMA-IVOS will be completely established when high correlations with fertility are obtained.

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Comparaison entre l'analyse d'image informatisée et l'analyse visuelle dans l'évaluation de différents paramètres biologiques de la semence de lapin - Afin de comparer l'analyse d'images assistée par ordinateur (HTMA-IVOS Hamilton-Thorn) avec les méthodes standard d'évaluation des caractéristiques biologiques de la semence de lapin, 47 ejaculats sont analysés. Le pH et les paramètres visuels classiquement utilisés pour l'observation de la semence ont été estimés immédiatement après récolte. Pour chaque éjaculat, 40 µl de semence diluée 5 fois ont été prélevés et déposés dans 3 tubes contenant 2,8 ml de dilueur, 3 gouttes de chaque tube et 4 champs sont analysés (c.a.d. 36 analyses) ou 2 tubes, 2 gouttes et 2 champs (c.a.d. 8 analyses). Les spermatozoïdes incorrectement identifiés par l'analyseur de semence ont été dénombrés, afin de connaître le nombre exact de cellules de chacun des champs. La concentration estimée par l'HTMA est hautement corrélée avec le nombre exact de cellules (r=0,999, P=0,0001) démontrant ainsi une bonne précision. Les corrélations calculées sur 36 ou 8 champs sont comparables. Le pourcentage de cellules progressives estimé par l'HTMA et VAP sont négativement corrélés au pH (respectivement, r=-0,31, P=0,03 et r=-0,32, P=0,02). Le pourcentage de cellules progressives estimées par l'HTMA est positivement corrélé à la motilité individuelle (r=0,52, P=0,0002). Malgré un délai de 2 heures, le pourcentage de cellules progressives estimé visuellement ou par l'HTMA sont significativement corrélés (r=0,79, P=0,0001). Les concentrations obtenues par analyse d'images sont corrélées à l'estimation sur hématimètre ou par densité optique (respectivement, r=0,68, P=0,001 et r=0,65, P=0,001). Les auteurs confirment l'intérêt des méthodes d'analyse d'images pour évaluer les caractéristiques biologiques de la semence de lapin, cependant, leur intérêt ne sera clairement établi que si elles permettent d'obtenir des critères prédicteurs de la fertilité.
