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WAVE PARAMETERS OF THE SPERM FLAGELLUM AS PREDICTORS OF HUMAN SPERMATOZOA MOTILITY

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SUMMARY

We characterized the undulatory movement of the sperm's flagellum as a sigmoid wave by measuring the absolute and linear speed of the sperm, period, amplitude and length of the flagellum's wave, and the segment comprised between the head and the origin of the movement in the flagellum. These parameters were correlated with traditional ones used to determine the pattern of movement of the sperm. Our results show that wave parameters are useful predictors of sperm motility. They correlate among themselves, and thus, a few wave parameter may characterize the sperm motility. The advantage of wave parameters is that they can be easily obtained and can be eventually associated to the sperms internal morphology.

INTRODUCTION

Sperm motility is thought to be an important factor in the biology of reproduction at both the systemic and cellular levels, and the quality of the sperm movement has been related to fertility in humans (Dredsner and Katz,1981) and other animals (Suarez et al.,1984). The internal structure of the flagellum is now well understood but not its consequences on the flagellum's undulations, which should be relevant in determining the quality of the movements (Fauci and Mc. Donald,1995). During the last years, seminal evaluation has been undertaken using the computerized image analysis of the sperm's movement. The computerized system considers the track of the head to classify the different types of movement of the spermatozoa . The lateral displacement of the sperm head is a characteristic that seems to be associated with its capacity to penetrate cervical mucus and progress into the female genital tract (Aitken, 1995). This parameter is related to minor changes in the amplitude of the lateral displacement of the sperm's head, which in turn is reflective of the undulatory movement of the flagellum, all of which determines the propulsive force necessary for fertilizing the egg (Aitken, 1995).

Considering that the sperm movement is originated in the flagellum, and thus, that direct measurements of the parameters of the flagellum's movement would be more convenient, we attempt here to characterize directly the undulatory movement of the sperm's flagellum as a sigmoid wave by measuring the absolute and linear speed of the sperm, period, amplitude and length of the flagellum's wave, and the segment comprised between the head and the origin of the movement in the flagellum. These parameters were correlated with traditional ones used to determine the pattern of movement of the sperm.

MATERIALS AND METHODS

Twelve semen samples were obtained by masturbation following 3 to 5 days of sexual abstinence, from 12 healthy donors. For each sample, motile sperms were isolated by overlaying the semen samples with an equal volume of warm BWW (Brinster 1965), supplemented with 0.3% Bovine Serum Albumin, incubating the mixture at 37° C for 60 min and then collecting the BWW medium sperm suspension. Each sample was analyzed with two different methods:

1- A semi automatic system (Comhaire & Vermeulen 1995) consisting in a single step for the assessment of sperm characteristics. A drawing tube was introduced between the objective and the ocular of the microscope, to observe motile spermatozoa and to track their movement manually with the help of a cursor on a digitizing tablet. This tablet generated data which were analyzed with a computer program (Hintnig et al. 1988). For each sample, a minimum of 50 spermatozoa were analyzed. The method allowed for the determination of sperm concentration, percentage of motility, sperm linear velocity, linear index, and motility grading. Motility grading had four categories: MG-A: sperms with rapid linear progressive motility with linear velocity in excess of 22 µm/s; MG-B: sperms with slow or sluggish linear or non linear movement and with linear velocity below 22 µm/s, MG-C: sperms with non-progressive motility and velocity below 5 µm/s: MG-D: immobile sperms (Comhaire & Vermeulen 1995).

2- The sperm suspensions were observed under phase contrast microscope and the sperm movement were registered with a video camera which was connected to the microscope. The images were registered with a VHS system. With this method we analyzed a minimum of 20 sperms per sample (total of 277 sperms) from the 12 different sample preparations. Then the sperm movement was analyzed by assuming that the flagellum's movement can be described by a sigmoid wave. The following characteristics of the wave were measured: amplitude, wavelength, speed and period. We also measured the length of the segment comprised between the head and the site of the flagellum where the undulatory movement was initiated. Only sperms that exhibited complete undulatory movement of the flagellum visible on the two dimensional plane of the screen were analyzed. When a sperm with these characteristics was identified, the VHS was stopped and the image was used to measure the variables in mm. Figure 1 illustrates the measurements taken. A graduated Neubauer chamber was used to measure and calculate the correspondence between the values in mm obtained in the monitor with the real values. The period was defined as the time necessary to realize a whole flagellum's wave or one cycle. To estimate the period we fixed an initial position of the sperm and measured the time taken to describe a whole undulatory movement of the flagellum, i.e. until the wave returned to the initial position. The period was expressed in seconds / cycle.

The statistical correlation between the different variables were obtained applying the Spearman rank order correlation analysis.

RESULTS

The results obtained for the different variables characterizing the wave movement of the sperm's flagellum (Figure 2-6), show that the variables have discontinuous values. That is, the variables seem to converge onto discrete values, where intermediate values have a very low probability to occur. This effect is strongest in variables measuring simple wave features. The movement of the flagellum rarely starts at its insertion into the head of the sperm. The site of the flagellum where the movement starts (i) seems to be related to the amplitude (a) and length (l) of the wave described by the flagellum. Consequently the wave parameters and the speed (s) of the sperm is also related to that site. Our results show that all wave parameters measured correlate among themselves (Table 1). Linear regressions between the variables (Table 2) may give us an idea how they relate. We found that the speed of the sperm can be predicted from any of the parameters with a reasonable precision (correlation coefficient $r = 0.895$ using i) and that it is inversely related to the period (p). Using all four variables to predict the speed improves only slightly the precision ($r = 0.908$). On the other hand, i can be predicted from a and l ($r = 0.966$) and p from a and l ($r = 0.934$). That is, the sperms movement can be described (using length measurements of a, l and i in µm and of p in ms), with the following formula :

$i = 0.27\,a + 0.14\,l$ (ß for $a = 0.32$, $l = 0.66$)

$p = 32\,a + 7\,l$ (ß for $a = 0.5$, $l = 0.45$)

$s = 6.8\,i$ (ß for $i = 0.9$)

$s = 3\,a + 1.2\,l - 29\,p$ (ß for $a = 0.47$, $l = 0.73$, $p = -0.3$)

The wave parameters measured correlated strongly with the motility indices calculated with the semi automated analysis system (Table 3), suggesting in fact that the wave parameters are reliable indicators for sperm motility.

DISCUSSION

Our results suggest that the flagellum is constricted in the type of movements it can perform to a finite number of states, probably reflecting the degrees of freedom the morphological structures allow regarding the expression of movement of the flagellum. This result seems to be related to the discrete nature of the activation process of microtubules (Kamimura and Mandelkow 1992), suggesting that the microtubules responsible for the movement of the flagella have a finite number of combinations in which they can express their activity. The movement of the flagellum of the sperm can be viewed as a string vibrating in a viscous medium, or as a flexible screw exerting force on a viscous medium. These kind of problems are best solved empirically, as no physical analytical solution to them is known.

David et al. (1981) studied the amplitude of the lateral movement of the sperm head as a reflective of the amplitude of the flagellum's wave. Here we report directly the characteristic of the flagellum's movement and correlate them with the physical parameters defining the sigmoid wave formed by the moving spermatozoa. Our results show that the wave parameters of the flagellum are useful predictors of sperm motility, as they correlate significantly among themselves, and thus, a single or a few wave parameter may characterize the movement of the sperm.

An advantage of wave parameters over other motility indices is that wave parameters can eventually be associated to the sperms internal morphology in more direct ways, as they represent real physically characteristics of a movement. If this can be confirmed independently, it suggests that wave parameters may be more helpful in relating sperm movement to sperm pathology and to fertility. One indication of the close relation between the wave parameters measured and their internal morphology and physiology is the quantum nature of these values. The wave parameters showed a wide variability and a tendency to cluster around discontinuous values, where intermediate values have a low probability to occur. It should be possible to explain this tendency taking into account the number of microtubules and their fixed pattern of relative movements (Aitken, 1995), which would determine the movement pattern of the flagellum. We suggest, thus, that the discrete distribution of the wave parameters reflects a discrete number of possible movements of the sperm, restricted by the limited array of possible interactions among the fixed number of microtubules in the flagellum..

We suggest that the new methodology described here is a useful tool for the characterization of the motility of spermatozoa. This method has several advantages : Wave parameters can be assessed without the help of computers and are thus easier to obtain than alternative indices ; results obtained in this way are comparable with the velocity obtained by the semi-automated seminal evaluation system; wave parameters can be obtain from any static photo of moving sperms and their measurement is very simple. In addition, automatization of these measurements is also possible and desirable as it increases the speed and precision of the measurements. The next step should to be to investigate the relation between the characteristics of the flagellum's wave parameters and fertility.

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Table 1: Correlation coefficients between wave parameters

STAT. BASIC STATS — Correlations: Marked correlations are significant at p < .05 N=277 (Casewise deletion of missing data)

Variable	Initial segment	Amplitude	Wavelength	Period	Speed
Initial segment	1.0000 p= ---	.2364* p=.000*	.2983* p=.000*	-.1443* p=.016*	.1592* p=.008*
Amplitude	.2364* p=.000*	1.0000 p= ---	-.1795* p=.003*	.2583* p=.000*	.0115 p=.849
Wavelength	.2983* p=.000*	-.1795* p=.003*	1.0000 p= ---	-.1494* p=.013*	-.0965 p=.109
Period	-.1443* p=.016*	.2583* p=.000*	-.1494* p=.013*	1.0000 p= ---	-.4520* p=.000*
Speed	.1592* p=.008*	.0115 p=.849	-.0965 p=.109	-.4520* p=.000*	1.0000 p= ---

Table II: Multiple Regression for the prediction of the speed of movement of sperm

STAT. MULTIPLE REGRESS.	Regression Summary for Dependent Variable: SPEED R= .90836130 R2= .82512025 Adjusted R2= .82255791 F(4,273)=322.02 p<0.0000 Std. Error of estimate: 13.321					
N=277	BETA	St. Err. of BETA	B	St. Err. of B	t(273)	p-level
Init. segm	.403307	.099064	3.0699	.754052	4.07116	.000061
Wavelength	.446764	.097343	.7257	.158118	4.58960	.000007
Amplitude	.324978	.078928	2.0465	.497047	4.11741	.000051
Period	-.265624	.071380	-26.1439	7.025526	-3.72128	.000241

STAT. MULTIPLE REGRESS.	Regression Summary for Dependent Variable: SPEED R= .90249818 R2= .81450296 Adjusted R2= .81247197 F(3,274)=401.04 p<0.0000 Std. Error of estimate: 13.694					
Amplitude	.471328	.072236	2.9682	.454903	6.52487	.000000
Wavelength	.728138	.070469	1.1827	.114466	10.33270	.000000
Period	-.296823	.072956	-29.2147	7.180688	-4.06851	.000062

STAT. REGRESS.	Regression for Dependent Variable: SPEED and each of the wave parameters one by one					
Init. segm	.895371	.026805	0.895	.204033	33.40300	0.00
Amplitude	.861216	.030592	0.861	.192655	28.15130	0.00
Wavelegth	.886093	.027900	0.886	.045319	31.75982	0.00
Period	.797145	.036344	0.797	3.577124	21.93350	0.00

Table III: Correlation between wave parameters and sperm motility indices
as given by Comhaire & Vermeulen (1995)

STAT. BASIC STATS	Correlations Marked correlations are significant at p < .05000 N=277 (Casewise deletion of missing data)				
Variable	Initial segment	Amplitude	Wavelegth	Period	Speed
Sperm Concentra.	.3405* p=.000*	-.0240 p=.691	.3458* p=.000*	-.2357* p=.000*	.2669* p=.000*
MG-A	.2960* p=.000*	.3101* p=.000*	-.1788* p=.003*	-.0729 p=.227	.2356* p=.000*
MG-B	.0959 p=.111	-.1047 p=.082	.0362 p=.549	-.1312* p=.029*	.3074* p=.000*
MG-C	-.1618* p=.007*	-.0806 p=.181	.1904* p=.001*	.1101 p=.067	-.3824* p=.000*
MG-D	-.2275* p=.000*	.0166 p=.783	-.2855* p=.000*	.1353* p=.024*	-.1160 p=.054
Sperm Speed	.2642* p=.000*	.3661* p=.000*	-.3310* p=.000*	-.0158 p=.794	.3329* p=.000*
Linear Speed	.2834* p=.000*	.3452* p=.000*	-.2988* p=.000*	-.0441 p=.465	.3497* p=.000*
Linear Index	.1714* p=.004*	-.1488* p=.013*	.3144* p=.000*	-.1563* p=.009*	.0599 p=.321

Figure 1 : Wave parameters of the movement of the flagellum, indicating the amplitude (a), distance from head where the wave movement starts (i) and the wavelength (l).

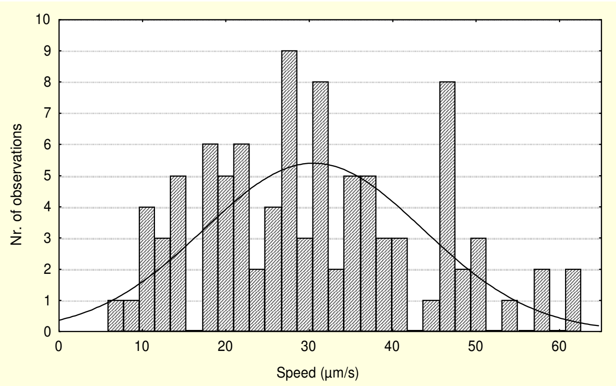
Figure 2 : Frequency distribution of values for spermatozoa for the distance from the head where the movement of the flagellum starts

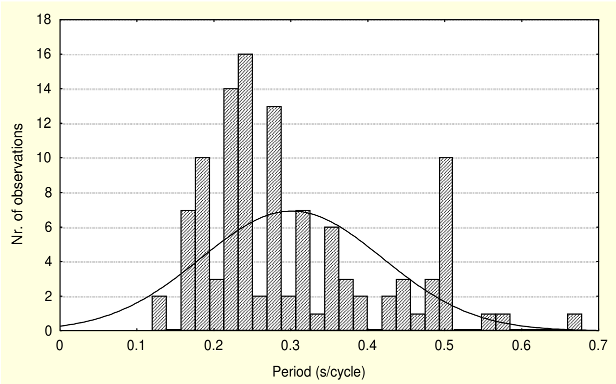
Figure 3 : Frequency distribution of values for spermatozoa for the wavelength of the movement of the flagellum.

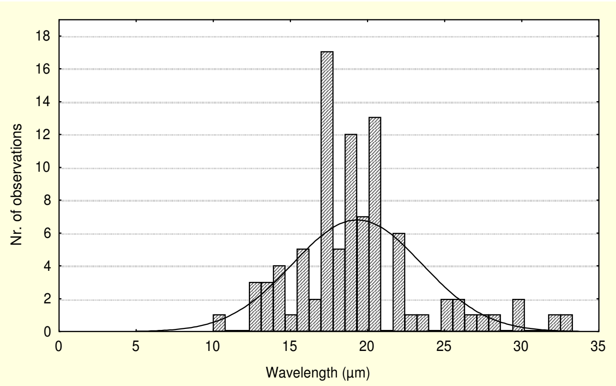
Figure 4 : Frequency distribution of values for spermatozoa for the amplitude of the wave length of the flagellum

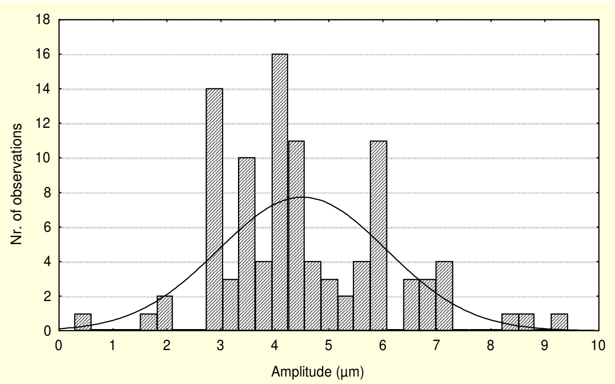
Figure 5 : Frequency distribution of values for spermatozoa for the period of the wave movement of the flagellum.

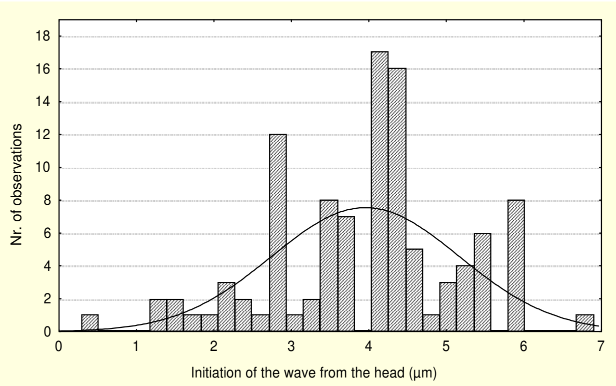
Figure 6 : Frequency distribution of spermatozoa velocities.


Nr. of observations
Speed (µm/s)
0
1
2
3
4
5
6
7
8
9
10
0
10
20
30
40
50
60


Nr. of observations
Period (s/cycle)
0
2
4
6
8
10
12
14
16
18
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7


Nr. of observations
Wavelength (µm)
0
2
4
6
8
10
12
14
16
18
0
5
10
15
20
25
30
35


Nr. of observations
Amplitude (µm)
0
1
2
3
4
5
6
7
8
9
10
0
2
4
6
8
10
12
14
16
18


Nr. of observations
Initiation of the wave from the head (µm)
0
2
4
6
8
10
12
14
16
18
0
1
2
3
4
5
6
7