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Evaluation of Human Sperm Hyperactivated Motility and Its Relationship with the Zona-Free Hamster Oocyte Sperm Penetration Assay

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ABSTRACT: The authors studied hyperactivated motility of human spermatozoa as a method of evaluating capacitation by examining its relationship to results of zona-free hamster oocyte sperm penetration assays (SPA) of semen samples from 50 men attending the infertility clinic. Hyperactivated motility was assessed in the seminal plasma and after swim-up preparation of spermatozoa at 1, 3, and 24 hours of incubation in capacitation media using a computer-assisted semen analysis system equipped with a hyperactivation module. Hyperactivated motility reached a peak at 1 hour and plateaued at 3 hours. The percentage of spermatozoa in seminal plasma with star-spin hyperactivated motility was significantly lower in the group showing no penetration in the SPA. The hyperactivated motility characteristics did not differ in the groups with positive or negative penetration. Correlation analysis failed to show any significant relationship between the hyperactivated motility parameters and SPA score. When the hyperactivated motility characteristics were compared in samples with normal and abnormal semen analyses, the total percentage of spermatozoa with hyperactivated motility and the percentage with star-spin at 3 hours were significantly lower in the group with abnormal semen analysis. The data indicate that lower hyperactivated motility of spermatozoa was found in patients with a score of zero for SPA and in patients with abnormal semen analysis. It was concluded that although no direct correlations were found between the results of SPA and hyperactivated motility, evaluating hyperactivated motility may still be useful as an early indicator of capacitation abnormalities of human spermatozoa not measured by SPA.

Key words: Motility, capacitation, fertilizing capacity.
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In most mammalian species, spermatozoa complete a final maturation process, usually in the female reproductive tract, before interaction with oocytes. This process, termed capacitation, is characterized by spermatozoa undergoing the acrosome reaction and developing hyperactivated motility (HA; Yanagimachi, 1988). Hyperactivated motility is a highly active but nonprogressive motility marked by wide amplitude of the flagellar beats. This flagellar movement leads to bending of the midpiece and the principle piece of the sperm cell and results in marked lateral head displacement, low forward progression, and "star-spin" or "whiplash" trajectories (Yanagimachi, 1988). The expression and movement parameters of HA have been defined and characterized in human spermatozoa using manual and computer-assisted semen analysis methods (Burkman, 1984, 1986a, b, 1991; Mortimer et al, 1984; Okada et al, 1985; Morales et al, 1988; Katz et al, 1989; Robertson et al, 1988; Mack et al, 1988, 1989; Mortimer and Mortimer, 1990; Grunet et al, 1990). At least two commercial computer-assisted semen analysis (CASA) systems are equipped with software programs to allow more objective study of HA in human spermatozoa. These systems were set up based on parameters derived from sperm head images tracked both manually and using CASA (Mack et al, 1988; Grunet et al, 1990; Burkman, 1991). These include the Cellsoft semen analyzer with the hyperactivation module (Cryo Resources Ltd, Montgomery, NY; Mack et al, 1988; Robertson et al, 1988) and the Hamilton-Thorn System (Hamilton-Thorn Research, Beverly, MA; Grunet et al, 1990).

Evaluating HA may be a method of assessing capacitation. Abnormalities in capacitation should cause poor sperm penetration in the zona-free hamster oocyte sperm penetration assay (SPA; Yanagimachi, 1984). In the current investigation, we studied HA characteristics of human spermatozoa in 50 samples from patients attending an in-

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fertility clinic. We compared the sperm HA in samples with a negative or positive SPA and examined whether HA characteristics were related to the outcome of SPA.

Materials and Methods

Semen Samples and Sperm Preparation

Semen samples were obtained from 50 untreated men attending the infertility clinic. Twelve of the subjects had oligospermia or asthenospermia as defined by the World Health Organization (WHO, 1987) standard criteria (sperm concentration $< 20 \times 10^6$/ml and sperm motility < 50%). Routine semen analyses, including sperm count, motility, and morphology, were performed according to the *WHO Laboratory Manual* (World Health Organization, 1987). Motile spermatozoa were harvested from the seminal plasma using the swim-up technique recommended by WHO (1987). Motile spermatozoa were incubated in Biggers, Whitten and Whittingham (BWW) medium (Biggers et al, 1971) supplemented with 0.35% (w/v) human serum albumin (HSA; Sigma Chemical Co., St. Louis, MO). The HSA was used as the protein source in the incubation media instead of maternal serum so that all semen samples could be treated with the same batch of HSA. A low protein concentration (0.35%) of HSA was chosen because the percentage of spermatozoa with HA decreases with increasing protein concentration in the capacitation medium (Mack et al, 1989). Preliminary experiments on 12 normal semen samples in our laboratory showed that the percentage of HA between 1 and 3 hours was 6.8 ± 1.7% (mean ± SE) in 0.35% HSA compared with 1.7 ± 0.4% in 7.5% heat-inactivated human serum. In addition, results of the SPA in these preliminary studies showed similar penetration rates of 26.4 ± 5.9% when 0.35% HSA was used as compared with 31.7 ± 8.9% when 7.5% heat-inactivated human serum was used in the media. These data demonstrated that HA was enhanced in media with low protein concentration without affecting the results of SPA.

Movement Analysis and Hyperactivated Motility

Movement analysis was performed on spermatozoa in seminal plasma and in the swim-up preparation after 1, 3, and 24 hours of incubation using the Cellsoft CASA system (Cryo Resources, New York, NY) with initial parameters as previously described (Chan et al, 1989). Hyperactivated motility was determined at the same time. Preliminary experiments showed that peak HA occurred between 1 and 3 hours, and analyses at 6 and 8 hours did not yield further information. The sperm suspension was adjusted to about 10 to 20 $\times 10^6$ motile spermatozoa/ml. According to the method described by Mack et al (1989), 32-μm deep preparations were made using dry transfer rings (Chartpak #RDC49, 13 mm circles; Chartpak, Leeds, MA). Hyperactivated motility was analyzed using a Cellsoft system equipped with a hyperactivation module (Cryo Resources). All sperm preparations were analyzed on a heated microscope stage (37°C) and videotaped for at least 5 minutes. Set-up parameters were similar to those described by Robertson et al (1988) and Mack et al (1989) for analysis of hyperactivated sperm movement. The following parameters were computed or calculated: curvilinear velocity (VCL, μm/s), straight line velocity (VSL, μm/s), linearity (LIN = VSL/VCL × 100), maximum amplitude of lateral head displacement (max ALH, μm), mean amplitude of lateral head displacement (mean ALH, μm), and head beat-cross frequency (BCF, Hz). In addition to the parameters described above, the derived parameter DANCEMEAN (mean ALH × VCL/VSL, μm) was calculated for each cell (Robertson et al, 1988). Hyperactivated sperm cells were classified as transitional if the VCL was greater than 80 μm/s, LIN was greater than 19 and less than or equal to 34, and DANCEMEAN was greater than or equal to 17 μm, and as star-spin if the VCL was greater than 80 μm/s, LIN was less than or equal to 19, and DANCEMEAN was greater than or equal to 17 μm (Robertson et al, 1988). Data from each individual cell track were recorded and printed. Mean and standard errors of the mean (SE) of movement parameters of both star-spin and transitional cells were calculated manually from individual cell data. At least 100 motile sperm cells were analyzed for each sample.

Zona-Free Hamster Oocyte Sperm Penetration Assay

The sperm penetration assay was performed according to the method described by the WHO (1987). After an overnight incubation of prepared semen samples at 37°C in BWW with 0.35% HSA, the motile sperm concentration was readjusted to 3.5 × 10^6/ml before incubation with zona-free hamster oocytes. The sperm–oocyte mixture was then incubated for 3 hours before assessment of penetration rate according to methods described in the *WHO Laboratory Manual* (1987). Semen samples were divided into two categories depending on the results of the SPA: no penetration-negative SPA (n = 15) and presence of some penetration-positive SPA (n = 35; mean ± SE penetration rate, 32.1 ± 4.5%; range, 2.7% to 100%).

Statistical Analysis

Statistical analyses, including analysis of variance, regression analysis, chi-squared test, and discriminant analysis, were performed using the Statistical Package for the Social Sciences (Nie et al, 1975).

Results

The mean total percentage of spermatozoa with HA from the 50 samples was 3.2 ± 0.5% (mean ± SE) in seminal plasma before sperm preparation. Most of the spermatozoa showed the transitional type of motility pattern (2.3 ± 0.4%). The percentage of spermatozoa with HA in both the positive and negative SPA samples peaked at 1 hour after sperm preparation (8.4 ± 0.8%) and consisted mainly of spermatozoa with star-spin trajectory (5.3 ± 0.5%). At 3 hours, the average percentage of spermatozoa with HA was 6.7 ± 0.8%, slightly lower than that at 1 hour ($P = 0.068$) but still higher than that before sperm preparation (3.2 ± 0.5%; $P < 0.001$) and 24 hours after sperm preparation (1.3 ± 0.3%; $P < 0.001$). This time-course pattern of HA in human spermatozoa occurred in semen samples both with positive and negative results in the SPA (Fig 1). In the negative SPA group, however, the percentage of sperma-

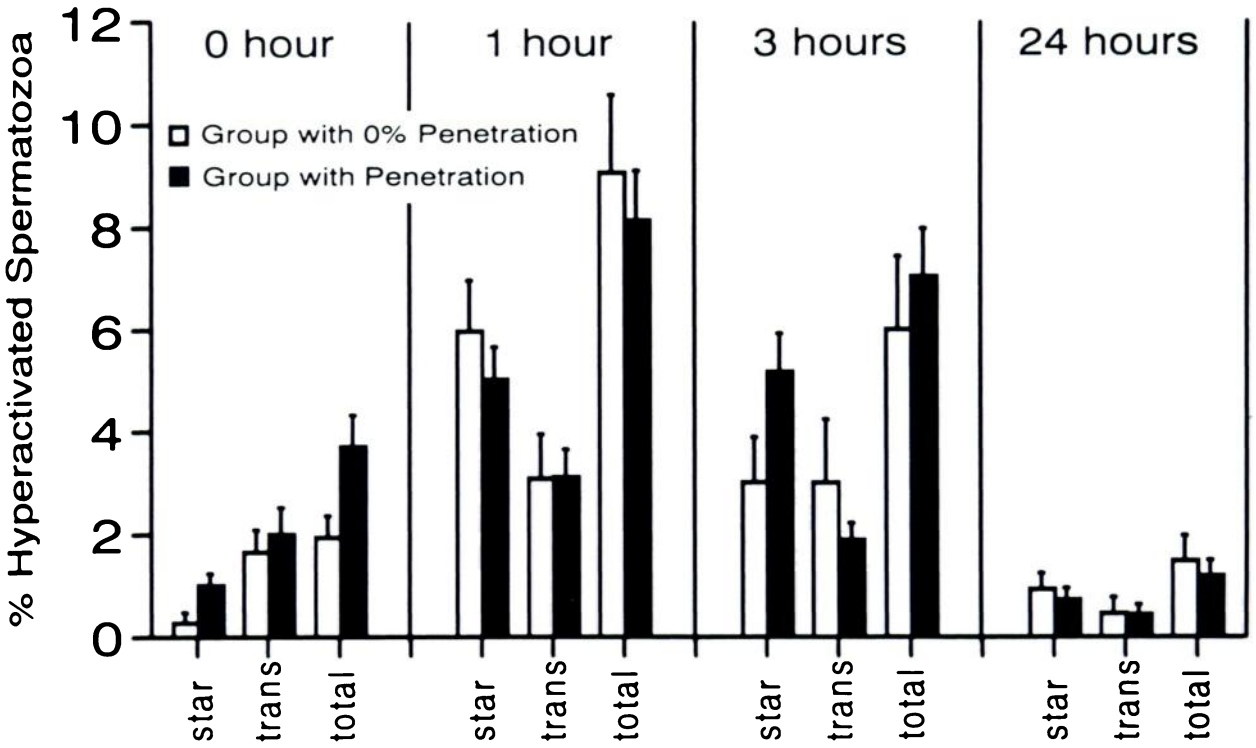

FIG. 1. Percentage (mean ± SE) of spermatozoa with hyperactivated motility in seminal plasma (ie, 0 hour) and 1, 3, and 24 hours after sperm preparation and incubation in BWW medium supplemented with 0.35% HSA at 37°C. Filled bars = samples with positive penetration, and open bars = samples with no penetration in the zona-free oocyte sperm penetration assay. Star = star-spin; trans = transitional; and total = star-spin plus transitional.

tozoa in seminal plasma with star-spin HA (0.3 ± 0.2%) was significantly higher than the positive SPA group (1.0 ± 0.2%; P = 0.05; Fig 1). The total percentage of spermatozoa with HA in seminal plasma in the negative SPA group was 1.9 ± 0.4%, which was not significantly lower than the positive SPA group (3.7 ± 0.6%; P = 0.08). Due to the large inter-subject variation, the percentage of spermatozoa with HA was not significantly different between the two groups at 1, 3, and 24 hours after incubation (Fig 1.).

The motility parameters of spermatozoa with star-spin or transitional HA were calculated from individual cell data and are presented in Table 1. In general, sperm cells with the star-spin pattern of HA had lower VSL and linearity and higher DANCEMEAN than cells with the transitional motility pattern. These HA parameters did not significantly differ between the groups with negative and positive SPA. Regression analysis did not demonstrate any significant relationship between the percentage of spermatozoa with HA or the movement parameters of the sperm cells showing HA and the results of the SPA. Multivariate discriminant analyses showed that inclusion of HA parameters in routine semen analysis or computer-derived movement characteristics gave no additional information for discriminating samples showing a positive or negative penetration in SPA (data and analyses not shown).

In the 12 samples with at least one abnormal semen parameter (sperm count = 66 ± 14 × 10^6/ml; motility = 45.0 ± 4.8%; morphology = 52.3 ± 4.6%; SPA result = 11.2 ± 6.8%), five of 12 (41.7%) had positive SPA result. This positive SPA rate was significantly lower (chi-square = 6.0; $P < 0.02$) than the normospermic samples (sperm count = 94 ± 17 × 10^6/ml; motility = 68.7 ± 1.4%; morphology = 70.5 ± 1.4%; SPA result = 26.0 ± 4.3%) in which 30 of 38 (78.9%) samples showed a positive SPA result. When the spermatozoal HA of the two groups of patients with normal or abnormal routine semen analysis was compared, the percentage of spermatozoa with HA at 3 hours and the percentage with star-spin trajectory at 0 and 3 hours were significantly lower in the patients with oligospermia (Table 2). Regression analysis showed no significant correlation between HA parameters and sperm count, motility, or morphology.

Discussion

We analyzed and studied HA of human spermatozoa in 50 semen samples using a CASA system equipped with a module for hyperactivation. These studies were performed using standardized conditions described by Robertson et al (1988)

Table 1. *Motility parameters of hyperactivated star-spin and transitional spermatozoa in subjects with and without penetration into zona-free hamster oocytes*

Time and motility parameters	Star-spin spermatazoa (% oocyte penetration)		Transitional spermatazoa (% oocyte penetration)	
	0	>0	0	>0
0 h				
VCL (μm/s)	104.7 ± 3.7	103.9 ± 4.6	102.7 ± 3.2	113.8 ± 7.7
VSL (μm/s)	9.4 ± 2.8	13.8 ± 2.4	29.5 ± 1.6	31.1 ± 1.9
Linearity	8.9 ± 2.5	12.7 ± 1.8	28.9 ± 1.4	27.6 ± 0.6
Mean ALH (μm)	5.7 ± 1.1	7.2 ± 0.7	7.8 ± 0.4	8.4 ± 0.7
BCF (Hz)	7.3 ± 1.1	7.3 ± 0.5	7.6 ± 1.0	8.3 ± 1.7
DANCEMEAN (μm)	65.6 ± 5.8	80.9 ± 15.7	28.2 ± 1.9	30.9 ± 3.2
1 h				
VCL (μm/s)	117.4 ± 6.3	119.8 ± 4.3	146.6 ± 0.5	132.3 ± 5.5
VSL (μm/s)	11.9 ± 1.0	12.9 ± 0.8	37.5 ± 3.2	36.9 ± 2.3
Linearity	10.0 ± 0.5	10.6 ± 0.6	25.9 ± 0.9	26.6 ± 0.7
Mean ALH(μm)	7.2 ± 0.5	7.0 ± 0.3	8.8 ± 0.6	11.3 ± 2.9
BCF (Hz)	7.7 ± 0.3	6.6 ± 0.2	9.1 ± 0.8	8.6 ± 0.6
DANCEMEAN (μm)	73.7 ± 5.6	68.4 ± 4.6	34.6 ± 2.0	40.1 ± 9.3
3 h				
VCL (μm/s)	113.8 ± 5.8	112.7 ± 3.4	133.1 ± 11.3	121.7 ± 5.5
VSL (μm/s)	11.6 ± 1.7	10.9 ± 0.6	33.4 ± 3.7	31.8 ± 2.1
Linearity	9.9 ± 1.7	9.5 ± 0.5	24.9 ± 1.6	25.9 ± 1.0
Mean ALH (μm)	6.8 ± 0.4	6.4 ± 0.3	8.1 ± 0.8	8.2 ± 0.5
BCF (Hz)	7.1 ± 0.4	6.4 ± 0.4	7.2 ± 0.8	8.5 ± 0.4
DANCEMEAN (μm)	86.0 ± 20.9	69.6 ± 4.0	33.8 ± 4.4	33.1 ± 1.9
24 h				
VCL (μm/s)	112.4 ± 13.1	121.5 ± 7.8	115.5 ± 20.3	115.2 ± 9.0
VSL (μm/s)	10.8 ± 3.6	13.3 ± 2.9	35.3 ± 7.4	28.5 ± 2.5
Linearity	8.7 ± 1.8	11.2 ± 2.2	30.4 ± 2.5	25.2 ± 1.9
Mean ALH (μm)	6.5 ± 1.3	7.3 ± 0.5	7.5 ± 0.7	8.1 ± 0.5
BCF (Hz)	7.1 ± 0.9	5.8 ± 0.8	10.9 ± 0.1	8.3 ± 0.8
DANCEMEAN (μm)	89.6 ± 25.4	96.5 ± 17.7	25.1 ± 3.6	34.0 ± 3.8

Values are presented as the mean ± SE.

and Mack et al (1988). A low protein concentration in the incubation media was used to optimize the percentage of spermatozoa with HA (Mack et al, 1989). Under these conditions, the percentage of spermatozoa with HA in the raw semen sample was very low, which is consistent with the findings reported by others (Burkman, 1986a, b; Robertson et al, 1988; Grunet et al, 1990). After swim-up preparation to remove seminal plasma, the total percentage of spermatozoa with HA increased at 1 hour and remained significantly higher at 3 hours when compared with basal conditions. The HA expressed by the spermatozoa was markedly decreased after a 24-hour incubation period. These results were similar to those reported by Burkman (1986a, b), Morales et al (1988), and Mortimer and Mortimer (1990) but differed from the report by Robertson et al (1988). This may be due to the low protein concentration in the culture media, the different types of media (Mack et al, 1989), or the different systems of motility analysis (Grunet et al, 1990; Mortimer and Mortimer, 1990). The rapid achievement of peak HA suggests that at least some human spermatozoa acquire the characteristic motility patterns of HA soon after separation from seminal plasma. This may indicate that HA in human spermatozoa is an early marker of capacitation and not an indicator of completed capacitation and readiness for interaction with the vestments of homologous oocytes. The early occurrence of HA in the sperm preparation may explain the low incidence (less than 2%) of HA reported by some investigators (Grunet et al, 1990) when observations of HA were made after 6 hours of incubation. Similar to previous reports and by definition, the percentage of spermatozoa with star-spin movement patterns had lower VSL and linearity and higher DANCEMEAN than those exhibiting the transitional pattern (Burkman, 1986a; Robertson et al, 1988; Mortimer and Mortimer, 1990). In the raw semen sample, spermatozoa with HA mainly exhibited the transitional pattern. After 1 to 3 hours of capacitation, the majority of the spermatozoa had star-spin trajectories.

When the percentage of spermatozoa with HA was compared in the samples with and those without penetration in the SPA, the differences were very small and not significant for most parameters. Only the percentage of spermatozoa with star-spin trajectory was significantly lower in the group that showed negative penetration in the SPA. When each individual HA motility parameter was compared in the groups of samples with positive or negative SPA scores,

Table 2. *Hyperactivated motility in samples with normal semen analysis or oligo/asthenospermia*

Time	Type of hyperactivated motility	Normal semen analysis (n = 38)	Oligo/ asthenospermia (n = 12)
0 h	Star-spin	1.0 ± 0.2	0.2 ± 0.2
	Transitional	2.7 ± 0.5	1.4 ± 0.6
	HA	3.7 ± 0.6	1.7 ± 0.7
1 h	Star-spin	5.8 ± 0.6	3.7 ± 1.0
	Transitional	3.0 ± 0.5	3.5 ± 1.1
	HA	8.8 ± 0.9	7.1 ± 1.8
3 h	Star-spin	5.2 ± 0.7	2.3 ± 0.9*
	Transitional	2.4 ± 0.6	1.7 ± 0.5
	HA	7.6 ± 0.9	3.9 ± 1.2*
24 h	Star-spin	0.7 ± 0.2	1.0 ± 0.4
	Transitional	0.4 ± 0.2	0.5 ± 0.4
	HA	1.2 ± 0.3	1.5 ± 0.6

Values are percentage, presented as mean ± SE.
HA = total percentage of spermatozoa with hyperactivated motility.
*$P < 0.05$ when compared to group with normal semen analysis.

none of the HA parameters showed a significant difference. Again, none of the HA parameters showed a significant correlation with the penetration rate in SPA, and multivariate discriminant analyses showed that HA parameters were not useful for predicting the outcome of SPA. The lack of correlation between HA measurement and SPA results may be related to the low percentage of spermatozoal cells displaying this movement pattern or the HA module not detecting all cells undergoing HA during the capacitation process. In this study, the SPA was not performed after 1 and 3 hours of incubation to establish a temporal relationship of HA with the results of the SPA. Although Morales et al (1988) showed that the results of SPA were correlated with some hyperactivated motility parameters (eg, sperm head rolling frequency and flagellar beat frequency reflective of sperm vigor in 10 infertile patients), examination of their data showed large variation and an overlap in both the fertile and infertile patients.

The number of samples with a positive SPA result was lower in oligo/asthenospermic compared with normospermic specimens. The HA parameters in samples with oligospermia also showed a lower percentage of HA, especially of the star-spin type at 0 and 3 hours. Our data suggest that although HA parameters had no direct relationship with SPA results, studying the HA of spermatozoa may still be useful as a method of evaluating abnormalities, especially in the early events of capacitation. Different time-related abnormalities in capacitation may be detected by evaluating the temporal relationship between SPA and HA. The relationship between human sperm HA and *in vitro* fertilization of human oocytes is not clear. Our laboratory is currently examining this relationship.

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