

HYPOOSMOTIC SWELLING: EVALUATION OF SPERM TAILS USING STRICT CRITERIA IN CASES OF GLOBOZOOSPERMIA

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The semen from two men with globozoospermia was evaluated for hypoosmotic swelling changes and morphologic evaluation of the tail using strict criteria. No abnormalities were noted. Globozoospermia is due to a genetic defect that results in the absence of acrosomal structures. The acrosomal vesicle in the Golgi region does not develop in close contact to the nucleus, which results in the formation of globe-shape heads. Although some semen parameters were abnormal in both cases, they were different types, suggesting no association with round-headed sperm.

Key Words: Globozoospermia; Round-headed sperm; HOS Test; Acrosome; Strict morphology.

INTRODUCTION

A rare cause of male infertility is known as globozoospermia or the round-head syndrome [4, 6]. Ultrastructurally, these spermatozoa have a complete absence of both an acrosome and postacrosomal sheath, as well as abnormal inner membranes [5]. The sperm were unable to penetrate zona-free hamster oocytes [7]. The ejaculates of globozoospermic men are normal, at least with regard to concentration and motility of spermatozoa. Syms et al. [7] used computerized digital image analysis to evaluate patterns of sperm motility, and the individual patterns were typical of the normal fertile population. Little is known about the pattern of motility of sperm tail using the new strict morphologic criteria [3]. Furthermore, there are no studies of the hypoosmotic swelling (HOS) test (which evaluates the functional integrity of the sperm membrane) in patients with this syndrome [2].

MATERIALS AND METHODS

Two cases of round-headed sperm were analyzed. Semen samples were collected by masturbation following 2-4 days of abstinence. Specimens were allowed to liquefy at 37°C in a microbiological incubator for 20 min, or longer if needed. Volume of each sample was noted and the following tests were conducted on each sample.

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Morphology. Semen smears were stained in a modified Wright's stain for 2 min. Sperm morphology was evaluated using Kruger's strict morphology [3]. Head defects were noted as previously described by Kruger et al. [3]. Tail morphology was evaluated as follows to be normal: uncoiled, approximately 45 mm (too long may be abnormal), absence of bend or breakage.

Viability Test. A mixture of 7 μL of liquefied semen and 7 μL of eosin stain (0.5 g eosin resuspended to 100 mL with D-PBS) was coverslipped and allowed to incubate for 1 min. Two hundred sperm cells were evaluated for stain absorption. Stained sperm were recorded as nonviable.

Hypoosmotic Swelling (HOS) Test. Liquefied semen (100 μL) was placed in 1 mL of hypoosmotic solution (0.7350 g sodium citrate and 1.3510 g fructose resuspended to 100 mL with sterile H_2O) in a 10 \times 75-mm test tube and mixed well. The tube was incubated in a 37°C dry heat block for 30 min. A drop of this suspension was placed on a slide and coverslipped. Two hundred sperm were evaluated and hypoosmotic changes were noted as a swelling of the sperm tail.

Computer-Assisted Semen Analysis. Concentration and motility patterns of spermatozoa were evaluated using a computer-assisted semen analyzer. Seven microliters of semen is placed on a Makler counting chamber. A heated stage (37°C) on a phase-contrast microscope is used to keep temperature conditions constant. At least 200 sperm were analyzed to give an accurate count ($\times 10^6/\text{mL}$) and percent motility. Several motility patterns of sperm were evaluated: curvilinear velocity, linearity, ALH, and beat cross-frequency (Table 1).

Anti-Sperm Antibodies. Five to ten million motile sperm were washed with a 0.5% BSA/BWW solution 3 times. Fifty microliters of a 1:10 dilution of bovine fetal serum to 0.5% BSA/BWW was used to resuspend the sperm pellet. Seven microliters of the above was mixed in 50 μL of GAM immunobeads (BIO-RAD) which is suspended in 1% BSA/BWW. The slide was coverslipped and allowed to incubate at room temperature for 5-10 min. Two hundred sperm were evaluated for bead attachments.

TABLE 1 Evaluation of Sperm Motility Patterns Using Computer-Assisted Semen Analysis

Motility Parameter	Definition/Evaluation
Curvilinear velocity ($\mu\text{m/s}$)	Evaluated by adding the distances between every two successive frames on a cell's track and dividing that number by the time interval during which the sperm was tracked.
Linearity	Evaluated as the difference of the actual sperm tracks from the vector that connects the beginning and the endpoint of the sperm track. The result is then multiplied by 10, giving a range of 0 to 10, with 10 being a straight line.
ALH	The lateral distance that the sperm head moves. An abnormal ALH is seen in sperm that do not rotate often but appear to glide through the semen. Maximum ALH is the greatest amount by which the sperm head deviates from a computer-calculated mean. The mean of the ALH maximum amplitudes is called the mean ALH.
Beat cross-frequency	The mean is calculated by the computer. The number of times the sperm crosses this mean in one second is counted.

TABLE 2 Semen Parameters and Motility Pattern as Evaluated by CASA, HOS Scores, and Strict Morphology in Two Men with Globozoospermia

Parameter	Normal Values	Patient 1	Patient 2
Age (years)		35	32
Abstinence (days)		2	4
Volume (mL)	> 2.0	2.0	5.6
Count ($\times 10^6$ /mL)	> 20	48.0	47.0
Motility (%)	> 50	29.0	29.0
Curvilinear velocity (μ m/s)	> 40	47.2	34.9
Linearity	> 5.5	4.4	5.8
ALH MAX (μ m)	> 2.3	3.5	2.7
ALH MEAN (μ m)	> 1.8	3.0	2.2
Beat cross-frequency (Hz)	> 13	11.4	16.0
Viability (%)	> 50	79	76
HOS (%)	> 50	72	69
Motile density ($\times 10^6$ /mL)	> 10	13.7	10.1
GAM ^a	< 20%	4.0%	2.0%
Strict morphology (%)	> 14%	0% normal	0% normal
Tail morphology		0% slightly abnormal	0% slightly abnormal
		87% normal	95% normal
Comments		Moderate bacteria	Some immatures
		Many WBCs	Some debris
		Moderate debris	

^aGAM, 2-immuno bead assay using micrometer-sized hydrophilic particles with covalently bound highly purified rabbit anti-human immunoglobulin (heavy and light chains) antibodies.

RESULTS

Patient 1 had a history of primary infertility for 11 years. He had had two previous semen analyses in hospital laboratories and was told that his semen analyses were normal. No known factors for their infertility could be found. Examination of his semen sample showed 100% round-headed sperm. The details of his semen parameters, including computer-assisted semen analysis, HOS scores, and strict morphologic criteria of the sperm tails, are summarized in Table 2. The HOS score and sperm viability were normal and only 13% of the sperm tails had morphological abnormalities.

Patient 2 had 3 years of primary infertility. His female partner had a luteal phase deficiency corrected by supplemental progesterone (P), but no pregnancy was achieved. A previous semen analysis had been interpreted as normal in a hospital laboratory. Semen analysis performed in our lab had shown that 100% of the sperm had round heads (Table 2). Similar to patient 1, the HOS score was normal and strict tail morphology showed 95% normal.

The curvilinear velocity from patient 1 was normal for our laboratory, but the linearity and straight-line velocity were low. In patient 2 the curvilinear velocity decreased but the linearity was normal. Also, the beat cross-frequency in patient 1 but not patient number 2 was subnormal. Leukocytospermia was noted in patient 1, but not in patient 2.

DISCUSSION

Round-headed sperm as judged by electron microscopy are globular with irregular surface membranes and have no acrosomal structure, compared to normal sperm, which are ovoid and have a smooth surface with a clear acrosomal region. Since our examinations were performed using phase-contrast microscopes we are basing our terminology of round-headed sperm solely on the lack of an acrosomal region. However, this defective membrane is not associated with any apparent functional impairment as evaluated by HOS scores in both patients. Although no standard morphologic abnormalities of the tail had been previously noted it would appear that more subtle abnormalities may be present. Both men had morphologically normal tails by strict criteria.

Both men had some sperm abnormalities based on the parameters measured by CASA. Since these sperm abnormalities were of different natures, it would appear that they are random and are not associated with this syndrome. Furthermore, in our previous studies we could not correlate the clinical significance to any specific defect [1].

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