MORPHOMETRIC AND VOLUMETRIC COMPARISONS OF HUMAN SPERMATOZOA

T. W. TURNER, S. M. SCHRADER, M. PEREZ-PELAEZ, R. F. KARUHN, H. H. VAN DER VEN, and R. S. JEYENDRAN

Morphometric measures and volumes of spermatozoa were determined for 28 human ejaculates which were previously analyzed for semen volume, sperm concentration, morphology, motility, and fertility by in vitro fertilization procedures (IVF). Morphometric measurements of sperm heads were analyzed using a Zeiss Videoplan computer, while spermatozoan volume was determined with an Elzone particle analyzer. Though a strong relationship was anticipated, correlations between the volumetric data and different morphometric measures revealed poor, insignificant values. This lack of correspondence may be due to individual differences in the thickness of the spermatozoa within a sample. Twenty-two of the ejaculates used in this study were classified as fertile and six were infertile according to the IVF procedure results. Correlations between the morphometric measurements and the volume determinations in the fertile group were all positive. In contrast, those of the infertile group were all negative with one exception (width vs. volume).

Key Words: Morphometry; Morphology; Sperm; Spermatozoan volume; Fertility; In vitro fertilization.

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INTRODUCTION

Morphometric sperm head analysis and volumetric determinations of spermatozoa are relatively new techniques that can be used quickly and accurately to classify sperm in an objective manner. While neither of these assays has been shown to be a sole predictor of fertility [3, 4], changes in sperm head measurements have been associated with exposure of workers to toxic chemicals [5]. This study was undertaken to see if any relationship exists between the spermatozoan volume and sperm head morphometry. Because the morphometric and volumetric measurements of spermatozoa are based on mathematical equations that are related to each other, a good to high correlation between these determinations is expected.

MATERIALS AND METHODS

Ejaculates from 28 men attending an in vitro fertilization (IVF) program were obtained by masturbation after at least 3 days of sexual abstinence. After liquifaction, these samples were analyzed for semen volume, sperm concentration, morphology, and motility according to WHO guidelines [1]. Air-dried

From the National Institute for Occupational Safety and Health, Division of Biomedical and Behavioral Sciences, Cincinnati, Ohio 45266 (T.W.T., S.M.S.); Institute of Reproductive Medicine, Chicago, Illnois (M.P-P., R.S.J.); Particle Data Labs, Elmhurst, Illinois (R.F.K.); and University of Bonn, Bonn, F.R. Germany (H.H.V.).

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semen smears on microscope slides were obtained by the Papanicolaou procedure and evaluated for sperm morphology and morphometry [3]. Aliquots from each sample were then taken for the IVF procedure and the remainder of the semen was frozen for spermatozoan volumetric determination.

In vitro Fertilization. IVF procedures were performed as published previously [2]. Fertilization was considered to have occurred when at least one preovulatory oocyte showed two or more pronuclei and/or normal embryonic development; that is as long as one oocyte was fertilized, the sperm sample was considered successful in fertilization.

Morphometry. Morphometric measurements were determined by tracing the sperm head outline of 200 different cells, selected randomly, for each of 28 different ejaculates, using a semiautomatic image analysis system [3]. The measurements included the area, perimeter, length, and width of the sperm head.

Volumetry. Spermatozoan volumes were determined by using an electronic particle analyzer (Elzone computerized Model 112, Particle Data, Inc., Elmhurst, Illinois) as described by Jeyendran et al. [4]. Briefly, the analyzer was calibrated with 10.18- and 3.01-μm diameter latex beads, and the lower- and upper-level discriminators were set to eliminate cellular debris present in the ejaculates. An electronic volume depends on shape in addition to size, and the volumes obtained for the spermatozoa were corrected for their ellipsoidal shape. The frozen thawed (killed) sperm were diluted with physiological saline. At least 10,000 sperm per ejaculate were analyzed and the population mode, median, and mean sperm volume were calculated.

Statistics. Pearson's correlation coefficients were used to determine the degree of relationship between sperm head morphometry and spermatozoan volumetric determinations for the total sample of ejaculates and for those classified as fertile and nonfertile as described below. Values of $p < 0.05$ were considered statistically significant.

RESULTS

The semen analysis results (mean ± SD) for sperm concentration, sperm motility, and sperm morphology (percentage normal) from the ejaculates used in this study (reported in [3]) are presented in Table 1 and classified in terms of fertilization status. That is, of the 28 ejaculates, spermatozoa from 22 samples fertilized at least one oocyte and were considered successful by the IVF procedure. Spermatozoa from the six remaining ejaculates produced no evidence of fertilization, and thus were considered unsuccessful.

TABLE 1 Summary Statistics for Semen Analysis Data[a]

Parameters	*N*	Concentration[b]	Motility[c]	Morphology[d]
Unsuccessful fertilization	6	64.7 ± 38.0	36.0 ± 23.0	42.7 ± 11.4
Successful fertilization	22	121.4 ± 93.2	49.3 ± 20.6	44.0 ± 12.9
Total	28	108.8 ± 86.8	46.8 ± 21.3	43.7 ± 12.4

[a]Data are expressed as means ± standard deviation.
[b]Sperm count (million/ml).
[c]Percentage of motile sperm.
[d]Percentage of normally shaped sperm.

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TABLE 2 Summary Statistics for Morphometry and Volumetry Data

	Successful Fertilization Group (N = 22)			Unsuccessful Fertilization Group (N = 6)		
Parameters[a]	Mean	SD	Range	Mean	SD	Range
Perimeter	11.464	0.540	10.156–12.46	11.528	0.334	11.04–11.96
Area	9.335	0.829	7.57–10.84	9.567	0.555	8.98–10.27
Length	4.366	0.249	3.74–4.83	4.342	0.180	4.07–4.54
Width	2.737	0.142	2.48–3.01	2.82	0.131	2.56–2.92
Volume	18.581	2.70	13.71–23.83	19.823	2.77	17.17–24.72

[a]Perimeter: distance measured around the sperm head (μm); area: measurement of the sperm head (μm^2); length: measurement of the major axis of the sperm head (μm); width: measurement of the minor axis of the sperm head (μm); volume: arithmetic mean of the spermatozoan volume (μm^3).

The mean sperm concentration, motility, and morphology were lowe in this unsuccessful fertilization group of samples as compared to the successful fertilization group, but no statistically significant differences were found. The mean values and Pearson's correlation coefficients between the various morphometric parameters and spermatozoan volumes are detailed in Tables 2 and 3 for both the total sample and by fertilization status. Figures 1–4 show the distribution of the successful vs. the unsuccessful groups, comparing area, perimeter, length, and width vs. arithmetic mean of the volume. These results reveal no significant correlations between the volumetric measures and the different morphometric measure in any instance.

DISCUSSION

It was reported that neither morphometric values nor the spermatozoan volumes are useful sole predictors of fertility based on IVF procedures [3, 4]. Morphometric and volumetric parameters may be related through additional, though currently unmeasurable, shape and thickness parameters. Two such equations could be:

$$\text{Volume} = K \times \text{area} \times \text{thickness}$$

TABLE 3 Correlation Coefficients of Morphometric Measurements vs. Spermatozoan Volumes

Parameters	N	Perimeter[a]	Area	Length	Width
Unsuccessful	6	r −0.394	−0.219	−0.492	0.148
		p 0.440	0.677	0.321	0.779
Successful	22	r 0.305	0.252	0.373	0.026
		p 0.167	0.258	0.087	0.908
Total	28	r 0.217	0.203	0.228	0.095
		p 0.268	0.301	0.242	0.631

[a]r: correlation coefficient; p: probability value; definitions for dimensions are given in footnote to Table 2.

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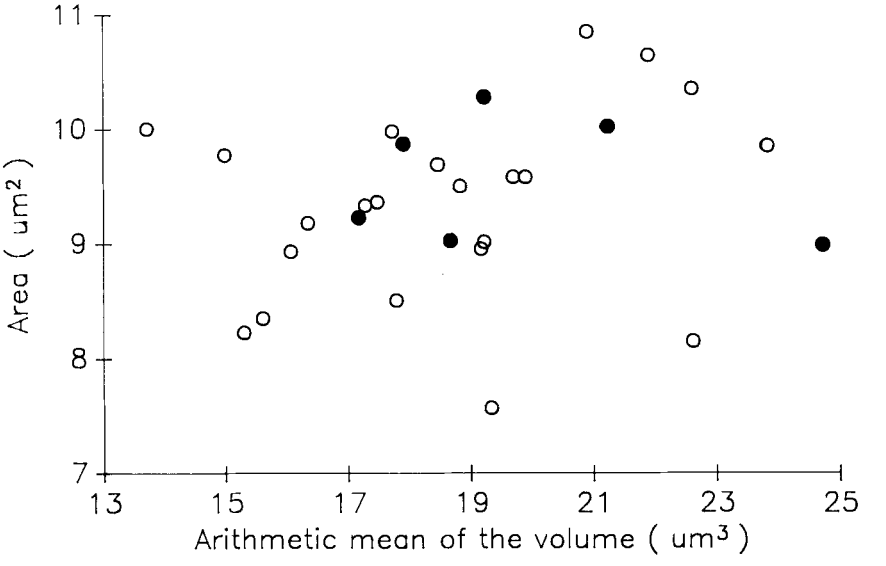

FIGURE 1 Distribution of sperm head area vs. spermatozoan volume for successful (○) and unsuccessful (●) samples. For successful samples, $r = 0.252$, $p = 0.258$; for unsuccessful samples, $r = -0.219$, $p = 0.677$.

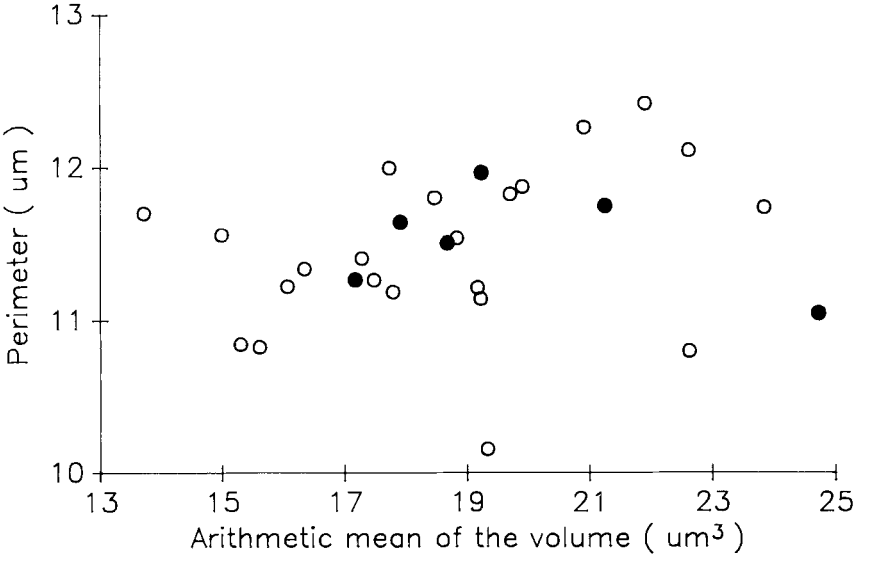

FIGURE 2 Distribution of sperm head perimeter vs. spermatozoan volume for successful (○) and unsuccessful (●) samples. For successful samples, $r = 0.305$, $p = 0.167$; for unsuccessful samples, $r = -0.394$, $p = 0.440$.

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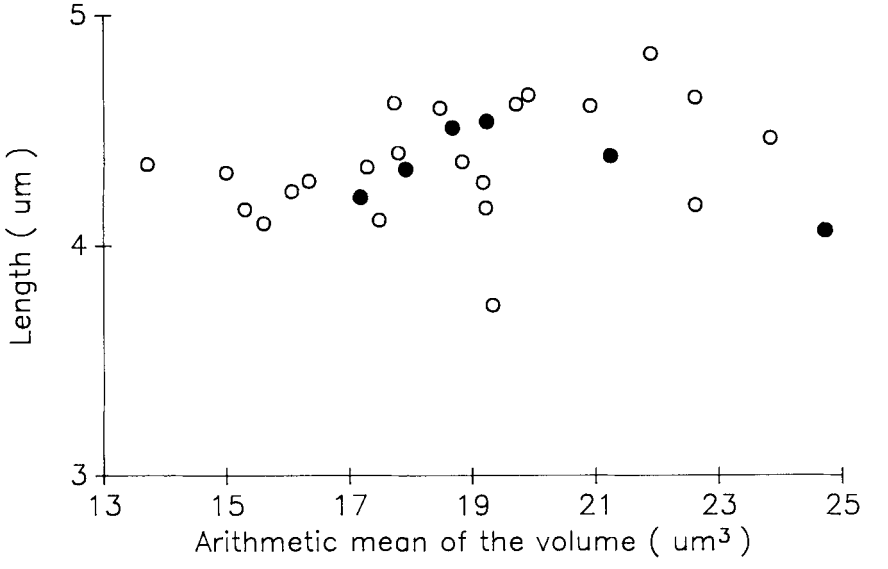

FIGURE 3 Distribution of sperm head length vs. spermatozoan volume for successful (○) and unsuccessful (●) samples. For successful samples, $r = 0.373$, $p = 0.087$; for unsuccessful samples, $r = -0.492$, $p = 0.321$.

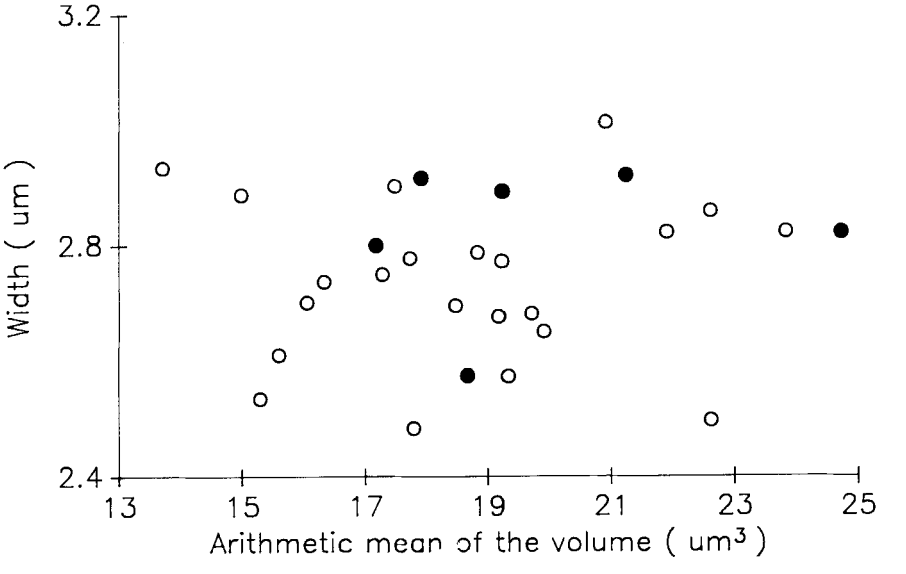

FIGURE 4 Distribution of sperm head width vs. spermatozoan volume for successful (○) and unsuccessful (●) samples. For successful samples, $r = 0.026$, $p = 0.908$; for unsuccessful samples, $r = 0.148$, $p = 0.779$.

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or

$$\text{Volume} = K \times \text{width} \times \text{length} \times \text{thickness}$$

Either of these equations might also be adjusted by a shape parameter that would tell how the sperm head shape deviates from an ellipsoidal shape. Therefore, a good to high correlation between the morphometric and volumetric values of the spermatozoa was anticipated. However, only a very poor relationship was observed (Table 3). This lack of relationship may be due to inherent differences between the electronic volume (volume obtained by the electronic analyzer) and the actual volume of spermatozoa, or to inaccuracy in the morphometric determinations.

However, shape is similar for most human spermatozoa; therefore, the lack of relationship between the morphometric and volumetric values cannot be totally explained because of sperm shape. Another possible explanation might be that volume is a three-dimensional measurement while morphometry represents only a two-dimensional parameter. Thus, the variation in the thickness of each individual sperm could be an explanation for the low correlations between morphometric and volumetric values found in this study. Although the number of ejaculates is too small to make any conclusive statements, the correlations between the morphometric measurements and the volume determinations in the successful fertilization group were all positive. In contrast, those of the unsuccessful fertilization group were all negative with the exception of width vs. volume.

Further investigations should be performed on a large number of ejaculates from successful and unsuccessful ejaculates to determine if the trends observed in the current study are reproducible and become significant in a larger population study. If these trends are found in a larger population, this information may provide important insights into differences between fertile and infertile sperm.

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