

## THE CORRELATION BETWEEN SPERM MORPHOLOGY AND MOTILITY IN FERTILE AND INFERTILE MEN

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### ABSTRACT

The swim-up procedure was used to collect a sperm fraction with improved motility and normal morphology from fertile and infertile semen samples. The untreated and swim-up fractions were analyzed with the Hamilton-Thorn Motility Analyser. Comparisons were made to see the effect of this sperm selection technique on sperm motility and morphology. They were then examined by transmission electron microscopy and the various sperm morphologies were recorded. Fertile and infertile samples were compared for differences in these parameters.

There were no significant differences between fertile and infertile samples in the frequencies of various sperm morphologies in the untreated and swim-up fractions. There was a trend towards fertile samples having a higher motility of the untreated ejaculate, as well as more normal heads and tails and fewer morphological abnormalities than infertile samples.

Sperm motility was found to be affected by sperm morphology, with an increase in normal sperm morphology resulting in an increase in motility. Normal sperm tail morphology appears to be the most important factor in motility, since this was the only morphology to show a significant increase in the swim-up fractions in all semen samples. Head morphology is less important to motility, but it is thought to play a major role in fertilization of the egg.

**Keywords:** Morphology, motility, sperm.

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### INTRODUCTION

The World Health Organization (1978) analyzed data from more than 7000 infertile couples and proposed the following criteria for classifying a normal semen sample. The sperm concentration must be greater than or equal to 20 million per mL, there should be no agglutination, at least 30% of the sperm must be morphologically normal and 75%

of the sperm must be alive. The seminal fluid must be normal in appearance and viscosity, and at least 50% of the sperm must be fully motile (WHO 1992). Most authors accept that a correlation exists between fertilizing ability and seminal parameters.<sup>9,11,13,26</sup> There is good evidence that morphologically normal spermatozoa offer the best fertilizing potential,<sup>4,20</sup> and morphologically abnormal spermatozoa are also functionally abnormal.<sup>4,7,16,17</sup>

# Correlation Between Sperm Morphology and Motility and Fertility

Table I. HTM data.

Donor	A*		B		C		D*		E		F*	
	UT	SUP	UT	SUP	UT	SUP	UT	SUP	UT	SUP	UT	SUP
total cell no.	842	164	347	49	2022	281	1078	52	1009	196	485	18
concentration (10 <sup>6</sup> /mL)	175	34	60	10	300‡	58	224‡	11	279‡	54	134	5
motile cell no.	471	109	36	13	600	185	642	45	347	179	202	15
concentration (10 <sup>6</sup> /mL)	98	23	6.2	2.7	88	38	130	9.3	96	50	56	4.2
% cells recovered		19.5		14.1		13.9		4.8		19.4		3.7
% motility	56	66	10	27	30	66	58	87	34	91	42	83
% difference in motility between UT and SUP		10		17		36		29		57		41
VAP (μm/s)	37	39	28	36	31	42	30	42	32	51	38	39
VSL (μm/s)	33	32	27	36	28	39	25	41	23	47	28	35
mean STR (%)	83	75	88	94	85	91	82	90	73	92	74	93
mean ALH (μm)	3	4.9	1.8	1.4	2.6	3.0	2.1	1.3	3.1	3.7	3.2	4.1

\* = fertile

‡ = excess density warning

UT= untreated

SUP= swim-up

VAP= mean path velocity

VSL= mean progressive velocity

STR= straightness

% cells recovered = SUP total cell no./UT total cell no. ×100

ALH= amplitude of lateral head displacement

The work of many *in vitro* fertilization (IVF) laboratories is therefore aimed at making use of procedures which isolate higher percentages of motile spermatozoa with normal morphology from fresh ejaculate. Of the many methods for semen preparation used in IVF, the swim-up procedure is the most commonly used for samples showing either normal or subnormal seminal parameters.<sup>2,12,23</sup>

There is much controversy over the importance of using motility or morphology in defining a sample as fertile or infertile.<sup>19</sup> The swim-up technique appeared to be successful in selecting a fraction of sperm with improved motility and normal morphology.

## MATERIALS AND METHODS

The semen samples (A, B, C, D) were obtained from donors attending the infertility clinic, and semen samples (E, F) were obtained from donors not attending the clinic. The semen was collected by masturbation after a period of at least forty-eight hours of abstinence. Each sample was divided into two: half was left untreated and half was used

Table II. Head morphology.

Donor	A*		B		C		D*	
	UT	SUP	UT	SUP	UT	SUP	UT	SUP
Normal head	25.6	44.9	7.2	24.6	11.5	14.6	3.2	3.9
Double head	0.9	0.0	0.0	0.5	1.0	0.0	0.0	0.5
Abnormal/multiple vacuoles	15.2	13.4	2.9	11.8	15.0	19.0	26.9	20.0
Nuclear inclusion	1.4	3.7	6.2	4.4	9.5	4.4	3.7	8.8
Macrocephalic	2.8	2.3	1.0	0.5	0.1	0.0	0.5	0.0
Microcephalic	0.0	4.2	0.5	0.0	0.1	0.0	0.0	0.0
Granulated nucleus	16.6	4.2	17.7	16.3	15.0	6.8	7.8	17.6
Abnormal acrosome	33.6	19.9	42.6	32.0	32.5	41.5	54.3	46.3
Cytoplasmic droplet	3.7	7.2	22.0	9.8	15.5	13.8	3.8	2.9

(%) Frequencies of the various sperm morphologies before and after swim-up in the four semen samples, when examined by transmission electron microscopy.

\*=fertile

UT= untreated

SUP=swim-up

Table III. Tail morphology.

Donor	A*		B		C		D*	
	UT	SUP	UT	SUP	UT	SUP	UT	SUP
Normal head	45.7	76.7	39.0	53.0	49.5	78.0	66.0	68.1
2 tails	0.9	4.8	3.8	2.3	1.4	0.0	2.0	0.5
3 tails	0.5	0.5	0.0	0.0	0.0	0.0	0.0	0.0
4 tails	0.5	1.0	0.0	0.5	0.0	0.0	0.5	0.0
Extra microtubule	14.6	3.8	8.5	5.1	7.1	8.5	1.0	2.3
Missing random microtubule	8.2	2.4	12.7	9.2	4.8	1.5	3.0	4.6
1/2 axoneme	10.0	3.8	12.2	9.2	12.9	2.5	9.5	5.6
Missing central microtubule	0.5	0.1	2.3	2.2	0.0	0.3	0.5	2.3
Deranged axoneme	11.3	5.7	11.7	11.1	12.4	4.5	10.5	7.0
Irregular plasma membrane	0.1	0.0	1.4	1.3	4.3	1.6	0.5	0.8
Irregular mitochondria	5.0	0.7	5.6	4.2	3.8	2.1	3.0	2.7

(%) Frequencies of the various sperm morphologies before and after swim-up in the four semen samples, when examined by transmission electron microscopy.

\*=fertile

UT= untreated

SUP=swim-up

in the swim-up procedure. Both untreated and swim-up fractions were first analyzed using the Hamilton-Thorn Motility Analyser (HTM) and then prepared for transmission electron microscopy (TEM). The analyzer measured the various seminal parameters, and when the sperm concentration was greater than 150 million sperm per mL or when the machine showed an excess density warning, 1:1 (v/v) dilution of the sample with human tubular fluid (HTF) was necessary. For swim-up, 1 mL of liquefied semen from the fresh ejaculate was placed in a test tube and 1 mL of HTF was layered on top and it was left in an incubator for 1 hour. A drop of this swim-up fraction was analyzed in the HTM and then prepared for TEM in the same way as the untreated ejaculate. MAR test was carried out on the samples to detect the presence of antisperm antibodies. Each sample was centrifuged and resuspended in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) for 2.5 hours. After fixation the samples were washed in cacodylate buffer (pH 7.2) twice for 5 minutes and postfixed in 1% osmium tetroxide for 1 hour. Dehydration using increasing concentrations of methanol was followed by infiltration in Spurr resin. The blocks were thin sectioned using a Reichert OMU3 ultramicrotome. Each grid was

stained by 2% uranyl acetate and 3% lead citrate. The sections were examined by TEM (Philips 400). 200 head and 200 tail morphologies were scored and examined separately from the swim-up and untreated preparations from donors. The microscopic field of view was moved up and down in an ordered way to cover the grid consistently and avoid counting any sperm heads or tails twice.

## RESULTS

### HTM Data

There was a dramatic decrease in the total cell number between the untreated and swim-up fractions in all samples analyzed, although no apparent difference was seen between fertile and infertile samples (Table I). In all samples there was an increase in percentage of motility following swim-up, but the amount varied. Normospermic Donor A showed the smallest increase (10%). Donor B who was asthenozoospermic showed an increase in motility of 17% after swim-up, but he was still below WHO criteria for normal motility even after sperm selection (27%). Donor C was also asthenozoospermic, but showed a larger increase of 36% motility following swim-up. Normospermic Donor

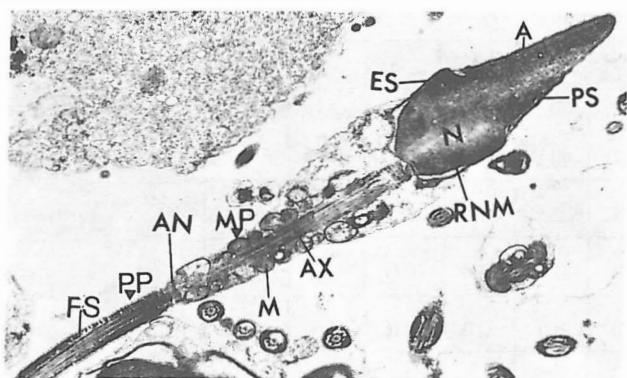


Fig. 1. Longitudinal section through sperm ( $\times 10,500$ ). The acrosome (A) surrounds the nucleus (N). The principle segment (PS) is thicker than the equatorial segment (ES). The redundant nuclear membrane (RNM) can be seen in the neck region. The annulus (AN) marks the boundary between the mid piece (MP) and the principle piece (PP). The axoneme (AX) is visible, as well as the ribs of the fibrous sheath (FS). Mitochondria (M) form a sheath around the mid piece of the flagella.

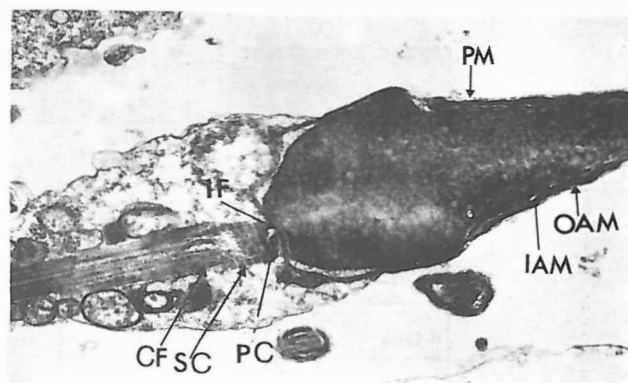


Fig. 2. Longitudinal section through head and neck region of sperm ( $\times 22,500$ ). The plasma membrane (PM) surrounds the entire sperm cell. The acrosome is bounded by the inner acrosomal membrane (IAM) and the outer acrosomal membrane (OAM). The nuclear membrane lines the implantation fossa (IF) and the proximal centriole (PC) is embedded in the capitulum. The segmented columns (SC) which make up the capitulum attach to the anterior end of the nine outer coarse fibers (CF).

D had the highest motility prior to swim-up (58%) but his motility increase was less than in Donor C, E and F. Asthenozoospermic Donor E showed the largest increase in percentage motility following swim-up (57%), followed by normospermic Donor F who showed the second largest increase in motility of 41%. Overall there was no significant difference in motility increase after swim-up between fertile and infertile donors. Donors A, C, E and F showed an increase in mean amplitude of lateral head displacement (ALH) after swim-up, the largest increase of 1.9% being for normospermic Donor A. Donors B and D showed a decrease in mean ALH after swim-up. All except A showed an increase in mean straightness (STR) after swim-up, and the highest increase of 19% was recorded for Donors E and F. There was an increase in mean progressive velocity (VSL) in all donors except A following sperm selection, together with a universal increase in mean path velocity (VAP). The largest increase in both of these was in Donor E. There were no significant differences between the motion characteristics after swim-up between clinically fertile and infertile donors.

### Electron micrographs

The acrosome (A) surrounds the anterior end of the nucleus (N). The principle segment (PS) of the acrosome is thicker than the equatorial segment (ES). The redundant nuclear membrane (RNM) can be seen in the neck region. The annulus (AN) marks the boundary between the mid piece (MP) and the principle piece (PP), the axoneme (AX) is visible, as well as the ribs of the fibrous sheath (FS). The mitochondria (M) form a sheath around the mid piece of the

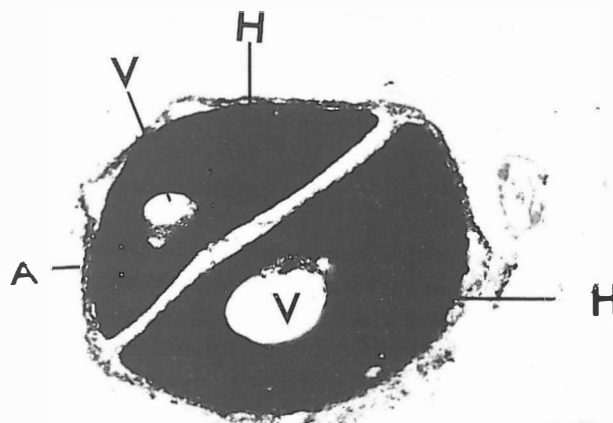


Fig. 3. Double headed sperm ( $\times 15,000$ ). The enlarged acrosome (A) and large vacuoles (V) deform the heads (H). Vacuoles can contain cytoplasmic organelles, lipid droplets or arrays of membranes.

flagella (Fig. 1). The acrosome is bounded by the inner acrosomal membrane (IAM) and the outer acrosomal membrane (OAM). Between the nuclear membrane and the inner acrosomal membrane is the subacrosomal space. The plasma membrane (PM) surrounds the entire sperm. In the neck region, the nuclear membrane lines the implantation fossa (IF) and the proximal centriole (PC) is embedded in the capitulum. The segmented columns (SC) which make up the capitulum attach to the anterior end of the nine outer coarse fibers (CF) (Fig. 2). In double heads, the acrosome (A) is partially enlarged and both heads (H) share the plasma membrane. Normal sperm can contain small

## DISCUSSION

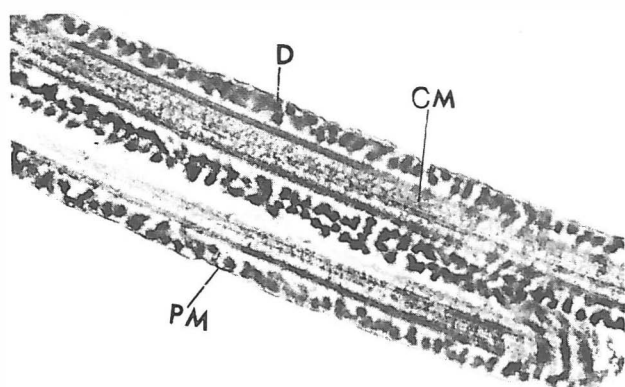


Fig. 4. Longitudinal section through double-tailed sperm ( $\times 45,000$ ). The double tail with ribs of fibrous sheath, microtubule doublets (D) and central microtubules (CM) are bounded by the same plasma membrane (PM).

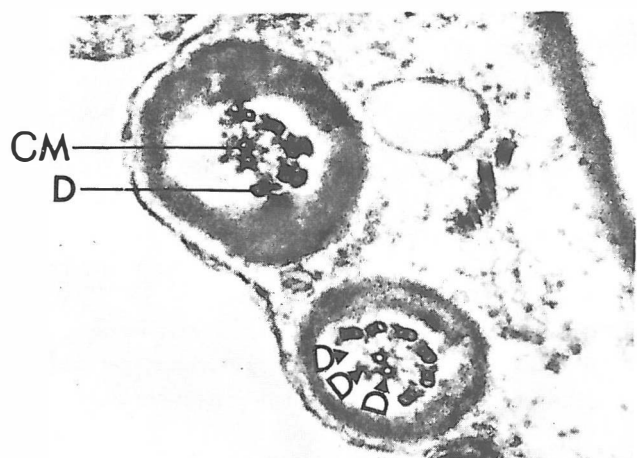


Fig. 5. Transverse section through principle piece of sperm tail with half an axoneme and sperm tail with missing microtubule doublets ( $\times 67,500$ ). Deletion involves microtubule doublets (D), but the central microtubule (CM) is not included.

vacuoles, but in some cases these vacuoles (V) may be so large that they deform the head or reduce the amount of nuclear chromatin (Fig. 3). Sperm were scored with 2, 3 and 4 tails, and axonemal abnormalities were the most common tail abnormality. The double tail with ribs of fibrous sheath, microtubule doublets (D) and central microtubules (CM) are bounded by the same plasma membrane (PM) (Fig. 4). Deletions involved the absence of one or more microtubule doublets from random position in the axoneme. Deletion of the central microtubules involved either the absence of both or one. In some flagella, exactly half of the axoneme is missing, although the central pair of microtubules (CM) is not included in this description (Fig. 5). Comparative TEM data about head and tail morphology are shown in Tables II and III. Although HTM data for donors E and F are included, there is no comparative TEM data for them since too few cells were recovered in swim-up fractions for TEM study.

Donor A has shown the smallest percentage increase in motility after swim-up because his sperm were initially of a good motility and exceeded the WHO criteria for a fertile sample. Donor C's motility increase of 36% transformed this semen sample into a fertile one, with an improved motility of 66%. The motility increase from 58 to 87% in Donor D was surprising because he had a high level of antisperm antibodies detected in the fresh ejaculate. Antisperm antibodies are thought to cause a decrease in sperm numbers and motility.<sup>3,22,25</sup> However Donor D's high percentage of motility after swim-up could be because antisperm antibodies were free in the semen.<sup>14,21</sup> Donor D was known to be only IgG positive which may explain why his sperm remained highly motile. Donor A showed the largest increase in normal heads after swim-up which was expected since this semen sample had the most normal parameters. However, the very small increase in normal head morphology seen in Donor C, together with the large increase in motility after swim-up, throws some doubt on the importance of normal head morphology to motility.<sup>8</sup> The high frequency of abnormal acrosomes in both the untreated and swim-up fractions of Donor D was perhaps related to the presence of antisperm antibodies. Also, since Donor C was the only sample to show an increase in abnormal acrosomes after swim-up despite a high increase in percentage of motility, these results for C and D suggest either that abnormal acrosomes do not affect motility or that they are artefacts of fixing.<sup>11</sup>

Surprisingly, Donor D had the highest percentage of nuclear inclusions and granulated nuclei after swim-up as well as a high percentage of motility. Therefore it appears that these head abnormalities do not affect motility. The fact that the majority of samples showed a decrease in deletions of microtubules reflects the deleterious effect of this abnormality on sperm motility. Derangement of the normal axonemal topography is thought to have an adverse effect on sperm motility as the tail cannot perform the sliding mechanism needed for propagation of flagellar waves.<sup>15,27</sup>

According to WHO criteria, Donor A and Donor D can be classified as fertile, and Donor B and Donor C are both asthenozoospermic. Normal head morphology does not correlate with motility because Donor B had a higher frequency of normal heads after swim-up than C and D, yet his motility after swim-up was less than half that of C's and a third of that of D's. The swim-up technique appeared to be successful in selecting a fraction of sperm with improved motility and normal morphology, although swim-up does not remove all the abnormal morphologies in an ejaculate, neither does *in vivo* selection in the female genital tract.<sup>1</sup> Thus swim-up is a good emulation of this sperm selection process. The main differences between a

## Correlation Between Sperm Morphology and Motility and Fertility

clinically fertile and infertile sample using WHO criteria are that a fertile sample has both a higher initial motility of untreated ejaculate and a lower percentage of ultrastructurally abnormal morphology, especially axonemal abnormalities, than an infertile sample. As expected, motility was seen to be affected by sperm morphology with an increase in normal morphology resulting in an increase in motility.<sup>6</sup> All samples show an increase in motility following swim-up which correlates with an increase in normal tail morphology and a decrease in the number of tail abnormalities. However tail morphology appears to be most important for sperm motility and the only significant difference was an increase in the number of normal tails after swim-up, while head morphology does not correlate with motility but plays a major role in fertilization of the egg.<sup>18</sup> The increase in normal tail, decrease in missing microtubules and decrease in deranged axonemes in all samples after swim-up is most likely to be responsible for increased motility after swim-up. This indicates a positive correlation between motility and normal tail morphology.

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