



## Development of a novel Computer Assisted system for human Sperm Head Morphometry based on open source software

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### ABSTRACT

Computer assisted sperm head morphology systems have been commercially available since the mid-1980s. The goal of these systems has been to obtain objective data on sperm head that can be used in research, human fertility clinics, and animal breeding programs. Widely used commercial systems include the Hamilton-Thorne, Bioscience, Beverly, Massachusetts, The commercial systems differ in their grayscale bit-depth, search region for finding the sperm in the image, grayscale thresholding method, image segmentation method to determine the pixel coordinates of the sperm head, A large amount of information necessary to reproduce data obtained with commercial systems is proprietary information not easily available, eventually limits the ability to conduct comparative evaluation and also its elevated cost has slowed down widespread adoption. In the present study, we have developed a plugin for sperm head morphology using open source software. Described the systems functionality and confirmed its validity with respect to the commercial softwares.

**Keywords-** Microscopic image, Spermatozoon, IUI, IVF, ICSI, WHO, IMAGEJ, Medea LAB CASA

### INTRODUCTION

From the first volume of the WHO manual for standardized analysis of human semen, morphology estimation had been given prompt importance and was noted sperm morphology had direct influence on to the pregnancy, though not explained with intricate details. Later strict criterion [3], their experiments to arrive at the strict criterion, their validation across the globe by various groups, gave us the information that morphology is the single most affecting parameter of the sperm that can influence highly the outcome of the live birth.

Though the data of strict criterion mostly gives us the influence sperm morphology has on the implantation rates and live birth rates but fails to

explain conclusively in detail the mechanism by way of which the influence of sperm morphology on the outcome. This is the exact point where many researchers especially people from molecular biology background jumped in and started postulating that Sperm Chromatin may hold the key for unexplained pregnancy loss. The point was well taken and many groups started performing their researches in this direction.

There are two methods for examining human sperm morphology, both based on the microscopic analysis of stained smears, either through visual observations (manual methods) or by using computer vision-derived methods. For either of these methods, a smear must be optimally stained to provide sharp

contrast for defining the sperm outline and cell details. Recognition of these features also depends on the final magnification generally x1000 with the manual method combining a x100 oil objective with a x10 ocular lens. Pre-analytical procedures have been described, and guidelines can be found in the fourth edition of WHO laboratory manual <sup>[40]</sup>.

Using a standard microscopic approach, the observer categorizes each sperm cell as normal or abnormal, and eventually classifies each anomaly encountered using strictly defined criteria. By contrast, computer-assisted technology measures different morphological features (mostly head parameters) for each selected sperm cell. The level of variability in the assessment of sperm morphology for either normal spermatozoa or for sperm defects is relatively extensive. However, it should be pointed out that the inter-observer variability found for several anomaly categories could be lower than for normal spermatozoa <sup>[4]</sup>.

Variability may be considerably reduced by standardizing the analytical methods, and by providing sufficient basic knowledge and training <sup>[4]</sup> with periodic internal and external quality control testing <sup>[42,43]</sup>. However, some of the variability is linked to the continuous nature of sperm shape and size, and thus cannot be eliminated, making the classification of subnormal shape and size difficult. The use of an eyepiece with a graduated reticle may be very useful to correctly assess sperm size defects (for example, distinguishing a marginally 'thin' head vs. a 'normal' head).

One major factor that renders visual assessment of sperm morphology difficult is the fact that this analysis depends on mechanisms of human vision and their integration in the brain. Visual observation is subject to several limitations. The eye-brain combination is a powerful tool in pattern recognition (far better than 'machine vision'), but is poorly adapted to measurement. This is reflected by the higher inter-observer variability in determining the total percentage of spermatozoa with size defects compared with spermatozoa with a qualitative anomaly <sup>[4]</sup>. In general, visual classification under the microscope of

morphologically normal and abnormal spermatozoa, categorizing all visible defects according to their definitions, requires the assessment of cellular and sub cellular sizes (sperm head size, tail length, residual cytoplasm area, etc.), size ratio (between-sperm size comparison) and pattern recognition (multiple heads or tails, absent tail, coiled tail, etc.). One way to replace the poor visual capacity for assessing the continuum of sperm sizes, shapes and textures would be to quantify sperm morphology with the assistance of a computer.

Over the last three decades, image cytometry has been increasingly used in cell biology. This approach allows for the precise and reproducible measurement of cell structure and function. Image cytometry generally relies on image analysis systems that combine microscopy, video and data processing. It is based on the measurement of absorbed light at each point of a sperm cell on a stained smear under a microscope. These measurements can then be reiterated by scanning all the points making up the cell or sub cellular compartment studied to give a representative image in the form of a numerical matrix (each point of the image source is 'represented' by its coordinates in the field of analysis and by a grey scale value). This is stored in the image analysis system to be subsequently processed by specific algorithms. Quantitative information describing this image can then be extracted.

## LITERATURE REVIEW

The basic semen parameters, e.g. motility concentration and morphology <sup>[11, 12, 5, 6]</sup> have been correlated with IVF success. The problem with the different semen parameters is that in the literature there is not a consistent threshold indicating fertility and subfertility, especially when using motility and concentration. There have, however, been attempts to establish thresholds for sperm morphology <sup>[11, 13]</sup>. By means of a structured literature review of the IVF situation studied. The impact of sperm morphology on fertilization and pregnancy rates <sup>[14]</sup>. A total of 216 articles were identified by the initial search, of which only 49 satisfied the selection

criteria. The selection criteria were (i) statistical associations between sperm morphology and IVF and/or pregnancy, (ii) abnormal/normal sperm morphology fertility thresholds, and (iii) whether descriptive data (per oocyte fertilization, per cycle/transfer pregnancy rates and pregnancy outcome) were presented. Odds ratio (OR) and 95% confidence interval (CI) analysis were performed on the number of oocytes fertilized and on the number of pregnancies within certain morphology thresholds<sup>[14]</sup>.

The majority of the articles (36/43 = 81.4%) concluded in their closing remarks that normal sperm morphology, including acrosomal morphology, had a role to play in the diagnosis of male fertility potential. Statistical analysis, however, could only be performed on 18 studies due to a lack of adequate descriptive data.

Using a 5% threshold (strict criteria), 10 studies provided data that could be analysed for the prediction of fertilization and 11 studies for the prediction of pregnancy. All the studies showed a positive predictive value for fertilization, with only one<sup>[15]</sup> not reaching significance. In the prediction of pregnancy (per cycle), nine studies obtained a positive predictive value with predictive value association. The studies of<sup>[5,16,14]</sup> reached significance.

Using a 14% threshold (strict criteria), five studies provided data that could be analysed for the prediction of fertilization and eight studies for the prediction of pregnancy. Similarly, all the studies analysed showed positive and significant predictive value with regard to fertilization. In the prediction of pregnancy, two studies<sup>[15,17]</sup> did not obtain a positive predictive value, while the studies of<sup>[13, 16]</sup> were both positive and significant.

When studying all the data in the 5% (strict criteria) threshold analysis, the no-transfer rate was 24.0% (86/359) in the 4% group compared to 7.4% (80/1088) in the >4% group.

Of the three studies using 'other'<sup>[18,19]</sup> normal sperm morphology classification criteria, three were positive with regard to fertilization and two with regard to pregnancy. Two of the studies reached

significance in the prediction of fertilization<sup>[17, 20]</sup>, while none reached significance in the prediction of pregnancy.

When looking at all the studies available, 92% of the articles evaluated showed a positive association between sperm morphology and IVF success. The association was not restricted to any one particular classification system and/or evaluation procedure. Some of these studies also showed that this association was independent of any of the other semen parameters<sup>[11,12,16,20,21]</sup>. It is of utmost importance to obtain good quality control in a laboratory evaluating sperm morphology. Adherence to these principles has helped to establish the strict criteria as a dependable diagnostic tool. While the strict morphology classification system has been refined with time [P (poor prognosis) pattern 4%, G (good prognosis) pattern 5-14% and N (normal) pattern >14%]<sup>[13]</sup>, the physiologically based criteria<sup>[22]</sup> clinically based threshold<sup>[11]</sup> have remained constant since 1986. This classification system has now been adopted and used successfully by authors world-wide. The majority of the studies<sup>[6,16,23,24]</sup> have confirmed the predictive value of normal sperm morphology within the established thresholds. In comparison, the WHO<sup>[25,26]</sup> guidelines, another of the major classification systems in use world-wide, have changed dramatically since 1980, becoming stricter with each revision. The result has been a high level of subjectivity and a lack of consensus, especially with regard to the clinical value and corresponding fertility thresholds of this classification<sup>[14]</sup>. Recently, new publications<sup>[27,34,35]</sup> dealing with strict morphology criteria and IVF outcome supported the conclusions of<sup>[14]</sup>. However, others did not get such clear thresholds and clinical help using this approach.

The advancement of infertility treatment with the introduction of the intracytoplasmic sperm injection (ICSI) procedure has made the correct classification of male fertility paramount, to ensure the best cost-benefit ratio. This is especially true in cases of severe male infertility. The ICSI procedure has been shown to consistently produce fertilization rates of

between 50 and 70% in severe male factor cases. This underlines the importance of being able to identify these severe cases so that they can be given the option of ICSI, or at least a diagnostic cycle (a cycle in which half the oocytes are fertilized by ICSI and the other half inseminated).

The importance of standard semen analysis, especially with reference to sperm morphology, is highlighted in the review by <sup>[14]</sup>. If laboratories adhere to the basic principles, do sperm morphology carefully, and if they are consistent in their evaluations, this parameter will be of use in the clinical arena on a day-to-day basis.

Conventional pattern recognition image analysis systems <sup>[28]</sup> To obtain more detailed information regarding the differences between the CellForm-Human instrument and the integrated visual optic system (IVOS; dimension system) from Hamilton-Thorn Research, we refer the reader to the following two articles <sup>[28,38]</sup>. Both systems take measurement of length and width of spermatozoa into consideration. A clear difference between the two systems is the evaluation of acrosomal size and shape of spermatozoa by the IVOS (dimension system), which was shown in previous studies to be of importance in clinical practice <sup>[12, 13]</sup>.

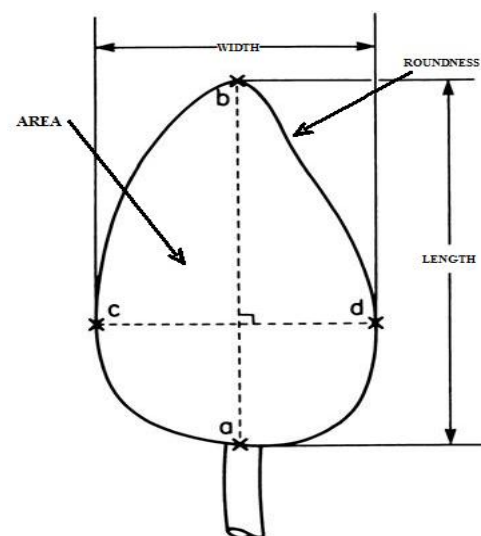
Automated sperm morphology analysis (ASMA) instruments: These work much like current versions of instruments for computer-aided sperm analyses for motion, except that no movement information is required <sup>[30,31,32,92,33]</sup>. The system consists of a microscope, a video camera, a computer frame grabber and morphology software. The video camera delivers the image to the computer's frame grabber which stores it for analysis <sup>[29,30,32]</sup>. The image is evaluated by the morphology software to determine whether spermatozoa are present. Sperm recognition is based on software specifications for size, shape, colour intensity and other characteristics. Once spermatozoa have been recognized and segregated from debris and other objects, metric measurements are performed on the head, midpiece, acrosome and other cytological features. These measurements are the basis for the sperm morphological classification. The accuracy

and precision of ASMA instruments depend on (i) the microscope optics, magnification and focusing capabilities; (ii) video camera quality; (iii) array size of the frame grabber; (iv) image processing techniques; (v) definitions of metric measurements <sup>[28,29,30]</sup> and (vi) staining methods used <sup>[32,36, 37]</sup>.

It is thus obvious from the above-mentioned data from different sections that the computer can become a helpful clinical tool in andrology laboratories and IVF centres. If careful slide preparation is adhered to, computerized analysis can bring more objectivity into morphology evaluation world- wide. More research in this field in the next few years is, however, mandatory to obtain definitive answers.

## MATERIALS AND METHODS

Sperm cells were displayed on the monitor at equivalent brightness and all the cells which did not present any overlap with debris or other cells were considered for analysis. From each sample heads were captured and analysed using Image J software. After treatment of the images some of the cells had to be discarded because of defective binarization as observed by incorrect correspondence between the original image and its mask. Each sperm head was measured for four out of nine primary parameters in the present work [head area (A), head length (L), head width (W), head roundness (R) Shown in the Fig. 1.



**Figure 1.** Morphometric parameters examined in this study.

## RESULTS AND ANALYSIS

The below tables [1- 5] shows measurement of four morphometric parameters form the five donors. And results using open source software checked for accuracy and Difference in Mean values of length, width, area and roundness obtained when compared with commercial software MedeaLAB CASA <sup>[9]</sup>

Table 1. Difference in Mean Values of four Morphometric Parameters for Donor-1

Donor-1					
Sperm Count	Commercial tool		Open source tool		Difference
29	Parameter (µm)	Mean	Parameter (µm)	Mean	
	Length	6.45	Length	6.25	-0.2 um
	Width	3.52	Width	3.21	-0.31 um
	Area	12.93	Area	12.67	-0.26 um
	Roundness	2.34	Roundness	2.21	-0.13 um

Table 2. Difference in Mean Values of four Morphometric Parameters for Donor-2

Donor-2					
Sperm Count	Commercial tool		Open source tool		Difference
89	Parameter (µm)	Mean	Parameter (µm)	Mean	
	Length	4.14	Length	3.85	<b>-0.29 um</b>
	Width	3.12	Width	2.81	-0.31 um
	Area	7.66	Area	7.35	-0.31 um
	Roundness	1.76	Roundness	1.50	-0.26 um

Table 3. Difference in Mean Values of four Morphometric Parameters for Donor-3

Donor-3					
Sperm Count	Commercial tool		Open source tool		Difference
94	Parameter (µm)	Mean	Parameter (µm)	Mean	
	Length	5.60	Length	5.33	<b>-0.27 um</b>
	Width	3.65	Width	3.67	-0.02 um
	Area	12.88	Area	12.58	-0.3 um
	Roundness	1.81	Roundness	1.55	-0.26 um

Table 4. Difference in Mean Values of four Morphometric Parameters for Donor-

Donor-4					
Sperm Count	commercial tool		open source tool		Difference
32	Parameter (µm)	Mean	Parameter (µm)	Mean	
	Length	5.48	Length	5.14	<b>-0.34 um</b>
	Width	3.58	Width	3.24	-0.34 um
	Area	11.39	Area	11.07	-0.32 um
	Roundness	1.79	Roundness	1.69	-0.1 um



Table 5. Difference in Mean Values of four Morphometric Parameters for Donor-5

Donor-5					
Sperm Count	Commercial tool		Open source tool		Difference
119	Parameter (µm)	Mean	Parameter (µm)	Mean	
	Length	5.74	Length	5.48	-0.26um
	Width	3.53	Width	3.25	-0.28um
	Area	12.41	Area	12.12	-0.29um
	Roundness	2.05	Roundness	1.80	-0.25um

## CONCLUSIONS AND FUTURE WORK

In the present study, we have developed a freely available sperm head morphology analyzer plug-in for open source software. Described the systems functionality and confirmed its validity with respect to the commercial softwares such as Sperm-Class Analyzer<sup>[10]</sup>, Sperm Morphometry Module of ISAS<sup>[11,14]</sup>, and the Metrix Oval Head Morphology software component of the Hamilton-Thorne CEROS system<sup>[12]</sup>. Out of nine morphological indices four are automatically measured in the present study (Length, Width, Area and Roundness)<sup>[13]</sup>. Remaining five morphological indices to be measured automatically in the future work.

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