Human Sperm in the Third Dimension

Claire Garrett[1], Joselito Chua[2], KG Tan[1], Eduard Sanchez[3] and HW Gordon Baker[1]

[1] *University of Melbourne Department of Obstetrics and Gynaecology, Royal Women's Hospital, Parkville, Australia*
[2] *University of Melbourne Department of Computer Science and Software Engineering, Parkville, Australia*
[3] *Microptics SL, Barcelona, Spain*

OBJECTIVE:

To make objective new parameters for assessment of three dimensional sperm morphology available for general evaluation in a commercial semen analysis system.

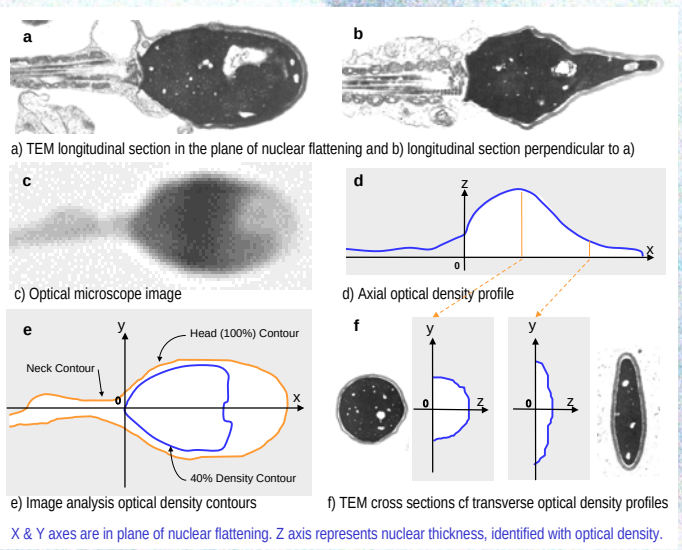
"THREE DIMENSIONAL" OPTICAL MORPHOMETRY

The human sperm is not elipsoidal. The TEM images of Figure 1 demonstrate that the three-dimensional configuration of the nucleus is flattened, with roundest cross section in the region posterior to the equatorial segment and gradually flattening toward the anterior tip, rather like a duck bill. The acrosome is a thin cap located on the surface of this bill like section and hence the misleading reference in morphology by light microscopy to the acrosome as the lighter stained region of the head.

In practice, the acrosome is not actually observed in light microscopy. Instead, when a sample has been washed of the viscous seminal plasma so that sperm heads in a smear readily lie in the plane of their nuclear flattening (x-y), the optical density of the sperm head can be interpreted as the shape of the head in the third dimension (z). Computer image analysis provides the unique opportunity to quantify and investigate the importance of this added dimension to sperm morphology.

Using a fully automated in-house morphometry system[1], we found that the human zona pellucida (ZP) preferentially binds sperm with specific densiometric or "third dimension" morphometries, irrespective of many conventional morphometric parameters such as head dimensions in the x-y plane[2]. The fact that the ZP favours sperm with a large pronounced "bill" probably reflects selection of sperm with physical attibutes more likely to make good surface contact on impact with the ZP and thus offering greatest opportunity for subsequent sperm-ZP binding and acrosome reaction to occur.

FIGURE 1:



a) TEM longitudinal section in the plane of nuclear flattening and b) longitudinal section perpendicular to a)

c) Optical microscope image

d) Axial optical density profile

e) Image analysis optical density contours

f) TEM cross sections cf transverse optical density profiles

X & Y axes are in plane of nuclear flattening. Z axis represents nuclear thickness, identified with optical density.

CLINICAL APPLICATION

A sample assessment (%Z) derived from the parameter values preferentially selected by the sperm-ZP interaction, many of which relate to the "third dimension", was found strongly related to natural pregnancy rates in a group of 1191 subfertile couples[3]. However, to be of clinical value, this %Z assessment needs to be transportable. It needs to be available for general evaluation in a commercial, fully automated semen analysis system which is robust to the variation inherent in application by different laboratories, despite working to similar protocols. To this end, Microptic SL(Spain) have agreed to trial our "third dimension" parameters in their Sperm Class Analyzer (SCA).

METHODS:

1. The densiometric parameters are not only crucial to the assessment of %Z, but also contribute to the automated identification of analyzable sperm head images in a microscope field. Decision tree learning is used to reject images of seminal debris and superimposed cells. The decision tree approach does not apply static rules for rejection as these do not serve well as recognitian algorithms, given the heterogeneity observed in the shape of human sperm. A training set of 1891 images containing balanced numbers of images scored manually as "accept" or "reject" for sperm head analysis, was used to create a decision tree using Weka (Waikato Environment for Knowledge Analysis) free software. The accuracy of the automated rejection was then tested on a set of 1920 scored images.

2. The potential sensitivity and normalization of the %Z assessment across semen smears using different staining techniques was evaluated by comparing results for the morphometric parameters of 100 sperm head images captured in each of 2 different locations on washed semen smears from 10 samples, using two different staining methods: haematoxylin & eosin (with blue filter) and spermBlue (with orange filter), and x100 objective.

RESULTS 1: Decision Tree Analysis

Table 1 is the confusion matrix for classification of images suitable for sperm head analysis, and corresponds to a 95% accuracy for the automated classification. The resultant decision tree of Figure2 illustrates the complexity of the classification process (79 decision nodes and 40 leaf endpoints) and the involvement of the stain derived morphometric parameters.

TABLE 1.	Automated classification	
Manual classification	Reject	Accept
Reject	208	1
Accept	97	1614

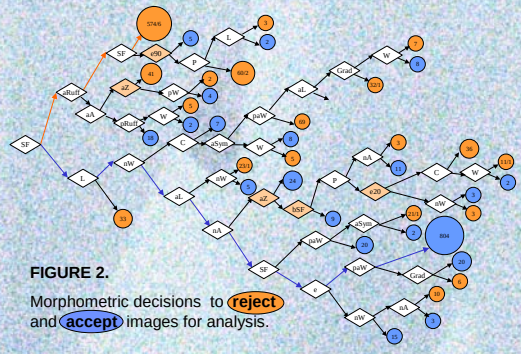


FIGURE 2.

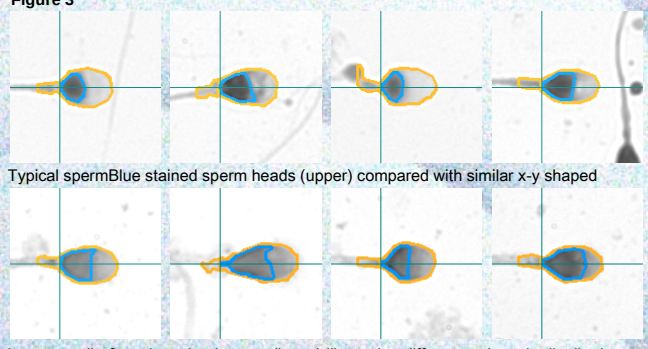
Morphometric decisions to **reject** and **accept** images for analysis.

RESULTS 2: Stain Normalization

All non stain dependent parameters were consistent with haematoxylin stained sperm heads being systematically larger than heads stained with spermBlue, but with shape in the x-y plane invariant to staining method. There is a scaling factor of 1.09(±0.01) between haematoxylin and spermBlue results for linear dimensions and 1.00(±0.03) for shape parameters.

However, the situation for the important stain dependent parameters is more complicated and there is no equivalent simple scaling factor to normalize between the two staining methods. As seen in Figure 3, haematoxylin stained sperm heads typically show less distinct differential staining between anterior and posterior regions and have a 40% smaller fraction of the head area exhibiting low stain density. The ratio of anterior to posterior stain density is a factor of 2 higher with haematoxylin, although the bill length parameter is only 10% smaller.

Figure 3



Typical spermBlue stained sperm heads (upper) compared with similar x-y shaped haematoxylin & eosin stained sperm (lower) illustrating differences in stain distribution.

CONCLUSIONS:

Our preliminary work suggests that sperm head morphometry in the x-y plane can be simply scaled between staining methods. However, the "third dimension" interpretation of optical morphometry is seriously compromised by differences in staining, possibly due to saturation effects or confounding from surface proteins.

Quantification of internal staining characteristics of human sperm heads is useful for automated discrimination of sperm heads from seminal debris and essential for the %Z assessment identifying sperm with fertilizing potential. However, without a means to calibrate densiometric parameters, interpretation and repeatability of fully automated sperm morphometry will be limited.

More work needs to be done to establish robust staining methods for invariant interpretation of optical density in terms of sperm morphometry "in the third dimension".

REFERENCES:

1. Garrett & Baker. Fertility & Sterility 1995;63:1306-17
2. Garrett, Liu & Baker. Fertility & Sterility 1997;67:362-71
3. Garrett, Liu, Clarke, Rushford & Baker. Human Reproduction;18:1643- 49