LASER LIGHT SCATTERING AS A PROBE OF SPERM MOTILITY

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SUMMARY

Practical aspects of assaying live and dead fractions of bull spermatazoa, as well as concentration effects and organism survival, are briefly discussed. The technique and physical basis for the use of laser light scattering in the assay is presented. Previous work assumes that the high frequency signal components arise from translation motion. Our recent work demonstrates that sperm rotation is responsible for the high frequency photomultiplier-detector signals.

INTRODUCTION

The quantitative measurement of sperm motility has been a long-standing problem, and visual assessment has become the only widely used routine technique.

Recently laser light beating spectroscopy has become recognized as an appropriate tool in this field (Berge *et al.*, 1967; Nossal, 1971; Dubois *et al.*, 1975; Cook *et al.*, 1976; Shimizu and Matsumoto, 1976; Finsey *et al.*, 1979). The only substantial application of this technique to bull sperm in the context of artificial breeding is that of Hallet *et al.* (1978).

The technique has been based on the principle that the speed of a microscopic object which scatters light can be determined by measuring the associated Doppler shift induced in the scattered light field.

Basic to all these studies have been the assumptions that the size and shape of the spermatazoon are unimportant, the swimming velocity distribution is isotropic, and the fluctuating photocurrent results from beats between Doppler components. However, recent studies (Harvey and Woolford, 1980) have cast serious doubt on the validity of these assumptions and the associated interpretations of previous light scattering results.

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This paper briefly describes the basic technique, outlines several important factors which must be considered, and presents some illustrative data.

METHODOLOGY

Sperm cells within a defined volume of the sample (typically < 0.5 mm^3) scatter light from an incident laser beam to a photodetector (Fig. 1).

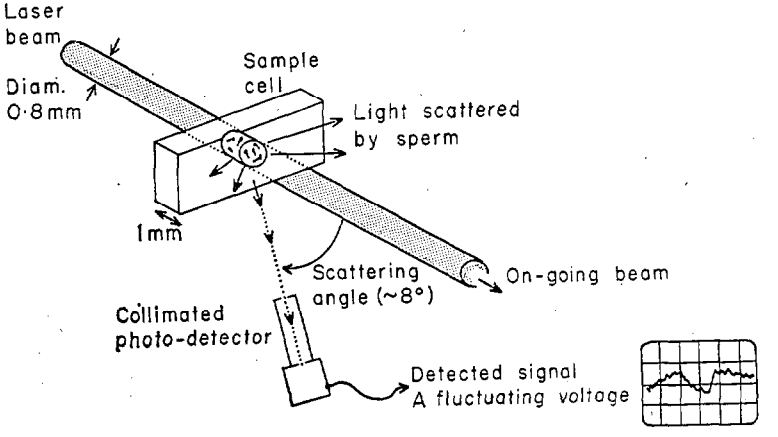

FIG. 1: *Geometry of the laser light scattering system.*

Harvey and Woolford (1980) have shown that the scattered light arises largely from the head region, which behaves as a spinning mirror, giving large intensity peaks as the head rotates.

The resultant photocurrent exhibits fluctuations which appear to arise not from the beating of Doppler shifts in the scattered light, but from intensity fluctuations generated as the cells rotate or otherwise move.

At small scattering angles the photocurrent fluctuations may be characterized by two overlapping frequency domains, a low range 0-10 Hz generated by immotile sperm and a high range 0-500 Hz generated by motile cells.

Analysis of this signal has been carried out in the time domain by forming the autocorrelation function, and in the frequency domain using spectral analysis to derive the power spectrum. Both the autocorrelation function and the power spectrum are of a two-component form arising from the motile and immotile fractions, and features of these functions can be related to certain

kinetic attributes of the sperm sample. In particular, the amplitude ratio (AAR) of the two autocorrelation components is related to the percent motile cells, the autocorrelation decay time is determined by random motions of dead cells, and the high frequency range of the power spectrum appears closely related to the head rotation rates among the motile fraction.

Sperm samples (both bull and ram sperm have been successfully used) are diluted to a standard concentration (usually $< 10^7$/ml) in an optically clear medium. Sodium citrate based buffer (2%) or citrate solubilized milk powder and egg yolk based extenders have been found satisfactory. Egg yolk concentrations up to 5% have been found usable provided the scattering angle is small ($< 10°$).

Large protein aggregates and contaminants must be excluded by filtering to 0.22 μm.

Sample temperature is controlled to close limits (± 0.05 °C) at 37°C to prevent convective motions of the medium.

RESULTS AND DISCUSSION

The two-component nature of the correlation function for a mixture of motile and immotile sperm is evident in Fig. 2. Motile cells generate a characteristic peak arising from the short correlation times for the high frequency components in the signal, and this peak progressively diminishes as the sample ages. The slowly

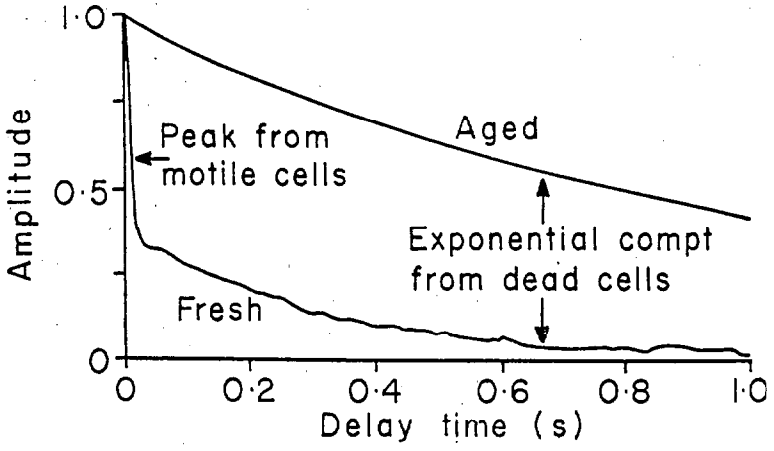

FIG. 2: *Correlation functions for fresh (2 h) and aged (incubated 72 h at 37°C) sperm samples.*

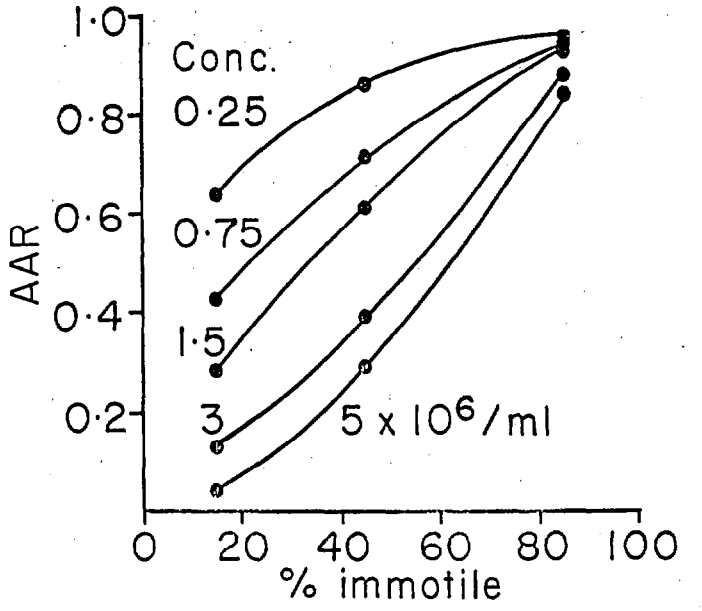

FIG. 3: *Relationship between the autocorrelation amplitude ratio (AAR) for the motile and immotile components and the percent dead cells, over a range of total sperm concentration.*

decaying exponential feature arises from motions of the dead or immotile cells.

The AAR of the two autocorrelation components has been found to give a useful measure of the percent immotile cells, although the relationship is strongly dependent on the total sperm concentration (Fig. 3). While the measurement is clearly empirical, repeatability is generally better than $\pm 5\%$ for a sample analysis time of 5.5 min.

The apparent increase in the percent immotile cells with decreasing sperm concentration is partially a real effect, since adding typically 5% seminal plasma to the diluent was generally found to give a decrease in AAR, particularly at high sperm dilutions. Concentration-dependent changes consistent with collisional perturbations of dead cells have also been observed in the immotile component of the autocorrelation function. Immotile or dead cells normally become vertically oriented, head down, due to geotaxis. Collisional interactions may then decrease the AAR as a consequence of the markedly anisotropic intensity distribution in the light scattered from spermatozoa (Harvey and Woolford, 1980),

since perturbed cells may become reoriented so that they do not contribute to the scattered intensity at the detector.

The scattering anisotropy has been found to be of such magnitude that the detector observes only those spermatozoa either aligned or swimming within $\pm 20°$ of the normal to the plane defined by the laser beam and the detector.

Spermatazoa have also been found to exhibit a remarkable tendency to swim in close proximity to the internal surfaces of the sample chamber. This results in a spatial separation of the motile and immotile cells in the sample, and with inappropriate cell geometry can contribute to both concentration and time dependence of the AAR. These effects confound the absolute measurement of the percent motile cells, unless they are appropriately considered in the experimental design.

Information on the vigour of the motile fraction is often more conveniently derived from the power spectrum than from the autocorrelation function.

Typical time-dependent changes in the spectrum are illustrated in Fig. 4 for samples incubated at both 37 and 20°C. The exponential feature extending to several hundred Hertz is generated by rotational motions of motile cells which produce rapid intensity fluctuations.

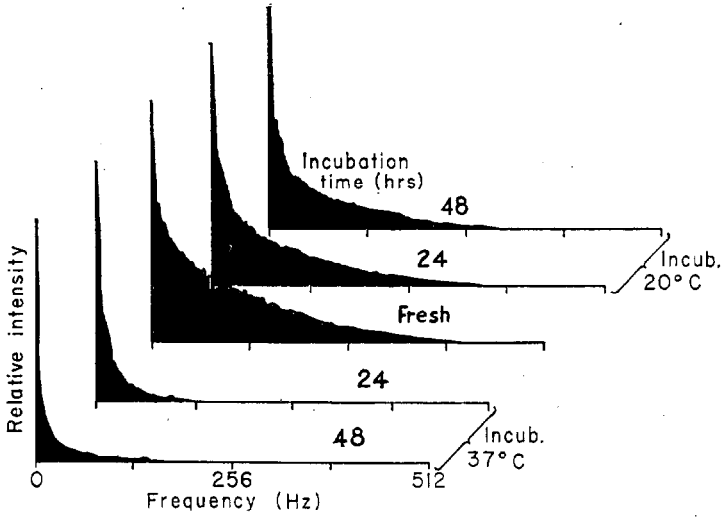

FIG. 4: *Power spectra for fresh and incubated samples.*

The decreasing prominence of the high frequency region with incubation is associated with a decrease in both the number and vigour of swimming cells. Changes in the spectrum are characterized by computing the power distribution between the frequency ranges 0-10 Hz (immotile cells), 10-100 Hz (defective or weak swimmers) and 100-500 Hz (vigorous swimmers).

Data collection and processing time is typically 5 min, allowing motility changes to be followed over a short time scale if desired.

Results to date clearly demonstrate that the light scattering technique gives a rapid, objective and repeatable measure of sperm motility. Further development of the optical geometry and data analysis should establish the technique as a routine laboratory assay.

ACKNOWLEDGEMENTS

The authors would like to thank Dr R. Sherlock for helpful discussions during the course of this work, and recognize the general support for the programme given by the University of Waikato.

REFERENCES

Berge, P.; Volochine, B.; Billard, R.; Hamelin, A., 1967. *C. R. Acad. Sci., Paris, 265*: 889, Series D.

Cook, D.; Hallet, F. R.; Barker, C. A. V., 1976. *J. Mechanochem. Cell Motility, 3*: 219.

Dubois, M.; Jouanet, P.; Berge, P.; Volochine, B.; Serres, C.; David, G., 1975. *Ann. Phys. Biol. Med., 9*: 19.

Finsey, R.; Peetermans, J.; Lekkerkerker, H., 1979. *Biophys. J., 27*: 187.

Hallet, F.; Craig, T.; Marsh, J., 1978. *Biophys. J., 21*: 203.

Harvey, J. D.; Woolford, M. W., 1980. *Biophys. J.* (in press).

Nossal, R., 1971. *Biophys. J., 11*: 341.

Shimizu, H.; Matsumoto, G., 1976. *Opt. Commun., 16*: 197.