Optimizing the MAS spinning rate for NMR Studies of Live Spermatazoa

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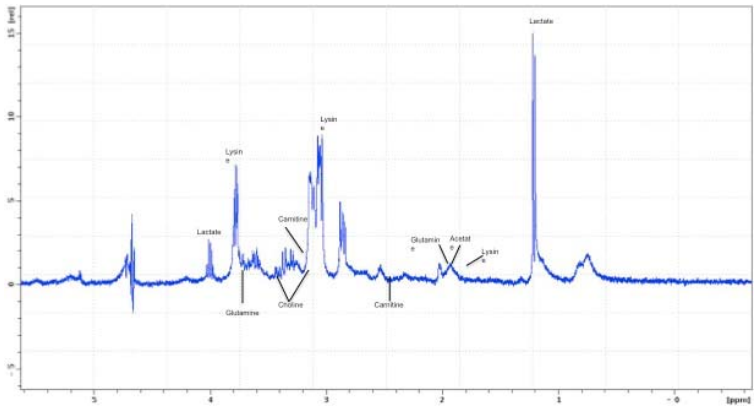
Introduction: The assessment of male fertility involves light microscopy by a trained technician of a freshly ejaculated semen sample that is compared against World Health Organisation reference values. This method has been used clinically since the 1950's, and although other putative tests of sperm function have been developed none have yet been adopted in the clinical setting. Studies of sperm metabolism have the potential to provide new diagnostic information about sperm function [1] we therefore used ^{1}H High Resolution Magic Angle Spinning (HR-MAS) NMR spectroscopy to identify metabolites in spermatozoa and determine a spinning speed that maximizes viability.

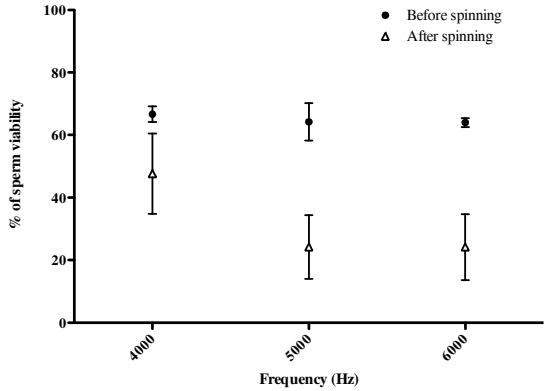
Methods: Boar spermatozoa of high quality and motility were prepared by washing with Percoll gradients followed by density centrifugation. ^{1}H MAS NMR spectra were obtained using a Bruker Avance III 9.4T scanner with HR-MAS probe, (zgppw5; watergate solvent suppression sequence; NS=8; DS=2; SWH=8223.685Hz; AQ=3.98s; D1=2s). Samples were spun at 4000, 5000 & 6000 Hz and 2D DQF-COSY spectra were obtained to aid identification of metabolites (NUC1=1H; NS=1280; TD= 24760, 64; DS=2; SWH 4201.68 Hz, 4202.73Hz; AQ 1st Dimension=2.9357s, 2nd Dimension 0.0076s; D1=1s). The data was processed using the Bruker Topspin package. Tentative assignment of metabolites was performed by comparison to literature [1, 2] and confirmation by cross peak identification on 2D spectra. Sperm viability was measured using a Live/Dead Sperm Viability Kit (Molecular probes 2001-L-7011).

Results: A representative 1D spectrum is shown below (A) and key metabolites which may play a role in fertility have been clearly identified including Lactate, Lysine, Glutamine, Choline, Carnitine & Acetate. Further spectral identification including the aromatic region (6.5-8.5ppm) is in progress. As the spinning frequency increased the sperm viability decreased markedly (B).

A



B



(A) Representative 1D ^{1}H NMR spectra for 10^{8} Spermatozoa: (B) Percentage spermatozoa viability before and after spinning at 4000, 5000 & 6000Hz (Data shown is mean ± SEM for three experiments)

Discussion: ^{1}H HR-MAS allows the acquisition of high-resolution spectra showing metabolites from live sperm. Important metabolites were identified from these spectra including lactate, a product of cellular metabolism. The 4000hz-spinning frequency provided high-resolution spectra, the highest overall percentage viability and avoided spinning associated artifacts found at lower frequencies. As a result, this would appear to be the optimum spinning frequency for acquisition in future experiments. It is likely that spermatozoa death at higher frequencies was caused by the increasing g-force. Future experiments will include measuring the effect of pharmacological agents on spermatozoa metabolism.

1. Lin, C.-Y., et al., *(1)H NMR to investigate metabolism and energy supply in rhesus macaque sperm.* Reproductive Toxicology, 2009. 28(1): p. 75-80.
2. Jones, A.R. and W.A. Bubb, *Substrates for endogenous metabolism by mature boar spermatozoa.* Journal of Reproduction and Fertility, 2000. 119(1): p. 129-135.