Identifying Intracellular Sperm Metabolites Using HR MAS NMR

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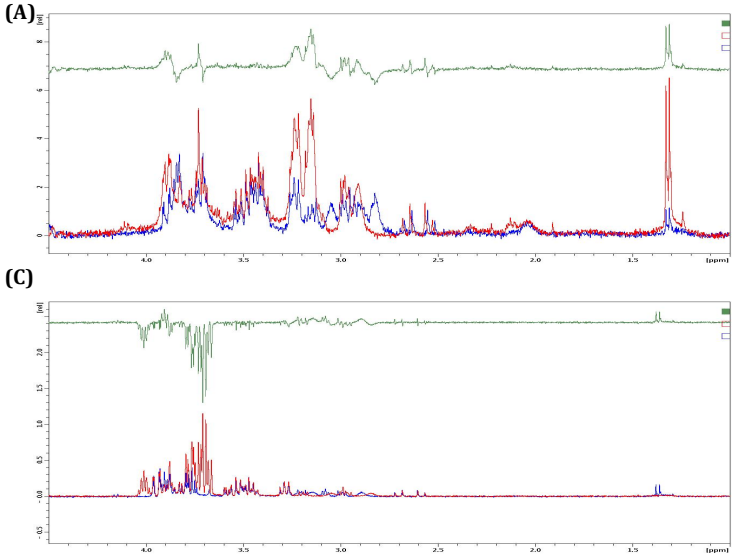
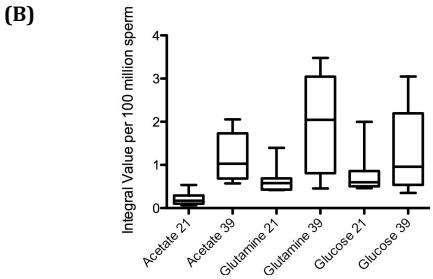
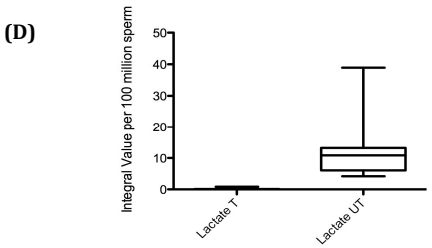
Purpose: There have been numerous attempts to develop sperm function tests to assist the diagnosis of male infertility, but none have been adopted clinically. ^{1}H NMR has previously been used to identify biomarkers of male fertility in seminal plasma [1] and to differentiate between spermatogenic failure and obstructive azoospermia [2]. NMR has also been used to observe the effects of environmental toxins on sperm function and semen quality in primates [3]. Given that there is a need for improved tests of sperm function, we used HR MAS to measure sperm metabolite concentrations after incubation at two temperatures and in the presence of absence of α-chlorohydrin (ACH) – an inhibitor of glycolysis, to see if changes in metabolite concentrations could be detected.

Methods: Boar sperm of high quality and motility were prepared by washing through isotonic Percoll gradients using density centrifugation before being re-suspended in 1ml Phosphate Buffered Saline (PBS). All experiments were conducted using a Bruker Avance III 9.4T Scanner with HR MAS probe. In all experiments 40µl of sperm sample was placed into a 4mm HR MAS zirconium rotor along with 10µl 20mM Trimethylsilyl Propionic Acid reference compound. Rotor frequency was 3000Hz at 39°C. ^{1}H Watergate excitation sculpting pulse sequence was used (zgppw5; NS=8; DS=2; SWH=8223.685; AQ=0.6; D1=0.6). For the temperature experiments, sperm from 8 individual boars were incubated at either 21°C or 39°C for 3 hours. For the inhibition experiments, sperm from 8 individual boars were incubated in 1ml 50mM ACH in PBS for 1 hour before scanning. The data were processed using the Bruker Topspin package. Assignment of metabolite peaks was performed by comparison to the literature [4, 5] and an online spectral database.

Results: Figure A shows representative spectra of boar sperm metabolites after 3 hour incubation at 21°C (Blue) & 39°C (Red), difference (Green). Figure B shows significant increases in acetate ($p<0.01$), glutamine ($p<0.05$) & glucose ($p<0.05$) were detected in sperm incubated at 39°C. Figure C shows representative metabolite spectra before (Blue), and after (Red) inhibition of glycolysis with ACH, difference (Green). Peaks seen at 3.7ppm in the ACH treated sample (Figure C- Red) are associated with ACH itself. There was a significant decrease in lactate ($p<0.001$) when motility was inhibited with ACH (T) compared to an untreated sample (UT) (Figure D).







Discussion: HR-MAS successfully detects changes in metabolite concentrations at different temperatures and following incubation with ACH. The significant decrease in lactate when ACH is added is consistent with the theory that glycolysis plays a significant role in pig sperm metabolism [6]. The significant increase in acetate when temperature is increased may be explained by its formation from pyruvate in the sperm of many species [5]. The significant increase in glutamine could be related to its influence on sperm motility in mammals [7].The difference in glucose peaks cannot be explained. Future work will examine metabolites in human sperm and their role as biomarkers of infertility, including assessing the intracellular metabolites of fertile and infertile men.

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