Better Understanding Maturation Arrested Men Through HR-MAS Spectroscopy of Human Testicular Biospy Tissue

R. B. Iman[1], M. Swanson[1], T. Walsh[2], D. Aaronson[2], S. Zhao[3], Y. Lu[3], and J. Kurhanewicz[1]

[1]Radiology, University of California, San Francisco, San Francisco, CA, United States, [2]Urology, University of California, San Francisco, [3]University of California, San Francisco

Intro:
[1]H magnetic resonance spectroscopy (MRS) may represent a non-invasive tool for identifying and localizing sperm production in azoospermic men. Previous studies have demonstrated significantly higher levels of phosphocholine (PC) in normal versus azoospermatic men (1). A third group, maturation arrest, has demonstrated varying levels of sperm production clinically as reflected in PC concentrations. Men diagnosed as maturation arrested have a wide gamut of spermatogenesis which is difficult to quantify clinically. To determine which maturation arrested men may be potential in vitro fertilization candidates, *ex vivo* [1]H high-resolution magic angle spinning (HR-MAS) spectroscopy was performed on human testicular biopsy tissues.

Methods:
Twenty-seven testicular biopsies were obtained from 27 different patients with normal (N=9), maturation arrested (N=9), or azoospermatic (N=9) diagnoses. Samples were weighed (mean 13.64 ± 6.89mg) and placed into custom designed 20 or 35 µl leak proof zirconium rotors containing 3.0 µl D_2O + 0.75% TSP. [1]H HR-MAS data were acquired at 11.7T, 1°C, and 2,250 Hz spin rate using a Varian INOVA spectrometer, equipped with a 4 mm gHX nanoprobe. Quantitative 1D spectra were acquired with 2s relaxation, 2s presaturation, 2s acquisition (TR = 6s), 40,000 points, 20,000 Hz spectral width, and 256 transients. The Electronic Reference To access In vivo Concentrations (ERETIC) (2) method was used as a quantitation standard. Data were quantified using HR-QUEST, a custom version of QUEST (ref) adapted for analysis of short-echo time HR-MAS spectra containing 40,000 points. Basis set spectra of 19 metabolites were collected in solution and incorporated into the HR-QUEST fitting routine. Peaks from known macromolecules and unidentified compounds were also included as part of the basis set. HR-QUEST estimated the background signal using an HLSVD algorithm and iterated between fitting the metabolites and modeling the background 6 times. Finally, concentrations were calculated relative to the peak area of the ERETIC signal. Logistic regression analysis used the binary dependent variable of being normal or azoospermatic and independent variables of age and a single metabolite. For metabolites that had age-adjusted p-value less than 5%, we further calculated the predicted probability of being normal for maturation arrested patients using the logistic regression equation for normal versus azoospermatic patients.

Results:
Figure 1 shows representative 1D presat HR-MAS spectra of A) normal, B) maturation arrest, and C) azoospermatic testicular tissue. Note the PC signal observed in the maturation arrest spectra falls in between the other two. The concentrations of phosphocholine (PC) in normal, maturation arrested, and azoospermatic testes are shown in Figure 2. The maturation arrest samples which fell into the normal range (1.56-4.15mmol/kg) of PC concentrations were found to contain at least one spermatid of mature sperm. Those that fell into azoospermatic range (.01-2.03mmol/kg) arrested early in spermatogenesis and contained no spermatids or sperm. The concentration of PC phosphoethanolamine (PE), and glutamine in normal tissue was found to be significantly different from azoospermatic tissue with p values of .023, .041, and .040, respectively. All three, with the exception of glutamine, were found to be greater in normal tissue. PC, myo-inositol, sinositol, and PE were found to correctly predict whether maturation arrest patients fall into a normal or azoospermatic range.
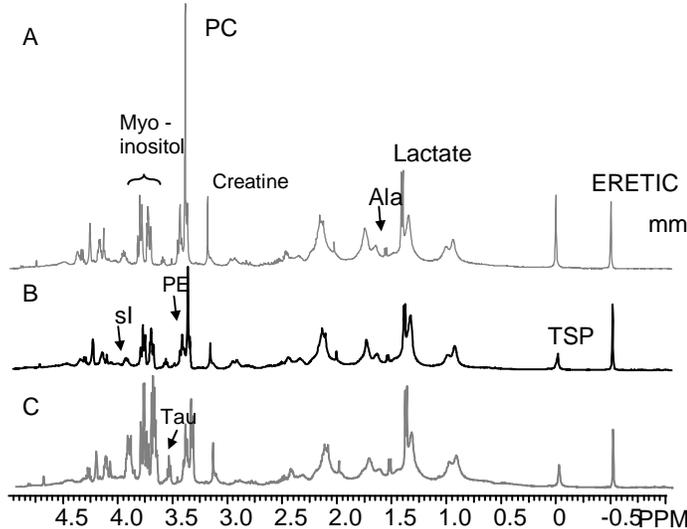

Figure 1. [1]H HR-MAS spectra of A) normal, B) maturation arrested, and C) azoospermatic human testicular tissue.
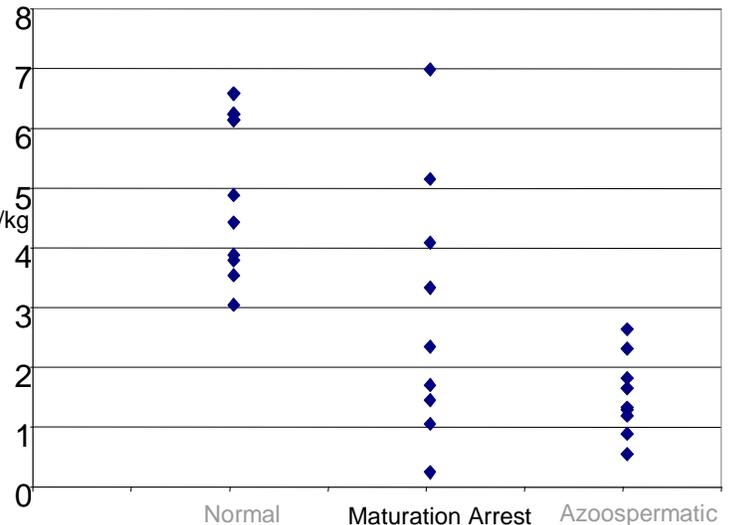
Figure 2. PC concentrations (mmol/kg) in normal, maturation arrested, and azoospermatic human testicular tissue.

Discussion/Conclusion:
The observation that PC concentrations were significantly higher in normal testes and did not overlap with azoospermatic testes in a larger cohort study further validates using PC measurements in MRS to provide sensitive quantitative assessments of male infertility. The observation that PC levels could predict whether maturation arrested patients had any viable sperm in any position of the cell cycle provides optimism that a classification of maturation arrest can be further delineated. Although there is a clear need to test this maturation predictive model on a larger patient cohort to authenticate these findings, the logistical model can be used to predict qualitative assessment of male infertility. This data suggest a unique chemical signature for spermatogenesis that may be used to develop a novel, non-invasive, diagnostic study for men with no sperm in the ejaculate due to poor or absent sperm production in the testis..

References
1) Iman R, et al. ISMRM 2008 2) Albers M. et al. (2008) Magn Reson Med "in press"; 3) Ratiney H, et al. (2005) NMR Biomed 18(1):1-13.