in H_2 (H_2+A) (n=42); with rotenone (200μM) in C (C+R) or in H_2 (H_2+R) (n=45); and with pentoxifylline (2 mg/ml) in C (Px) (n=85). ATP content was measured with an luminometer. Sperm concentration and motility were measured with Makler chamber and Sperm Class Analyser (SCA).

RESULTS: In terms of sperm ATP amount, Sperm treated in the 75% H_2-saturated medium showed increased amount compared with in the medium only with antimycin A (H_2+A vs. C+A: 305.58 $\pm$ 204.89 vs. 186.65 $\pm$ 111.26, n = 30, $P < 0.001$), with rotenone (H_2+R vs. C+R: 297.37 $\pm$ 157.58 vs. 171.11 $\pm$ 77.36, n = 30, $P < 0.001$), with pentoxifylline (H_2 vs. Px: 547.01 $\pm$ 324.89 vs. 358.84 $\pm$ 190.12, n = 45, $P < 0.001$), without agents (H_2 vs. C: 437.06 $\pm$ 291.47 vs. 306.92 $\pm$ 200.01, n = 105, $P < 0.001$), respectively (pmol /10^6 sperm: data are shown as mean $\pm$ SD). On sperm motility parameters with SCA, sperm treated in C+A showed significantly lower than in H2+A in the parameters of VCL, VSL, VAP, ALH, and BCF ($P < 0.05$, n = 12). Sperm treated in C+R showed significantly lower than in H_2+R in the parameters of VCL and ALH ($P < 0.05$, n = 15). Sperm treated in Px showed significantly lower than in H_2 in the parameters of VSL, VAP and BCF ($P < 0.01$, n=40), LIN, STR, WOB ($P < 0.05$, n=40).

CONCLUSIONS: The findings of this study indicate that H_2 treatment increases the intracellular ATP content of the normospermic patients' sperm. Possibly, men with severe sperm dysfunction could select IVF instead of ICSI by using H_2 treatment. It may also be useful in the selection of good quality sperms for ICSI. This is a basic study on a relatively small sample size with limited conditions. The confirmation using larger samples under various conditions may be required. Furthermore, we need to check the safety of H_2 treated sperm use in ART.

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P-522 Wednesday, October 19, 2016

SEGMENTED HAIRPIN-LOOP ORGANIZATION OF CHROMOSOMES IN SPERM NUCLEI: IMPLICATIONS FOR FERTILIZATION AND EMBRYOGENESIS. D. Ioannou[a] H. G. Tempest.[b] [a]Human and Molecular Genetics, Assistant Professor, Miami, FL; [b]Human and Molecular Genetics, Florida International University, Miami, FL.

OBJECTIVE: Genomes are nonrandomly organized within nuclei. Sperm cells are proposed to have a unique hairpin-loop arrangement that is hypothesized to be critical for the ordered exodus of the paternal genome following fertilization. This model describes centromeres clustering in the center (chromocenter), with p- and q-chromosome arms stretching toward the nuclear periphery. This study examines whether evidence can be provided to support this model of organization in sperm using 3D modeling.

DESIGN: Transversal study in a laboratory environment.

MATERIALS AND METHODS: This study was approved by the local IRB, five normozoospermic males were recruited. Three color fluorescence in situ hybridization targeted the centromere and p- and q-arms of eight different chromosomes (2, 3, 6, 8, 10, 12, 16, and 18). 3D modeling was employed to investigate the radial organization of each targeted region by measuring the geometric center of each target to the nearest nuclear periphery. Furthermore, hairpin-loop configurations were determined by the angle created between p- and q-arms. A minimum of 30 cells per subject, per chromosome were studied. Nonrandom organization of was established using the Chi-squared goodness-of-fit test ($p<0.05$).

RESULTS: Distinct reproducible chromosome-specific patterns of organization emerge. All chromosomes were found to possess nonrandom radial organization ($p<0.05$). Chromosome arms were found to form discrete hairpin-loop configurations. Three reproducible categories of chromosome loops were observed: narrow (<40°: 10, 12), intermediate (>40°<60°: 2, 8, 18), and wide (>60°: 3, 6, 16). Four centromeres (3, 6, 12, and 18) were found to be localized closer to the nuclear periphery than their chromosome arms.

CONCLUSIONS: We report reproducible nonrandom hairpin-loop organization of chromosomes that supports the proposed model. However, our findings do not support the existence of a centralized chromocenter with four centromeres being more proximal to the nuclear periphery than their chromosome arms. This suggests the sperm nucleus is more segmentally organized; resulting in specific genomic regions being exposed, remodeled and activated first following fertilization. The sequential exodus and remodeling could impact patterns of gene activation observed within the early embryo, perturbations in which, could negatively impact fertilization and early embryogenesis.

P-523 Wednesday, October 19, 2016

CAPACITATION TIMING VARIES AMONG MEN, BUT IS CONSISTENT AMONG EJACULATES WITHIN INDIVIDUALS. A. J. Travis,[a] A. Simpson,[b] M. A. Moody,[b] C. Cardona,[b] G. C. Ostermeier.[b] [a]Cornell University, Ithaca, NY; [b]Androvia LifeSciences, Mountainside, NJ.

OBJECTIVE: If inseminated too late in relation to ovulation, sperm may not obtain fertilizing ability before egg quality declines, preventing pregnancy. For fertilization to succeed, sperm must first capacitate, a process dependent upon sterol efflux and altered dynamics of ganglioside G_{M1}. Cap-Score^{TM} reflects the percent of capacitation competent sperm as determined by G_{M1} distribution patterns and thus used to assess capacitation timing.

DESIGN: The percentage of capacitated sperm was compared among and within fertile men, controlling for presence of capacitating stimuli and timing of incubation and fixation.

MATERIALS AND METHODS: Semen was collected from presumed fertile men (pregnant partner or recent father), as approved by WIRB (20152233). Samples were liquefied, washed, and aliquots incubated under non-capacitating (NC) and capacitating (CAP) conditions for 3hrs. The sperm were fixed and analyzed immediately (Day0) or after overnight incubation in fix (Day1). In another trial, NC and CAP samples were incubated for 3 hrs and 24 hrs prior to fixation. Cap-Score was compared using T-Tests (MS Excel 2013).

RESULTS: An increase in Cap-Score was noted in fixed CAP samples from Day0 to Day1 (p=1.0E-4; n=53). To test if this was physiological, a second trial was done (n=25). Cap-Score was greater for Day1 CAP than Day0 CAP (p=6.6E-5), but was the same for samples incubated overnight in fix or CAP media (p=0.44). These data are consistent with prior work, demonstrating that physiologic membrane lipid changes associated with capacitation can occur in the presence of certain fixatives. Variation in capacitation timing among ejaculates was evaluated by scoring 124 samples from 53 men on Day0 and Day1. An increase in Cap-Score was observed in 82% (102/124) of CAP samples, with 44% (54/124) increasing more than 1 SD (7.8; presumed fertile men). These data support capacitation timing differences among ejaculates. The reproducibility of capacitation timing was assessed by classifying 52 ejaculates from 11 men as either early or late capacitators (Day1-Day0>7.8). The average concordance was 84%, supporting the view that capacitation timing is consistent within men.

CONCLUSIONS: Capacitation timing differs among men, but is consistent among ejaculates within individuals. This information could be used to optimize insemination timing in procedures such as IUI, IVF, and natural conception leading to personalized management of infertility.

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P-524 Wednesday, October 19, 2016

IDENTIFICATION OF COMMON UNDERLYING PATHOLOGIES ASSOCIATED WITH MALE INFERTILITY AND DIABETES USING DATA MINING AND *IN SILICO* ANALYSES. N. Kothandaraman,[a] A. Agarwal,[a] L. Samanta,[b,a] M. Assidi.[c] [a]American Center for Reproductive Medicine, Department of Urology, Cleveland Clinic, Cleveland, OH; [b]Redox Biology Laboratory, School of Life Sciences, Ravenhaw University, Orissa, India; [c]Centre for Excellence in Genomic Medicine Research, Jeddah, Saudi Arabia.

OBJECTIVE: Recent reports indicate that men with diabetes have abnormal semen parameters, which could affect their fertility status. The objective of the current study was to evaluate the functional relevance of genes and proteins common to diabetes and those associated with male infertility by performing *insilico* analysis.

DESIGN: The study considered all genes and proteins from published data representing infertility and diabetes and proteins reported from studies using seminal plasma and spermatozoa from patients with high, medium and low levels of reactive oxygen species in their semen samples.

MATERIALS AND METHODS: Through data mining, genes and the associated proteins they encode were identified and used for further downstream *in silico* analysis to identify their functionalities.

RESULTS: A total of 11,454 proteins were identified. Gene sets associated with oxidative phosphorylation, fatty acid metabolism, and ROS pathways were more represented in the gene sets common to these two diseases. Tissue localization analysis showed that most genes (cell surface receptors, TFs, translocated genes) were localized in the adult prostate and testis. Functional annotation of the genes using DAVID showed acetylation, proteinase and hydrolase to be overrepresented. Lipid metabolism, PTM, cytoskeleton and signal transduction mechanisms share common ground with the two