when added after 30-120min of initiated capacitation induced by dbcAMP-IBMX. TSP promoted a greater toxic effect of hydrogen peroxide on sperm viability that was abolished by D-penicillamine (PEN) suggesting the production of an oxidative stress by inhibition of 2-Cys PRDXs. MJ33 also impaired sperm viability but its effect was not prevented by PEN, suggesting that not only peroxidase by also PLA2 activity of PRDX6 is necessary to guarantee sperm viability. In conclusion, 2-Cys PRDXs and PRDX6 PLA2 activity are necessary to allow spermatozoa to achieve fertilizing ability. PRDXs are important to allow phosphorylation of PKA substrates and of tyrosine residues associated with capacitation. PRDXs maintain low levels of ROS for capacitation, thus avoiding the toxic effects of oxidative stress that would occur if these enzymes are inactivated.

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REGULATION OF PROTEIN PHOSPHATASE 2A DURING MOUSE SPERM CAPACITATION

Maria Gracia Gervasi, PhD and Pablo Visconti, PhD
Department of Veterinary and Animal Sciences, University of Massachusetts, Amherst, Massachusetts
(Presented By: Maria Gracia Gervasi, PhD)

Introduction: After ejaculation mammalian sperm acquire the ability to fertilize an egg during their transit through the female reproductive tract. The changes that occur on the sperm in that period are collectively named capacitation, and they can be mimicked in vitro in a defined media. At a molecular level, this process is related to the cAMP-dependent activation of protein kinase A (PKA) and a consequent increase in tyrosine phosphorylation (pY). Our laboratory has previously shown that a downregulation of serine/threonine phosphatases is required in parallel to PKA activation for the increase in pY related to capacitation. We hypothesized that during sperm capacitation Src Family Kinases (SFKs) are activated and phosphorylate specific phosphatases that are inhibiting the downstream effectors of PKA. The present work was aimed to identify the protein phosphatase involved in this signaling pathway and to evaluate its regulation during sperm capacitation.
Methods: In vitro sperm capacitation, western blot, immunofluorescence, and affinity purification tandem to mass spectrometry were performed.
Results: Protein phosphatases PP1, PP2A, and calcineurin were found expressed in sperm and, based on experiments with pharmacological inhibitors, PP2A is our candidate for regulation during sperm capacitation. The activity of the PP2A holoenzyme can be regulated by phosphorylation or methylation of the catalytic subunit, therefore the phosphorylation and methylation status of this subunit during sperm capacitation was evaluated by western blot. Our results show that PP2A is phosphorylated during sperm capacitation and this phosphorylation is SFKs-dependent. PP2A is also methylated during capacitation which could result in changes of the regulatory subunit conforming the holoenzyme. To evaluate this, we used a custom-made affinity purification method coupled with tandem mass spectrometry analysis. Our results suggest a phosphatase interaction with members of the testis specific serine/threonine kinase family.
Conclusion: Altogether, our data support the hypothesis of a pathway parallel to PKA that regulates sperm capacitation and bring new insight into the participation of phosphatases in this process.

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RELIABILITY ESTIMATES FOR A TEST OF SPERM CAPACITATION, A FUNCTIONAL COMPLEMENT TO SEMEN ANALYSIS

G. C. Ostermeier, PhD[1]; C. Cardona, PhD[1]; T. T. Smith, PhD[2]; M. A. Moody, MS[1]; A. J. Simpson, BS[1] and A. J. Travis, VMD/PhD[3],[1]
[1]Androvia LifeSciences, NJ; [2]ARTWorks Consulting, NY; [3]Cornell Univ, NY
(Presented By: G. Charles Ostermeier, II, PhD)

Introduction: Evaluations of semen quality are subjective. This can add unwanted variability into fertility assessments. To augment traditional semen evaluation, samples were evaluated for their ability to capacitate and become fertilization competent using localization patterns of the ganglioside GM1, the basis of the laboratory-developed Cap-ScoreTM Sperm Function Test. Here, Cap-Score precision and its variation when determined by the same and different operators were assessed.
Methods: Following liquefaction of semen samples from consenting men, sperm were washed, incubated, fixed and then evaluated via fluorescence microscopy for GM1 localization patterns. Student's t-Test employing unequal variance was done using Microsoft Excel (2013).
Results: Precision was evaluated by comparing the percent change about Cap-Score values (%Δ=(y2-y1)/y2) when 50, 100, 150 and 200 sperm were evaluated. Changes in values of 11, 6 and 5% were observed for each addition of 50 sperm ($n \geq 23$). This supports the view that Cap-Score precision was only modestly improved by counting more than 100 sperm. To be conservative, Cap-Score was determined by counting the GM1 localization patterns of at least 150 cells. To assess variation within and between readers, 8 large image files containing up to 5,000 sperm were generated. Two different readers were trained and determined Cap-Scores by randomly resampling the image 20 times, counting 150 cells each time. When scoring the same sample, individual readers reported an average SD of 3 Cap-Score units. The difference between readers when scoring the same sample ranged from 0.00 to 1.52, with an average difference of 1 between the readers for any given sample. Applying the Bonferroni correction, no difference between readers was observed for any image file (p-values ranged from 0.02 to 0.99). These data demonstrate that the same and independent readers can replicate Cap-Score values when repeatedly evaluating the same semen donor.
Conclusion: Common measures of semen quality are subjective and can vary within and among readers, making the assessment of male fertility challenging. The Cap-Score sperm Function test evaluates the ability of sperm to capacitate, a necessity for male fertility. The data presented here show that the Cap-Score Sperm Function Test is highly reproducible and reliable within and between readers, which are key considerations when attempting to diagnose male infertility.
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