pregnancy. Patients undergoing VR should be counseled on the impact that past and continued tobacco use may have on reproductive outcomes.

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MEMBRANE POTENTIAL FLUORIMETRY ASSESSMENT AS A PREDICTOR TOOL FOR HUMAN SPERM FERTILIZING CAPACITY

Carolina Baro Graf[1,2], Carla Ritagliati[1], Valentina Torres Monserrat[3], Cintia Stival[1,2], Carlos Carizza[3], Mariano Buffone[4] and Dario Krapf[1,2]
[1]Insituto de Biologia Molecular y Celular de Rosario (CONICET-UNR), Rosario, Argentina; [2]Facultad de Ciencias Bioquimicas y Farmaceuticas de la Universidad Nacional de Rosario, Rosario, Argentina; [3]Fertya Medicina Reproductiva, Rosario, Argentina; [4]Instituto de Biologia y Medicina Experimental (CONICET), Buenos Aires, Argentina
Presented By: Carolina Baro Graf, MS

Introduction & Objective: Mammalian sperm cannot fertilize the egg without suffering certain physiological changes triggered during its course through the female reproductive tract, known as capacitation. Plasma membrane hyperpolarization associated to capacitation is well described for mouse sperm, and shown to be both necessary and sufficient for sperm to undergo the acrosome reaction (AR). Considering this, our aim was to answer the following questions: What happens with the human sperm membrane potential (Em) during capacitation? Is it involved in acquisition of acrosomal responsiveness and in fertilization competence?
Methods: Samples were classified according to the World Health Organization parameters and capacitated for 3 hours. Em measurements were performed using the potentiometric dye $DISC_3(5)$ in a fluorimetric set-up which results in an absolute Em value. The AR was evaluated using progesterone as an inductor and sperm were stained with PSA-FITC. IVF patients had normal spermograms and had no female conditions related to diminished ovarian reserve.
Results: Among normospermic donors a significant Em hyperpolarization was evidenced during capacitation (from –42.6±13.4 mV to –48.9±16.3 mV, $P < 0.05$). In addition, we found a strong negative correlation between Em values obtained after capacitation and the sperm capacity to undergo induced AR (Pearson coefficient r = -0.8234, $P < 0.01$), meaning that depolarized sperm showed less induced AR. Finally, we performed Em measurements from IVF patients. Sperm samples with successful IVF rates (fertilized oocytes >60%) exhibited a significant Em hyperpolarization during capacitation (from −41.7 mV±13.5 mV to −55.8 mV±12.2 mV, $P < 0.05$), while those with unsuccessful IVF rates did not (from −53.0 mV±3.0 mV to −38.6 mV±7.3 mV, ns). Moreover, Em values from capacitated sperm correlated significantly with IVF rates (Pearson coefficient r=-0.4556, $P < 0.05$). Interestingly, a predictive analysis showed that hyperpolarizing samples have significantly higher IVF rates (87.4±3.8%) in comparison with depolarizing samples (54.3±13.7%, $P < 0.01$). The area under the curve in a ROC analysis was 0.8571 ($IC_{95\%}$: 0.6529; 1), indicating that Em from capacitated sperm is a good predictor for IVF outcomes.
Conclusion: Normospermic samples hyperpolarize during capacitation. This Em shift is important for the acquisition of acrosomal responsiveness. Accordingly, our study shows that Em hyperpolarization is associated with successful IVF rates. Results presented herein set up the basis for the usage of Em measurements as a useful tool to predict the success rate of IVF procedures.

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PERCENTAGE OF SPERM THAT CAPACITATE PROSPECTIVELY PREDICTS PREGNANCY AND IDENTIFIES REDUCED FERTILIZATION CAPACITY IN A HIGH PERCENTAGE OF MEN CONSIDERED NORMOZOOSPERMIC

Alexander Travis[1,2], Fady Sharara[3], Eric Seaman[4], Randy Morris[5], Jay Schinfeld[6], Jennifer Nichols[6], Michael Sobel[6], Annette Lee[6], Stephen Somkuti[6], Steven Hirshberg[6], Tara Budinetz[6], Larry Barmat[6], Gianpiero Palermo[7], Zev Rosenwaks[7], Natan Bar-Chama[8], Joshua Bodie[9], John Nichols[10], John Payne[10], Travis McCoy[10], Edward Tarnawa[10], Gail Whitman-Elia[10], Lauren Weissmann[11], Maria Doukakis[12], Josua Hurwitz[12], Mark Leondires[12], Cynthia Murdock[12], Ilana Ressler[12], Spencer Richlin[12], Shaun Williams[12], Matthew Wosnitzer[13], Michael Butcher[14], James Kashanian[15], Peter Ahlering[16], Mira Aubuchon[16] and G Ostermeier[2]
[1]Master of Public Health Program, Cornell University, Ithaca, NY, USA; [2]Androvia LifeSciences, Mountainside, NJ, USA; [3]Virginia Center for Reproductive Medicine, Reston, VA, USA; [4]New Jersey Urology, Millburn, NJ, USA; [5]IVF1, Naperville, IL, USA; [6]Abington Reproductive Medicine, Abington, PA, USA; [7]Weill Cornell Medicine, Ronald O. Perelman & Claudia Cohen Center for Reproductive Medicine, New York, NY, USA; [8]RMA New York, New York, NY, USA; [9]University of Minnesota, Urology, Minneapolis, MN, USA; [10]Piedmont Reproductive Endocrinology Group, Greenville, SC, USA; [11]South Jersey Fertility Center, Marlton, NJ, USA; [12]RMA Connecticut, Norwalk, CT, USA; [13]Yale New Haven Health/Northeast Medical Group, Fairfield, CT, USA; [14]Park Nicollet Sexual Medicine & Male Infertility Clinic, St. Louis Park, MN, USA; [15]Weill Cornell Medicine, Urology, New York, NY, USA; [16]MCRM Fertility, Chesterfield, MO, USA
Presented By: Alexander Travis, PhD, VMD

Introduction & Objective: Infertility is often viewed as a "women's health" problem, although men contribute to approximately half the cases. Current evaluations leave many infertile men diagnosed as having idiopathic infertility, resulting in repeated calls for the development of a test of sperm fertilizing ability. Cap-Score™, the percentage of sperm that can capacitate, prospectively identified low versus normal male fertility. An equation explaining the relationship between Cap-Score and the probability of generating a pregnancy (PGP) within 3 cycles (Schinfeld et al, 2018; n = 124; 5 clinics) was established. Here, we prospectively tested the equation and assessed the

prevalence of reduced sperm function/fertilizing ability in men questioning their fertility (MQF).
Methods: PGP and pregnancy outcomes from 6 clinics (n = 128 couples with idiopathic infertility; women had largely non-remarkable workups; 33.5 ± 3.6 mean female age ± SD; men screened by semen analysis (SA)) were rank ordered by Cap-Score and then evenly divided into 5 groups. The previously defined equation prospectively predicted each man's PGP. A chi-square goodness of fit compared the observed and predicted pregnancies. Evaluation of the Akaike Information Criterion determined the best model. Cap-Score was performed on 2,155 MQF from 22 clinics (traditional SA data available for 1,948), which were binned by PGP (≤19, 20–29, 30–39, 40–49, 50–59, ≥60) and then compared to 76 fertile men (Chi-Square and t-test).
Results: No difference was detected between predicted and observed pregnancies ($P = 0.809$). The best prediction model used Cap-Score alone, with SA measures contributing minimally. MQF had reduced PGP and Cap-Score distributions when compared to fertile men ($P < 0.001$). For illustrative purposes, 64% of normozoospermic MQF (757/1,183), had PGP ≤39% (Cap-Scores ≤31), versus 25% of fertile men. Low Cap-Scores were equally likely among men passing single or multiple SA metrics, or having $>10 \times 10^6$ total motile cells ($P = 0.987$).
Conclusion: Sperm capacitation ability accurately predicted PGP and provides clinicians and patients with new information to inform discussions regarding their best pathway to parenthood. Cap-Score alone was the best predictor of pregnancy outcome, indicating that capacitation serves as a key male fertility metric. Reduced sperm function/fertilizing ability is common in MQF and cannot be detected by SA. These data show that a test of sperm capacitation offers a powerful complement to SA, capable of identifying normozoospermic men with reduced sperm fertilizing ability.

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VARICOCELE ALTERS CAPACITATION ON VIABLE SPERM

Rhayza Andretta[1], Letícia Castro[2], Jheysson Moura[1], Renata Carvalho[1], Fatima Okada[1] and Ricardo Bertolla[1]
[1]Federal University of Sao Paulo, Sao Paulo, Brazil; [2]University of Sao Paulo, Sao Paulo, Brazil
Presented By: Rhayza Andretta, PhD

Introduction & Objective: Varicocele is defined as an abnormal dilation of the testicular veins in the pampiniform plexus with retrograde blood flow in the internal spermatic veins. It is considered one of the main causes of male infertility, and is responsible for alterations to semen quality, leading to failures in the processes associated with fertilization, such as sperm capacitation. Many changes occur during sperm capacitation, such as cholesterol efflux, calcium and bicarbonate influx, changes in motility pattern and acrosome reaction. However, studies associated to the varicocele and viable sperm capacitation are scarce in the literature. Objective: To evaluate the effects of varicocele on sperm capacitation in viable sperm.
Methods: Semen samples from controls (n = 19) and men with varicocele (n = 57) were collected by masturbation after 2–5 days of ejaculatory abstinence. Semen samples were submitted to a discontinuous density gradient (layers 90 and 45%), 300 xG, for 20 minutes, 37°C. The pellet was then washed in culture media HTF supplemented with BSA (1%) for 10 minutes. . An aliquot was used to evaluate motility by computer-assisted sperm analysis (CASA). Capacitation was then induced using culture media HTF supplemented with BSA 1% (w:v) for 2 hours at 37°C, 5% CO_2. Following capacitation induction, the following measures were assessed: computer – assisted sperm analysis (CASA), capacitation state, acrosome reaction, membrane integrity, intracellular superoxide anion activity and mitochondrial activity. For statistical analysis, groups were compared using a Student's t test for independent samples. CASA results, which were assessed before and after capacitation were assessed using a General Linear Model (GLM) for repeated measures test. For acrossomal status analyses, which were assessed after sperm capacitation, GLM test was applied. Results were considered statistically significant when $P < 0.05$.
Results: After processing, the varicocele group showed lower mitochondrial activity and capacitation than control group. The capacitation period acts on motility, in path velocity and straightness both groups (control and varicocele). On the other hand, the other variables evaluated no significant differences were observed
Conclusion: Varicocele is associated with lower mitochondrial activity and lower sperm capacitation even after sperm selection by discontinuous density gradient method. Induction of capacitation altered motility, by increasing path velocity and decreasing straightness. There was no interaction of capacitation effects in men with varicocele.

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SINGLE-CELL RNA SEQUENCING REVEALS MARKERS OF STEM/PROGENITOR SPERMATOGONIA IN HIGHER PRIMATES

Sarah Munyoki[1], Adrienne Shami[2], Qianyi Ma[2], Xianing Zheng[2], Jun Li[2], Sue Hammoud[2] and Kyle Orwig[1]
[1]Department of Obstetrics, Gynecology and Reproductive Sciences, Integrative Systems Biology Graduate Program, Magee-Womens Research Institute, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA; [2]Department of Human Genetics, University of Michigan, Ann Arbor, MI, USA
Presented By: Sarah Munyoki

Introduction & Objective: Spermatogonial stem cells (SSCs) are essential for continuous sperm production throughout an adult males' reproductive life. Mouse SSCs can be transplanted into the testis of an infertile male mouse, regenerating spermatogenesis and restoring fertility. Thus, SSC transplantation is a promising fertility therapy for the human clinic. However, while the basic processes of spermatogenesis are conserved from rodents to primates, there is divergence in SSC phenotype and spermatogenetic lineage development. Furthermore, mouse SSC culture conditions do not support the survival and/or expansion of primate SSCs. Identifying the specific molecular mechanisms regulating primate SSC function, will enable the development of human SSC based fertility therapies.

