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THE EXTRACELLULAR CUMULUS MATRIX DOUBLES THE SPERM ZONA-ADHESION IN NORMOZOOSPERMIC PATIENTS. Rumiana Ganeva, MSc, Dimitar Parvanov, PhD, Kristina Nikolova, MSc Magdalena Vasileva, MSc, Ferihan Shaban, MSc, Georgi Stamenov Stamenov, MD/PhD. Nadezhda Women's Health Hospital, Sofia, Bulgaria.

OBJECTIVE: The aim of this study was to observe the effect of the cumulus extracellular matrix on the sperm zona-adhesion rate in healthy fertile men.

DESIGN: Comparison of the zona-adhesion rate between spermatozoa treated with cumulus extracellular matrix and non-treated spermatozoa.

MATERIALS AND METHODS: The cumulus matrix proteins used in this study were isolated from 150 cumulus complexes that were obtained from 16 donors during oocyte retrieval procedures. The cumulus cells and their extracellular matrix were separated by pipetting followed by centrifugation. The protein content in the pool of isolated cumulus matrixes (CM) was measured by Bradford method. Semen samples were obtained from 30 normozoospermic donors. After sperm washing, the motile spermatozoa were isolated by swim-up and diluted to $0.5x10^6$ cells/ml. Each sample was divided into four aliquots and incubated with (1) 0.5 mg/ml CM, (2) 1.25 mg/ml CM, (3) 2.5 mg/ml CM and (4) wash medium for 30 min at 37ºC. The zona-adhesion rate was evaluated by counting the adhered spermatozoa to immobilized acid-solubilized zonae pellucidae from healthy donors. Results are presented as number of adhered spermatozoa per 1 mm^2 of the immobilized surface (sp/mm^2). Statistical analysis was performed with paired t-test using IBM SPSS Software ver.21.

RESULTS: The zona-adhesion rate of the untreated spermatozoa was 81 ± 17 sp/mm^2 (Mean ± SD) and ranged between 54 sp/mm^2 and 116 sp/mm^2. CM treatment of the spermatozoa dose-dependently and significantly increased the zona-adhesion rate in every patient ($p<0.05$). When spermatozoa were treated with 2.5 mg/ml CM, 1.25 mg/ml CM and 0.625 mg/ml the mean sperm zona-adhesion was 128 ± 28 sp/mm^2, 107 ± 37 sp/mm^2 and 99 ± 27 sp/mm^2, respectively.

CONCLUSIONS: The results from this study show the important role of the cumulus matrix in the preparation of the spermatozoa before meeting the oocyte and confirm that the cumulus effect should be considered during sperm processing for ICSI.

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VARICOCELE DIMINISHES SPERM CAPACITATION FUNCTION AND THE CHANCES OF GENERATING A PREGNANCY. Philip Xie, B.S.,[a] Alessandra Parrella, M.Sc.,[a] Alexander J. Travis, VMD, PhD,[b] Zev Rosenwaks, M.D.,[a] James A. Kashanian, MD,[c] Gianpiero D. Palermo, M.D., Ph.D.[a] [a]The Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Cornell Medicine, New York, NY; [b]Cornell University, Ithaca, NY; [c]Weill Cornell Medicine, Department of Urology, New York, NY.

OBJECTIVE: To determine whether varicocele can adversely affect sperm capacitation and therefore the probability of generating a pregnancy (PGP).

DESIGN: In 8 consenting men with grade 2 varicoceles or larger, we assessed functional semen characteristics by using Cap-Score™ to measure the percentage of sperm that can capacitate, and calculated the related PGP calculation. Ten men with normal semen parameters, no varicoceles, and proven fertility served as a control. Cap-Score was determined in a blind fashion regarding presence/absence of varicocele.

MATERIALS AND METHODS: Semen analyses were performed on fresh ejaculates of 18 consenting men. Those men in the control group did not have a history of varicocele nor pertinent urological issues. Presence of a varicocele was determined by physical exam in the standing position. All men in the varicocele group were diagnosed with a unilateral or bilateral varicocele of grade 2 or higher. Sperm capacitation was measured by Cap-Score™ assay (Androvia LifeSciences) with a normal threshold of >27.6%. To quantify the actual number of capacitated spermatozoa, Cap-Score x volume x concentration was calculated. PGP was determined by the corresponding Cap-Score™, with a threshold of >32.7% considered normal. Semen parameters, Cap-Score™, total capacitated spermatozoa and PGP were compared between control and varicocele group using unpaired *t* tests at 0.05, with 0.05 considered significant.

RESULTS: Men in the control group (n=10) and those with varicocele (n=8) were of comparable age (34.7 ± 2.8 years and 36.0 ± 7.0 years, respectively). Semen parameters including volume (2.9 ± 0.9 ml), concentration (69.8 ± 26.3 $x10^6$/ml), motility (47.7 ± 2.8%) and normal morphology (3.1 ± 0.7%) in the control also did not differ from those men with varicocele (volume of 3.1 ± 1.1 ml, concentration of 45.4 ± 30.9 $x10^6$/ml, motility of 45.6 ± 1.0% and normal morphology of 2.8 ± 1.0%). The control group had an average Cap-Score™ of 31.4 ± 4.8% while that of the varicocele group was 26.4 ± 3.7% ($P = 0.03$). There was a significantly higher number of capacitated spermatozoa in the control group (67.1 ± 40.1 $x10^6$) when compared to the varicocele group (32.8 ± 22.4 $x10^6$) ($P = 0.04$). In the control group there was an average PGP of 39.7 ± 9.1%, while men with varicocele yielded a PGP of 31.0± 6.0% ($P = 0.03$).

CONCLUSIONS: Scrotal varicocele is known to induce male infertility by impairing sperm production and inducing sperm chromatin fragmentation. While the impact of varicocele on actual sperm function is unclear, in this study, we demonstrate for the first time that venostasis may be responsible for lessening one of the prominent sperm functions such as capacitation.

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IMPACTS OF TEST (TES AND TRIS) YOLK BUFFER AND COOLING ON THE ABILITY OF HUMAN SPERM TO CAPACITATE. G. Charles Ostermeier, PhD,[a] Cristina Cardona, PhD,[a] Melissa A. Moody, MS,[a] Alana J. Simpson, BS,[a] Romeo Mendoza, MT,[a] Alexander J. Travis, VMD, PhD.[b] [a]Androvia LifeSciences, Mountainside, NJ; [b]Cornell University, Ithaca, NY.

OBJECTIVE: Studies across several mammalian species show that G_{M1} localization patterns are indicative of capacitation at the single cell level. The Cap-Score™ Male Fertility Assay reports the proportion of sperm displaying G_{M1} localization patterns consistent with capacitation. Using clinical pregnancy outcomes, Cap-Score was previously shown to prospectively predict a man's fertility and the relationship between Cap-Score and a man's probability of generating a pregnancy was established. TEST (TES and Tris) yolk buffer (TYB) can prolong the fertilization capacity of sperm. Here, we evaluated whether incubation in TYB overnight at a cool temperature affected human sperm capacitation.

DESIGN: To evaluate the impact of semen extension with TYB and cooling on sperm capacitation, ejaculates were split into control and test samples for a repeated measure design.

MATERIALS AND METHODS: Studies approved by WIRB (20152233). Semen was collected, liquefied and split into control and test samples. Control samples were processed normally for Cap-Score. Test samples were extended in TYB at 1:1 (n=5), 1:6 (n=7) or 8:5 (n=5; volume ratio of semen:TYB) and cooled overnight in a Styrofoam box with an ice pack. The next day, samples were washed, exposed to non-capacitating (NC) or capacitating (CAP) conditions for 3 hrs, and then fixed overnight before Cap-Score determination. Test-samples were compared to controls using paired t-tests.

RESULTS: In all experiments, Cap-Score was greater for control-CAP when compared to control-NC ($p<0.05$). No differences were observed between the control-CAP and the test-CAP for any dilution (1:1 ratio: 39.7±0.04 vs 40.0±0.02%; p=0.87; 1:6 ratio: 32.0±0.04 vs 34.0±0.03%; p=0.33; 8:5 ratio: 36.0±0.02 vs 34.2±0.01%; p=0.5).

CONCLUSIONS: A good capacitation response was observed in the controls for all experiments, suggesting proper stimulus by the CAP condition. The ratios of semen:TYB were chosen to mimic typical ejaculate volumes, such that a constant volume of extender could potentially be utilized in an at home semen collection kit that maintains sperm capacitation ability. Addition of a fixed volume of TYB to varying ejaculate volumes would limit user input. Similar Cap-Score values between the control-CAP and test-CAP, no matter the ratio, indicates that ejaculates can be maintained overnight in varying concentrations of TYB with minimal impact on next-day function. At home sample collection could lessen the burden of processing samples at clinics with limited resources. It could also encourage pursuit of workup by men whose main barrier is privacy in producing samples at clinics or bringing them to clinics. It could also broaden the geographical availability of sperm function tests to those living far from clinics, and reduce financial burdens associated with travel and time away from work.

SUPPORT: Androvia LifeSciences.

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RELATIONSHIP AMONG INTRACELLULAR SUPEROXIDE DISMUTASE ACTIVITY, GLUTATHIONE PEROXIDASE ACTIVITY, MOTILITY AND MORPHOLOGY IN HUMAN SEMEN. Luchezar Vasilev Jelezarski, PhD, Dimitar Parvanov, PhD, Vilyana Georgieva, MSc, Rumiana Ganeva, MSc,