detected more sperm heads with fluorescent DNA in samples with a high fixed particle background. Entrained software (Celeste) could distinguish between sperm heads and somatic cell nuclei and quantify each. However, with some cross-over between the lobed nuclei of granulocytes and sperm heads in the 5% of post-vasectomy specimens contain cells. Immunostaining the post-vasectomy specimens containing cells distinguishes the class of leukocytes from other somatic cells.

Conclusion: Fluorescent-staining sperm head DNA in aldehyde-fixed, mail-in semen specimens improves the accuracy of sperm counts in post-vasectomy specimens with a high background. In addition, the identity of fixed cells in the specimens may reveal valuable information about male reproductive health after vasectomy.

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MODIFYING CAPACITATION ABILITY THROUGH LIFESTYLE CHANGES

Fady Sharara[1], Anna Lysenko-Brockman[1], Haneen Taha[1], Melissa Moody[2], G Ostermeier[2], Alexander Travis[2,3]

[1]*Virginia Center for Reproductive Medicine, Reston, VA, USA,* [2]*Androvia LifeSciences, Mountainside, NJ, USA,* [3]*Cornell University, Ithaca, NY, USA*

Presented By: Fady Sharara, MD

Introduction & Objective: Reports suggest that traditional semen analysis parameters have been declining, although impacts on male fertility are unclear. Lifestyle practices and environmental exposures might be contributing to such declines. Traditional semen analysis (SA) assesses spermatogenesis, accessory gland contributions, and descriptive parameters of motility, concentration, and morphology. However, it fails to evaluate sperm function and to diagnose at least half the cases of male infertility. To fertilize, sperm must complete the process of capacitation. Cap-Score™ not only quantifies capacitation ability and functionally assess male fertility, it also prospectively predicts a man's ability to generate pregnancy.

Objective: Determine the ability of lifestyle changes to moderate capacitation ability and SA measures.

Methods: Cap-Score was determined in blinded fashion for 38 men seeking fertility assistance. Their lifestyle was subsequently changed by quitting use of tobacco, marijuana, or alcohol; avoiding laptops on laps or Jacuzzis/saunas; losing weight if obese; increasing Vitamin D intake (at least 2k/day); and starting supplements (Androferti (n = 30; vitamins C, E, B12, Folate, Zinc, selenium L-carnitine, and coenzyme Q10), or Conception XR (n = 8; vitamins C, E, D, Folate, Zinc, selenium, and Lycopene)). A second blinded analysis was done approximately 10 weeks after starting this change. To determine supplement impact, two-sample t-tests were done on the difference between the first and second reading. The impacts of lifestyle changes were determined using paired t-tests comparing the first to the second analysis. Linear regression was used to assess the relationship between Cap-Score and strict normal morphology.

Results: Both supplements had a similar impact on all measures ($p > 0.05$). An increase in Cap-Score from 24.2±1.2 to 27.9±1.2 ($p = 0.016$), corresponding to a 20% increase in a man's probability of generating a pregnancy, was observed after lifestyle changes. Lifestyle changes had no impact on the following SA measures: semen volume ($p = 0.527$), sperm concentration ($p = 0.547$), sperm motility ($p = 0.202$), and total motile sperm ($p = 0.535$). In contrast, strict normal morphology improved (1.7±0.2 to 3.3±0.5; $p = 0.001$). No relationship was detected between Cap-Score and strict normal morphology before ($p = 0.566$) or after ($p = 0.156$) lifestyle changes.

Conclusion: These data support the view that promoting a man's overall health by quitting smoking, drinking, marijuana and losing weight, in combination with nutritional supplements, is linked to changes in sperm capacitation ability and an increase in male fertility.

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PROTEOMIC ANALYSIS OF TESTICULAR AND EPIDIDYMAL SPERM FROM BULLS WITH A HIGH PERCENTAGE OF MIDPIECE ABNORMALITIES

Arabela Viana[1], Edgar Andrés Díaz-Miranda[1], Nara Clara Lazaroni Merchid[2], Alexandre Dutra[3], André Ferreira[4], Jonas Aguiar[4], José Guimarães[1], Mariana Machado-Neves[1,2]

[1]*Department of Veterinary, Universidade Federal de Viçosa, Viçosa, MG, Brazil, Viçosa, Brazil,* [2]*Department of General Biology, Universidade Federal de Viçosa, Viçosa, MG, Brazil., Viçosa, Brazil,* [3]*Federal University of Jequitinhonha Valley and Mucuri, Brazil., Teófilo Otoni, Brazil,* [4]*Laboratory of Toxicology, FIOCRUZ, Brazil., Rio de Janeiro, Brazil*

Presented By: Arabela Viana, DVM, MS

Introduction & Objective: Morphological abnormalities in ejaculated sperm indicate alterations in testicular and epididymal functions. Midpiece defects including aplastic midpiece defect (AMD) may compromise male fertility by reducing sperm motility and generating oxidative stress. Herein, we aimed to characterize the protein profile of bovine sperm with AMD, from their production in the testis to their maturation in the epididymis.

Methods: Testis and caput and cauda epididymis from six bulls (*Bos indicus*) were included in this study. The animals were divided into two groups, classified as satisfactory potential breeders (control; n = 3) and unsatisfactory potential breeders (AMD; n = 3), due to the presence of AMD in the semen. The organ fragments were placed in Petri dishes containing BWW media. After incubation, spermatozoa were recovered, filtered, and centrifuged in Percoll® 30%. The cells were washed in red blood cell lysis buffer and BWW medium until removing contaminating cells. Spermatozoa from control and AMD bulls were pooled, and their proteins were extracted and analyzed by LTQ Orbitrap XL mass spectrometry. Data were processed (Peaks Studio 8.5) and proteins were identified using Uniprot and NCBI repositors. A log 2-fold change (log2FC) was carried out to identify differentially expressed sperm proteins between control and AMD bulls. The enrichment of metabolic pathways was accessed using the DAVID platform.