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NORMAL EJACULATES, AS DEFINED BY WHO, CAN HAVE ABNORMAL CAPACITATION ABILITY.

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Introduction: The diagnosis of male infertility is based predominantly on the results of standard semen analysis. However, standard semen analysis provides little information on sperm functional competence. Localization of the ganglioside GM1 (Cap-ScoreTM) identifies sub-populations of sperm capable of undergoing the functional maturation process known as capacitation and tracks strongly with fertility (Paniza et al., ASRM 2014). Here Cap-Score results were compared to standard semen analysis parameters obtained from men questioning their fertility.
Methods: Semen samples from consenting patients were liquefied, washed and incubated under both non-capacitating and capacitating conditions. Semen analysis was performed according to WHO guidelines. Cap-Score values were obtained via fluorescence microscopy. Statistical analyses were done using Microsoft Excel (2013) and XLSTAT (2015).
Results: Samples from 84 men referred to an infertility specialist were analyzed and had Cap-Scores ranging from 13 to 52% (mean from a population with known fertility was 39, ±7% (n=41)). An analysis of variance was done to compare Cap-Scores and sperm morphology. Samples were classified as having 0, 1, 2, 3, or ≥ 4% normal forms (scores ≥ 4% are considered normal, WHO) and mean Cap-Scores were compared among the groups. No relationship between Cap-Score and morphology was observed (P=0.67). Next, sperm concentration (range 3x106 to 210x106/mL) was compared to Cap-Score using the Pearson product-moment correlation coefficient and no connection was found (r=0.01, P=0.90). Lastly, Cap-Score was compared to total % motility (range 15 to 80%) and the two measures were determined to be independent (r=0.14 P=0.21). Multiple donors who were classified as normal by WHO criteria had Cap-Scores more than 2 SD below the normal mean, supporting the view that even normal appearing sperm can have functional abnormalities.
Conclusion: Traditional semen analysis identifies only 50% of male infertility cases. This study shows that there is little relationship between Cap-Score™ and standard semen analysis parameters. Since capacitation is necessary for fertilization, the addition of Cap-Score to traditional semen evaluations could both identify cases of idiopathic infertility and help clinicians counsel couples towards the most appropriate treatment.
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IDENTIFICATION OF BOVINE SPERM ACROSOMAL PROTEINS THAT INTERACT WITH A 32KDA ACROSOMAL MATRIX PROTEIN

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Mammalian fertilization is accomplished by the interaction between sperm and egg. Previously, we have identified a stable acrosomal matrix assembly from the bovine sperm acrosome termed the outer acrosomal membrane-matrix complex (OMC). This stable matrix assembly exhibits precise binding activity for acrosin and N-acetylglucosaminidase. A highly purified OMC fraction is comprised of three major (54, 50, and 45kDa) and several minor (38-19kDa) polypeptides. The set of minor polypeptides (38-19kDa) termed "OMCrpf polypeptides" is selectively solubilized by high-pH extraction (pH 10.5) while the three major polypeptides (55, 50 and 45kDa) remain insoluble. Proteomic identification of the OMC32 polypeptide (32kDa polypeptide isolated from high-pH soluble fraction of OMC) yielded two peptides that matched the NCBI database sequence of acrosin-binding protein. Anti-OMC32 recognized an antigenically related family of polypeptides (OMCrpf polypeptides) in the 38-19kDa range with isoelectric points ranging between 4.0 and 5.1. Other than glycohydrolases, OMC32 may also be complexed to other acrosomal proteins. The present study was undertaken to identify and localize the OMC32 binding polypeptides and to elucidate the potential role of the acrosomal protein complex in sperm function. OMC32 affinity chromatography of a detergent soluble fraction of bovine cauda sperm acrosome followed by mass spectrometry-based identification of bound proteins identified acrosin, lactadherin, SPACA3, and IZUMO1. Co-immunoprecipitation analysis also demonstrated the interaction of OMC32 with acrosin, lactadherin, SPACA3, and IZUMO1. Our immunofluorescence studies revealed the presence of SPACA3 and lactadherin over the apical segment; whereas, IZUMO1 is localized over the equatorial segment of Triton X-100 permeabilized cauda sperm. Immunoblot analysis showed that a significant portion of SPACA3 was released after the lysophosphatidyl choline (LPC)-induced acrosome reaction; whereas, the IZUMO1 and lactadherin polypeptides remain associated to the particulate fraction. Almost entire population of bovine sperm IZUMO1 relocates to the equatorial segment during the LPC-induced acrosome reaction. We propose that the interaction of OMC32 matrix polypeptide with detergent soluble acrosomal proteins regulates the release of hydrolases/other acrosomal protein(s) during the acrosome reaction.