Journal of Andrology, Vol 14, Issue 4 289-297, Copyright © 1993 by The American Society of Andrology

JOURNAL ARTICLE

Validation of an acrosomal stain for equine sperm that differentiates between living and dead sperm

P. J. Casey, R. B. Hillman, K. R. Robertson, A. I. Yudin, I. K. Liu and E. Z. Drobnis

Department of Reproduction, School of Veterinary Medicine, University of California, Davis 95616.

An acrosomal staining technique that can differentiate between living and dead sperm was developed for equine sperm. The fluoresceinated lectin Pisum sativum agglutinin (FITC-PSA) was used to identify the presence or absence of acrosomal contents, while the supravital nuclear dye Hoechst 33258 (H258) was used to assess viability. The accuracy of the FITC-PSA acrosomal stain was tested by comparing the percentage of sperm that had lost their acrosomal contents, detected by the staining method, with that detected by transmission electron microscopy (TEM). Following capacitation in vitro, the acrosomal status of sperm induced to acrosome react with A23187 and of control sperm were very similar with the staining technique and TEM, confirming the accuracy of the FITC-PSA acrosomal stain. We investigated the relationship between viability as measured by exclusion of H258 and motility as measured by three methods: one subjective and two objective. Although there was a good correlation between viability and motility as measured by all three methods (r = 0.88, 0.85, 0.75), there was always a proportion of viable sperm that were nonmotile. The physiology of the viable, nonmotile sperm was further investigated by comparing for individual sperm the viability as measured by exclusion of H258 with the mitochondrial function as measured by rhodamine 123. A good correlation (r = 0.99) was found to exist between viability and mitochondrial function, indicating that viable, nonmotile sperm possess functional mitochondria and confirming the ability of supravital staining to distinguish between living and dead sperm. We determined that 29-81% of the sperm in semen that had lost their acrosomal contents were in fact dead. Thus, this acrosomal staining technique can provide more relevant endpoints for future investigations of capacitation, the acrosome reaction, and sperm handling techniques in the horse.