Bulletin UASVM, Veterinary Medicine 67(1)/2010
ISSN 1843-5270; Electronic ISSN 1843-5378

Canine Sperm Simultaneous Fluorometric Assessment of Plasma and Mitocondrial Membranes

J. DORADO[1], M.J. GÁLVEZ[1], M.R. MURABITO[1], S. DEMYDA[2,4], M. MORENO[3], M. HIDALGO[1]

Animal Reproduction Group, University of Cordoba, Spain[1]; Dairy Production Department, National University of Lomas de Zamora, Argentina[2]; Department of Genetics, University of Cordoba, Spain[3]; MAEC-AECID grant holder[4]

SUMMARY

This study was conducted to apply a simple technique for the simultaneous integrity evaluation of plasma membrane and mitochondrial function in canine spermatozoa using the association of fluorescent probes. Nine ejaculates from 4 dogs were collected by masturbation and pooled (3 ejaculates/pool). The pooled semen samples were diluted in Tris buffer added to 20% centrifuged egg yolk and cooled to 5ºC over 96 h. Samples were stained with propidium iodine (PI), acridine orange (AO) and rhodamine 123 (R123) association and evaluated by epifluorescence microscopy. Motility parameters assessed objectively by a CASA system (Sperm Class Analyzer) were: total and progressive motility, curvilinear velocity, average path velocity, progressive speed, lateral head displacement and beat cross frequency. Each semen sample was evaluated at 0 and 96 h of preservation. Data were statistically analysed by independent samples t-test and bilateral correlation was established between those parameters. The association of fluorescent probes resulted in the classification of sperm cell into 4 categories: intact plasma membrane and mitochondrial function (PI-/AO+/R123+); intact plasma membrane and without mitochondrial function (PI-/AO+/R123-), damaged plasma membrane and mitochondrial function (PI+/AO-/R123+), and damaged plasma membrane and without mitochondrial function (PI+/AO-/R123-). Significant effects ($P<0.001$) were observed between fresh and cooled-rewarmed semen samples for the mean percentage of spermatozoa showing plasma membrane integrity and mitochondrial function. The majority of CASA-derived parameters showed a high correlation with PI-AO-R123-stained sperm. We concluded that this association of fluorescent probes reflects the functional status of plasma membrane and mitochondria of dog semen and it is able to separate 4 cell populations.