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#### ACCEPTED MANUSCRIPT

### POST ARTIFICIAL INSEMINATION ENDOMETRIAL INFLAMATION AND ITS CONTROL IN DONKEYS

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#### Abstract

Donkey frozen semen shows good spermatozoa (spz) survival results, however fertility results are very bad in jennies. Donkey frozen semen is able to penetrate oocytes in vitro and to fertilize mares. Jennies express a high endometrial inflammatory response 6h after AI with frozen semen. High amount of polymorphonuclear neutrophils (PMNs) cytological smears and biopsies, and intense cyclooxygenase-2 (COX-2) in immunohistochemical labelling were detected. In vivo and in vitro experiences showed that seminal plasma (SP), eliminated during semen freezing process, has an important role in modulating this response. SP reduces the PMN-spz binding in vitro. Moreover, SP decreases COX-2 expression and eosinophils and neutrophils migration in vivo. In vitro, PMN-spz incubation showed that SP is able to activate PMNs and preserves sperm survival and motility. After 3-4h of incubation, the main part of spz attached to PMNs were alive, and several attached spz set free had good motility parameters when assessed by CASA system. Fractioning SP attending proteins molecular weight (MW), we observed that low MW fractions (3-10 kDa) induced a rapid spz death, however fractions between 30-100 kDa showed an interesting maintenance of survival and motility as well as a control of reactive oxygen species (ROS) production. Moreover, donkey SP has melatonin and donkey spz have melatonin receptors. Spz produce ROS but PMNs produce much more. Therefore, in understanding the role of SP fractions on sperm survival and the antioxidant effect of SP, melatonin and other antioxidant SP compounds are very important to increase donkey frozen semen AI results.

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