ABSTRACTS

ISCFR 2012

July 26-29, Whistler, Canada

7th International Symposium
on
Canine and Feline Reproduction

In a joint meeting with

EVSSAR 2012

15th Congress of the
European Veterinary Society for Small Animal Reproduction

Editors: Gary England, Michelle Kutzler, Pierre Comizzoli, Wojciech Nizanski, Tom Rijsselaere and Patrick Concannon

Reprinted in IVIS with the permission of the ISCFR Organizers
Sperm ubiquitination in epididymal feline semen

Vernocchi, V1; Varesi, S1; Sartori, R2; Maffioli, E2; Tedeschi, G2 and Luvoni GC1

1Dipartimento di Scienze Cliniche Veterinarie, 2Dipartimento di Patologia Animale, Igiene e Sanità Pubblica Veterinaria - Università degli Studi di Milano, Via Celoria 10, 20133 Milano, Italy
valentina.vernocchi@unimi.it

INTRODUCTION: Ubiquitin is a 8.5 kDa peptide that tags other proteins for proteasomal degradation and it is also involved in the regulation of protein function. Ubiquitin has been discovered as a normal component of human blood, ovarian follicular fluid and seminal plasma (1). Ubiquitination might be responsible of the elimination of defective spermatozoa during transit through epididymis in humans and cattle (2, 3). Results indicated that the increase of sperm ubiquitin was inversely associated with spermatogenic concentration, motility and normal morphology indicating that ubiquitination could be considered as a biomarker of poor semen quality (3). Conversely, other authors (4) found a positive correlation between sperm ubiquitin and good semen parameters suggesting a different role for sperm ubiquitination. These conflicting data indicate that the exact biological function of this peptide in seminal plasma has not yet been clarified. In the domestic cat (Felis catus) no positive or negative correlations between semen quality and ubiquitination have been observed, although no definitive conclusions about its role have been drawn (5). Magnetic cell separation techniques, based on the use of antibodies or proteins-coated magnetic beads as magnetic ubiquitin beads, may allow the removal of ubiquitinated spermatozoa from the sample contributing to the identification of a potential correlation between ubiquitin and abnormal spermatozoa.

OBJECTIVES AND METHODS: The present study was designed to investigate whether ubiquitin could be considered a biomarker of quality of epididymal feline semen. Morphology and acrosomal integrity of spermatozoa were correlated with ubiquitination of the protein patterns in semen samples treated with magnetic ubiquitin beads. Moreover, the modification of the two major cytoskeletal proteins (actin and tubulin), and of prohibitin, a protein involved in cell cycle which is ubiquitinated in bovine semen (6), was evaluated in details to investigate the possible correlation of sperm morphological alterations and proteins ubiquitination.

Semens samples from 10 healthy and sexually mature cats were collected by squeezing isolated caudae epididymis after routine orchectomy. Samples were divided into two aliquots. Magnetic ubiquitin beads (Li Starfish S.r.l., Cernusco S/N, Milan, Italy) were added to one aliquot at the final concentration of 5 particles/spermatozoa. The suspension in the tube was gently mixed for 15 min before placing it on an external laboratory magnet for 15 min. The sample treated with beads (S+B) was collected while the tube was still in the magnetic field, whereas the “ubiquitinated” spermatozoa bound to the beads remained attached to the wall of the tube as long as the magnet was in place. The second semen aliquot was used as a control and was treated under the same conditions except for the presence of the beads (S-B). Before and after the treatment, sperm cell concentration was determined with a Bürker chamber and semen morphology was assessed following staining with Peanut agglutinin (PNA) conjugated with a fluorescein isothiocyanate (FITC) and propidium iodide (PI). Staining with Bengal Rose and Victoria Blue B on at least 100 spermatozoa per slide. Acrosome integrity was analyzed by assessment of the ubiquitination of sperm proteins was carried out by Western Blot analysis using specific antibodies, namely anti-ubiquitin, anti-actin, anti-tubulin and anti-prohibitin. Each sample was solubilized in lysis buffer (50 mM Tris HCl pH 8.0) and quantified for its protein content by the Bradford method. Mean±SD of sperm characteristics were analyzed by Student’s t-test (p<0.05).

RESULTS: Proportions of morphologically normal spermatozoa were similar in samples treated with ubiquitin beads compared to the control samples (S+B: 52.9±11.9% vs. S-B: 44.5±16.4%). Treated samples did not show an increase of spermatozoa with intact acrosome (S+B: 81.3±13% vs. S-B: 76.5±15.1%), whereas a significant decrease in the total number of spermatozoa (1 x 10^6sp) was observed after the treatment compared to the control (S+B: 6.7±5.2 vs S-B: 12±7.5; p=0.002). Western Blot analysis using anti-ubiquitin antibodies clearly showed that the same pattern of protein ubiquitination was present before and after treatment with the beads. Actin and prohibitin, and not tubulin, were target of ubiquitin in cat semen and the extent of this post-translational modification was comparable in samples treated with beads and in the control.

CONCLUSIONS: The present data suggest that sperm ubiquitination, morphology and acrosomal integrity of feline epididymal spermatozoa are not related. Sperm anomalies at subcellular level, not revealed by the analyses performed in the
present study, should be further investigated before stating that the use of ubiquitin is not a reliable biomarker of semen quality in the domestic cat.

(5) Mota PC, Ramalho-Santos J. Comparison between different markers for sperm quality in the cat: Diff-Quik as a simple optical technique to assess changes in the DNA of feline epididymal sperm. Theriogenology 2006;65:1360–1375.