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## Veterinarni Medicina

**Demonstration of the effect of epidermal growth factor on ram sperm parameters using two fluorescent assays**

Makarevich AV, Kubovicova E, Sirotkin AV, Pivko J:

Veterinarni Medicina, 55 (2010): 581-589

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**ABSTRACT:** The goal of this study was to examine the effect of epidermal growth factor (EGF) on sperm viability using two fluorescent techniques and to analyze the obtained results in relation to sperm motility, determined by subjective estimation. Fresh ram semen diluted in a Biladyl commercial extender was cooling stored (at 4 °C in a fridge) for four days in the presence of EGF at doses of 0, 10, 100, 200 or 400 ng/ml. Thereafter, sperm samples were analyzed for progressive motility (Motility test) and membrane integrity using two fluorescent techniques: SYBR-14/PI (Method 1) or PI/DAPI (Method 2). Application of Method 1 did not detect an effect of EGF

at any concentration on sperm membrane integrity. A positive effect of EGF (200 ng/ml) on sperm membrane integrity was found using Method 2 of staining, and this result was confirmed by the sperm motility test, which demonstrated an EGF-stimulating effect (200 or 400 ng/ml) on a percentage of progressively moving spermatozoa. Strong positive correlations between Methods 1 and 2 ( $r = 0.785$ ), Method 1 and Motility ( $r = 0.803$ ), Method 2 and Motility ( $r = 0.699$ ), as well as between both techniques taken together and the Motility test ( $r = 0.853$ ) were found. Regression analysis confirmed that Method 2 was more exact than Method 1, and the results obtained with Method 2 are comparable with those of the Motility test. Dependence of the viability or motility on EGF concentrations (linear regression function) was significant only for Method 2 or the Motility test. The obtained results suggest a stimulating effect of EGF (at higher concentrations) on ram sperm functions (viability/membrane integrity and motility). Furthermore, they indicate substantial differences between two fluorescent techniques in the determination of sperm

membrane integrity. Only the data obtained using PI/DAPI were confirmed by a functional Motility test. These findings suggest that the technique chosen for analysis of sperm viability can influence the conclusion concerning the effects of the treatment on sperm function.

### **Keywords:**

viability; motility; membrane integrity

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