



# Flow cytometric evaluation of the effect of two homeopathic selenium preparations on mitochondrial activity of bovine spermatozoa



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## INTRODUCTION

Spermatozoa are highly differentiated cells and rich in mitochondria, generating energy especially to support their motility (1). Therefore they seem as a suitable cell model to test the effect of certain drugs, which enhance the mitochondrial activity. Positive results could have two advantages: At first as a possibility to stimulate the oxidative energy metabolism of different body cells and secondly especially that of the sperm cells, because their mitochondrial activity is an important parameter concerning their motility and fertilization capacity (2). Because there are reports about a close correlation between the selenium status or Se-content of sperms and their morphology and motility (3,4), we were interested to investigate the effect of homeopathic selenium containing drugs on the mitochondrial activity of sperm cells.

## MATERIALS AND METHODS

Fresh semen with normal characteristics from two mature bulls was diluted 1:40 with three different diluents (Hepes, Hepes + normal saline and Hepes + homeopathic drugs, 8 samples for each group). The final concentration of the tested homeopathic preparations (Selenium-Injeel forte<sup>®</sup>, Selenium-Homaccord<sup>®</sup>, Heel, Baden Baden) in the diluents were 1:9 and 1:4. The samples were evaluated by flow cytometer (Becton Dickinson, Heidelberg, Germany). The mitochondrial activity was determined by the mean of green fluorescence of Rhodamine 123 (5). To register possible side effects of the drugs, SYBR-14 + PI, LYSO-G and Acridine orange were used to detect the effects on viability, acrosomal integrity and chromatine structure of sperms, respectively(5-7). The data were compared with those of two control groups (one without any addition and one with addition of normal saline). The results were subjected to one way repeated measures analysis of variance using Jandel Sigmastat statistical software V2.0.

## RESULTS AND DISCUSSION

The mitochondrial activity of the sperms was enhanced clearly by both homeopathic drugs and in both concentrations. These distinct increases proved to be statistically significant ( $P < 0.001$ ). But there was no significant difference in the degree of stimulation between the two drugs and their two concentrations (Figure 1). The explanation for these results could be an antioxidative effect of these selenium containing drugs against spontaneous lipid peroxidation and protection of the mitochondria and the other membrane systems of the sperms via increased glutathionic peroxidase activity, as Kendall et al (8) supposed from their experiments with Se-supplementation in rams, however they were not Se-deficient. Another interpretation of the results are the principles of Hormesis or Homeopathy which mean, that toxic agents as Se become stimulatory or protective, if they are used in high or ultra-high dilutions (9).

The results of the simultaneously measured sperm parameters: percentage of sperm with active mitochondria (Figure 2), viability (Figure 3), acrosomal integrity (Figure 4) and sperm chromatine structure (Figure 5) did not show an important and significant difference to the control samples. This suggests, that the addition of the drugs had no negative side effects on the sperms. This seems important, because Se in other form and concentration, such as sodium selenite, tightly adhered to the sperms and decreased their motility, when it was added to the semen extender (10).

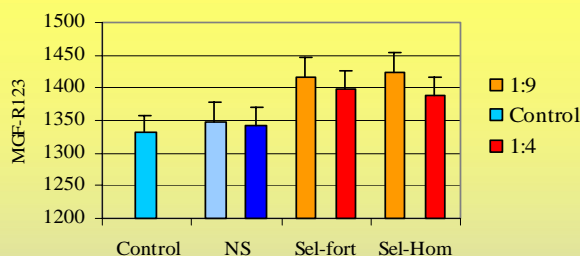


Figure 1: MGF-R123 of the three tested groups, (n=8).

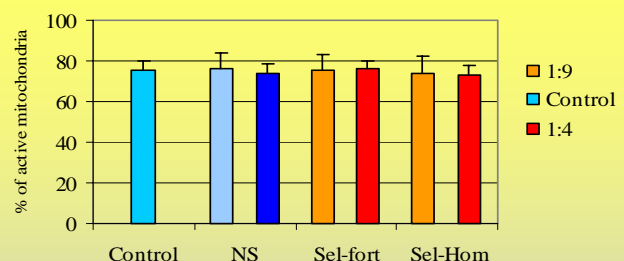


Figure 2: Percentage of sperm with active mitochondria of the three tested groups, (n=8).

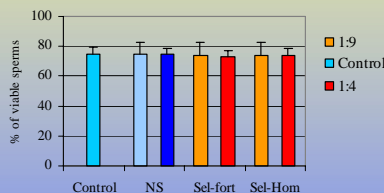


Figure 3: Percentage of viable sperm of the three tested groups, (n=8).

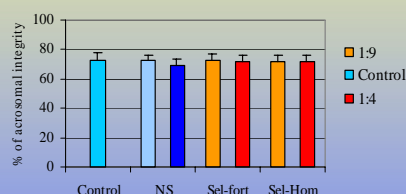


Figure 4: Acrosomal integrity of the three tested groups, (n=8).

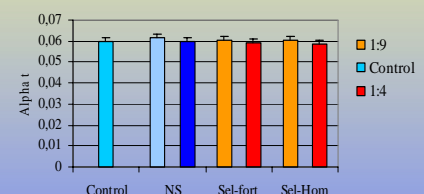


Figure 5: SCSA (Alpha t) of the three tested groups, (n=8).

## CONCLUSION

From the presented results we conclude, that the application of the tested homeopathic selenium-containing drugs offers an effective and safe way to stimulate the mitochondrial activity of cells. This can be used: 1. In the context with artificial insemination. 2. In any case, where the energy metabolism needs support. 3. This investigation is also contribution to reduce the use of experimental animals.

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