



# Bovine spermatozoa a suitable cell model for pharmacological studies: effects of some homeopathic drugs on mitochondrial activity and other important parameters of cell function

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## INTRODUCTION

Animal protection requires efforts for expanding the range, where pharmacological studies with animals can be replaced by appropriate cell models. According to our considerations bovine spermatozoa seemed to be a suitable cell model, especially for testing drugs, which should influence the oxidative energy metabolism.

Their special properties respective advantages are: They are primary, mature, differentiated mammalian cells with all essential cell elements and especially rich in mitochondria (1). From their central role in the integrative coordination of metabolism pathways and production of oxidative energy results their comprehensive importance for the health, the fitness and the development of different pathogenic processes in man, animals and plants. Therefore the results we get in pharmacological studies with spermatozoa must be transferable to other

mammalian cells respective organs, especially to those with a high metabolic rate, as liver cells, nerve cells, muscle cells etc.

Semen contains naturally single homogenous and equivalent cells, so that the researcher needs no further procedures for isolation, culture or other processing steps and their different functions can be easily tested flow cytometrically. Furthermore the interested scientist can get them in sufficient quantities from nearby artificial insemination stations in fresh or deep frozen aliquots.

**Aim of the study:** In this controlled study we tested the influence of three commercial homeopathic combination drugs on their effect on the mitochondrial activity of bovine spermatozoa. These drugs are used in the human and veterinary medicine as intermediate catalysts to stimulate or regulate and coordinate an insufficient oxidative energy metabolism in a wide range of indications (2).

## MATERIALS AND METHODS

**Drugs and preparation of samples:** Three Homeopathic drugs (Ubichinon Comp.<sup>®</sup>, Coenzyme Comp.<sup>®</sup> and Selenium-Homaccord<sup>®</sup>, Heel, Baden-Baden, Germany) were added to a commercial extender in two concentrations; 9:1 and 8:2 extender:drug (Vol.:Vol.). Simultaneously three control samples were used; control A (extender alone), control B and C containing 9:1 and 8:2 extender:normal saline (Vol.:Vol.). Finally we have 9 different samples: three control samples and two samples for each of the three tested homeopathic drugs.

**Addition of drugs to the semen samples:** Fresh semen was collected from 10 bulls (Rinder Union West, Borken). The semen from each bull was divided to 9 aliquots and diluted with the above 9 samples to a final concentration of 60 X 10<sup>6</sup> sperm/ml. These different semen samples were processed and frozen (liquid nitrogen) in 0.25 ml plastic straws.

**Evaluation of the mitochondrial activity:** These frozen semen samples (3 samples from each of the 10 bulls) were thawed and stained with R-123 during an incubation at 37 C for 30 min before flow cytometric examination (FACScan flow cytometer, Becton Dickinson, Heidelberg, Germany) (3).

**Estimation of potential side effects of the drugs:** Simultaneously the prepared frozen semen samples from 6 of the 10 bulls were thawed and stained with SYBR-14 (4), Lyso Tracker Green DNA-20 (5) and Acridine orange (6) to evaluate the sperm viability, acrosomal integrity and sperm chromatin structure, respectively.

**Statistics:** ANOVA Test was used to compare the normal distributed data, and significant difference were determined by the Duncan's Multiple Range Test (SigmaStat, Jandel scientific software V2.0).

## RESULTS

- Representative examples of the outcome from flow cytometer are shown in Figure 1.
- The effects of the tested drugs on mitochondrial activity are shown in Figure 2. A significant increase of mitochondrial activity was observed in the samples, which were treated with 9:1 Coenzyme Comp., 8:2 Ubichinon Comp., 9:1 and 8:2 Selenium-Homaccord, while no significant changes were recorded in the percentage of active mitochondria (Table 1).
- No significant side effects were observed in the other tested sperm parameters (Table 1).

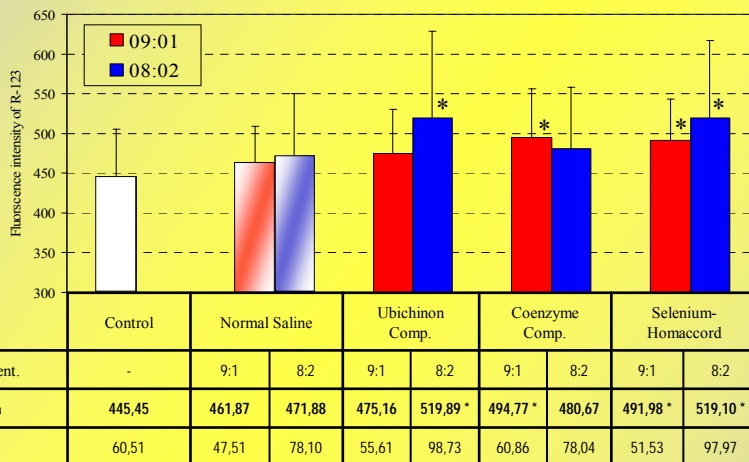


Figure 2: The changes in the mitochondrial activity of sperm. (\* P<0.05).

Table 1: The effects of homeopathic drugs on the tested sperm parameters.

Groups	Conc.	% of Active Mit. (R-123)	Sperm viability (SYBR-14)	Acrosomal integrity (Lyso-T-G)	SCSA (Acridin Orang)	
					Alpha t	COMP alpha t
Control	-	58,66 ± 3,51	57,66 ± 4,31	58,60 ± 3,85	231,78 ± 8,18	15,76 ± 4,05
Normal Saline	1:9	56,96 ± 3,19	56,11 ± 4,81	59,34 ± 3,90	233,12 ± 8,04	16,70 ± 3,78
	2:8	57,29 ± 3,31	56,07 ± 4,22	53,98 ± 5,85	232,41 ± 7,94	16,21 ± 3,97
Ubichinon Comp.	1:9	58,18 ± 3,92	58,07 ± 5,00	57,27 ± 4,19	229,32 ± 7,58	14,66 ± 3,79
	2:8	59,15 ± 3,69	59,57 ± 5,65	54,03 ± 5,86	230,98 ± 9,09	15,49 ± 4,55
Coenzyme Comp.	1:9	60,78 ± 4,37	57,81 ± 5,24	58,84 ± 3,98	232,59 ± 8,33	16,49 ± 3,97
	2:8	58,10 ± 4,90	57,45 ± 4,11	59,57 ± 3,77	231,50 ± 9,16	15,75 ± 4,58
Selenium-Homaccord	1:9	60,53 ± 4,55	58,03 ± 5,46	55,96 ± 4,84	227,11 ± 8,01	13,82 ± 3,71
	2:8	55,92 ± 5,55	57,31 ± 4,15	55,47 ± 5,03	227,95 ± 9,48	13,98 ± 4,74

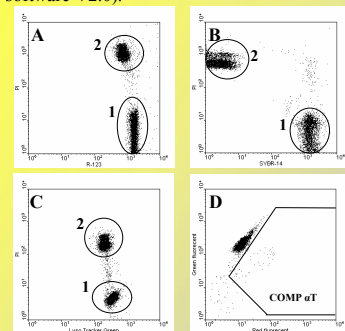


Figure 1: (A) Dot plots of spermatozoa stained with R-123/PI, (1) Sperms with active mitochondria and (2) Dead sperms. (B) Dot plots of sperms stained with SYBR-14/PI, (1) Live sperms and (2) Dead sperms. (C) Dot plots of sperms stained with LYSO-G/PI, (1) Sperms with intact acrosom and (2) Dead sperms. (D) Dot plots of sperms stained with acridine orange. COMP αT show the percentage of spermatozoa with denaturated DNA.

## CONCLUSION

➤ The results indicate that the tested homeopathic drugs can be used to stimulate the mitochondrial activity of the semen samples without any side effects on the viability, acrosomal integrity and chromatin structure of the sperms, which were not unexpected because we tested homeopathic drugs and a substantial energy supply is also essential for the integrity of the other tested cell functions. Whether this positive effect can be utilized in artificial insemination to meliorate the fertilizing capacity of sperms must be analyzed in the future.

➤ The results also confirm the above mentioned positive experiences with these drugs in the human and veterinary medicine in those indications, where a disturbed energy metabolism seems to be involved in the generation of a special disease process.

➤ Furthermore bovine spermatozoa proved to be a suitable cell model for testing the influence of drugs on vital cell functions. They are also useful to find the side effects of a certain drug.

➤ So we can suggest from these experiments, that sperm cells offer an innovative, very sensitive and promising supplement or alternative to the use of cell cultures and laboratory animals in pharmacological studies.

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