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Short Communication

Preliminary Evaluations of the effects of *Cuminumcyminum* and *Coriandrumsativum* essential Oils on Swine Spermatozoa

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- Essential oils
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Abstract

In the last years, Essential Oils are commanding the attention of the scientific world in a variety of fields because of their peculiar characteristics. Among the reasons behind this growing interest, there is their capability of interfering with bacteria, viruses and fungi. Moreover, they can also act as effective antioxidant and spermicidal agents. The aim of this preliminary study was to analyze the effects of the Essential Oils extracted by *Cuminumcyminum* and *Coriandrumsativum* on the membranes of spermatozoa using porcine ejaculates as model, with the future prospective of possible applications in reproductive medicine. Four different concentrations of the oils mix (1:1) were tested on samples of swine spermatozoa alongside with two controls (one with and one without Penicillin). The prepared samples were incubated at 16°C (± 1°C) in a refrigerated bath, and evaluated for Viability and Acrosome Status at three different time points (24, 72 and 120 h). When compared to the control samples, the two lower tested concentrations (0.1 and 0.2 mg/ml) do not seem to alter viability nor acrosome reaction percentage. On the other hand, the two remaining concentrations impair both parameters in a concentration-dependent manner. Overall, these preliminary results prove how this Essential Oils mix can interact with the spermatozoa membranes, both cytoplasmic and acrosomal.

INTRODUCTION

In the last years, Essential Oils (EOs) are commanding the attention of the scientific world in a variety of fields because of their peculiar characteristics [1]. These compounds are oily aromatic liquids extracted from aromatic plant, and can be biosynthesized as secondary metabolites in different organs of the plan [1]. Among the reasons behind this growing interest, there is their capability of interfering with bacteria [2], viruses [3] and fungi [4], that could be exploited in several fields of medicine. Regarding reproductive medicine, especially in its veterinary branch, the antibacterial capabilities of EOs might be helpful when searching for alternatives to the use of antibiotics in artificial insemination doses that are currently mandatory to prevent the transmission of diseases [5]. Moreover, it has to be acknowledged the some EOs show strong protective effects against oxidative damage [6], and could therefore help during spermatozoa cryopreservation [7]. Finally, alongside the above-mentioned positive effects, EOs might also act as contraceptives since most of the spermicidal compounds actually derive from plants [8]. In the light of all of these exploitable effects of EOs in reproductive medicine, studies aimed to analyze their effects of spermatozoa are highly necessary. Literature suggests how EOs of *Cuminumcyminum* and *Coriandrumsativum* show strong synergistic antibacterial and antioxidant activities, again potentially proving to be of reproductive interest [9]. The aim of this preliminary study was to analyze the effects of the EOs extracted by *Cuminumcyminum* and *Coriandrumsativum* on the membranes of spermatozoa using porcine ejaculates as model, with the future prospective of possible applications in reproductive medicine.

MATERIALS AND METHODS

Analyses were performed on four ejaculates (n=4) collected from two commercial hybrid boars [(Large White x Landrace) x Duroc] housed in the Physiology Piggery of the DIMEVET (Department of Veterinary Medical Sciences-University of Bologna). Ejaculates were collected once a week using the hand-gloved technique by an experienced operator, and only the sperm rich fractions (SRF) were used for the experimental purposes. Inclusion criteria of the ejaculates were: Viability >80%, Acrosome reaction <5%.

Before starting the experiments, *Cuminumcyminum* and *Coriandrumsativum* EOs were qualitative and quantitative characterized using Gas chromatography- Flame Ionization Detector (GC-FID) analysis. The EOs were than equally combined (1:1) and, in order to guarantee even diffusion in our medium,

added with two emulsifiers: dimethylsulfoxide (DMSO) and Tween 80 (respectively 0.5 and 0.02% v/v)as suggested by literature [9]. The medium of choice was the Swine Fertilization Medium (SFM), already well validated by literature[10].

Experimental samples were prepared by diluting $15x10^7$ spermatozoa in 5 ml of SFM without any antibiotic, with the four different concentrations of the EOs mix: 0.2 mg/ml, 0.4mg/ml, 0.6mg/ml and 0.8 mg/ml. In addition, two control samples, one with Ampicillin at the dose of 1mg/ml (AB+) and one without (AB-) were prepared. After preparation, the doses were incubated at 16°C (± 1°C) in a refrigerated bath and subsequently evaluated for Viability, by means of eosin-nigrosin staining [11], and Acrosome Status, by means of Comassie-blue staining [5], at three different time points (24, 72 and 120 h).

RESULTS AND DISCUSSION

The results of the chemo-typing of the two EOs used in the study are reported in Table 1. This step is crucial for the interpretation of the results as EOs are constituted by different compounds and their composition is strongly influenced by a variety of factors including place and technology of both production of the plant and extraction of the EO itself [12]. Moreover, it will be important to separately test each of the most representative compounds in order to try and identify the accountable for any noticed effects. Before discussing any result, it has to be acknowledged that prior to the actual trial, the authors tested the effects of the emulsifiers alone on the spermatozoa in order to exclude any interference. No effects on the morph-functional parameters of these cells were noticed.

The results of the descriptive statistics of the effects of the mix of EOs on the spermatic membranes are reported in Figure 1 for Viability, and (Figure 2) for Acrosome Status. The morphological aspect of the spermatozoa after the two staining techniques are reported in Figure 3. The first noticeable results, applicable for both parameters, are that the mix of EOs acts on the spermatozoa in a concentration-dependent manner and already within the first 24 hours of incubation. The concentration-dependent pattern of action is very important, and somehow implies that lower concentrations may be non-harmful on spermatozoa but still effective when it comes to bacteria on oxidative stress.

The two lower tested concentrations (0.1 and 0.2 mg/ml) do not seem to alter viability, which represents the status of the cytoplasmic membrane, with values higher than 85% throughout the entire trial as the controls. On the other hand, the other two concentrations, 0.4 and 0.8mg/ml, strongly alter viability, taking it way under the common required standards for swine semen.

Regarding Acrosome Status, the effect of the mix seems to be identical. Indeed, the two lower concentrations never determine a percentage of acrosome reaction higher than 5 %, considered as normal in swine ejaculates. The concentrations of 0.4 and 0.8 mg/ml of EOs mix, on the other hand, increase acrosome reaction in the experimental samples not only in a concentration-dependent but also time-dependent manner.

Overall, these preliminary results prove how the mix of the two tested EOs can interact with the spermatozoa membranes, both cytoplasmic and acrosomal. According to these results, this mix should not be used at concentrations higher than 0.2 mg/ml unless a spermicidal effect is wanted.

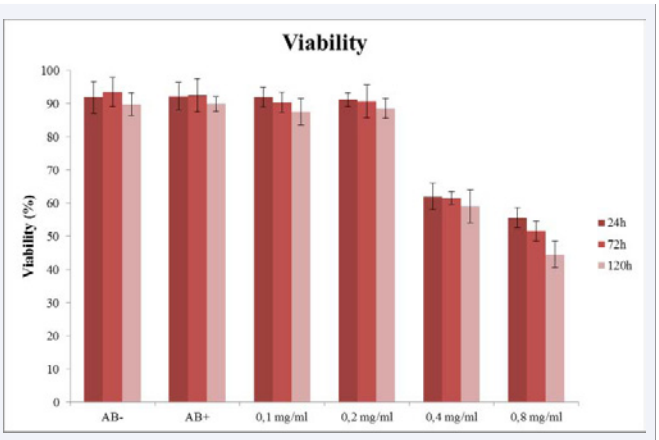

Figure 1 Viability of the experimental samples at the three time points expressed as means and standard deviations. (AB: Antibiotic).

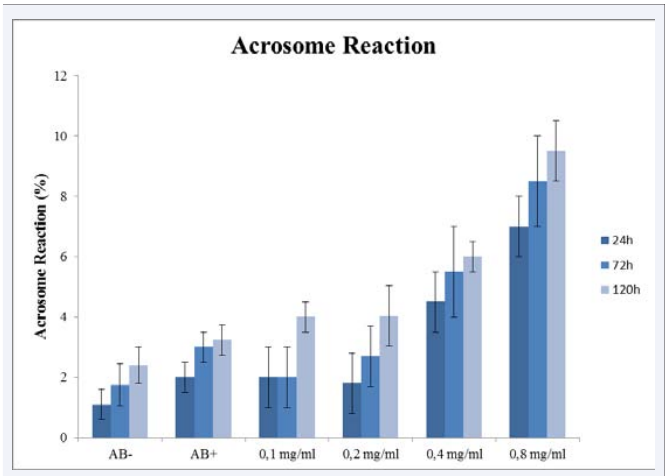

Figure 2 Acrosome reaction percentage of the experimental samples at the three time points expressed as means and standard deviations. (AB: Antibiotic).

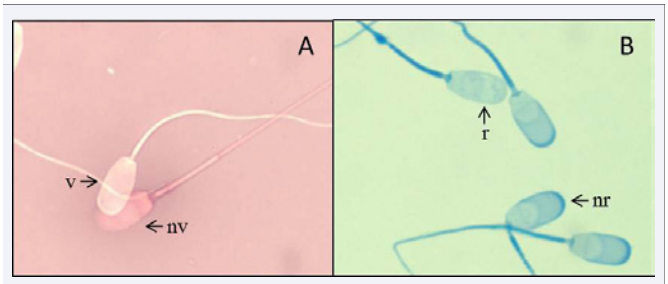

Figure 3 Morphological aspect of the porcine spermatozoa after the staining techniques:
A: Eosin-Nigrosin for Viability (v=viable; nv=non-viable); B: Comassie blue for Acrosome Status (r= reacted; nr= non-reacted).

CONCLUSION

In conclusion, this preliminary study proves how spermatozoa react to the Essential Oils in a concentration-dependent manner, and that the lowest tested concentration does not seem to alter the cytoplasmic and the acrosomal membranes. Further studies, including more morpho-functional evaluations of treated

Table 1: Characterization of the Essential Oils used in the study by Gas Chromatography.

Cumino cyminium			***Coriandrumsativum***		
COMPONENT	LRI	%	COMPONENT	LRI	%
α-tujene	928	0.21	α-tujene	927	0.09
α-pinene	934	2.74	α-pinene	934	13.73
β-pinene	978	13.47	camphene	947	2.11
β-myrcene	993	0.69	sabinene	973	0.27
p-cymene	1027	13.13	β-pinene	976	0.94
1,8-cineole	1030	1.35	β-myrcene	992	1.68
γ-terpinene	1062	15.66	α-terpinene	1017	0.13
α-terpinolene	1089	0.12	*p*-cymene	1025	3.48
camphor	1144	0.15	limonene	1029	4.82
terpinen-4-ol	1179	0.13	γ –terpinene	1060	7.61
myrtenale	1196	0.38	*cis*-linalooloxide	1073	0.45
cuminaldehyde	1253	43.31	terpinolene	1089	1.3
geranial	1279	0.25	linalool	1111	42.3
α-terpinen-4-ale	1289	5.32	camphor	1147	8.76
γ-terpinen-4-ale	1294	1.02	borneol	1167	0.31
β-caryophyllene	1425	0.33	terpinen-4-ol	1179	0.31
			α-terpineol	1192	0.74
			geraniol	1261	4.07
			myrtenyl acetate	1330	0.22
			ciclosativene	1369	0.1
			geranyl acetate	1388	5.93
			β-caryophyllene	1421	0.11
TOTAL		98.28	TOTAL		99.46

Abbreviations: LRI: Linear Retention Indices

spermatozoa, will be needed to further investigate the potential capabilities of such compounds in all the fields of medicine and reproduction. Once the action/toxicity mechanisms and effects will become more clear, preclinical and clinical application protocols will be performed safely.

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