EFFECT OF NATURAL SUBSTANCES ADDED TO SEMEN EXTENDER ON THE BOAR SEMEN SURVIVAL TIME

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Abstract

The objective of the experiment was to investigate the effect of natural substances as the potential substitute for antibiotics in the boar extender on sperm survival time. Thirteen natural substances were tested. Natural substances were dissolved in 4% DMSO whit a minimum bactericidal effective concentration of between 300 and 4,800 μg/ml. The control sample was diluted with a semen dilution ratio of 1:1 in an APS extender without antibiotics. The sperm survival was assessed according to sperm motility. Sperm motility was evaluated at 0h, 1h and 24h after semen dilution. The sperm motility was significantly decreased over the storage time for all tested natural substances ($p < 0.01$). The sperm motility after a 24h storage period was only recorded in hydroquinone monomethylether 10 % and *Foeniculi aetheroleum* 5 %. The results of the present study indicate a negative effect of the concentrations of natural substances added to semen extender on the boar semen survival time. At present, practical utilization of the natural substances we tested as a potential substitute for antibiotics in boar extenders is not possible owing to its reduced sperm motility.

Key Words: Boar, sperm, natural substance, motility, survival time

Fresh semen is widely used for artificial insemination (AI) in swine production. Microorganisms contaminating boar semen are one of the most important factors that negatively affect the biological quality of spermatozoa. Their strong biochemical activity leads to the decrease in energetic sources of seminal plasma and produces toxic metabolites to reproductive cells (Mazurová et al., 2007). Therefore, insemination with sperm doses that have high numbers of bacteria can interfere with fertilization, resulting in higher numbers of sows returning to oestrus (Kuster and Althouse, 1997).

Curative effects of natural substances (NS) have been known for many centuries. Their biological activity includes antimicrobial, antimycotic, antivirus and antiparasitic effects. The systematic screening of antibacterial plant extracts represents a continuous effort on the part of many laboratories to find new compounds with the potential to replace antibiotics (Mazurová et al., 2006).

This study is connected to the work of Mazurová et al. (2006; 2007) focused on the bactericidal activity of various NS against microorganisms contaminating raw boar ejaculates. Therefore these NS were chosen for our experiment: essential oils (*Carvi aetheroleum, Caryophylli aetheroleum, Terebinthinae aetheroleum, Lavandulae aetheroleum, Foeniculi aetheroleum, Rosmarini aetheroleum, Eucalypti aetheroleum),* organic acid (ethylgallate), natural monoterpene phenol (thymol), organic compounds (hydroquinone, hydroquinone monomethylether), monoterpens (carvacrol) and diterpene (cnicin).

The objective of this study was to investigate the effect of NS as the potential substitute for antibiotics in boar extenders on the boar sperm survival.

Material and Methods

The NS used in this study were *Carvi aetheroleum, Caryophylli aetheroleum, Terebinthinae aetheroleum, Lavandulae aetheroleum, Foeniculi aetheroleum, Rosmarini aetheroleum, Eucalypti aetheroleum* (prepared in the laboratory of the Department of Pharmaceutical Botany and Ecology, Pharmaceutical Faculty in Hradec Králové, Charles University in Prague), ethylgallate, thymol, hydroquinone, hydroquinone monomethylether, carvacrol, cnicin (Sigma-Aldrich Co., Praha, Czech Republic). The tested effective minimum bactericidal concentrations of NS are summarized in Table 1. For solution NS were used 4% DMSO (Sigma-Aldrich Co., Praha, Czech Republic) that was prepared by dilution in extender APS (Gerlich Ondry s.r.o., Czech Republic) without antibiotics.

Fresh semen collected using the gloved-hand technique from one fertile boar (3 ejaculates) aged less than two years with 85 % motile and 16 % morphologically abnormal spermatozoa was used in the experiment. The control sample was prepared from native boar semen and APS extender without antibiotics with a dilution ratio of 1:1 and was stored at a temperature 17°C for up to 24h. The pH of fresh semen, APS extender and semen dilution samples were measured at 17°C by means of

Microprocessor pH Meter 211 (Martes, Praha, Czech Republic) calibrated with pH 4.01 and 9.21 standard solutions.

Boar sperm survival in NS was evaluated according to sperm motility. The sperm motility was evaluated subjectively by microscopic estimation of the number of sperm moving in a visual filed of phase-contrast microscopy with a heating stage (38 °C) at 100× magnification. Each sample was examined at three different microscopic fields, and motility was expressed as a percentage of sperm showing normal forward progressive movement. Sperm motility was evaluated at 0h, 1h and 24h after semen dilution.

The basic statistical characteristics of the results (arithmetic means and significance) were calculated by the QC Expert program (TriloByte Statistical Software, s.r.o., Pardubice, Czech Republic). Statistical significance analysis was checked using the variance ANOVA - Tukey test and t-test at significance levels of $p < 0.05$ and $p < 0.01$.

Results and Discussion

Results of tested NS as a potential substitute for antibiotics in boar semen extender APS without antibiotics are presented in Table 2. Sperm motility was significantly decreased over the storage period for all the tested NS ($p < 0.01$). *Terebinthinae aetheroleum* and cnicin showed the best results with respect to sperm motility after dilution. They had about 4 % lower sperm motility than the control sample (62.5 %). Sperm motility in the other NS was lower than 40 %. Reduced sperm motility ($p < 0.01$) was noted during the first hour after semen dilution with NS. Rapidly decreasing sperm motility in NS was recorded after 24h ($p < 0.01$), where sperm motility was assessed in hydroquinone monomethylether 10 % and *Foeniculi aetheroleum* 5 %. The other NS tested had zero sperm motility. The pH of semen dilution samples in NS are shown in Table 2. The values of semen dilution samples in NS were between 7.52 and 7.76 pH compare to control sample (7.28).

Boar sperm viability in NS was evaluated according to sperm motility, because sperm motility is one of the main parameters of boar sperm quality. Sperm motility is a good indicator of an active metabolism and the integrity of membranes (Johnson et al., 2000), and is considered to be of great importance to fertilization.

The presented results document that the addition of the NS we tested has a negative effect on boar sperm viability during short storage time, because sperm motility was reduced. In particular, the critical period in which the reduction in the number of motile spermatozoa was noticed ($p < 0.01$) was after 24h of storage of the diluted semen. Sperm motility before dilution averaged 85 %, which is typical for fresh boar ejaculates (Strzeżek et al., 1998). Britt et al. (1999) stated that sperm motility scores of ≤ 60 % lead to fewer fertilized eggs and lower farrowing rates. Smital (2003) noted average values of sperm motility between 63 and 69 % after a 24h storage time for different breeds of boars. In this study, hydroquinone monomethylether and *Foeniculi aetheroleum* showed only the sperm motility after a 24h storage period. Sperm motility in other NS had zero sperm motility.

In our study, we were also able to rule out the influence of 4% DMSO as an NS solution on sperm motility. The quantity of 4% DMSO that was used had no influence on sperm motility ($p < 0.01$). The fresh boar ejaculate had 7.22 pH. Johnson et al. (2000) mentioned that the pH of freshly ejaculated boar semen varies between 7.2 and 7.5 and also found a reduction of sperm motility and metabolism for pH values < 7.2. In the semen dilution samples in NS was assessment pH in range 7.52 - 7.76 compared to control sample (7.28). The slightly higher values pH could affect the sperm motility. Sperm motility could be also influenced by the incomplete solubility of NS, because thymol formed the suspension, essential oils formed the emulsion and cnicin was insoluble in 4% DMSO. However, the minimum bactericidal concentrations of NS used against microorganisms contaminating boar ejaculates probably also reduced the viability of boar spermatozoa.

Table 1. Tested minimum bactericidal concentration of natural substances on boar sperm motility

Natural substance	Concentration (μg/ml)
Carvi aetheroleum	4,800
Caryophylli aetheroleum	4,800
Terebinthinae aetheroleum	4,800
Lavandulae aetheroleum	4,800
Foeniculi aetheroleum	4,800
Rosmarini aetheroleum	4,800
Eucalypti aetheroleum	4,800
Ethylgallate	4,800
Thymol	1,200
Hydroquinone monomethylether	2,400
Hydroquinone	4,800
Carvacrol	300
Cnicin	3,000

Table 2. Effect of natural substances added to semen extender APS without antibiotics on sperm survival rate and pH (mean values)

Natural substance	pH	Sperm motility (%)		
		0h	1h	24h
Carvi aetheroleum [1]	7.52	$35^{b,A}$	7.5^{B}	$0^{c,C}$
Caryophylli aetheroleum [1]	-	0^{c}	0^{b}	0^{c}
Terebinthinae aetheroleum [1]	7.53	60^{A}	15^{C}	$0^{c,C}$
Lavandulae aetheroleum [1]	7.61	$42.5^{b,A}$	$2.5^{b,B}$	$0^{c,C}$
Foeniculi aetheroleum [1]	7.76	45^{A}	5^{bC}	5c,C
Rosmarini aetheroleum [1]	7.67	$40^{b,A}$	$2.5^{b,B}$	$0^{c,C}$
Eucalypti aetheroleum [1]	7.62	$35^{b,A}$	$0^{b,C}$	$0^{c,C}$
Ethylgallate	-	0^{c}	0^{b}	0^{c}
Thymol [2]	7.73	0^{c}	0^{b}	0^{c}
Hydroquinone monomethylether	7.75	$40^{b,A}$	30^{B}	10^{c}
Hydroquinone	-	$20^{b,A}$	10^{B}	$0^{c,C}$
Carvacrol	-	0^{c}	0^{b}	0^{c}
Cnicin [3]	7.65	60^{A}	$0^{b,C}$	$0^{c,C}$
Control sample	7.28	62.5^{a}	50^{a}	62.5^{a}

^{a,b,c} means within the column ^{a,b} $p < 0.05$, ^{a,c} $p < 0.01$
^{A,B,C} means within the row ^{A,B} $p < 0.05$, ^{A,C} $p < 0.01$
[1]Emulsion
[2]Suspension
[3]Insoluble

Conclusion

The present study showed the negative effect of the NS we tested on boar sperm motility when being stored. Higher sperm motility would need to be achieved for practical AI utilization. At present, practical utilization of NS as a potential substitute for antibiotics in boar semen extenders is not possible, owing to its reduced sperm motility. Therefore, it is necessary to research other NS and effective concentrations thereof for a possible replacement for antibiotics in boar extenders.

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