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REGULAR ARTICLE

IN VITRO STUDY OF THE EFFECT OF 17β-ESTRADIOL AND 4-NONYLPHENOL ON BOVINE SPERMATOZOA

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ABSTRACT

Nonylphenol (NP), an environmental endocrine disruptor, is a final metabolite of nonylphenol ethoxylate (NPE) that is able to interfere with hormonal system of numerous organisms. Estrogens play a central role in female reproduction, but also affect the male reproductive system. In males, stimulate mammalian spermatozoa capacitation, acrosome reaction and fertilizing ability. The aim of this *in vitro* study was to determine the effect of 17β-estradiol and nonylphenol (NP) on the spermatozoa motility. Specifically, we examined the dose- and time-dependent effect of nonylphenol (1, 10, 100 and 200 μg/mL) with the addition 1 μg/mL of 17β-estradiol on the motility and progressive motility of bovine spermatozoa during two time periods (0 h and 6 h). The spermatozoa motility was determined by CASA (Computer Assisted Semen Analyzer) system using the Sperm Vision™ program. The results showed decreased average values of motility in all experimental groups during 0 h of *in vitro* cultivation. The motility and the progressive motility of bovine spermatozoa increased in the experimental groups using concentrations 10, 100 and 200 μg/mL after 6 h

of cultivation and significant differences (P<0.05) were detected between these groups and the control group. The results suggest that the addition of 17β-estradiol could positively affect spermatozoa motility during the short-term cultivation of spermatozoa with NP.

Keywords: nonylphenol, 17β-estradiol, spermatozoa, motility, CASA

INTRODUCTION

Nonylphenol (NP) belongs to the group of alkylphenols, which are degraded products of alkylphenolpolyethoxylates (APEs), a well known class of environmental endocrine disruptors **(Raecker *et al*., 2011).** NP is a non-ionic surfactant widely used as component of detergents, paints, herbicides and many other synthetic products **(Gong and Han, 2006)**, which is capable of interfering with hormonal system of numerous organisms **(Soares *et al*., 2008)**.

Estrogens are steroid hormones playing a central role in female reproduction, but also affecting the male reproductive system **(Hess *et al*., 1997).** For many decades, estrogens have been considered to be primarily female hormones, contributing to female health and fertility. However, more recently estrogens have also been shown to play important roles in males **(Korach *et al*., 1996; Hess *et al*., 1997; Luconi *et al*., 2002).** In males, it is present in low concentrations in blood but is present at extraordinarily high levels in ejaculate **(O´ Donnell *et al*., 2001).** Estrogens stimulate mammalian spermatozoa capacitation, acrosome reaction and fertilizing ability **(Adeoya-Osiguwa and Fraser, 2003).** Estrogen effects are mediated by 2 distinct nuclear receptors, estrogen receptor α (ER α) and ER β **(O´ Donnell *et al*., 2001).** ER α is present in postacrosomal, midpiece and tail regions and ER β midpiece and tail regions and mitochondria of ejaculated human sperm **(Durke *et al*., 1998; Aquila *et al*., 2004; Solakidi *et al*., 2005).** The two receptors share common structural and functional domains, bind estrogens with high affinity and bind to estrogen response elements **(Korach, 2000).**

The estrogenic effect of NP, such as induced expression of estrogen receptor (ER) and inhibiting estrogen binding to ER, might cause endocrine disruption **(Fekadu *et al*., 1999; Kwack *et al*., 2002)**.

The objective of this study was to evaluate the positive effect of 17β-estradiol dissolved in 1% dimethyl sulfoxide (DMSO) against the influence of nonylphenol (NP)

dissolved in 1% dimethyl sulfoxide (DMSO) on the motility and progressive motility of bovine spermatozoa during two time periods.

MATERIAL AND METHODS

Semen samples and *in vitro* culture

Bovine semen samples were obtained from 10 adult breeding bulls (Slovak Biological Services, Nitra, Slovak Republic). The samples had to accomplish the basic criteria given for the corresponding breed. The semen was obtained on a regular collection schedule using an artificial vagina. After collecting the samples were stored in the laboratory at room temperature (22-25°C). Each sample was diluted in physiological saline solution (PS) (sodium chloride 0.9% w/v, Bieffe Medital, Grosotto, Italia), using a dilution ratio of 1:40, depending on the original spermatozoa concentration.

Spermatozoa were incubated with various concentrations of nonylphenol (4-*n*-NP; Fluka, Buchs, Switzerland) dissolved in 1% dimethyl sulfoxide (DMSO, Sigma-Aldrich, Bratislava, Slovakia) (group A – 1; B – 10; C – 100; D – 200 µg/mL of NP) with the addition 1 µg/mL of 17β-estradiol (Sigma-Aldrich, Buchs, Switzerland) dissolved in 1% dimethyl sulfoxide (DMSO). The control spermatozoa (Ctrl) group was cultured with physiological saline solution.

Spermatozoa were cultivated in the laboratory at 37°C in incubator. The control group (medium without NP) was compared to the experimental groups (exposed to different concentrations of NP with the addition 17β-estradiol) during 0 h and 6 h of *in vitro* cultivation.

Computer-assisted semen analysis (CASA)

The spermatozoa motility was evaluated using a CASA (Computer Assisted Semen Analyzer) system – SpermVision^{TM} program (MiniTűb, Tiefenbach, Germany) with the Olympus BX 51 microscope (Olympus, Tokyo, Japan) at cultivation times 0 h and 6 h. Each sample was placed into the Makler Counting Chamber (deph 10 µm, Sefi-Medical Instruments, Haifa, Izrael) and the following parameters evaluated: the percentage of motile spermatozoa (motility > 5 µm/s; MOT) and the percentage of progressively motile

spermatozoa (motility > 20 μm/s; PROG). This study was performed in ten replicates at each concentration (n = 10). At least 1000 spermatozoa were analyzed in each sample.

Statistical analysis

Obtained data were statistically analyzed using PC program GraphPad Prism 3.02 (GraphPad Software Incorporated, San Diego, California, USA). Descriptive statistical characteristics (mean, minimum, maximum, standard deviation and coefficient of variation) were evaluated. T-test and Wilcoxon matched pairs test were used for statistical evaluations. The level of significance was set at *** (P<0.001); ** (P<0.01) and * (P<0.05).

RESULTS AND DISCUSSION

Nonylphenol (NP) is one of the most abundant alkylphenolpolyethoxylates (APE) derivates that can induce cell death in gonads and changes to other reproductive parameters **(Cardinali *et al.*, 2004).**

Bovine spermatozoa motility after exposure to various concentrations of NP dissolved in 1% DMSO with the addition 1 μg/mL of 17β-estradiol is shown in the Table 1. The initial values of spermatozoa motility in all experimental groups were lower in comparison to the control group during time 0 h of *in vitro* cultivation**.** The average values of spermatozoa motility increased in the experimental groups B, C and D after 6 h of *in vitro* cultivation and significant differences (P<0.05) were found between these groups (65.83%; 65.02% and 72.20%) and the control group (61.02%).

Table 1 Bovine spermatozoa motility (MOT; %) exposed to NP with the addition 17β-estradiol dissolved in 1% DMSO in various time periods

Groups	**Control**	**1**	**10**	**100**	**200**
	Ctrl	**A**	**B**	**C**	**D**
	µg/mL of NP with the addition of 1 µg/mL of 17β-estradiol				
Time 0					
x	92.66	91.41	87.57	91.27	89.90
minimum	80.26	78.94	60.78	80.51	73.52
maximum	97.59	97.79	98.46	97.14	98.66
S.D.	3.20	3.85	9.04	3.91	5.99
CV (%)	3.46	4.21	10.32	4.28	6.66
Time 6					
x	61.02	64.12	65.83^{C}	65.02^{C}	72.20^{C}
minimum	40.62	41.66	40.35	41.66	46.15
maximum	87.05	89.58	90.54	92.30	95.65
S.D.	14.78	12.60	12.26	14.04	11.48
CV (%)	16.53	11.68	11.31	16.23	19.85

Legend: x – mean, SD – standard deviation, CV (%) – coefficient of variation

$^{A}P<0.001$; $^{B}P<0.01$; $^{C}P<0.05$

The similar results were also detected for the average values of progressive motility of bovine spermatozoa. The results are shown in the Table 2.

Table 2 Bovine progressive spermatozoa motility (PROG; %) exposed to NP with the addition 17β-estradiol dissolved in 1% DMSO in various time periods

Groups	**Control**	**1**	**10**	**100**	**200**
	Ctrl	**A**	**B**	**C**	**D**
	µg/mL of NP with the addition of 1 µg/mL of 17β-estradiol				
Time 0					
x	90.43	89.59	85.50	88.54	84.41
minimum	78.94	75.28	55.55	72.09	44.11
maximum	96.38	97.05	98.46	96.96	97.33
S.D.	3.33	4.34	9.33	5.45	11.71
CV (%)	3.68	4.84	10.92	6.16	13.87
Time 6					
x	59.17	62.77	63.89^{C}	63.18^{C}	70.48^{C}
minimum	40.00	42.85	47.05	44.00	54.16
maximum	84.72	83.33	86.48	85.71	87.27
S.D.	13.94	10.02	10.82	12.03	6.31
CV (%)	13.17	15.96	16.93	19.04	8.96

Legend: x – mean, SD – standard deviation, CV (%) – coefficient of variation

$^{A}P<0.001$; $^{B}P<0.01$; $^{C}P<0.05$

Many authors described the negative impact of NP on the reproductive parameters on fish **(Tollefsen and Nilsen, 2008)**, amphibians **(Feng *et al*., 2011)** and mammals **(Hamdy *et al*., 2012).** Our previous *in vitro* study **(Lukáčová *et al*., 2012)** confirmed the negative effect of NP on spermatozoa motility. The data obtained from this study indicate, that the addition of 17β-estradiol has positive effect on spermatozoa motility. Our opinion confirms the study of **Adeoya-Osiguwa *et al*. (2003)** that found out the addition of 17β-estradiol significantly stimulated capatitation and increased mouse spermatozoa motility.

NP can induce apoptosis in a wide variety of cells **(Roy *et al*., 1997)**, including rat primary germ and Sertoli cell cultures, while 17β-estradiol was without that effect **(Raychouhury *et al*., 1999)**. **Aravindakshan and Cyr (2005)** also confirm that 17β-estradiol didn´t cause apoptosis of mouse Sertoli cells. While exposure of the cells to estradiol did not alter intracellular communication, exposure to nonylphenol dramatically reduced intercelullar communication **(Tapiero *et al*., 2002).**

The results are not clear, because **Uguz *et al*. (2009)** observed that the addition 1 μg/mL of 17β-estradiol dissolved in 1% ETOH significantly inhibited rat spermatozoa motility after 3 h of *in vitro* cultivation, while 1 μg/mL of 17β-estradiol dissolved in 1% DMSO decreased the motility, but it didn´t have significant effect.

CONCLUSION

The data obtained from this study suggest the positive role of 17β-estradiol on spermatozoa motility and progressive motility during the short-term *in vitro* cultivation of spermatozoa with nonylphenol.

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