

INHIBITORY EFFECT OF CADMIUM AND TOBACCO ALKALOIDS ON EXPANSION OF PORCINE OOCYTE-CUMULUS COMPLEXES

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SUMMARY

Studies aimed at the influence of smoking on reproductive functions have found out fertility disorders in smokers occurring at any stage of reproductive processes. In our experiments the role of cadmium, nicotine and anabasine was investigated in the expansion of oocyte-cumulus complexes (OCC) isolated from large antral porcine follicles. Suppression of FSH-induced cumulus expansion and significant inhibition of synthesis and accumulation of hyaluronic acid in the cell/matrix compartment of the OCC was observed in the presence of different concentrations of tested compounds. The suppressive effect of cadmium and tobacco alkaloids on the cumulus expansion was accompanied by decreased progesterone production by cumulus cells during 42 h incubation of the OCC with FSH.

Key words: porcine follicles, cumulus expansion, hyaluronic acid, progesterone, tobacco alkaloids

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INTRODUCTION

Although smoking cigarettes is a widely recognized health hazard, consumption of tobacco remains prevalent in human society. Studies aimed at the influence of smoking on female fertility have shown fertility disorders occurring at any stage of reproductive processes and manifesting as conception delay, significantly lower pregnancy rate at *in vitro* fertilization followed by poor embryo development (1), more prevalent spontaneous abortion, and in an advance in the age of menopause in smoking women.

The effects of cigarette smoke components absorbed into the organism on intrafollicular processes may in part explain the negative impact of smoking on female fertility. Heavy metal cadmium, and alkaloids nicotine and anabasine are significant compounds of tobacco and smoke (1).

Because the reproductive system is complex, vulnerability to disruption of reproduction may occur at many sites, from hypothalamic-pituitary axis to the germinal cells. In response to the preovulatory surge of gonadotropins, cumulus cells synthesize and deposit hyaluronic acid forming an extensive extracellular matrix. Expansion of the oocyte-cumulus complex (OCC) is a process necessary for the release of mature oocyte into the oviduct and the compounds of the matrix contribute to successful fertilization by stabilizing structure of the egg zona pellucida (2).

In the present study, the effects of cadmium, nicotine and anabasine on FSH-induced expansion of OCC isolated from porcine follicles were investigated.

METHODS

Transport of porcine ovaries, isolation and treatment of OCC were conducted as described previously (3). The OCC were cultured with or without FSH and cadmium, nicotine or anabasine. The degree of expansion was assessed after 24 h incubation according to a subjective score system from 0 to +4 (3). At the end of the incubation period, the OCC media were collected for progesterone determination by [¹²⁵I]-progesterone radioimmunoassay. Hyaluronic acid (HA) synthesis was measured using a procedure of Eppig with slight modifications (4). The data were analyzed by analysis of variance (ANOVA).

RESULTS

After 24 h incubation with 1 µg/ml FSH (a positive control group), more than 61 % of the complexes expanded to the +4 and +3 stage (Table 1). Suppression of cumulus expansion stimulated by FSH was observed in the presence of different cadmium, nicotine and anabasine concentrations ($p < 0.05$). At the end of the culture period, the mucified cumuli of FSH, FSH+cadmium and FSH+nicotine groups were dispersed with Streptomyces hyaluronidase and the amount of HA present in the medium (total, T) and in cell/matrix compartment (retained, R) was determined using ³H-glucosamine hydrochloride as a metabolic precursor of HA synthesis and distribution. (Fig. 1). Treatment of the OCC with cadmium resulted in significant decrease ($p < 0.01$) of total and retained HA. However, nicotine failed to affect total accumulation

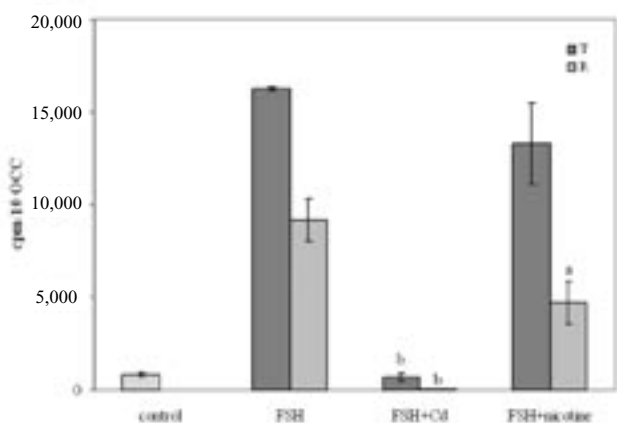


Fig. 1. Hyaluronic acid synthesis (total, T and retained, R) by follicular OCC after 24 h incubation in the absence (control, C) or presence of FSH (1 µg/ml), cadmium (0.5×10⁻⁴M) and nicotine (2×10⁻⁶M). The values are the means ± S. E. M. of three estimations (^a p<0.05, ^b p<0.01 versus FSH).

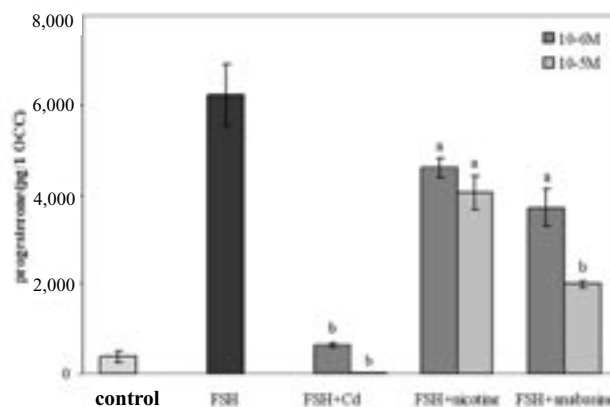


Fig. 2. Effect of cadmium, nicotine and anabasine on FSH-stimulated progesterone production by the OCC after 42 h incubation of the OCC (^a p<0.05, ^b p<0.001 versus FSH).

Table 1. Effect of cadmium, nicotine and anabasine on FSH-induced cumulus expansion of the oocyte-cumulus complexes (OCC) isolated from 5-8 mm porcine follicles.

Treatment	n	Degree of expansion					Number of expanded OCC (%)
		+4	+3	+2	+1	0	
Control	30	0	0	0	0	30	0.0
FSH (1 µg/ml)	70	22	21	15	5	7	61.4
FSH+ cadmium (10 ⁻⁶ M)	14	3	1	6	4	0	28.6
FSH+ cadmium (10 ⁻⁵ M)	14	0	3	8	2	1	21.4
FSH+ cadmium (0.5×10 ⁻⁴ M)	29	0	5	13	7	4	17.2
FSH+ nicotine (2×10 ⁻⁶ M)	9	1	2	2	2	2	33.3
FSH+ nicotine (2×10 ⁻⁵ M)	8	0	2	2	2	2	25.0
FSH+ nicotine (2×10 ⁻⁴ M)	8	1	2	1	1	3	37.5
FSH+ anabasine (10 ⁻⁶ M)	10	2	1	2	5	0	30.0
FSH+ anabasine (10 ⁻⁵ M)	12	0	4	4	1	3	33.3
FSH+ anabasine (10 ⁻⁴ M)	11	0	0	3	2	6	0.0

Number of expanded OCC = number of the OCC reaching stages +3 or +4 after 24 h incubation.

of HA but significantly decreased (p<0.05) the amount of HA retained within the FSH-treated complex. The inhibitory effect of tested compounds on the cumulus expansion was accompanied by decreased cumulus cell progesterone production during 42 h incubation of the OCC (Fig. 2). Cadmium, nicotine and anabasine caused a significant decrease in FSH-induced progesterone secretion by the OCC.

DISCUSSION

The present study provides the first report of the toxic effects of substances from cigarette smoke on the cumulus cell expansion. The data obtained revealed suppression of FSH-stimulated cumulus expansion of the oocyte-cumulus complexes in the presence of cadmium, nicotine and anabasine.

Cadmium is absorbed into the organism in low doses through consumption of contaminated drinking water, inhaling polluted air and primarily from cigarette smoke (1). It is accumulated in the body over a period of years and easily incorporated into the reproductive tissues. Study on human granulosa cells showed cadmium-diminished progesterone biosynthesis and alterations in cellular morphology (5), what correlates with inhibition of progesterone production observed in our study.

Nicotine, the principle alkaloid in tobacco, is quickly absorbed through the respiratory tract. The study of Blackburn et al. (6) demonstrated toxic effect of nicotine on ovulation and fertilization rate in PMSG-primed rat ovary. Experiments on human granulosa cells have found out even cytotoxic potential of tobacco alkaloids (7). Another possible mechanism that could lead to modifying of female reproductive processes is the effect of these alkaloids on steroidogenesis in follicular cells. The presence of nicotine and

anabasine in our studies markedly inhibited FSH-induction of cumulus cells progesterone production. The results of experiments aimed at influence of alkaloids on steroidogenic function of granulosa cells, however, are contradictory (7, 8).

Numerous findings demonstrate the negative influence of smoking on reproductive health. The present study has shown that the expansion of the oocyte-cumulus cell complex could be another site of reproduction potentially disrupted by the effects of cadmium, nicotine and anabasine as cigarette smoke constituents.

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ANTIOXIDANT VITAMIN POOL IN SENIOR POPULATION

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SUMMARY

Aim: To compare plasma concentration of α -tocopherol and ascorbic acid in healthy seniors (age over 65 years), senior patients with either diabetes mellitus, acute myocardial infarction or dyslipidemia and recommended values of these vitamins. **Methods:** Studied groups included 30 patients with diabetes mellitus (DM); 30 patients 1 – 2 weeks after acute myocardial infarction (AMI); 11 patients with lipid metabolism disorder (LD, total cholesterol > 6.2 mM); and control group of 27 healthy persons. **Results:** Concentration of α -tocopherol in DM group was $14.6 \pm 5.3 \mu\text{M}$, in AMI group $13.7 \pm 5.6 \mu\text{M}$, in LD group $15.9 \pm 5.6 \mu\text{M}$ and in control group $12.9 \pm 4.1 \mu\text{M}$. No statistically significant differences were found. However, comparison of determined values with levels recommended for prevention revealed remarkable low plasma concentration of α -tocopherol in the Czech population. Plasma concentration of ascorbic acid in DM group was $47.07 \pm 22.80 \mu\text{M}$, in AMI group $33.15 \pm 12.81 \mu\text{M}$, in LD group $45.59 \pm 23.02 \mu\text{M}$ and in control group $43.28 \pm 26.57 \mu\text{M}$. No statistically significant differences were found between the controls and individual groups of patients. Plasma concentrations of vitamin C reached the recommended value in all cases except the AMI group, where it was significantly lower. **Conclusion:** Seniors in the Czech population were proved to be significantly short of α -tocopherol, minor shortage of vitamin C was found only in group of patients with myocardial infarction.

Key words: vitamin E, vitamin C, antioxidants, seniors

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INTRODUCTION

Ischemic heart disease as well as diabetes mellitus is accompanied by increased oxidative stress, which plays an important role in the pathogenesis of vascular complications. Oxidative stress occurs

when the reactive oxygen species (ROS) evolution overcomes the capacity of antioxidative system and the balance between their production and elimination becomes impaired. Antioxidative system comprises of both endogenous part – antioxidative enzymes and exogenous part – especially vitamins E, C, β -carotene.