Slovak J. Anim. Sci., 42, 2009, SUPPLEMENT 1: 35-41

ISSN 1337-9984

Scientific paper formerly presented

THE RELEASE OF INSULIN–LIKE GROWTH FACTOR - I BY OVARIAN GRANULOSA CELLS OF PREGNANT SOWS AFTER LEAD AND MERCURY ADMINISTRATION *IN VITRO*

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ABSTRACT

Lead (Pb) and mercury (Hg) are reproductive and developmental toxicants even at very low doses. The general objective of this study was to examine the secretory activity of porcine ovarian granulosa cells of pregnant sows after the lead and mercury *in vitro* administration. Ovarian granulosa cells were incubated with/without lead acetate, mercury chloride and follicle-stimulating hormone (FSH) for 18 hours. A maximal dose of $Pb(CH_3COO)_2.3H_2O$/ $HgCl_2$ was 0.5 mg ml^{-1}; group A - 0.25 $mg.ml^{-1}$, group B - 0.083 $mg.ml^{-1}$, group C - 0.063 $mg.ml^{-1}$, group D - 0.046 $mg.ml^{-1}$; in case of FSH: group E - 1.0 $ng.ml^{-1}$, group G - 100 $ng.ml^{-1}$. Group F received FSH (1.0 $ng.ml^{-1}$), lead acetate (0.083 $mg.ml^{-1}$) and mercury chloride (0.083 $mg.ml^{-1}$); group H received FSH (100 $ng.ml^{-1}$), lead acetate (0.063 $mg.ml^{-1}$) and mercury chloride (0.063 $mg.ml^{-1}$). Concentrations of IGF–I were determined in 25–100 µL incubation medium by RIA. In our study a significant release of IGF-I by ovarian granulosa cells from pregnant sows was revealed after lead administration at doses of 0.5 $mg.ml^{-1}$ and 0.25 $mg.ml^{-1}$. No significant changes at doses of 0.083 $mg.ml^{-1}$, 0.063 $mg.ml^{-1}$ and 0.046 $mg.ml^{-1}$ were demonstrated. IGF-I was stimulated by the Hg addition at dose of 0.083 $mg.ml^{-1}$. The concentrations 0.25 $mg.ml^{-1}$, 0.063 $mg.ml^{-1}$ and 0.046 $mg.ml^{-1}$did not affect the release of IGF-I by granulosa cells. IGF-I release was not significantly stimulated by FSH (1 $ng.ml^{-1}$), Pb (0.083 $mg.ml^{-1}$) and Hg (0.083 $mg.ml^{-1}$). Non-significant changes in the IGF-I output by granulosa cells after FSH (100 $ng.ml^{-1}$), Pb (0.063 $mg.ml^{-1}$) and Hg (0.063 $mg.ml^{-1}$) administrations were noted. These observations suggest a possible involvement of heavy metals – Pb and Hg and pituitary hormone - FSH in a regulation of the growth factor IGF-I release by porcine ovarian granulosa cells of pregnant sows. Our results contribute to knowledge of mechanisms of lead and mercury effects on porcine ovarian granulosa cells of pregnant sows. However, uncontrolled growth of follicles by increased IGF-I level can be a possible risk factor of cancer.

Key words: pregnancy; IGF–I; lead; mercury; FSH; granulosa cells; sows

INTRODUCTION

Knowledge of normal levels of trace elements (Cd, Pb, Hg, Cu, Zn, and Se) in the population serves, among others, in design of regulations concerning health protection, determination of exposition limits and prevention of diseases caused by deficiency of trace elements (Benes et al., 2002). Essential metals can affect the metabolism of non–essential metals (Min et al., 2008). Changes in the endocrine system might be a useful indicator of metal exposure and its potential toxicity in animals (Baos et al., 2006).

Lead (Pb) is an ubiquitous environmental and industrial pollutant (Shan et al., 2009; Silberstein et al., 2006) which is found in water, brass plumbing fixtures, soil (Zhuang et al., 2009; Chrastný et al., 2009), fish

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(Perelló et al., 2008), vegetables and rice (Zhuang et al., 2009). The environmental lead is known as reproductive toxicant (Nampoothiri and Gupta, 2006; Silberstein et al., 2006; Priya et al., 2004) which accumulates in female follicular fluid (Silberstein et al., 2006; Al-Saleh et al., 2008), in granulosa cells of the rat ovaries (Nampoothiri and Gupta, 2006), sheep ovaries (Bires et al., 1995), rat kidneys and testes (Massanyi et al., 2007), the liver and kidney of pig (López-Alonso et el., 2007) and brown hares (Kolesarova et al., 2008b; Kramarova et al., 2005; Massanyi et al., 2003) and muscle of pigs (López-Alonso et el., 2007).

Mercury (Hg) is a non-essential element for plant and animal nutrition. Its presence in agricultural systems is of concern due to its high potential toxicity. Mercury is persistent in the environment and has been listed as a pollutant by several environmental organizations (Tazisong and Senwo, 2009). Skin-lightening creams and dental amalgam are important contributors to mercury exposure (Al-Saleh et al., 2008). The highest concentrations of Hg (raw and cooked samples) were mainly found in fish (Perelló et al., 2008). Exposure to a high concentration of metallic mercury vapour may cause an increase in reproductive problems (Schuurs, 1998). Hg is a potent neurotoxic agent and neurotoxicant (Pugach and Clarkson, 2009) which accumulates in female follicular fluid (Al-Saleh et al., 2008), liver and kidney of pig (López-Alonso et el., 2007) and brown hares (Kolesarova et al., 2008b; Kramarova et al., 2005; Massanyi et al., 2003) and muscle of pigs (López-Alonso et el., 2007). The cooking process is only of a very limited value as a means of reducing metal (Pb, Hg) concentrations. This hypothetical reduction depends upon cooking conditions (time, temperature and medium of cooking) (Perelló et al., 2008).

The **insulin-like growth factor (IGF)** system plays an important role in folliculogenesis (Alexiadis et al., 2006). Insulin–like growth factor–I (IGF–I) is produced by porcine ovarian granulosa cells (Budacova et al., 2001; Sirotkin et al., 2009). It controls growth and lactation in swine (Lucy, 2008). The IGF system is also thought to contribute to the pathogenesis of many cancers, including those of the ovarian epithelium (Alexiadis et al., 2006). IGF-I promotes the FSH-stimulated synthesis (Nemcova et al., 2007).

Follicle-stimulating hormone (FSH) activates proliferation and inhibits apoptosis in rabbit ovarian cells, and that these effects can be mediated via cAMP/protein kinase A/CREB- or MAP kinase/CREB-dependent intracellular mechanisms (Lauková and Sirotkin, 2007). Hormonal interactions of the hypothalamic-pituitary-ovarian-uterine axis are accountable for a normal reproduction in female pigs. It is of importance to have knowledge of estrous symptoms and hormonal profiles around ovulation (Madej et al., 2005).

Mechanism of effects of lead and mercury in connection to IGF-I on ovarian granulosa cells of pregnant sows has not been examined previously. General objective of this *in vitro* study was to examine secretory activity of porcine ovarian granulosa cells from pregnant sows after lead, mercury and FSH additions.

MATERIAL AND METHODS

Preparation, culture and processing of granulosa cells from ovaries

Pregnant sows (n=12) of Slovakian White breed were kept under standard conditions at the Experimental Station of the Animal Production Research Centre Nitra. Conditions of their care, manipulations and use were in accordance to the instruction of EC no. 178/2002 and related EC documents, and they were approved by a local ethic commission. Porcine ovaries at the early and mid–follicular phase of the estrous cycle without visible reproductive abnormalities were obtained at slaughter house from healthy sows at fourth days of pregnancy (Olexiková et al., 2008). Ovaries were transported to the laboratory at 4ºC and washed in sterile physiological solution. Follicular fluid was aspirated from 3–5 mm follicles. Granulosa cells were isolated by centrifugation at 200g for 10 min followed by washing in sterile DMEM/F12 1:1 medium (BioWhittaker™, Verviers, Belgium) and resuspended in the same medium supplemented with 10 % fetal calf serum (BioWhittaker™) and 1 % antibiotic–antimycotic solution (Sigma, St. Louis, Mo, USA) at a final concentration of 10^6 cells.ml^{-1} of medium (determined by haemocytometer). Portions of the cell suspension were dispensed into 24–well culture plates (Nunc™, Roskilde, Denmark, 1 ml.well^{-1}). The granulosa cells in plate wells were incubated at 37.5 ºC and 5 % CO_2 in humidified air until a 75 % confluent monolayer was formed (5–7 days). At this point the medium was renewed and ovarian granulosa cells were incubated with the same supplements (10 % fetal calf serum, 1 % antibiotic–antimycotic solution) and with/without lead acetate - acetate trihydrate $Pb(CH_3COO)_2.3H_2O$, mercury chloride – $HgCl_2$ and FSH administration described in Table 1 as follows: maximum used dose of $Pb(CH_3COO)_2.3H_2O$/$HgCl_2$ was 0.5 mg.ml^{-1}; group A - 0.25 mg.ml^{-1}; group B - 0.083 mg.ml^{-1}; group C - 0.063 mg.ml^{-1}; group D - 0.046 mg.ml^{-1}; in case of FSH: group E - 1.0 ng.ml^{-1} and group G - 100 ng.ml^{-1}. Group F received FSH (1 ng.ml^{-1}) + lead acetate (0.083 mg.ml^{-1}) + mercury chloride (0.083 mg.ml^{-1}). Group H received FSH (100 ng.ml^{-1}) + lead acetate (0.063 mg.ml^{-1}) + mercury chloride (0.063 mg.ml^{-1}). Further culture was performed for 18 hrs and then the samples of culture media from plate wells were aspirated and kept at –20 ºC to await further RIA.

Table 1: Lead acetate, mercury chloride and FSH concentrations used in the study

Group	Lead acetate ($mg.ml^{-1}$)		Mercury chloride ($mg.ml^{-1}$)	FSH ($ng.ml^{-1}$)
Control	-	/	-	-
Max	0.500	/	0.500	-
A	0.250	/	0.250	-
B	0.083	/	0.083	-
C	0.063	/	0.063	-
D	0.046	/	0.046	-
E	-		-	1.0
F	0.083		0.083	1.0
G	-		-	100.0
H	0.063		0.063	100.0

Immunoassay

Concentrations of IGF–I were determined in 25–100 µL incubation medium by RIA using RIA kits (Immunotech SAS, Marseille Cedex, France) according to the manufacturer's instructions. RIA assay was validated for use in samples of culture medium (Makarevich and Sirotkin, 1999; Massanyi et al., 2000). The sensitivity of the assay was 2 $ng.ml^{-1}$, inter- and intra-assay coefficients of variation did not exceed 6.8%, and – 6.3%, respectively.

Statistical analysis

Each experimental group was represented by four culture wells of cultured granulosa cells. Assays of substances in incubation medium were performed in duplicates. Significant differences between the control (without administration of lead acetate, mercury chloride and FSH) and experimental groups (with lead acetate, mercury chloride and FSH administration – Max, A, B, C, D, E, F, G, H) were evaluated by paired t–test using Sigma Plot 9.0 statistical software (Jandel, Corte Madera, USA). The data are means±SEM. Differences were compared for statistical significance at the level $P<0.05$.

RESULTS

Release of IGF-I by ovarian granulosa cells from pregnant sows after the lead addition

IGF–I was released by the ovarian granulosa cells in the control group without lead administration and in the experimental groups with lead addition. Granulosa cells of the control group released 3.61±0.03 $ng.ml^{-1}$ of IGF-I, in case of group D - 4.33±4.29 $ng.ml^{-1}$, group C - 3.75±0.1.78 $ng.ml^{-1}$, group B - 5.33±4.54 $ng.ml^{-1}$ and group Max - 13.81±0.32 $ng.ml^{-1}$. Granulosa cells of groups Max and A released significantly ($P<0.05$) higher concentrations of IGF–I than the control group. The highest amount of IGF-I was released by ovarian cells of the experimental group Max with the highest Pb administration used in this study. In case of groups B, C, and D no significant ($P>0.05$) changes were noted in comparison to the control group (Fig. 1).

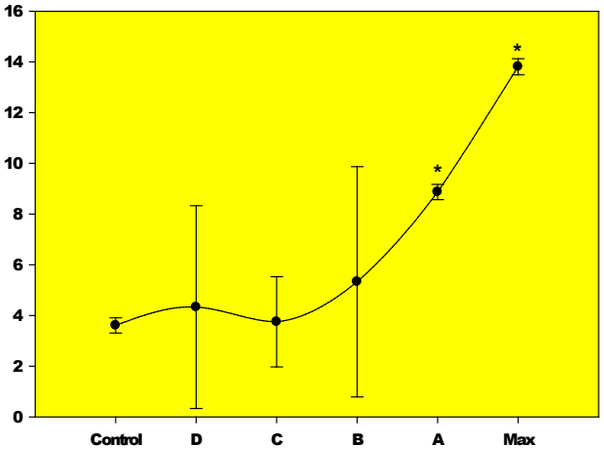

Fig. 1: Effect of lead acetate administration on IGF-I release by porcine ovarian granulosa cells of pregnant sows. Control represents culture medium without lead acetate administration. Group Max received lead acetate at 0.5 $mg.ml^{-1}$. Group A - 0.25 $mg.ml^{-1}$, group B - 0.083 $mg.ml^{-1}$, group C - 0.063 $mg.ml^{-1}$, group D - 0.046 $mg.ml^{-1}$. Values are means±SEM. * Significant differences from control $P<0.05$ were evaluated by paired t-test

Release of IGF-I by ovarian granulosa cells of pregnant sows after mercury addition

The release of IGF-I by porcine ovarian granulosa cells was 3.65±0.03 $ng.ml^{-1}$ in the control group. Release of IGF-I by granulosa cells of experimental group B after mercury addition showed significant differences ($P<0.05$) in comparison to the control group (Fig. 2). The highest amount of IGF-I was released by ovarian cells in the experimental group B (9.08±2.46 $ng.ml^{-1}$) with 0.083 $mg.ml^{-1}$ Hg administration used in this study. No significant differences ($P>0.05$) in released IGF-I by ovarian granulosa cells of pregnant sows in groups A (5.84±0.88 $ng.ml^{-1}$), C (0.72±0.05 $ng.ml^{-1}$) and D (0.79±0.01 $ng.ml^{-1}$) were noticed.

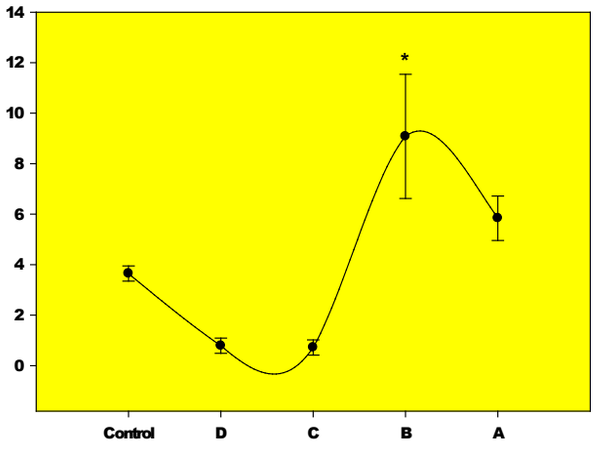

Fig. 2: Effect of mercury chloride administration on IGF-I release by porcine ovarian granulosa cells of pregnant sows. Control represents culture medium without mercury chloride administration. Group A received mercury chloride at 0.25 mg.ml^{-1}, group B - 0.083 mg.ml^{-1}, group C - 0.063 mg.ml^{-1}, group D - 0.046 mg.ml^{-1}. Values are means±SEM. *Significant differences from control P<0.05 were evaluated by paired t-test

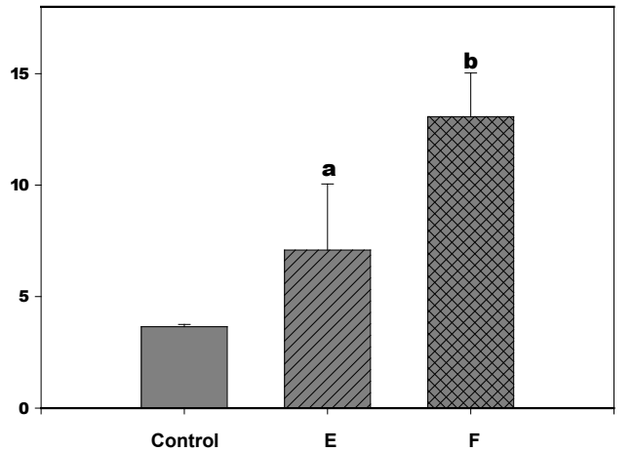

Fig. 3: Effect of FSH (1.0 ng.ml^{-1}), lead acetate and mercury chloride administration on IGF-I release by porcine ovarian granulosa cells of pregnant sows. Control represents culture medium without additions. Group E received FSH at 1 ng.ml^{-1}, group F received FSH (1 ng.ml^{-1}) + lead acetate (0.083 mg.ml^{-1}) + mercury chloride (0.083 mg.ml^{-1}). Values are means±SEM. a - no significant (P>0.05) differences between control and group E (paired t-test). b - no significant (P>0.05) differences between group E and F (paired t-test)

Release of IGF-I by ovarian granulosa cells of pregnant sows after FSH addition

It was found that IGF-I was released by granulosa cells of pregnant sows after FSH, Pb and Hg additions (Fig. 3, 4). Release of IGF-I was not significantly (P>0.05) stimulated by FSH addition at the concentration of 1.0 ng.ml^{-1} (group E, 6.20±1.51 ng.ml^{-1}) in comparison to the control (3.65±0.03 ng.ml^{-1}). It was found that IGF-I release was not significantly (P>0.05) stimulated by FSH + Pb + Hg (group F, 5.70±3.66 ng.ml^{-1}) in comparison to the group E with FSH addition 1 ng.ml^{-1} (6.20±1.51ng. ml^{-1}). Release of IGF-I (7.09±2.96 ng.ml^{-1}) by ovarian cells was not significantly (P<0.05) stimulated by FSH administration at 100 ng.ml^{-1} (group G) in comparison to the control (3.65±0.03 ng.ml^{-1}) without hormonal addition. FSH + Pb + Hg (group H) did not significantly (P>0.05) stimulate IGF-I release (13.07±1.96 ng.ml^{-1}) by granulosa cells of pregnant sows in comparison to the group G only with FSH addition.

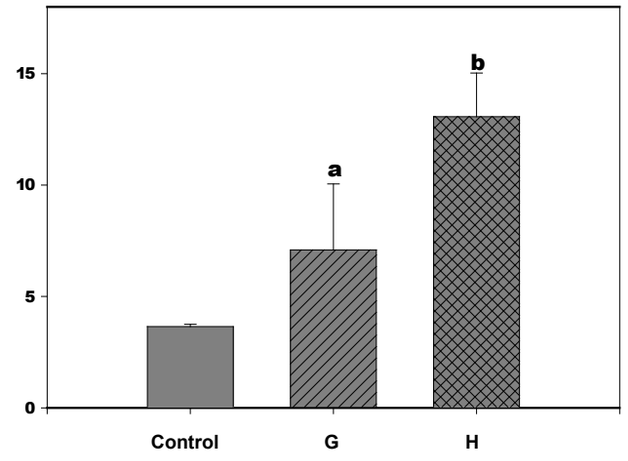

Fig. 4: Effect of FSH (100 ng.ml^{-1}), lead acetate and mercury chloride administration on IGF-I release by porcine ovarian granulosa cells of pregnant sows. Control represents culture medium without additions. Group G received FSH 100 ng.ml^{-1}, group H received FSH (100 ng.ml^{-1}) + lead acetate (0.063 mg.ml^{-1}) + mercury chloride (0.063 mg.ml^{-1}). Values are means±SEM. a - no significant (P>0.05) differences between control and group G (paired t-test). b - no significant (P>0.05) differences between group G and H (paired t-test)

DISCUSSION

Our observations describe the influence of Pb, Hg, as environmental risk factors and effect of FSH on the release of the growth factor (IGF-I) by ovarian granulosa cells of pregnant sows. Our reports confirm previous data about influence of heavy metals on cellular processes (Silberstein et al., 2006). The ovaries were the organs that accumulated Pb (Bires et al., 1995) what is in accordance with our investigation. Our study shows an accumulation of Pb in ovarian granulosa cells of pregnant sows, as it was previously reported in human follicular fluid (Silberstein et al., 2006; Al-Saleh et al., 2008), rat ovarian granulosa cells (Nampoothiri and Gupta, 2006) and sheep ovaries (Bires et al., 1995).

IGF-I release by granulosa cells of pregnant sows after Pb addition was determined. Significantly higher release of IGF-I by ovarian granulosa cells of pregnant sows after lead administration at doses of 0.5 $mg.ml^{-1}$ and 0.25 $mg.ml^{-1}$ was demonstrated in this study. Our report confirm previous data on a direct influence of Pb, as a reproductive toxicant (Nampoothiri and Gupta, 2006; Silberstein et al., 2006; Priya et al., 2004) on granulosa cell function (Priya et al., 2004). Histological changes in the number of ovarian follicles and the increased occurrence of primary atretic follicles indicate alterations in the membrane structures and organelles of oocytes and in the follicular cells of the *stratum granulosum* in sheep (Bires et al., 1995). Pre–pubertal female rats maternally exposed to Pb exhibit suppressed serum levels of IGF–I and delayed puberty (Pine et al., 2006). But the time to puberty onset in mice was markedly influenced by exposure to dietary lead (Iavicoli et al., 2004). Our investigation shows that higher doses of Pb contribute to the induction of IGF-I output by ovarian granulosa cells of pregnant sows.

Our reports on toxicity of Hg to ovarian granulosa cells of pregnant sows confirm previous studies concerning accumulation of Hg in human follicular fluid (Al-Saleh et al., 2008). Neurodevelopmental toxins, such as heavy metals, interrupt growth factor signalling (Waly et al., 2004). In our experiment a release of IGF-I by granulosa cells of pregnant sows was significantly stimulated after a mercury addition at the concentration 0.083 $mg.ml^{-1}$. Our study shows that mercury can be a potent regulator of the IGF-I release by ovarian granulosa cells of pregnant sows. This heavy metal, as an environment risk factor, can be a potential risk factor for normal growth of follicles during pregnancy of pigs.

The IGF-I signalling axis is important for the cell growth, differentiation and survival and increased serum IGF is a risk factor for cancers (Kaplan-Lefko et al., 2008). Evidence suggests that activation of the hypothalamic-pituitary-adrenal axis may hamper normal gonadotropin secretion and as a consequence, the ovarian function (Madej et al., 2005). IGF-I in conjunction with gonadotropins are important stimulators of mitosis by granulosa and theca cells, which are required for normal oocyte development and hormonal feedback signaling to the hypothalamus and pituitary (Grado-Ahuir et al., 2009). In this study IGF-I release by granulosa cells of pregnant sows after FSH, Pb and Hg additions was found. Release of IGF-I was not significantly stimulated by FSH addition in all used concentrations - 1.0 $ng.ml^{-1}$ and 100 $ng.ml^{-1}$. FSH reduced IGF-I (at 1-100 $ng.ml^{-1}$) in transgenic rabbits (Sirotkin et al., 2008). IGF-I promotes the FSH-stimulated synthesis in porcine cumulus-oocyte complexes (Nemcova et al., 2007). It was found that IGF-I was not significantly stimulated by FSH (1.0 $ng.ml^{-1}$), Pb (0.083 $mg.ml^{-1}$) and Hg (0.083 $mg.ml^{-1}$). Release of IGF-I by ovarian cells of pregnant sows was not significantly stimulated by the FSH (100 $ng.ml^{-1}$), Pb (0.063 $ng.ml^{-1}$) and Hg (0.063 $ng.ml^{-1}$) administration. These investigations suggest that effect of heavy metals Pb and Hg in pregnant sow depends on the secretion of FSH by the pituitary.

Our observations suggest possible involvement of heavy metal – Pb and Hg and hormone of pituitary FSH, in the regulation of IGF-I release by porcine ovarian granulosa cells of pregnant sows. Our results contribute to knowledge of mechanisms of lead and mercury effect on porcine ovarian granulosa cells of pregnant sows. However, uncontrolled growth of follicles by increased IGF-I level can be a potential risk factor of cancers.

ACKNOWLEDGMENT

The authors are thankful to Katarína Tóthová, Ing. Žofia Kuklová and Ing. Peter Čupka for skilful technical assistance. This work was financially supported by the APVV project 0299–06 and VEGA scientific grant 1/0696/08.

REFERENCES

ALEXIADIS, M. – MAMERS, P. – CHU, S. – FULLER, P. J. 2006. Insulin-like growth factor, insulin-like growth factor-binding protein-4, and pregnancy-associated plasma protein-A gene expression in human granulosa cell tumors. *Int. J. Gynecol. Cancer*, vol. 16, 2006, no. 6, p. 1973-1979.

AL-SALEH, I. – COSKUN, S. – MASHHOUR, A. – SHINWARI, N. – EL-DOUSH, I. – BILLEDO, G. – JAROUDI, K. – AL-SHAHRANI, A. – AL-KABRA, M. – EL DIN MOHAMED, G. 2008. Exposure to heavy metals (lead, cadmium and mercury) and its effect on the outcome of in-vitro fertilization treatment. *Int. J. Hyg. Environ. Health.* vol. 211, 2008, no, 5-6, p. 560-579.

BAOS, R. – BLAS, J. – BORTOLOTTI, G. R. – MARCHANT, T. A. – HIRALDO, F. 2006. Adrenocortical response to stress and thyroid hormone status in free-living nestling

white storks (Ciconia ciconia) exposed to heavy metal and arsenic contamination. *Environmental Health Perspectives*, vol. 114, 2006, p. 1497-1501.

BENES, B. – SPĚVÁCKOVÁ, V. – SMÍD, J. – CEJCHANOVÁ, M. – KAPLANOVÁ, E. – CERNÁ, M. – GAJEWSKÁ, V. – BLATNÝ, J. 2002. Determination of normal concentration levels of Cd, Pb, Hg, Cu, Zn and Se in urine of the population in the Czech Republicjavascript:AL_get(this, ‚jour', ‚Cent Eur J Public Health.'); *Cen.t Eur. J, Public Health*., 2002, vol. 10, 2002, no. 1-2, p. 3-5.

BIRES, J. – MARACEK, I. – BARTKO, P. – BIRESOVA, M. – WEISSOVA, T. 1995. Accumulation of trace elements in sheep and the effects upon qualitative and quantitative ovarian changes. *Veterinary and human toxicology,* vol. 37, 1995, p. 349-356.

BUDACOVA, A. – SIROTKIN, A. – KRAMAROVA, M. – KOVACIK, J. – FLORKOVICOVA, I. – SANISLO, P. 2001. Changes in function and responses of porcine ovarian granulosa cells during sexual maturation. *Reproduction Abstract Series,* vol. 27, 2001, p. 14.

HABAN, M. – HABANOVA, M. – OTEPKA, P. – LUKAC, N. – MASSANYI, P. 2008. Concentration of heavy metals in various children's herbal tea types and their correlations. *Journal of environmental science and health B,* vol. 43, 2008, p. 533-538.

CHRASTNÝ, V. – KOMÁREK, M. – HÁJEK, T. 2009. Lead contamination of an agricultural soil in the vicinity of a shooting range. In: *Environmental Monitoring and Assessment*, 2009, Feb 20 [Epub ahead of print]

GRADO-AHUIR, J. A. – AAD, P. Y. – RANZENIGO, G. – CALONI, F. – CREMONESI, F. – SPICER, L. J. 2009. Microarray analysis of insulin-like growth factor-I-induced changes in messenger ribonucleic acid expression in cultured porcine granulosa cells: possible role of insulin-like growth factor-I in angiogenesis. *J. Anim. Sci.* vol. 87, 2009, no. 6, p. 1921-1933.

IAVICOLI, I . – CARELLI, G. – STANEK, E. J. 3rd – CASTELLINO, N. – CALABRESE, E. J. 2004. Effects of low doses of dietary lead on puberty onset in female mice. *Reproductive Toxicology, vol.* 19, 2004, P. 35-41.

KAPLAN-LEFKO, P. J. – SUTHERLAND, B. W. – EVANGELOU, A. I. – HADSELL, D. L – BARRIOS, R. J. – FOSTER, B. A. – DEMAYO, F. – GREENBERG, N. M. 2008. Enforced epithelial expression of IGF-1 causes hyperplastic prostate growth while negative selection is requisite for spontaneous metastogenesis. *Oncogene,* vol. 27, 2008, no. 20, p. 2868-76.

KOLESAROVA, A. – SLAMECKA, J. – JURCIK, R. – TATARUCH, F. – LUKAC, N. – KOVACIK, J. – CAPCAROVA, M. – VALENT, M. – MASSANYI, P. 2008. Environmental levels of cadmium, lead and mercury in brown hares and their relation to blood metabolic parameters. *Journal of environmental science and health, Part A Toxic/Hazardous Substances and Environmental Engineering*, vol. 43, 2008, p. 646-650.

KRAMÁROVÁ, M. – MASSÁNYI, P. – SLAMECKA, J. – TATARUCH, F. – JANCOVÁ, A. – GASPARIK, J. – FABIS, M. – KOVACIK, J. – TOMAN, R. – GALOVÁ, J. - JURCIK, R. 2005. Distribution of cadmium and lead in liver and kidney of some wild animals in Slovakia. *Journal of environmental science and health, Part A Toxic/Hazardous Substances and Environmental Engineering*, vol. 40, 2005, p. 593-600.

LAUKOVÁ, M. – SIROTKIN, A.V. 2007. Follicle-stimulating hormone: effects and possible mechanisms of action in rabbit ovarian cells. *Slovak J. Anim. Sci.,* vol. 40, 2007, no. 2, p. 66 – 71.

LÓPEZ-ALONSO, M. – MIRANDA, M. – CASTILLO, C. – HERNÁNDEZ, J. – GARCÍA-VAQUERO, M. – BENEDITO, J. L. 2007. Toxic and essential metals in liver, kidney and muscle of pigs at slaughter in Galicia, north-west Spain. *Food Additives and Contaminants*, vol. 24, 2007, p. 943-54.

LUCY, M. C. 2008. Functional differences in the growth hormone and insulin-like growth factor axis in cattle and pigs: implications for post-partum nutrition and reproduction. *Reproduction in Domestic Animals*, vol. 43, 2008, p. 31-39.

MADEJ, A. – LANG, A. – BRANDT, Y. – KINDAHL, H. – MADSEN, M. T. – EINARSSON, S. 2005. Factors regulating ovarian function in pigs. *Domest. Anim. Endocrinol.*, vol. 29, 2005, no. 2, p. 347-361.

MAKAREVICH, A. V. – SIROTKIN, A. V. 1999. Development of sensitive radioimmunoassay for IGF-I determination in samples from blood plasma and cell-conditioned medium. *Veterinary Medicine*, vol. 44, 1999, p. 71-78.

MASSANYI, P. – TATARUCH, F. – SLAMECKA, J. – TOMAN, R. – JURCIK, R. 2003. Accumulation of lead, cadmium, and mercury in liver and kidney of the brown hare (Lepus europaeus) in relation to the season, age, and sex in the west Slovakian lowland. *Journal of environmental science and health Part. A*, vol. 38, 2003, p. 1299-1309.

MASSANYI, P. – LUKAC, N. – MAKAREVICH, A. V. – CHRENEK, P. – FORGACS, Z. – ZAKRZEWSKI, M. – STAWARZ, R. - TOMAN, R. - LAZOR, P. - FLESAROVA, S. 2007. Lead-induced alterations in rat kidneys and testes in vivo. *Journal of environmental science and health, Part A Toxic/Hazardous Substances and Environmental Engineering*, vol. 42, 2007, p. 671-676.

MASSANYI, P. – UHRIN, V. – SIROTKIN, A. – PAKSY, K. – FORGACS, Z. S. – TOMAN, R. – KOVACIK, J. 2000. Effect of Cadmium on Ultrastructure and Steroidogenesis in Cultured Porcine Ovarian Granulosa cells. *Acta Veterinaria Brno*, vol. 69, 2000, p. 101-106.

MIN, K. S. – UEDA, H. – KIHARA, T. – TANAKA, K. 2008. Increased hepatic accumulation of ingested Cd is associated with upregulation of several intestinal transporters in mice fed diets deficient in essential metals. *Toxicological Sciences*, vol. 106, 2008. 284-289.

NAMPOOTHIRI, L. P. - GUPTA, S. 2006. Simultaneous effect of lead and cadmium on granulosa cells: a cellular model for ovarian toxicity. *Reproductive Toxicology*, vol. 21, 2006, p. 179-185.

NEMCOVÁ L. – NAGYOVÁ, E. – PETLACH, M. – TOMÁNEK, M. – PROCHÁZKA, R. 2007. Molecular mechanisms of insulin-like growth factor 1 promoted synthesis and retention of hyaluronic acid in porcine oocyte-cumulus complexes. *Biol. Reprod.*, vol. 76, 2007, no. 6, p. 1016-1024.

OLEXIKOVÁ, L. – MAKAREVICH, A. V. – KUBOVIČOVÁ, E. – PIVKO, J. 2008. The effect of antiserum to heat-shock proteins 70 (Hsp70) on the *in vitro* development of porcine

embryos exposed to hyperthermia. *Slovak J. Anim. Sci.,* vol. 41, 2008, no. 3, p. 105-108.

PERELLÓ, G. – MARTÍ-CID, R. – LLOBET, J. M. – DOMINGO, J. L. 2008. Effects of various cooking processes on the concentrations of arsenic, cadmium, mercury, and lead in foods. *J Agric Food Chem.*, vol. 56, 2008, no. 23, p. 11262-11269.

PINE, M. D. – HINEY, J. K. – DEARTH, R. K. - BRATTON, G. R. – DEES, W. L. 2006. IGF-1 administration to prepubertal female rats can overcome delayed puberty caused by maternal Pb exposure. *Reproductive Toxicology*, vol. 21, 2006, p. 104-109.

PRIYA, P. N. – PILLAI, A. – GUPTA, S. 2004. Effect of simultaneous exposure to lead and cadmium on gonadotropin binding and steroidogenesis on granulosa cells: an *in vitro* study. *Indian J Exp Biol.*, vol. 42, 2004, p. 143-148.

PUGACH, S. – CLARKSON, T. 2009. Prenatal mercury exposure and postnatal outcome: clinical case report and analysis. *Clin Toxicol (Phila)*, Apr 21:1-5. [Epub ahead of print]

SHAN, G. – TANG, T. – ZHANG, X. 2009. The protective effect of ascorbic acid and thiamine supplementation against damage caused by lead in the testes of mice. *Journal of Huazhong University of Science and Technology Medical Sciences*, vol. 29, 2009, p. 68-72.

SCHUURS, A. H. Working with mercury: cause of infertility in women? *Ned. Tijdschr. Tandheelkd.*, vol. 105, 1998, no. 11, p. 401-403.

SILBERSTEIN, T. – SAPHIER, O. – PAZ-TAL, O. – TRIMARCHI, J. R. – GONZALEZ, L. – KEEFE, D. L. 2006. Lead concentrates in ovarian follicle compromises pregnancy. *J. Trace Elem. Med. Biol.*, vol. 20, 2006, 205-207.

SIROTKIN, A. V. – CHRENEK, P. – DARLAK, K. – VALENZUELA, F. – KUKLOVÁ, Z. 2008. Some endocrine traits of transgenic rabbits. II. Changes in hormone secretion and response of isolated ovarian tissue to FSH and ghrelin. *Physiol Res.*, vol. 57, 2008, no. 5, p. 745-751.

SIROTKIN, A. V. – RAFAY, J. – KOTWICA, J. – DARLAK, K. – VALENZUELA, F. 2009. Role of ghrelin in regulating rabbit ovarian function and the response to LH and IGF-I. *Domestic Animal Endocrinology*, vol. 36, 2009, p. 162-172.

TAZISONG, I. – SENWO, Z. 2009. Mercury concentration and distribution in soils impacted by long-term applied broiler litter. *Bull Environ. Contam. Toxicol.*, vol. 83, 2009, no. 2, p. 291-294.

WALY, M. – OLTEANU, H. – BANERJEE, R. – CHOI, S. W. – MASON, J. B. – PARKER, B. S. – SUKUMAR, S. – SHIM, S. – SHARMA, A. – BENZECRY, J. M. POWER-CHARNITSKY, V. A. – DETH, R. C. 2004. Activation of methionine synthase by insulin-like growth factor-1 and dopamine: a target for neurodevelopmental toxins and thimerosal. *Mol. Psychiatry*, vol. 9, 2004, no. 4, p. 358-370.

ZHUANG, P. – ZOU, B. – LI, N. Y. – LI, Z. A. 2009. Heavy metal contamination in soils and food crops around Dabaoshan mine in Guangdong, China: implication for human health. *Environmental Geochemistry and Health*, Feb 13 [Epub ahead of print]