# ANTIOXIDANT STATUS OF HENS OVARIAN GRANULOSA CELLS AFTER MOLYBDENUM TREATMENT IN VITRO

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## ABSTRACT

The objective of this study was to determine the total antioxidant status (TAS) of medium and lysate of hens ovarian granulosa cells cultured *in vitro* after molybdenum (Mo) treatment. Portions of the cell suspension were dispensed to 24-well plates. The plates were incubated until a confluent monolayer was formed. At this point the medium (1 ml.well$^{-1}$) was renewed and ovarian granulosa cells were incubated with ammonium molybdate administrations as follows: group A (0.05 mg.ml$^{-1}$), group B (0.33 mg.ml$^{-1}$), group C (0.17 mg.ml$^{-1}$), group D (0.09 mg.ml$^{-1}$) and the control group without Mo additions for 18 h. In medium of granulosa cells higher values of TAS were recorded in D and control group. Significantly ($P<0.05$) higher TAS in lysate of granulosa cells was determined in D group.

**Keywords**: hens, granulosa cells, molybdenum, total antioxidant status

## INTRODUCTION

The reproductive health of animals could be affected by a number of endogenous as well as exogenous factors, such as exposure to heavy metals **(Massanyi et al., 2010).** Female reproductive functions can be compromised by exposure to toxic chemicals at a variety of sites, including ovary or reproductive tract **(Mlynarcikova et al., 2005; Kolesárová et al., 2009).** As it was published previously, the exposure of hens and other animals to xenobiotics caused various alternations of zootechnical parameters **(Arpasova et al., 2007; Kalafová et al., 2009a)** as well as imbalance in internal milieu **(Schneidgenová et al., 2007, 2008; Capcarova et al., 2008; Kolesarova et al., 2008, Skalická et al., 2009; Koréneková et al., 2009; Martiniaková et al., 2009; Capcarova et al., 2010; Kolesarova et al., 2010a, 2010b; Kňažická et al., 2010).** Internal milieu of animals is very important indicator of certain changes **(Schneidgenova and Kalafova, 2005; Kalafová et al., 2009b)** caused by various factors. Recent studies indicate that transition metals act as catalysts in the oxidative reactions of biological macromolecules, and therefore, the toxicities associated with these metals might be due to oxidative tissue damage **(Ercal et al., 2001)**. Oxidative stress occurs as a consequence of excessive production of reactive oxygen species (ROS) or impaired antioxidant defence systems **(Sugino, 2007)**. The antioxidant power of biological fluids can be evaluated either by quantification of individual antioxidants or by assessing their aggregate, cumulative action and synergic effect. This latter concept is known as the total antioxidant capacity or status (TAC or TAS). The quantification of individual antioxidants is a complicated, expensive and time consuming task. Therefore the idea of a single measurement of TAS is very attractive **(Fingerova et al., 2007)**. TAS represents the level of cumulative antioxidant reserve of the body and enables evaluation of the average antioxidant potential. Thus, the overall antioxidant status will give more relevant biological information compared to that obtained by the measurement of individual components **(Miller et al., 1993)**. Heavy metals cause oxidative stress by inducing the generation of ROS, reducing the antioxidant defense system of cells via depleting glutathione, inhibiting sulfhydryl-dependent enzymes, interfering with some essential metals needed for antioxidant enzyme activities, and/or increasing susceptibility of cells to oxidative attack by altering the membrane integrity and fatty acid composition. Consequently, it is plausible that impaired oxidant/antioxidant balance can be partially responsible for the toxic effects of lead. Data suggest that antioxidants may play an important role in abating some hazards of heavy metals **(Capcarova et al., 2009; Dalton et al., 1999)**.

The purpose of this study was to evaluate the effect of molybdenum treatment on antioxidant status of hens ovarian granulosa cells cultured *in vitro*.

## MATERIAL AND METHODOLOGY

**Preparation, culture and processing of granulosa cells from ovaries**: White Leghorn hens (n=12) about 500 days old, with an egg laying rate of more than 75 %, were held under standard conditions at the Experimental Station of the Slovak Agricultural University in Nitra. Conditions of their care, manipulations and use did correspond the instruction of EC no. 178/2002 and related EC documents, and they were approved by local ethic commission. Birds were decapitated between 9:00 and 11:00 and the largest (F1-F2) follicles were isolated from the ovary. The stage of folliculogenesis was determined by recording the time of the last oviposit and by the size and position of the next ovarian follicle. Granulosa cells were isolated by centrifugation for 10 min at 200xg 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 to 24-well culture plates (Nunc™, Roskilde, Denmark, 1 ml.well$^{-1}$). The well plates were incubated at 38.5$^{o}$C and 5 % CO in humidified air 2 until a 75 % confluent monolayer was formed (4 days). At this point the medium (1 ml.well$^{-1}$) was renewed and ovarian granulosa cells were incubated with the 1 % antibiotic-antimycotic solution and with ammonium molybdate administrations as follows: group A (0.05 mg.ml$^{-1}$), group B (0.33 mg.ml$^{-1}$), group C (0.17 mg.ml$^{-1}$), group D (0.09 mg.ml$^{-1}$) and the control group without Mo additions for 18

h. The culture media from well plates were aspirated and kept at -70°C to await further analysis. Cells from plates were manually smashed and lysate was obtained. The total antioxidant status of porcine ovarian granulosa cells (medium/lysate) was assayed by spectrophotometer Genesys 10 (using antioxidant RANDOX kits (Randox Labs., Crumlin, UK) according to the manufacturer's instructions.

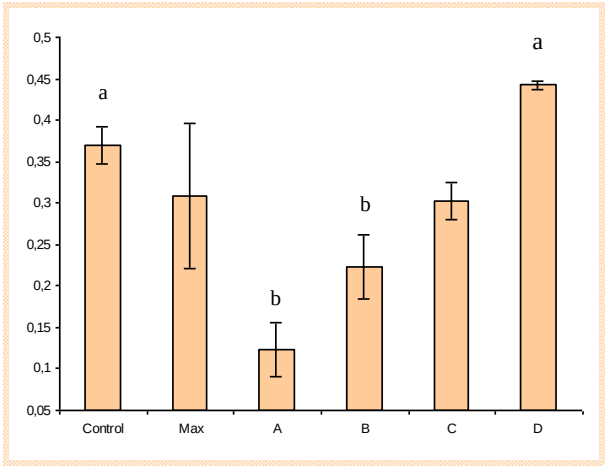
**Statistical analysis:** Each experimental group was represented by four culture wells of cultured granulosa cells. Assays of substances in incubation medium were performed in duplicate. Significant differences between the control and experimental groups were evaluated by one-way ANOVA test using statistical software Sigma Plot 11.0 (Jandel, Corte Madera, USA). The data are expressed as means ± SEM. Differences were compared for statistical significance at the levels $P < 0.05$.

## RESULTS AND DISCUSION

The comparisons of TAS (medium and lysate) among individual groups are shown in Figure 1-2. The mechanisms of alleviating oxidative stress in porcine oocytes are very complex and supplementing maturing oocytes with anti-oxidants may enhance enzyme activities and eliminate free radicals **(Whitaker and Knight, 2008).**

In the medium of granulosa cells higher values of TAS were recorded in D group (the lowest molybdenum exposure) and control group (without molybdenum treatment). Statistical analyses showed significant ($P<0.05$) differences between control and A groups, than between D and A groups, and between D and B groups.

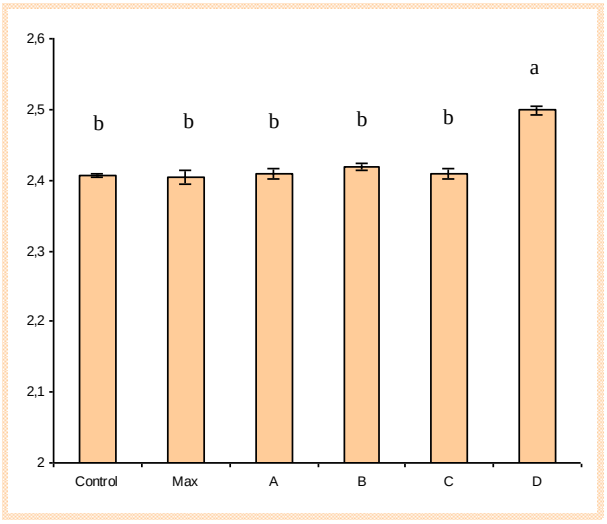
**Figure 1 Effect of molybdenum on total antioxidant status in medium of hens granulosa cells**




[a,b] Means with different superscripts differ significantly ($P<0.05$), values shown are mean±SEM

The significantly ($P<0.05$) higher TAS in lysate of granulose cells was determined in D group with the lowest molybdenum treatment in comparison with other groups.

**Figure 2 Effect of molybdenum on total antioxidant status in lysate of hens granulosa cells**




[a,b] Means with different superscripts differ significantly ($P<0.05$), values shown are mean±SEM

Total antioxidant status is defined as the sum total of endogenous and food derived antioxidants of the extracellular fluids of an individual. Cooperation of all the different antioxidants provides greater protection against attack by ROS or nitrogen radicals than any single compound alone **(Miller et al., 1993).** In this study the higher TAS release by granulosa cells was observed in the control group and in the group with the lowest molybdenum treatment (group D). It could indicate the presence of oxidant/antioxidant imbalance due to lead acetate addition in ovarian granulosa cells after molybdenum addition. TAS seems to correlate with decreased fertilization potential **(Tatone et al., 2008)**. It seems that antioxidant system inside of cells is stronger that this in medium. This may be due to activation and involvement of antioxidant mechanisms.

## CONCLUSION

In conclusion, molybdenum may have negative effect on hens ovarian granulosa cells when added to culture medium. TAS in medium was decreased by virtue of molybdenum. Results of this study provide a foundation for further analysis and researches of heavy metals impact on living cells and the system of possible protection against its effects as well as evaluation of various dose dependencies on antioxidant status of cells.

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