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CYTOCHROME P450 1A INDUCTION IN CHICKEN OVARIAN FOLLICLES EXPOSED TO 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN

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Cytochrome P450 monooxygenases (CYPs) comprise a large family of proteins that are involved in catalyzing the oxidation of wide variety of xenobiotics, including dioxins and dioxin-like compounds. Chickens have two CYP1A isoforms (CYP1A4 and CYP1A5) which are orthologous to mammalian CYP1A1 and CYP1A2, respectively. It is well-known that the chicken liver expresses both CYP1As, and dioxins induce CYP1A4 and A5 activities in this organ. Recently, we have found that chicken ovarian follicles express *CYP1A4* and *CYP1A5* mRNA. The aim of the present experiment was to measure *in vitro* CYP1A activity in control and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) exposed ovarian follicles of the laying hen.

The experiment was performed on Hy-Line Brown hens at the age of 25 weeks. Birds were fed *ad libitum* and kept in individual cages at a neutral temperature (18-20°C), and under a photoperiodic regime of 14L:10D. Hens were decapitated 2 h after ovulation. Small (1-4 mm) and large (4-8 mm) white follicles and 3 the largest preovulatory follicles (20-36 mm; F3-F1; F3<F2<F1) were isolated from the ovary. In respect to the preovulatory follicles, the granulosa layer was separated from the theca one by Gilbert's method. The white follicles and fragments of granulosa and theca layers were incubated for 24 h at 39°C in 1 ml Eagle's medium supplemented with TCDD (Dr. Ehrenstorfer GmbH, Germany) at concentrations of 0 (control) or 10 nM, ovine LH (oLH; 10 ng/ml) and combination of oLH (10 ng/ml) with TCDD (10 nM). After incubation, tissues were collected and kept at -80°C till the measurement of the CYP1A enzyme activity. CYP1A activity was determined by fluorometric MROD assay using 7-methoxyresorufin as a substrate. The methoxyresorufin metabolite, resorufin, was measured using the excitation wavelength of 530 nm and the emission wavelength of 590 nm. Protein concentration was measured using fluorescamine at the excitation wavelength of 400 nm and the emission wavelength of 460 nm. The measurements were done in triplicates using 96-well plates with BioTek FLx-800TBI microplate reader. MROD activity was expressed as nmol resorufin/mg protein/min. The statistical analysis of the results was performed by ANOVA for repeated measures followed by Tukey test. Significance of differences was considered at the level of $P<0.05$.

The MROD activity was detected in all investigated ovarian follicles. The basal MROD activity ranged from 0.18 in the granulosa layer of the F3 follicle to 2.5 nmol resorufin/mg protein/min in the theca layer of the F1 preovulatory one ($P<0.01$). In comparison to control group, TCDD or oLH significantly ($P<0.05$-0.01) increased the MROD activity in all investigated follicles and tissues. The highest MROD activity following TCDD exposure was found in the granulosa layer of F3 preovulatory follicle (14.0 nmol resorufin/mg protein/min; $P<0.01$) while following oLH treatment in the granulosa layer of the F2 follicle (11.2 nmol resorufin/mg protein/min; $P<0.01$). Incubation of these follicles in medium supplemented with both oLH and TCDD revealed that TCDD did not affect oLH stimulated MROD activity.

In conclusion, the basal and TCDD inducible CYP1A activity in ovarian follicles suggest that the chicken ovary is involved in the process of xenobiotic detoxication.

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