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ACTIVITY OF ALKALINE AND ACIDIC PHOSPHATASE IN GLANDULAR CELLS OF UTERINE ENDOMETRIUM OF PUERPERAL EWES AFTER EXPOSURE TO POLYCHLORINATED BIPHENYLS

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The study is focused on the observation of alkaline and acidic phosphatase activity in the glandular cells of uterine endometrium in puerperal ewes after exposure to polychlorinated biphenyls. Ewes of Slovak merino breed (n=25) divided into 2 groups were included in the experiment. The animals in the experimental group (n=14) and control group (n=11) were euthanised on day 17, 25 and 34 postpartum. The ewes in the experimental group were given per os capsules of the chemical preparation Delor 105 of domestic proveniance containing polychlorinated biphenyls (PCB) for a period of 30 days. This preparation is equivalent to the foreign preparation Aroclor 1254. A dose of 100 μg/kg of Delor 105 was given to the animals of the experimental group. These animals were euthanised on day 17 postpartum (n=4) i. e. 5 days from the end of a 30-day period of application; on day 25 postpartum (n=5) i.e. 17 days from the last application of PCB; on day 34 postpartum (n=5), which was equivalent to day 28 from the last application. The ewes from the control group were euthanised on day 17 (n=3), day 25 (n=4) and on day 34 (n=4) postpartum. When evaluating alkaline phosphatase (ALP) activity in the glandular cells of the endometrium in the control group, a statistically significant increase (P<0.01) was observed on day 25 and on day 34 (P<0.001) compared to day 17 postpartum. No statistically significant differences in alkaline phosphatase (ALP) activity were observed (P>0.05) in the experimental group. The mean values of its activity in the observed period were below the level of values of day 17 in the control group. Acidic phosphatase activity in the glandular cells of the ewes' endometrium showed a statistically conclusive increase between day 17 and day 25 as well as day 34 postpartum (P<0.001). Acidic phosphatase density in the experimental group of ewes showed no statistically marked change (P>0.05) at the observed intervals postpartum.

The discussion is focused on PCB effect on the activity of alkaline and acidic phosphatase in the glandular cells of the endometrium of ewes in the puerperal period.

Key words: puerperium, ewe, alkaline phosphatase, acidic phosphatase, polychlorinated biphenyls.

INTRODUCTION

The uterus is an organ with a metabolically active tissue which is subject to functional and morphological changes at the time of the estrous cycle, pregnancy and puerperium. During the puerperal period, regressive and regenerative processes occur in the uterine mucosa, particularly the regeneration of the surface epithelium. The localization of enzymes (alkaline and acidic phosphatase) in the cells of surface epithelium and glandular uterine cells plays an important role in physiological tissue regression and transphosphorilation reactions.

Several authors gained knowledge of enzyme activity using histochemical reactions during the estrous cycle in various animal species (Schnurrbusch *et al*., 1988; Schroder, 1987; Uhrín, 1992). The rate of the acidic phosphatase reaction begins to increase significantly as early as the onset of the follicular phase (Uhrín, 1992) of the estrous cycle in cows. An increasing trend of alkaline and acidic phosphatase activity with the progressing of the postpartal period in goats was reported by Krajničáková *et al*. (2002). Marjanov *et al*. (1985) observed that the activity of alkaline phosphatase decreased in the endometrium of sows after administration of medrooxyprogesterone. Exposure to polychlorinated biphenyls (PCB) had a negative effect on sperm motility, *in vitro* fertilization and embryo development in mice (Lu-Shuiyuan *et al*., 2000; Fielden *et al*., 2001). Rats exposed to polychlorinated biphenyls (Aroclor 1248) exhibit a decrease in androgen serum levels. These data were also confirmed by *in vitro* studies which showed that micromolar concentrations of A 1248 inhibit the conversion of δ - 4 – androstendione into testosterone (Andric *et al*., 2000). The effect of polychlorinated biphenyls on decrease in aromatase activity was reported by Gerstenberger *et al*., (2000).

Available data suggest that polychlorinated biphenyls have an inhibitive effect on the enzymatic system in animal organisms.

The aim of this study was to observe the activity of alkaline and acidic phosphatase in the glandular cells of endometrium in puerperal ewes after exposure to polychlorinated biphenyls from Delor 105 preparation and to detect a possible effect of PCB on enzyme activity during regenerative and regressive processes in the above stated period.

MATERIALS AND METHODS

Experimental animals

The experiment was carried out on the basis of accreditation of State veterinary and food administration of the Slovak republic (accreditation number 10115/02-220).

Slovak merino ewes (n=25) divided into 2 groups were included in the experiment. The experimental ewes (n=14) and control group ewes (n= 11) were euthanised on day 17, 25 and 34 postpartum. The experimental ewes were given *per os* PCB preparation Delor 105 in capsules of home production (Chemko, Slovakia), which is equivalent to the foreign preparation Aroclor 1254 (CAS 11097691). The ewes were weighed individually before exposure to the chemical. The application of the chemical - 100 μg/kg was made on the basis of the body weight. Lambing period of the ewes lasted 21 days.

The experimental animals were euthanised 5 days after the last Delor 105 application, on day 17 postpartum (n=4), on day 25 postpartum (n=5), which was equivalent to 17 days from the last PCB application and on day 34 postpartum (n=5), which corresponded to day 28 from the last PCB application. Animals in the control group were euthanised on day 17 (n=3), day 25 (n=4) and day 34 (n=4) postpartum. Feed ration per animal/day consisted of meadow hey (1.5 kg), fodder concentrate (250 g), straw (1.5 kg) and root crops (300 g). A mineral supplement (Se, Zn, J, Cu, Co, Mg) and water were available *ad libitum* (Sommer *et al*., 1994).

The reproductive tract of the ewes was excised after euthanasia (Thiopental ® inj., Léčivá CZ). Samples of the endometrium from the uterine body and uterine horns were frozen in liquid nitrogen vapour (-196 ^{o}C) and stored in a freezer at -20 ^{o}C until processed.

The determination of alkaline phosphatase was performed by a modified method as described by Lojda *et al*. (1979). The incubation medium contained naphtol- AS-BI-phosphate (Fluka, Germany), stable diazonium salt Fast Blue BB (Sigma, USA) and veronal acetate buffer (pH 9.2). The incubation was carried out at 37 ^{o}C for 15 minutes.

Acidic phosphatase was determined using the procedure described by Lojda *et al*. (1979). The incubation medium contained naphtol-AS-MX-phosphate (Fluka, Germany), hexazo-p-rozaniline (Serva, Germany). Sodium acetate (pH 5.3) was used as buffer. The incubation was performed at 37^{o} C for 30 minutes.

Absorbance was measured at a wavelength of 480 μm and 520 μm. Enzyme density was analysed cytophotometrically with a VICKERS 85 (UK) micro-densitometer. The measurements were accomplished using a x40 objective in scanning area of 28.3 μm^3 and 0.5 μm scanning spot. Masking shield was placed on at least 30 places along uterine sections. Activity of the observed enzymes was calculated as absorbance values min/μm^3 recorded by the instrument in the glandular uterine epithelial cells.

Statistic evaluation

Statistical evaluation of the enzyme activity (ALP, AP) was accomplished using a one-way variance analysis (ANOVA). Variance significance between the groups and statistical significance on individual days of euthanasia in the ewes' postpartum period was determined by Tukey test.

RESULTS AND DISCUSSION

Alkaline phosphatase activity in the glandular cells of the endometrium (Tab. 1) had a rising tendency with a statistical significance between day 17 and day 25 postpartum ($P<0.01$). The range of values of its activity showed significant difference ($P<0.001$) between day 17 and day 34 of the evaluated period in the control group. Similar results were reported by Krajničáková *et al*., (2002) in regressive changes occurring in the uterus during the postpartum period of goats. Zamiri *et* Blackshaw (1979) claim that alkaline phosphatase activity at the surface uterine glands of ewes gradually increased with peak values between day 14 and day 21 of the estral cycle. The proliferation process of endometrial structures of sheep and goats as observed by Krajničáková *et al*., (1999, 2002) is consistent with the occurrence of alkaline phosphatase activity and connected with their role in transformation mechanisms and metabolic processes occurring in the postpartum uterus.

Table 1. The mean values of the activity of alkaline phosphatase in the endometrial glandular cells of the control and experimental groups of ewes after parturition

DAYS POSTPARTUM	ALKALINE PHOSPHATASE	
	CONTROL	EXPERIMENTAL
17	5.394 ± 0.243 ^{a}	5.088 ± 0.63 ^{a}
25	6.240 ± 0.497 ^{b**}	5.169 ± 0.61 ^{b}
34	6.664 ± 0.396 ^{b***}	5.228 ± 0.62 ^{b}

Statistical significance compared to day 17, 25 and 34 days after parturition
** = $P<0.01$; *** = $P<0.001$
Significance of differences between the control and experimental groups during the observed days after parturition
a : a = $P>0.05$; b : b = $P< 0.001$

No significant statistical difference was noted ($P>0.05$) when evaluating alkaline phosphatase activity in the glandular cells of the endometrium (Tab. 1) in the experimental group of ewes. Mean values of its density in the evaluated period were below the level of values on day 17 in the control group.

The effect of polychlorinated biphenyls on hepatic microsomal oxidases and toxic impact of pentachlorbiphenyls on homeostasis defects were reported by Yoshimura *et al*., (1985). Bose *et al*., (1996) observed the inhibitory effect on the enzymatic system of the ovaries after endosulphane application. Khan *et* Thomas

(2001) claim that exposure to PCB was the cause of decrease in concentration of hypothalamic 5-hydroxytriptamine and inhibitory effect on hypothalamic tryptophan hydroxylase activity.

If the above stated facts on PCB inhibitory effect on the enzymatic system are taken into account, then the stagnation of alkaline phosphatase activity observed by the authors after exposure of ewes to PCB confirms the above facts.

The evaluation of acidic phosphatase activity in the glandular cells of the endometrium in the control group of ewes is given in Tab. 2. Its activity showed a statistically conclusive increase between day 17 and day 25 as well as day 34 postpartum ($P<0.001$). It is evident that acidic phosphatase activity observed in the glandular cells of the endometrium of puerperal ewes in the control group had a tendency to increase. The results of acidic phosphatase activity are comparable with mean values of its activity in postpartal period as reported by Krajničáková *et al*., (2003). A statistically conclusive increase in acidic phosphatase activity in the goat endometrium during the follicular phase was confirmed by Bhattacharya and Saigal (1985). The authors presume that the increasing tendency of acidic phosphatase activity in the postpartum period is due to the onset of gradual postpartum restoration of the uterine endometrium, which plays an important role in physiological regressions. Acidic phosphatase activity in the glandular cells of the ewes' endometrium in the experimental group (Tab. 2) did not vary significantly at the observed intervals of the postpartum period ($P>0.05$). Its values were below the level on day 17 in the control group. The range of mean values of acidic phosphatase in the experimental group was on evaluated days of the puerperal period lower ($P<0.001$) when compared to the control group.

Table 2. The mean values of the activity of acidic phosphatase in the endometrial glandular cells of the control and experimental groups of ewes after parturition

DAYS POSTPARTUM	ACIDIC PHOSPHATASE	
	CONTROL	EXPERIMENTAL
17	7.860 ± 0.32 ^{b}	6.78 ± 0.48 ^{b}
25	9.017 ± 0.63 ^{b***}	6.86 ± 0.45 ^{b}
34	10.17 ± 0.35 ^{b***}	7.13 ± 0.28 ^{b}

Statistical significance compared between day 17, 25 and 34 after parturition *** = $P<0.001$
Significance of differences between the control and experimental groups of ewes during observation days post partum. b:b = $P<0.001$

Experiments performed on mice (Lu-Shuiyuan *et al*., 2000), rats (Andric *et al*., 2000) and sheep (Jan *et al*., 1999) showed that exposure to polychlorinated biphenyls markedly affect: sperm motility, follicular growth and maturation, embryonal development with inhibitory effect on the enzymatic system and conversion of circulating hormones. Acidic phosphatase activity in the experimental group is probably linked with the above mentionded facts.

The authors concluded from the available data and results that exposure of animals to polychlorinated biphenyls (Delor 105) had an inhibitory effect on acidic and alkaline phosphatase activity in reparative and regressive processes occurring in the uterus during the puerperal period in ewes.

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AKTIVNOST ALKALNE I KISELE FOSFATAZE U GLANDULARNIM ĆELIJAMA ENDOMETRIJUMA OVACA U PUERPERIJUMU POSLE DAVANJA POLIHLORISANIH BIFENILA

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SADRŽAJ

Ova istraživanja su sprovedena sa ciljem da se odredi aktivnost alkalne i kisele fosfataze u glandularnim ćelijama endometrijuma ovaca u puerperijumu posle aplikacije polihlorisanih bifenila. Ogled je sproveden na 25 ovaca, rase slovački merino podeljenih u dve grupe. Životinje u eksperimentalnoj grupi (n=14) i u kontrolnoj grupi (n=11) su bile žrtvovane 17., 25. i 34. dana posle jagnjenja. Ovce iz eksperimentalne grupe su peroralno dobijale kapsule hemijskog preparata Delor 105 domaće proizvodnje, koji sadrži polihlorne bifenile (PCB), i koji je ekvivalentan stranom preparatu Aroclor 1254, tokom perioda od 30 dana u dozi od 100 μg/kg. Ove ovce su bile žrtvovane 17. dan posle jagnjenja (n=4), što znači pet dana posle završetka 30-o dnevne aplikacije, 25. dan posle jagnjenja (n=5), što je 17 dana od poslednje aplikacije PCB i na 34. dan posle jagnjenja (n=5), što je 28. dana od poslednje aplikacije PCB. Ovce iz kontrolne grupe su bile žrtvovane 17. (n=3), 25. (n=4) i 34. dana (n=4) posle jagnjenja. Merenjem aktivnosti alkalne fosfataze (ALP) u glandularnim ćelijama endometrijuma u kontrolnoj grupi je dokazana statistički signifikantna razlika ($P<0,01$) u odnosu na 25. dan kao i na 34. dan ($P<0,001$) u odnosu na 17. dan posle porođaja. U okviru ek-

sperimentalne grupe nisu dokazane statistički značajne razlike. Prosečna vrednost ove aktivnosti tokom praćenog perioda je bila ispod vrednosti koje su registrovane 17. dana u kontrolnoj grupi. Aktivnost kisele fosfataze u glandularnim ćelijama endometrijuma ovaca ukazuje na statistički signifikantno povećanje između 17. i 25. dana, kao i do 34. dana posle jagnjenja ($P<0,001$). Razlike u aktivnosti kisele fosfataze u eksperimentalnoj grupi ovaca nisu bile statistički značajne ($P>0.05$) u praćenom periodu.