Activity of alkaline phosphatase, acidic phosphatase and nonspecific esterase in the oviducts of puerperal ewes after exposure to polychlorinated biphenyls

I. Valocky[1], J. Legath[2], L. Lenhardt[3], G. Lazar[1], F. Novotny[1]

[1]Department of Gynaecology, Obstetrics and Andrology, [2]Department of Toxicology, [3]Department of Pathological Anatomy, University of Veterinary Medicine, Kosice, Slovak Republic

ABSTRACT: The objective of this study was to examine the alkaline, acidic phosphatase and nonspecific esterase activity in the epithelial cells of oviducts after exposure to polychlorinated biphenyls (PCBs) at the time of puerperium. PCBs were administered in the last days of pregnancy and during early puerperium. Animals in the experimental group were exposed to Delor 105 at a dose of 100 μg/kg/day and were euthanised on Day 17 postpartum (n = 4), i.e. 5 days after the termination of 30-day PCB administration; on Day 25 postpartum (n = 5), i.e. 17 days from the last PCB administration and on Day 34 postpartum (n = 5), which corresponded to Day 28 from the completion of PCB administration. Ewes in the control group were euthanised on Day 17 (n = 3), Day 25 (n = 4) and Day 34 (n = 4) postpartum. The authors demonstrated the inhibitory effect of PCB on the enzymatic system of the oviduct during the puerperal period. The alkaline phosphatase, acidic phosphatase and nonspecific esterase activity in the oviductal epithelial cells during a 34-day observation period exhibited a rising trend ($P < 0.001$ vs. $P < 0.001$ vs. $P < 0.01$) in the control group of animals. Experimental animals exposed to the 30-day PCB administration (Delor 105) showed a stagnant tendency ($P > 0.05$) in alkaline phosphatase while acidic phosphatase and nonspecific esterase activity ($P > 0.05$) dropped even below the level of their activity values in the control group. It is essential to continue to monitor the effect of pollutants in exposed industrial areas on reparative and regenerative processes in puerperium and their possible impact on reproductive performance.

Keywords: oviduct; puerperium; ewe; alkaline phosphatase; acidic phosphatase; nonspecific esterase; polychlorinated

The oviduct is a female reproductive organ with the metabolically active tissue in which enzymes involved in transphosphorylation functions in physiological regressions of the epithelial cells are localized. Cell activity and particularly localization of enzymes which catalyse various processes during the oestrous cycle and puerperal period of animals were investigated by several authors (McDaniel et al., 1968; Pivko and Majerciak, 1978; Bhattacharya and Saigal, 1984; Schnurrbusch et al., 1988; Uhrin, 1992; Krajnicakova et al., 2002). The highest alkaline phosphatase activity in the cells of the ampullar section of oviducts of cows during the follicular phase was reported by Uhrin (1992). He also found out that the intensity of acidic phosphatase reaction during the oestrous period was low and peaked during dioestrus. The rising trend of alkaline phosphatase and acidic phosphatase with the progressing postpartum period of goats was observed by Krajnicakova et al. (2002, 2004). The activity of the cytoplasmic enzyme nonspecific esterase attained a peak in oestrus and it decreased to a later proestral stage in non-ciliary oviduct cells (Steffl et al., 2003). An intensive reaction was observed in the surface

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epithelial cells and surface endometrial glands in cows during the follicular phase.

Polychlorinated biphenyls (PCBs) as pollutants are persistent bioaccumulative and toxic chemicals hazardous to the environment. Biological effects of polychlorinated biphenyls including inhibitory ones on the enzymatic systems were investigated on laboratory animals (Iwamoto, 1982), birds (Ahmed et al., 1978) and fish (Perez-Lopez et al., 2002). The increased hepatic microsomal aniline hydroxydase activity in chicks after exposure to PCB was reported by Ruprich and Piskac (1990). The effect of the long-term feeding of commercial PCB of domestic provenance to infantile sows on selected metabolic parameters of blood plasma was studied by Guoth et al. (1984). The authors found a marked inhibitory effect of PCBs on the enzymatic system. Transfer and distribution of PCBs from mother's body to the foetus via the placenta and via mother's milk to sucking offspring were investigated by Masuda et al. (1979), Hori et al. (1981), Lee et al. (2002).

Disruptions of steroid metabolism due to PCB exposure can cause pathological changes in the reproduction of farm animals (Kacmar et al., 1985). Chemical compounds that mimic or block some of the actions of the steroid hormone oestradiol have evoked public concern primarily because of potential adverse reproductive effects on wildlife and humans (Voharova et al., 2005). Many studies, both *in vivo* and *in vitro*, have revealed an abnormal reproductive function following the exposure to these compounds (Katoh et al., 2004). The number of chemicals known to have the potential to modulate endocrine functions is increasing. Endocrine disrupting agents may interfere with the reproductive processes of both males and females at several points of the reproductive cycle and through a range of physiological mechanisms. The exposure of domestic ruminants occurs via contaminated water and pasture on contaminated grazing land (Boerjan et al., 2002). Polychlorinated biphenyls belong to the group of endocrine disruptors (Safe, 2004). International interest is focused on the selection of suitable tests for their utilization in environmental risk assessment (Hutchinson et al., 2003).

However, at present there is a paucity of data on PCBs effects on the dynamics of functional changes in the reproductive tract of ewes during puerperium. For this reason experimental ewes were exposed to a precisely defined PCB concentration which would reliably imitate the load of the environment contaminated by polychlorinated biphenyls.

The objective of this study was to investigate changes in enzymatic activities of the epithelial cells of the oviduct in puerperal ewes after their exposure (during the last days of pregnancy and during early puerperium) to Delor 105 containing congeners of PCBs. The activity of the enzymes alkaline phosphatase, acidic phosphatase and non-specific esterase in relation to regenerative and reparative processes in the epithelial cells of the oviducts was examined during puerperium.

MATERIAL 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). The production of products containing PCB congeners was banned by law in Slovakia in 1982, however, their utilization continued until the supplies ran out.

Twenty-five Slovak Merino ewes divided into 2 groups were included in the experiment. Animals in the experimental group (n = 14) and control group (n = 11) were euthanised on Day 17, 25 and 34 postpartum. The experimental group ewes were given for 30 days perorally (once a day) a Slovak-made PCB of the trade name Delor 105 (Chemko, Slovak Republic), which is equivalent to the foreign product Aroclor 1254. The animals were weighed individually before exposure to PCB. The administration of the chemical at a dose of 100 μg/kg per day was established on the ewes' body weight. The preparation was administered in one gelatine capsule placed directly at the root of the tongue. Deglutition reflex was induced followed by a thorough examination of the oral cavity. The lambing period lasted 21 days. The experimental animals were euthanised 5 days after the termination of Delor 105 administration: on Day 17 postpartum (n = 4), on Day 25 postpartum (n = 5), which corresponded to 17 days from the last PCB administration and on Day 34 postpartum (n = 5), which corresponded to Day 28 from the completion of PCB administration. The control group animals were euthanised on Day 17 (n = 3), Day 25 (n= 4) and Day 34 (n = 4) postpartum. During the experiment the ewes were kept in an experimental centre. Feed ration per animal/day contained meadow hay, fodder concentrate, and root crops. A mineral supple-

ment and water were available *ad libitum* (Sommer et al., 1994). The reproductive tract of the ewes was excised after euthanasia (Thiopental® inj., Lečiva, Czech Republic) and bleeding was done according to an advance schedule at the above intervals of the postpartum period. Samples of the oviducts collected from the ampullar section were frozen in liquid nitrogen vapour (–196°C) and stored at a temperature of –20°C until processed.

Enzyme assay

The demonstration of alkaline phosphatase (AlP) activity was performed by the modified simultaneous azo-coupling method (Lojda et al., 1979) with naphthol AS-BI phosphate (Sigma, Deisenhofen, Germany) and Fast Blue BB (Sigma, Deisenhofen, Germany). Acidic phosphatase (AcP) activity was established by the simultaneous azo-coupling method (Lojda et al., 1979) with naphthol-AS-MX-phosphate (Fluka, Germany) and hexazo-*p*-rosaniline (Serva, Germany) and nonspecific esterase (NsE) activity with naphthol-AS-D-acetate (Fluka, Germany) and ferrocyanide (Lojda et al., 1979).

The enzyme activity was analyzed cytophotometrically with a Vickers M85 microdensitometer. The measurements were performed with a 40× objective, an effective scanning area of 28.3 μm^3 and a scanning spot of 0.5 μm. The integrated absorbance was measured at a wavelength of 480 nm for AlP and 540 nm for AcP and NsE. The mask was set over in at least 30 areas of the oviduct in five tissue sections. The activity of enzymes was calculated as the absorbance values recorded by the instrument /min/μm^3 in the oviductal epithelial cells ± S.E.M. and these mean values were referred to one animal.

Statistical evaluation

The data were expressed as mean ± S.E.M. Statistical evaluation of the observed enzyme activities (AlP, AcP, NsE) was carried out by two-way analysis of variance (ANOVA). Variance significance between the groups and statistical significance on individual days of euthanasia in the ewes' postpartum period were determined by Tukey's test. Sigma Stat statistical software (Jandel Scientific 2000, San Rafael, CA) was used for the analysis of data.

RESULTS

The evaluation of alkaline and acidic phosphatase density in the oviductal epithelial cells of ewes in the late puerperal phase is shown in Table 1 and Table 2. The alkaline phosphatase activity in the oviductal epithelial cells (Table 1) had a rising tendency with statistical significance between Day 17 and Day 25 postpartum ($P < 0.01$). The range of the values of its activity showed significance of difference ($P < 0.001$) between Day 17 and Day 34 of the evaluated period in the control group.

No statistically significant differences were noted ($P > 0.05$) when evaluating the alkaline phosphatase activity in the oviductal epithelial cells (Table 1) in the experimental group of ewes. Mean values of its density in the evaluated period were below the level of the values of day 17 in the control group.

The evaluation of acidic phosphatase activity in the oviductal epithelial cells in the control group of ewes is documented in Table 2. Its activity showed a statistically significant increase between Day 17 and Day 25 as well as Day 34 postpartum ($P < 0.001$). It is evident that the acidic phosphatase activity

Table 1. Alkaline phosphatase activity in the epithelial cells of the oviduct in the control and experimental group of postpartal sheep (means ± S.E.M)

Days after parturition	Alkaline phosphatase	
	control	experimental
17	5.39 ± 0.29[a]	5.10 ± 0.43[a]
25	6.24 ± 0.37[b**]	5.17 ± 0.23[b]
34	6.66 ± 0.21[b***]	5.23 ± 0.32[b]

AlP activity is given as the integrated absorbance in min/μm^3 of oviductal endothelial cells at a wavelength of 480 nm; $^{**}P < 0.01$, $^{***}P < 0.001$. Statistical significances compared between Day 17 and other days postpartum; $^{a}P < 0.01$, $^{b}P < 0.001$. Significance of differences between the control and experimental group during the days of observation postpartum

Table 2. Acidic phosphatase activity in the epithelial cells of the oviduct in the control and experimental group of postpartal sheep (means ± S.E.M)

Days after parturition	Acidic phosphatase	
	control	experimental
17	7.87 ± 0.37[b]	6.78 ± 0.27[b]
25	8.92 ± 0.18[b***]	6.87 ± 0.31[b]
34	10.17 ± 0.38[b***]	7.12 ± 0.29[b]

AcP activity is given as the integrated absorbance in min/μm^3 of oviductal endothelial cells at a wavelength of 540 nm; ***$P < 0.001$. Statistical significance compared between Day 17 and other days after parturition; [b]$P < 0.001$. Significance of differences between the control and experimental group during the days of observation postpartum

Table 3. Nonspecific esterase activity in the epithelial cells of the oviduct in the control and experimental group of postpartal sheep (means ± S.E.M)

Days postpartum	Nonspecific esterase	
	control	experimental
17	9.87 ± 0.37[b]	7.26 ± 0.29[b]
25	11.27 ± 0.35[b*]	7.53 ± 0.42[b]
34	11.56 ± 0.46[b**]	7.86 ± 0.12[b*]

NsE activity is given as the integrated absorbance in min/μm^3 of oviductal endothelial cells at a wavelength of 540 nm; *$P < 0.05$, **$P < 0.01$. Statistical significance compared between Day 17 and other days after parturition; [b]$P < 0.001$. Significance of differences between the control and experimental group of ewes during the days of observation postpartum

observed in the oviductal epithelial cells of puerperal ewes in the control group had an increasing tendency.

The acidic phosphatase activity in the oviductal epithelial cells in the experimental group (Table 2) did not vary significantly at the observed intervals of the postpartum period ($P > 0.05$). Its values were below the level of Day 17 in the control group. The range of the mean values of acidic phosphatase in the experimental group was statistically significantly lower ($P < 0.001$) on the evaluated days of puerperal period when compared to the control group.

The NsE activity (Table 3) increased significantly from Day 17 to Day 34 in the control group ($P < 0.001$). During the same time interval the NsE activity rose insignificantly ($P > 0.05$) in the ewes exposed to PCB and retained its mean values below the level of NsE activity in the unexposed ewes.

When the AlP, AcP, and NsE activity in the oviductal epithelial cells of ewes on Day 25 and Day 34 postpartum was compared, a statistically significant difference ($P < 0.001$) was found between the group of ewes exposed to PCB and the control group of ewes. The unexposed ewes showed a markedly higher activity of the examined tissue enzymes with a progressive trend of increase in their activity. The ewes exposed to PCB showed a substantially lower activity in all evaluated parameters than the unexposed ones as early as on Day 1 of the observation (Day 17 postpartum). The statistical analysis explicitly confirmed a specific effect of exposure to Delor 105 on the AlP, AcP and NsE activity in the oviductal epithelial cells in puerperal ewes.

DISCUSSION

The results achieved in this experiment showed an upward trend of AlP, AcP and NsE activity in the epithelial cells of the oviduct of puerperal ewes in the control group between Day 17 and Day 25 as well as between Day 25 and Day 34 postpartum.

Similar results were reported by Krajnicakova et al. (2002) in regressive changes occurring in the

oviduct during the puerperal period. The proliferation process of oviductal structures in sheep and goats observed by Krajnicakova (1998) is consistent with the incidence of AlP activity and is related to their task in transformation mechanisms and metabolic processes occurring in the oviduct after parturition. The results achieved by the authors are comparable with the mean values of AcP activity in postpartum goats reported by Krajnicakova et al. (2003). A statistically provable rise in the AcP activity in the epithelial cells of the oviduct of goats during the follicular phase was confirmed by Bhattacharya and Saigal (1984). The authors suppose that the upward trend of AcP activity in the puerperal period is caused by the onset of gradual postpartal restoration of the oviductal epithelial cells which plays an important role in physiological regressions.

An intensive response of NsE localized in the apical parts of the glandular cells was observed by Schroder (1987) in cows during dioestrus while a less intensive response was recorded in the epithelial cells of the uterus. Boshier and Katz (1975) claim that oestrogens stimulate the incidence of NsE in surface epithelial and glandular cells. From this point of view, the results of changes in the activity of NsE observed in this experiment indicate their possible involvement in the processes of energy transformation.

The results of this experiment showed significant stagnation of AlP, AcP and NsE activity in the epithelial cells of the oviducts of puerperal ewes exposed to Delor 105 between day 17 and day 25 as well as between day 25 and day 34 postpartum, which accords with the observations of other authors, suggesting an inhibitory influence of PCBs on both the enzymatic and neuroendocrine systems. The effect of polychlorinated biphenyls on hepatic microsomal oxidases and toxic impact of pentachlorobiphenyls on homeostasis defects were reported by Yoshimura et al. (1985), Ruprich and Piskac (1990). Bose et al. (1996) observed an inhibitory effect on the enzymatic system of the ovaries after endosulphane application.

Prenatal exposure of rats to a low concentration of Aroclor 1254, a mixture of EDs, results in a perturbing effect on the battery of hypothalamic androgen metabolising enzymes involved in the control of sexual differentiation of the developing brain, namely aromatase and 5 alpha-reductase type 1 and 2. Altered polyadenylation levels of aromatase mRNA in the brain of male as well as female EDs treated rats were demonstrated (Brevini et al., 2005).

Aroclor 1254 inhibited GnRH nuclear mRNA levels at high dosages, and stimulated GnRH mRNA at low doses, suggesting a post-transcriptional mechanism of regulation. Qualitatively, Aroclor 1254 caused the retraction of GT1-7 cell (hypothalamic GT1-7 cells, which synthesize and secrete the key hypothalamic hormone, gonadotropin-releasing hormone) processes and neurotoxicity in mice (Gore et al., 2002).

Acute exposure to ortho-PCB congeners 95 (2,3,6-2',5') or 101 (2,4,5,-2',5') causes changes in the performance of the hypothalamo-pituitary-thyroid (HPT)-axis. These congeners interfere with the HPT-axis by causing a subnormal response of the pituitary and thyroid to TRH stimulation (Khan and Hansen, 2003).

Experiments conducted on mice (Lu et al., 2000), rats (Andric et al., 2000; Wang et al., 2002) and sheep (Jan et al., 1999) showed that the exposure to polychlorinated biphenyls markedly affected sperm motility, follicular growth and maturation, embryonal development with inhibitory effect on the enzymatic system at circulating hormone conversion, and oocyte competence (Pocar et al., 2003, 2006).

The authors conclude from available data and their current results that the exposure of animals to polychlorinated biphenyls (Delor 105 given to the experimental group of ewes in this experiment) had an inhibitory effect on the AlP, AcP and NsE activity in reparative and regressive processes occurring in the oviduct during the puerperal period. The produced results can be utilized as input data for environmental risk assessment.

It is highly probable that also ewes exposed to PCB in an industrially polluted environment as well as in an environment contaminated by PCB pollutants exhibit the inhibition of hydrolytic enzyme activity in the epithelial cells of the oviduct during puerperium.

The effect of the above pollutants on the regeneration of reproductive activity of puerperal ewes (in the following breeding season) and fertility level necessitates further study.

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Corresponding Author:

MVDr. Igor Valocky, PhD., Department of Gynaecology, Obstetrics and Andrology, University of Veterinary Medicine, Komenskeho 73, 041 81 Kosice, Slovak Republic

E-mail: bodnarova@uvm.sk