Polish Journal of Veterinary Sciences Vol. 24, No. 1 (2021), 5–12

DOI 10.24425/pjvs.2020.135813

Original article

The effect of DDE and dieldrin and their parental substances (DDT and aldrin) on PGE2 and PGF2α release from bovine endometrial explants collected during 120 to 180 days of the gestational period

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Abstract

Dieldrin and DDE are environmental metabolites of the organochlorine pesticides aldrin and DDT, respectively. During pregnancy, these chemicals can quickly infiltrate through the placental barrier, accumulate in amniotic fluid and fetus, and act as endocrine disruptors (EDs). The aim of this study was to investigate the effect of DDE and dieldrin and their parental substances at concentrations of 1 and 10 ng/ml on secretion of PGE2 and PGF2α from bovine endometrial explants (120-150 and 151-180 days of pregnancy) after 24 hr of incubation with EDs. The mRNA expression of *COX2, PGES* and *PGFS* and the concentrations of PGE2 and PGF2α were measured. EDs did not affect ($p>0.05$) *COX2* gene expression, but DDT and DDE decreased ($p<0.05$) *PGES* expression and PGE2 secretion in the explants from 120-150 days of pregnancy. Depending on the dose, DDT and DDE increased ($p<0.05$) *PGFS* expression and PGF2α secretion from the explants from 120-150 days and decreased PGF2α secretion ($p<0.05$) from the explants from 151-180 days of pregnancy. Aldrin and dieldrin decreased ($p<0.05$) *PGFS* expression and PGF2α secretion from all explants. In summary, EDs disrupt the secretion of PGE2 and PGF2α by influencing the gene expression of *PGES* and *PGFS*.

Key words: pesticides, pregnancy, endometrium, prostaglandin secretion, cattle

Introduction

Pesticides such as DDT (1-chloro-4-[2,2,2-trichloro-1-(4-chlorophenyl)ethyl]benzene) and aldrin (1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4:5,8-dimethanonaphthalene) are one of the most frequently detected environmental pollutants. These organochlorine pesticides have biological activity and can act as endocrine disrupters (EDs). Although they are resistant to biodegradation, these compounds gradually biotransform to DDE (1,1-dichloro-2,2-bis(4-chlorophenyl)ethene) and dieldrin (1aR,2R,2aS,3S,6R,6aR,

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7S,7aS)-3,4,5,6,9,9-hexachloro-1a,2,2a,3,6,6a,7,7a-octahydro-2,7:3,6-dimethanonaphtho[2,3-b]oxirene, which has even stronger activity than the parental substances. Although their use was banned in the 1980s, they are still used in Southeast Asian and African countries to fight mosquito-spreading malaria (Van den Berg et al. 2017, Tangena et al. 2020). All these lipophilic compounds easily penetrate into humans and animals and then accumulate in tissues and organs of the reproductive tract, where they act as endocrine disruptors (EDs). During pregnancy, these molecules can quickly infiltrate through the placental barrier and accumulate in the amniotic fluid and fetus (Hirako et al. 2005).

We observed that DDT and DDE caused disorders in structural proteins, such as connexins (Cx23, Cx32, Cx38) and keratin-1 (Wojciechowska et al. 2017). These substances affect the secretion of prostaglandins (PG) F2α, E2 and I2 from the endometrium and myometrium during the estrus cycle as well as during pregnancy (Wrobel et al. 2012, Wrobel and Mlynarczuk 2017). Moreover, studies carried out on the cotyledon scrap model have shown that DDT and DDE at concentrations present in the environment can result in secretion of PGF2α and PGE2, altering their mutual proportions (Wojciechowska et al. 2017). Prostaglandins F2α and E2 play significant roles in the regulation of blood flow, especially in the fetal part of the placenta (Maigaard et al. 1986, Poston 1997), and are also regulators of uterine contractile activity (Ruckebusch and Bayard 1975). Furthermore, PGE2 together with progesterone regulates the growth and development of the placentomes (Robertson et al. 1979, Janowski et al. 1996). In bovine placenta, PGs are synthesized and released within cotyledons and endometrium in interplacentomal areas. In the first stages of pregnancy, more than 40% of PGs originate from these areas (Slama et al. 1994, Arosh et al. 2004).

However, at the beginning of the 5th month of pregnancy, the bovine placenta is remodeled from the epithelial type placenta towards the connective-epithelial type placenta. At this time, cotyledons quickly grow (Pfarrer et al. 2003) and remain the main source of PG production in the ruminant placenta (McLaren et al. 2000). Since PGs can easily diffuse from the maternal part of the placenta to the fetal part and vice versa (Kelleman et al. 1992), disruption in PG secretion even in one part of the placenta may adversely affect the normal course of pregnancy. Nonetheless, the influence of chlorinated EDs on the secretion of prostaglandins from the endometrium in the middle stage of pregnancy has not been previously studied.

The aim of this study was to investigate the effect of DDE and dieldrin and their parental substances (DDT and aldrin) at concentrations of 1 and 10 ng/ml (at the range measured in animal and human tissues) on the synthesis and release of PGE2 and PGF2α from explants of the bovine endometrium obtained from before to the beginning of placental remodeling (from 120 to 150 days of pregnancy) and after the start of the remodeling process (from 151 to 180 days).

Materials and Methods

Endometrial explant preparation

Fragments of pregnant uterus were collected during the 120 to150 days and 151 to 180 day of gestational period (n=4 of each) in an abattoir 15 to 20 min after the animal was killed. The tissues were then transported to the laboratory in an ice bath (4°C) within 1 h of the time of collection in 0.9% NaCl. At the first, period of pregnancy was identified after crown-rump length measurement of fetus using Keller's formula: $x(x + 2) =$ = length of fetus, where "x" was the gestational age. Then, the inspection of fetus present in the uterine horn, appearance of the uterine horn and degree of cotyledons development were done (Jainudeen and Hafez 1980) to exactly determine of the gestation period. They were taken into account: horn pits apparent and tooth eruption - as < 120 days of pregnancy, the first hair around eyes and muzzle - as < 150 days of pregnancy and good developed of hair around eyes and muzzle and the first sensory hairs (vibrissaes) as <180 days of pregnancy. As the length of pregnancy in cow 280 days were assumed. Uterine fragments were excised from the interplacentomal areas of each uterus, and then, the maternal part of the placenta (endometrium) was separated from the fetal part (smooth chorion). Isolated fragments of the endometrium were divided into explants (60-80 mg) and washed in a cold (4°C) 0.9% NaCl solution supplemented with penicillin (10 IU/ml), streptomycin (100 µg/ml), amphotericin B (2 µg/ml) and L-glutamine (100 µg/ml).

Tissue explants viability

The viability of the endometrial explants was assessed using an AlamarBlue test (Life Technologies, Waltham, MA, USA), which is based on the measurement of mitochondrial dehydrogenase activity. Briefly, endometrial explants (20–40mg) from four cows were placed in triplicate in 24-well plates in 1 ml of medium with a composition identical to use in main experiments, and they were incubated for 48 hrs with DDT, DDE, aldrin and dieldrin at a dose of 100 ng/ml each. As a negative control, explants treated with 0.004% paraformaldehyde (PFA) were used. Colorimetric measurements were performed at two wavelengths (570

Table 1. Primer sequences used in RealTime-PCR reactions.

Gene	Primers sequence (5'→ 3') (forward – F, reverse - R	Amplicon lenght (bp)	Access number (GeneBank)
COX-2	**F**: GCCTGATGACTGCCCAACA **R**: GCAAAGAATGCAAACATCAGATTT	140	AF004944.1
PGES	**F:** TGGGCGGACGACTGGTT **R:** GGTGGTGCCTGCGTTTG	151	NM_001166554.1
PGFS	**F**: TGTGGTGCACGTATCACGACA **R**: AATCACGTTGCCGTCCTCATC	160	S54973
TBP	**F**: CAGAGAGCTCCGGGATCGT **R**: ACACCATCTTCCCAGAACTGAATAT	194	NM_001075742.1

and 600 nm) after 4 hrs. of incubation with AlamarBlue (Epoch Microplate Spectrophotometer, BioTek, Winooski, VT, USA). The results are expressed as the percentage of AlamarBlue reduction per 1 mg of tissue.

Hormone determination

The PGFM (13,14-dihydro-15-keto-PGF2α; a stable metabolite of PGF2α) concentration in culture medium reliably reflects the quantity of PGF2α that is secreted from these explants. Therefore, PGFM and PGF2α were used interchangeably (Skarzynski et al. 1999). Its concentrations were determined in culture media using an enzyme immunoassay (EIA) method. The standard curve range, intra-, and inter-assay coefficients of variation, and relationship between the added and measured hormone concentrations, which were expressed as coefficients of regression, were as follows: PGFM: 62.5 to 2000 pg/ml, 10.1%, 12.1%, and $r = 0.92$; PGE2: 78 to 20,000 pg/ml, 10.6%, 12.7%, and $r = 0.92$. Working dilutions of the primary antibodies were as follows: anti-PGFM (gift from prof. W. Silvia): 1:50 000; anti-PGE2 (gift from prof. W.W. Thatcher): 1:35 000. All assays were performed in 96-well plates coated with ovine anti-rabbit γ-globulin, which was obtained from our department. The absorbance was measured at a wavelength of 450 nm using a Multiscan MX plate reader (Thermo Scientific), and the results were analyzed using Genesis Lite 3.0 (Labsystems, Vantaa, Finland). The final hormone concentrations were expressed per mg of tissue explants.

Total RNA isolation and real-time PCR analysis

Total RNA was isolated using a Total RNA kit (A&A Biotechnology, Gdynia, Poland) after the frozen explants of tissues were pulverized (Retsch, Haan, Germany) in liquid nitrogen and covered with phenozol (A&A Biotechnology). The concentration and purity of the isolated RNA samples were determined using a NanoDrop spectrophotometer and NanoDrop 1000 V.3.7.1 software (Thermo Scientific, Waltham, MA, USA). One microgram of mRNA from each sample was reverse-transcribed to generate cDNA (42°C for 60 min) in a 20 µl reaction. The obtained complementary DNA was stored at -20°C until further analysis. Primers against cyclooxygenase-2 (*COX-2)*, prostaglandin F synthase (*PGFS)*, prostaglandin E synthase (*PGES)* were designed using Primer Express software (Thermo Scientific) based on gene sequences available in GenBank (NCBI). Real-time PCR was performed using a KAPA SYBR FAST qPCR Master Mix kit (KapaBiosystems, Wilmington, NC, USA) and a 7900HT Fast Real-Time PCR System (Applied Biosystems, Carlsbad, USA) with a 384-well block. The total volume of each reaction was 10 µl (3 µl containing 200 ng of complementary DNA, 6 µl of qPCR Master Mix, and 0.5 µl of each 0.2 mM primer). The reaction conditions were as follows: initial denaturation (95°C at 10 min), followed by 40 cycles of denaturation (95°C for 15 sec), annealing and extension (60°C for 60 sec). All reactions were performed in duplicate. After each PCR, melting curves were obtained by increasing the temperature from 60°C to 95°C in a stepwise manner to ensure single-product amplification. In addition, the specificity of each real-time PCR product was confirmed by electrophoresis on a 2% agarose gel (EuroGenTec, Köln, Germany). The data were normalized using TATA box binding protein (*TBP*) mRNA as previously reported (Wojciechowska et al. 2017). Primers sequence are shown in Table 1.

Statistical analysis

The mean values for endometrial explants viability (n=8) were compared by one-way analysis of variance followed by a Newman-Keuls post hoc test. The raw results of the real-time PCR analyses were calculated based on the real-time PCR Miner algorithm. The mean values for hormone secretion (n=8) and mRNA expression analyses (n=8) were compared by two-way analysis of variance followed by Newman-Keuls post hoc

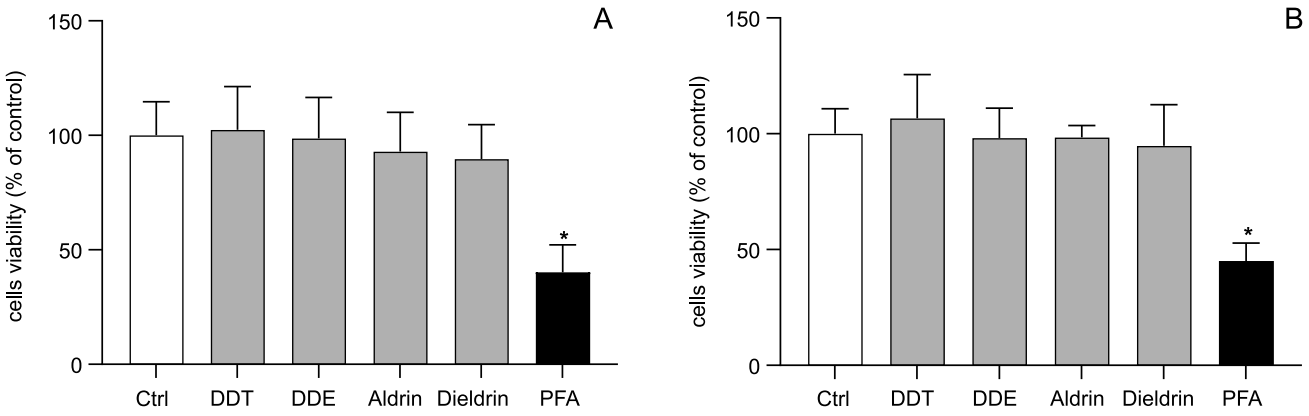

Fig. 1. Effects of EDs (at the dose 100 ng/ml) on cells viability of bovine endometrium explants collected between 120 and 150 days (A), and 151 to 180 days (B) of the gestational period, after 48 hrs of incubation. Paraformaldehyde (PFA) in concentration 0.004% was used as a negative control. Different superscripts above bars are indicative of differences among values ($p<0.05$; ±SD).

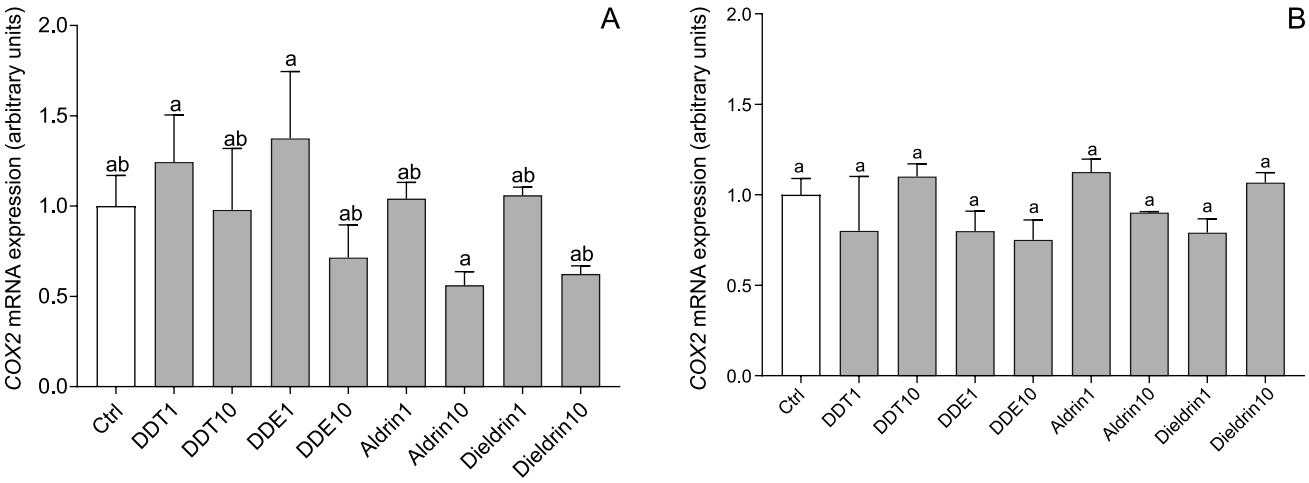

Fig. 2. Effects of DDT, DDE, aldrin and dieldrin at the doses 1 and 10 ng/ml on the mRNA expression of *COX2* in endometrium explants collected between 120 and 150 days (A), and 151 to 180 days (B) of gestational period. Different superscripts above bars are indicative of differences among values ($p<0.05$; ±SEM).

test (GraphPad PRISM 6.0; Graph Pad Software, San Diego, CA, USA). All data are expressed as the mean (±SEM), and differences of $p<0.05$ were considered significant.

Results

There was no negative ($p>0.05$) effect of the xenobiotics evaluated in this study on cell viability in endometrial explants collected during 120 and 150 days (Fig. 1A) and 151 to180 days of the gestational period (Fig. 1B).

In explants of the endometrium, none of the EDs used affected ($p>0.05$) the mRNA expression of *COX2* in the explants collected between 120 and 150 days and 151 to180 days of the gestational period (Fig. 2). The mRNA expression of *PGES* was inhibited ($p<0.05$) in the endometrial explants collected between 120 and 150 days of the gestational period by DDT at a dose of 10 ng/ml and both doses of DDE. In contrast, in these explants, the mRNA expression of the *PGES* gene increased ($p<0.05$) after treatment of aldrin and dieldrin at both doses (Fig. 3A). Only DDE exhibited the same effect ($p<0.05$) on the mRNA expression of *PGES* in the endometrial explants collected between 151 to 180 days of the gestational period (Fig. 3B). In the explants obtained between 120 and150 days of the gestational period, an increase in the expression of this gene ($p<0.05$) was noted after DDT (10 ng/ml) treatment and both doses of DDE. In contrast, dieldrin at 1 ng/ml caused a decrease ($p<0.05$) in the mRNA expression of the *PGFS* gene (Fig. 3C). In the explants collected between 151 and 180 days of the gestational period, the mRNA expression ($p<0.05$) of *PGFS* was less when DDT (1 ng/ml), DDE (1 and 10 ng/ml), aldrin (10 mg/ml), and dieldrin at 10 ng/ml was added to the media. However, aldrin (1 ng/ml) and dieldrin at 10 ng/ml

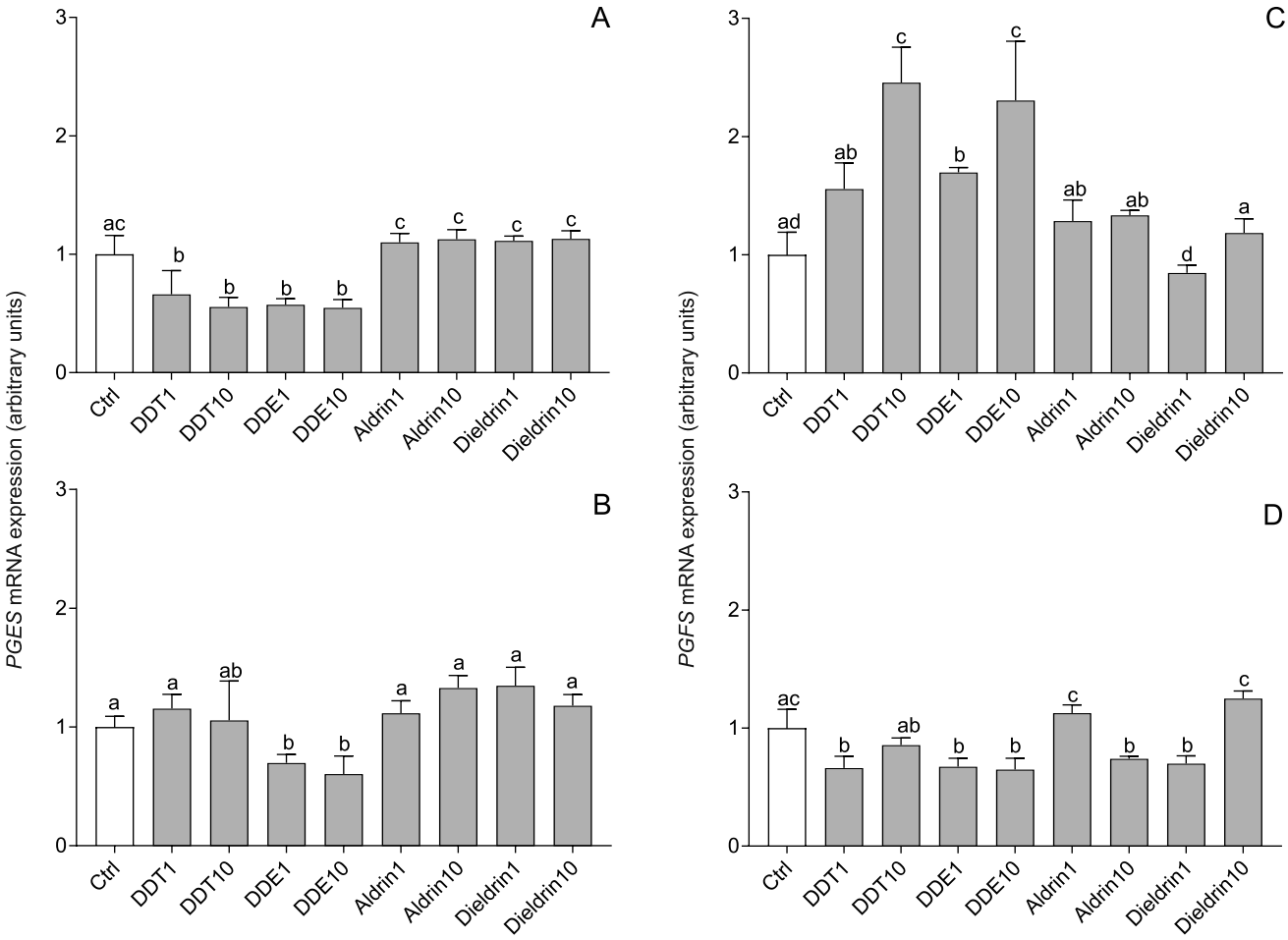

Fig. 3. Effects of DDT, DDE, aldrin and dieldrin at the doses 1 and 10 ng/ml on mRNA expression of *PGES* (A, B), and *PGFS* (C, D) in endometrium explants collected between 120 and 150 days (A, C) and 151 to 180 days (B, D) of the gestational period. Different superscripts above bars are indicative of differences among values ($p<0.05$; ±SEM).

caused an increase in the expression of this gene ($p<0.05$) in the same explants (Fig. 3D).

All doses of DDT and DDE decreased ($p<0.05$) of the PGE2 release from the endometrial explants collected during 120 to150 days of the gestational period (Fig. 4A), whereas in the explants collected between 151 to 180 days of the gestation period, this effect was observed ($p<0.05$) after treatment with DDT and DDE at a dose of 10 ng/ml or dieldrin at a dose of 1 ng/ml (Fig. 4B). Aldrin and DDE (both at 1 ng/ml) increased ($p<0.05$) secretion of PGF2α, but 10 ng/ml of aldrin decreased ($p<0.05$) its release from the endometrial explants collected between 120 and 150 days of gestational period (Fig. 4C). In contrast, both doses of aldrin and dieldrin at 1 ng/ml decreased ($p<0.05$) the release of PFG2α in the explants collected between 151 and 180 days of pregnancy (Fig. 4D).

Discussion

The metabolites of pesticides and their parental substances used did not affect the viability of the endometrial explants. Thus, it can be assumed that the observed changes in the mRNA expression of the genes responsible for enzyme and hormone secretion were not evoked by the acute cytotoxic effects of exposure to these compounds.

The results obtained indicate that the tested xenobiotics affect the synthesis and release of PGE2 and PGF2α at relatively low doses and short incubation times. The changes caused by DDT/DDE and aldrin/dieldrin are different and indicate that both investigated pairs of EDs may affect the secretion of PGs in different ways. Moreover, in contrast to experiments carried out on endometrial explants and endothelial cells of the cyclic uterus (Wrobel et al. 2009, Wrobel et al. 2014) and fallopian tube epithelium (Wrobel et al. 2012), none of the studied EDs significantly changed *COX2* expression in both examined periods of pregnancy. These disruptive influences on gene expression focused on the PG syn-

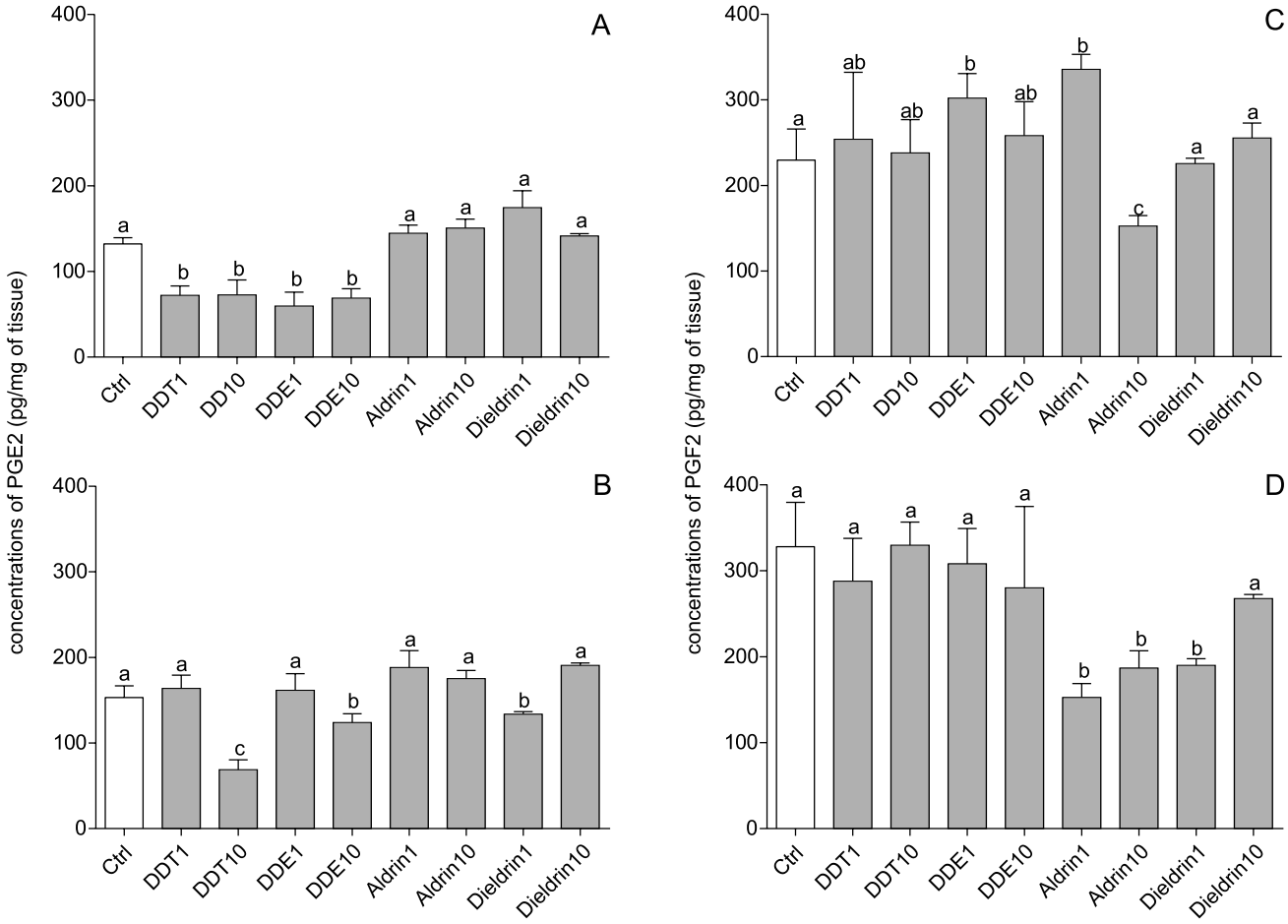

Fig. 4. Effects of DDT, DDE, aldrin and dieldrin at the doses 1 and 10 ng/ml on release of prostaglandins E2 (A, B) and prostaglandin F2α (C, D) from endometrium explants collected between 120 and 150 days (A, C) and 151 to 180 days (B, D) of the gestational period. Different superscripts above bars are indicative of differences among values ($p<0.05$; ±SEM).

thase genes *PGES* and *PGFS*. The lack of effect on *COX2* expression may be due to the physiological decrease in the expression of this gene caused by interferon-tau (IFNτ). This gene is produced in the trophoblast area from the first days of pregnancy (Thatcher et al. 2001). Hence, it is possible that the studied EDs at the indicated doses could not disrupt this effect.

The factors regulating the expression of the *PGES* and *PGFS* genes and the enzymatic activity of the respective prostaglandin synthases in the bovine endometrium are peroxisome proliferator-activated receptors (PPARs) α, β and γ (MacLaren et al. 2006). The selective activation of individual receptors changes the gene expression and enzymatic activity of both PG synthases (Aleshin et al. 2009). DDT and DDE act as PPARγ antagonists (Routti et al. 2016), while aldrin decreases the levels of PPAR receptors, especially the PPARγ izotype, in cells (Moreno-Aliaga et al. 1999). The results obtained indicate that changes in the expression of the *PGES* and *PGFS* genes can be an effect of blocking or decreasing the activity of PPARs caused by EDs.

During pregnancy, the mutual proportions between individual PPAR isotypes are changed (Vitti et al. 2016). For example, in ewe endometrium, PPAR*α* expression declined between day 7 and day 17 of pregnancy, whereas PPAR*β* was constantly expressed at all developmental stages, and PPAR*γ* expression was erratically regulated (Yang et al. 2008). This phenomenon may be a reason for the different effects of DDT and DDE on *PGFS* expression and PGF2α secretion from the endometrial explants collected between 120 and 150 days, and 151 to 180 days of the gestational period.

Both prostaglandins participate in the regulation of the bloodstream on the chorion area (Poston 1997). Furthermore, PGs secreted by the endometrium easily penetrated through the placental barrier and can change the PGE2:PGF2α ratio in the chorion. This change may result in disturbed motoric activity of chorionic blood vessels (Maigaard et al. 1986). Moreover, PGs in fetal circulation have a longer half-life than those in peripheral circulation due to their less efficient vascular trans-

membrane uptake process, which can unfavorably affect blood flow in the fetal part of the placenta (Boura and Walters 1991). Consequently, the delivery of nutrients to the fetus, gas exchange and removal of the metabolic products between maternal and fetal parts of the placenta can be disturbed. It is believed that chloroorganic pesticides can increase the risk of abortion or premature deliveries in cows.

In summary, the EDs used affect the release of PGE2 and PGF2α from endometrial explants by influencing the expression of genes of their synthases, *PGES* and *PGFS,* in both studied stages of pregnancy.

Acknowledgements

We would like to thank Professors W.J. Silvia (University of Kentucky, Lexington, KY, USA) and W.W. Thatcher (University of Florida, Gainesville, FL, USA) for donation of the PGFM and PGE2 anti-sera, respectively.

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