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DDT- AND DDE-INDUCED DISRUPTION OF OVARIAN STEROIDOGENESIS IN PREPUBERTAL PORCINE OVARIAN FOLLICLES: A POSSIBLE INTERACTION WITH THE MAIN STEROIDOGENIC ENZYMES AND ESTROGEN RECEPTOR β

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We evaluated impact of DDT isomers, o, p'- DDT [1, 1-dichloro-2, 2-bis (p, p'-chlorophenyl) ethylene] and p, p'-DDT [1, 1, 1-trichloro-2, 2-bis (p-chlorophenyl) ethane], and their metabolites, o, p'-DDE and p, p'-DDE, on ovarian steroidogenesis. All these compounds, except for p, p'-DDT, demonstrated estrogenic effects on steroid secretion in co-cultures of porcine prepubertal granulosa and theca cells. p,p'-DDT decreased progesterone and estradiol release, which was reversed by the addition of testosterone. In contrast, o, p'-DDT inhibited progesterone secretion with parallel stimulation of basal and testosterone-stimulated estradiol release. DDEs stimulated progesterone and estradiol secretion. The fluorometric assay confirmed that p,p'-DDE, o,p'-DDT, and o,p'-DDE stimulated aromatase activity. Western blots indicated that o,p-DDT and o,p'-DDE diminished the expression of estrogen receptor β (ERβ). This study demonstrated the isomer-dependent action of DDT in pig ovarian cells. We propose that DDT could disrupt ovarian steroidogenesis either by interfering with main steroidogenic enzymes or affecting ERβ.

Key words: *prepubertal ovarian follicle, DDT, DDE, steroidogenesis, aromatase, estrogen receptor*

INTRODUCTION

DDT (dichlorodiphenyltrichloroethane), a well-known organochlorine pesticide has been described as an endocrine-disrupting chemical. Both DDT and

its major and most stabile metabolite, dichlorodiphenyldichloroethylene (DDE), have been found to exert the endocrine effects on male and female reproductive system. These compounds have been detected in the follicular fluid of human ovaries (1, 2) and there is evidence that exposure to these compounds is associated with altered ovarian function (3) and decreased female fertility (4, 5).

There are several mechanisms by which DDT and DDE could act as endocrine disruptors in cells: through steroidogenic pathway (6, 7), through receptor-mediated changes in protein synthesis (8) or through their anti-androgenic and estrogenic actions (9). A lot of endocrine effects of DDT and its metabolite DDE result from their ability to mimic estradiol-17β (10, 11).

In the ovary, the estrogenic responses are mediated through two separate estrogen receptors (ER): ERα and ERβ (12, 13), but the ERβ is the most abundant (14). Estradiol and ERβ are important for the regulation of follicular development (14, 15, 16). Healthy follicles in the ovary show P450aromatase (CYP19) activity and are capable of estradiol synthesis (16, 17). ERβ controls this enzymatic activity and capacity to convert an androgen precursor to estradiol (15, 18). Until now there have been no studies which investigated the mechanism of estrogenic DDT action in the ovary. Therefore, the aim of present study was to investigate the mechanism of action of two DDT isomers: p, p'-DDT and o, p'-DDT as well as their metabolite (DDE) in ovarian follicular cells. We investigated impact of these compounds on secretion and conversion of steroid hormones (progesterone and estradiol) as well as on expression of ERβ. Moreover, aromatase activity was measured using fluorogenic substrate dibenzylfluorescein (DBF). The specifity of aromatase-dependent production of DBF metabolite was verified with the selective aromatase inhibitor, CGS 16949A. Cytotoxic effects of the above compounds were determined with LDH-released from damaged cells.

MATERIALS AND METHODS

Reagents

M199, trypsin, and calf serum (FBS), antibiotic/antimycotic solution (100x) TRIS, Na-deoxycholate, Nonidet NP-40, sodium dodecyl sulfate SDS, protease inhibitor (EDTA-free), EDTA, DTT, Tween 20 and bromophenol blue, dibenzylfluorescein (DBF), NADP, glucose 6-phosphate and glucose-6-phosphate dehydrogenase were purchased from Sigma (Chemical Co. St. Louis, MO, USA). DDT compounds (p, p'-DDT, o, p'-DDT, p, p'-DDE and o, p'-DDE) were purchased from Reference Standards, EPA, Research Triangle Park, N. C, USA. Stock solutions of these test compounds were prepared in DMSO and added to M199 supplemented with 5% FBS. The final concentration of DMSO in the medium was always 0.1%. The non-steroidal aromatase enzyme system inhibitor, CGS 16949A (fadrozole) [4-(5,6,7,8-tetrahydroimidazol [1,5-α] pyridin/5-yl)benzonitrile monochloride] was generously provided by Ciba-Geigy Ltd., Switzerland.

Cell cultures

Prepubertal porcine ovaries were obtained from a local abattoir and transported to the laboratory. Approximately 1.5 h elapsed from slaughter to arrival to the laboratory. Follicles (4-5

mm in diameter) were excised from ovaries. Granulosa cells (Gc) and theca interna cells (Tc) from the same follicles were subsequently prepared according to the technique described by Stoklosowa *et al*. (19, 20). After isolation, Gc and Tc were collected and resuspended in M199 supplemented with 10% FBS. For co-culture experiments, the viability of granulosa and theca cells was determined by the Trypan blue exclusion test and subsequently they were inoculated at a concentration of 6 x 10^4 and 1.5 x 10^4 cells/well, respectively, in Nunc 96-well tissue culture plates. Therefore, the ratio of both types of cells was comparable to that observed *in vivo* (Gc: Tc = 4:1) according to Stokłosowa *et al*. (20). The cultures were maintained at 37°C in a humidified atmosphere of 5% CO_2 /95% O_2.

Experimental procedure

Experiment 1 was designed to study dose-response effect of the test compounds on estradiol secretion. Cells were initially cultured in the medium without test compounds for 24 h to allow for cell attachment. After 24 h, the medium was discarded and the fresh M199 medium supplemented with 5 % FBS was added to the control culture, while to the experimental culture o,p'-DDT, p,p'-DDT, o,p'-DDE or p,p'-DDE were added at the concentrations of 4, 40, 400 ng/ml and 4 mg/ml. These concentrations cover the range of concentrations of DDT and DDE reported to be present in ovarian follicular fluid and serum (21) and also they were used in our previous study on ovarian steroidogenesis of cycling animals (22). After 24h, the cultures were terminated and 100 ml of fresh media were collected for the LDH activity assay. The remaining amount of the media was frozen for estradiol determination. Every treatment was conducted in 4 wells and each experiment was repeated 3 times.

Experiment 2 was conducted to examine the effect of the test compounds on progesterone (P4) and estradiol (E2) secretion by follicular cells. Cells were initially cultured without test compounds for 24 h and then the medium was discarded. The fresh M199 medium supplemented with 5 % FBS was added to the control culture, while to the experimental culture, o,p'-DDT, p,p'-DDT or their metabolites were added at the concentration of 4 µg/ml medium. Media were collected after 24 h and frozen for steroids determination. Every treatment was conducted in 4 wells and each experiment was repeated 3 times.

Experiment 3 was performed to demonstrate the effect of DDT isomers and their metabolites on aromatase activity by 1) measuring conversion of testosterone to estradiol and 2) study the direct action on aromatase activity.

The experimental cultures were maintained in the presence of 4 µg/ml o,p'-DDT, p,p'-DDT or their metabolites with or without 10^{-7} M of testosterone. Culture media were collected after 24 h of exposure, and frozen for estradiol determination.

In separate experiments, cells were cultured in the presence of 4 µg/ml of o,p'-DDT, p,p'-DDT or their metabolites with or without 10 µM aromatase inhibitor CGS 16949A (fadrozole hydrochloride). After 24 h of exposure, media were discarded and cells were frozen for aromatase activity measurement.

Experiment 4 was conducted to demonstrate the effect of the test compounds on estrogen-β receptor protein expression. Following 24 hrs of pre-incubation, the culture medium was changed and replaced with M199 supplemented with 5% FBS and o,p'-DDT, p,p'-DDT or their metabolites at the 4 mg/ml concentration. After 3 h of treatment, the culture media were removed and cells were lysed in 50 µl of ice-cold lysis buffer, containing 50 mM Tris HCl (pH 7.5) 100 nM NaCl, 0.5% Na-deoxycholate 0.5% Nonidet NP-40, 0.5% sodium dodecyl sulfate SDS and protease inhibitor (EDTA-free). Lysates were sonicated and centrifuged at 10 000 x g at 4°C for 20 min. Supernatants were collected and stored at -20°C for immunoblotting analyses.

LDH assay

Cytotoxicity detection kit (Roche Applied Science) is a colorimetric assay for quantification of cell death and cell lysis based on the measurement of lactate dehydrogenase (LDH) activity released from the cytosol of damaged cells into supernatant. After 24-h treatment of the cells with DDT and its metabolites, culture supernatants were collected and incubated with the reaction mixture from the kits. After 30 min, the reaction was stopped by adding 1 N HCL and absorbance was measured at wavelength of 490 nm with reference wavelength of 600 nm in microELISA plate reader.

Immunoblotting

Protein concentration of lysates was determined with Bradford reagent (Bio Rad Protein Assay; Bio Rad Laboratories, Munchen, Germany) using bovine serum albumin (BSA) as the standard. The 20 μg of total protein was separated on 7.5% SDS-polyacrylamide gel electrophoresis in BIO-RAD Mini-Protean II Electrophoresis Cell. After electrophoretic separation, proteins were transferred to nitrocellulose membranes using Bio Rad Mini Trans-Blott apparatus. Following the transfer, membranes were washed and non-specific binding sites were blocked with 5% milk and 0.2%, Tween 20 in 0.02 M TBS for two hours with shaking. Then the membranes were incubated with anti-ER-β rabbit polyclonal antibody (H-150; sc-8974, Santa Cruz Biotechnology, Inc, Santa Cruz, CA) diluted 1:200 in TBS/Tween at room temperature for 3 hrs. After incubation with the primary antibody, the membranes were washed and incubated for 1 h with horseradish peroxidase-conjugated antibody (anti-rabbit IgG-HRP, sc-2004, Santa Cruz Biotechnology) diluted at 1:500 in TBS/Tween. Signals were developed by chemiluminescence (ECL) using Western Blotting Luminol Reagent; sc-2048, Santa Cruz Biotechnology) and visualized with the use of PhosphoImager FujiLas 1000. Immunoreactive bands were quantified by an image analyzer (Image Gauge V4.0; Fuji Photo Film Co., LTD).

Steroid analysis

Testosterone (T) and 17β-estradiol (E2) concentrations in the media were determined by using EIA kits (IBL, Germany).

Progesterone in the culture medium was assayed by using a direct enzyme immunoassay (EIA) as described previously by Okuda *et al*. (23). The standard curve range was from 0.39 to 100 ng/ml and effective dose for 50% inhibition (ID50) of the assay was 4.5 ng/ml. The intra- and inter- assay coefficients of variation were 6.6% and 9.2%, respectively.

Aromatase (CYP19) activity measurement

Aromatase (CYP19) converts C19 androgens to aromatic C18 estrogenic steroids. We estimated the activity of CYP19 enzyme using the fluorometric substrate dibenzylfluorescein (DBF). The fluorescence assay using dibenzylfluorescein for aromatase activity on the 96-well plates was performed according to Stresser *et al*. (24). Briefly, cells were thawed at room temperature for 10 min and then lysed with 0.5 M Tris-HCL. After this time, 2.6 mM NADP, 6.6 mM glucose 6-phosphate and 0.4 U of glucose-6-phosphate dehydrogenase/ml were added and plates were incubated for 15 min at 37°C. Then DBF in acetonitrile was added at a final concentration of 2 μM. After 30 min the reaction was terminated by the addition of 2 M sodium hydroxide. The DBF metabolite, fluorescein, was measured by using an excitation wavelength of 485 nm and an emission wavelength of 538 nm.

Apart from CYP19 (aromatase), DBF is also a substrate for the other cytochoromes such as: CYP2C8, CYP2C9, CYP2C19, CYP3A. However, there is no specific substrate for fluorescent measurement of CYP19 activity. Both CYP2C and CYP3A families are involved in drug/xenobiotic and steroid metabolism. In contrast to them, CYP19 (aromatase) is engaged in steroid biosynthesis

only. In order to verify if aromatase was involved in production of the DBF metabolite, fluorescein, we used the selective aromatase inhibitor, CGS 16949A. We observed that CGS 16949A did not affect the control fluorescein production, whereas it inhibited p,p'-DDE-, o,p'-DDT'-, o,p'-DDE-induced fluoescence production.

Statistical analysis

Each treatment was repeated three times in quadruplicates and thus the total number of replicates was 12. Since the variations between the experiments were small, those 12 results were averaged and analyzed by one-way analysis of variance (ANOVA) followed by Tuckey honestly significant difference (HSD) multiple range test. All data (n = 12) are expressed as the mean ± SEM.

RESULTS

Dose-dependent action of o,p'-DDT and p,p'-DDT and their metabolites o,p'-DDE, p,p'-DDE on estradiol secretion

In control cultures, estradiol secretion into the medium during 24 h of culture was 29.11 ± 2.9 pg/ml (*Fig.1*). A significant decrease in estradiol secretion was noted after the addition of 400 ng/ml and 4 µg/ml of p,p'-DDT. The amounts of estradiol in the experimental media were 21.9 and 20.2 pg/ml, respectively *vs*.

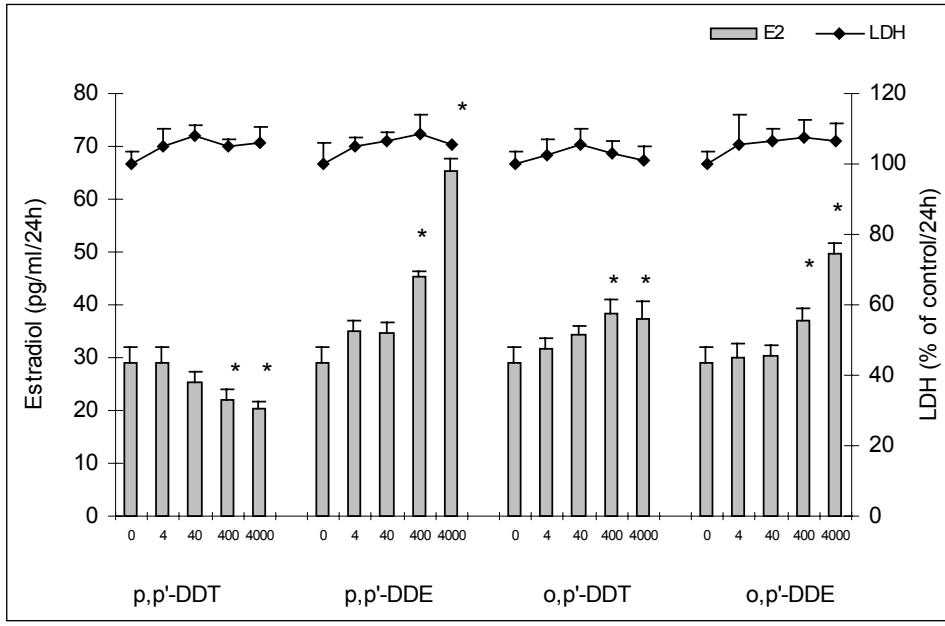

Fig .1. Dose-dependent effects of p, p-DDT, p, p-DDE, o, p'-DDT and o, p'-DDE on estradiol secretion (bars) and LDH activity by co-cultured granulosa and theca cells. Each point represents the mean ± S.E.M. of results of three independent experiments, each of which consisted of four replicates per treatment group. (*) indicate statistically ($p<0.05$) significant differences between groups.

29.1 pg/ml in the control culture ($p<0.01$). Unlike p,p'-DDT, the second isomer, o,p'-DDT, increased estradiol secretion to the values of 38.4 and 37.3 pg/ml, in 400 ng/ml and 4 ug/ml o,p'-DDT-treated cells, respectively ($p<0.01$).

Both DDE isomers: p, p'-DDE and o, p'-DDE increased estradiol secretion. At a dose of 400 ng/ml these compounds elevated estradiol level 1.5-fold and 2.9-fold, respectively. At a higher dose, 4 µg/ml, they elevated estradiol secretion 1.2-fold and 2.3-fold, respectively ($p< 0.01$).

The investigated pesticides which did not affect cell viability, thus suggesting that the observed changes in estradiol secretion were not affected by pesticide-induced cytotoxicity (*Fig.1*).

The action of 4 µg/ml of o, p'-DDT and p, p'-DDT and their metabolites, o,p'-DDE, p, p'-DDE, on progesterone and estradiol secretion.

In control cultures, progesterone secretion into the medium during 24 h incubation was 2727.5 ± 303.6 pg/ml (*Fig.2A*). A significant decrease in progesterone secretion was noted after the addition of both DDT isomers: p,p'-DDT and o,p'-DDT as well as p,p'-DDE.

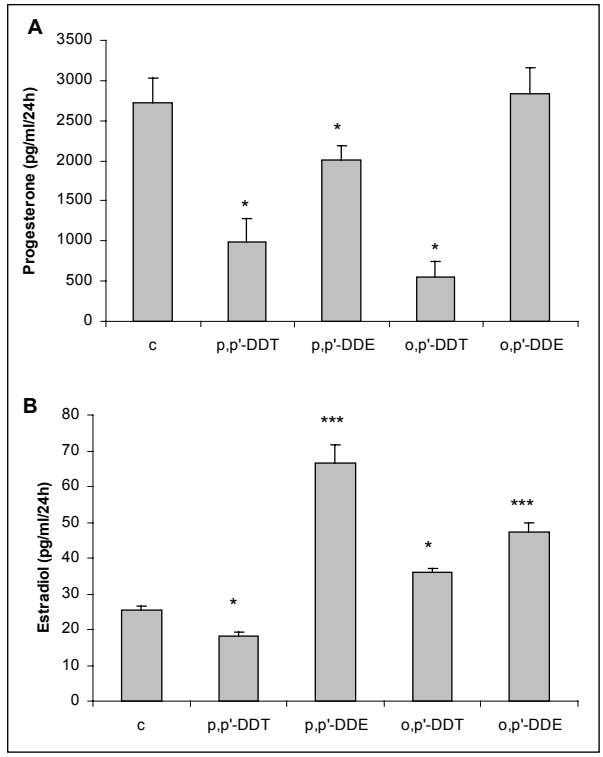

Fig. 2. Effect of 4 µg/ml of p, p-DDT, p, p-DDE, o, p'-DDT and o, p'-DDE on (**A**) progesterone and (**B**) estradiol secretion by co-cultured granulosa and theca cells. Each point represents the mean ± S.E.M. of results of three independent experiments, each of which consisted of four replicates per treatment group. (*) indicate statistically ($p<0.05$) significant differences between groups.

In p,p'-DDT, o,p'-DDT and p,p'-DDE-treated cell cultures progesterone concentrations were 980.7 ± 302.57 pg/ml and 546.38 ± 92,07 pg/ml and 2013.13 ± 170,06pg/ml, respectively; ($p<0.01$); (*Fig. 2A*).

The amount of estradiol in the control culture was 25.49 ± 0.9 pg/ml (*Fig. 2B*). The addition of 4 µg/ml of p,p'-DDT caused a significant decrease in estradiol secretion (18.09 ± 1.32 pg/ml *vs*. control; $p<0.01$). In contrast, the treatment with 4 ng/ml of o,p'-DDT, p,p'-DDE and o,p'-DDE caused a significant increase in estradiol secretion (36.08 ± 0.97 pg/ml, 66.6 ± 5.1 pg/ml and 47.3 ± 2.6 pg/ml respectively, *vs*. 25.49 ± 0.9 pg/ml; $p<0.01$). (*Fig.2B*).

The action of 4 µg/ml of o, p'-DDT and p, p'-DDT and theirs metabolites o,p'-DDE, p, p'-DDE on conversion of testosterone into estradiol and on aromatase activity.

a) Conversion of testosterone to estradiol

All investigated compounds significantly increased the conversion of testosterone into estradiol ($p<0.01$); (*Fig. 3A*).

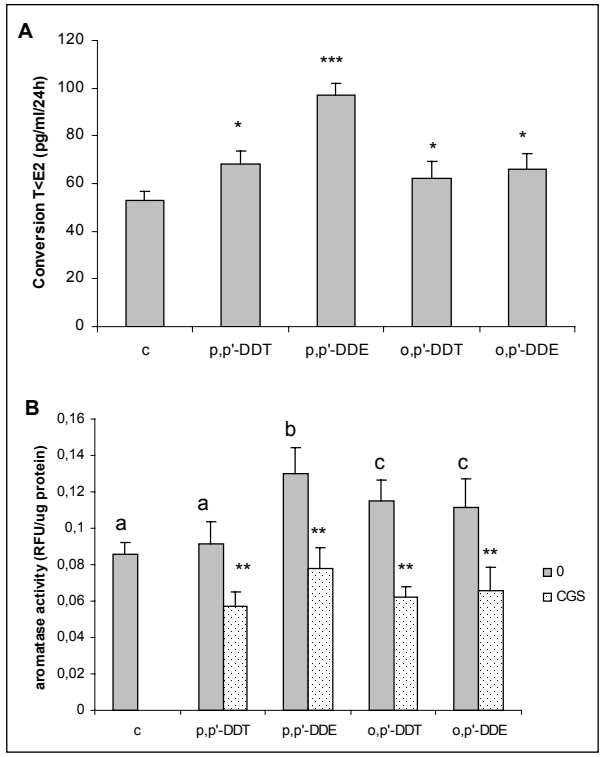

Fig. 3. Effect of p, p-DDT, p, p-DDE, o, p'-DDT and o, p'-DDE on (**A**) conversion of testosterone to estradiol and (**B**) aromatase activity. Each point represents the mean ± S.E.M. of results of three independent experiments, each of which consisted of four replicates per treatment group. (*) indicate statistically ($p<0.05$) significant differences between reagents and CGS 16949A treated cells. Significant statistically differences between groups (control and particular reagents treated) are indicated with different letters. Sam letter indicating no significant difference, with a <b<c.

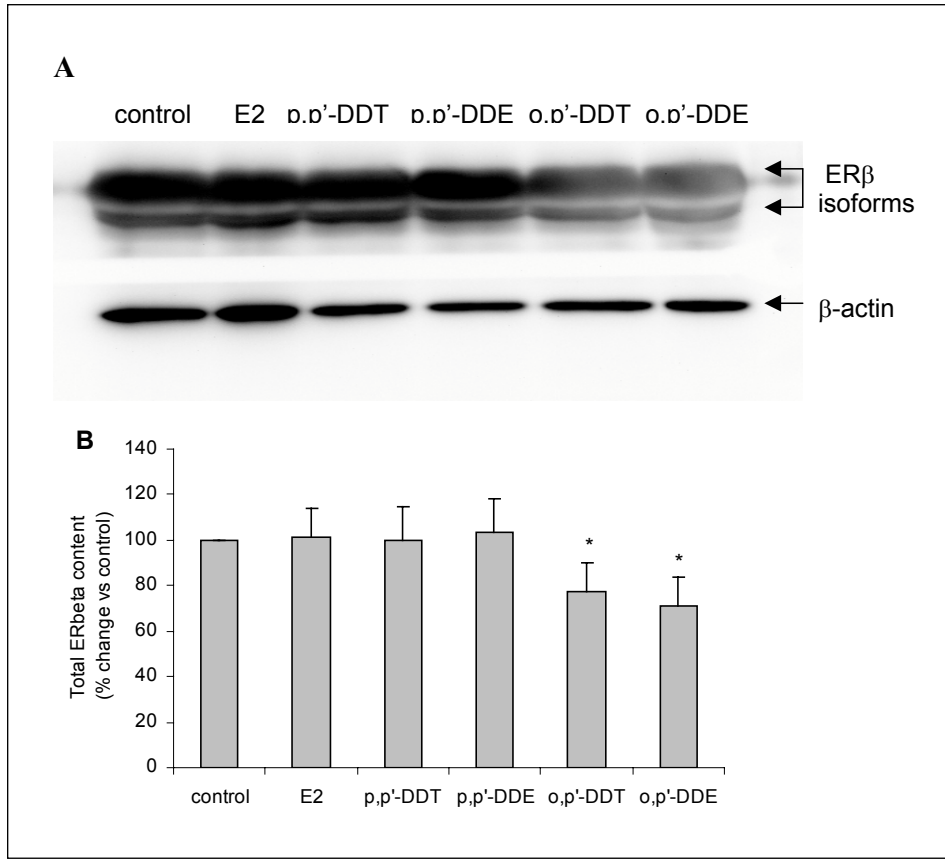

Fig. 4. Effect of p, p-DDT, p, p-DDE, o, p'-DDT and o, p'-DDE on the expression of the b estradiol receptor protein (ERβ) in follicular cells. (**A**) Representative Western blot analysis of ERβ protein levels in co-cultured granulosa and theca cells without treatment (control), estradiol (E2) and p,p-DDT, p,p-DDE, o,p'-DDT and o,p'-DDE treated cells. Blots were stripped and re-probed with anti actin antibody to control the amounts of protein loading.
(**B**) Densitometric analysis of total ERb content, expressed as percent changes with respect to the control group, which has been set at 100%. Each bar represents the mean ±S.E.M. of the three independent experiments. Statistically significant ($p<0.05$) differences among groups are indicated by (*)

b) Direct effect on aromatase (CYP19) activity

The aromatase activity was determined by fluorescence measurement of fluorescein as described in Materials and Methods. In cells exposed to o, p'-DDT, o, p'-DDE and p, p'-DDE, aromatase activity was increased by 1.3-; 1.3- and 1.5-fold, respectively (*Fig. 3B*). CGS 16949A did not affect the control fluorescein production, but it inhibited p,p'-DDE-, o,p'-DDT'-, o,p'-DDE-induced aromatase activity ($p <0,05$).

The effect of o, p'-DDT and p, p'-DDT and their metabolites o, p'-DDE, o, p'-DDD, p, p'-DDE on ER-β protein expression

Immunoblott analysis of ERβ showed a strong band in p, p'-DDT and p, p'-DDE-treated cells similar to the estradiol-treated and control cells. (*Fig.4*). However, contrast, a decrease in the expression of ERb protein was noted in o, p'-DDT and o, p'-DDE-treated cells (*Fig.4*).

DISCUSSION

In the present study, we demonstrated the action of DDT isomers, p,p'-DDT and o,p'-DDT, and their metabolites, p,p'-DDE and o,p'-DDE, on ovarian follicular cells isolated from immature, prepubertal pigs. We found that all these compounds, except for o,p'-DDE, decreased progesterone secretion, thus confirming their action as disrupters of ovarian steroidogenesis. These results are in agreement with those of Lindeau *et al*. (25) who reported that female rabbits treated with p,p'-DDT showed a significantly reduced ovulation rate because of low level of progesterone. Chedrese and Feyles (26), who used a high concentrations of DDE mixture (p,p'-DDE and o,p'-DDE) observed also a decreased progesterone synthesis in pig ovarian cells. Like in our study, Haney *et al.* (27) indicated a diminished progesterone production elicited by o,p-DDT and p,p-DDT in pig granulosa cells. Crellin *et al*. (11) have suggested anti-estrogenic properties of a DDT metabolite, since they observed that DDE inhibited the 8-bromo-cAMP- or cholera toxin-stimulated progesterone synthesis, thus acting similarly to estradiol in the primary culture of porcine granulosa cells. However, in contrast to our study, the authors did not observe any alterations in progesterone levels after treatment with DDE. In another study, these authors reported that the higher doses of DDE (300 and 10 000 ng/ml) were able to inhibit progesterone production in stable granulosa cell line JC-410 (6).

Rate of progesterone formation depends on the rate of cholesterol transport from intracellular stores inside the inner mitochondrial membrane and loading of cholesterol side-chain cleavage enzyme ($P450_{scc}$) with cholesterol (28, 29). Therefore, the DDT/DDE-induced decrease in progesterone secretion, which was observed in our study, could be due to the lack of the substrate for progesterone synthesis or inhibition of $P450_{scc}$. Similar mechanism, related to the inhibition of an early step of steroidogenesis, was proposed for another pesticide, methoxychlor, and its metabolite: 2,2-bis(p-hydroxyphenyl)-1,1, 1-trichloroethane. Akingbemi *et al*. (30) found that metoxochlor-mediated decrease in testosterone biosynthesis, observed in rat Leydig cells, was due to suppression of steady-state mRNA levels of $P450_{scc}$ enzyme.

Apart from the inhibition of an early step of steroidogenesis, the DDT-induced decrease in progesterone secretion may be related to estradiol synthesis, a process where progesterone serves as a precursor. Indeed, in our study the p,p'-DDE-, o,p'-

DDT-induced decrease in progesterone secretion was followed by the increase in secretion of estradiol. Nevertheless, in case of o,p'-DDE, an increase in estradiol secretion was not preceded by an alteration in progesterone secretion. In contrast to these compounds which stimulated estradiol secretion, p,p'-DDT-inhibited it. This points to the inhibition of early steps of steroidogenesis as the main reason of p,p'-DDT-induced decrease in progesterone secretion. It is well known that progesterone serves as a substrate for testosterone synthesis, which is subsequently aromatized to estradiol. In our study, addition of testosterone reversed the inhibitory effect of p,p'-DDT which indicated insufficient amount of the substrate for aromatization to estradiol. However, addition of testosterone accelerated the stimulatory effects of p,p'-DDE, o,p'-DDT, and o,p'-DDE. These findings, related to the secretion of progesterone and estradiol, were verified by measurement of aromatase activity, which confirmed the ability of p,p'-DDE, o,p,-DDT and o,p'-DDE to stimulate steroidogenesis by affecting aromatase. Indeed, aromatase appeared to be affected by the above compounds, since these pesticide-induced aromatase activity was inhibited by CGS 16949A. Our results are in agreement with the study of Younglai *et al*. (7) who reported that concentrations of p,p'-DDE, similar to those present in human follicular fluid, enhanced basal and FSH-stimulated aromatase activity in human granulosa cells. DDE has also been directly shown to increase aromatase activity and expression in hepatic microsomes (31) and endometrial stromal cells (32). The estrogenic activity of DDT and DDE have been demonstrated in many mammalian and fish systems (33, 34, 35). Moreover, Danzo *et al*. (36) observed that the treatment of the castrated guinea pigs with p,p'-DDE stimulated reproductive tract in the manner similar to the endogenous estrogens. In addition to the above-mentioned studies, we indicated for the first time the isomer-dependent action of DDT in pig ovarian cells, namely p,p'-DDT was anti-estrogenic, but o,p'-DDT was estrogenic. Moreover, we demonstrated that both DDE isomers showed strong estrogenic properties, p,p'-DDE being most effective in stimulation of estradiol production.

Another interesting achievement of the present study was demonstration for the first time that in ovarian follicular cells, DDT isomers could modulate ER β activity/expression in different ways. It is well known that estrogen receptors are present in ovarian tissue, mainly in granulosa cells (37, 38). On the other hand, estrogens regulate granulosa cell function. (39). A number of studies have found that p,p'-DDT binds to ER although this effect may be relatively weak (40, 41, 42). Some studies have reported that o,p'-DDT mimics the endogenous estrogen 17-β estradiol (E2) (42) and can bind to the ER, however, its affinity is much lower than that of E2. It was also demonstrated that p,p'-DDE could weakly bind to the ER (41,43). Tiemann *et al*. (44) showed that the o,p-DDT could bind to the cytoplasmic estrogen receptor and, moreover, it could inhibit the binding of radiolabeled estradiol to the bovine uterine endometrial explants. In our study, p,p'-DDT and p,p'-DDE did not change the ERb protein expression, whereas o,p'-DDT and o,p'-DDE decreased ERb protein level. A possible explanation could

follow the hypothesis of di Lorenzo *et al*. (42) who studied tissue-specific effects of DDT isomers in adult and suckling newborn mice. They suggested that the two isomers displayed opposite effects: pp-DDT acted as an ER agonist but o,p'-DDT as an ER antagonist. Dauvois *et al*. (45) and Mueller *et al.* (46) showed that the level of ER protein and its turnover were dependent on hormone-binding domain of the receptor. For this reason, the ER agonists increased, while ER antagonist decreased expression of mRNA and protein level (45, 46).

In conclusion, our study demonstrated for the first time the isomer-dependent action of DDT in pig ovarian cells. We suggest that DDT as well as DDE may block or activate ER transcriptional activity, depending on the target tissue and isomer specificity towards estrogen receptor. On the basis of the presented results, we propose two different potential mechanisms by which DDT and DDE could interfere with ovarian steroidogenesis. First, DDT and DDE can affect the main steroidogenic enzymes, such as P-450$_{scc}$ or P-450arom, and second they can act as ERβ receptor agonist or antagonist.

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