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THE EFFECTS OF DEXAMETHASONE TREATMENT OF PREGNANT RATS ON MATERNAL, FETAL AND NEONATAL ACTH-CELLS

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Adrenocorticotropic hormone (ACTH) is secreted in response to a number of stimuli and it influences growth, differentiation and adrenal steroidogenesis. Rat pituitary ACTH-cells are differentiated at 14-16 days of fetal life, while synthesis of adrenal glucocorticoids starts from the 17th day of gestation as a result of increased synthesis and release of ACTH. The aim of this work was to examine the effect of dexamethasone (Dx), administered to gravid females, on the synthetic ability of their pituitary ACTH-cells as well as those of their fetal and neonatal offspring. The experimental group of pregnant females received Dx (1.5 mg/kg bw) on day 16 of gestation, while the control group received an equal volume of diluent at the same time. Plasma ACTH and cortisol concentrations were determined by RIA. The ACTH-cells were examined under light and electron microscopes.

The results obtained indicate that single Dx-treatment of pregnant rats suppresses differentiation of fetal adrenocortical cells and the release of ACTH. However, in neonatal rats the number of ACTH- and precursor cells and their proliferation increased, as well as ACTH and cortisol synthesis and release, i. e. stimulation of synthesis and secretion of ACTH was noticed after suppression in the fetal period.

The numerous changes observed in the fetuses and early neonates of dams treated with a single dose of Dx during pregnancy had disappeared in 30-day-old offspring.

Key words: pituitary, ACTH-cells, fetal period, neonatal period, adult period, dexamethasone.

INTRODUCTION

Corticotropin-releasing hormone (CRH), a hypothalamic neuropeptide (41-amino acids), plays the major role in regulating the function of the hypothalamic-pituitary-adrenocortical axis (HPA). CRH, together with other neuropeptides and neurotransmitters, stimulates synthesis and release of adrenocorticotropin (ACTH) from anterior pituitary ACTH-cells.

CRH and CRH binding sites in the brain and pituitary have been demonstrated in 16-day-old rat fetuses (Insel et al., 1988). Pituitary receptors for CRH, which are present in the prenatal period, are sensitive to glucocorticoid feedback inhibition at the end of gestation (Brooks et al., 1989).

Immunocytochemical investigations of fetal rat pars distalis have shown that ACTH-cells are the first cell type which can be identified. ACTH-cells are differentiated at 14-16 days of fetal life (Setalo and Nakane, 1976, Nemeskéri et al., 1989), while synthesis of adrenal glucocorticoids starts from the 17th fetal day as a result of increased synthesis and release of ACTH (Nemeskéri and Halasz, 1989, Kachaturian et al., 1983; 1991).

Placental-fetal metabolic interrelationships are very important in the control and determination of many aspects of fetal development (Hay, 1995). Maternal corticosteroids can cross the placental barrier to reach the fetuses before delivery, but fetuses have lower physiological levels of glucocorticoids than their dams (Kittinger, 1974).

Treatment with dexamethasone (Dx) during ontogenesis induced: retardation of fetal growth (Hristić et al., 1995, 1997a, Slotkin et al., 1992), alteration in the rate of maturation of some organs such as the lung (Seckl and Miller; 1997), kidney (Wintour et al., 1994), hypothalamic nuclei PVN, and adrenal atrophy (Hristić et al., 1995, 1997a, 1997b). Studies in baboons demonstrated that placental corticosteroid metabolism dictates the amount of cortisol arriving in the fetuses and regulates fetal pituitary adrenocortical function (Pepe, 1994).

The aim of this work was to examine the effect of Dx, administered during gestation as a single dose on day 16 of gestation, on the synthetic activity of pituitary ACTH-cells of the fetuses and neonatal offspring. In addition, analysis of ACTH-cell synthetic activity was performed on the dams.

MATERIAL AND METHODS

Treatment of animals

These investigations involved pregnant Wistar strain rats, their male fetuses (20-day-old) and neonatal offspring (3- 14- and 30-day-old). Three-month-old rats were mated in the laboratory. The day when females were sperm-positive was considered as the first day of pregnancy. Pregnant rats were housed individually in conditions of controlled heating (22^0 C) and lighting (12 h light/dark cycle). Food and water were offered ad libitum. On day 16 of gestation pregnant females were housed individually and divided into two groups: experimental and control.

Experimental procedure

The experimental group received Dx (Krka, Novo Mesto, Slovenia) ip (1.5 mg/mg/kg bw) on day 16 of gestation, while the control group received an equal volume of diluent at the same time. Fetuses (20-day-old) and neonatal offspring (3-, 14- and 30-day-old males) from both control and experimental rats were divided into 3 subgroups consisting of 5-10 animals. Their blood was used for measurement of ACTH and cortisol concentrations (subgroups I and II), and their pituitary glands for light and electron microscopy (subgroups III). Plasma ACTH concentration was also determined in experimental and control dams 3, 14 and 30 days after delivery (subgroup IV). All rats in the study were sacrificed by decapitation under light ether narcosis between 9h and 11h.

Plasma ACTH determination

After killing the animals, trunk blood was collected and, after separation, plasma was frozen and kept at -20^0 C. Plasma concentrations of ACTH were determined by radioimmunoassay (RIA). The blood collected from six fetuses or 3-day-old neonatal rats was pooled and considered as one sample. Blood samples from 14- and 30-day-old offspring were examined individually.

Plasma cortisol determination

Cortisol concentrations were determined using a commercial RIA kit (Cortisol-RIA kit. INEP, Zemun).

Electron microscopy

The pituitary glands were removed quickly and 1mm^3 slices were fixed in 3% glutaraldehyde buffered to 7.2 with 0.1 M phosphate. Tissue was postfixed in 1% osmium tetraoxide in 0.1 M phosphate buffer (7.2), dehydrated in a graded series of acetone and embedded in araldite. Semithin and thin sections were cut with glass knives on an LKB ultramicrotome III and stained with toluidine blue-azur II and methylene blue or uranylacetate and lead citrate, respectively.

Statistical analysis

Data were expressed as group means ±SD. The difference between two means was evaluated by Student's t-test and between three means by one-way analysis of variance followed by the least significant difference test (LSD).

RESULTS

Concentration of ACTH in the plasma

In fetuses and offspring of the control females, the values for plasma ACTH concentration were approximately the same in all four tested periods (Table 1.).

Table 1. Plasma ACTH concentration (pmol/L) in fetuses (20-day-old) and offspring (3-, 14- and 30-day-old) of dams injected with saline (C) or dexamethasone (Dx) (1.5 mg/kg bw) on day 16 of gestation.

	20-day-old fetuses	3-day-old neonates	14-day-old neonates	30-day-old rats
C	40.81 ± 5.47	38.97 ± 3.67	42.39 ± 2.83	43.33 ± 2.55
Dx	35.23 ± 3.99	55.38 ±3.37 [a]	58.97 ±3.08** [a]	40.45 ± 2.52

Results are expressed as mean ±SD for 5 samples in each group.

*P< 0.01 and **P< 0.005 vs C

[a]P< 0.01 vs 20-day-old fetuses

The mean plasma concentration of ACTH in 3- and 14-day-old offspring of Dx-treated dams was markedly increased compared with fetal values (3-day-old rats vs. fetuses - 57%; 14-day-old rats vs. fetuses - 67%) and compared with control values for the same period. Thus, in 3- and 14-day-old pups from Dx-treated dams ACTH concentration in plasma was significantly increased by 42% and 39%, respectively (Table 1.).

Moreover, the mean value for ACTH concentration was higher on the 3rd day after delivery in Dx-treated dams than in control dams (58%) (Table 2.). In the control dams, a significant decrease in plasma ACTH concentration was observed in the period from 3 to 30 days after delivery (14 day vs. 3 day - 24%; 30 day vs. 3 day - 36%). In Dx-treated dams this decrease in plasma ACTH concentrations was more marked (14 day vs. 3 day - 60% and 30 day vs. 3 day - 51%).

Table 2. Plasma ACTH concentration (pmol/L) in dams injected with saline (C) or dexamethasone (Dx) (1.5 mg/kg bw) on day 16 of gestation.

	Days after delivery		
	3	14	30
C	69.30 ± 4.64	52.33 ± 5.17 [a]	44.62 ± 5.70 [b]
Dx	102.49 ± 10.83 *	41.17 ± 3.20 [c]	50.34 ± 3.11 [c]

Results are expressed as mean ± SD for 5 animals in each group
*P< 0.025 vs C
[a]P< 0.05 vs dams 3 days after delivery
[b]P< 0.01 vs dams 3 days after delivery
[c]P< 0.01 vs dams 3 days after delivery

Cortisol concentration in plasma

In the 14-day-old offspring from control dams plasma cortisol concentration was greatly increased compared to 3-day-old pups (14-day-old vs. 3-day-old - 77%). In 3- and 14-day-old pups from Dx-treated dams the values of plasma cortisol concentration were much higher than in 30-day-old offspring (63% and 62%, respectively). Mean plasma concentration of cortisol in 3-day-old neonates of Dx-treated dams was about 2.5 fold higher than in the corresponding controls (Table 3.).

ACTH-cells.

Adrenocorticotrophic-cells of the anterior pituitary gland of perinatal control rats were usually irregular in shape and localized among the somatotropin and prolactin-cells. Secretory granules of ACTH-cells (diameter 120-200nm) were distributed mainly near the plasma membrane. Some of them, near the Golgi apparatus appeared solid and some had central cores. The mitochondria, endoplasmatic reticulum and Golgi apparatus were localized near the nucleus.

Table 3. Plasma cortisol concentration (nmol/L) in offspring (3-, 14- and 30-day-old) of dams injected with saline (**C**) or dexamethasone (**Dx**) (1.5 mg/kg bw) on day 16 of gestation.

	3-day-old neonates	14-day-old neonates	30-day-old rats
C	12.09 ± 0.98	21.40 ± 3.42 [a]	12.94 ± 1.18 [b]
Dx	30.64 ± 1.58*	29.69 ± 4.52 [c]	11.40 ± 1.22 [c d]

Results are expressed as mean ± SD for 5 samples in the group.
***P** < 0.01 vs **C**
[a]**P** < 0.05 vs 3-day-old pups
[b]**P** < 0.05 vs 14-day-old pups
[c]**P** < 0.01 vs 3-day-old pups
[d]**P** < 0.01 vs 14-day-old pups

In ACTH-cells of fetuses from Dx-treated dams, an increased number of secretory granules was noted in the region of the Golgi apparatus. In 3- and 14-day-old neonatal pups the secretory granules were localized near the plasma membrane, and their formation in the Golgi apparatus was observed (Fig. 1.). In addition, an increase number of mitoses in precursor cells was found (Fig. 1).

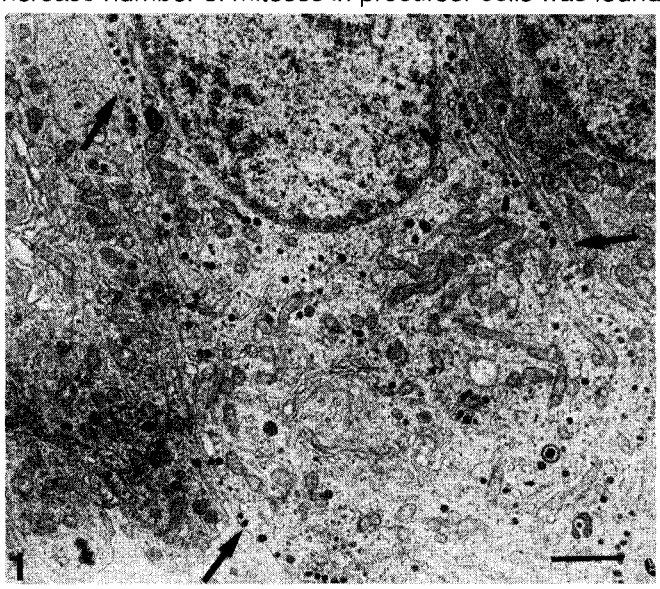

Figure 1. Electron microscopy of ACTH-cell of 14-day-old pup from Dx-treated dam. The secretory granules are localized in the vicinity of the plasma membrane. The Golgi apparatus is well developed and numerous mitochondria are present. (Bar 1μm).

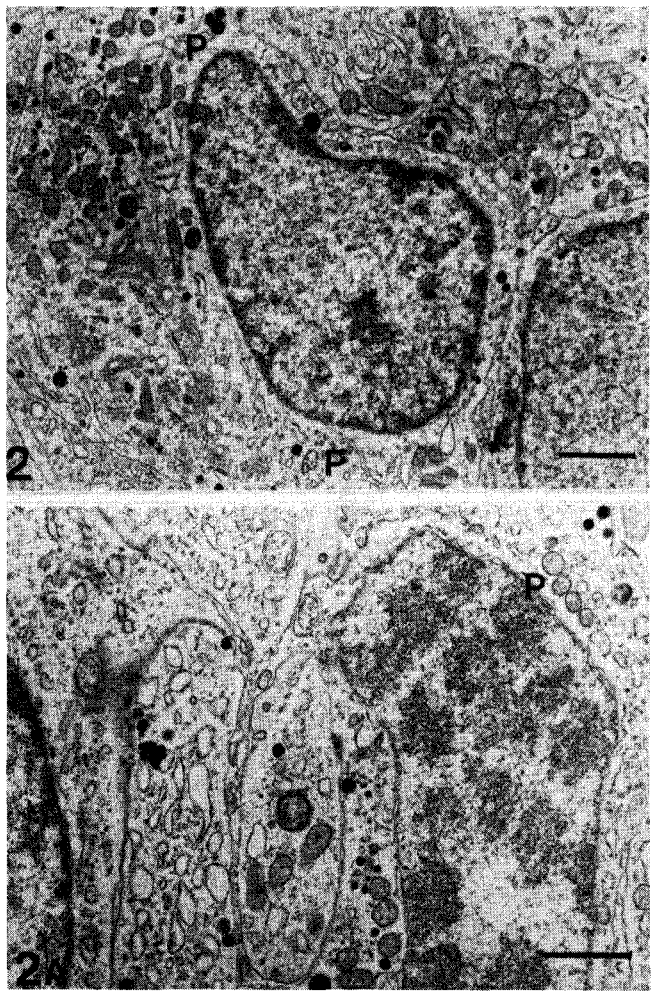

Figure 2. Electron microscopy of precursor cells of 3-day-old offspring of Dx-treated dams. A. Precursor cell in the process of mitosis. (Bar 1μm).

There were no differences in structure of ACTH-cells between 30-day-old offspring from Dx-treated dams and the corresponding controls (Fig. 2, A)

ACTH-cells of experimental dams, 3 days after delivery were almost without secretory granules but with hypertrophic nuclei. However, 30 days after delivery such alterations in the structure of these cells were not seen (Fig. 3A, B).

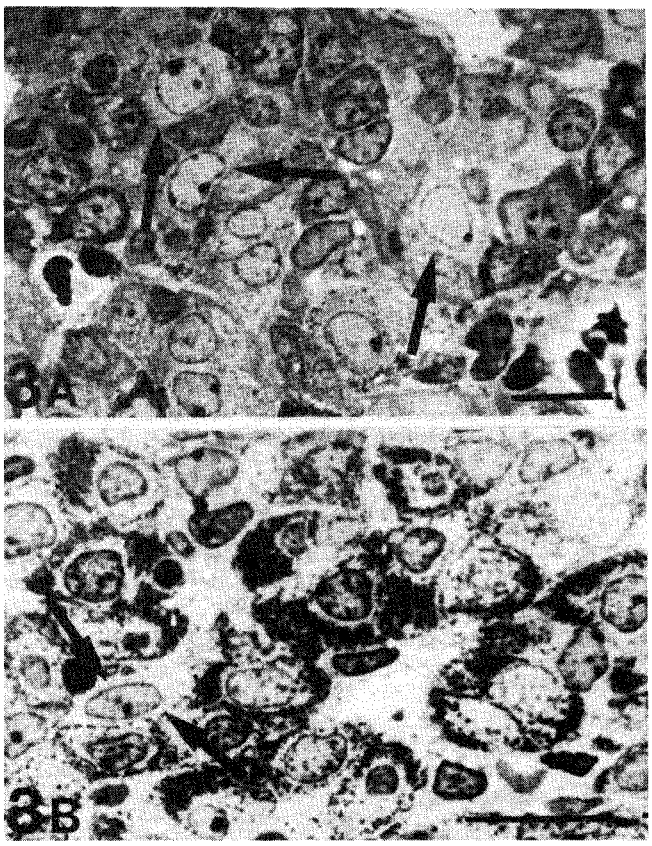

Figure 3. ACTH-cells of an experimental dam (semithin sections, stained with toluidine blue-azur II and methylene blue). A. 3 days after delivery with hypertrophied nuclei and with scarce secretory granules. B. ACTH-cell of experimental dam 30 days after delivery.

DISCUSSION

The results of this study demonstrate that a single dose of Dx, given to pregnant rats on day 16 of gestation, changed the synthetic activity of ACTH-cells in the anterior lobe of the pituitary gland of the dams and their offspring.

Our previous examinations showed that a single maternal Dx-treatment (1.5 mg/kg bw) led to a significant decrease in body and adrenal weight of the fetal and neonatal offspring, and also to a reduction of cortical cell number due to decreased cell proliferation (Hristić et al., 1997a, Manojlović et al., 1998, Kalafatić et al., 1996, 1998). Maternal or fetal stress stimulates placental secretion of CRH which might have stimulated the fetal HPA axis to secrete glucocorticoids. This increases the level of glucocorticoids in plasma and reduces average birth weight (Seckl, 1994). Concerning prenatal alterations in glucocorticoid level, the single dose of Dx given to pregnant rats in our experiments affected different

subpopulations of PVN neurosecretory cells indicating changes in the synthesis of several peptides and/or neurotransmitters. These results confirm and extend the data concerning the dependence of brain development on prenatal levels of glucocorticoids (Kalafatić et al., 1998, Hristić et al., 1997b). Clinical experience suggests that there are no teratogenic changes in children of mothers treated with pharmacological doses of prednisone during pregnancy, but low birth weight of babies may occur (Lockshin and Sammartano, 1998).

The results of this work show that plasma ACTH concentrations were approximately equal in fetuses and neonates of control females in all four tested periods. The hypothalamic CRH mRNA levels did not change significantly during the period from day 20 of fetal life to the day 15 of postnatal life (Emanuel et al., 1989). The fetal ACTH concentration in the experimental group was lower (14%), but the difference was not statistically significant in comparison with control values. However, plasma ACTH concentration was significantly increased in neonatal pups (3- and 14-day-old) of dams treated with Dx (40% and 45%, respectively). These results indicate that ACTH release, during the early neonatal period, is increased, i.e. stimulation of secretion of ACTH occurs after suppression in the fetal period. The differences in ACTH concentration disappeared in 30-day-old offspring.

In the experiment in which the dose of 1.5 mg/kg bw was divided into five consecutive doses of 0.3 mg/kg bw given from fetal days 16-20 i.e. in the period of development of the hypothalamo-pituitary-adrenal system, a significant reduction in ACTH concentration in the plasma of fetuses and neonatal offspring was found (Kalafatić et al., in press). This prolonged treatment with Dx caused changes in the hypothalamic paraventricular nuclei and adrenal gland that were of appreciably greater intensity than the changes induced by 1.5 mg Dx given as a single dose (Hristić et al., 1997a, 1997b). Dx-treatment decreased the level of CRH in the paraventricular nuclei (PVN) and median eminence of rats (Jessop et al., 1990). Dexamethasone treatment of pregnant rhesus monkeys during late gestation also evoked a marked decrease of fetal plasma cortisol levels (Challis et al., 1974, Walsh et al., 1979).

The present study also demonstrates that an increase of ACTH concentration in experimental neonatal pups is followed by markedly increased plasma cortisol concentrations in the early neonatal period compared with 30-day-old offspring (3-day-old vs. 30-day-old - 63%; 14-day-old Vs 30-day-old - 62%). Previous examinations showed that repeated maternal dexamethasone treatment caused the opposite change. Namely, a significant decrease of plasma cortisol concentration in fetuses and 3- and 14-day-old offspring was recorded in comparison with corresponding controls (Kalafatić et al., in press).

Electron microscopical analysis of ACTH- and precursor cells are in agreement with the results obtained for plasma ACTH concentration. An increased number of secretory granules in the Golgi apparatus and their localization near the plasma membrane in neonatal pups of experimental dams point to elevated pituitary ACTH secretion. Membrane binding sites for glucocorticoid have been found on pituitary cells (Koch et al., 1978, Sakly and Koch, 1981). Glucocorticoids are involved in the autoregulation of glucocorticoid receptors (Okret et al., 1986). It has also been suggested that glucocorticoid receptors which bind dexamethasone are localized in the rat brain (Meaney et al., 1985) and Dx-treatment induces changes in the level of tissue-specific glucocorticoid receptors mRNA in the brain of 14-day-old rats (Kalinyak et al., 1989).

Analysis of ACTH concentration in the plasma of control and experimental dams showed that the increase in ACTH 3 days after delivery was more expressed in Dx-treated dams than in corresponding controls. Thus, we can suppose that, as a result of Dx-treatment, ACTH is increased after suppression in late gestation. The ultrastructure of ACTH-cells in the same period, also confirms our hypothesis.

In nonpregnant mammals, ACTH is synthesized predominantly by anterior pituitary ACTH-cells and regulates glucocorticoid synthesis in the adrenal cortex. During pregnancy ACTH was found in both the fetal and maternal circulation (Carr et al., 1981). ACTH, which is detectable in the fetal circulation, may originate from fetal pituitary ACTH-cells or from the syncytiotrophoblast cells of the placenta (Chen et al., 1986, Al-Timini and Fox, 1986). ACTH from the fetal pituitary regulates primarily the development of human fetal adrenals (Mesiano and Jaffe, 1997). Maternal glucocorticoids cross the placental barrier in late pregnancy (Kittinger, 1974). The protective placental enzyme 11β-hydroxysteroid dehydrogenase type 2 (11βHSD2) regulates fetal exposure to maternal glucocorticoids, catalyzing rapid metabolism of cortisol and/or corticosterone to inert 11-keto forms. The synthetic steroid, dexamethasone, which is a poor sub/strate for 11β HSD2, readily crosses the feto-placntal barrier, and causes much greater effects on the fetal HPA system than natural glucocorticoids (Seckl, 1997).

Glucocorticoids, given to pregnant rats, suppressed the synthetic activity of fetal pituitary ACTH-cells, but in the early neonatal period this suppression was followed by stimulation of ACTH secretion.

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EFEKAT DEKSAMETAZONSKOG TRETMANA GRAVIDNIH ŽENKI PACOVA NA MATERNALNE, FETALNE I NEONATALNE ACTH-ĆELIJE

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SADRŽAJ

Adrenokortikotropni hormon (ACTH) se oslobađa kao odgovor na brojne stimuluse i utiče na rast, diferencijaciju i sintezu steroidnih hormona adrenalne žlezde. ACTH ćelije u hipofizi pacova diferenciraju se između 14. i 16. dana fetalnog života, dok sinteza glukokortikoidnih hormona nadbubrežne žlezde počinje 17. fetalnog dana kao rezultat povećane sinteze i oslobađanja ACTH. Cilj ovoga rada je bio da se ispita efekat deksametazonskog (Dx) tretmana gravidnih ženki na ACTH ćelije fetusa i potomaka tokom neonatalnog perioda. Paralelno je ispitivana i sintetska aktivnost ACTH ćelija majki. Eksperimentalna grupa gravidnih ženki tretirana je Dx (1.5 mg/kg/tt) 16. dana gestacije, dok je kontrolna grupa u istom periodu primila odgovarajuću količinu fiziološkog rastvora. Koncentracija ACTH u plazmi određena je RIA metodom. ACTH ćelije fetusa, neonatalnih pacova i gravidnih ženki ispitivane su svetlosnom i elektronskom mikroskopijom. Rezultati ovog istraživanja ukazuju da jednokratni Dx tretman gravidnih ženki inhibitorno utiče na diferencijaciju ACTH ćelija hipofize fetusa i oslobađanje ACTH. Međutim, u neonatalnom periodu broj prekursorskih ACTH ćelija i njihova proliferacija su povećani, kao i sinteza i oslobađanje ACTH i kortizola, tj nakon perioda inhibicije u fetalnom dobu dolazi do stimulacije u pogledu sinteze i sekrecija ACTH ćelija tokom neonatalnog perioda. Brojne promene opisane tokom fetalnog i neonatalnog perioda kod potomaka majki jednokratno tretiranih Dx 16. dana gestacije iščezavaju kod potomaka starih mesec dana.