Int Arch Occup Environ Health (2002) 75 (Suppl): S36–S44
DOI 10.1007/s00420-002-0351-3

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Assessment of steroid disruption using cultures of whole ovary and/or placenta in rat and in human placental tissue

Published online: 3 July 2002

Abstract ***Objectives***: The paper presents results of collaborative research on cadmium as an endocrine disruptor. To detect steroidogenic alterations in cycling and pregnant rats following cadmium exposures in vivo (at 3 or 5 mg/kg as a single s.c. dose) and in vitro (from 0 through 2,000 μM Cd^{2+}) whole-ovary culture was used. To evaluate steroid productions in rats fed low iron (10 ppm) and concomitantly exposed to cadmium (5 mg/kg total dose by s.c.-implanted osmotic pumps) during 19 days of pregnancy whole-placenta culture was also used. In human placental tissue cadmium and progesterone concentrations were assessed in relation to cigarette smoking. ***Methods***: Cultures of minced ovaries were evaluated for 1-h basal steroid production and following 1-h production stimulated with either human chorionic gonadotropin (hCG) or hCG and pregnenolone. Placental cultures were evaluated for average 1-h progesterone production following 3 h of unstimulated production. Steroid hormones were evaluated by specific radioimmunoassay. Placental cadmium concentrations were analyzed by atomic absorption spectrometry. ***Results***: In-vivo cadmium exposure interfered with normal steroidogenesis in cycling rats and in early pregnancy, with ovarian estradiol production the most affected. Under in-vitro cadmium exposure the most affected was ovarian production of progesterone and testosterone in cycling (proestrous) rats with medial inhibitory concentrations under 500 μM Cd^{2+}. Cadmium interfered with the steroidogenic pathway at more than one site. Linear and additive effects of low-iron feeding and concomitant cadmium exposure during pregnancy on placental progesterone production were found. In humans, we found that the placentas of smoking mothers contained twice as much cadmium and approximately half the amount of progesterone than did the placentas of non-smoking mothers. ***Conclusions***: Results of the research on cadmium-induced steroidogenic effects using cultures of whole rat ovary and/or placenta as well as human placental tissues point to cadmium as an endocrine disruptor that may compromise pregnancy outcome and fetal viability.

Keywords Cadmium · Endocrine disruptors · Placenta · Steroidogenesis · Whole-ovary culture

Work presented at the 29th Congress on Occupational and Environmental Health in the Chemical Industry (Medichem 2001), 3–6 September 2001, Prague, Czech Republic

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Introduction

Exposure to toxicants in both occupational and ambient environment may be associated with alterations in steroid hormone production. In the 1990s Laskey and Berman introduced whole-ovary culture as a rapid and convenient in-vitro assay for steroidogenic activity following in-vivo toxicant exposure (Berman et al. 1990, 1991; Laskey et al. 1991a, 1991b). Since then, various chemicals suspected of interfering with normal steroidogenesis in non-pregnant (cycling) and pregnant rats, such as dibutylphthalate, diethylhexyl phthalate, ethane dimethane sulfonate, epostane, methoxychlor, aminoglutethamide phosphate, and fungicide fenarimol, have been tested by Laskey, Berman and coworkers using whole-ovary culture (Berman et al. 1990, 1991; Laskey et al. 1991a, 1991b, 1995; Cummings and Laskey 1993; Berman and Laskey 1993; Laskey and Berman 1993).

Assessment of cadmium effects on steroidogenesis

The existing evidence in humans is not sufficient to consider female reproductive effects as critical effects of cadmium exposure (Clarkson et al. 1985; Kostial 1986; FAO and WHO 1989; WHO 1992; ATSDR 1993, Järup et al. 1998). However, further facts argue that cadmium is an element of concern as a potential reproductive toxicant in women. Cadmium is a ubiquitous toxic metal. Main sources of exposure in the general population are food (shellfish and high-fiber meals) and cigarette smoke. Smokers expose themselves to twice the cadmium concentration than do non-smokers. Cadmium is a cumulative toxicant that accumulates during the lifetime in the internal organs. It also accumulates in the tissues of the reproductive system including the placenta, more in women habitually eating a diet rich in cadmium (Järup et al. 1998; Moberg Wing et al. 1992) and/or in cigarette smokers (Varga et al. 1993; Pereg et al. 2001; Piasek et al. 2001). Women are at higher risk of cadmium intoxication then men because they have a greater cadmium body burden then men, mostly acquired during the generative period when they are prone to essential element (iron, calcium) deficiencies (ATSDR 1993; Järup et al. 1998). Experimental studies have shown that pregnant, lactating and/or nutritionally deficient animals absorb and retain more cadmium from the gastrointestinal tract (Järup et al. 1998; Kostial et al. 1991a, 1991b; Bhattacharyya et al. 2000).

Till the 1990s only limited literature data were available on the effects of cadmium exposure on ovarian steroid production. Perturbations in serum concentrations of steroid hormones due to cadmium exposure have been reported in a few studies on female rats (Laskey et al. 1980; Paksy et al. 1989, 1990a, 1990b) and in one study on hamsters (Saksena and Salmonsen 1983). At that time there were no data in the literature on the effects(s) of cadmium on steroid production and/or possible sites of action in the steroidogenic pathway either in the ovary or in the placenta using in-vitro assay.

We started our collaborative research in 1992/1993 to evaluate the effect(s) of cadmium exposure on selected reproductive parameters in sexually mature female rats, with special emphasis on steroidogenesis (Piasek et al. 1993). The scope of our investigation was to detect steroidogenic alterations after both in-vivo and in-vitro exposures to cadmium in cycling and pregnant animals and to identify the general site of altered steroidogenesis. We used cultured whole rat ovaries to assess ovarian steroidogenesis. Three specific reproductive stages were chosen for evaluation. The first stage was diestrus–proestrus, the time prior to ovulation, when maximum ovarian hormone production occurs. Gestation day 7 to 8 was chosen as the second stage, the time subsequent to implantation and with onset of increased ovarian progesterone production. The third stage chosen was gestation day 16 to 17, which is after organogenesis and during a second peak of ovarian progesterone production in pregnancy.

A further step in our investigation was to assess placental steroid production in rats after in-vivo cadmium exposure in cultured whole placentas near to term, on gestation day 19, after subchronic cadmium exposure and concomitant low-iron diet during pregnancy. And finally, concentrations of progesterone were assessed and correlated with cadmium concentrations in human placental tissue of healthy parturients exposed to cadmium (and other toxicants) in cigarette smoke vs. non-smoking parturients.

Material and methods

Animals

Mature female 60 to 90-day-old cycling and time-pregnant Sprague-Dawley rats (Charles River Laboratories, Raleigh, N.C., USA) were used in the experiments. The rats were maintained in the animal facility under standard indoor condition and at 12-h light/dark cycles. In all experiments the required principles of laboratory animal care and use according to the institutional research guidelines on the protection of animal welfare were followed.

To identify regular 4-day estrus cycles or sperm positivity, vaginal lavages and cervicovaginal smear cytological analyses by light microscopy were performed (Fox and Laird 1970).

Cadmium exposure

In-vivo exposure (acute exposure)

Cadmium ($CdCl_2$, Fisher Scientific) was administered subcutaneously at doses of 0, 3 or 5 mg/kg body weight (bw) to cycling rats (on day of diestrus), in early pregnancy (on day 7), or late pregnancy (on day 16), 24 h prior to ovarian steroid hormone assay.

Exposure in vitro

Cadmium ($CdCl_2$, Fisher Scientific) was added at concentrations of 0, 100, 500, 1,000, 1,500, and 2,000 μM Cd^{2+} to the culture medium with minced whole ovaries of cycling rats (in proestrus) or pregnant rats in early (gestation day 6) or late pregnancy (gestation day 16).

Subchronic exposure during pregnancy

Cadmium ($CdCl_2$, Fisher Scientific) was administered continuously from gestation day 1 through 19 by subcutaneously implanted osmotic mini-pumps (MP2ML4 Alzet, Alza, Palo Alto, Calif., USA). In this experiment, rats were concomitantly fed on test diets (Teklad) with either high iron (240 ppm) or low iron (10 ppm) content.

Subjects

Healthy parturients (urban women, $n = 56$, median age 28 years, 1st or 2nd gestation) with normal pregnancies and deliveries at term, whose placentas were included in the study, were interviewed after delivery by a physician. The results were recorded in the pre-designated questionnaire form (Piasek et al. 2001). The data from the medical history files were also used. Informed consent was obtained from every participant. The investigation was approved by the ethical committees of the Institute for Medical Research and Occupational Health in Zagreb and the Maternity Hospital of the University of Zagreb Clinic.

Culture conditions and sampling

Whole-ovary culture

The method comprises several steps (Fig. 1). In a 1.5-ml polystyrene vial is placed 1 ml of culture medium (containing M-199, supplemented with $NaHCO_3$, *N*′-hydroxyethyl piperazine-*N*′-2-ethanesulfonic acid, bovine serum albumin, and soy-bean trypsin inhibitor, diluted to 1 l) and whole ovary. Each ovary is minced with scissors in 2–3 mm particles. Cultures are incubated at 34 °C and 5% CO_2 for 1 h while being slowly shaken. The vials are then centrifuged (at 1,500 rpm, ~200 *g* for 3 min), and the supernatant is decanted and used for specific radioimmunoassay (RIA) of steroid hormones.

Supernatants were collected from each culture after three consecutive hours. The 1st incubation hour was basal (unstimulated), the 2nd hour was stimulated with human chorionic gonadotropin (hCG), and the 3rd hour was stimulated with both hCG and substrate pregnenolone. The culture of whole rat ovary proved to be better for steroid hormone assay than serum-hormone assays for characterizing steroid production in the normal ovary, as well as for confidently detecting alteration(s) and identifying the general site(s) of altered steroidogenesis (Berman et al. 1990, 1991; Laskey et al. 1991a, 1991b, 1995; Berman and Laskey 1993; Laskey and Berman 1993).

Whole-placenta culture

Maternal and fetal portions of placentas were placed separately in 1.5-ml vials in culture medium in proceeding similar to those for cultured whole ovaries. Placental samples were first "washed" with medium (cultures were incubated for 1 h, the vials were spun and the supernatant decanted), and then tissue particles were re-suspended in fresh media, incubated, centrifuged (at 1,500 rpm, ~200 *g* for 3 min), and supernatants decanted two more times, for two consecutive hours. Average 1-h (unstimulated) placental production of progesterone in maternal and fetal placental portions was evaluated by specific RIA. In this paper the values of average total placental progesterone productions are presented.

Sampling of human placental tissue

Placentas were collected at the Maternity Hospital in Zagreb and kept deep-frozen at –20 °C until required for analysis. Three pairs of representative samples – one for metal and one for hormone assessment – from each partially thawed placenta (excluding chorionic plate and decidua basalis) were taken. One pair of samples was taken from a section from the center, avoiding the region with umbilical cord insertion, and two pairs of samples were taken from the sections between the central region and periphery, within a minimum 3 cm of outer placental margin.

Samples (cubes of 2–3 g wet tissue weight) for metal assessment were prepared following recommendations for metal analysis in human placentas by atomic absorption spectrometry (Miller et al. 1988). For steroid extraction from placental tissue, an original method was applied. Samples (cubes of ca. 1 g wet tissue weight) were homogenized and lyophilized after steroid extraction with ethanol (Piasek et al. 2001). This method enables assessment of steroid disruption in both fresh and stored material. Values determined by this procedure fall within the range reported in the literature for progesterone in human term placenta (Wilson et al. 1984; Pasqualini and Kincl 1985; Kalenga et al. 1991).

Steroid hormone assay

Medium samples from cultured rat ovaries were assayed for progesterone, testosterone, and estradiol and from rat placentas for progesterone. Samples of placental tissue lyophilizates reconstituted in deionized water were assayed for progesterone. For all these analyses specific radioimmunoassay (RIA Coat-A-Count kits TKP25, TKTT5 and/or TKE25, Diagnostic Products, Los Angeles, Calif., USA) was used as described earlier (Berman and Laskey 1993; Laskey and Berman 1993; Laskey et al. 1995; Piasek and Laskey 1994, 1999; Piasek et al. 2001).

Statistical analyses

Data were analyzed by both one-way and two-way analyses of variance (ANOVA by PROC GLM) available in the 1985 edition of Statistical Analysis System (SAS 1985). When necessary, data were analyzed after being logarithmically transformed to normalize distribution and eliminate heterogeneity of variance. Where the overall analysis of variance was significant ($P < 0.01$), the least-square means (LSMEANS) option of the PROC GLM was used to make two-tailed *t*-test comparisons (at level of significance $P < 0.05$). Linear trends and correlations were tested post-hoc occasionally.

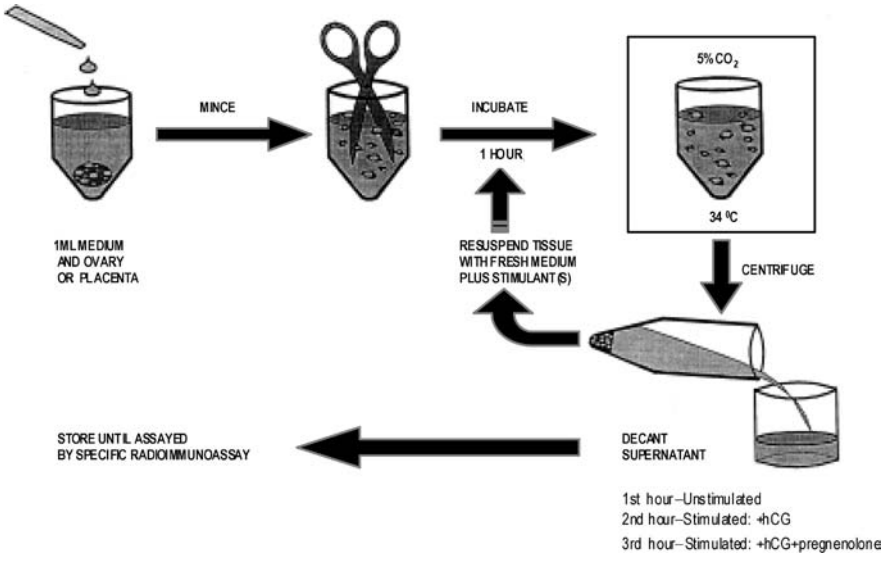

Fig. 1. Culture of whole rat ovary or placenta

Results

Effects of acute cadmium exposure in vivo in different reproductive stages

Perturbations in whole-ovary steroid productions (expressed in nanograms per ovary per hour) after acute cadmium exposures are presented in Figs. 2 and 3.

Whole-ovary progesterone production in proestrous rats decreased at 5 mg/kg cadmium dose in basal, unstimulated culture and in hCG-stimulated culture. With hCG and pregnenolone stimulation, progesterone production increased and did not differ between control and cadmium-exposed animals (Fig. 2). No consistent pattern in the cadmium effects on progesterone production in ovaries of pregnant rats was observed. On gestation day 8, at 5 mg/kg cadmium dose, progesterone production was significantly reduced only in cultures stimulated with hCG and pregnenolone. On gestation day 17, in the 5-mg/kg cadmium-exposed rats, unstimulated progesterone production was higher, and hCG-stimulated production was lower than in controls.

Figure 3 shows that whole-ovary estradiol production in proestrous rats decreased at 5 mg/kg cadmium dose in unstimulated and in both hCG- and hCG plus pregnenolone-stimulated cultures. On gestation day 8, unstimulated and hCG-stimulated estradiol production decreased in both 3 and 5-mg/kg cadmium-exposed rats. On gestation day 17, whole-ovary estradiol production was not affected by cadmium exposure.

Similar reductions as for whole-ovary estradiol production were found in testosterone production of unstimulated and both hCG- and hCG plus pregnenolone-stimulated cultures of proestrous and gestation-day-8 rats at both cadmium doses (Piasek and Laskey 1994). Serum estradiol concentrations were also significantly lower in proestrous and on gestation day 8 at both cadmium doses, and serum progesterone was not affected at all.

Effects of cadmium exposure in vitro in different reproductive stages in rats

For each ovarian steroid hormone, the effective cadmium concentration for a medial inhibitory effect, i.e., 50% reduction in steroid production (Cd-EC_{50}) was calculated using a single exponential equation:

$$\text{Steroid production} = B_0 \times e^{(B_1 \times [Cd])}$$

where B_0 is the steroid production with no cadmium addition and B_1 is the slope of the dose-response. Evaluations for perturbations in ovarian steroidogenesis were made on progesterone, testosterone and estradiol individually and presented in Fig. 4 as Cd-EC_{50} values for each hormone.

Figure 4 shows that in-vitro cadmium exposure exerted perturbations in ovarian steroidogenesis, with progesterone and testosterone most affected primarily in proestrous rats. During the 1st hour (in unstimulated culture), ovaries from cadmium-exposed pregnant rats on both gestation days 6 and 16 were less affected for progesterone production, with Cd-EC_{50} approximately 2,400 μM compared with 630 μM in proestrous rats.

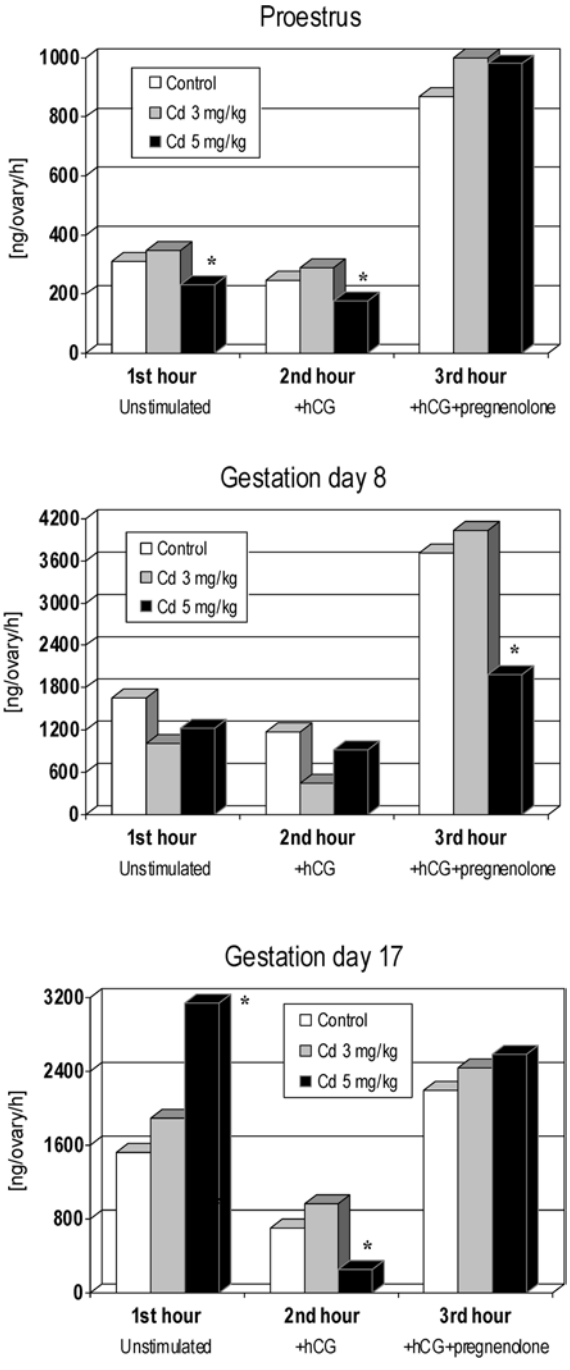

Fig. 2. Progesterone production (ng/ovary/h) in proestrus, gestation day 8 and gestation day 17 in rats 24 h after a single s.c. dose of cadmium (as $CdCl_2$; 0, 3 or 5 mg/kg bw) by whole-ovary cultures: *1st hour* unstimulated (basal); *2nd hour* stimulated with hCG; *3rd hour* stimulated with hCG plus pregnenolone *Significant difference from respective control value at $P < 0.05$ (n = minimum 5 per group)

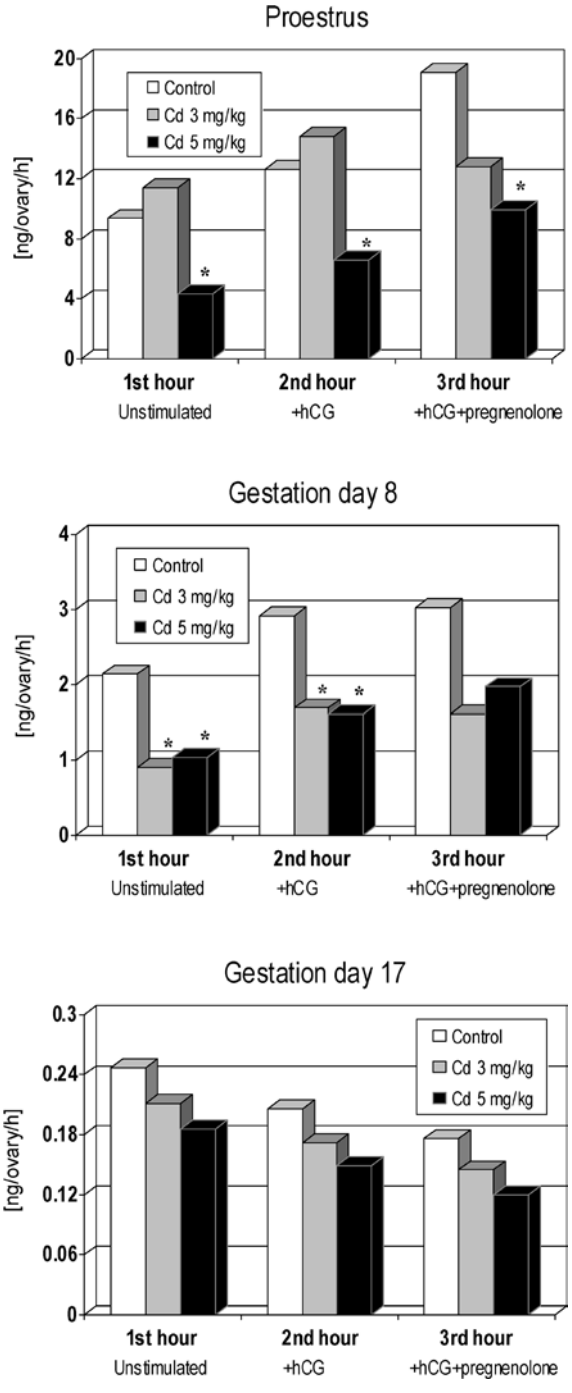

Fig. 3. Estradiol production (ng/ovary/h) in proestrus, gestation day 8 and gestation day 17 in rats 24 h after a single s.c. dose of cadmium (as $CdCl_2$; 0, 3 or 5 mg/kg bw) by whole-ovary cultures: *1st hour* unstimulated (basal); *2nd hour* stimulated with hCG; *3rd hour* stimulated with hCG plus pregnenolone, *Significant difference from respective control value at $P < 0.05$ (n = minimum 5 per group)

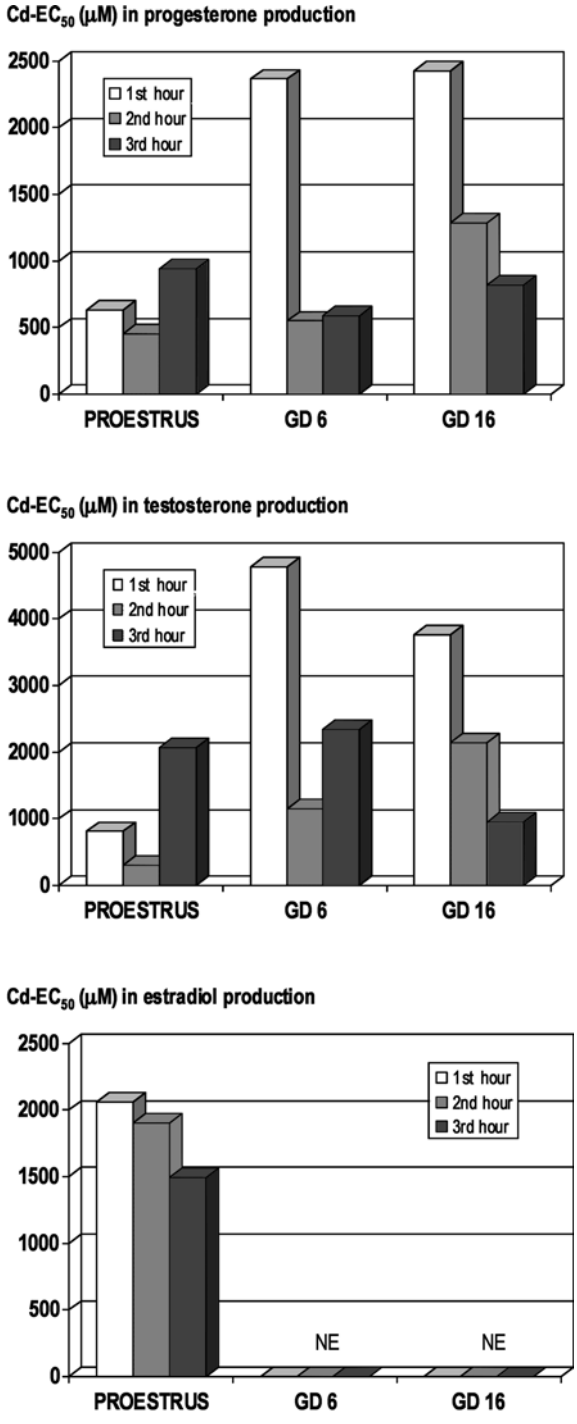

Fig. 4. Production of progesterone, testosterone and estradiol by cultured ovaries from proestrous and pregnant rats on gestation day (*GD*) 6 and GD 16 (n = minimum 6 per group): *1st hour* unstimulated (basal); *2nd hour* stimulated with hCG; *3rd hour* stimulated with hCG plus pregnenolone. Effects of in-vitro cadmium exposure are expressed as Cd-EC_{50}, determined from 0 (control) through 2,000 μM Cd^{2+}. *NE* no effective cadmium concentration calculated

This inhibition in proestrous ovaries was overcome (mitigated) in the 3rd hour with hCG plus pregnenolone stimulation to a Cd-EC_{50} value above 900 μM. In cultured ovaries from the rats on gestation day 6, Cd-EC_{50} with hCG or hCG plus pregnenolone stimulation was similar, approximately 550 μM. In the hCG-stimulated ovaries from the rats on gestation day 16, Cd-EC_{50} was approximately 1,300 μM, while with hCG plus

pregnenolone stimulation it was slightly reduced to around 800 μM.

Similarly as for progesterone production, in the proestrous rats, Cd-EC_{50} for unstimulated testosterone production was lower (4–5 times) than for ovaries of pregnant rats. In both the 1st hour (unstimulated) culture and the 2nd hour (hCG-stimulated) culture, Cd-EC_{50} was under 500 μM. The low Cd-EC_{50} value in proestrous rats was overcome by hCG and pregnenolone stimulation, with Cd-EC_{50} increasing to above 2,000 μM. At the same time, in both stimulated cultured ovaries from pregnant rats, Cd-EC_{50} values dropped in the 2nd and 3rd hours to 1,000–2,000 μM.

In cultured ovaries from proestrous rats, all Cd-EC_{50} values of estradiol were between 1,500 and 2,000 μM. Ovaries from pregnant rats produced only small amounts of estradiol, and no effect of cadmium exposure on estradiol production was found (Fig. 4, marked as "NE").

Effects of subchronic cadmium exposure and concomitant low-iron diet during pregnancy

Total placental progesterone production near term, on gestation day 19, decreased in the group at 5 mg/kg cadmium dose and fed on low (10 ppm) iron diet, compared with the control group. There was a cadmium main effect and the effect was linear (Fig. 5). A low-iron diet and concomitant cadmium exposure had an additive effect on reduction of progesterone production.

Concentrations of placental cadmium and progesterone in relation to cigarette smoking

Based on the self-reported data on potential sources of metal exposure (occupational and environmental sources, dietary history, smoking habits – active and passive smoke exposure), the parturients were divided into two groups according to different smoking habits: 29 non-smokers and 27 smokers. "Non-smokers" comprised persons who "never smoked", "did not smoke during pregnancy" and "former smokers" who stopped smoking more than 12 months before the onset of the last pregnancy. "Smokers" comprised "smoked during pregnancy" and "former smokers" who stopped smoking at most 12 moths before the onset of the last pregnancy.

Figure 6 shows that in average placental tissue, cadmium concentration was twice as high in smokers than in non-smokers. Placental tissue progesterone was significantly lower in smokers, with the average value almost half the value of that in non-smokers.

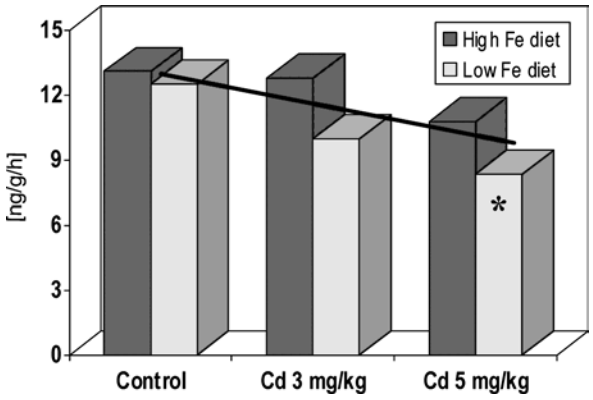

Fig. 5. Average placental progesterone production (ng/g/h) from rats on gestation day (GD) 19 after exposure to cadmium (as $CdCl_2$; by s.c.-implanted osmotic mini-pumps in total dose 0, 3 or 5 mg/kg bw) from GD 1 through GD 19 and fed on high-iron (*Fe*) (240 ppm) or low-Fe (10 ppm) test diet *Significant difference from respective control value at $P < 0.05$ (n = minimum 7 per group). Line denotes significant linear trend of main effect of cadmium exposure

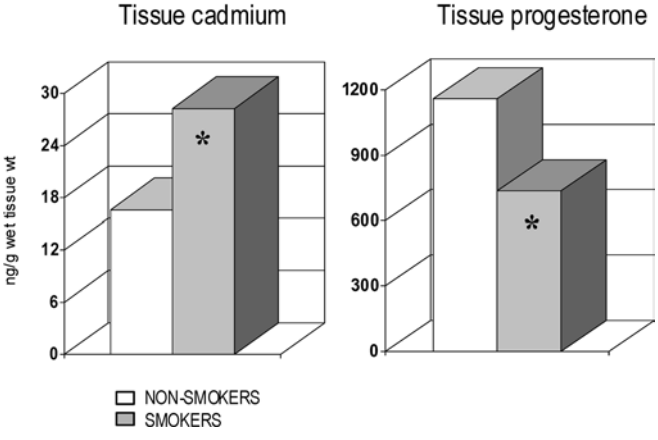

Fig. 6. Tissue concentrations (ng/g wet tissue weight) of cadmium and progesterone in human placentas in relation to cigarette smoking. *Significant differences in smokers ($n = 27$) vs. non-smokers ($n = 29$) at $P < 0.05$

Discussion

The use of cultures of whole ovary and whole placenta to detect changes in steroid production brought about by in-vivo or in-vitro exposure to cadmium in our investigation has been shown in both cycling (non-pregnant) and pregnant rats. The in-vitro assessment provided the means to identify possible site(s) of cadmium toxic action in the steroidogenic pathway. Detailed examination of the production of progesterone, testosterone and estradiol in ovaries from proestrous rats in previous investigations has shown that hCG maintains active steroidogenesis following the 1st hour in culture, which was linear through 3 h (Berman and Laskey 1993; Laskey and Berman 1993; Laskey et al. 1995). Steroid production in the 1st hour has been identified as a combination of the steroid present in the ovarian or placental tissue at killing and the steroid produced during the period of incubation.

The actual mechanisms of the effect(s) of cadmium ions on the female reproductive system have still not

been clarified. Studies in rodents have shown that cadmium-induced gonadal alterations are highly dependent on species, strain, age, gender and physiological status. The general pattern is of reversible microvascular damage, with hemorrhages, follicular degeneration and necroses in steroid-sensitive reproductive organs (ovary, testis, placenta, uterus), these being a target for acute parenteral cadmium exposure (Kar et al. 1959; Pařízek 1983; Levin et al. 1987; Rehm and Waalkes 1988; Laskey and Phelps 1991).

The hormonal environment modulates responsiveness to acute cadmium exposure in both genders. Adult male rats and mice develop overt testicular lesions at cadmium doses between 0.7 and 1 mg/kg bw, while testes of immature rats are not affected (Gunn et al. 1965; Phelps and Laskey 1989). Laskey and Phelps (1991) have shown that in adult rats a dose as low as 0.18 mg/kg bw caused depression of both steroid and sperm production without apparent morphological changes. However, in most strains of adult, sexually mature female rats, the parenteral administration of cadmium salts at 1 mg/kg cadmium dose does not result in ovarian damage and/or sterility. This cadmium dose causes hemorrhages and necroses in anovulating and immature rats (with the exception of mature F344 and WF rats) and in mature female hamsters and mice (Saksena and Salmonsen 1983; Kar et al. 1959; Pařízek 1983; Levin et al. 1987; Rehm and Waalkes 1988). Our investigation corroborates these findings. We found that cadmium doses of 3 and 5 mg/kg were associated with perturbation in ovarian and placental steroidogenesis with no concurrent morphological changes in ovaries of mature rats (Piasek and Laskey 1994). By both in-vivo and in-vitro cadmium exposures, most severe cadmium effects were found in proestrus and in early pregnancy (gestation days 6 to 8). In late pregnancy (gestation days 16 or 17), ovarian steroidogenesis was relatively unaffected.

It is possible that inhibited steroid hormone production is the result of cadmium effects on the hypothalamus and/or pituitary. Paksy et al. (1989) and Varga and Paksy (1991) have reported effects of cadmium (5 and 7.5 mg/kg bw) on the rat hypothalamus. The result was anovulation that was preventable with stimulation by a luteinizing hormone releasing hormone (LHRH), and an effect on the pituitary resulting in reduced concentrations of follicle stimulating hormone (FSH) and luteinizing hormone (LH) in the serum. Some support to this theory was the complete lack of cadmium-related effects on adrenal progesterone production found in our study on acute cadmium effect on rat steroidogenesis (Piasek and Laskey 1994).

Actual cadmium concentrations in rat ovaries following a single s.c. administration of 3 and 5 mg/kg bw were 1.2 and 1.6 μg/g wet tissue wt, respectively (Piasek and Laskey 1994). Under in-vitro cadmium exposure of rat ovaries to substantially higher concentrations of cadmium ions (100 to 2,000 μM Cd^{2+}), the most affected were production of progesterone and testosterone in proestrous rats and less in pregnant, whereas estradiol was not affected at all. These results agree with the findings of Paksy et al. (1992, 1996, 1997) on in-vitro cadmium-exposed rat and/or human ovarian cells. Paksy et al. (1997) reported that the lowest cadmium concentration able to reduce progesterone production in cultured human granulosa cells was 16 μM (1.8 μg/l), that is ca. 3.5 times greater than concentrations reported in the ovaries of smokers (up to 0.5 μg/g wet tissue weight).

By subchronic cadmium exposure during 19 days of pregnancy at a total dose of 5 mg/kg bw in rats, placental progesterone production was significantly reduced, and average placental cadmium was (mean ± SEM) 1.18 ± 0.109 μg/g wet tissue weight (Piasek et al. 2000). Our finding in human placentas on maternal smoking-related reduction of placental progesterone content could not be causally related to cadmium exposure. Average cadmium concentrations in human placentas were ca. 50 times lower than the above values in rat placentas. Placental cadmium (mean ± SEM) in smoking vs. non-smoking parturients was 0.029 ± 0.002 vs. 0.016 ± 0.001 μg/g wet tissue weight (Piasek et al. 2001). Our investigation supports the established association of smoking and placental cadmium, and showed that mothers who smoke up to 20 cigarettes a day during pregnancy or had stopped fewer than 12 months before the last pregnancy, accumulate twice as much cadmium in their placentas than do non-smokers. Animal data indicate that cadmium, as a cigarette-smoke constituent, can contribute to cigarette smoking-related perturbation in placental progesterone that can compromise pregnancy outcome.

The in-vitro assessment can identify possible site(s) of toxic action in steroidogenic pathway. Theoretically, the absence of functional cytochrome P450 cholesterol side-chain cleavage enzyme would prevent ovarian/testicular, placental or fetal adrenal steroidogenesis. The hypothesis has been proven in vitro by Laskey and Phelps (1991) on isolated Leydig cells from male rats. It was found that cadmium ions inhibited testosterone production at the site(s) prior to cholesterol side-chain cleavage. Cadmium ions also stimulated testosterone production at the site(s) between cholesterol side-chain cleavage and the 3-beta-hydroxysteroid dehydrogenase/isomerase conversion of pregnenolone to progesterone.

In our investigation, the effects on progesterone and testosterone production, for the most part, were moderated by the provision of pregnenolone to the culture medium, while estradiol production was unaffected. Such results suggest that cadmium acts at site(s) prior to cholesterol side-chain cleavage. The depression of testosterone and estradiol production by cadmium exposure indicates that there are other site(s) affected in the pathway between progesterone and aromatase-conversion of testosterone or androstenedione to estrogens.

The results obtained in human placentas so far, showing that the placenta is a site for toxic action of cadmium, include alterations in hCG production in vitro

(Wier et al. 1990) and imbalance between thromboxane A_2 and prostacyclin, a disturbance seen in late gestation in pre-eclampsia (Eisenmann and Miller 1995). Recently Jolibois et al. (1999a, 1999b) have shown dose- and time-dependent bioaccumulation of cadmium by purified term human trophoblast cells in primary culture associated with a concomitant significant inhibition of progesterone (up to 35% of control values). Assessment of the potential role of cadmium as a transcriptional regulator has shown a significant dose-dependent reduction in the abundance of low-density lipoprotein (LDL) receptor mRNA in trophoblast cells in vitro (Jolibois et al. (1999a, 1999b). The finding suggests that receptor concentrations may be depressed via transcriptional regulation potentially interfering with the uptake of the requisite substrate (LDL-cholesterol) necessary for placental progesterone production. Other mechanisms by which cadmium may exert its effects on progesterone synthesis and release in human placenta is by competing with other calcium-dependent pathways in trophoblast cells (Lin et al. 1997), altered intracellular trafficking of cholesterol into mitochondria, activities of the mitochondrial P450 side-chain cleavage enzyme, cytoplasmic 3-beta-hydroxysteroid dehydrogenase enzymes, and/or DNA transcription by cadmium interference with the zinc-finger motif.

In conclusion, our investigation using cultures of whole rat ovary and whole rat placenta showed perturbation in steroidogenesis after cadmium exposures in vivo and/or in vitro. Cadmium exposure and concomitant dietary iron deficiency in rats during pregnancy had additive and linear effects on placental steroidogenesis. Placentas of expectant mothers who smoked cigarettes contained twice as much cadmium and approximately half the amount of progesterone than did the placentas of non-smokers. Our results, together with other authors' data from the studies on laboratory animals and human ovaries and placentas lead to the following conclusion. Cadmium, as a cigarette smoke constituent and ubiquitous (mainly food) contaminant, could be considered to be an endocrine disruptor or a contributing factor for disruption of ovarian and placental steroidogenesis that can compromise pregnancy outcome and fetal viability in humans.

Acknowledgements The experimental part of the study was conducted in the Reproductive Toxicology Branch, Developmental Toxicology Division of, at that time, Health Effects Research Laboratory (HERL) in the United States Environmental Protection Agency, Research Triangle Park, N.C. during the fellowships of Martina Piasek, sponsored by the International Research and Exchanges Board Inc. (IREX) Washington, D.C., USA and NIH Fogarty International Center, Bethesda, Md. Our special thanks are due to the late Dr. Ezra Berman for his consultation and help during experiments, Ms Janet M. Ferrell for her skilled technical assistance and Dr. Ralph L. Cooper in providing human placental progesterone analyses. The authors are grateful for the technical help of Ms Đurđa Breški and Ms Marija Ciganović in metal analyses. The research on human placentas has been financially supported by The Ministry of Science and Technology of the Republic of Croatia grant No. 00220102.

References

ATSDR (1993) Toxicological profile for cadmium. Agency for Toxic Substances and Disease Registry Update TP-92/06. US Department of Health and Human Services, Public Health Service, Atlanta, Ga

Berman E, Laskey JW (1993) Altered steroidogenesis in whole-ovary and adrenal culture in cycling rat. Reprod Toxicol 7:349–358

Berman E, Laskey JW, Carter H, Ferrell J (1990) Whole ovary culture as an in vitro assessment of in vivo toxicant exposure affecting ovarian steroidogenesis. Toxicologist 10:210

Berman E, Laskey JW, Ferrell J, Carter H (1991) Steroid profiles from whole-ovary culture following in vivo chemical exposure in cycling rats. Biol Reprod 44 [Suppl 1]:134

Bhattacharyya MH, Wilson AK, Rajan SS, Jonah M (2000) Biochemical pathways in cadmium toxicity. In: Zalups RK, Koropatnick J (eds) Molecular biology and toxicology of metals. Taylor & Francis, London New York, pp 34–74

Clarkson TW, Nordberg GF, Sager PR (1985) Reproductive and developmental toxicity of metals. Scand J Work Environ Health 11:145–154

Cummings AM, Laskey JW (1993) Effect of methoxychlor on ovarian steroidogenesis: role in early pregnancy loss. Reprod Toxicol 7:17–23

Eisenmann CJ, Miller RK (1995) Cadmium and glutathione – effect on human placental thromboxane and prostacyclin production. Reprod Toxicol 9:41–48

FAO and WHO Working Groups (1989) Cadmium. WHO food additives series 24:163–219

Fox RR, Laird CW (1970) Sexual cycles. In: Hafez ESE (ed) Reproduction and breeding techniques for laboratory animals. Lea and Febinger, Philadelphia, Pa, pp 116–117

Gunn SA, Gould TC, Anderson WAD (1965) Protective effect of estrogen against vascular damage to the testis caused by cadmium. Proc Soc Exp Biol Med 119:901–905

Järup L, Berglund M, Elinder CG, Nordberg G, Vahter M (1998) Health effects of cadmium exposure – a review of the literature and a risk estimate. Scand J Work Environ Health 24 [Suppl 1]:1–52

Jolibois LS Jr, Shi W, George WJ, Henson MC, Anderson M (1999a) Cadmium accumulation and effects on progesterone release by cultured human trophoblast cells. Reprod Toxicol 13:215–221

Jolibois LS Jr, Burrow ME, Swan KF, George WJ, Anderson M, Henson MC (1999b) Effects of cadmium on cell viability, trophoblastic development, and expression of low density lipoprotein receptor transcripts in cultured human placental cells. Reprod Toxicol 13:473–480

Kalenga MK, De Hertogh R, Whitebread S, Vankrieken L, Thomas K, De Gasparo M (1991) Distribution of the concentrations of angiotensin-II, angiotensin-II receptors, hPL, prolactin, and steroids in human placenta, amnion and chorion (in French). Rev Fr Gynecol Obstet 86:585–591

Kar AB, Das RP, Karkun JN (1959) Ovarian changes in prepuberal rats after treatment with cadmium chloride. Acta Biol Med Ger 3:372–399

Kostial K (1986) Cadmium. In: Mertz W (ed) Trace elements in human and animal nutrition, vol. 2. Academic Press, New York, pp 319–345

Kostial K, Blanuša M, Kargačin B, Piasek M, Maljković T, Kello D (1991a) Sex-related differences in genetic susceptibility to toxic metals. In: Grandjean EC (ed) Ecogenetics: genetic predisposition to the toxic effects of chemicals. WHO, Regional Office for Europe, Copenhagen, Denmark, Chapman & Hall, London, pp 111–122

Kostial K, Blanuša M, Maljković T, Kargačin T, Piasek M, Momčilović B, Kello D (1991b) Age and sex influence the metabolism and toxicity of metals. In: Momčilović B (ed) Trace elements in man and animals 7. IMI, University of Zagreb, Croatia, pp 11.1–11.5

Laskey JW, Phelps PV (1991) Effect of cadmium and other metal cations on in vitro Leydig cell testosterone production. Toxicol Appl Pharmacol 108:296–306

Laskey JW, Berman E (1993) Steroidogenic assessment using ovary culture in cycling rats: effects of bis(2-diethylhexyl) phthalate on ovarian steroid production. Reprod Toxicol 7:25–33

Laskey JW, Rehnberg GL, Favor MJ, Cahill DF, Pietrzak-Flis Z (1980) Chronic ingestion of cadmium and/or tritium. II. Effects on growth, development, and reproductive function. Environ Res 22:466–475

Laskey JW, Berman E, Carter H, Ferrell J (1991a) Identification of toxicant induced alterations in steroid profiles using whole ovary culture. Toxicologist 11:111

Laskey JW, Berman E, Gray LE Jr, Ferrell J, Carter H (1991b) Steroid profiles from whole-ovary culture following in vivo chemical exposure in pregnant rats. Biol Reprod 44 [Suppl 1]:132

Laskey JW, Berman E, Ferrell JM (1995) The use of cultured ovarian fragments to assess toxicant alterations in steroidogenesis in the Sprague-Dawley rat. Reprod Toxicol 9:131–141

Levin AA, Kilpper RW, Miller RK (1987) Fetal toxicity of cadmium chloride: the pharmacokinetics in the pregnant Wistar rat. Teratology 36:163–170

Lin FJ, Fitzpatrick JW, Ianotti CA, Martin DS, Mariani BD, Tuan RS (1997) Effects of cadmium transport on trophoblast calcium transport. Placenta 18:341–356

Miller RK, Mattison DR, Plowchalk D (1988) Biological monitoring of the human placenta. In: Clarkson TW, Nordberg GF, Sager PR (eds) Biological monitoring of toxic metals. Plenum Press, New York, pp 567–602

Moberg Wing A, Wing K, Tholin K, Sjöström R, Sandström B, Hallmans G (1992) The relation of the accumulation of cadmium in human placenta to the intake of high-fibre grains and maternal iron status. Eur J Clin Nutr 46:585–595

Paksy K, Varga B, Horváth E, Tátrai E, Ungváry G (1989) Acute effects of cadmium on preovulatory serum FSH, LH, and prolactin levels and on ovulation and ovarian hormone secretion in estrous rats. Reprod Toxicol 3:241–347

Paksy K, Náray M, Varga B, Kiss I, Folly G, Ungváry G (1990a) Uptake and distribution of Cd in the ovaries, adrenals, and pituitary in pseudopregnant rats: effect of Cd on progesterone serum levels. Environ Res 51:83–90

Paksy K, Varga B, Folly G (1990b) Long-term effects of a single cadmium chloride injection on the ovulation, ovarian progesterone and estradiol-17β secretion in rats. Acta Physiol Hung 76:245–252

Paksy K, Varga B, Lázár P (1992) Cadmium interferes with steroid biosynthesis in rat granulosa cells in vitro. Biometals 5:245–250

Paksy K, Varga B, Lázár P (1996) Zinc protection against cadmium-induced infertility in female rats. Effect of zinc and cadmium on the progesterone production of cultured granulosa cells. Biometals 10:27–35

Paksy K, Rajczy K, Forgáscs Z Lázár P, Bernard A, Gati I, Kaali GS (1997) Effect of cadmium on morphology and steroidogenesis of cultured human ovarian granulosa cells. J Appl Toxicol 17:321–327

Pařízek J (1983) Cadmium and reproduction: a perspective after 25 years. In: Clarkson TW, Nordberg GF, Sager PR (eds) Reproductive and developmental toxicity of metals. Plenum Press, New York, pp 301-313

Pasqualini JR, Kincl FA (1985) Hormones and the fetus. Volume I: Production, concentration and metabolism during pregnancy. Pergamon Press, Oxford

Pereg D, Lagueux J, Dewailly É, Poirier GG, Ayotte P (2001) Cigarette smoking during pregnancy: comparison of biomarkers for inclusion in epidemiological studies. Biomarkers 6:161–173

Phelps PV, Laskey JW (1989) Comparison of age-related changes in in vivo and in vitro measures of testicular steroidogenesis after acute cadmium exposure in Sprague-Dawley rats. J Toxicol Environ Health 27:95–105

Piasek M, Laskey JW (1994) Acute cadmium exposure and ovarian steroidogenesis in cycling and pregnant rats. Reprod Toxicol 8:495–507

Piasek M, Laskey JW (1999) Effects of in vitro cadmium exposure on ovarian steroidogenesis in rats. J App Toxicol 19:211–217

Piasek M, Laskey JW, Berman E (1993) Effects of cadmium on steroid production in female rats. Second International Meeting on Molecular Mechanisms of Metal Toxicity and Carcinogenicity, Madonna di Campiglio, Italy. Abstracts, p 97

Piasek M, Kostial K, Laskey JW, Ferrell JM (2000) Low iron diet and cadmium exposure disrupt steroidogenesis in the rat. In: Roussel AM, Anderson RA, Favier AE (eds) Trace elements in man and animals 10. Kluwer Academic/Plenum Publishers, New York, pp 809–812

Piasek M, Blanuša M, Kostial K, Laskey JW (2001) Placental cadmium and progesterone concentrations in cigarette smokers. Reprod Toxicol 15:673–681

Rehm S, Waalkes MP (1988) Cadmium-induced ovarian toxicity in hamsters, mice, and rats. Fundam Appl Toxicol 10:635–647

Saksena SK, Salmonsen R (1983) Effects of cadmium chloride on ovulation and on induction of sterility in the female golden hamster. Biol Reprod 29:249–256

SAS (1985) User's guide: statistics. Statistical Analysis System, SAS Institute, Cary, N.C.

Varga B, Paksy K (1991) Toxic effects of cadmium on LHRH-induced LH release and ovulation in rats. Reprod Toxicol 5:199–203

Varga B, Zsolnai B, Paksy K, Náray M, Ungváry G (1993) Age dependent accumulation of cadmium in the human ovary. Reprod Toxicol 7:225–228

Wier PJ, Miller RK, Maulik D, di Sant'Agnese PA (1990) Toxicity of cadmium in the perfused human placenta. Toxicol Appl Pharmacol 105:156–171

WHO (1992) Cadmium. The environmental health criteria no. 134. International Programme on Chemical Safety, World Health Organization, Geneva

Wilson EA, Jawad MJ, Powell DE (1984) Effect of estradiol and progesterone on human chorionic gonadotropin secretion in vitro. Am J Obstet Gynecol 149:143–148