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IN UTERO AND LACTATIONAL EXPOSURE TO DIOXIN ALTERED THE REPRODUCTIVE FUNCTION IN FEMALE RAT OFFSPRING BEFORE AND AFTER PUBERTY

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ABSTRACT:

To evaluate effects of in *utero* and lactational 2,3,7,8-tetrachlorodibenzo-rho-dioxin (TCDD) exposure on the reproductive function in female rat offspring, before and after puberty. The pregnant Sprague Dawely rat administered 0, or 1.0 mg TCDD/kg on Gestation Day (GD) 8 and 15. Female offspring were examined at the post-weanling before puberty on posnatal day (PND) 21 and in young adult stage of development on PND42. Ovulation assessment, radioimmunoassay for serum gonadotropins, steroids and histo-morphmetric analysis to the ovaries were evaluated. The analysis included a count, measurement and classification of preantral and antral follicles throughout the entire ovary on PND 21. The results indicate that TCDD treatment significantly reduced the ovulation rate, serum gonadotropins, steroids levels and the number of antral and preantral follicles of certain size classes. The histopathological examination revealed small preovulatory follicles displaying an atretic morphologic difference among the ovaries of rats exposed to TCDD treatments. These data support the hypothesis that TCDD results in adverse effects on female reproductive function. However, the age of animals before or after puberty play an important role in the difference between results. Moreover, TCDD exposure on the GD 8 or 15 has a great concern in the results observed.

***Keywords*: Dioxin; Ovary; LH; FSH; Estrogen; Ovarian Follicles.**

INTRODUCTION:

2,3,7,8-Tetrachlorodibenzo p-dioxin (TCDD) is a persistent environmental contaminant inadvertently produced as a by-product of herbicide and pesticide manufacturing (Kulkarni et al., 2008; Hites, 2011). TCDD is also released during the bleaching process at tree pulp and paper mills, and during the burning of municipal solid waste (Frakes et al., 1993; Tuppurainen et al., 2003). TCDD is the most toxic member of a class of chemicals called dioxins. Dioxins, like TCDD, have a long environmental half-life, bioaccumulate in the food chain, and can be found in human fat tissue, blood serum, breast milk and ovarian follicular fluid Schecter et al., 2006; Tsutsumi et al., 2011; (Humblet et al., 2011; Ulaszewska et al., 2011). The resistance of TCDD to metabolism, as well as its lipophilic chemical nature, contributes to bioaccumulation in animals and humans, as well as biomagnification within the ecosystem. Its half-life can be more than 10 years in humans (Aylward et al., 2005). Human TCDD exposure at low levels com Monly occurs through the consumption of contaminated meats and dairy products (Travis and Hattemer-Frey, 1991). Toxic effects of TCDD include teratogenesis, immune impairment, carcinogenesis, and developmental and reproductive dysfunction (Pohjanvirta et al., 1994).

The dioxin/aryl hydrocarbon receptor (AhR) mediates most, if not all, effects of dioxins. TCDD, the most well characterized ligand of AhR, has for a long time been regarded as having antiestrogenic properties such as inhibition of 17b-estradiol (E2)-induced uterine weight increase and decreased levels of estrogen and progesterone receptors in uterus of rats and mice (Safe, 1995). In Ah-responsive MCF-7 human breast cancer cells, TCDD inhibits 17b-estradiol-induced cell proliferation and decreases the mRNA levels of Cathepsin D (Romkes et al., 1987; Gierthy et al., 1988). One of the suggested mechanisms of inhibitory AhR-estrogen receptor (ER) crosstalk involves direct interaction of the AhR

complex with functional DREs within the regulatory part of estrogen responsive genes, such as Cathepsin D (Safe, 1995). Interestingly, ligand activated AhR was more recently shown to be a member of an ubiquitin ligase complex promoting proteosomal degradation of ERa (Ohtake et al., 2007). On the contrary, estrogenic effects of TCDD have also been reported (Abdelrahim et al., 2003; Watanabe et al., 2004).

There is an increasing evidence of TCDD's endocrine disrupting effects on immature female reproductive system. In the hypothalamus, TCDD has been shown to decrease the responsiveness to estradiol (Gao et al., 2001). A direct stimulatory effect of TCDD on pituitary gonadotropin secretion in immature female rats was demonstrated by Li et al. (1997). In utero and lactational exposure to TCDD has been shown to disrupt estrus cycles and inhibit ovulation rate (Salisbury and Marcinkiewicz, 2002). Furthermore, maternal exposure to TCDD has potency to directly disturb ovarian steroidogenesis of female progeny (Chaffin et al., 1997) and TCDD has been shown to alter the ;activity and expression of several ovarian steroidogenic enzymes either directly or indirectly (Dasmahapatra et al., 2000; Gregoraszczuk et al., 2001; Myllym¨aki et al., 2005). Reproductive organs and endocrine system are sensitive to TCDD during development and sexual maturation Mably et al., 1992) (Gray and Ostby, 1995; Compared to placental transfer, exposure via lactation plays a major role in offspring receiving maternal PCDD/Fs and dioxin-like PCBs (Chen et al., 2001). Female Sprague–Dawley rats appear to be more sensitive to dioxin-like PCBs than males (Chu et al., 1995). It has been proposed, that the increased susceptibility of females to TCDD toxicity may be due to the sex-specific kinetics of TCDD (Petroff et al., 2000). The sensitivity to TCDD has been demonstrated to vary with the alterations in ovarian hormonal status (Petroff et al., 2001), and estrogens have been shown to modulate the effects exerted by TCDD (Petroff et al., 2000; Wyde et al., 2001).

TCDD exposure causes compromised ovarian function through alteration of follicle development,

alteration of steroidogenesis and inhibition of ovulation (Petroff et al., 2001b). To date, the majority of studies conducted with the purpose of understanding the effect of in *utero* and lactation TCDD exposure on ovarian development and function have been conducted using animals before or after puberty. Additionally, the results reported vary widely and sometimes are conflicting depending on animal model, dose of TCDD, length of exposure, and age of the animals being used. Thus, in this study, we set out to determine the effect of in *utero* and lactation single oral dose of TCDD on ovarian development and function in female rat offspring before and after puberty. Moreover, we objected to test whether the perinatal exposure to TCDD on GD8 or on GD 15 can develop different response.

Material and methods:

Chemical:

2,3,7,8-teterachlorodibenzo-p-dioxin(TCDD)solution(99%purity)was purchased from Grey Hound company (England).TCDD was dissolved in a vehicle (corn oil: acetone; 19: 1 [v:v].

Animals:

Nine pregnant, eight- week-old, female Sprague Dawely rats 200- 250g were purchased from laboratory animal's house, Assiut University, Egypt. The day following overnight mating with the evidence of the vaginal plug is designated as day zero of pregnancy GD 0; animals were housed individually in clean plastic cages with constant temperature (22 ± I °C), humidity (55 + 5%), and light schedule (12 L: 12 D). The animals were provided with commercial food (Rat Chow) and water ad libitum. All rats were handled in accordance with the standard guide for the care and use of laboratory animals.

Experimental design:

The pregnant females were divided into three groups, 3 dams each, the first group received a single oral dose of 1.0 µg TCDD in vehicle/kg of maternal body mass by gavage on gestation day eight (GD8). The second group received the same previous dose of TCDD on gestation day fifteen (GD15). The third group was served as a control and received a dose of vehicle

(corn oil: acetone; 19: 1 [v: v] 2 mL/kg).

The dose used in the present study was based on extensive published work by previous studies involving in *utero* and lactational exposure of rats to TCDD Mably et al., 1992). (Chen et al., 2001; Gregoraszczuk et al., 2001; GD 8 was chosen because it is an important time in fetal ovarian development and major fetal organogenesis. During this time, primordial germ cells migrate to the genital ridge and begin to colonize the indifferent gonad (Hirshfield, 1991). GD 15 was used because TCDD exposure at this time has been shown to induce a number of unusual reproductive alterations, as delayed vaginal opening and vaginal threads (Gray and Ostby, 1995). At birth, postnatal day (PND) 0, live offspring were counted and sexed. On PND 1, litters were adjusted to sex females for each dam to allow for a similar nutritional environment and lactational exposure to TCDD.

Nine female offspring from each group were terminated on PND 21, before puberty, for ovarian histology. Ovaries were analyzed on PND 21 because rat primordial follicles enter the growing pool during this time period (Hirshfield, 1991). PND 21 was chosen because it is prior to rat vaginal opening, and thus provides a time to determine whether abnormalities occur before puberty. Another nine offspring from each group were terminated on PND42 for measurement of serum estradiol, progesterone, FSH and LH. PND 42 was chosen because it is just after normal puberty and vaginal opening in the rat (Flaws et al., 1997). Moreover, postnatal days 25 and 42 were chosen in order to follow the pups' exposure through lactation. All female offspring were visually inspected and their body weight was recorded immediately before termination.

Sample collections:

on postnatal days 21 and 42, individual body weight was recorded and 54 female pups, 18 from each treated group and 18 from the control group were anesthetized with CO, and decapitated. Two pups were randomly picked from each litter. Trunk blood was collected after decapitation and allowed to clot at 4°C. Sera were

collected and stored at -80°C until determination of hormones by radioimmunoassay. Ovaries were carefully removed from the body cavity, cleaned of adhering tissue and weighed. Ovaries from the control group and from the treated groups were prepared for the histological analyses.

Ovarian histology and microscopy:

Ovaries were collected upon sacrifice, weighed, and fixed in Bouin's solution. Fixed tissues were embedded in paraffin, sectioned at 6 Mm and stained with hematoxylin and eosin for examination of ovarian morphology by light microscopy.

Morphmetric analysis of the ovarian follicles:

Follicles were analyzed in a double-blind paradigm. The analysis included a count classification and measurement of area (μm^2) of preantral and antral follicles serially throughout the entire ovary at PND 25. The greatest cross sectional area of each follicle was calculated using the equation for an ellipse, rr (A) (B), in which A and B are one-half the greatest length and width of the section containing the germinal vesicle (Heimler et al.,1998).

Ovulation assessment:

Oviducts were dissected and ova flushed from the oviduct using normal saline containing 0.1% hyaluronidase according to Li et al. (1995). The number of ova/rat was counted and the ovulation rate was calculated (%) at PND42.

Radioimmunoassay:

Serum Gonadotropins

Serum concentrations of lutinzing hormone (LH) and follicular stimulating hormone (FSH) were determined by radioimmunoassay (Taya et al., 1981).

Serum Steroid

Serum concentrations of progesterone (P_4) and estradiol (E_2) were measured as previously described by Terranova and Garza (1983).

Statistical analysis:

Data are expressed as means ± SD. Statistical analysis was performed to compare treated groups with respective control groups using one-way analysis of variance (ANOVA), followed by the Duncan's multiple range test when appropriate. Values of

$P < 0.05$ were considered statistically significant.

Results:

Body weight:

TCDD treatment significantly retarded the increase in body weight seen in the control animals in this experiment. There is no significant difference between GD 8 and GD15 on PND 21 or 42 (Table 1).

Ovarian weights:

A significant reduction in the ovarian weight was obtained in TCDD treated group in comparison with the control animals. There was not a significant difference between GD 8 and GD15 on PND 21 or 42 (Table 1).

Table 1: Effects of in utero and lactational exposure to TCDD (1.0 µg/kg) or vehicle control on the body and ovarian weights in female rat offspring.

	Body Weight (g)	Ovarian Weight (mg/rat)
Control PND21 PND42	36.2 ± 8.9 65.5 ±3.5┼	43.96±2.3 65.8±3.7┼
GD8 PND21 PND42	28.7±5.4* 51.4±4.7*┼	38.16±1.3* 53.50±1.6*┼
GD15 PND21 PND42	29.3±4.3* 50.7±4.0*┼	39.2 ±1.5* 50.5±1.7*┼

***Significantly different from controls ($P < 0.05$)**
┼ Significantly different between the PND21 and PND42 ($P < 0.05$).

Serum gonadotrophins:

The serum LH and FSH levels in immature female rats were found to be higher at 21 day of age than later at day 42 in control and exposed rats.

TCDD reduced the LH and FSH surges significantly reduced (by 75–80%) than the control in GD8 and 15 on PND21 and 42. There was not a

significant difference between GD 8 and GD15 on PND 21 or 42 (Table 2).

Serum steroid:

Serum progesterone

ANOVA confirmed a significant inhibitory effect of TCDD on concentrations of serum P_4. This inhibition was obtained in female rat offspring on PND 42 in both GD8 and 15. Moreover, there was a significant difference in P_4 concentrations between female rat offspring sacrificed on PND 21 and 42. The inhibitory effect was more obvious on PND 42 than PND21 (Table 2).

*Serum 17*b-*estradiol concentrations*

In female rat offspring treated with TCDD, serum E_2 concentrations were significantly reduced when compared with control in GD 8 and 15 on PND 21 and 42. Moreover, there was significant decrease in estrogen level in GD8 on PND21and 42 than in GD 15 on PND 21 and 42. In addition, there was a significant difference in E_2 levels between female rat offspring sacrificed on PND 21 and 42 (Table 2).

Table 2: Effects of in utero and lactational exposure to TCDD (1.0 µg/kg) or vehicle control on the serum gonadotrophine and steroids in female rats offspring on PND25and PND42.

	FSH IU/L	LH IU/L	E_2 pg/mL	P_4 ng\ml
Control				
PND21	0.66±0.03	0.59±0.02	25.5±0.4	8.80±0.12
PND42	0.43±0.04┼	0.49±0.04┼	55.5 ±0.2┼	19.89±0.12┼
GD8				
PND21	0.45±0.04*	0.49±0.02*	21.5 ±0.2*b	6.30+0.10*
PND42	0.29 ±0.02*┼	0.44±0.04*┼	39.2±0.2*d┼	14.30+0.10*d┼
GD15				
PND21	0.47±0.04*	0.46±0.02*	26.2±0.7a	7.30+0.10
PND42	0.30 ±0.02*┼	0.40±0.04*┼	47.4±0.2c┼	16.30+0.10*c┼

*Significantly different from controls (P < 0.05)
Different letters indicate significance between the GD8 and GD15 (P < 0.05)
┼ Significantly different between the PND21 and PND42 (P < 0.05)

Morphmetric analysis of the ovarian follicles:

Classification, count and measurement of the ovarian follicles

As observed from morphmetric analysis of the ovarian sections of TCDD(versus vehicle-treated controls) reduced significantly the number of antral follicles less than 75,000 μm^2 and greater than 100,000 μm^2 and preantral follicles less than 50,000 μm^2 in female rat offspring in GD 8 and 15 on PND21. There was a significant difference between GD 8 and 15 in the number of antral and prantral follicles less than 50,000, 50,000-75,000, greater than 100,000 μm^2 and less than 50,000, respectively (Table 3).

Ovulation assessment:

There was a significant inhibition of ovulation by TCDD, hence it alter the number of ova shed in comparison with control group. This effect on the ovulation and the decrease in the ova number is more obvious in GD8 than GD 15 on PND 42 (Table 4).

Table 3: Ovarian follicle number in various size categories: effects of in-*utero* and lactational exposure to TCDD or vehicle control on PND21.

	Control		TCDD	
Area (μm^2)	GD8	GD15	GD8	GD15
Antral <50,000	364	377	216**a*	299**b*
50~75,000	177	165	115 **a*	140**b*
75 ~100,000	70	68	48*	54*
> 100,000	85	82	75**a*	55**b*
Preantral <50,000	2100	2110	1760**a*	1880**b*
50-75,000	19	20	20	25
>75,000	4	6	2*	4*

***Significantly different from controls (P < 0.05)**
Different letters indicate significance between the GD8 and GD15 at (P < 0.05)

Table 4: Effects of in utero and lactational exposure to TCDD on ovulation in female rat offspring on PND 42.

	Ovulation (ova/rat)	Ovulation rate (%)
Control PND 42	8.3 ± 1.3	100 (18\18)
GD8 PND 42	2.2 ±2.0'**a*	33 (6\18)**a*
GD 15 PND 42	6.2±1.7**b*	70 (12\18)**b*

***Significantly different from controls (P < 0.05)**
Different letters indicate significance between the GD8 and GD15 (P < 0.05).

Ovarian morphology:

There were marked morphologic differences among the ovaries of different treatments regarding macroscopic and microscopic appearance. Treatment with TCDD (1.0 µg/kg) on PND 21 of GD8 resulted in an aberrant morphology consisting of small ovaries (approximately 1.5 to 2.5 mm) bearing numerous small preovulatory follicles displaying an atretic morphology (i.e., pyknotic nuclei, scattered granulosa) at the ovarian periphery in the rat (Fig. 1a,b). On PND 42 of GD8, TCDD exposure was associated with smaller ovaries (approximately 2.3-3 mm) bearing fewer corpora lutea (2 to 4) and a noticeable cohort (15 to 20) of small preovulatory follicles in rats. There were some degenerated ovum accompanied with an atresia in the secondary follicles (Fig. 2. a). There were few, if any, corpora lutea in these ovaries and the hilar region appeared edematous (Fig. 2. b). The morphological alteration of the ovary was more obvious in GD8 on PND 21 and 42 than in GD 15 on PND 21and 42. Moreover, TCDD exposure induced pronounced atretic effect in the ovaries on PND 42 in both GD 8 and 15; it was more obvious on PND42 than on PND21.

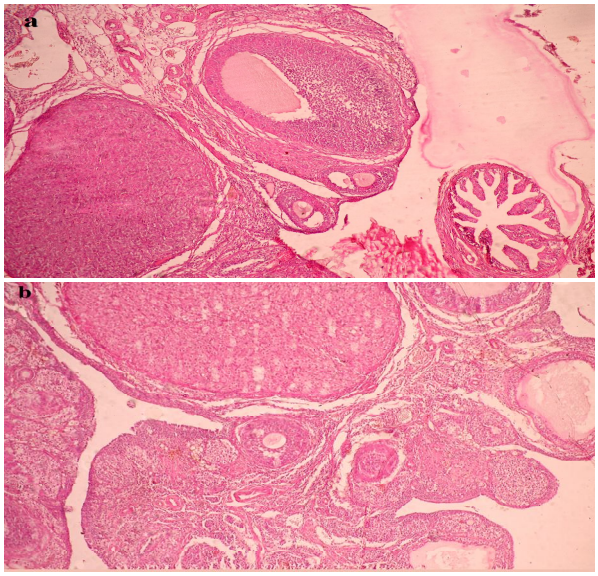

Fig.1: Ovaries from TCDD- exposed female rat offspring on PND21 of GD8 showed a) Atresia in the secondary and vesicular follicles along with congestion of the ovarian blood vessels X25. b) Numerous small preovulatory follicles displaying an atretic morphology (i.e., pyknotic nuclei, scattered granulosa) at the ovarian periphery X 25.

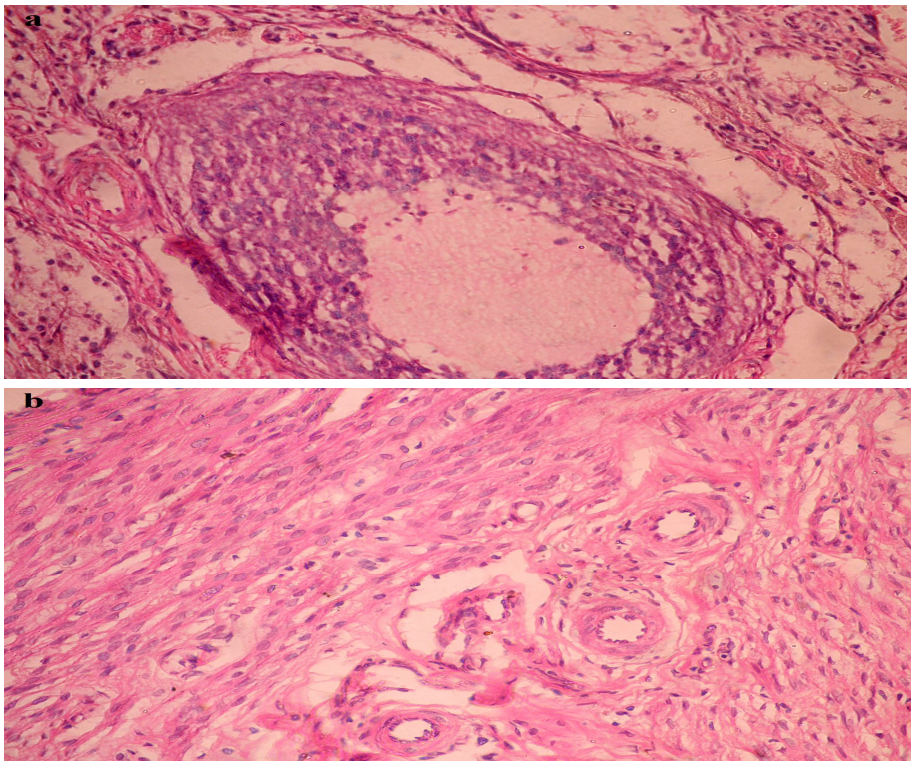

Fig.2: Ovaries from TCDD- exposed female rat offspring on PND 42 of GD8 showed a) Degeneration in the ovum accompanied with an atresia in the secondary follicle X 40. b) An increase in the thickness of the ovarian epithelium. There are few corpora lutea in these ovaries and the hilar region appeared edematous X 40.

DISCUSSION:

Much attention has been paid to the effects of dioxin in offspring exposed in- *utero* and lactationally, since the dose level required eliciting these effects is very low (Yonemoto, 2000). TCDD exposures have been linked to delayed puberty and early onset of menopause in women (Eskenazi et al., 2005; Warner et al., 2007). Similarly, TCDD exposures lead to early puberty, irregular estrous cycles, reduced or blocked ovulation, decreased circulating E2 levels, and early reproductive senescence in female rodents Gray and Ostby, 1995; Li et al., 1995; (Chaffin et al., 1996; Myllymäki et al., 2005; Franczak et al., 2006; Shi et al., 2007) Jablonska et al., 2010;.

In the present manuscript, an in- *utero* and lactational TCDD exposure at a dose of 1.0 µg/ kg resulted in over toxicity in female rat's offspring as shown by the decreased rate of body weight gain during the experimental period. And also our results of perinatal TCDD exposure suggest that TCDD reduced ovarian weights vs. controls on PND21 and 42. Exposure to PCDDs was associated with significantly lower ovarian weights vs. controls in adult Sprague-Dawley rats exposed acutely just prior to PMSG-induced follicular development (Gao et al., 1999a; Son et al., 1999).

The results of the present study are in accordance with previous studies on dioxin-exposed rodents in that an activated AhR (here CA-AhR) leads to antiestrogenic effects in the presence of estrogen, but to estrogenic effects in the absence of estrogen. (Brunnberg et al., 2006). This finding is consistent with the hypothesis that the activated AhR may play an estrogen like role in the absence of estrogen (Ohtake et al., 2003; Boverhof et al., 2006). Activation of the aryl hydrocarbon receptor (AhR) by the highly toxic, prototypical ligand, TCDD or other dioxin-like compounds compromises ovarian function by altering follicle maturation and steroid synthesis (Tischkau et al ., 2011). In both an earlier and the present study, we had shown that an in-*utero* and lactational exposure of

female rats to TCDD results in a significant reduction in serum estrogen concentrations on PND 21 (Chaffin et al., 1996). Where they observed tissues specific regulation of estrogen receptors (ER) and decreased circulating estrogen levels in female offspring of Holtzman rats given 1 µg/kg of TCDD on GD15. In contrast, Gray et al. (1997) stated that in female rats exposed to TCDD perinatally, no effects on estrus cyclicity, ovary function or serum estradiol concentrations were observed. When the pregnant Long Evans rats exposed to TCDD on GD8, female offspring had enhanced incidence of constant estrus cycle and reduced fertility from second to fourth litter when monitored by continuous breeding. This decrease in estrogen levels resulted in altered ER mRNA levels. Proposed mechanisms for the antiestrogenic action(s) of TCDD include oxidative metabolism of estrogens via products of the AhR gene battery, inhibition of expression and: or efficacy of estrogen receptors and suppression of estradiol-induced gene expression (Safe, 1995). Moreover, the decrease in estrogen levels may be attributed to a significant reduction in certain classes of ovarian follicles which considered the major source of estrogen (the antral follicles in particular) (Heimler et al., 1998). This explanation is agreed with our findings of the decrease in the number of antral and preantral follicles of certain size-classes. MacLuskyet al. (1998) suggested that the mechanism that underlies the alteration of reproductive function in offspring exposed perinatally to TCDD may be due to incomplete sexual differentiation of the CNS. Such alteration is probably not mediated by blocking the estrogen response, but may instead involve subtle developmental changes in other parts of the endocrine system, perhaps also affecting the feedback control of the adenocortical function. More recent studies confirmed this criterion; Effects of TCDD on ovarian function are likely mediated indirectly through the hypothalamic–pituitary axis, as well as directly at the level of the ovary Gao et al., 2001; Mizuyachi et al., 2002; Petroff et al., 2001a, 2003). Miller et al., 2004; Hernandez-Ochoa et al., 2009; (Cao et al., 2011; The herein

presented results are also compatible with the assumption that TCDD alters reproductive function by a direct action at the level of the ovary as well as an indirect action via the hypothalamic-pituitary axis. Our results showed a marked reduction in antral follicles less than 75,000 μm^2 and greater than 100,000 μm^2, and preantral follicles less than 50,000 μm^2, following TCDD exposure. TCDD causes a slowing of the movement of follicles from the preantral stage that are greater than 50,000 μm^2 into the antral stage, and from small primordial or primary follicles to preantral follicles smaller than 50,000 μm^2 (Heimler et al.,1998). TCDD attenuated follicular maturation as well, reducing the number of antral and preantral follicles without inducing apoptotic cell death in rats exposed to TCDD in *utero* and during lactation (Heimler et al., 1998). In this regard, antral follicles are the main functional unit of the ovary, housing the gametes and serving as the primary source of sex steroid hormones, such as E_2. Follicles grow from the primordial to the antral stage in a process called folliculogenesis. In the process of folliculogenesis, follicles increase in size mainly due to proliferation of granulose cells. It has been proposed that TCDD decreases circulating E_2 levels in part by inhibiting the growth of follicles from the preantral to antral stage and by disrupting the synthesis and metabolism of sex steroid hormones Heimler et al., 1998; (Dasmahapatra et al., 2000; Gregoraszczuk et al., 2001; Grochowalski et al., 2001; Morán et al., 2000, 2003a, 2003b; Myllymäki et al., 2005; Pesonen et al., 2006). In the ovary, E_2 synthesis requires both the thecal and granulosa cell compartments. In the rodent, the thecal cells are responsible for producing P_4 and androstenedione (A_4). A_4 then diffuses to nearby granulosa cells where it is converted to testosterone (T) and E_2. In antral follicles, the rate limiting steps in steroid hormone production are the mobilization of cholesterol from the cytoplasm to the inner mitochondrial membrane via steroidogenic acute regulatory protein (StAR) and the conversion of cholesterol to pregnenolone by the mitochondrial enzyme cytochrome P_{450}, family 11, subfamily a, polypeptide 1

(P_{450}scc).Pregnenolone is then converted to P_4, and then by a multistep process to the androgens, and finally to E_2 (Edson et al., 2009).

Moreover, our results showed a significant inhibition of ovulation by TCDD exposure, hence it alter the number of ova shed in comparison with control group. This an ovulatory effect of PCDDs is seen in hypophysectomized Sprague-Dawley rats induced to ovulate with exogenous gonadotropins (Gao et al., 1999a; Petroff et al., 2000) and direct application of TCDD to the ovary blocks ovulation as well (Petroff et al., 2000). Thus PCDDs have direct effects on the ovulatory follicle that are sufficient to block ovulation.

The significant decrease in the serum level of gonadotropin was observed in female offspring in the present study, after perinatal TCDD exposure suggest that TCDD act indirectly on the ovary through the hypothalamic–pituitary axis. Previous studies indicated that the patterns of preovulatory LH and FSH secretion were altered in female rats receiving TCDD (Li et al., 1995; Gao et al., 1999b). TCDD blocked the LH and FSH surges (Gao et al., 1999b). Chaffin et al. (1997) documented that a significant reduction of pituitary FSH mRNA was observed. Despite the reduced E_2 levels, neither serum FSH nor LH concentration increased significantly. Results of perinatal TCDD exposure suggest that it does not act on serum gonadotropin. Reduced serum E_2 levels appear to result from direct or indirect action on the ovary at some point following androstenedione production. In contrast, previous studies suggested that TCDD augmented the feedback action of steroids on the hypothalamic-pituitary axis (Bookstaff, 1990). Ovulation was reduced when TCDD was administered on the morning of estrus, which is right after the surge in FSH inducing the next cohort of follicles to ovulate in the next proestrus-estrus (Smith et al., 1975). This suggests that TCDD had a direct effect on the ovary in preventing ovulation. On the other hand the extension of diestrus indicates that gonadotropin release was insuffi-

cient to produce estrogen for subsequent cycles (Li et al., 1995).

In the present study, in- *utero* and lactational TCDD exposure alters the morphological appearance of ovaries of female offspring on PND21 and 42. The TCDD exposure resulted in an aberrant morphology consisting of small ovaries bearing numerous small preovulatory follicles displaying an atretic morphology, pyknotic nuclei and scattered granulose at the ovarian periphery. There were few corpora lutea in these ovaries and the hilar region appeared edematous on PND 42. Diminution in the number of antral and preantral follicles of certain size-classes was observed on PND 21. These ovarian effects of PCDDs are evident histologically as smaller ovaries with fewer corpora lutea and a cohort of unruptured antral follicles still containing their oocytes (Gao et al., 1999a,b; Petroff et al., 2000). While the combined direct and indirect effects of PCDDs on ovarian steroidogenesis appear somewhat equivocal, PCDD treatment clearly induces aberrations in periovulatory proteolysis and follicular tissue remodeling as evidenced by the failure of ovulation seen following acute exposure to these compounds (Gao et al., 1999a,b;Petroff et al., 2000). The mechanisms of this interruption of degradation of the follicular wall are as yet unknown and may involve modulation of steroid action or direct alteration of gene transcription by the PCDDs.

The results of the present study are in accordance with Gray et al. (1997) in that TCDD treatment on GD 8 has more toxic effect on the ovulation, ovary foliculogenisis, and ovary morphology and steroids levels in female offspring than on GD 15. Administration of 1 mg TCDD/kg on GD 15 of pregnancy induces a high incidence of external genitalia malformations in Holtzman female rat progeny. Treatment with TCDD earlier in gestation on GD 8 is generally less effective in inducing malformations of the genitalia of the female offspring, but it does induce functional reproductive alterations (Gray and Ostby, 1995).

The examination of the ovarian follicles on PND 21 (before puberty)

or assessment of the ovulation rat by counting the fully mature shed ova on PND 42 (after puberty) give the same indication of in-utero and lactional exposure to TCDD induce inhibition in the ovulation rate in both times related to TCDD effect on floculogenesis. The results of serum steroid hormones (E_2 and P_4) and serum gonadotrophen (LH and FSH) revealed a significant decrease in both times than control. Alterations in the morphological appearance of ovaries of female offspring on PND 21 and 42 were observed. In comparison between the effects of TCDD exposure in female rat offspring on PND 21 and PND 42, there were significant differences in body, ovaries weight and serum levels of E_2, P_4, LH and FSH. Moreover, the obtained alterations in the ovaries more pronounced on PND 42 than on PND 21.

These findings may be due to difference in the age of the animals and related to the puberty stage. In conclusion, in-utero and lactional exposure to TCDD induce disturbance in the female offspring reproductive function through direct and indirect effect on the ovaries. TCDD exerts its toxic effect on both GD 8 and 15 but more pronounced in GD 8 than 15. In addition, TCDD toxic effect is more obvious on PND 42 than PND 21. This may be related to time of puberty.

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التعرض إلى الديوكسين عن طريق الرحم و الرضاعة يغير الوظيفة الإنجابية في ذرية الفئران الإناث قبل وبعد سن البلوغ

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قد أجريت هذه الدراسة للتعرف علي إمكانية تقييم آثار التعرض للديوكسين عن طريق الرحم والرضاعة على الوظيفة الإنجابية في ذرية الفئران الإناث، قبل وبعد سن البلوغ. قد تم إعطاء الديوكسين للجرذان الحوامل بجرعات ٠ أو ١،٠ ميكروغرام / كغ في يوم الحمل الثامن و الخامس عشر. وقد بدا فحص ذرية الإناث في فترة ما بعد الفطام قبل سن البلوغ في اليوم الواحد والعشرون بعد الولادة وأيضا تم فحص ذرية الإناث في مرحلة ما بعد البلوغ في اليوم الثاني و الأربعين بعد الولادة. وقد تم تقييم لمستوي الإباضة، و أيضا وقد تم عمل تحليل كيميائي حيوي لقياس نشاط كل من الهرمونات الخاصة بالجونادوتروبين وأيضا قياس مستوي هرمون الاستروجين والبروجستيرون في المصل وذلك باستخدام المقايسة المناعية الشعاعية، كما تم فحص هستومورفولوجي للمبيضين. وشمل التحليل العد والقياس والتصنيف لكل أنوع البصيلات والجريبات الغارية في جميع أنحاء المبيض بالكامل. وتشير النتائج إلى أن التعرض إلى الديوكسين قد أدي إلي خفض معنوي ملحوظ فى معدل التبويض، وأيضا في مستوي الهرمونات الخاصة بالجونادوتروبين في المصل، وأيضا في مستويات هرمون الاستروجين والبروجستيرون في المصل، وقد أدي التعرض للديوكسين إلي نقصاً معنوياً ملحوظاً في عدد الجريبات الغارية والبصيلات من فئات معينة حجم عندما تم الفحص الهستومورفولوجي للمبيض. كما أظهر الفحص المجهرى للمبيض من ذرية الفئران المعرضة للديوكسين، تحلل وفقد و تغير فى شكل الخلايا التي تتكون منها أنسجة البصيلات المسئولة عن الإباضة. بالنظر لهذة النتائج يمكن أن نستنتج أن هذه البيانات تدعم الفرضية القائلة بأن التعرض للديوكسين قد يؤدي الي آثار ضارة على الوظيفة التناسلية للإناث. مع الوضع في الاعتبار، إن عمر الحيوانات قبل أو بعد سن البلوغ تلعب دوراً هاماً في الفرق بين النتائج. وعلاوة على ذلك، قد تم ملاحظة أن وقت التعرض للديوكسين أثناء الحمل في اليوم الثامن أو الخامس عشر كان له تأثير واضح للفرق بين النتائج.