

PRESENCE OF DIOXINS IN HUMAN FOLLICULAR FLUID: THEIR POSSIBLE STAGE-SPECIFIC ACTION ON THE DEVELOPMENT OF PREIMPLANTATION MOUSE EMBRYOS

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Introduction

Polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are ubiquitous environmental pollutants that become concentrated in animal tissues, climb the food chain, and cross the placenta into the mammalian embryos (1,2). These substances are also deposited in the eggs of egg-laying animals (3). Their toxic effects on the reproductive system include a reduced fertility, decreased litter size, diminished uterine weight, and altered ovarian function in several animal species (4-6). However, the reproductive consequences of human exposure to these contaminants in the general environment are largely unknown.

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is the most toxic of the halogenated aromatic hydrocarbons that have been shown to interact with a cytosolic receptor, the aryl hydrocarbon receptor (AhR) (7). It was recently reported that AhR mRNA and protein are present in mouse preimplantation embryos (8). This raises the concern that TCDD or TCDD-like compounds may also be present in human reproductive fluid and may affect embryonic development, which is exquisitely sensitive to a variety of agents (9), causing a declined female fertility. Our objective was to evaluate human follicular fluid for the presence of PCDDs and PCDFs and determine the possible effects of TCDD on the preimplantation embryo at the earliest stages of development.

Materials and Methods

Follicular fluid was aspirated from follicles of 6 women with informed consent during oocyte collection for *in vitro* fertilization. Cleanup procedures were modified after the method of Ono et.al. (10). The gas chromatography-mass spectrometry analysis was performed on a JEOL JMS-700 high performance double focusing mass spectrometer. Eight- to 10-week-old Crj; CD-1 (ICR) female mice were superovulated with 5 IU of pregnant mare serum gonadotropin, followed 48 h later by 5 IU of human chorionic gonadotropin (hCG). Two-cell embryos, obtained 44 h after hCG administration by flushing the oviducts, were placed in 2 ml of modified BWB medium (11), and cultured for 24 and 48 h to obtain 8 cell embryos and

blastocysts *in vitro*, respectively. TCDD (crystallin solid, Cambridge Isotope Laboratories, MA) was added to the medium at concentrations between 0.5 pM and 100 nM. Chi-square analysis was performed to compare the percentage of embryos that had developed to specific stages with or without TCDD.

Results and Discussion

PCDDs and PCDFs were detected in the follicular fluid from the infertile women who were undergoing the *in vitro* fertilization procedure (Table 1). The toxic equivalency (TEQ) value, which is used to express the toxic potency of complex mixtures of PCDDs and PCDFs (2), was approximately 0.01 pg TEQ/ml, equivalent to 0.031 pM of TCDD. The contamination of the follicular fluid with these chemicals indicates that the reproductive organs have been exposed to these substances, and that the germ cells and early embryos may be affected, as those substances can adversely affect mammalian fetal development (1).

The developmental rates to 8-cell embryos were significantly decreased by exposure to concentrations of TCDD between 1 and 5 pM as compared with the control group (Fig.1, ○). No inhibitory effect, however, was observed at higher concentrations between 10 pM and 10 nM. On the other hand, exposure to TCDD at concentrations between 0.5 pM and 10 nM increased the rates of blastocyst formation from 8-cell embryos, while significant decrease was observed at 100 nM (Fig.1). The highest rate of blastocyst formation more than 90% was observed at a concentration of 2 pM TCDD, which was significantly higher than that of the control 8-cell embryos (Fig. 1, □). No significant differences, however, were observed in the overall rates of blastocyst formation from 2-cell embryos in the 48-h cultures except for 100 nM (Fig. 1, ●). Although the blastocysts that developed appeared morphologically normal, and could not be distinguished from those not exposed to TCDD, they exhibited a significant, and dose-dependent increase in the number of cells (Fig. 2). Putting altogether, TCDD may have a dual effect on preimplantation embryos, that: 1) a low concentration of TCDD may inhibit the proliferation of the 2-cell embryos possibly on an "all or nothing" basis; and 2) TCDD may accelerate the differentiation of embryos that survive to the 8-cell or later stage.

The mechanism of the TCDD-induced positive and negative alterations observed during preimplantation development is not clear. These alterations may be mediated by a cytosolic AhR, in that both AhR mRNA and protein are present in mouse preimplantation embryos (8). It is also possible that TCDD modulates the stimulatory effect of epidermal growth factor (EGF) on embryonic development, since it down-regulates the function of EGF receptor (12). It is of interest to note that, in newborn mice, TCDD, like EGF, causes early opening of the eyelids and early tooth eruption (12). This precocious differentiation of epithelial tissue may parallel the stimulatory effect of TCDD on embryonic development observed in the present study.

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Table 1. Presence of polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) in the follicular fluid.

| Sample | Total PCDDs | | Total PCDFs | | Total PCDDs+PCDFs | |
|---------|------------------|----------------|------------------|----------------|-------------------|----------------|
| | conc. (pg/ml) | TEQ (pg/ml) | conc. (pg/ml) | TEQ (pg/ml) | conc. (pg/ml) | TEQ (pg/ml) |
| 1 | 1.3 | 0.0077 | 0.088 | 0.0018 | 1.4 | 0.0095 |
| 2 | 0.68 | 0.0054 | 0.026 | 0.0067 | 0.71 | 0.012 |
| average | 1.0 | 0.0066 | 0.057 | 0.0043 | 1.1 | 0.011 |

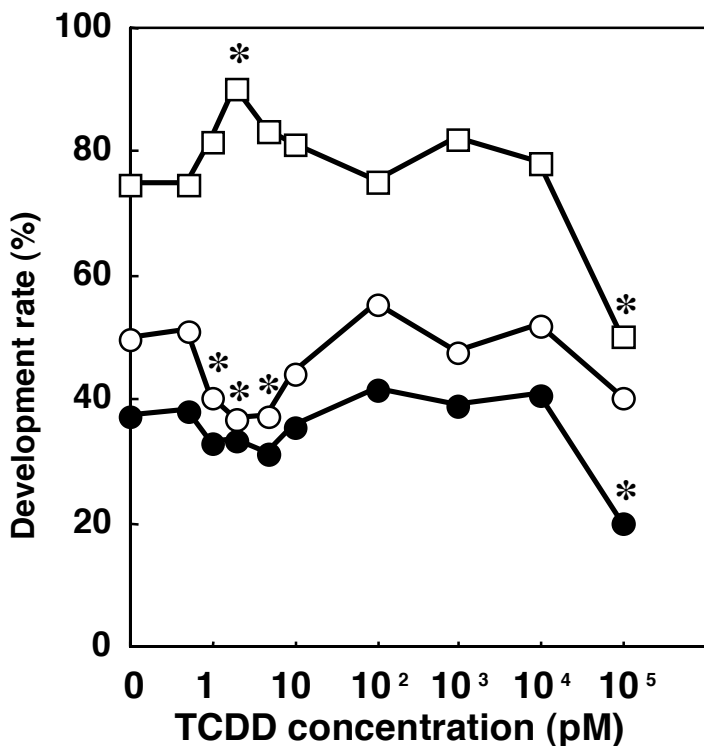


Figure 1. Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on the development of 2-cell mouse embryos to 8-cell embryos (○) and blastocysts (●). Percentage of blastocysts developed from 8-cell embryos in the latter 24 h is also illustrated (□). * indicates p<0.01, as compared with that of control embryos.

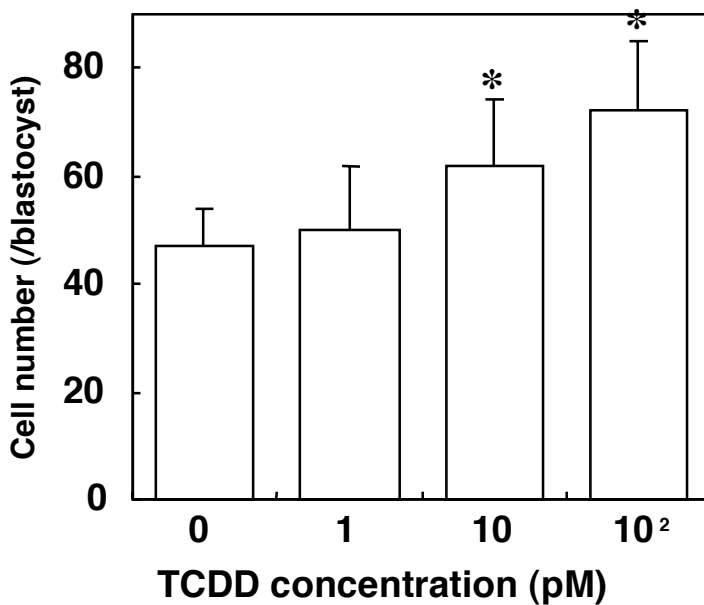


Figure 2. Dose-response relation between concentration of 2,3,7,8-tetrachloro-dibenzo-p-dioxin (TCDD) and the number of cells in the blastocysts developed from 2-cell embryos *in vitro*. Results are expressed as a mean \pm SD. * indicates p<0.01, as compared with blastocysts not exposed to TCDD.