Environmentally relevant doses of di (2-ethylhexyl) phthalate (DEHP) increases the rate of arrest in cell divisions of the zygote

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INTRODUCTION

Di(2-ethylhexyl) phthalate (DEHP) is present in many commonly used products such as paints, medical devices, food and beverage containers, toys, clothing, and personal care products, posing a risk to human health. The phthalate DEHP is the most widely used in the PVC plastics industry. Since DEHP is not covalently bound to polymers, it leahes easily from plastics. Thus, plastics represent the main source of exposure to DEHP in human populations (Clausen et al., 2004). In fact, DEHP levels have been detected in several organs including amniotic fluid, suggesting that DEHP is common (Wu et al., 2010).

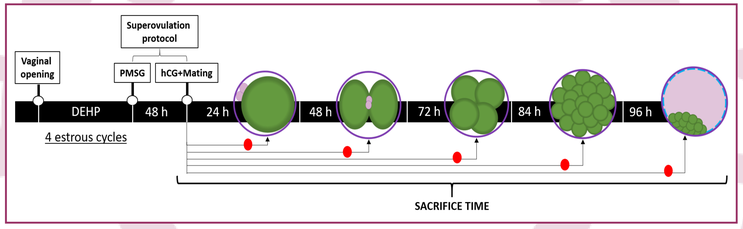
Studies in laboratory animals have shown that exposure to DEHP causes adverse effects on different systems that could be similar to those observed in human populations. DEHP exposure has been correlated with implantation failure, fetal loss, poor sperm quality, and decreased sex steroid hormones such as testosterone (Wu et al., 2010). Furher, DEHP has been related to alterations in ovarian function, including decreased ovulation, decrease in the size of granulosa cells (crucial cells in the ovary), Suppression of estradiol levels produced by the ovarian follicles, aromatase suppression, prolongation of the estrous cycle, decreased folliculogenesis, delayed age of onset of puberty and endometriosis (Ernest et al., 2014). In in vitro assay maturation studies, DEHP (10 nM-1200 µM) and its main metabolite, the MEHP (50-400 mM), negatively modulate the progression of meiosis (prophase I to metaphase II) oocyte. Finally, DEHP has been reported to slow the progression of 2-cell zygote to blastocyst maturation in an in vitro system (Huang et al., 2012).

Although many studies have reported adverse effects on the quality of the oocyte, the mechanism by which it is generated is not established. Thus, the objective of this study is to determine the effects of exposure to DEHP on the quality of oocytes and zygotes in female mice in an *in vivo* system.

MATERIAL AND METHODS

Female mice CD-1 were used; vaginal cytologies were performed daily from the day of vaginal opening. Mice were dosed orally with 20, 200 and 2000 µg/kg/day DEHP or corn oil daily for a period that targeted the first three reproductive cycles (12-15 days). When females were on estrus, they were administered one dose of 5 IU equine chorionic gonadotrophin (eCG), and 48 h later they received one dose of 5 IU Human chorionic gonadotropin (hCG). They were mated with males of proven fertility, and zygotes were recovered at different stages of maturation (Figure 1).

The results are presented as mean ± standard error (SEM). Comparison between two groups were performed using the Student's t-test. Comparisons for more than two groups were performed using one-way analysis of variance (ANOVA) followed by the Bonferroni's post hoc multiple comparison test. P-values < 0.05 were considered statistically significant.



RESULTS

Tabla 1. The effect of DEHP on developmental stages of the zygote.

Time of euthanasia/ expected stage[a]	Treatment (µg/kg/day)	Recovered	Arrest phase	Recovered N (m/SEM)	Percentage (%)[b]	Arrest phase	Recovered N (m/SEM)	Percentage (%)[b]	Arrest phase	Recovered N (m/SEM)	Percentage (%)[b]	Arrest phase	Recovered N (m/SEM)	Percentage (%)[b]
2-Cell (48h)	CONTROL	211	1-Cell	15 (3/0.8)	7.5									
	20	188		16 (3.2/0.7)	8.8									
	200	162		14 (2.8/0.7)	9.9									
	2000	153**		37 (7.4/1.21)	25.12**									
4-Cell (72h)	CONTROL	200	1-Cell	5 (1/0.4)	4.3	2-Cell	7 (1.4/0.8)	5.5						
	20	175		6 (1.6/0.5)	7.8		9 (1.8/0.4)	8.5						
	200	180		7 (1.4/0.5)	6.3		13 (2.6/0.5)	12.4**						
	2000	134**		12 (2.4/0.7)	14.6**		28 (5.6/.08)	35.1**						
Mórula (84h)	CONTROL	208	1-Cell	3 (0.6/0.3)	1.4	2-Cell	6 (1.6/0.4)	4.6	4-Cell	2 (0.4/0.4)	1.62			
	20	189		8 (0.2/0.2)	0.7		8 (1.6/0.5)	7.0		1 (0.2/0.2)	0.74			
	200	160**		7 (1.4/0.2)	4.3**		32 (6.4/0.7)	33.8**		16 (3.2/0.8)	16.9**			
	2000	169**		5 (1.0/0.5)	3.1**		21 (4.2/0.7)	21.0**		5 (1/0.5)	5.08			
Blastocyst (96)	CONTROL	189	1-Cell	3 (0.6/0.3)	3.1	2-Cell	5 (1.7/0.7)	5.6	4-Cell	1 (0.2/0.2)	1.3	Morula	2 (0.4/0.4)	2.38
	20	147		2 (0.4/0.3)	2.5		9 (3.0/1.2)	11.4		0 (0/0)	0		1 (0.2/0.2)	1.15
	200	138**		4 (0.8/0.2)	6.8**		8 (2.7/1.1)	13.2**		0 (0/0)	0		5 (1/0.5)	10.28**
	2000	124**		6 (1.2/0.2)	13.7**		13 (4.3/1.8)	29.8**		1 (0.2/0.2)	1.7		13 (2.6/0.4)	30.13**

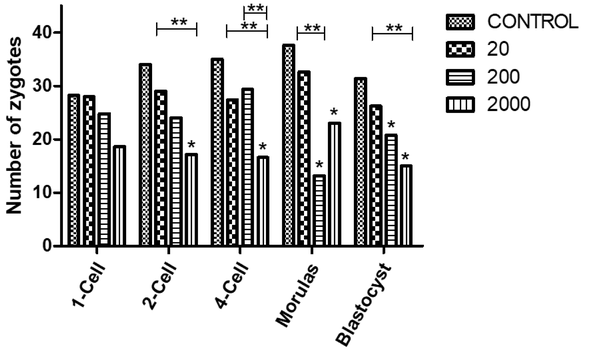


Figure 1. DEHP decreases the number of pre-implantation embryos. Following the DEHP or vehicle dosing period, the females were euthanized at specific times and pre-implantation embryos were recovered. A. Number of 1-cell zygotes obtained at specific times for each state of development. The 100% corresponds to zygotes of the previous stage of development. Data are expressed as mean ± SEM from 6 mice per group. * p<0.05 compared to control, and **p<0.05 between groups, according to one-way ANOVA- Bonferroni's post hoc.

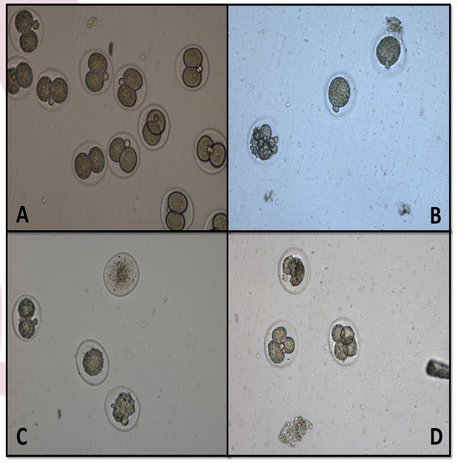


Figure 2. Zygotic specific stages obtained from mice exposed to DEHP 2000 µg/kg/day. A. Two cell zygotes obtained from the control group (Corn oil tocopherol free). B. Specific stage of 1 cell zygotes mostly showing alterations that compromise their viability. C. Zygotic specific stages of two cells mostly showing disrupted cells. D. Zygotic specific stages of four cells mostly showing cells arrested in three- and two-cell. Micrographs in phase contrast microscopy. Magnification 20 X.

CONCLUSIONS

1. The dose of 2000 µg/kg/day DEHP impaired fertilization and arrested zygotic division .

2. Exposure to DEHP (2000 µg/kg/day) may cause fragmentation in zygotes. Further studies are warranted to elucidate the consequences on embryo development.

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