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Urinary phthalates metabolite concentration effects on endometrial receptivity in infertile Iraqi women

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SUMMARY

AUTHORS' CONTRIBUTION: (A) Study Design · **(B)** Data Collection . **(C)** Statistical Analysis · **(D)** Data Interpretation · **(E)** Manuscript Preparation · **(F)** Literature Search · **(G)** No Fund Collection

Background: Phthalate are esters of phthalic acid mainly used as plasticizers (primarily to soften Polyvinyl Chloride (PVC)). Exposure occurs primarily through food consumption, personal care product usage, and contact with dust.

Applications: Food packaging, processed food, water bottles, toys, wires, medical devices (IV lines, gloves, NG tubes), nail polish, liquid soap and shampoo, the recommended airborne exposure limit (REL) is 5 mg/m3 averaged over a 10-hour work shift. The tolerable daily limit is 0.05 mg/kg body weight. In adults, Phthalate are significantly related to altered seminal parameters, Insulin resistance, high blood pressure and reproductive system problems including early menopause, low birth weight, pregnancy loss and preterm birth. Phthalate causes endocrine disruption; it interferes with normal hormonal mechanism. Modify the release of hypothalamic pituitary and peripheral hormones, change GNRH hormone secretion in hypothalamus and promote pituitary proliferation.

Objectives: To investigate the association of urinary phthalate level and endometrial parameters (thickness, pattern) in infertile women (spontaneous, IUI).

Results: The results showed there is negative coefficient indicates that an increase in exposure to diethyl phthalate is associated with a decrease in the odds of achieving pregnancy, indicating a potential adverse effect of diethyl phthalate on pregnancy outcomes. There is not enough evidence to conclude that exposure to diethyl phthalate significantly affects the likelihood of achieving pregnancy. This means that higher levels of diethyl phthalate may be associated with slightly lower odds of pregnancy, However, since 95% CI includes 1 (indicating no effect), and given the insignificant P value, this association is not statistically significant Participants reported a lack of significant medical history highlighting an opportunity to focus on the impact of lifestyle and environmental factors, such as exposure to diethyl phthalate, on fertility. At the same time, the presence of specific conditions such as PCOS presents the need to consider these possible confusing factors in such analyzes. Understanding the interaction between PCOS and environmental exposure can provide deeper insights into its collective impact on reproductive health. Among the group without PCOS, indicating no statistically significant association between PCOS and endometrial pattern.

Conclusions: The higher concentrations of Diethyl phthalate are associated potentially impacting reproductive health and fertility outcomes. Phthalate exposure can induce reproductive disorders at various regulatory levels. The Diethyl phthalate is not a significant predictor of fertility history. Phthalates represent a serious concern for female reproductive health and an economic burden. Diethyl phthalate did not have a significant effect on the odds of observing the Non-trilaminar category of Endometrial Pattern; The McFadden R-squared value calculated for this model was 0.02. Diethyl phthalate did not significantly affect the odds of having ET measurements in the <7 mm or >10 mm categories compared to the 7-10 mm category.

Keywords: Phthalate; Endometrial; Diethyl phthalate; Pregnancy; PCOS; Fertility

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INTRODUCTION

Phthalate are esters of phthalic acid mainly used as plasticizers (primarily to soften polyvinyl chloride (PVC)). They are inexpensive, colorless, non-corrosive, odorless liquids. They are added to plastics to increase their flexibility, transparency, durability and longevity. Exposure occurs primarily through food consumption, personal care product usage, and contact with dust [1]. They are labeled "Type 3" Phthalate are esters of phthalic acid mainly used as plasticizers (primarily to soften polyvinyl chloride (PVC). Exposure occurs primarily through food consumption, personal care product usage, and contact with dust. Applications: food packaging, processed food, water bottles, toys, wires, medical devices (IV lines, gloves, NG tubes), nail polish, liquid soap and shampoo, the recommended airborne exposure limit (REL) is 5 mg/m^3 averaged over a 10-hour work shift [2].

The tolerable daily limit is 0.05 mg/kg body weight. In adults, Phthalate are significantly related to altered seminal parameters, Insulin resistance, high blood pressure and reproductive system problems including early menopause, low birth weight, pregnancy loss and preterm birth [3]. Phthalate causes endocrine disruption; it interferes with normal hormonal mechanism. Modify the release of hypothalamic pituitary and peripheral hormones, change GNRH hormone secretion in hypothalamus and promote pituitary proliferation.

Applications: food packaging, processed food, water bottles, toys, wires, medical devices (IV lines, gloves, NG tubes), nail polish, liquid soap and shampoo [4]. The recommended airborne exposure limit (REL) is 5 mg/m^3 averaged over a 10-hour work shift. The tolerable daily limit is 0.05 mg/kg body weight [5]. In adults, Phthalate are significantly related to altered seminal parameters, Insulin resistance, high blood pressure and reproductive system problems including early menopause, low birth weight, pregnancy loss and preterm birth. While in children, early exposure may cause neurodevelopment by disrupting thyroid function, reducing gonadal hormone levels, or altering fatty acid concentrations in the brain [6].

METHODS

The study was designed to include 99 female infertile patients in high institute for infertility diagnosis and assisted reproductive technologies/Baghdad Governorate. Group of patients with spontaneous and IUI program enrolled in the

present study comprised of 99 patients, from age (18-44) with duration of infertility about (1 - 9 year) with primary infertility female and secondary infertility female. All patients were exposed in their daily lives' food packaging, processed food, water bottles, toys, wires, medical devices (IV lines, gloves, NG tubes), nail polish, liquid soap and shampoo, … Questionnaire formula applied to the patients including their age, BMI, socio- economic status, educational level, primary or secondary infertility and using high Phthalate products.

The parameter: - includes: At cycle Day 2, Patient is selected, consent is taken and inclusion-criteria is applied. Tran's vaginal ultrasound is done, and antral follicle count is documented. Ovulation induction and ovarian stimulation started by giving Letrezol and (FSH or hMG) injection.

Measurement of Endometrium: Patients is followed and ultrasound done at mid cycle of endometrial receptivity is obtained by measuring (endometrium thickness and pattern) ovulation trigger is given and IUI or spontaneous (Timed intercourse) is schedule.

Urine collection: Patients were asked to give a urine sample at mid cycle day. Some instructions were given to the Participant: To take a mid-stream sample of urine in the containers, to screw the led of the containers to present specimen leakage and decreased possible contamination. Sample is collected in urine specimen cap of 150-250 ml sterile hand plastic. The sample container of urine was labeled and the name and sample number are written on it. The sample directly after collection was turned into a glass tube to decrease exposure and contamination with plastic material. 2 ml of urine sample was stored by deep freezing at a temperature of -20 °C or below for 1-2 month prior to analysis by HPLC.

Extraction of phthalate from urine: At time of extraction of phthalate, we take the samples from deep freezing wait until thawing, 2 ml of urine is centrifuging for 10 min PRM 5000. 400 ml was taken, deleted with equal amount of distal water 800 micro-Diethyl ether. Then we used a mixture vortex for 5 min, leave solvent for two hours to dry and to let the Diethyl ether exposure. Take 100 ml (micromole) from the residue and delete with methanol for 20 times. Volume of injection was 20 ml from each sample to be injected in HPLC. Ultrasound done at mid cycle of check follicle size and number, endometrial receptivity is obtained by measure (endometrium thickness and pattern, sonography was done.

RESULTS

Data analysis

Demographic and Baseline Characteristics: See Tab. 1.

Age and BMI: The age of participants ranged from 18 to 43 years, with a median age of 30 years. The mean age, along with a standard deviation of 30.91 ± 6.42, indicates a moderately wide age distribution among the study participants. Body Mass Index (BMI) values varied from 21.9 to 40, with a mean BMI of 26.93 ± 3.77, situating the average participant in the overweight category according to World Health Organization (WHO) standards.

Fertility-related endometrial thickness: Tab. 2. presents crucial fertility markers respective Endometrial Thickness means of 8.51± 1.60. The range and distribution of these markers underscore the variability in reproductive potential within the population. The average of endometrial thickness, with a mean of 8.51 ± 1.60 mm, falls within the range considered conducive for implantation and pregnancy, suggesting that a majority of the study population had endometrial lining of adequate thickness for successful embryo implantation.

Diethyl phthalate exposure: Exposure levels to diethyl phthalate ranged significantly across the study population, from 0.037 to 3.30 ug/ml, with a mean exposure level of 1.43 ± 1.15 ug/ml. **Tab. 3.** This wide range indicates substantial variability in environmental exposure among participants. The median exposure level (1.48 ug/ml) suggests that half of the study population had exposure levels above this value, emphasizing the prevalence of diethyl phthalate exposure and its potential significance in evaluating fertility outcomes diethyl phthalate descriptive statistics of the participants.

Endometrial patterns among the study participants: The distribution of endometrial patterns among the study participants, as presented, is a critical component of assessing fertility outcomes, particularly in the context of Assisted Reproductive Technologies (ART) such as Intrauterine Insemination (IUI). The endometrial pattern is an essential factor in predicting implantation success, with the trilaminar pattern often associated with higher rates of pregnancy. The breakdown of endometrial patterns in your dataset is as follows: Homogeneous Endometrial patterns Observed in 13 participants, accounting for 13.1% of the study population. This pattern is characterized by a uniform appearance of the endometrium, which, depending on

Tab. 1. Descriptive statistics of Age and BMI of all subjects in the study.

Statistic	Range	1st Quartile	Median	3rd Quartile	Mean ± SD	SEM
Age	25.00	26.00	30.00	36.50	30.91 ± 6.42	0.65
BMI	21.90	24.60	27.30	28.95	26.93 ± 3.77	0.38

Tab. 2. Fertility markers in the study participants.

Statistic	Range	1st Quartile	Median	3rd Quartile	Mean ± SD	SEM
Endometrial Thickness	10.00	7.30	8.30	9.35	8.51± 1.60	0.16

other factors, might suggest varying implications for fertility outcomes. Thin Endometrial patterns Found in 10 participants, representing 10.1% of the total. A thin endometrium can be a concern for implantation success, as it may indicate insufficient endometrial development for optimal embryo attachment and growth.

Trilaminar Endometrial patterns Seen in 76 participants, making up 76.8% of the study cohort. The trilaminar pattern, featuring a distinct triple-line appearance typically observed in the mid-follicular phase, is generally considered favorable for embryo implantation and a positive predictor for pregnancy outcomes in ART cycles. The predominance of the trilaminar pattern in 76.8% of the study participants is noteworthy, as it suggests a majority of the cohort exhibited an endometrial pattern associated with higher implantation and pregnancy success rates. The presence of a trilaminar pattern aligns with the optimal endometrial receptivity window, enhancing the likelihood of positive fertility outcomes. Conversely, the thin endometrial pattern observed in 10.1% of participants may indicate a potential barrier to successful implantation. Factors contributing to a thin endometrium include insufficient estrogen stimulation, prior endometrial injury, or inherent uterine factors, all of which might necessitate specific interventions to improve fertility outcomes. The homogeneous pattern, while not typically highlighted as the most conducive for implantation compared to the trilaminar pattern, still represents a significant portion of the cohort (13.1%). The impact of this pattern on fertility outcomes can vary, underscoring the need for a personalized approach in fertility treatments, considering other clinical and hormonal markers.

Pregnancy outcomes

The data on pregnancy outcomes within the study population reveals a stark contrast between the number of failed attempts and successful pregnancies. The distribution is as follows:

Failed: A significant majority, 90 participants (90.9%), experienced failed pregnancy attempts. This high rate of unsuccessful attempts is indicative of the challenges faced by individuals or couples within this cohort, emphasizing the need to explore underlying factors that may contribute to these outcomes, including but not limited to environmental exposures, infertility issues, and the efficacy of fertility treatments.

Pregnant: A small fraction of the cohort, 9 participants (9.1%), achieved pregnancy. This outcome, while representing a success for those individuals or couples, also highlights the low overall success rate within the study population. The successful pregnancies can serve as a comparative baseline to investigate what factors might have contributed to positive outcomes *vs.* the failed attempts.

Correlation between diethyl phthalate levels and fertility markers: The correlation coefficient of -0.085 with a P-value of 0.4019 (**Tab. 4.**) indicates a small and not statistically significant negative relationship between diethyl phthalate levels and endometrial thickness. This suggests that diethyl phthalate exposure does not have a significant impact on the thickness of the endometrial lining. The analysis also reveals that there are no substantial relationships with other key reproductive markers, implying that diethyl phthalate exposure may not significantly affect endometrial development at the levels found in this sample. It is important to consider a comprehensive approach to fertility assessment, incorporating environmental exposures with traditional fertility examinations, given the lack of significant relationships with other indicators. These findings add to the growing knowledge about the reproductive health effects of environmental toxins and emphasize the need for ongoing research to understand the full range of consequences associated with diethyl phthalate exposure. This will help in establishing guidelines and strategies to minimize potential risks for individuals undergoing fertility treatment.

The Chi-squared test was used to analyze the relationship between fertility history (primary *vs.* secondary) and method of conception (IUI *vs.* spontaneous). The results showed that there is no statistically significant association between fertility history and the chosen method of conception within the data collected. This means that the preference for or effectiveness of IUI over spontaneous conception does not significantly differ between those experiencing primary *vs.* secondary infertility in this cohort. The Contingency Coefficient also indicates a very weak association between fertility history and method of conception, reinforcing the Chi-squared test's findings. It's important to carefully consider the clinical relevance and practical implications of these findings.

DISCUSSION

Diethyl Phthalate (DEP) is nominated by the National Institute of Environmental Health Sciences for reproductive toxicity studies based on widespread exposure to the general population and inadequate data to evaluate its potential reproductive hazard [7]. DEP is extensively used in consumer products, and recent bio monitoring data indicate that DEP and its metabolites are found at higher

Tab. 3. Diethyl phthalate ranged significantly across the study population.

Statistic	Range	1st Quartile	Median	3rd Quartile	Mean± SD	SEM
Diethyl phthalate ug/ml	3.30	0.22	1.48	2.38	1.43± 1.15	0.12

Tab. 4. Correlation between diethyl phthalate levels and fertility markers.

Fertility Markers	Pearson correlation	Diethyl phthalate ug/ml
ET	Correlation coefficient Significance Level P	-0.085 0.4019

concentration in the urine of the general population than several other phthalates [8]. Significant concern has been raised for phthalates regarding their endocrine disrupting properties and adverse effects on reproductive development in experimental animals [9,10]. In a continuous breeding study in mice, there was a reduction in litter size at the highest dose level tested and a decrease in sperm concentration in offspring with no such effects in the parental generation. The mouse is known to be less sensitive to the reproductive effects of phthalates compared to rats, and a multigenerational reproductive study in rats has only recently been published [11]. There were few developmental effects and no effects on reproductive performance observed in this well-conducted study. Limitations in the design of this study however, leave the question of potential reproductive hazard of DEP unanswered [12,13]. Therefore, a second multigenerational reproductive toxicity study in rats that incorporates modern endocrine related end points and sufficient numbers of animals retained in the F1 generation is needed to adequately define the dose response for reproductive toxicity and particularly the potential for effects in the F1 generation [14]. Design parameters of a second multigenerational reproductive toxicity study should include assessment of the androgen status of F1 male offspring (e.g. measurement of anogenital distance and nipple retention perinatally and in adults) and retaining a minimum of two males and females per litter in the F1 generation. Metabolism after oral administration of DEP to rats can result in hydrolysis, with monoethyl phthalate (MEP) as the principal urinary metabolite and phthalic acid as the minor secondary urinary metabolite (0.10). After oral human use, DEP turns into a new ingredient called MEP that is the dominant metabolite observed after oral administration. On this basis, MEP concentration measurements seen in urine were used as a biomarker to determine the amount of exposure to DEPs in humans [15].

The analysis of Diethyl phthalate concentrations based on endometrial pattern revealed notable differences between the Non-trilaminar and Trilaminar groups. The non-trilaminar group exhibited a higher average concentration of Diethyl phthalate (1.70) compared to the Trilaminar group (1.35). Although the statistical significance of this difference has not been assessed within this section, the observed difference in averages suggests a potential relationship between endometrial pattern and Diethyl phthalate exposure [16]. The higher average concentration of Diethyl phthalate in the Non-trilaminar group could indicate that exposure to this compound might influence the development or maintenance of a non-trilaminar endometrial pattern. This pattern is often associated with different reproductive outcomes compared to the trilaminar pattern, which is typically considered more favorable in fertility contexts. This research could contribute to a better understanding of the impact of environmental toxins on reproductive health and inform guidelines to minimize exposure risks. Other outcomes had slight, inconsistent or a lack of results: malformation of testes, spontaneous abortion, time to pregnancy, early onset puberty, indicators of cardiovascular health, kidney function, oxidative stress, atopic dermatitis, bone health, and inflammation [17].

CONCLUSION

One of the causes of current reproductive disorders could be environmental chemicals, such as phthalates. Our findings highlight the nuanced relationship between Diethyl phthalate exposure and various reproductive parameters. While some results did not reach statistical significance, the observed trends underscore the need for further research. Phthalate exposure can induce reproductive disorders at various regulatory levels. The Diethyl phthalate is not a significant predictor of fertility history. Phthalates represent a serious concern for female reproductive health and an economic burden. Diethyl phthalate did not have a significant effect on the odds of observing the non-trilaminar category of Endometrial Pattern; The McFadden R-squared value calculated for this model was 0.02. Diethyl phthalate did not significantly affect the odds of having ET measurements in the <7 mm or >10 mm categories compared to the 7-10 mm category.

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