Significant changes in follicular fluid phthalate metabolite levels reflect the lifestyle changes brought about by the strict COVID-19 lockdown in India

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Objective: To assess if the unprecedented changes in lifestyle because of the lockdown initiated by the COVID-19 pandemic, which altered human behavior, and influenced purchase and consumption patterns, may have had an impact on the exposure to phthalates in Indian women undergoing in vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI). To evaluate if the effects of the strict and lengthy lockdown in India, which promoted the new norms of stay-at-home and work-from-home, closure of beauty parlors, and restriction on public gatherings, may have contributed to a decrease in the exposure to phthalates like dibutyl phthalate and diethyl phthalate. These chemicals are found in many personal care products (PCPs) which include cosmetics and fragrances. To investigate if the extensive use of flexible single-use plastic in personal protective equipment like face masks/gloves and in plastic packaging used for online purchases, food takeaways, and home deliveries of essentials and groceries during the COVID-19 pandemic, in an attempt to provide a contact-free delivery system may have inadvertently led to an increase in exposure to phthalates like di(2-ethylhexyl) phthalate, di-isononyl phthalate, and di-isodecyl phthalate which are plasticizers used in manufacturing flexible plastic.
Design: A comparative study of the levels of six phthalate metabolites detected in follicular fluid (FF) of Indian women undergoing IVF/ICSI 1 year before and immediately after the lockdown initiated by the COVID-19 pandemic.
Setting: In vitro fertilization center in a large referral hospital in India.
Patient(s): A total of 176 Indian women seeking treatment for infertility and undergoing oocyte retrieval were included after obtaining consent. Each woman contributed one FF sample to the study. Group A (n = 96) women (mean age, 34.0 [±3.9] years, and mean BMI, 25.4 [±4.8]) had their FF samples collected and screened between January 2019 and mid-March 2020, 1 year before the lockdown. Group B (n = 80) women (mean age, 33.9 [±4.1] years, and mean BMI, 25.0 [±4.4]) had their FF collected and screened post the lockdown between October 2020 and June 2021. Both groups were matched by age and BMI.
Intervention(s): The cryopreserved FF samples of 176 women were processed using enzymatic deconjugation as well as the solid-phase extraction technique, and analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) to detect the total levels of six phthalate metabolites.
Main Outcome Measure(s): To evaluate the impact of the COVID-19 lockdown on the change in the phthalate metabolite levels in the FF of Indian women undergoing IVF/ICSI pre and post the lockdown.
Result(s): The median levels of mono-n-butyl phthalate (1.64 ng/ml in group A vs. 0.93 ng/ml in group B; $P < .001$) and mono-ethyl phthalate (5.25 ng/ml in group A vs. 3.24 ng/ml in group B; $P < .001$) metabolites of dibutyl phthalate and diethyl phthalate found in PCPs including cosmetics and fragrances were significantly higher in the FF of 96 women (group A) compared with the levels seen in

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the FF of 80 women (group B). However, the median levels of mono-isononyl phthalate (0.11ng/ml in group A vs. 0.13 ng/ml in group B; $P<.001$), mono-isodecyl phthalate (0.11 ng/ml in group A vs. 0.14 ng/ml in group B; $P<.001$), and mono(2-ethyl-5-oxohexyl) phthalate (0.13 ng/ml in group A vs. 0.14 ng/ml in group B; $P=.023$) metabolites of di-isononyl phthalate, di-isodecyl phthalate, and di(2-ethylhexyl) phthalate used as plasticizers were significantly higher in the FF of women in group B compared with women in group A.

Conclusion(s): The significant drop in mono-n-butyl phthalate and mono-ethyl phthalate levels, accumulated in the FF of 80 Indian women in group B reflects a decrease or absence of usage patterns of PCPs, including cosmetics and fragrances, thereby suggesting that these women may have deprioritized their use during the COVID-19 pandemic giving preference to personal hygiene and safety. Whereas the unprecedented increase in the use of flexible single-use plastic that became our first line of defense against the coronavirus during the COVID-19 pandemic might be responsible for the accumulation of significantly higher levels of mono-isononyl phthalate, mono-isodecyl phthalate, and mono(2-ethyl-5-oxohexyl) phthalate in FF of the same group. (Fertil Steril Sci® 2022;3:237–45. ©2022 by American Society for Reproductive Medicine.)

Key Words: Follicular Fluid, COVID-19 lockdown, lifestyle changes, phthalates, endocrine-disrupting chemicals

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Modern-day lifestyle choices and the ubiquitous presence of endocrine-disrupting chemicals like phthalates have resulted in universal exposure of humans and animals to them. Phthalates are a group of man-made chemicals that are produced in high volumes worldwide. High molecular weight phthalates like di-(2-ethylhexyl) phthalate (DEHP), di-isononyl phthalate (DiNP), and di-isodecyl phthalate (DiDP) are used as plasticizers while manufacturing polyvinyl chloride plastics to make them soft, flexible, and durable and are present in consumer products like food packaging film, food storage/ takeaway containers and general plastic packaging (1–7). Flexible plastic is used in the manufacture of medical tubing, blood storage bags, intravenous bags, and intravenous lines (2, 7, 8). With the onset of the COVID-19 pandemic, the use of personal protective equipment like face masks and gloves by health care professionals and the general population became a necessary precaution. It is estimated that globally, 129 billion single-use face masks and 65 billion gloves were used monthly (9). Most single-use face masks and gloves are made from plastic polymers like polypropylene. Because polymers require the addition of phthalates during the manufacturing process, the extensive use of single-use face masks/gloves could have resulted in an increase in human exposure to phthalates during the COVID-19 pandemic (10–14).

Low molecular weight phthalates like dibutyl phthalate (DBP) and diethyl phthalate (DEP) are added to personal care products (PCPs) like cosmetics and fragrances, in which they act as solvents and fixatives for color and fragrances and also serve as humectants, emollients, or skin penetration enhancers (15–19). Women who use more PCPs such as perfumes, deodorants, nail polish, and skin and hair care products are at a higher risk of exposure to DBP and DEP.

Phthalates enter the human body either by inhalation, ingestion, or through dermal absorption. Although their metabolism patterns differ, most phthalates undergo a phase I hydrolysis, followed by a phase II conjugation. In phase I, the diester phthalates are rapidly hydrolyzed to their monoesters by lipases and esterases found in the saliva, gut, and liver (20). The monoesters of high molecular weight phthalates like DEHP are further metabolized to secondary or oxidative metabolites (20). In phase II, the monoesters and their oxidative metabolites can be transformed to their glucuronide conjugate which is catalyzed by the enzyme uridine 5′-diphosphoglucuronyl transferase (20). Both the phthalate monoesters and their oxidative metabolites are eliminated from the body in urine and feces either in the conjugated or free forms. However, intravenous exposure to phthalates from blood transfusions and IV lines are known to bypass the first-pass metabolism in the gut and liver and are responsible for high levels of diester phthalates reaching different organs including the ovary (21).

Phthalates are known as the "everywhere chemical," being detected in human tissues and body fluids and are labeled as the 21st century's plague for reproductive health (22). While many phthalates have been classified as reproductive toxicants because of their ability to adversely affect normal reproductive functioning in both men and women (23, 24), phthalate-induced ovarian toxicity poses new challenges in preserving female fecundity (25, 26). It has been indicated that phthalate diesters entering the bloodstream through dermal absorption (possibly during usage of PCPs), could directly reach the ovary escaping the first-pass metabolism (27). Recent evidence indicates the capability of mouse ovaries to metabolize these phthalate diesters (28), thereby suggesting that if the human ovary also possessed this function, it could be exposed to higher levels of phthalates than was initially thought. This could explain why in recent years, young Indian women are presenting with a diminished ovarian reserve, low antimüllerian hormone values, and poor reproductive potential (29). Because of this, ovarian exposure to phthalates would be best assessed by screening the follicular fluid (FF).

The World Health Organization declared COVID-19 as a global pandemic on March 11, 2020 (30). This was followed by India implementing the world's largest lockdown on March 25, 2020 (31). Our ongoing study of screening the FF of Indian women undergoing in vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI) for levels of phthalate metabolites had to be halted for several months. During the lockdown we had the opportunity to see peacocks dancing on the once busy streets of our city, monkeys entering our houses and whales and dolphins on our seashores. Having observed these changes in animal behaviour, once the study was resumed, we realized that the pandemic may have given us an opportunity to investigate whether these lifestyle changes had an impact on the exposure to phthalates in Indian women undergoing IVF/ICSI.

Since we had already screened the FF of several women undergoing IVF/ICSI for phthalate metabolites before the COVID-19 lockdown, the objective of this study was to screen the FF of women undergoing IVF/ICSI in the immediate post-lockdown period to assess if there was a drop in the levels of mono-n-butyl phthalate (MBP) and mono-ethyl phthalate (MEP) metabolites of DBP and DEP, assuming that lifestyle changes and restrictions would have discouraged the use of cosmetics and fragrances by women. Additionally, we aimed to assess any increase in the levels of mono-isononyl phthalate (MiNP), mono-isodecyl phthalate (MiDP), mono(2-ethyl-5-oxohexyl) phthalate (MEOHP), and mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP) metabolites of DiNP, DiDP, and DEHP in the FF of the same group of women undergoing IVF/ICSI post the COVID-19 lockdown. This would reflect the extensive use of single-use flexible plastic during the pandemic.

MATERIALS AND METHODS

Patient Selection

Between January 2019 and mid-March 2020, 96 women (mean age, 34.0 [±3.9] years, and mean BMI, 25.4 [±4.8]) who sought treatment for infertility and had undergone an oocyte retrieval were included in the study (group A). From Mid-March 2020, we stopped IVF/ICSI cycles for approximately 6 months. Once the COVID-19 protocols were put in place, the study resumed, and between October 2020 and June 2021, 80 women (mean age, 33.9 [± 4.1] years, and mean BMI, 25.0 [±4.4]) were included in the study (group B).

All women signed a written informed consent allowing for collection and cryopreservation of their FF samples at the time of oocyte retrieval and provided one FF sample for the study. Women in both the study groups filled out a survey questionnaire in which the frequency of use of three most commonly used PCPs; lipstick, perfume/deodorant, and nail polish was documented by them as either used daily, used occasionally, or never used. This information was sought for a time frame of up to 6 months before the IVF/ICSI treatment to assess if the difference in the usage patterns of PCPs could have influenced the accumulation of levels of phthalate metabolites in the FF. The study was approved by the Scientific Advisory and Ethics Committees.

Study Design

A comparative study of the median levels of 6 phthalate metabolites detected in the FF of 96 women (group A) who underwent IVF/ICSI 1 year before the COVID-19 lockdown and 80 women (group B) who underwent IVF/ICSI immediately after the lockdown. The metabolites were: MBP metabolite of DBP, MEP metabolite of DEP, MiNP metabolite of DiNP, MiDP metabolite of DiDP, MEOHP metabolite of DEHP and MEHHP metabolite of DEHP.

Collection of FF Samples

Follicular fluid samples were collected on the day of oocyte retrieval. On completion of the identification and isolation process of the oocyte cumulus complexes, the FF were pooled together. Fluid contaminated with blood was not used. For each woman, approximately 20 ml FF was centrifuged (1500 g for 20 minutes) after which 4 ml of the supernatant FF was aliquoted into 5 plain glass vacutainers (5 ml), coded, and stored at −20 °C till analysis. Coded FF samples were transported in dry ice to Thyrocare Technologies Limited, a CAP and ISO 9001:2015 accredited laboratory for the screening of total levels of 6 phthalate metabolites.

Measurement of Levels of Phthalate Metabolites in FF

The total levels (free + conjugated) of six phthalate metabolites MBP, MEP, MEHHP, MEOHP, MiNP, and MiDP were quantified (ng/ml) in the FF using enzymatic deconjugation followed by solid-phase extraction (SPE) and liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) using an established protocol (32) with some modifications. Briefly, 125 μl of 1M phosphoric acid (CAT No.100552; Merck) was added to a 500 μl FF sample and vortex mixed for 10 minutes at 1,000 rpm to inactivate the hydrolytic enzymes. Next, 200 μl of the acid pretreated FF samples were spiked with 10 μl isotopically labeled internal standards (Cambridge Isotope Laboratories, Inc.) and 4-methylumbelliferone glucuronide (CAT No.M1381; Sigma-Aldrich). This was followed by the addition of 150 μl ammonium acetate buffer solution (pH 5) (CAT No.V800034; HPLC-grade, Sigma-Aldrich [Vetec]), 100 μl water (CAT No.14-650-357; HPLC-grade, J.T. Baker), and 20 μl of enzyme solution β-glucuronidase (CAT No.G0751; Helix Pomatia, H1 Sigma-Aldrich). The spiked FF samples were mixed and incubated at 37 °C for 3 hours to allow for de-glucuronidation. After incubation, 10 μl formic acid (CAT No. 0128-01; HPLC-grade, J.T. Baker) was added to stop the enzyme activity and samples were further diluted with 400 μl of HPLC-grade water.

After enzymatic deconjugation, the FF samples were loaded on an SPE workstation (Agilent Vac Elut 12, Agilent Technologies) equipped with SampliQ C18 (3 ml) SPE cartridges (Agilent Technologies) for automated SPE. The SPE cartridges were washed with 1.5 ml HPLC-grade water and samples were eluted with 1 ml of acetonitrile (CAT No. 02-002-180; HPLC-grade, J.T. Baker). The eluate was evaporated to dryness using a Speedovap LV (low volume) Nitrogen Evaporator (Takahe Analytical Instruments) at 40 °C under a gentle nitrogen stream. The dry residue was reconstituted in 150 μl 0.1% acetic acid (CAT No. 193402; Merck Emplura) in acetonitrile/water (1:9, v/v). Next, 50 μl of this mixture was injected into the Shimadzu LCMS-8045 Triple Quadrupole Liquid Chromatograph Mass Spectrometer (LC-MS/MS) system equipped with an electrospray ionization source. The chromatographic separation was performed on a 75 mm × 4.6 mm, 2.6 μm particle Kinetex HPLC column (Phenomenex, Torrance, CA) at a flow rate of 0.5 ml/min. The analytes were quantified using electrospray ionization tandem mass spectrometry (ESI-MS-MS) with product/precursor ion scans unique for each analyte.

Each analytic batch of 35 FF samples included 5 standards along with 2 FF samples spiked with known amounts of the

TABLE 1

Total levels of six phthalate metabolites (ng/ml) detected in FF of 176 Indian women undergoing IVF/ICSI treatment along with a comparison of levels of metabolites accumulated in the FF of 96 women before and 80 women after the COVID-19 initiated lockdown.

Parent phthalate	Total levels of metabolite screened in the FF	% > LOD	Total study Group A + B (n=176) median (IQR) (ng/ml)	Group A (n=96) before COVID-19 lockdown median (IQR) (ng/ml)	Group B (n=80) after COVID-19 lockdown median (IQR) (ng/ml)	*P* value[a]
DBP	MBP	100	1.27 (0.92, 1.74)	1.64 (1.26, 2.11)	0.93 (0.70, 1.16)	< .001[b]
DEP	MEP	100	4.13 (2.92, 5.61)	5.25 (3.58, 6.12)	3.24 (2.46, 4.41)	< .001[b]
DEHP	MEOHP	100	0.13 (0.11, 0.16)	0.13 (0.11, 0.15)	0.14 (0.12, 0.16)	.023[b]
DEHP	MEHHP	100	0.17 (0.14, 0.20)	0.16 (0.14, 0.20)	0.17 (0.14, 0.20)	.71
DiNP	MiNP	100	0.12 (0.11, 0.14)	0.11 (0.10, 0.14)	0.13 (0.12, 0.15)	< .001[b]
DiDP	MiDP	98.9	0.12 (0.11, 0.15)	0.11 (0.09, 0.13)	0.14 (0.12, 0.16)	< .001[b]

DBP = dibutyl phthalate; DEHP = di(2-ethylhexyl) phthalate; DEP = diethyl phthalate; DiDP = di-isodecyl phthalate; DiNP = di-isononyl phthalate; FF = follicular fluid; ICSI = intracytoplasmic sperm injection; IQR = interquartile range; IVF = in vitro fertilization; LOD = limit of detection; MBP = mono-n-butyl phthalate; MEHHP = mono(2-ethyl-5-hydroxyhexyl) phthalate; MEOHP = mono(2-ethyl-5-oxohexyl) phthalate; MEP = mono-ethyl phthalate; MiDP = mono-isodecyl phthalate; MiNP = mono-isononyl phthalate.

[a] Mann-Whitney *U* test.

[b] $P < .05$ was considered statistically significant.

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target phthalates (0.5 ng/ml and 5 ng/ml) which served as quality controls to determine the accuracy and precision of the method by calculating the recovery. Additionally, blank controls comprising deionized distilled water were run after every 5 FF samples to access contamination during sample analysis. The recovery of all the internal standards in the standards and samples was 80%–120%, with a relative SD of <10.0%. The linearity correlation coefficient of calibration standards at concentrations ranging from 1–20 ng/ml was >0.98. The limit of detection (LOD) for all 6 phthalate metabolites was calculated as 0.001 ng/ml. Samples with concentrations below the LOD were assigned a value of the LOD divided by $\sqrt{2}$.

HPLC-grade reagents were used and screened to ensure that they were free from the six phthalate metabolites of interest. The glassware used in the sample processing was methanol-rinsed and dried. Glass vacutainers were used to store the FF samples, thereby reducing the possibility of contamination with parent phthalates at the time of collection and storage.

Statistical Analysis

Data were entered on MS Excel (Microsoft) and analyzed using Stata Version (15.1 StataCorp, College Station, Texas). We estimated the means and SD, and median and interquartile range for continuous variables. We estimated the proportions for categorical variables. For non-normal data, we used the Mann-Whitney *U* test. The proportions were compared using the χ^2 test or Fisher's exact test for low expected cell counts. A $P < .05$ was considered statistically significant.

RESULTS

The 176 FF samples collected from 176 Indian women undergoing IVF/ICSI showed the accumulation of all six phthalate metabolites detected at a rate of 98.9 %-100 % (Table 1). MEP (a metabolite of DEP) was detected at the highest concentration with a median level of 4.13 ng/ml followed by MBP (a metabolite of DBP) detected with a median level of 1.27 ng/ml. The remaining 4 phthalate metabolites, MiNP (a metabolite of DiNP), MiDP (a metabolite of DiDP), MEOHP, and MEHHP (oxidative metabolites of DEHP) were detected in the 176 FF samples at median levels ranging from 0.12 ng/ml to 0.17 ng/ml.

A comparative study of the median levels of the six phthalate metabolites detected in the FF of 96 women (group A) who underwent IVF/ICSI 1 year before the COVID-19 lockdown and 80 women (group B) who underwent IVF/ICSI in the immediate period post the lockdown is presented in (Table 1). The median levels of MBP (1.64 ng/ml group A vs. 0.93 ng/ml group B; $P < .001$) and MEP (5.25 ng/ml group A vs. 3.24 ng/ml group B; $P < .001$) were significantly higher in the FF of 96 women (group A) as compared with the levels seen in the FF of 80 women (group B). However, the median levels of MiNP (0.11 ng/ml group A vs. 0.13 ng/ml group B; $P < .001$), MiDP (0.11 ng/ml group A vs. 0.14 ng/ml group B; $P < .001$), and MEOHP (0.13 ng/ml group A vs. 0.14 ng/ml group B; $P = .023$) metabolites of DiNP, DiDP, and DEHP were significantly higher in the FF of women in group B compared with women in group A.

The frequency of use of three commonly used PCPs; lipstick, perfume/ deodorant, and nail polish by women in both the study groups during the 6 months before their IVF/ICSI treatment is presented in Table 2. The daily usage patterns for all three PCPs were found to be significantly higher for the 96 women in group A (prepandemic period). Lipstick was used daily by 49.0% of women in group A vs. 13.8% of women in group B ($P < .001$). Perfume/deodorant was used daily by 64.6% of women in group A vs. 43.8% of women in group B ($P = .006$). Similarly, nail polish was used daily by 17.7% of women in group A vs. 6.2% of women in group B ($P = .02$). For the 80 women in group B (lockdown period), while the daily usage pattern for lipstick and fragrances had declined, there was a significant increase in the occasional usage patterns for the PCPs. Lipstick was used occasionally by 75.0% of women in group B vs. 49.0% of women in group A ($P = .001$). Perfume/deodorant was used occasionally by 45.0% of women in group B vs. 28.1% of women in group A ($P = .027$). Additionally, it was also observed that in comparison to group A, a higher number of women in group B

TABLE 2

Frequency of use of 3 commonly used PCPs: lipstick, perfume/ deodorant, and nail polish by 96 women before and 80 women after the COVID-19 initiated lockdown.

Frequency of use in the last 6 months	Group A (n=96) before COVID-19 lockdown	Group B (n=80) after COVID-19 lockdown	*P* value[a]
Lipstick	n (%)	n (%)	
Used Daily	47 (49.0)	11 (13.8)	< .001[b]
Used Occasionally	47 (49.0)	60 (75.0)	.001[b]
Never used	2 (2.0)	9 (11.2)	.024[b]
Perfume / Deodorant	n (%)	n (%)	
Used Daily	62 (64.6)	35 (43.8)	.006[b]
Used Occasionally	27 (28.1)	36 (45.0)	.027[b]
Never used	7 (7.3)	9 (11.2)	.363
Nail Polish	n (%)	n (%)	
Used Daily	17 (17.7)	5 (6.2)	.02[b]
Used Occasionally	72 (75.0)	60 (75.0)	>.99
Never used	7 (7.3)	15 (18.8)	.022[b]

[a] Fisher's exact test or χ^2 test.
[b] $P< .05$ was considered statistically significant.

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had opted not to use any lipstick, fragrances, or nail polish at all. In all, 11.2% of women in group B vs. 2.0% of women in group A ($P=.024$) had not used any lipstick, 11.2% of women in group B vs. 7.3% of women in group A ($P=.363$) had not used any fragrances, and 18.8% women in group B vs. 7.3% women in group A ($P=.022$) had not used any nail polish at all. Interestingly, although a higher number of women in group B had chosen to refrain from using any nail polish, 75% of women had continued to use nail polish despite the ongoing pandemic.

DISCUSSION

Although phthalates have short half-lives (33, 34), this study has demonstrated the accumulation of all six phthalate metabolites in the FF of 176 women with a detection rate of 98.9%-100% (Table 1), affirming their presence in the ovarian microenvironment (35–38). Follicular fluid comprises a delicate balance of hormones, growth factors, reactive oxygen species, and antioxidants working in tandem toward the developmental potential of the oocyte (39). Disruption of this environment by phthalates can be detrimental to the reproductive process. Daily urinary excretion results in the rapid elimination of phthalate metabolites. However, their extended accumulation in the FF is a cause of concern throughout folliculogenesis (25). Besides, the metabolite clearance rate for FF is not established. Our study results indicated that MEP was detected in the FF of 176 women at the highest concentration with a median level of 4.13 ng/ml, followed by MBP with a median level of 1.27 ng/ml. Their parent phthalates DEP and DBP are predominantly found in PCPs like cosmetics and fragrances (15–19). Women undergoing IVF are counseled to refrain from using PCPs even before starting ovarian stimulation and the IVF procedure. Hence, the FF levels of MEP and MBP are more likely to be a result of bioaccumulation because of previous exposures to DEP and DBP rather than any recent exposure. The possibility of earlier exposure of all women was assessed from the survey questionnaire in which the frequency of use of three commonly used PCPs; lipstick, perfume/deodorant, and nail polish were sought for a time frame of up to 6 months before the initiation of IVF/ICSI treatment. The remaining 4 phthalate metabolites; MiNP, MiDP, MEOHP, and MEHHP were detected in the FF of 176 women at median levels ranging from 0.12 ng/ml to 0.17 ng/ml and reflect the habitual use of plastics that increased during the COVID-19 pandemic, thereby facilitating the exposure to plasticizers like DiNP, DiDP, and DEHP.

Interestingly, the levels of phthalate metabolites detected in the FF in our study (n=176) were comparable to the levels reported in three earlier studies (Table 3). All three studies have emphasized that phthalate metabolites in FF can adversely impact reproductive potential even if detected at relatively low levels. Yuan XQ et al. (36), indicated a positive association of phthalate metabolites like MEP, MBP, MEHHP, and MEOHP with oxidative stress levels in the FF of 332 women undergoing IVF. An increase in oxidative stress levels can impair follicle growth, affect oocyte development, maturation, and quality and can subsequently hamper ovulation (40, 41), thereby impacting reproductive outcomes. The same research group in an earlier study by Du Y et al. (35), showed that even at low concentrations, certain phthalate metabolites, including MEP, MBP, and metabolites of DEHP in the FF of (n = 194) women could alter the levels of intrafollicular reproductive hormones, suggesting the adverse effect of phthalate exposure on theca and granulosa cell function. In a more recent study, Barnett-Itzhaki Z et al. (38) showed a significant correlation between relatively low levels of phthalate metabolites such as MEP and metabolites of DiNP and DiDP detected in the FF of (n=105) women and follicular extracellular vesicle microRNAs profiles, suggesting impairment of pathways involved in the oocyte development, maturation, and fertilization. It is therefore imperative that women who are trying to conceive should

TABLE 3

Comparing median levels of phthalate metabolites (ng/ml) detected in the FF of (n = 176) Indian women in our study with three other studies.

Parent phthalate	Metabolite screened in the FF	Current study Parikh et al. (n = 176) median (ng/ml)	Barnett-Itzhaki Z et al. (38) (2021) (n = 105) median (ng/ml)	Yuan XQ et al. (36) (2020) (n = 332) median (ng/ml)	Du Y et al. (35) (2019) (n = 194) median (ng/ml)
DBP	MBP	1.27	1.88	1.32	1.68
DEP	MEP	4.13	1.66	0.28	1.24
DEHP	MEOHP	0.13	0.04	0.03	1.07
DEHP	MEHHP	0.17	0.08	0.72	1.12
DiNP	MiNP	0.12	0.04	Not Screened	Not Screened
DiDP	MiDP	0.12	Not Screened	Not Screened	Not Screened

DBP = dibutyl phthalate; DEHP = di(2-ethylhexyl) phthalate; DEP = diethyl phthalate; DiDP = di-isodecyl phthalate; DiNP = di-isononyl phthalate; FF = follicular fluid; MBP = mono-n-butyl phthalate; MEHHP = mono(2-ethyl-5-hydroxyhexyl) phthalate; MEOHP = mono(2-ethyl-5-oxohexyl) phthalate; MEP = mono-ethyl phthalate; MiDP = mono-isodecyl phthalate; MiNP = mono-isononyl phthalate.

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reduce their exposure to phthalates by modifying their lifestyle and consumption patterns.

India experienced one of the lengthiest and most stringent lockdowns in the world. We observed a significant decline in the median levels of MEP (5.25 ng/ml group A vs. 3.24 ng/ml group B; $P < .001$) and MBP (1.64 ng/ml group A vs. 0.93 ng/ml group B; $P < .001$) in the FF of 80 women (group B) who were screened in the immediate post-lockdown period (Table 1). Our results indicated that the drastic and unprecedented change in lifestyle by these women may have either dissuaded, decreased, or deprioritized the use of cosmetics and fragrances, thereby reducing their exposure to the parent phthalates DEP and DBP. This was further supported by an evident change that was observed in the usage pattern of lipstick, perfume/deodorant, and nail polish by the 80 women in group B in the 6 months (lockdown period) before their IVF/ICSI treatment (Table 2). It was observed that in comparison to group A, fewer women in group B had used lipstick (49.0% in group A vs. 13.8% in group B; $P < .001$) and perfume/deodorant (64.6% in group A vs. 43.8% in group B; $P = .006$) daily, a significantly higher percentage of women had used lipstick (49.0% in group A vs. 75.0% in group B; $P = .001$) and perfume/ deodorant (28.1% in group A vs. 45.0% in group B; $P = .027$) occasionally. This significant shift in the usage pattern in these 80 women (group B) could be due to restrictions such as work-from-home, stay-at-home routines, and stopping of social activities along with compulsory face mask usage. Hence it is observed that the daily use pattern dropped significantly, whereas the occasional use pattern increased significantly. Additionally, we observed that a higher percentage of women in group B preferred not to use any lipstick (2.0% in group A vs. 11.2% in group B; $P = .024$) or fragrance (7.3% in group A vs. 11.2% in group B; $P = .363$) at all. Because cosmetics and fragrances contain DBP and DEP (15–19) and elevated urinary levels of their metabolites MBP and MEP have been previously reported in the women using these products (16, 19, 42–44), a shift from a daily use pattern to a more occasional use pattern along with the decision to refrain from using either lipstick or fragrances by a higher number of women in group B is suggestive of the decrease in the exposure to DEP and DBP, thus, contributing to a decline in the accumulation of FF levels of MEP and MBP being reported.

Leonard Lauder, of Estee Lauder, introduced the concept of the “lipstick effect” when he saw a spike in the sales of lipstick during the 2001 recession. He explained that although extravagant luxuries were out of reach and unaffordable during economic duress, the sale of lipstick still stayed strong because it was considered a more affordable indulgence that could boost a woman’s self-image instantly (45). However, this economic indicator was challenged during the recession in 2020 caused by the COVID-19 pandemic. The global cosmetic industry witnessed a significant drop in lipstick purchases especially in the first 4 months of the pandemic because of the mask mandate and women finding themselves home-bound (46, 47). Interestingly, the surge in the online purchase of nail care products in the UK was reported as nail care possibly becoming the “COVID-19 Lipstick effect” and could be giving rise to the “nail-polish effect” (48). In this Indian study, we observed that a section of women in group B had completely refrained from using nail polish (7.3% in group A vs. 18.8% in group B; $P = .022$) suggesting that they had probably emphasized hand hygiene during the COVID-19 pandemic. In reality, 75% of women in group B had continued using nail polish even though there was an ongoing pandemic. This suggests that nail care may have helped generate a feeling of well-being among Indian women who found themselves restricted to their homes during the lockdown and, therefore nail polish could be the “COVID-19 lipstick effect” for them.

Most of the products used at beauty parlors, spas, and hair and nail salons contain phthalates. Women frequenting these facilities are at constant risk of exposure. Indoor air samples from hair and nail salons have shown the highest levels of phthalates specifically DEP and DBP (49, 50). Occupational exposure to phthalates is also seen in nail and hair salon workers (51). While higher urinary levels of MBP have been reported in manicurists (52, 53), hairdressers have higher urinary levels of MEP when compared with office workers because DEP is found in many hair care products (54). The COVID-19 lockdown shut down many businesses dealing with beauty care. This is relevant to this study. Unlike the 96 women (group A) who underwent IVF/ICSI before the lockdown or in the prepandemic period, the 80 women (group B) who underwent IVF/ICSI in the immediate period post the lockdown did not have an opportunity to visit these facilities, thereby reducing any possible exposure to DEP and DBP. This could explain the decline in the levels of MEP and MBP in their FF. Another study from India reported that there was a significant drop in the sale of beauty products and cosmetics along with an increase in the demand for personal hygiene products in the first 5–6 months of the lockdown (55). These findings suggest that Indian women deprioritized the use of beauty products during the pandemic which further supports our observations.

The excessive use of single-use plastic during the pandemic was adopted as a necessary precaution. As a result of this, the impact of the COVID-19 pandemic on plastic pollution has become a global concern (9, 56–58). The lockdown promoted online purchases and emphasis was given to plastic packaging of commodities bought online or delivered from a local supermarket or grocery (59). Although the plastic packaging may have offered a contact-free delivery system it probably resulted in an increase in exposure to phthalates like DiNP, DiDP, and DEHP. Restaurants and eateries were encouraged to follow the takeaway system. Plastic food packaging material, storage and takeaway containers are a source of exposure to phthalates, allowing migration of phthalates into the food during storage and transportation (3–6, 60, 61). In the food packaging industry, DiNP and DiDP are known to have gradually replaced DEHP; therefore, fast food and food items that are plastic wrapped or packaged in plastic containers have been associated with human exposure to DiNP and DEHP (4, 62–64). Because there is a high probability that the 80 women (group B) would have used more plastic packaging for their online purchases, food takeaways, and home deliveries of essentials, nonessentials, and groceries, this could have increased their exposure to DiNP, DiDP, and DEHP, thereby resulting in the accumulation of significantly higher median levels of MiNP (0.11 ng/ml group A vs. 0.13 ng/ml group B; $P<.001$), MiDP (0.11 ng/ml group A vs. 0.14 ng/ml group B; $P<.001$), and MEOHP (0.13 ng/ml group A vs. 0.14 ng/ml group B; $P=.023$) in their FF (Table 1). Recent studies suggest that single-use face masks are a potential source of phthalate exposure (10–13). A common observation in these studies was that most single-use face masks contained a mixture of phthalates with DEHP being predominantly present in all mask samples and often at the highest level while DBP and DEP were also found at varying levels (10–13). Based on these findings, it can be assumed that excessive and extended use of most single-use polymer-based face masks by women in group B resulted in an exposure to higher levels of DEHP reflected by significantly higher levels of MEOHP in their FF. Additionally, DEHP and DiNP have been detected in vinyl gloves that were commonly used as personal protective equipment (14) and extensive use of such gloves during the pandemic could have also contributed to the higher levels of MEOHP and MiNP found in the FF of the 80 women (group B).

The COVID-19 pandemic has provided a real-world scenario that demonstrated how a significant change in the lifestyle of 80 women (group B) during the lockdown where the use of single-use plastic had drastically increased and the use of cosmetics and fragrances had significantly decreased had subsequently altered their exposure to phthalates. While the use of selected PCPs in the previous 24–48 hrs had been associated with elevated urinary levels of MEP and MBP (16, 42–44), in this study, we were able to show that the use of selected PCPs 6 months before the pandemic by 96 women (group A) was associated with the accumulation of higher levels of MEP and MBP, while the use of the same PCPs during the 6 months of the lockdown by 80 women (group B) was associated with the accumulation of significantly lower levels of MEP and MBP, suggesting that chronic long term exposure to phthalates may reduce the rate of metabolic clearance.

Because the women in group B did not show a decline in the levels of all the six phthalate metabolites in their FF but instead showed a simultaneous decline in the levels of MBP and MEP along with increased levels of MiNP, MiDP, and MEOHP owing to the change in lifestyle, it is possible that the impact on embryological parameters and reproductive outcomes between the two groups might not be significantly different but is being investigated by us.

There were several limitations in the study. First, the small study size was a major limitation. It was difficult to recruit many women after the lockdown because of safety concerns. We could include 80 women who underwent IVF/ICSI from October 2020 to June 2021. By July 2021, our city had completed a phase-wise opening up of the lockdown. Women were not recruited after the COVID-19 lockdown restrictions were relaxed because the levels of phthalate metabolites in their FF would not be representative of the lifestyle changes brought about during the lockdown. Hence we had to limit our study to 80 women. Second, we were able to access the usage patterns of only three commonly used PCPs; lipstick, perfume/deodorant, and nail polish. We may have had a better representation of the utilization pattern of PCPs before and during the COVID-19 pandemic had we assessed the utilization of some more PCPs. Third, because the questionnaire filled by women in group A, which was before the COVID-19 pandemic, was the same as that filled by women in group B, we had not asked about the extensive use of single-use plastic which was a phenomenon observed only during the pandemic. In fact, before the pandemic, our city was in the process of gradually phasing out the use of single-use plastic. Finally, our observation of findings for the group of infertile women may not hold for the general population. However, because the infertile population is more susceptible to the adverse impacts of phthalates, it is helpful to study the findings in these women.

CONCLUSION

This study shows the effects of lifestyle changes that resulted because of the COVID-19 lockdown in altering human behavior. This greatly influenced purchase and consumption patterns and had an impact on the exposure to phthalates in Indian women undergoing IVF/ICSI. The significant drop in the accumulated levels of MBP and MEP in the FF of women undergoing IVF/ICSI immediately after the lockdown reflects a decrease or absence of usage patterns of PCPs, including cosmetics and fragrances, suggesting that women may have deprioritized their use during the COVID-19 pandemic, giving a priority to safety and hygiene. The accumulation of significantly higher levels of MiNP, MiDP, and MEOHP in the FF of the same group indicated an increase in the use of single-use plastics during the COVID-19 pandemic. To our knowledge, this is the first study in the world to show how lifestyle changes brought about by stringent COVID-19 lockdown measures influenced the levels of MBP, MEP, MiNP, MiDP, and MEOHP in the FF of women undergoing IVF.

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