Reviewer’s report

Title: Parameters of ovarian reserve in relation to urinary concentrations of parabens.

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Reviewer: Mariana Fernandez

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Environmental Health ENHE-D-19-00047R1

Title: Parameters of ovarian reserve in relation to urinary concentrations of parabens.

General comments:

Jurewicz et al., have examined the association of urinary paraben concentrations with markers of ovarian reserve in a large study of women seeking fertility treatment at several infertility clinics.

The study explores an important and understudied question. Fortunately, there are a growing number of studies that aim to examine the impact of non-persistent endocrine pollutants exposure, such as parabens, on female reproductive health. In this work associations between urinary exposure to several parabens and ovarian reserve, used as variable to evaluate the woman's response to ovarian stimulation, were examined. Serum concentration of follicle-stimulating hormone (FSH), anti-Müllerian Hormone (AMH), and estradiol, as well as antral follicle count (AFC), were also assessed. Only one previous epidemiological study has explored a similar research aim.

I feel that this article may contribute to the literature on chemical exposure and female reproductive health but still need to improve several aspects, outlined below:

1. To improve the English (a native English speaker should review the manuscript for clarity).

2. To include more information about the urinary assessment of the selected parabens

3. To expand the results and the discussion

Materials and Methods:

1. Participants.

- Please include geographical study area, recruitment period, number of infertility clinics, and its public or private condition (it is not clear how many centers participated because among the strengths authors say that all study subjects were recruited in the same center)
- Participant's age: the range is different in the abstract (20-39) than in the method section (25-39)

- All the participants (511) gave one urine sample prior to the measurement of the markers of ovarian reserve, and a quarter of them (120) gave an additional urine sample during infertility treatment cycles. How was calculated the urinary paraben concentrations for each women? How was computed the within-person concentration in those women with two urinary samples? Was this variation applied to all study population?

2. Measurement of urinary paraben concentrations

- Add: Time and conditions (fasting, first of the morning) of the urine sample collection
- Add: Strict control and validation procedures: Methods for preparation of samples, standard solutions and quality controls as well as the instrumental analysis and measures taken to avoid external contamination of samples during collection and processing, is needed

3. Statistical analysis:

- Please clarify why the multivariate model only included three covariates for adjustment (age, smoking habit and BMI). In this large study, female varied on the main cause of infertility diagnosis (male, idiopathic, female factor). This covariate should also be explored/included in the multivariate statistical model, for example in a sensitivity analysis. Please, assess whether cause of infertility had further influence on the effect estimates.
- Because general population is exposure to a large number of xenoestrogens, we suggest taking also into account the potential effect of mixtures and at least consider exploring the association between Σ parabens with the outcome.

Results

- There were great differences between the urinary parabens concentrations in the first and second set of urine samples; please explain the biological reasons. Could the infertility treatment cycles affect paraben levels in the second set of urinary samples (n=120)?

- Table 1: Translate Wykształcenie

- Table 3: In the previous similar epidemiologic they found that nearly 100% of the women included had detectable urinary concentrations of MP and PP, and >75% of BP concentrations. However, Jurewicz et al have found lower detectable level (mainly for BP) in this study population. Some discussion should be added in this regards (detection levels showed in the text differ, lines 136, 137, from the levels included in table 3)
- Table 6 and 7: Which urinary paraben concentrations were used in this statistical assessment for each woman? Please add appropriate information in the table legend explaining the statistical model used in addition to the adjusted covariates

Discussion

Lately analytical method based on sample treatment using dispersive liquid-liquid microextraction (DLLME) followed by ultrahigh performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) analysis are the proposed methodology to assess human exposure to non-persistent endocrine disruptors such as the selected parabens instead Gas chromatography. Please discuss it.

The data is discussed within the context of these existing studies as well as within the context of the epidemiological literature (there are some important missing articles). For example, authors compared urinary concentrations of parabens found with those reported among USA populations. Several studies have also been conducted among European population (see Jiménez-Díaz et al. Urinary levels of bisphenol A, benzophenones and parabens in Tunisian women: A pilot study. Sci Total Environ. 2016;562:81-88).

Results should interpret with caution because, for example some limitations have not been considered: the cross-sectional design of this study does not allow the inference of causal relationships and, consequently, reverse causality issues cannot be ruled out. A further limitation is that you did not collect the use of cosmetic product before urine sampling. Additionally, you only considered paraben exposure, and women are exposed to a wide range of environmental chemicals that may contribute to the cocktail effect.

References

The references are cited by numbers in the reference list at the end of the article but the reference style inside the text is different. Please clarify

Minor:

Line 58: the reference is lacking

Line 83: I do not agree with this sentence: "This study adds to the previous human studies of parabens exposure more statistical power, by including additional parabens (five parabens- ethyl paraben (EP), butyl paraben (BP), methyl paraben (MP), iso-butyl paraben (iBu-P)"
Level of interest
Please indicate how interesting you found the manuscript:

An article of importance in its field

Quality of written English
Please indicate the quality of language in the manuscript:

Needs some language corrections before being published

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