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- Comments from the reviewers and editors (email to author requesting revisions)
- Response from the author (cover letter submitted with revised manuscript)*

**The corresponding author has opted to make this information publicly available.*

Personal or nonessential information may be redacted at the editor's discretion.

Questions about these materials may be directed to the *Obstetrics & Gynecology* editorial office: obgyn@greenjournal.org.

Date: 12/08/2023
To: "Rebecca Troisi" [redacted]
From: "The Green Journal" em@greenjournal.org
Subject: Your Submission ONG-23-2031

RE: Manuscript Number ONG-23-2031

Prenatal Diethylstilbestrol Exposure and High-Grade Squamous Cell Neoplasia of the Lower Genital Tract

Dear Dr. Troisi:

Thank you for sending us your work for consideration for publication in Obstetrics & Gynecology. Your manuscript has been reviewed by the Editorial Board and by special expert referees. The Editors would like to invite you to submit a revised version for further consideration.

If you wish to revise your manuscript, please read the following comments submitted by the reviewers and Editors. Each point raised requires a response, by either revising your manuscript or making a clear argument as to why no revision is needed in the cover letter.

To facilitate our review, we prefer that the cover letter you submit with your revised manuscript include each reviewer and Editor comment below, followed by your response. That is, a point-by-point response is required to each of the EDITOR COMMENTS (if applicable), REVIEWER COMMENTS, and STATISTICAL EDITOR COMMENTS (if applicable) below. The revised manuscript should indicate the position of all changes made. Please use the "track changes" feature in your document (do not use strikethrough or underline formatting). Upload the tracked-changes version when you submit your revised manuscript.

Your submission will be maintained in active status for 21 days from the date of this letter. If we have not heard from you by 12/29/2023, we will assume you wish to withdraw the manuscript from further consideration.

EDITOR COMMENTS:

Please note the following:

* Help us reduce the number of queries we add to your manuscript after it is revised by reading the Revision Checklist at https://journals.lww.com/greenjournal/Documents/RevisionChecklist_Authors.pdf and making the applicable edits to your manuscript.

* As of January 2024, only certain article types will appear in the print version of the journal. All accepted articles will continue to publish online. All articles will be indexed in PubMed as an official article of Obstetrics & Gynecology. Additional information is available in the Instructions for Authors (https://journals.lww.com/greenjournal/Pages/InformationforAuthors.aspx#II).

* All submissions that are considered for potential publication are run through CrossCheck for originality. The following lines of text match too closely to previously published works or need to be cited:

- Large portions of text from your submission are already posted online at https://obgynkey.com/prenatal-diethylstilbestrol-exposure-and-high-grade-squamous-cell-neoplasia-of-the-lower-genital-tract/. It is not clear to the Editorial Office if this is a legitimate website or not, and if you did not intend to have your study posted online, please contact the webmaster of OBGYN Key to have it removed. If you did intend to post the other study, please disclose the previous publication on your title page.

REVIEWER COMMENTS:

Reviewer #1: The authors report an update to the NCI coordinated cohort of patients with exposure to antenatal DES as well as a cohort of unexposed patients. The authors are presented updated outcomes data with six additional years of follow up. The authors describe results consistent with prior reports that antenatal DES exposure is associated with

increased risk of high-grade cervical dysplasia. No significantly new findings were reported.

Reviewer #2: Overall this study highlights important aspects of management of DES exposed patients, which is less familiar to practitioners today as we veer further from the years of DES exposure.

1. Can you state what the objective of this study was in the abstract and the methods body? (i.e. This was a follow-up study of x to evaluate y)

2. Can you define "Deikman" and "DESAD" and "VED" terminology?

3. Title of table mentioned "cervical, vaginal and vulvar" but I don't see any results reported for vaginal and vulva dysplasia in the table nor mentioned in the body of the text.

4. Could there be a statement regarding clinical implications and practical information about management of DES offspring? I would imagine most offspring would not have any idea whether their mother took DES < 8 weeks or were taking low or high dose DES. Clinically, any history of DES probably would warrant increased screening given that level of detailed information would not be available.

Reviewer #3: The authors analyzed the risk of CIN 2+ among women exposed to DES in utero and found that elevated risk persisted up until age 45 compared to non-exposed women.

From the introduction: Patients exposed to DES in utero were not recommended to have ongoing screening with cytology for the risk of CIN 2+ so much as for the risk of cervical and vaginal clear cell adenocarcinoma. The authors should clarify this. The one study they cite shows a doubled RR of CIN 2+ but not beyong age 45—so the lifelong screening recommended for these patients with DES exposure in utero is due to the multiple studies demonstrating increased risks of CCAM.

The first paragraph in the methods section is not clear. The authors used NCI data but should clarify that in the first paragraph because as written it is confusing. Where did the additional exposed and unexposed cases come from? Could the authors clarify where they obtained information from the patient history and medical charts? I think that the first 2 paragraphs of the methods section just need to be elaborated upon a little bit more to give information to people who may not be familiar with the NCI data and DESAD cohorts.

When discussing the models that used dose, the authors should clarify that they are referring to dose of DES.

In the discussion, I would change lower genital tract neoplasia to CIN 2+ because the authors only examined CIN2 + rates. The findings of increased risk with VEC should be highlighted, as it is significant.

However, at this point in time, all of the women exposed to DES in utero are beyond age 45. To make the discussion/recommendation stronger, the authors should address this point and perhaps could suggest that for those women who have not had a history of CIN 2+ or CCAM, that screening can be stopped at age 65, just as we do with non-DES exposed patients. Right now, the "why" of this study provides little applicability to real life patient care as these patients are all greater than age 45. But it could be used to postulate changes in screening, as other papers have argued for previously (see Wamakima et al in the Journal of Lower Genital Tract Disease).

STATISTICAL EDITOR COMMENTS:

lines 13-14 and 17-19: Need to reconcile the statements: "through age 44, but not after 45" vs ""through age 45".

line 81: Should include this data in supplemental material.

lines 81-82: Should include in Table the analysis for all ages aggregated thru age 44 yrs. In the Table, only the age < 30 had a statistically significantly elevated aHR for CIN2+ occurrence. The various age categories individually had relatively few counts and hence wide *and mostly NS) CIs. No doubt limited stats power played a large role. Similarly, the counts of those age 45* were relatively small. Although one cannot excluded. Therefore, one cannot conclude from these data that the risk is not increased for those women age 45+. Should include in Table or in supplemental the extent of missing

data, exclusion due to hysterectomy or loss to follow-up for the various groups, particularly by age.

lines 86-87: Neither comparison (with vs without VEC) reached statistical significance at .05 inference threshold.

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Sincerely,
Jason D. Wright, MD
Editor-in-Chief

The Editors of Obstetrics & Gynecology

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We thank the reviewers for their thoughtful comments and believe the paper has improved because of them.

Reviewer #1: The authors report an update to the NCI coordinated cohort of patients with exposure to antenatal DES as well as a cohort of unexposed patients. The authors are presented updated outcomes data with six additional years of follow up. The authors describe results consistent with prior reports that antenatal DES exposure is associated with increased risk of high-grade cervical dysplasia. No significantly new findings were reported.

We would like to thank the reviewer for their careful reading of our paper. We agree that the findings are similar with additional follow-up. We believe this information is still relevant as it informs cervical cancer screening recommendations for DES exposed females. Long-term follow-up is essential to determine whether disease rates/risk have changed over time, and the follow-up findings are helpful for the DES-exposed women and their providers.

Reviewer #2: Overall this study highlights important aspects of management of DES exposed patients, which is less familiar to practitioners today as we veer further from the years of DES exposure.

1. Can you state what the objective of this study was in the abstract and the methods body? (i.e. This was a follow-up study of x to evaluate y)

   This has now been added to the abstract: "We report the results of a follow-up study of prenatal DES exposure and risk of CIN2 and greater." In addition, we added this as the first sentence to the methods section on p.3, "We conducted a prospective study of prenatal DES exposure and risk of CIN2+."

2. Can you define "Deikman" and "DESAD" and "VED" terminology?

   We thank the reviewer for identifying this omission. We now include on p.4, third paragraph, information from the supplemental materials defining the Dieckmann and DESAD cohorts, and the source of information on VEC (vaginal epithelial changes).

   "Members of two original cohorts (the Dieckmann cohort consisting of females whose mothers participated in a clinical trial of DES in 1951-52, and the females who participated in the National Cooperative Diethylstilbestrol Adenosis Project ([DESAD] cohorts from Boston, California, Minnesota, Wisconsin, Texas) underwent a comprehensive gynecologic examination with identical screening protocols for exposed and unexposed females around the time of recruitment in the mid-1970s that systematically identified vaginal epithelial changes (VEC) by means of

colposcopy or iodine staining in exposed females. Identical screening protocols were used for the exposed and unexposed females. VEC is glycogen poor squamous epithelium found in the vagina or exocervix that presumably reflects glandular epithelium undergoing transformation over time to glycogenated, normal adult type squamous epithelium. These changes were more frequent in females prenatally exposed to DES early in pregnancy who also had large cumulative doses of DES by the end of pregnancy. "

3. Title of table mentioned "cervical, vaginal and vulvar" but I don't see any results reported for vaginal and vulva dysplasia in the table nor mentioned in the body of the text.

   We have replaced in the table title - "cervical, vaginal and vulvar" with "lower genital tract," and now give the number of cervical (n=173; 138 exposed and 35 unexposed), vaginal (n=12 (10 exposed and 2 unexposed), and vulvar (n=5; 3 exposed and 2 unexposed) cases in the footnote.

4. Could there be a statement regarding clinical implications and practical information about management of DES offspring? I would imagine most offspring would not have any idea whether their mother took DES < 8 weeks or were taking low or high dose DES. Clinically, any history of DES probably would warrant increased screening given that level of detailed information would not be available.

   The reviewer raises an important and complicated issue. We at NCI have discussed cervical cancer screening recommendations for prenatally DES exposed females with experts from the CDC and academic physicians. There are risks of not identifying advanced CIN and risks of unnecessary procedures and the emotional toll of false positive results. In our data, as the statistical reviewer pointed out, the relative risk among females 45 and older did not include 1.0 so we cannot rule out an increased risk. Therefore, we wrote in the Discussion: "Whether females older than 45 years should continue to have increased screening would require careful weighing of possible risks and benefits." We have been told by the study's exposed participants that medical schools' curricula no longer cover the effects of DES. Consequently, gynecologists may not be able to effectively manage these patients. It's possible, however, that the new cervical cancer screening guidelines incorporating clinical history of HPV status, a necessary (but not sufficient) cause of cervical cancer, and previous CIN results is adequate for DES exposed women. If the editor agrees, we could add this last point to the Discussion.

Reviewer #3: The authors analyzed the risk of CIN 2+ among women exposed to DES in utero and found that elevated risk persisted up until age 45 compared to non-exposed women.

From the introduction: Patients exposed to DES in utero were not recommended to have ongoing screening with cytology for the risk of CIN 2+ so much as for the risk of cervical and vaginal clear cell adenocarcinoma. The authors should clarify this. The one

study they cite shows a doubled RR of CIN 2+ but not beyond age 45—so the lifelong screening recommended for these patients with DES exposure in utero is due to the multiple studies demonstrating increased risks of CCAM.

> *We have addressed this comment in the Introduction with this sentence: "Females with prenatal diethylstilbestrol (DES) exposure were exempted from less frequent screening[1] due to their increased incidence of vaginal and cervical neoplasia,* ***including clear cell adenocarcinoma****.[2]*

The first paragraph in the methods section is not clear. The authors used NCI data but should clarify that in the first paragraph because as written it is confusing. Where did the additional exposed and unexposed cases come from? Could the authors clarify where they obtained information from the patient history and medical charts? I think that the first 2 paragraphs of the methods section just need to be elaborated upon a little bit more to give information to people who may not be familiar with the NCI data and DESAD cohorts.

> *We appreciate the need for more details pointed out by the reviewer. We put most of the study methods in the supplemental materials to remain within the word limit for a research letter. If the editor allows, we will now add this information, below, to the Methods section.*
>
> *"The NCI DES Combined Cohort Follow-up consists of the following: 1) females who participated in the National Cooperative Diethylstilbestrol Adenosis Project ([DESAD] cohorts from Boston, California, Minnesota, Wisconsin, Texas)1 2) females whose mothers participated in a clinical trial of DES in 1951-52 (Dieckmann Cohort),2 3) females whose mothers were treated in a large private infertility practice in Massachusetts, USA (Horne Cohort) and 4) females from Massachusetts, New Hampshire, Maine and the Mayo Clinic whose mothers participated in the Women's Health Study ([WHS] Cohort), a study of the subsequent health effects of DES in females who were administered DES during their pregnancy.3*
>
> *From 1975-1983, DESAD cohort members were examined annually; medical records and pathology reports were collected for cancers and gynecologic neoplasia; annual questionnaires were administered from 1984-1989. The Dieckmann cohort was followed by questionnaire from the late 1970s to 1990. The Horne cohort was mailed annual questionnaires through the 1980s. The follow-up of the four combined cohorts by NCI began in 1994 with a mailed questionnaire, with subsequent questionnaires mailed in 1997, 2001, 2006, 2011 and 2016 (responses were received through 2017). Follow-up of daughters from the WHS cohort began with the NCI combined follow-up in 1994.*
>
> *For all combined cohort participants, prenatal exposure to DES, or the lack thereof, was documented by the medical record or a physician's note. Gestational week of first DES use was available for 75% of all exposed females, and for 80.2% of the*

DESAD and Dieckmann exposed. Because data for total cumulative DES dose were available for only 38% of the females, we classified the individual cohorts as high- or low-dose based on differences in prescribing practices by U.S. region (unknown for a subgroup of the WHS). Agreement between the dose categories and individual doses was excellent among those with complete data.6 Information on highest level of education, smoking status, and frequency of routine medical examinations, including Pap smears, in the last 5 years was collected on the questionnaire. Smoking status was updated on the 2006 and 2016 questionnaire, and menopausal status and frequency of Pap smears were ascertained on all six questionnaires. Screening by Pap smears was treated as time-dependent in the analysis.

In this report, all neoplasias, including those of the vulva and vagina (VIN), are referred to using the CIN nomenclature. Reports of CIN2+ were available from four sources, records from the original cohorts (1982-1988), the NCI Combined Cohort Study (1989-2016), state cancer registry searches (invasive and CIS) and the National Death Index (NDI) Plus (invasive cancer only); the methods for confirmation of cases were similar. The NCI Combined Cohort Study questionnaires ascertained new diagnoses of neoplasia, and biopsies of the cervix, vagina or vulva that indicated a precancerous condition (dysplasia or carcinoma in-situ). Pathology records were obtained for reported biopsy-confirmed, genital-tract neoplasia of any grade (including HPV infection). Slides also were requested for pathology-confirmed diagnoses of intraepithelial neoplasia grade 2 and above (CIN2+), and were reviewed by one pathologist (SJR), blinded to DES-exposure status. Cases were assigned their highest grade at diagnosis. We identified CIS cases that were not included in our previous reports from the state cancer registry searches. "

When discussing the models that used dose, the authors should clarify that they are referring to dose of DES.

We have clarified for every use of dose in the manuscript that we are referring to DES dose.

In the discussion, I would change lower genital tract neoplasia to CIN 2+ because the authors only examined CIN2 + rates. The findings of increased risk with VEC should be highlighted, as it is significant.

We have changed "lower genital tract neoplasia" to CIN2+ on p.6 and 7 at the end of the Discussion. While the increased CIN2+ risk with VEC was suggestive, as the statistical reviewer notes, it was not statistically significant as the confidence interval included 1.0.

However, at this point in time, all of the women exposed to DES in utero are beyond age 45. To make the discussion/recommendation stronger, the authors should address this point and perhaps could suggest that for those women who have not had a history of

CIN 2+ or CCAM, that screening can be stopped at age 65, just as we do with non-DES exposed patients. Right now, the "why" of this study provides little applicability to real life patient care as these patients are all greater than age 45. But it could be used to postulate changes in screening, as other papers have argued for previously (see Wamakima et al in the Journal of Lower Genital Tract Disease).

We appreciate the reviewer pointing us to the paper by Wamakima et al. (Bridgette W Wamakima[1], Sara McKinney, Laura Bookman, Annika Gompers, Michele R Hacker, Huma Farid. Postmenopausal Vaginal and Cervical Cancer Risk Related to In Utero Diethylstilbestrol Exposure. J Low Genit Tract Dis.2023 Jan 1;27(1):35-39.) This study used a retrospective chart review at one institution of females prenatally exposed to DES 50 years and older. Out of 503 charts, 28 cases of gynecologic cancer occurrence were identified: 10 cervical cancers and one vaginal cancer. Only 1 woman of 503 developed a DES-related cervical or vaginal malignancy after age 50 years, and none after age 65 years. The authors concluded that DES related cancers are rare in women older than 50 years, and therefore that screening recommendations could be changed for these patients to align with current screening guidelines.

We have added this to the second paragraph of the Discussion: "A recent paper by Wamakima et al.[12] using a retrospective chart (n=503) review of females 50 years of age and older prenatally exposed to DES found that DES-related cancers were rare in women older than 50 years leading the authors to conclude that screening recommendations for DES exposed females could be changed to align with current guidelines."

STATISTICAL EDITOR COMMENTS:

lines 13-14 and 17-19: Need to reconcile the statements: "through age 44, but not after 45" vs ""through age 45".

Thank you for pointing out this inconsistency. The sentences now read: "Risk was significantly elevated into the mid-40s, confirming that more frequent cytological screening among DES-exposed females is appropriate through at least age 44. Whether females 45 years and older should continue to have increased screening would require careful weighing of possible risks and benefits."

line 81: Should include this data in supplemental material.

Line 81 reads "Limited cases of CIN3+ precluded meaningful analysis (not shown)." We had included this at the beginning of the results section: "Of these, the cumulative incidence of CIN3+ was 3.7% (CI 2.7%-4.7%) and 2.3% (CI 1.1%-3.5%), respectively." We thank the reviewer for this comment because we realized that we should include this information.

We have now deleted the phrase indicating that the number of cases of CIN3+ was limited and changed the text to the following:
"The fully adjusted HR for prenatal DES exposure and CIN3+ was 1.59 (CI 1.02-2.49). The HR for age at CIN3+ diagnosis <45 was 1.44 (CI 0.87-2.37) and 1.74 (CI 0.65-4.68) for 45+."

We have also added this finding to the abstract and discussion (p.7).

lines 81-82: Should include in Table the analysis for all ages aggregated thru age 44 yrs. In the Table, only the age < 30 had a statistically significantly elevated aHR for CIN2+ occurrence. The various age categories individually had relatively few counts and hence wide *and mostly NS) CIs. No doubt limited stats power played a large role. Similarly, the counts of those age 45* were relatively small. Although one cannot excluded. Therefore, one cannot conclude from these data that the risk is not increased for those women age 45+.

We have added the HR for <45 years to the Table (the HR for 45+ was already reported). We agree with the reviewer that a possible association in women 45+ cannot be ruled out. *We added this to the Discussion, p.9: Although the CI was wide, the HR for CIN2+ in women 45+ was elevated, so we cannot exclude the possibility of increased risk in older women."*

Should include in Table or in supplemental the extent of missing data, exclusion due to hysterectomy or loss to follow-up for the various groups, particularly by age.

We have added this to the Methods section: "The analyses focused on the first occurrence of pathology confirmed squamous cell CIN2+. Person-years at risk for each woman were computed from 1/1/82 (except for the WHS, for which follow-up started in 1994-1995) until the date of first documented diagnosis of CIN2+, date of last known follow-up, or date of last questionnaire response. The start of follow-up was chosen to correspond to the end of follow-up in the original cohort study of incident dysplasia among the DESAD cohort,[4] which comprises 70% of the current study population. Females were censored at the reported date of hysterectomy, except when CIN2+ was diagnosed at the time of that surgery. We did not censor for hysterectomy during follow-up because females were still at risk for vaginal and vulvar cancer. Participants who reported dysplasia on the questionnaire but for whom we were unable to obtain pathology records were censored at their reported diagnosis year.

We have added Table 1 to the manuscript with covariates by DES exposure status including percent missing. In addition, we have added tables to the supplement that includes loss to follow-up of DES exposed and unexposed participants by calendar year and by age. Loss to follow-up by exposure status did not differ by either calendar year or age.

lines 86-87: Neither comparison (with vs without VEC) reached statistical significance at .05 inference threshold.

> *We have revised the description of the analyses by VEC as follows: "Compared with unexposed females, CIN2+ risk was more evident in females with DES exposure early in gestation [<8 weeks (2.24; CI 1.43-3.52)], and those exposed to high DES doses (1.52; CI 1.04-2.22). Prenatal DES exposure and CIN2+ risk appeared positively associated among DES-exposed females with evidence of vaginal epithelial changes [(VEC) (1.48; CI 0.96-2.30)] but the confidence interval included 1.0, and there was no association among those without VEC (1.0; CI 0.62-1.62)."*