Effect of chemotherapy on the uterus of young adult cancer survivors

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Objective: To investigate the impact of chemotherapy on the uterus.
Design: Cross-sectional pilot study.
Setting: Single university fertility clinic.
Patient(s): Twelve patients with a history of alkylating agent chemotherapy exposure after Hodgkin lymphoma (cancer) vs. 12 normally menstruating women (controls).
Intervention(s): The inclusion criteria were age of 18–45 years and consent for endometrial biopsy. The exclusion criteria were the absence of the uterus, completed pelvic radiation, uterine or cervical cancer, and metastatic cancer. Each participant underwent endometrial biopsy and pelvic ultrasound. All study visits were conducted in the late proliferative phase of the menstrual cycle.
Main Outcome Measure(s): Uterine volume, blood flow, endometrial thickness, histology, deoxyribonucleic acid methylation pattern, and relative ribonucleic acid (RNA) expression level during the same phase of the menstrual cycle.
Result(s): In the study group, visits were conducted at a median of 31.5 (13.5–42.5) months after chemotherapy. The median uterine volume among cancer survivors was 36 (11.3–67) cm^3, and that of the general population controls was 39 (13–54) cm^3. On histologic examination, there were no cytologic or architectural atypia. The RNA-sequencing analysis revealed poor clustering of both control and treatment samples. However, we identified 3 differentially expressed genes on RNA-sequencing, but there was no concordance found among the differentially expressed genes and deoxyribonucleic acid methylation changes suggesting most likely false-positive results.
Conclusion(s): Approximately 2.5 years after chemotherapy, a time at which several survivors of Hodgkin lymphoma may resume family-building, endometrial thickness and endometrial histology were not significantly affected by a history of alkylating agent chemotherapy exposure. (Fertil Steril Rep® 2022;3:198–203. ©2022 by American Society for Reproductive Medicine.)
Key Words: Alkylating agent chemotherapy, Hodgkin lymphoma, uterus, cancer survivors

At the time of a new cancer diagnosis, adolescent and young adult patients cite future fertility as one of their top priorities (1). According to 2018 SEER statistics, approximately 125,000 reproductive-age women are newly diagnosed with cancer each year in the United States (2). Although we have developed numerous methods to protect the ovary, there is no information about how to protect the uterus from potential damage from chemotherapy (3). Recent epidemiologic studies have demonstrated increased risks of obstetric complications after exposure to chemotherapy (4–6). In these pregnancy studies, ovarian function was present after chemotherapy, yet untoward pregnancy outcomes were noted, including increased risks of low birth weight and preterm birth. As fertility preservation for cancer survivors evolves, increased focus is being applied to not simply achieving "a pregnancy" but doing our best to assure the healthiest pregnancy possible. The uterus is sensitive to chemotherapeutic agents especially endometrium and possibly myometrium (7, 8). However, uterine health is often overlooked with regard to fertility preservation in the context of chemotherapy.

Endometrial injury after chemotherapy has not been adequately studied; however, radiation-induced endometrial injury has been more systematically assessed. Exposure to radiation therapy has been associated with decreased uterine volume and elasticity, increased uterine fibrosis, and decreased uterine blood flow (9). Women treated with abdominopelvic

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radiation have an increased rate of uterine dysfunction leading to miscarriage, preterm labor, low birth weight, and placental abnormalities (9). Thus, because uterine cells are mitotically active, it is possible that chemotherapeutic agents have similar effects.

The effects of chemotherapy on the uterus may be detected at an ultrasonographic, histologic, or molecular level. In this study, we investigate the impact of Adriamycin, Bleomycin, Vinblastine, and Dacarbazine (ABVD) chemotherapy on uterine physical characteristics, blood flow, endometrial histology, and deoxyribonucleic acid (DNA) methylation and ribonucleic acid (RNA) sequencing (RNA-seq).

MATERIALS AND METHODS

This study was approved by the University of Utah Institutional Review Board. Because it was a study of patients with a history of cancer, the University of Utah's Cancer Protocol Review and Monitoring Committee also approved the study protocol.

Aims and Main Outcome Measures

This pilot study aimed to accomplish 3 distinct but related goals. First, we described the structural phenotype of uterine/endometrial injury after alkylating chemotherapy sonographically and histologically. Second, we investigated the molecular impact of alkylating chemotherapy exposure on messenger RNA expression and epigenetic changes in endometrial biopsy (EMB) tissue homogenates. Third, we investigated the feasibility of performing ultrasonographic and EMB-based assessments in reproductive-age cancer survivors. Because of limited information available in this area of inquiry, this approach is exploratory and should prove to be hypothesis-generating.

The main outcome measures of this study included endometrial thickness (EMT) and uterine volume on transvaginal ultrasound. Endometrial histology, DNA methylation, and RNA-seq on EMB samples, and pain scale assessment before and after EMB.

Study Subjects

Twelve patients who were diagnosed with Hodgkin lymphoma and had been exposed to ABVD chemotherapy and 12 "healthy endometrium" age-matched controls were recruited in this study. There is agreement in the literature that alkylating agents are the drug class most strongly associated with epithelial atypia (10). Decrabazine is an alkylating agent in ABVD regimen.

The inclusion criteria included women between 18 and 45 years who gave consent to undergo EMB and transvaginal ultrasound and had a negative pregnancy test result during the study visit.

The exclusion criteria included the absence of uterus (current or planned); pelvic radiation (completed or planned); uterine, cervical, or any metastatic cancer; suspicion for endometrial polyps, submucosal fibroids, or adenomyosis on ultrasound; estrogen and progesterone receptor (+) breast cancer; or other contraindications to hormone replacement therapy. We also excluded women who could not be followed up after chemotherapy or if they had any intrauterine surgery within the last 3 months. Ten participants in the study group had regular menstrual cycles, and 2 were given hormonal replacement therapy to induce menstrual cycles. All participants in the control group had regular menstrual cycles (mean, 28 days).

Study Procedures

Patients were recruited by convenience sampling, on the basis of the aforementioned inclusion and exclusion criteria. Informed consent was obtained at the intake visit to the University of Utah Fertility Clinic. Ultrasound measurements and endometrial biopsies were performed during the late proliferative phase of menstrual cycle to minimize the effect of different phases of menstrual cycle. The late proliferative phase of the menstrual cycles was determined to be between cycle days 9 and 12 in patients with a mean menstrual cycle of 28 days. Tissues were taken from the EMB performed in the late proliferative phase of menstrual cycle after menses and before ovulation to decrease the possibility of including an early pregnancy or contamination of the red blood cells from the menses. For women with ovarian dysfunction and amenorrhea related to their recent chemotherapy exposure, menstrual cycle was induced with a physiologic dose of estradiol patches (0.1 mg/24 hours) for at least 14 days, followed by 14 days of estrogen patches and micronized progesterone (200 mg orally daily). Three-dimensional ultrasound was performed to measure the following parameters: uterine volume; EMT; and endometrial volume. For each EMB, pipelle was advanced, and a maximum of 3 passes were allowed into the uterus to obtain adequate endometrial sample. The specimen was evaluated for any histopathological, DNA methylation, or RNA expression changes. One pathologist blinded to the study read these histopathology samples.

Histopathological Evaluation

For each patient, tissue had been fixed in 10% neutral-buffered formalin, embedded in paraffin, and stained with hematoxylin and eosin. Routinely processed tissue sections from the EMB specimens were evaluated for a variety of histopathological findings associated with chemotherapy-induced cytotoxic effects. The histologic findings included cellular atypia, nuclear cytoplasmic ratio, nuclear enlargement, nuclear membrane irregularity, and metaplasia in the endometrial glandular epithelium.

DNA Extraction and Bisulfite Conversion

Frozen endometrial tissue samples were simultaneously thawed at room temperature. Once thawed, the tissue was mechanically disrupted to prepare the samples for DNA extraction. Deoxyribonucleic acid was extracted using the DNeasy kit (Qiagen, Valencia, CA) following the manufacturer's recommendations. Once extracted, the endometrial DNA was bisulfite converted with the EZ DNA Methylation Kit (Zymo Research, Irvine, CA) following the manufacturer's recommendations specifically optimized for use with Illumina's

(Irvine, CA) methylation arrays. Once converted, DNA was delivered to the University of Utah Genomics Core Laboratory for hybridization and array processing.

The raw intensity values were used to determine the fraction methylation of endometrial DNA using the R package "minfi" (11). The β-values, the fraction methylation at each CpG site, were calculated by examining the methylation or lack of methylation intensities at each CpG site, using the following formulation: β-value = methylated/(methylated + unmethylated). The β-values ranged from 0–1, with 1 representing a fully methylated CpG site and 0 representing a fully unmethylated CpG site. These values reflected the population mean of methylation.

Differential Methylation Analysis

Several statistical analyses were used to perform differential methylation analysis with the R and USEQ software, including point data and global methylation analyses using R and regional methylation analysis using the methylation array scanner application in the USEQ software package. Point data assessed differential methylation at each individual CpG site between the control and treatment groups, and global analysis assessed any differences in the mean methylation signal across all CpGs tiled on the array. Regional methylation analysis was performed with a sliding window approach (12). In brief, the sliding window analysis used a 1-kb window and a Wilcoxon's signed rank analysis to identify the regions of differential methylation between controls and treated patients. We used the following thresholds in our determination of statistical significance: a phred-scaled false discovery rate (FDR) of at least 13 (the equivalent of an adjusted P value of.05) and an absolute log2 ratio of at least 0.2.

Epigenetic Age Calculation

The epigenetic age of the samples was calculated using the freely available DNA methylation age calculator from Dr. Steve Horvath's laboratory (13). This predictive model uses a total of 353 CpG sites that have been shown to be highly predictive of chronological age within a wide variety of cell types. We compared the epigenetic age to the actual (chronological) age to assess whether age acceleration patterns were present in tissues recently exposed to chemotherapy by subtracting the actual age from the epigenetic (calculated) age.

Endometrial Tissue RNA Isolation and RNA-Seq

Ribonucleic acid isolation from endometrial tissue was conducted using the Qiagen RNeasy Mini Kit according to the manufacturer's instructions. Briefly, tissue that had been stored at −80 °C was thawed at room temperature. Approximately 20 mg of tissue was submerged in RLT lysis buffer containing 1% β-mercaptoethanol. The tissue was disrupted by pipetting using a 1-mL pipette tip and homogenized by passing the lysate 10 times through a blunt 18-gauge needle, followed by 10 times through a 28-gauge needle. The homogenized lysate was centrifuged, and the supernatant or cleared lysate was collected and processed using the RNeasy Mini Kit according to the manufacturer's instructions, including an on-column DNase digestion. Isolated RNA quality was assessed using the Agilent 2200 TapeStation assay, and RNA quantity was measured using the Qubit fluorometer. The RNA integrity number for the samples ranged from 3.0–9.4 with a mean of 6.3.

Sequencing libraries were prepared by the Huntsman Cancer Institute High-Throughput Genomics Core Facility. Moreover, 300 ng of RNA from each sample was used for library preparation with the Illumina Stranded Total RNA Library Prep Kit with Ribo-Zero Plus. The libraries were sequenced on the Illumina NovaSeq platform to generate 150-bp paired-end reads. Sequencing generated between 4.0 $\times$ 10^7 and 7.2 $\times$ 10^7 million reads per sample, with a mean of 5.1 $\times$ 10^7 million reads.

RNA-Seq Analysis

We completed all RNA-seq analyses in R. First, the quality control of the 24 paired-end RNA-seq samples was performed using FastQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Using the package Rsubread, we then aligned RNA-seq reads to the human reference genome GRCh38, assigned features, and generated a read count matrix (14). Next, we normalized the raw read counts and calculated the differential expression using the package DESeq2 (15). Also using DESeq2, we performed a principal component analysis and plotted the results to analyze the variance between the treatment and control samples. Significantly differentially expressed genes were identified using an adjusted P value of $<.05$ and an absolute log2 fold change of >1 as the cutoffs.

Time Since Chemotherapy Analysis

Gene expression and DNA methylation vs. time since cancer treatment regression analysis () Time since cancer treatment in the treatment group varied from 13.5–42.5 months; therefore, we assessed the relationship between gene expression and DNA methylation patterns and time since cancer treatment. To conduct this, we performed a linear regression analysis in R between all genes or significantly differentially methylated region and the number of months since cancer treatment for each treatment sample. For gene expression analysis, we normalized the read counts across samples using the median of ratio method employed by the package DESeq2. Similarly, we calculated the mean DNA methylation across each significant region. We then performed regression analysis using the expression levels or fraction methylation values of each gene or genomic region and the number of months since cancer treatment for each treatment sample and plotted the results. P values and linear models were calculated for each regression analysis.

Pain Scale

We used the previously validated 11-point numeric scale with ranges from "0" representing 1 pain extreme (e.g., "no pain") to "10" representing the other pain extreme (e.g., "pain as bad as you can imagine" or "worst pain imaginable").

TABLE 1

Demographics of the study participants in medians and 25th and 75th percentiles.

	Case (n = 12)	Control (n = 12)	*P* value
Age	29.6 (25.1–39.7)	23.9 (22.5–28.7)	.10
BMI	22.2 (20.9–24.3)	22.9 (20.9–29.8)	.56
Ethnicity			.01
Non-Hispanic	12	7	
Hispanic	0	5	
Smoking			.30
Yes	1 (past smoker)	0	
No	11	12	
Gravida	1.25	0.33	.24
No. of living children	0.83	0.33	.41

Note: BMI = body mass index.

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Sample Size Calculation

Because of resource limitations in this pilot study, we were able to recruit 12 women into each group. Given 12 women in each group, a β of 0.2, α of 0.05, and expected standard deviation in ultrasound measurements in the postcancer treatment setting of 1.1 mm, we were powered to detect a difference of 1.3 mm (16–18).

Statistical Analyses

Statistical analysis of the demographic characteristics was performed in STATA (v.15) with 2-sided *P* values of $<.05$ considered significant. The unpaired *t* tests and Wilcoxon's rank sum tests were used to compare continuous data. Categorical data were compared using the χ^2 test. Parametric data were reported as means and standard deviations. Nonparametric data were reported as medians and 25th–75th percentiles. The univariate general linear model was used to determine whether time since chemotherapy predicted change in EMT. Notably, we excluded 1 treatment sample and 1 control sample from all downstream analyses because of progesterone levels exceeding 1.5 ng/mL, indicating a high probability of being in postovulation phase at the time of RNA extraction, which could confound results.

RESULTS

There was no difference in the age, body mass index, ethnicity, smoking, gravida, and number of living children between the study and control groups (Table 1). In the cancer group, study visits were conducted at a median of 31.5 (13.5–42.5) months after chemotherapy. There was no relationship between time since chemotherapy and EMT (linear model coefficient, 0.005 mm/month from chemotherapy completion; 95% confidence interval, 0.028–0.038; $P=.76$). The median uterine volumes for cancer survivors and the general population controls were 36 (11.3, 67) and 39 (13, 54) cm^3, respectively ($P=.70$, Table 2). The median EMT was 7.05 mm in the study group vs. 7.3 mm in the control group ($P=.78$). On histologic examination of the EMB slides, there was no cytologic or architectural atypia. None of the patients in the control and cancer groups reported substantial pain.

Histologic Findings

Most patients had proliferative-phase endometrium. One patient was reported as in the early secretory phase, and 2 were reported as in the menstrual pattern. Nuclear cytoplasmic ration (8% vs. 0%), nuclear enlargement (8% vs. 0%) and metaplasia (92% vs. 67%) were higher in the study group than in the control group; however, the difference was not statistically significant.

DNA Methylation

We identified no statistical significance between the groups in our single CpG or global differential methylation analysis. However, we were able to identify 4 different sites with modestly regional differential methylation: Chr11:34460107-34461029 (FDR, 14.65; located at the promoter of *CAT*) Chr17:8055360-8055889 (FDR, 16.78; located in the promoter and gene body of *PER1*); Chr12:115134148-115135701 (FDR, 16.01, which is not associated with a gene); and Chr6:32063619-32064957 (FDR, 20.22, located in the gene body of *TNXB*). These regions averaged 1,085 base pairs in length, and all displayed a loss of methylation on average in the treatment group (Supplemental Fig. 1, available online). We performed enrichment analysis using the Genomic Regions Enrichment of Annotations Tool (19). We identified no significant gene ontology term or pathway enriched in our regions of interest.

TABLE 2

Results of transvaginal ultrasound and blood work.

	Case (n = 11)	Control (n = 11)	*P* value
EMT (mm)	7.05 (5.9–10.2)	7.3 (6.2–8.8)	.78
Uterine volume (cm^3)	36 (11.3–67)	39 (13–54)	.70
FSH (IU/L)	4.94 (4.51–18.69)	6.69 (4.9–8.7)	.53
LH (IU/L)	12.95 (5.09–30.20)	12.56 (7.27–20.48)	.71
Estradiol (E2) (pg/mL)	106.26 (67.8–145.2)	66.53 (54.28–201.5)	.57
Progesterone (P4) (ng/mL)	0.44 (0.30–0.82)	0.62 (0.37–0.72)	.57
AMH (ng/mL)	0.61 (0.13–3.02)	2.89 (2.82–3.38)	.05

Note: AMH = antimüllerian hormone; EMT = endometrial thickness; FSH = follicle-stimulating hormone; LH = luteinizing hormone.

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Epigenetic Age Calculation

The Horvath epigenetic age calculator successfully predicted the chronological age in our samples with an R^2 of 0.7031 considering the control and sample groups together (Supplemental Fig. 2, available online). There was no statistical difference in the age acceleration patterns between the chemotherapy-exposed and control groups.

RNA-seq

Principal component analysis revealed little variance between the 2 groups (Supplemental Fig. 3A, available online). However, we identified 3 significantly differentially expressed genes: *STMN2*; *ADRA1B*; and *CTD-2349P21.9*, each with an adjusted *P* value of.0248 (Supplemental Fig. 3B). STMN2 was up-regulated in treatment samples (log2 fold change, 1.1305), whereas both ADRA1B and CTD-2349P21.9 were down-regulated (log2 fold changes, −2.7673 and −3.3225, respectively), but these genes all had very low total read counts in the samples, increasing the probability that the apparent changes are because of stochastic variation or a false-positive result. Because there were only 3 differentially expressed genes, gene set enrichment analysis could not be conducted. Manual inspection of the raw read counts showed that most patients had <20 reads. However, 1–2 patients had over 100 reads in each gene. Thus, it appears that the signal was driven completely by stochastic variation during sequencing or rare outliers and do not represent a uniform change on the basis of time since treatment.

Time Since Cancer Treatment Regression Analysis

A linear regression analysis of expression levels of significantly differentially expressed genes and time since cancer treatment showed only a very weak positive correlation for *ADRA1B* (slope, 0.71; adjusted R^2, 0.27) and *CTD-2349P21.9* (slope, 0.047; adjusted R^2, 0.046) (Supplemental Fig. 4A–C, available online). Therefore, while these genes are down-regulated after cancer treatment, their expression may slowly increase with time. Additionally, we assessed the DNA methylation levels at the genomic loci found to be differentially methylated in the treated group and found no significant correlation with time since chemotherapy exposure (Supplemental Fig. 4D–G). In fact, the nonsignificant trend moved away from the control mean in all 4 sites.

Pain Scale

None of the participants have a pain score that caused significant distress during the research visit.

DISCUSSION

To our knowledge, this is the first study to systematically evaluate the effects of alkylating agent chemotherapy on the human uterus and endometrial lining. We found no difference in EMT, uterine volume, blood flow to the uterus on pelvic ultrasound, and any histologic changes on the EMB slides in cancer survivors compared with those in the control group. On RNA-seq analysis, there were differentially expressed genes, and DNA methylation showed different sites with modest regional differential methylation. There was no concordance among the differentially expressed genes on RNA-seq and DNA methylation changes in our patient population signifying that DNA methylation changes may not be because of change in the RNA expression. It very likely that these changes in RNA-seq and DNA methylation are false positives. The pain scores were not different before and after EMB during the study visit in both groups, suggesting that it is feasible to study the endometrium among cancer survivors.

Chemotherapy exposure can cause epithelial atypia throughout the body (7). Over the last 65 years, the uterine endometrium has only been sporadically assessed for chemotherapy-induced cellular changes. Previous case reports and case series have suggested that chemotherapy causes injury to the uterine endometrium (20–23). Postchemotherapy endometrial cells also showed strong immunoreactivity for the cell death marker bcl-2 and the cell proliferation marker *MIB-1* (22). These case reports have noted histologic changes in form of fragmented endometrial glands with high numbers of mitotic figures that were arrested in metaphase, degenerative chromatin patterns, abundant microvacuolated cytoplasm, and enlarged nuclei (7). These changes were noted to be more focal, unlike radiation-induced changes, which tend to be more widely dispersed. In contrast, there was a lack of any significant histologic changes in the endometrium of the study group in this study. This may be because of the small sample size of this pilot study. Estradiol levels may be lower one may expect in the late proliferative phase of menstrual cycle. However, they were comparable between the 2 groups, indicating the similar phase of menstrual cycle during EMB.

We cannot generalize the results of our study to all cancer treatment types because our study group included a homogeneous population of White females who were all treated with ABVD chemotherapy for Hodgkin lymphoma. In addition, our controls included women from a heterogeneous population. Future research could focus on prospective analysis, where patients before chemotherapy serve as their own controls after chemotherapy. We also excluded 2 samples, 1 from the study group and other from the control group, because of elevated progesterone levels indicating high probability of being in the postovulation phase. In the future, studies should ideally match the same phase of the menstrual cycle during the study visit by the hormonal panel and temporal phase of the menstrual cycle.

There are several confounding factors that may have impacted our results. For example, chemotherapy regimens usually include multiple agents. The ABVD treatments given to all of our patients for hodgkin's lymphoma included 4 different types of drugs (Adriamycin, also known as doxorubicin/hydroxydaunorubicin, bleomycin, vinblastine, and dacarbazine) with differing doses and mechanisms of action. Therefore, it is difficult to make a conclusive statement about the impact of alkylating agent chemotherapy in isolation. However, we would expect the alkylating effects to predominate, as is seen in both ovarian biology and the aforementioned case series (7). In addition, age, ethnicity, gravida, or the overall health status of the patients after cancer treatment

may influence the endometrium. In this pilot study, we did not have sufficient numbers of patients to adequately control for such factors. Despite conducting ultrasound analysis, some patients may have undetected uterine pathologies. We did not have information if any pregnancies in these patients after chemotherapy could have altered uterine characteristics, vascularity, histology, DNA methylation, or RNA-seq findings. Ultrasound was performed on the same day when laboratory samples were drawn, and the elevated estradiol level in the study group suggests that the endometrium in this group may be intrinsically different. The estradiol levels may be lower one may expect in the late proliferative phase of menstrual cycle. However, they were comparable between the 2 groups, indicating the similar phase of menstrual cycle during EMB. Finally, the nature of this study does not allow for pregnancy outcomes to be assessed. If similar work is conducted in the future, larger sample sizes and tightly controlled sample collection with plan for long-term longitudinal follow-ups to monitor pregnancy outcomes will be essential.

This study has several strengths. First, the exposure (alkylating agent chemotherapy) was known to precede the measured outcome (change in the uterus). Thus, the temporal relationship between alkylating agent chemotherapy and changes in the uterus is clear. Second, the timeframe for the ultrasound and EMB after chemotherapy was also standardized in the late follicular phase with a time window of 4 days to minimize the effect of menstrual cycle phase on the results. Lastly, we included women who were at least 6 months out of their chemotherapy. Our mean follow-up time was 2.5 years. This is similar to the typical 2-year waiting time suggested by oncologists before trying to get pregnant (24).

CONCLUSION

In conclusion, in this exploratory study, we did not identify significant changes in the uterine volume or EMT after ABVD chemotherapy treatment. We also did not identify any DNA methylation alterations that appeared likely to affect gene expression. This pilot study also demonstrates that it is feasible to study the endometrium of cancer survivors with safe but relatively invasive procedures, such as pelvic ultrasound and EMB.

REFERENCES

1. Klosky JL, Simmons JL, Russell KM, Foster RH, Sabbatini GM, Canavera KE, et al. Fertility as a priority among at-risk adolescent males newly diagnosed with cancer and their parents. Support Care Cancer 2015;23:333–41.
2. National Cancer Institute. SEER*Explorer: an interactive website for SEER cancer statistics. Available at: https://seer.cancer.gov/explorer/. Accessed September 25, 2018.
3. Murphy D, Orgel E, Termuhlen A, Shannon S, Warren K, Quinn GP. Why healthcare providers should focus on the fertility of AYA cancer survivors: it's not too late! Front Oncol 2013;3:248.
4. Anderson C, Engel SM, Mersereau JE, Black KZ, Wood WA, Anders CK, et al. Birth outcomes among adolescent and young adult cancer survivors. JAMA Oncol 2017;3:1078–84.
5. Black KZ, Nichols HB, Eng E, Rowley DL. Prevalence of preterm, low birthweight, and small for gestational age delivery after breast cancer diagnosis: a population-based study. Breast Cancer Res 2017;19:11.
6. Shliakhtsitsava K, Romero SAD, Dewald SR, Su HI. Pregnancy and child health outcomes in pediatric and young adult leukemia and lymphoma survivors: a systematic review. Leuk Lymphoma 2017;59:381–97.
7. Kim EK, Yoon G, Kim HS. Chemotherapy-induced endometrial pathology: mimicry of malignancy and viral endometritis. Am J Transl Res 2016;8:2459–67.
8. Garg D, Johnstone EB, Lomo L, Fair DB, Rosen MP, Taylor R, et al. Looking beyond the ovary for oncofertility care in women: uterine injury as a potential target for fertility-preserving treatments. J Assist Reprod Genet 2020;37:1467–76.
9. Wo JY, Viswanathan AN. Impact of radiotherapy on fertility, pregnancy, and neonatal outcomes in female cancer patients. Int J Radiat Oncol Biol Phys 2009;73:1304–12.
10. Slavin RE, Millan JC, Mullins GM. Pathology of high dose intermittent cyclophosphamide therapy. Hum Pathol 1975;6:693–709.
11. Aryee MJ, Jaffe AE, Corrada-Bravo H, Ladd-Acosta C, Feinberg AP, Hansen KD, et al. Minfi: a flexible and comprehensive Bioconductor package for the analysis of Infinium DNA methylation microarrays. Bioinformatics 2014;30:1363–9.
12. Wang Z, Li X, Jiang Y, Shao Q, Liu Q, Chen B, et al. swDMR: a sliding window approach to identify differentially methylated regions based on whole genome bisulfite sequencing. PLoS One 2015;10:e0132866.
13. Horvath S. DNA methylation age of human tissues and cell types. Genome Biol 2013;14:R115.
14. Liao Y, Smyth GK, Shi W. The Subread aligner: fast, accurate and scalable read mapping by seed-and-vote. Nucleic Acids Res 2013;41:e108.
15. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol 2014;15:550.
16. Letur-Könirsch H, Guis F, Delanian S. Uterine restoration by radiation sequelae regression with combined pentoxifylline-tocopherol: a phase II study. Fertil Steril 2002;77:1219–26.
17. Momeni M, Rahbar MH, Kovanci E. A meta-analysis of the relationship between endometrial thickness and outcome of in vitro fertilization cycles. J Hum Reprod Sci 2011;4:130–7.
18. Greco E, Litwicka K, Arrivi C, Varricchio MT, Caragia A, Greco A, et al. The endometrial preparation for frozen-thawed euploid blastocyst transfer: a prospective randomized trial comparing clinical results from natural modified cycle and exogenous hormone stimulation with GnRH agonist. J Assist Reprod Genet 2016;33:873–84.
19. McLean CY, Bristor D, Hiller M, Clarke SL, Schaar BT, Lowe CB, et al. GREAT improves functional interpretation of cis-regulatory regions. Nat Biotechnol 2010;28:495–501.
20. Weston JT, Guin GH. Epithelial atypias with chemotherapy in 100 acute childhood leukemias. Cancer 1955;8:179–86.
21. Irving JA, McFarland DF, Stuart DS, Gilks CB. Mitotic arrest of endometrial epithelium after paclitaxel therapy for breast cancer. Int J Gynecol Pathol 2000;19:395–7.
22. Clement PB, Young RH. Endometrioid carcinoma of the uterine corpus: a review of its pathology with emphasis on recent advances and problematic aspects. Adv Anat Pathol 2002;9:145–84.
23. Wales C, Fadare O. Chemotherapy-associated endometrial atypia: a potential diagnostic pitfall. Int J Surg Pathol 2018;26:229–31.
24. Hartnett KP, Mertens AC, Kramer MR, Lash TL, Spencer JB, Ward KC, et al. Pregnancy after cancer: does timing of conception affect infant health? Cancer 2018;124:4401–7.