Analysis of Differential Effects of Hormonal and Copper Ion Intrauterine Devices on the Endometrial Transcriptome

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Abstract

The contraceptive effectiveness of intrauterine devices (IUDs) relies in part on a foreign body reaction in the endometrium. In a paper published by Nature in April 2020, Dr. Smith-McCune and colleagues analyze the mechanisms of action of various forms of contraceptives through measurement of their effects on both endometrial and cervical transcriptomes.[1] Biopsies were collected from women using levonorgestrel-releasing intrauterine system (LNG-IUS, n = 11), copper intrauterine devide (cu-IUD, n = 13), or levonorgestrel-containing combined oral contraceptives (COC, n = 12), as well as from women not using contraceptives (control, n=11). The study performs transcriptional profiling using Affymetrix arrays, Principal Component Analysis, and the 'limma' BioConductor package. EnrichR and Reactome 2016 are used to perform pathway analysis. Analysis of endometrial samples from cu-IUD users showed no genes with statistically significant differential expression when compared to controls. In LNG-IUS users, 2509 genes showed differential expression and were mapped primarily onto inflammatory and immune-related pathways. In COC users, 133 genes showed significant dysregulation, primarily in pathways relating to metal ion regulation. In cervical samples, no groups showed statistically significant differential gene expression compared to controls. In conclusion, hormonal and copper IUDs differ significantly in their effects on the endometrial transcriptome, while cu-IUD endometrium transcriptomes are indistinguishable from luteal phase endometrium transcriptomes. Together, the findings of the paper argue against the foreign body reaction as a common mechanism of intrauterine device action.

Purpose

In this analysis, we seek to confirm that the commonly understood mechanism of foreign body reaction does not present itself in the data analysis of endometrial and cervical transcriptomes in the Smith-McCune study by replicating the procedures described.

Introduction

The intrauterine devide (IUD) is among the most popular forms of contraception, used by approximately 14% of women globally.[2] Among the most common types of IUD are the levonorgestrel-realeasing hormonal IUD and a non-hormonal alternative which releases copper ions. Previous studies show that the IUD's contraceptive effectiveness is attributed in part to the imitation of these secretions as foreign bodies that trigger foreign body response in the form of local inflammation, as well as the adverse effects the secretions have on sperm and cervical mucus.[3][4]

Recent studies have detected altered expression in host defense-related genes of the endometrium and cervix after administration of progestin-containing contraceptives.[5] The local immune microenvironment of the upper female reproductive tract suggest that some foreign body, whether it be the IUD itself, the hormone, or a combinaton of both, is directly responsible for this discrepancy and the resulting effectiveness of the contraceptive. This study sought to isolate and quantify the effect of these variables through the use of a

contraceptive-free control group, a hormone-free cu-IUD group, an orally administered contraceptive group, and the LNG-IUS group.

Methods

Design

Dr. Smith-McCune's cross-sectional study compared the transcriptomes from the endometrium or cervical transformation zone from samples donated by 4 groups of women using: no hormonal or intrauterine contraception (control group), a cu-IUD (Paragard T 380 A, Cooper Surgical), LNG-IUS (Mirena, Bayer Healthcare Pharmaceuticals Inc.), or LNG-containing COC (not specified). The study protocol, materials, and procedures were performed in accordance with UCSF Human Research Protection Program and Institutional Review Board regulations.

Sample Collection Procedures

All participants whose samples were used in this study were first checked to ensure they had sufficient amounts of endometrial and/or cervical tissue. The women were healthy, aged 18-45 years, and recruited from the San Francisco Bay Area. COC participant used a cyclic 28-day regiment of ethinyl estrogen and 0.10-0.15 mg of LNG per tablet. Control and cu-IUD participants had to have regular periods (every 21-35 days). Several exclusin criteria were considered to reduce confounding. These included: hysterectomy, breast-feeding, being within 6 months of parturition, abnormal cervical cytology in the past year, and use of systemic corticosteroids or immune-modulating therapies. During screening, written informed consent was obtained from all participants, urine tests were administered for pregnancy, Chlamydia trachomatis and Neisseria gonorrheae, and blood tests for HIV serology; clinical evidence of vaginitis, vaginosis or pelvic inflammatory disease were exclusionary factors. During a follow-up study visit, participants were given kits and instructions for urine testing for luteinizing hormone (LH) (ClearBlue Ovulation Test Digital, Proctor and Gamble). Women in the control and cu-IUD groups underwent biopsies 7 to 11 days after a positive LH test result. Women using the LNG-IUS underwent biopsies 7 to 11 days after a positive LH test or after 2 months with no positive result, whichever came first. COC users underwent biopsies on day 12–16 of their pill pack.

To collect samples, the posterior vaginal fornix was swabbed with Q-tips to measure of pH and prostate specific antigen (Abacus Diagnostics), a marker of recent vaginal intercourse; a pH above 6.0 or positive PSA test led to exclusion of the sample from analysis. Endometrial biopsies were obtained with a 3mm biopsy cannula (Softflex Endometrial Biopsy Cannula, Integra Miltex) and tissue was collected with 1 to 2 passes. A Tischler forceps biopsy was performed at the cervical TZ, identified as the junction between Lugol's staining and non-staining epithelium; if the TZ was not seen, the biopsy was obtained with one of the biopsy prongs within the operating site. The group then collected blood for measurement of plasma progesterone level. Finally, the biopsies were snap frozen and stored at -80 degrees Fahrenheit for further analysis.

Sample Preparation

Once retrieved from the freezer, biopsy samples were minced into small fragments and total RNA was extracted and treated with DNAase using the NuceloSpin RNA II Kit (Marcherey-Nagel Inc.). RNA quality was assessed using Bioanalyzer 2100 (Agilent Technologies) and quantity and purity determined with a NanoDrop Spectrometer. The Ribo-SPIA technology NuGen Pico V2 was used for amplification, fragmentation and biotin-labelling. Labeled cDNA was hybridized to Human Gene 1.0 microarrays (Affymetrix) at UCSF. The signal intensity fluorescent images produced during the resulting Affymetrix GeneChip hybridizations were read using an Affymetrix Model 3000 Scanner. They were then converted into GeneChip probe results files (.cel) using Command and Expression Console software from Affymetrix.

Data Preprocessing

Finally, analyses were performed separately comparing each of the contraceptive groups with the control group for the cervical and the endometrial samples using a design matrix. The raw .cel files were read using the

read.celfiles function within the 'oligo' BioConductor package. The probes were matched to their corresponding gene symbols and ensembl ids using 'annotate' and 'hugene10sttranscriptcluster.db' Bioconductor packages. The expression of all genes were normalized across all the samples in the chosen contraceptive and control group using the Robust Multi-array Average (RMA) procedure. The resulting raw .cel files from the publication were then deposited in the NCBI Gene Expression Omnibus and accessed through GEO Series accession number GSE137765 (at the following link: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE137765).

Statistical Model

The top 500 most variable (across all samples) genes were chosen using the normalized data. The data corresponding to these 500 genes were used to perform Principal Component Analyses (PCA) using the prcomp function in R. The association of the expression of genes with the particular contraceptive use was estimated using the bioconductor package limma. The p-values reported by limma are adjusted for multiple testing using the False Discovery Rate (FDR) procedure.

For pathway analysis, the list of differentially expressed genes with adjusted $p <= 0.05$ were entered into EnrichR, a gene list enrichment analysis tool, and subsequent pathway analysis was performed through https://reactome.org/PathwayBrowser/. The outputs displaying pathways ranked by combined score, a calculation based on the p-value and the Z score $z * log(p)$, can be found in the Results section.

Results

Below are all packages used in the analysis which follows.

```
BiocManager::install("affy")
BiocManager::install("oligo")
BiocManager::install("limma")
BiocManager::install("Biobase")
BiocManager::install("hugene10sttranscriptcluster.db")
BiocManager::install("annotate")
BiocManager::install("enrichR")
```

Next, raw .cel data was imported. Root Mean-Adjusted Normalization was used to normalize the data, then the exprs function measured expression.

```
library("oligo")
celData=list.files("/Users/keanusida/Documents/BIOL599/Final Project/GSE137765_RAW", full=T)
setwd("/Users/keanusida/Documents/BIOL599/Final Project")
rawData=read.celfiles(celData)
normData=rma(rawData)
expData=exprs(normData)
```

Design Matrices

Next, 2 design matrices were created that incorporated each of the 3 treatment groups for cervical and endometrial tissue, respectively.

```
setwd("/Users/keanusida/Documents/BIOL599/Final Project")
designData=read.csv("DesignData.csv", header = FALSE, col.names = c("Sample", "Treatment"))

cervIndex=grep("cervical", designData$Treatment)
cervExprs=expData[,cervIndex]
cervCUIndex=grep("cu", designData$Treatment[cervIndex])
cervLNGIndex=grep("LNG", designData$Treatment[cervIndex])
cervCOCIndex=grep("COC", designData$Treatment[cervIndex])
cervDesign=matrix(data = 0, nrow = length(cervIndex), ncol = 4)
```

```
colnames(cervDesign)=c("Control", "Copper", "Hormonal", "Oral")
cervDesign[,1]=1
cervDesign[cervCUIndex,2]=1
cervDesign[cervLNGIndex,3]=1
cervDesign[cervCOCIndex,4]=1
```

```
endoIndex=grep("endo", designData$Treatment)
endoExprs=expData[,endoIndex]
endoCUIndex=grep("cu", designData$Treatment[endoIndex])
endoLNGIndex=grep("LNG", designData$Treatment[endoIndex])
endoCOCIndex=grep("COC", designData$Treatment[endoIndex])
endoDesign=matrix(data = 0, nrow = length(endoIndex), ncol = 4)
colnames(endoDesign)=c("Control", "Copper", "Hormonal", "Oral")
endoDesign[,1]=1
endoDesign[endoCUIndex,2]=1
endoDesign[endoLNGIndex,3]=1
endoDesign[endoCOCIndex,4]=1
```

Linear Modelling

Next, a linear model was produced using the cervical and endometrial expression data and their respective design matrices. The limma package was used to perform eBayes analysis, and a TopTable was subsequently produced for each treatment group. Gene IDs were imported using the corresponding Affymetrix packages.

```
library("limma")
library("annotate")
library("hugene10sttranscriptcluster.db")
annodb="hugene10sttranscriptcluster.db"
ID=featureNames(normData)
Symbol=as.character(lookUp(ID, annodb, "SYMBOL"))
Name=as.character(lookUp(ID, annodb, "GENENAME"))
Entrez=as.character(lookUp(ID, annodb, "ENTREZID"))
```

```
cervFit=lmFit(cervExprs, cervDesign)
cervFit=eBayes(cervFit)
cervFit$gene=Name
cervFit$symbol=Symbol
cervFit$ID=Entrez
```

```
endoFit=lmFit(endoExprs, endoDesign)
endoFit=eBayes(endoFit)
endoFit$gene=Name
endoFit$symbol=Symbol
endoFit$ID=Entrez
```

Differential Expression Analysis

```
cervCopperP=topTable(cervFit, coef = 2, adjust.method = "fdr", sort.by = "p", genelist=cervFit$gene, nu
length(which(cervCopperP<0.05))
```

```
## [1] 0
```

```
cervHormoneP=topTable(cervFit, coef = 3, adjust.method = "fdr", sort.by = "p", genelist=cervFit$gene, n
length(which(cervHormoneP<0.05))
```

```
## [1] 0
```

```
cervOralP=topTable(cervFit, coef = 4, adjust.method = "fdr", sort.by = "p", genelist=cervFit$gene, numb
length(which(cervOralP<0.05))
```

```
## [1] 0
```

As shown in the original study, the cervical tissue expression data contain no differential gene expression with a significant ($p < 0.05$) adjusted P value.

```
cervCopperIDs=topTable(cervFit, coef = 2, adjust.method = "fdr", sort.by = "p", genelist = cervFit$gene
cervCopperIDs=cervCopperIDs[-which(cervCopperIDs == "NA")]
cervCopperIDs[1:10]
```

```
##  [1] "zinc finger protein, FOG family member 2"
##  [2] "protein phosphatase 4 catalytic subunit"
##  [3] "H1.6 linker histone, cluster member"
##  [4] "malate dehydrogenase 1B"
##  [5] "small nucleolar RNA, C/D box 114-2"
##  [6] "LZTS1 antisense RNA 1"
##  [7] "xylosyltransferase 2"
##  [8] "tetratricopeptide repeat domain 26"
##  [9] "NOVA alternative splicing regulator 1"
## [10] "male germ cell associated kinase"
```

```
cervHormoneIDs=topTable(cervFit, coef = 3, adjust.method = "fdr", sort.by = "p", genelist = cervFit$gen
cervHormoneIDs=cervHormoneIDs[-which(cervHormoneIDs == "NA")]
cervHormoneIDs[1:10]
```

```
##  [1] "carbonic anhydrase 5A"
##  [2] "uncharacterized LOC101928068"
##  [3] "prostaglandin E receptor 3"
##  [4] "laminin subunit beta 1"
##  [5] "noggin"
##  [6] "deoxyuridine triphosphatase"
##  [7] "apolipoprotein D"
##  [8] "inhibitor of DNA binding 4, HLH protein"
##  [9] "four and a half LIM domains 5"
## [10] "TAFA chemokine like family member 1"
```

```
cervOralIDs=topTable(cervFit, coef = 4, adjust.method = "fdr", sort.by = "p", genelist = cervFit$gene, 
cervOralIDs=cervOralIDs[-which(cervOralIDs == "NA")]
cervOralIDs[1:10]
```

```
##  [1] "neuroligin 1"
##  [2] "formin homology 2 domain containing 1"
##  [3] "8-oxoguanine DNA glycosylase"
##  [4] "potassium voltage-gated channel interacting protein 4"
##  [5] "hes related family bHLH transcription factor with YRPW motif like"
##  [6] "collagen type V alpha 1 chain"
##  [7] "long intergenic non-protein coding RNA 597"
##  [8] "apolipoprotein E"
##  [9] "small nucleolar RNA, C/D box 113-4"
## [10] "collagen type XXVIII alpha 1 chain"
```

The genes exhibiting the most significant p-values do not seem to share a common function or pathway. This is expected, as the adjusted P value analysis above suggests there is no significant differential expression.

```
endoCopperP=topTable(endoFit, coef = 2, adjust.method = "fdr", sort.by = "p", genelist=endoFit$gene, nu
length(which(endoCopperP<0.05))
```

```
## [1] 15
```

```
endoHormoneP=topTable(endoFit, coef = 3, adjust.method = "fdr", sort.by = "p", genelist=endoFit$gene, n
length(which(endoHormoneP<0.05))
```

```
## [1] 2963
```

```
endoOralP=topTable(endoFit, coef = 4, adjust.method = "fdr", sort.by = "p", genelist=endoFit$gene, numb
length(which(endoOralP<0.05))
```

```
## [1] 191
```

The above data once again corroborate the data professed in the Smith-McCune study. Though exact numbers vary slightly likely due to lack of access to demographic data (the age of participants was used as an additional factor in the design matrix), the endothelial tissue expression data contain numerous genes with significant ($p < 0.05$) adjusted P values. As shown in the Smith-McCune study, the copper IUD shows very few differentially expressed genes (15), while the oral contraceptive shows several hundred (191), and the hormonal IUD has the greatest gene expression disturbance with several thousand significant genes (2963).

```
endoCopperIDs=topTable(endoFit, coef = 2, adjust.method = "fdr", sort.by = "p", genelist = endoFit$gene
endoCopperIDs=endoCopperIDs[-which(endoCopperIDs == "NA")]
endoCopperIDs
```

```
##  [1] "PDZK1 interacting protein 1"
##  [2] "C-C motif chemokine ligand 20"
##  [3] "serpin family B member 7"
##  [4] "complement factor B"
##  [5] "complement factor B"
##  [6] "baculoviral IAP repeat containing 3"
##  [7] "ubiquitin D"
##  [8] "complement factor B"
##  [9] "ATP binding cassette subfamily A member 13"
## [10] "TNF alpha induced protein 3"
## [11] "vanin 3"
## [12] "ubiquitin D"
## [13] "haptoglobin"
```

```
endoHormoneIDs=topTable(endoFit, coef = 3, adjust.method = "fdr", sort.by = "p", genelist = endoFit$gene
endoHormoneIDs=endoHormoneIDs[-which(endoHormoneIDs == "NA")]
endoHormoneIDs[1:12]
```

```
##  [1] "cannabinoid receptor 1"
##  [2] "epiphycan"
##  [3] "metallothionein 1F"
##  [4] "metallothionein 1G"
##  [5] "C-C motif chemokine ligand 22"
##  [6] "mannose receptor C-type 1"
##  [7] "mannose receptor C-type 1"
##  [8] "metallothionein 1M"
##  [9] "cysteine rich secretory protein 3"
## [10] "glucosaminyl (N-acetyl) transferase 3, mucin type"
## [11] "interleukin 1 receptor like 1"
## [12] "metallothionein 1H"
```

```
endoOralIDs=topTable(endoFit, coef = 4, adjust.method = "fdr", sort.by = "p", genelist = endoFit$gene, n
endoOralIDs=endoOralIDs[-which(endoOralIDs == "NA")]
endoOralIDs[1:12]
```

```
##  [1] "metallothionein 1G"               "metallothionein 1M"
##  [3] "metallothionein 1L, pseudogene"   "metallothionein 1F"
##  [5] "metallothionein 1X"               "metallothionein 2A"
##  [7] "metallothionein 2A"               "metallothionein 1D, pseudogene"
##  [9] "metallothionein 1H"               "metallothionein 1J, pseudogene"
## [11] "metallothionein 2A"               "solute carrier family 5 member 1"
```

For endometrial tissues, the above genes were differentially expressed in their corresponding treatment group. Thus, I stored them for pathway analysis. It is worth noting that both the above results and those found in the Smith-McCune study contain strong presence of differentially expressed metallothionein family proteins, specifically 1F/1G.

Pathway Enrichment Analysis

Finally, the list of differentially expressed genes is entered into the enrichR function. Results are analyzed using the Reactome 2016 database.

```
library("enrichR")
dbs=listEnrichrDbs()
endoCopperSyms=topTable(endoFit, coef = 2, adjust.method = "fdr", sort.by = "p", genelist = endoFit$symb
endoCopperSyms=endoCopperSyms[-which(endoCopperSyms == "NA")]
endoCopperSyms
```

```
##  [1] "PDZK1IP1" "CCL20"    "SERPINB7" "CFB"      "CFB"      "BIRC3"
##  [7] "UBD"      "CFB"      "ABCA13"   "TNFAIP3"  "VNN3"     "UBD"
## [13] "HP"
```

```
endoHormoneSyms=topTable(endoFit, coef = 3, adjust.method = "fdr", sort.by = "p", genelist = endoFit$sy
endoHormoneSyms=endoHormoneSyms[-which(endoHormoneSyms == "NA")]
endoHormoneSyms[1:20]
```

```
##  [1] "CNR1"     "EPYC"     "MT1F"     "MT1G"     "CCL22"    "MRC1"
##  [7] "MRC1"     "MT1M"     "CRISP3"   "GCNT3"    "IL1RL1"   "MT1H"
## [13] "CCL2"     "PHYHIPL"  "C6orf141" "SLC5A1"   "CD24"     "HGD"
## [19] "HGD"      "DTNA"
```

```
endoOralSyms=topTable(endoFit, coef = 4, adjust.method = "fdr", sort.by = "p", genelist = endoFit$symbol
endoOralSyms[1:20]
```

```
##  [1] "MT1G"   "MT1M"   "MT1L"   "MT1F"   "NA"     "MT1X"   "MT2A"   "MT2A"
##  [9] "MT1DP"  "MT1H"   "MT1JP"  "MT2A"   "SLC5A1" "MT1HL1" "MT1E"   "GCNT3"
## [17] "HEY1"   "BNC2"   "S100P"  "DHCR24"
```

```
copperPaths=enrichr(endoCopperSyms, "Reactome_2016")
```

```
## Uploading data to Enrichr... Done.
##   Querying Reactome_2016... Done.
## Parsing results... Done.
```

```
i1=which(copperPaths$Reactome_2016$Adjusted.P.value<0.05)
copperPaths=copperPaths$Reactome_2016$Term[i1]
hormonePaths=enrichr(endoHormoneSyms, "Reactome_2016")
```

```
## Uploading data to Enrichr... Done.
```

```
##   Querying Reactome_2016... Done.
## Parsing results... Done.
```

```
i2=which(hormonePaths$Reactome_2016$Adjusted.P.value<0.05)
hormonePaths=hormonePaths$Reactome_2016$Term[i2]
length(hormonePaths)
```

```
## [1] 77
```

```
hormonePaths[1:10]
```

```
##  [1] "Immune System Homo sapiens R-HSA-168256"
##  [2] "Hemostasis Homo sapiens R-HSA-109582"
##  [3] "Innate Immune System Homo sapiens R-HSA-168249"
##  [4] "Platelet activation, signaling and aggregation Homo sapiens R-HSA-76002"
##  [5] "Immunoregulatory interactions between a Lymphoid and a non-Lymphoid cell Homo sapiens R-HSA-198
##  [6] "GPVI-mediated activation cascade Homo sapiens R-HSA-114604"
##  [7] "Cytokine Signaling in Immune system Homo sapiens R-HSA-1280215"
##  [8] "Adaptive Immune System Homo sapiens R-HSA-1280218"
##  [9] "Metabolism Homo sapiens R-HSA-1430728"
## [10] "Cell surface interactions at the vascular wall Homo sapiens R-HSA-202733"
```

```
oralPaths=enrichr(endoOralSyms, "Reactome_2016")
```

```
## Uploading data to Enrichr... Done.
##   Querying Reactome_2016... Done.
## Parsing results... Done.
```

```
i3=which(oralPaths$Reactome_2016$Adjusted.P.value<0.05)
oralPaths=oralPaths$Reactome_2016$Term[i3]
oralPaths
```

```
## [1] "Response to metal ions Homo sapiens R-HSA-5660526"
## [2] "Metallothioneins bind metals Homo sapiens R-HSA-5661231"
```

The above pathway enrichment analysis shows pathway enrichment for both the hormonal IUD and oral contraceptive treatment groups. In the hormonal IUD treatment group, a total of 77 pathways showed significant ($p < 0.05$) enrichment, most notably those involved in Immune response and homeostasis. In the oral contraceptive treatment group, metal ion response and metallothionein pathways showed significant ($p < 0.05$) enrichment.

Discussion

The Smith-McCune study demonstrated that, of the three contraceptive methods examined, the LNG-IUS had the strongest effect on the endometrial transciptome, causing significant alterations in genes regulating a number of immune and inflammatory pathways. The study was performed in part to test the hypothesis that different forms of IUDs would ellicit common changes in endometrial transcriptomes. However, while the LNG-IUS resulted in differential expression of over 2509 genes, the cu-IUD group showed no significant differential expression or pathway enrichment. These findings suggest that the action behind the inflammatory response observed in the LNG-IUS is due to the levonorgestrel released by the IUD rather than the foreign body of the IUD itself. Previous studies show LNG plays a role in oxidative stress and apoptosis, which corroborates effects observed in the endometrial transcriptome.[6] Participants were required to have had the IUD in for >= 6 months, so we do not know how or if transcriptional differences are related to length of use of the device. Prior studies have shown foreign body reactions to cu-IUDs and subsequent leukocyte infiltration in the endometrium[7]; that this effect wasn't reflected in our transcriptome analysis can be attributed to the large surface area of the uterine cavity sampled. Small portions of stroma tissue accompanying the endometrial biopsy could have blunted observable immunohistochemistry.

Prior endometrial RNA expression analysis in users of a Lippes loop IUD (an inert IUD, similar to the cu-IUD) demonstrated significant differential expression in 147 genes in the first month after IUD insertion.[8] In this study, no significantly differentially expressed genes appeared in cu-IUD users with a median use of 23 months. The increased length of IUD exposure in this study suggests that the effects observed in the prior study might be attenuated over time. The statistical methods used to analyze gene expression were also different. Nonetheless, temporal effects may be worth investigating in future studies. These results suggest that the effects of the cu-IUD on the endometrial transcriptome are subtle and could be dominated by a natural process such as the progesterone effects of the luteal phase.

As shown by both the UCSF analysis and my own, the effect of COCs on the endometrial transcriptome was intermediate between the cu-IUD and LNG-IUS. Unlike the LNG-IUS, COCs did not cause changes in inflammatory pathways. Similarly, however, it did cause differential expression in several members of the metallothionein (MT) gene family. MT has well-documented roles in binding of heavy metals including copper for homeostasis and detoxification, and is increasingly being recognized for its role in immunomodulation, apoptosis and the stress response.[9] The contraceptive efficacy of copper ions from the cu-IUD can be attributed to its spermicidal effects but we did not observe a change in MT gene expression in the cu-IUD group. The effects on metallithionein gene expression in both hormone-containing contraceptive groups but not in the cu-IUD group supports a common effect of LNG on the stress response functions of endometrial MT transcription.

The parallel processing of samples from 4 groups of women provided direct comparisons between contraceptive transcriptomes that had up to the point of the UCSF study never been previously performed. The nuanced and rigorous experimental design of the sample preparation portion of the study allowed for distinct detection of differences between the groups. However, it also shrunk the sample sizes ($10 < n < 14$) of the treatment groups, which may have concealed subtle differences between some of the groups. Finally, it is worth noting that the cu-IUD can not be compared without bias to the LNG-IUS. Rather, the ideal comparison would be between identical IUDs with and without LNG. Nevertheless, the above findings indicate a strong effect of local LNG on gene expression in the endometrium.

Finally, in support of these findings, my analyses found similar significant numbers, genes, and pathways. Minor discrepancies between the results of my analysis and that of the team at UCSF were a result of the lack of publicly accessible age data that sought to reduce confounding in the UCSF study. This confounding made it appear as though there were slightly more significant genes in each treatment group than with a more refined experimental design, but did not drastically change the relative intensities of each treatment group's differential expression significance. Collectively, these results suggest that the presence of a foreign body has less effect on the endometrium than locally released LNG and argue against a foreign body reaction as a common mechanism of action of IUDs.

Appendix

R code can be found in the *.Rmd file attached to this report.

Raw TAR.gz data can be downloaded from the Gene Expression Omnibus at the following link:

https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE137765

References

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