Check for updates

# Macrophages display proinflammatory phenotypes in the eutopic endometrium of women with endometriosis with relevance to an infectious etiology of the disease

Júlia Vallvé-Juanico, Ph.D.,[a,b,c] Xavier Santamaria, M.D., Ph.D.,[b,c,d] Kim Chi Vo, B.Sc.,[a] Sahar Houshdaran, Ph.D.,[a] and Linda C. Giudice, M.D., Ph.D.[a]

[a] Center for Reproductive Sciences, Department of Obstetrics, Gynecology and Reproductive Sciences, University of California, San Francisco, California; [b] Department of Gynecology, IVIRMA Barcelona S.L., and [c] Group of Biomedical Research in Gynecology, Vall Hebron Research Institute and University Hospital, Barcelona, Spain; and [d] Igenomix, Valencia, Spain

**Objective:** To phenotype transcriptomically M1 macrophages (Mϕ1) and M2 macrophages (Mϕ2) in the endometrium of women with endometriosis.
**Design:** Prospective experimental study.
**Setting:** University research laboratory.
**Patient(s):** Six women with endometriosis and five controls without disease, in the secretory phase of the menstrual cycle.
**Intervention(s):** Mϕ1, Mϕ2, uterine natural killer, and T regulatory cells were isolated from human endometrium using a uniquely designed cell-specific fluorescence activating cell sorting panel. Transcriptome profiles were assessed by RNA high sequencing, bioinformatics, and biological pathway analyses.
**Main Outcomes Measure(s):** Differential gene expression between Mϕ1 and Mϕ2 in women with and without endometriosis and in Mϕ1 versus Mϕ2 in each group was determined and involved different biologic and signaling pathways.
**Result(s):** Flow cytometry analysis showed no significant differences in total numbers of leukocytes between control and endometriosis groups, although Mϕ1 were higher in the endometriosis group versus controls. Statistical transcriptomic analysis was performed only in Mϕ1 and Mϕ2 populations due to larger sample sizes. Bioinformatic analyses revealed that in women with endometriosis, endometrial Mϕ1 are more proinflammatory than controls and that Mϕ2 paradoxically have a proinflammatory phenotype.
**Conclusion(s):** As Mϕ are phenotypically plastic and their polarization state depends on their microenvironment, the altered endometrial environment in women with endometriosis may promote endometrial Mϕ2 polarization and an Mϕ1 proinflammatory phenotype. Moreover, aberrant phenotypes of Mϕ may contribute to abnormal gene expression of the eutopic endometrium and a proinflammatory environment in women with endometriosis relevant to the pathophysiology of the disease and compromised reproductive outcomes. (Fertil Steril® 2019;112:1118-28. ©2019 by American Society for Reproductive Medicine.)
**El resumen está disponible en Español al final del artículo.**

**Key Words:** Endometriosis, macrophages, inflammation, endometrium, infection

**Discuss:** You can discuss this article with its authors and other readers at **https://www.fertstertdialog.com/users/16110-fertility-and-sterility/posts/52535-28440**

Received June 10, 2019; revised August 1, 2019; accepted August 19, 2019.
J.V.-J. has nothing to disclose. X.S. has nothing to disclose. K.C.V. has nothing to disclose. S.H. has nothing to disclose. L.C.G. has nothing to disclose.
Funding was provided by National Institutes of Health *Eunice Kennedy Shriver* National Institute of Child Health and Human Development grant no. HD055764-12 (to L.C.G.), the Kerfuffle Foundation (to L.C.G.), an SRI International Training Grant (to J.V.-J.), Industrial Doctorate ID-2015-074 (AGAUR, Spain, to J.V.-J.), and National Institutes of Health grant no. S10 1S10OD021822-01 supporting the Aria Fusion Jabba the Hutt in the University of California, San Francisco, Cytometry Core.
S.H. and L.C.G. should be considered similar in author order.
Reprint requests: Linda C. Giudice, M.D., Ph.D., 513 Parnassus Avenue, HSE 1619, Center for Reproductive Sciences, Department of Obstetrics, Gynecology, and Reproductive Sciences, University of California, San Francisco, California 94153 (E-mail: linda.giudice@ucsf.edu).

Fertility and Sterility® Vol. 112, No. 6, December 2019 0015-0282/$36.00
Copyright ©2019 American Society for Reproductive Medicine, Published by Elsevier Inc.
https://doi.org/10.1016/j.fertnstert.2019.08.060

Endometriosis is an estrogen-dependent inflammatory disease that results in pelvic pain and/or infertility. It affects approximately 10% of reproductive-age women (1–5) and is characterized by the presence of endometrium-like tissue outside the uterus where it elicits an inflammatory response (1, 3–5). The eutopic endometrium of women with endometriosis has been widely studied with regard to dysfunctionality of steroid hormone response, stem cell populations, and recruitment of immune populations for immune tolerance and overall tissue homeostasis and pregnancy success versus women without disease (1–5). However, there are scant data about the function and phenotypes of eutopic endometrial immune cells in women with and without endometriosis. As the endometrial immune niche involves multiple cell types with varying degrees of activation and communications among immune and nonimmune cells that dictate functionality of the tissue, characterizing the endometrial immune niche is of great relevance to understanding endometrial function and dysfunction.

Uterine natural killer cells (uNK) secrete angiogenic factors that contribute to the maturation of blood vessels that have a role in embryo implantation and successful pregnancy (6, 7). In healthy endometrium, their cytotoxic activity diminishes during the secretory phase of the menstrual cycle, which allows embryo implantation (8–10). However, in infertile patients with endometriosis, uNK have high cytotoxicity in the eutopic endometrium that could lead to an inhospitable environment for embryo implantation (11). Other immune cells, such as T regulatory cells (Treg), have also been described to behave differently in the endometrium of women with endometriosis. In healthy endometrium, they increase in the proliferative and decrease in the secretory phase, with the latter creating an immunotolerant environment allowing embryo implantation. However, in infertile women with endometriosis, Treg are increased in the peri-implantation endometrium, leading to an implantation failure (12).

Endometriosis has been referred to as "a disease of the macrophage" (13), based mainly on a replete literature on the roles and functionality of this cell type in the peritoneal fluid of women with disease and in the establishment of endometriosis lesions and associated processes of angiogenesis and fibrosis. Mϕ are key effector cells in both innate and humoral immunity as they phagocytose pathogens, act as antigen-presenting cells, and have a role in tissue regeneration, angiogenesis, and wound healing (14). In the eutopic endometrium of women without endometriosis, their numbers vary throughout the menstrual cycle, increasing in the secretory and menstrual phases (15). This increase may be attributed to their phagocytic properties and role in clearing cell debris and apoptotic cells during endometrial shedding (16). Cycle variation among endometrial Mϕ does not occur in women with endometriosis (17), suggesting that the survival of shed and refluxed endometrial cells may be enhanced, enabling them to migrate to the peritoneal cavity and establish disease. Mϕ are classified as either "classically activated" Mϕ (Mϕ1) or "alternatively activated" Mϕ (Mϕ2) (14), and, depending on the microenvironment, they can switch from one type to the other (18). Mϕ1 have a role in proinflammatory responses, whereas Mϕ2 are involved in angiogenesis, anti-inflammatory reactions, and tissue repair (14, 19). In healthy endometrium, the predominant population is Mϕ2 (19, 20), suggesting that the normal environment is anti-inflammatory. Taken together, most studies of the eutopic endometrium have focused on the number of immune cells in this tissue and how they fluctuate throughout the cycle, but little is known about their functionality in women with endometriosis.

Herein, we designed a novel flow cytometry panel to isolate Mϕ1, Mϕ2, Treg, and uNK from the eutopic endometrium of women with endometriosis and those with no evidence of disease. RNA high sequencing (RNA-seq) was used to elucidate Mϕ1 and Mϕ2 phenotypes and possible functions in disease. Overall, the data support a phenotypic switch of the common anti-inflammatory Mϕ2 to the proinflammatory Mϕ1 phenotype and a more exaggerated proinflammatory phenotype of the Mϕ1 population in women with endometriosis.

## MATERIALS AND METHODS

### Sample Collection and Processing

Eleven endometrial biopsies in the secretory phase were collected: six from women with endometriosis (stages I–IV) and five from women with no evidence of endometriosis at the time of the surgery for benign gynecologic disorders. The mean age was 37 and 42 (23–49 years), respectively. To evaluate whether the age could confound the results, a nonparametric *t* test with a subsequent Mann-Whitney test ($P<.05$) was performed, and no significant differences between groups were found ($P=.2641$). Patients had not used hormone therapy for at least 3 months before the study. Endometrial samples were obtained through the University of California, San Francisco, National Institutes of Health Human Endometrial Tissue and DNA Bank under approval of the University of California, San Francisco, Committee on Human Research (IRB no. 10-02786), and written informed consent was obtained from all participants. Endometrial tissue was digested as described elsewhere (21). Briefly, it was minced mechanically and incubated for 1 hour at 37°C in digestion media, which contained collagenase type I and hyaluronidase (21). Subsequently, the single-cell suspension was filtered using a 40 $\mu$m mesh to discard cell clumps, and single cells were cryopreserved in liquid nitrogen until use.

### Flow Cytometry Panel Design

A cytometry panel of 10 conjugated antibodies able to separate the immune populations of interest (Mϕ1, Mϕ2, Treg, and uNK) was designed. Specific membrane markers of resident and blood infiltrating immune cells were included to avoid sorting cells derived from the peripheral circulation. The brightest colors were used for the markers with the lowest antigen density. Minimum overlapping of 11 colors (10 antibodies plus the live/dead dye) was achieved. The markers and lasers used for each population are shown in Table 1, and the gating strategy is shown in Figure 1A. First, the cells were

**TABLE 1**

**Antibodies used for the FACS.**

| Marker | Fluorochrome (excitation/emission) | Conjugated antibody | Manufacturer and reference | FACS Aria Laser |
|---|---|---|---|---|
| Live/dead | Aqua (405 nm/512 nm) | — | ThermoFisher (L34957) | Violet E |
| CD45 | BV605 (405 nm/600 nm) | CD45-BV605 | BD Bioscience (BDB 564047) | Violet |
| CD14 | PE (496 nm/578) | CD14-PE | Biolegend (301805) | YGD |
| CD163 | PE-Cy7 (561 nm/785 nm) | CD163-PE-Cy7 | Biolegend (333614) | YGA |
| CD80 | PerCP-Cy5.5 (488 nm/695 nm) | CD80-PerCP-Cy5.5 | Biolegend (305231) | Blue A |
| CD3 | BUV737 (355 nm/737 nm) | CD3-BUV737 | BD Bioscience (BDB 564307) | UVA |
| CD4 | BUV395 (355 nm/395 nm) | CD4-BUV395 | BD Bioscience (BDB 563550) | UVC |
| CD25 | BB515 (488 nm/515 nm) | CD25-BB515 | BD Bioscience (564468) | Blue B |
| CD69 | APC-Cy7 (640 nm/785 nm) | CD69-APC-Cy7 | BD Bioscience (BDB 560737) | Red A |
| CD56 | BV421 (405 nm/421 nm) | CD56-BV421 | BD Bioscience (562752) | Violet F |
| CD16 | APC-Cy7 (640 nm/660 nm) | CD16-APC | Biolegend (302011) | Red C |

*Vallvé-Juanico. Endometrial macrophages in endometriosis. Fertil Steril 2019.*

gated with CD45 (leukocyte marker), conjugated with brilliant violet 605 (BV605). In the case of Mϕ, usually this population is a resident tissue population, and thus no specific tissue markers were used. It is challenging to differentiate between the Mϕ1 and Mϕ2 subpopulations, as they have some common markers and can polarize from one type to the other. Our strategy was as follows: for both types, CD14 marker conjugated with phycoerythrin (PE) was used. For Mϕ2, CD163, a specific marker for this population, was used conjugated with PE-cyanin7 (PE-Cy7). As there are no specific markers for Mϕ1 and it was suspected that the concentration of activated Mϕ1 would be low in the endometrium (as Mϕ2 are higher than Mϕ1 in normal endometrium) (19), CD80 (activation marker) conjugated with peridinin chlorophyll protein complex-cyanin 5.5 (PerCP-Cy5.5) antibody, a bright dye, was used. Treg express CD3 and CD4 markers. The most accepted specific marker for Treg is Foxp3. This is an intracellular marker and thus could not be used for sorting. However, Treg also express CD25. To be able to discern between tissue Treg and Treg deriving from circulation, CD69, an activation marker for Treg that is expressed in tissue, was included. Thus, $CD3^{+}CD4^{+}CD25^{+}CD69^{+}$ cells (tissue Treg) were isolated. The CD3 antibody was conjugated with ultraviolet B 737 (BUV737), as was the CD4 antibody with ultraviolet B 395 (BUV395). CD25 has a low antigen density; therefore, brilliant blue 515 (BB515) was used, which is one of the brightest dyes. Then CD69, a tissue activation Treg marker, was conjugated with allophycocyanin-cyanin7 (APC-Cy7), assuring that only resident Treg were isolated. For uNK cells, which are $CD56^{+}$, as it is known that blood NK are $CD16^{+}$, whereas uNK are $CD16^{Low/-}$ (22–25), $CD56^{+}CD16^{-}$ were collected. For CD56, brilliant violet 421 (BV421), was used, and in the case of CD16, APC conjugated antibody was used. Finally, to separate between live and dead cells, aqua dye was used (sources of all antibodies are listed in Table 1).

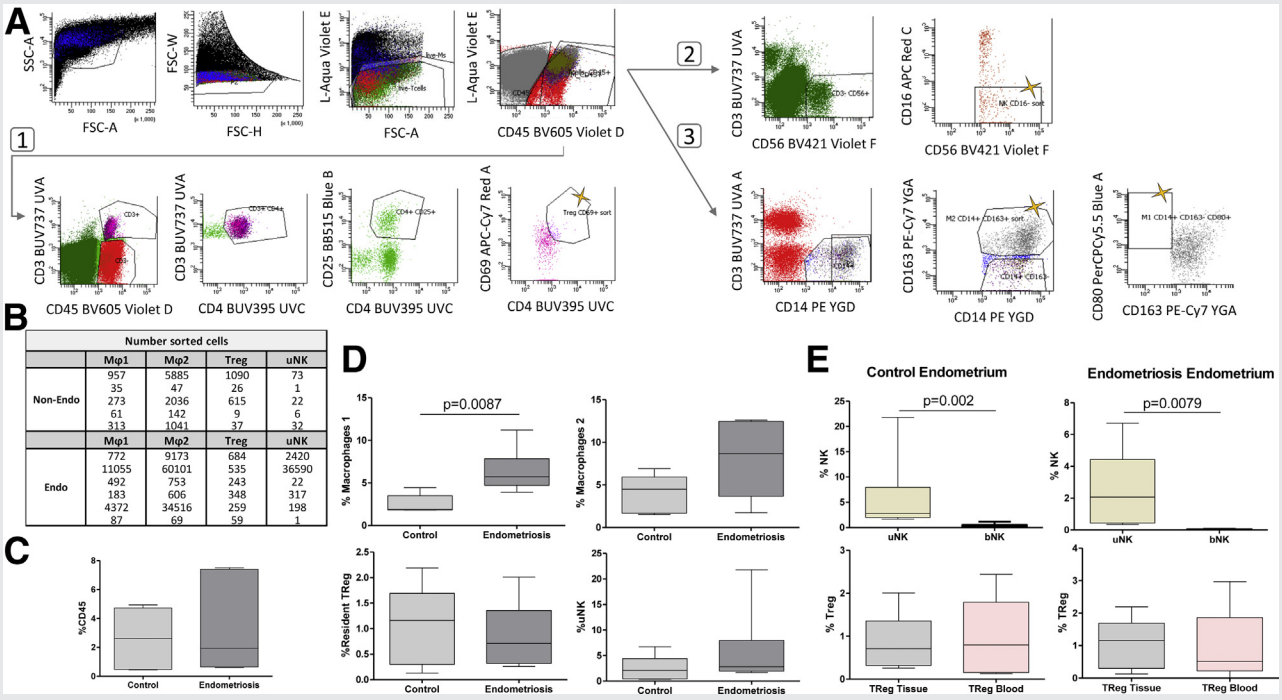
## Fluorescence Activated Cell Sorting (FACS)

Endometrial samples were thawed at 37°C. After centrifugation at 1,300 rpm for 5 minutes, the supernatant was discarded and the pellet was washed with 1× phosphate-buffered saline (PBS). After another centrifugation, cells were resuspended with 1× PBS + 5% bovine serum albumin and incubated at room temperature for 30 minutes. A minimum of 100,000 unlabeled cells were separated as a negative control. Ten conjugated antibodies were used to label the samples (Table 1). One microliter of antibody per million cells was used in all cases except for CD45 and CD4, where 2 μL per million cells were needed for optimal cell labeling. A solution of all fluorochromes minus one was prepared for each antibody to assess any overlap among the channels in the FACS instrument. After 1 hour of incubation at 4°C in 1× PBS + 3% bovine serum albumin and in the dark, cells were washed with 1× PBS and centrifuged for 5 minutes at 1,300 rpm. The pellet was resuspended with 500 μL of 1× PBS and labeled with 1 μL of LIVE/DEAD Fixable Aqua Dead cell labeling dye (ThermoFisher). On the other hand, UltraComp eBeads compensation magnetic beads (ThermoFisher) were labeled with each of the 10 antibodies following the manufacturer's instructions, to allow the correction of the spectral overlap between fluorochromes. Using the gating strategy (Fig. 1A), each population was sorted in the FACS Aria Jabba the Hutt (BD Biosciences) instrument and collected in 1× PBS. Flow cytometry analysis of the sorted cells was performed using FlowJo.v10 software (FlowJo LLC), and statistical analyses (Mann-Whitney test, $P<.05$) were conducted using GraphPad software (GraphPad Software).

## RNA Extraction

As low yields of cells were obtained after FACS (Fig. 1B), RNeasy micro kit (Qiagen) was used to isolate RNA from Mϕ populations (note yields from uNK and Treg cells were too low and were not used for RNA-seq). From the 22 sorted Mϕ samples (Mϕ1 and Mϕ2 populations of each of the 11 endometrial samples), RNA was extracted following the manufacturer's instructions to perform the total RNA-seq library prep from samples containing >900 cells (nine samples). The library preparation from the remaining samples, which contained at least 20 cells, was performed directly from cells in 1× PBS. RNA was eluted in 10 μL of RNase-free water, and the quality (RNA integrity numbers) and concentration were measured using a Tapestation4200 System (Agilent).

## FIGURE 1




Cytometry panel and flow cytometry analyses. **(A)** Gating strategy. The different gates used to sort the four desired populations are shown. The *x* and *y* axes show the lasers that were used to gate the cells. In addition, the name of the marker and the fluorochrome used have been inserted on the corresponding axes. First, cells were gated by their complexity (SSC-A/FSC-A). Then single cells were gated (FSC-W/FSC-H) as well as live cells (L-aqua violet E/FSC-A). In this case, two different gates were made: one for T cells and uNK and a bigger one for Mϕ due to the autofluorescence present in the latter. The next step was to gate live $CD45^+$ cells (L-aqua violet E/CD45 BV605 violet D). Thereafter, three strategies were used: [1] gating resident Treg ($CD45^+CD3^+CD4^+CD25^+CD69^+$); [2] gating uNK ($CD45^+CD3^-CD56^+CD16^-$); and [3] gating Mϕ2 ($CD3^-CD14^+CD163^+$) and Mϕ1 ($CD3^-CD14^+CD163^-CD80^+$). The final sorted populations are marked with a yellow star. **(B)** Number of sorted cells. The table shows the number of cells of Mϕ1, Mϕ2, resident Treg, and uNK cells obtained after FACS in patients without and with endometriosis. **(C)** Percentage of $CD45^+$ cells. The figure shows that no significant differences were found in the percentage of $CD45^+$ cells between controls ($n = 5$) and patients with endometriosis ($n = 6$). **(D)** Percentage of each subpopulation comparisons between control and endometriosis groups. Mϕ1 increased significantly in numbers in endometriosis endometrium, whereas no significant differences were found for Mϕ2, uNK, and Treg. **(E)** Percentage of uNK and Treg coming from blood circulation. It can be observed that in both control (left panel) and endometriosis (right panel) endometrium, there is a significant increase of resident uNK ($CD16^-$) compared with circulating NK ($CD16^+$), which indicates that there is almost no NK contamination from the peripheral circulation. In the case of Treg, it can be observed that there is an increase of Treg coming from blood in both groups, although it is not significant.

*Vallvé-Juanico. Endometrial macrophages in endometriosis. Fertil Steril 2019.*

### RNA-Seq and Statistical and Bioinformatic Analyses

SMART-Seq v4 Ultra Low Input RNA Kit for Sequencing (Clontech) was used to perform the RNA-seq library preparation. It allows RNA-seq to be performed with very low concentrations of RNA or to use whole cells to preserve sample integrity. In total, library preparations for 22 samples (10 from control and 12 from endometriosis) were performed. The quality of fastq files was tested using the FastQC (v0.11.5) (26) and the Qualimap (rnaseq module v2.2.1) software (27). Reads were aligned with the STAR mapper (v2.5.2a) (28) to release 88 of the Homo sapiens ENSEMBL version of the genome (GRCh38/hg38 assembly) (29). A raw count of reads per gene was also obtained with STAR (28). To overcome the heterogeneity between samples, samples were first removed from the analysis if they had <5 million uniquely mapped reads, and the remaining samples were down-sampled to 30 million mapped reads when needed. The data have been deposited in the NCBI GEO database (accession number GSE130435). The R/Bioconductor package DESeq2 (v1.20.0) (30–32) was used to assess differential expression between experimental groups (Wald statistical test + false discovery rate [FDR] correction). Statistically significant differentially expressed genes (DEGs) were considered when FDR < .05 and log fold change (LogFC) > 2. Different comparisons performed using Mϕ populations are shown in Table 2. Biologic significance analyses were conducted using Ingenuity Pathway Analyses (IPA) software (Ingenuity Systems), and significant molecular functions were established with an activation *Z* score > |2.00|.

## RESULTS

### FACS and Flow Cytometry Analyses

After FACS, low cell numbers were obtained (Fig. 1B) that subsequently guided further analyses. Cytometry analyses

**TABLE 2**

**Comparisons of the Mϕ populations analyzed by RNA-seq.**

| Comparisons | Sample size | DEG (LogFC ≥ 2/FDR < 0.05) |
|---|---|---|
| 1. Mϕ Endo vs. Mϕ Control | 11 vs. 7 | 1,567 |
| 2. Mϕ1 Control vs. Mϕ2 Control | 3 vs. 4 | 1,260 |
| 3. Mϕ1 Endo vs. Mϕ2 Endo | 5 vs. 6 | 705 |
| 4. Mϕ1 Endo vs. Mϕ1 Control | 5 vs. 3 | 1,422 |
| 5. Mϕ2 Endo vs. Mϕ2 Control | 6 vs. 4 | 1,544 |

*Note:* The first column shows each comparison performed after quality control analyses. The second column shows the number of samples used in each population to perform each comparison. The last column shows the number of DEGs obtained in each comparison by Wald statistical test and FDR. Endo = endometriosis.

*Vallvé-Juanico. Endometrial macrophages in endometriosis. Fertil Steril 2019.*

from all the immune populations showed that $CD45^+$ cells corresponded to an average of 6.8% of the total sample, in agreement with other studies wherein leukocytes comprise 10%–20% of total endometrial cells (33–38). No significant differences in $CD45^+$ cells were observed between the control and endometriosis groups (Fig. 1C). Statistical analyses comparing the percentage of each subpopulation between controls and endometriosis were performed, and no significant differences were observed except for Mϕ1, which was significantly higher in the endometriosis group ($P=.0087$; Fig. 1D). Because resident tissue markers were included in the cytometry panel, contamination of immune populations from the peripheral circulation could also be calculated. In both the control and endometriosis groups, uNK ($CD16^-$) were significantly higher compared with blood NK ($CD16^+$) (Fig. 1E), demonstrating that there was almost no contamination with blood NK cells. The percentage of Treg coming from blood ($CD69^-$) was higher than tissue Treg ($CD69^+$), although it was not significant (Fig. 1E). Due to the low number of uNK and Treg cells obtained, RNA-seq was only performed in the Mϕ populations that had significantly greater numbers of cells. Thus, from the 44 original FACS-sorted immune populations, 22 samples (Mϕ populations) from the endometriosis and control patients were used for the transcriptome study.

## RNA Extraction and Gene Expression Analyses (RNA-Seq)

RNA concentrations extracted from Mϕ ranged between 5 and 45 ng/μL. After RNA-seq and quality controls, we excluded any FastQ sequences for which the number of reads did not reach our threshold of 5 million reads/sequence. Thus, the populations analyzed were five Mϕ1 endometriosis, three Mϕ1 control, six Mϕ2 endometriosis, and four Mϕ2 control. After statistical analysis, DEGs (FDR < 0.05 and LogFC ≥ 2) were found in all comparisons (Table 2; Supplemental Table 1).

Biologic significance of the DEG analyses revealed significant molecular functions, relevant molecules secreted by Mϕ1 and Mϕ2, activation/inhibition of upstream regulators, and deregulated networks in each comparison (activation *Z* score ≥ 2.00; Table 3). The 25 top deregulated networks are shown in Supplemental Table 2. Increase in cell-cell contact was observed along with repression of RNA molecular functions, when comparing Mϕ1 endometriosis versus Mϕ1 control (comparison 4, Table 2). Increased cell-cell contact is consistent with, for example, increased adhesion to bacteria to accomplish bacterial engulfment. Top deregulated networks showed overexpression of cellular development, growth, and proliferation as well as overexpression of immune response–related networks, such as infectious disease and antimicrobial and inflammatory responses (Table 3). These data indicate that Mϕ1 in endometriosis has a more extensive proinflammatory phenotype than Mϕ1 in the control group.

In contrast, molecular functions upregulated in Mϕ2 in endometriosis (comparison 5, Table 2) included an accumulation of $Ca^{2+}$, increase in carbohydrate transport, and internalization of bacteria (Table 3). When comparing the Mϕ1 of women with versus without endometriosis (comparison 4, Table 2), the upstream regulator TNFα was predicted to be increased (Table 3). Increased internalization of bacteria is consistent with the phagocytic properties of the proinflammatory Mϕ1 phenotype. The top networks in endometrial Mϕ2 from women with endometriosis included deregulation of connective tissue disorders, endocrine system development and function, lipid metabolism, inflammatory disease/response, and drug metabolism (Table 3). These data overall demonstrate that Mϕ2 in the eutopic endometrium of women with endometriosis have a proinflammatory phenotype, compared with Mϕ2 in control women.

## DISCUSSION

In the current study, we developed a cytometry panel that allowed for separating circulating immune cells and tissue resident cells and different immune cell types within human endometrium. Thus, the analyzed immune populations were purely tissue-activated resident cells devoid of contamination by circulating immune cells. One goal was to develop and optimize this panel for the current study. However, it will also have value for other researchers aiming to separate these tissue-specific populations, since it is a challenging panel to design due to the multiple colors used and the possible overlap between channels.

After cytometry analyses, where Mϕ1 were found to be significantly higher in endometriosis, Mϕ were studied in more detail by transcriptomic analyses. To our knowledge this is the first RNA-seq data set of Mϕ in the eutopic endometrium of women with endometriosis. Abnormal distribution of Mϕ within the eutopic endometrium of women with disease could contribute to the aberrant distribution of immune cells in the pelvic cavity and the abnormal development and gene expression of this tissue. While Mϕ maintain organ homeostasis and facilitate host defense and wound healing, they also underlie the pathogenesis of many chronic inflammatory diseases (39).

The increased deregulated molecular functions and networks in Mϕ1 in the endometrium of women with endometriosis indicate these cells have a more proinflammatory phenotype than Mϕ1 in the control group. In addition, a

## TABLE 3

**Deregulated molecular functions, networks and upstream regulators in macrophages comparisons.**

| | Molecular functions | *Z* score | Genes | Deregulated networks | Upstream regulators | *Z* score |
|---|---|---|---|---|---|---|
| Mϕ Endo vs. Mϕ Ctr | Senescence of fibroblast cell line | –2.09 | ARHGAP10, DPY30, EREG, HYAL1, IL1A, LATS1, MAPK1, MATN4, ME2, PBRM1, POT1, PTEN, PTTG1, RBL1 | Hematologicsystem development and function, **immune cell trafficking**, **inflammatory response** | ↑ERG | 2,333 |
| | Activation of cells | 1.95 | ANGPT1, C3, CADM1, CD1D, CD4, CHRNA7, CSF2, DDIT4, EGF, EIF3A, GP1BA, HLAB1, IL13, IL1A, IL1R1, IL33, ITGA3, LBP, LTA, MYO18A, NOS3, NT5E, PSEN1, PTEN, PTGDR2, PTPN22, RAB5B, SBNO2, SIRPG, SPI1, STIM1, THBS1, TOP2A, VAMP4, VEGFA, WASL | **Infectious diseases**, **antimicrobial response**, **inflammatory response**<br>Organismal injury and abnormalities, cell morphology, cellular development<br>Cellular development, cellular growth and proliferation, **reproductive system development and function**<br>Dermatologic diseases and conditions, **inflammatory disease**, **inflammatory response** | | |
| Mϕ1 Endo vs. Mϕ1 Ctr | Cell-cell contact | 2.25 | ACTN4, ANGPT1, ARF6, ARHGAP19, CBLL1, CLDN2, CTNNB1, DNM2, DSP, ESRP2, F2RL2, GDF15, GSK3B, IL13, ING4, ITGA4, NRDC, NT5E, PLCB1, RAPGEF3, SYK, TJP1 | Cellular development, cellular growth and proliferation, **lymphoid tissue structure and development** | ↑TNF | 2,587 |
| | Repression of RNA | 2.19 | CUL3, DR1, ERCC2, FOXG1, LCOR, MECP2, TAF1 | **Infectious diseases**, **antimicrobial response**, **inflammatory response**<br>cardiac arrythmia, cardiovascular disease, hereditary disorder<br>Cell morphology, cellular movement, organismal injury and abnormalities<br>Cell signaling, cellular assembly and organization, cellular function and maintenance | | |
| Mϕ2 Endo vs. Mϕ2 Ctr | Concentration of $Ca^{2+}$ | 2.36 | DRD1, ITGAL, KLRD1, LPAR4, PLG, TRPV5, VIPR2 | Connective tissue disorders, **inflammatory disease**, **inflammatory response** | ↓LH | –2,169 |
| | Transport of carbohydrates | 2.21 | ABCB1, AQP2, C3, CSF2RA, NPC1, RALBP1, SLC23A1, SLC2A3, SLC45A1, SLC5A1 | Drug metabolism, endocrine system development and function, lipid metabolism | | |
| | Internalization of bacteria | 2.15 | C3, CEACAM6, ERBB2, PLG, PRKCA | Cell-to-cell signaling and interaction, hematologic system development and function, **immune cell trafficking**<br>Cell death and survival, hematologic system development and function, **lymphoid tissue structure and development**<br>Behavior, nervous system development and function, endocrine system disorders | | |

*Vallvé-Juanico. Endometrial macrophages in endometriosis. Fertil Steril 2019.*

## TABLE 3

**Continued.**

| | Molecular functions | *Z* score | Genes | Deregulated networks | Upstream regulators | *Z* score |
|---|---|---|---|---|---|---|
| Mϕ1 vs. Mϕ2 Ctr | Cytotoxicity of leukocytes | –2.18 | BMPR1A, CBLB, CD244, FCAR, KIR2DL4, KIR3DL1, KLRC1, NOTCH2 | Cancer, cell death and survival, hematologic disease | ↓miR-483-3p | –2,000 |
| | | | | Cellular function and maintenance, cellular movement, carbohydrate metabolism | ↑TGFB1 | 2,195 |
| | | | | Cell death and survival, cancer, organismal injury and abnormalities | | |
| | | | | developmental disorder, hereditary disorder, organismal injury and abnormalities | | |
| | | | | Hematologic system development and function, **lymphoid tissue structure and development,** tissue morphology | | |
| Mϕ1 vs. Mϕ2 Endo | Growth of *S. Cerevisiae* | -2.00 | LIG1, PARP1, POLR2K, ZMPSTE24 | Cell-to-cell signaling and interaction, hematologic system development and function, **immune cell trafficking** | ↑TNF | 2,385 |
| | Engulfment of cells | 2.12 | AP2B1, ATP6V1E1, AXL, CD47, CDC5L, CERK, CSK, DNM2, EGR1, ERBB2, LMBRD1, NR1H3, RHOG, SNX5, STK4, SYK, USPL1 | Cell death and survival, **embryonic development**, organismal injury and abnormalities | ↓SATB1 | -2,400 |
| | | | | Cell death and survival, **inflammatory response**, cancer | ↑INHBA | 2,449 |
| | | | | Cellular assembly and organization, cellular function and maintenance, neurologic disease | ↑NFkB | 2,784 |
| | | | | Cancer, cell death and survival, organismal injury and abnormalities | ↑IFN$\alpha$ | 2,186 |
| | | | | | ↑IL15 | 2,224 |
| | | | | | ↓VCAN | –2,000 |
| | | | | | ↓TGFB1 | –2,348 |

*Note:* The table shows the significant molecular deregulated molecular functions (*Z* score ≥ 2) obtained after IPA analyses of different macrophages comparisons, as well as the differentially expressed genes of the RNA-seq data set involved in these functions. The fifth column shows the five top deregulated networks in each comparison, where the immune-related networks are bolded. Finally, the sixth column shows the upstream regulators predicted to be activated (↑) or inhibited (↓) in the IPA analysis of each comparison. Endo = endometriosis; Ctr = control; ERG = ETS-related gene; TNF = tumor necrosis factor; miR-483-3p = micoRNA-483-3p; TGFB1 = transforming growth factor beta 1; SATB = stabilin 1; INHBA = inhibin subunit beta A; NFkB = nuclear factor kappa beta; IFN$\alpha$ = interferon alpha; IL15 = interleukin 15; VCAN = Versican.

*Vallvé-Juanico. Endometrial macrophages in endometriosis. Fertil Steril 2019.*

significantly higher number of sorted Mϕ1 was observed in patients with endometriosis (Fig. 1D), confirming a previous report (40). Moreover, these results suggest that the eutopic endometrium of women with endometriosis is more proinflammatory than control endometrium, consistent with findings from other groups (17, 41).

An unexpected finding herein was the proinflammatory phenotype exhibited by endometrial Mϕ2 from women with endometriosis. Mϕ2 in other tissues generally display an anti-inflammatory phenotype (39), and, importantly, Mϕ are phenotypically plastic with regard to their polarization state depending on their microenvironment (42). Moreover, Mϕ1 and Mϕ2 gene expression signatures often overlap, and the resultant phenotype depends on the tissue microenvironment (40). Thus, the endometrial Mϕ2 of women with endometriosis could undergo polarization in situ to Mϕ1, adopting a proinflammatory phenotype, due to an altered environment. The paradigm of different subpopulations of Mϕ is controversial in the immunology literature. Specifically, it is unclear whether there are unique Mϕ populations (as Mϕ1, Mϕ2) or whether Mϕ comprise a unique population that alters its phenotype depending on environmental cues. Herein, we have referred to Mϕ as two different subpopulations (Mϕ1 and Mϕ2), although the dynamics and mechanisms driving proinflammatory and anti-inflammatory Mϕ functional phenotypes remain to be determined.

Notably, tumors take advantage of macrophage plasticity. For example, in the early phases of cancer, high production of Mϕ1 inflammatory mediators activates the adaptive immune response capable of eliminating nascent neoplastic cells and also supports neoplastic transformation (40). In contrast, once the tumor is established, the main population of Mϕ is Mϕ2, producing an anti-inflammatory environment, which allows tumor growth. Endometriosis it is not a malignancy, although it shares some characteristics with cancers. In endometriotic lesions and peritoneal fluid of women with endometriosis, for example, Mϕ2 are increased (43), indicating that, as in cancer, an anti-inflammatory environment prevails that favors development and growth of the endometriotic lesions. In addition, that Mϕ2 have a role in angiogenesis further supports this paradigm. Finally, Mϕ2 are also involved in nerve growth, suggesting they may also have a role in endometriosis-related pain (44).

The initial proinflammatory phenotype of Mϕ in cancer increases NFkB and downstream events and increases transcription of proinflammatory cytokines such as TNF$\alpha$, IL12, IL23, IL1$\beta$, IL6, and reactive oxygen species. In the current study, the NFkB pathway was activated in Mϕ1 of endometriosis, which does not occur in Mϕ1 of control women (Table 3). Indeed, it has been described that the NFkB pathway is deregulated in the eutopic endometrium of women with endometriosis (45), which also indicates that the microenvironment in the endometrium of women with disease is more proinflammatory than heathy tissue.

Notably, an increase of transport of carbohydrates was observed in Mϕ2 of women with endometriosis. It is known that glycolysis is high in Mϕ1 and is decreased in Mϕ2 and that Mϕ polarization may derive from a reprogramming of glucose metabolism (46). Several studies have suggested that altering nutrient availability or blocking specific metabolic pathways skews the Mϕ phenotype and alters their effector functions in chronic inflammatory diseases (47). In this regard, Mϕ metabolism modulation could open a new therapeutic window for treating inflammatory diseases including endometriosis.

Finally, the upstream regulator TNF$\alpha$ was increased in IPA analysis when comparing Mϕ1 of women with versus without endometriosis (comparison 4, Table 2); in addition, increased $Ca^{2+}$ accumulation was activated in Mϕ2 (Table 3). It has been noted that a transient increase of $Ca^{2+}$ plays a role in the expression of TNF$\alpha$ by Mϕ1 (48). Intracellular $Ca^{2+}$ oscillations are likely to induce permanent changes in Mϕ physiology, and a supraphysiologic elevation of $Ca^{2+}$ in mitochondria can be cytotoxic and induce apoptosis in the long term (48). Whether TNF$\alpha$-mediated events play a role in Mϕ function awaits further studies.

Over the past decade, high-throughput sequencing techniques have challenged the dogma of the sterility of the uterine endometrium (49–54), and in particular an altered endometrial microbiome in women with endometriosis has been proposed (55). In addition, the endometrial microbiome also correlates with IVF outcomes (52), although whether this occurs in women with endometriosis awaits further study. However, treatment with antibiotics resulted in reduced numbers of endometriosis lesions in a mouse model, with concomitant alteration of the gut microbiome (56), although the endometrial microbiome was not reported in this study (54). Interestingly, a recent systematic review supports the use of antibiotics before oocyte retrieval in patients with endometriosis, among other gynecologic disorders (57). The presence of pathogenic, noncommensal bacteria in the endometrium may induce an altered immune cell profile and activation (increased numbers and activation of Mϕ1 and activation of Mϕ2) that could impact the production of cytokines by immune resident cells that adversely affect embryo implantation (58). In addition to effects on reproductive outcomes, the observed greater proinflammatory endometrial environment herein could be related to the pathophysiology of the disease. While attractive, we are aware that the sample size of the study is small. Therefore, these results should be taken with caution. Finally, whether the proinflammatory phenotype of the Mϕ2 population reported herein is in response to commensal bacteria or pathogens, or whether Mϕ populations are implicated in reproductive outcomes, is not clear. However, it is anticipated that this important area of research could have profound implications clinically and diagnostically.

## Conclusions

Overall, the results of the current study lead to the conclusion that both Mϕ1 and Mϕ2 in the eutopic endometrium of women with endometriosis display a higher proinflammatory phenotype compared with controls without disease. Endometrial Mϕ2 appear to be predisposed to Mϕ1 polarization in women with endometriosis, thus increasing their inflammatory phenotype. These findings suggest that eutopic

endometrium has different Mϕ gene signatures depending on the presence or absence of disease and that the endometrial environment of women with endometriosis is more proinflammatory than control endometrium. Whether subtypes of the disease are associated with different subsets of immunity and whether the macrophage proinflammatory status is related to bacteria in the endometrium of women with disease are yet to be determined. Finally, the results herein may have implications regarding the impact of the macrophage phenotypes on reproductive outcomes and possible novel therapeutics for microbiome-related symptoms and response for fertility and pain in women with endometriosis.

**Acknowledgments:** We thank Sarah Bonnin from the Centre for Genomic Regulation, Barcelona, for her help in the bioinformatics analyses; and Dr. Marielle Cavrois for her advice during the development of the cytometry panel.

## REFERENCES

1. Giudice LC. Clinical practice: endometriosis. N Engl J Med 2010;362:2389–98.
2. Becker CM, Laufer MR, Stratton P, Hummelshoj L, Missmer SA, Zondervan KT, et al. World Endometriosis Research Foundation Endometriosis Phenome and Biobanking Harmonisation Project: I. Surgical phenotype data collection in endometriosis research. Fertil Steril 2014;102:1213–22.
3. Lessey BA, Kim JJ. Endometrial receptivity in eutopic endometrium of women with endometriosis: it is affected, let me show you why. Fertil Steril 2017;108:19–27.
4. Zondervan KT, Becker CM, Koga K, Missmer SA, Taylor RN, Viganò P. Endometriosis. Nat Rev Dis Prim 2018;4:9.
5. Burney RO, Giudice LC. Pathogenesis and pathophysiology of endometriosis. Fertil Steril 2012;98:511–9.
6. Li XF, Charnock-Jones DS, Zhang EKO, Hiby S, Malik S, Day K, et al. Angiogenic growth factor messenger ribonucleic acids in uterine natural killer cells. J Clin Endocrinol Metab 2015;86:1823–34.
7. Lysakova-Devine T, O'Farrelly C. Tissue-specific NK cell populations and their origin. J Leukoc Biol 2014;96:981–90.
8. Salamonsen LA, Lathbury LJ. Endometrial leukocytes and menstruation. Hum Reprod Update 2000;6:16–27.
9. Yovel G, Shakhar K, Ben-Eliyahu S. The effects of sex, menstrual cycle, and oral contraceptives on the number and activity of natural killer cells. Gynecol Oncol 2001;81:254–62.
10. Souza SS, Castro FA, Mendonça HC, Palma PVB, Morais FR, Ferriani RA, et al. Influence of menstrual cycle on NK activity. J Reprod Immunol 2001;50:151–9.
11. Giuliani E, Parkin KL, Lessey BA, Young SL, Fazleabas AT. Characterization of uterine NK cells in women with infertility or recurrent pregnancy loss and associated endometriosis. Am J Reprod Immunol 2014;72:262–9.
12. Chen S, Zhang J, Huang C, Lu W, Liang Y, Wan X. Expression of the T regulatory cell transcription factor FoxP3 in peri-implantation phase endometrium in infertile women with endometriosis. Reprod Biol Endocrinol 2012;10:34.
13. Capobianco A, Rovere-Querini P. Endometriosis, a disease of the macrophage. Front Immunol 2013;4:9.
14. Mantovani A, Sica A, Sozzani S, Allavena P, Vecchi A, Locati M. The chemokine system in diverse forms of macrophage activation and polarization. Trends Immunol 2004;25:677–86.
15. Salamonsen LA, Zhang J, Brasted M. Leukocyte networks and human endometrial remodelling. J Reprod Immunol 2002;57:95–108.
16. Khan KN, Masuzaki H, Fujishita A, Kitajima M, Sekine I, Matsuyama T, et al. Estrogen and progesterone receptor expression in macrophages and regulation of hepatocyte growth factor by ovarian steroids in women with endometriosis. Hum Reprod 2005;20:2004–13.
17. Berbic M, Schulke L, Markham R, Tokushige N, Russell P, Fraser IS. Macrophage expression in endometrium of women with and without endometriosis. Hum Reprod 2009;24:325–32.
18. Gordon S, Taylor PR. Monocyte and macrophage heterogeneity. Nat Rev Immunol 2005;5:953–64.
19. Jensen AL, Collins J, Shipman EP, Wira CR, Guyre PM, Pioli PA. A subset of human uterine endometrial macrophages is alternatively activated. Am J Reprod Immunol 2012;68:374–86.
20. Cominelli A, Gaide Chevronnay HP, Lemoine P, Courtoy PJ, Marbaix E, Henriet P. Matrix metalloproteinase-27 is expressed in CD163+/CD206+M2 macrophages in the cycling human endometrium and in superficial endometriotic lesions. Mol Hum Reprod 2014;20:767–75.
21. Barragan F, Irwin JC, Balayan S, Erikson DW, Chen JC, Houshdaran S, et al. Human endometrial fibroblasts derived from mesenchymal progenitors inherit progesterone resistance and acquire an inflammatory phenotype in the endometrial niche in endometriosis. Biol Reprod 2016;94:118.
22. King A. Uterine leukocytes and decidualization. Hum Reprod Update 2000;6:28–36.
23. Koopman LA, Kopcow HD, Rybalov B, Boyson JE, Orange JS, Schatz F, et al. Human decidual natural killer cells are a unique NK cell subset with immunomodulatory potential. J Exp Med 2003;198:1201–12.
24. Salamonsen LA, Woolley DE. Menstruation: induction by matrix metalloproteinases and inflammatory cells. J Reprod Immunol 1999;44:1–27.
25. Yang Z, Kong B, Mosser DM, Zhang X. TLRs, macrophages, and NK cells: our understandings of their functions in uterus and ovary. Int Immunopharmacol 2011;11:1442–50.
26. Andrews S. FastQC—A quality control tool for high throughput sequence data; 2010.
27. Okonechnikov K, Conesa A, García-Alcalde F. Qualimap 2: advanced multi-sample quality control for high-throughput sequencing data. Bioinformatics 2015;32:292–4.
28. Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, et al. STAR: ultrafast universal RNA-seq aligner. Bioinformatics 2013;29:15–21.
29. Ftp://ftp.ensembl.org/pub/release-88/fasta/homo_sapiens. ENSEMBL: Homo sapiens, release 88.
30. Huber W, Carey VJ, Gentleman R, Anders S, Carlson M, Carvalho BS, et al. Orchestrating high-throughput genomic analysis with Bioconductor. Nat Methods 2015;12:115–21.
31. Foundation for Statistical Computing, Vienna A. R Core Team (2014). R: A language and environment for statistical computing. http://www.R-project.org/.
32. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol 2014;15:1–21.
33. Evans J, Salamonsen LA. Inflammation, leukocytes and menstruation. Rev Endocr Metab Disord 2012;13:277–88.
34. Flynn L, Byrne B, Carton J, O'Farrelly C, Kelehan P, O'Herlihy C. Menstrual cycle dependent fluctuations in NK and T-lymphocyte subsets from non-pregnant human endometrium. Am J Reprod Immunol 2000;43:209–17.
35. Givan AL, White HD, Stern JE, Colby E, Gosselin EJ, Guyre PM, et al. Flow cytometric analysis of leukocytes in the human female reproductive tract: comparison of fallopian tube, uterus, cervix, and vagina. Am J Reprod Immunol 1997;38:350–9.
36. King AE, Critchley HOD. Oestrogen and progesterone regulation of inflammatory processes in the human endometrium. J Steroid Biochem Mol Biol 2010;120:116–26.
37. Wira CR, Rodriguez-Garcia M, Patel MV. The role of sex hormones in immune protection of the female reproductive tract. Nat Rev Immunol 2015;15:217–30.
38. Yeaman G, Collins J. CD8 + T cells in human uterine endometrial lymphoid aggregates: evidence for accumulation of cells by traficking. Immunology 2001;2:434–40.
39. Parisi L, Gini E, Baci D, Tremolati M, Fanuli M, Bassani B, et al. Review article. Macrophage polarization in chronic inflammatory diseases: killers or builders? J Immunol Res 2018;2018:8917804.
40. Takebayashi A, Kimura F, Kishi Y, Ishida M, Takahashi A, Yamanaka A, et al. Subpopulations of macrophages within eutopic endometrium of endometriosis patients. Am J Reprod Immunol 2015;73:221–31.
41. Vallvé-Juanico J, Suárez-Salvador E, Castellví J, Ballesteros A, Taylor HS, Gil-Moreno A, et al. Aberrant expression of epithelial leucine-rich repeat containing G protein–coupled receptor 5–positive cells in the eutopic

endometrium in endometriosis and implications in deep-infiltrating endometriosis. Fertil Steril 2017;108:858–67.
42. Das A, Sinha M, Datta S, Abas M, Chaffee S, Sen CK, et al. Monocyte and macrophage plasticity in tissue repair and regeneration. Am J Pathol 2015;185:2596–606.
43. Bacci M, Capobianco A, Monno A, Cottone L, Di Puppo F, Camisa B, et al. Macrophages are alternatively activated in patients with endometriosis and required for growth and vascularization of lesions in a mouse model of disease. Am J Pathol 2009;175:547–56.
44. Morotti M, Vincent K, Brawn J, Zondervan KT, Christian M. Peripheral changes in endometriosis-associated pain. Hum Reprod Update 2015;20:717–36.
45. Zhou B, Rao L, Peng Y, Wang Y, Qie M, Zhang Z, et al. A functional promoter polymorphism in NFKB1 increases susceptibility to endometriosis. DNA Cell Biol 2010;29:235–9.
46. Blagih J, Jones RG. Polarizing macrophages through reprogramming of glucose metabolism. Cell Metab 2012;15:793–5.
47. Di Conza G, Ho PC. Metabolic adaptation of macrophages in chronic diseases. Cancer Lett 2018;414:250–6.
48. Clapham DE. Calcium signaling. Cell 1995;80:259–68.
49. Kyono K, Hashimoto T, Kikuchi S, Nagai Y, Sakuraba Y. A pilot study and case reports on endometrial microbiota and pregnancy outcome: an analysis using 16S rRNA gene sequencing among IVF patients, and trial therapeutic intervention for dysbiotic endometrium. Reprod Med Biol 2019;18:72–82.
50. Verstraelen H, Vilchez-Vargas R, Desimpel F, Jauregui R, Vankeirsbilck N, Weyers S, et al. Characterisation of the human uterine microbiome in non-pregnant women through deep sequencing of the V1-2 region of the 16S rRNA gene. Peer J 2016;4:e1602.
51. Fang R-L, Chen L-X, Shu W-S, Yao S-Z, Wang S-W, Chen Y-Q. Barcoded sequencing reveals diverse intrauterine microbiomes in patients suffering with endometrial polyps. Am J Transl Res 2016;8:1581–92.
52. Moreno I, Codoñer FM, Vilella F, Valbuena D, Martinez-Blanch JF, Jimenez-Almazán J, et al. Evidence that the endometrial microbiota has an effect on implantation success or failure. Am J Obstet Gynecol 2016;215:684–703.
53. Baker JM, Chase DM, Herbst-Kralovetz MM. Uterine microbiota: residents, tourists, or invaders? Front Immunol 2018;9:208.
54. Franasiak JM, Scott RT. Endometrial microbiome. Curr Opin Obstet Gynecol 2017;29:146–52.
55. Khan KN, Fujishita A, Hiraki K, Kitajima M, Nakashima M, Fushiki S, et al. Bacterial contamination hypothesis: a new concept in endometriosis. Reprod Med Biol 2018;17:125–33.
56. Chadchan SB, Cheng M, Parnell LA, Yin Y, Schriefer A, Mysorekar IU, et al. Antibiotic therapy with metronidazole reduces endometriosis disease progression in mice: a potential role for gut microbiota. Hum Reprod 2019;34:1106–16.
57. Pereira N, Hutchinson AP, Lekovich JP, Hobeika E, Elias RT. Antibiotic prophylaxis for gynecologic procedures prior to and during the utilization of assisted reproductive technologies: a systematic review. J Pathog 2016;2016:1–8.
58. Sirota I, Zarek SM, Segars JH. Potential influence of the microbiome on infertility and assisted reproductive technology. Semin Reprod Med 2014;32:35–42.

**Los macrófagos muestran fenotipos proinflamatorios en el endometrio eutópico de mujeres con endometriosis con relevancia para un etiología infecciosa de la enfermedad**

**Objetivo:** Realizar transcriptómicamente el fenotipo de macrófagos M1 (Mϕ1) y macrófagos M2 (Mϕ2) en el endometrio de mujeres con endometriosis.

**Diseño:** Estudio prospectivo experimental.

**Lugar:** laboratorio de investigación universitario.

**Paciente (s):** Seis mujeres con endometriosis y cinco controles sin enfermedad, en la fase secretora del ciclo menstrual.

**Intervención (es):** se aislaron del endometrio humano Mϕ1, Mϕ2, células natural killers uterinas y T reguladoras utilizando un único panel diseñado específicamente de clasificación de células activado por fluorescencia. Los perfiles del transcriptoma se evaluaron mediante ARN alta de secuenciación, análisis bioinformático y de patrones biológicos.

**Principales medidas de resultados:** se determinó la expresión diferencial de genes entre Mϕ1 y Mϕ2 en mujeres con y sin endometriosis y en Mϕ1 versus Mϕ2 en cada grupo e involucró diferentes vías biológicas y de señalización.

**Resultado (s):** el análisis de citometría de flujo no mostró diferencias significativas en el número total de leucocitos entre el grupo control y el grupo de endometriosis, aunque los Mϕ1 fueron mayores en el grupo de endometriosis versus controles.
Debido a tamaños de muestra más grandes solo se realizó un análisis estadístico transcriptómico en poblaciones Mϕ1 y Mϕ2. Los análisis bioinformáticos revelaron que en mujeres con endometriosis, los Mϕ1 endometriales son más proinflamatorios que los controles y, paradójicamente, los Mϕ2 tienen un fenotipo proinflamatorio.

**Conclusión (es):** como las Mϕ son fenotípicamente plásticos y su estado de polarización depende de su microambiente, el ambiente endometrial alterado en mujeres con endometriosis puede promover la polarización de los Mϕ2 endometriales y un fenotipo proinflamatorio en los Mϕ1.