Thin endometrium transcriptome analysis reveals a potential mechanism of implantation failure

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Abstract
Aim: Although a thin endometrium has been well recognized as a critical factor in implantation failure, little information is available regarding the molecular mechanisms. The present study investigated these mechanisms by using genome-wide mRNA expression analysis.

Methods: Thin and normal endometrial tissue was obtained from a total of six women during the mid-luteal phase of the menstrual cycle. The transcriptomes were analyzed with a microarray. Differentially expressed genes were classified according to Gene Ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways.

Results: The study identified 318 up-regulated genes and 322 down-regulated genes in the thin endometrium, compared to the control endometrium. The GO and KEGG pathway analyses indicated that the thin endometrium possessed aberrantly activated immunity and natural killer cell cytotoxicity that was accompanied by an increased number of inflammatory cytokines, such as IFN-γ. Various genes that were related to metabolism and anti-oxidative stress were down-regulated in the thin endometrium.

Conclusion: Implantation failure in the thin endometrium appears to be associated with an aberrantly activated inflammatory environment and aberrantly decreased response to oxidative stress.

KEYWORDS
implantation failure, infertility, oxidative stress, thin endometrium, uterine natural killer cells

1 | INTRODUCTION
Adequate growth of the endometrium is indispensable for a successful pregnancy. Women with thin endometria have lower pregnancy rates, largely related to implantation failure. The authors recently found high blood impedance in the uterine radial artery in patients with a thin endometrium and that vitamin E, L-arginine, and sildenafil citrate treatments, which increase the blood flow of the uterine radial artery, helped to thicken the endometrium. This suggests that a low level of blood flow to the endometrium reduces its thickness, although it remains unclear why this would result in implantation failure. In order to answer this question, the authors compared the transcriptomes of thin and normal endometrial tissues with a microarray.
2 | MATERIALS AND METHODS

2.1 | Tissue sampling

In total, six women with a history of infertility were recruited into the study. All the patients were diagnosed with unexplained infertility after excluding any obvious cause of infertility, such as uterine fibroid, endometriosis, tubal obstruction, and uterine malformation. The patients were classified into two groups, based on the endometrial thickness and level of blood flow impedance in the uterine radial artery. The endometrial thickness was measured at the maximal distance between each myometrial–endometrial interface by using vaginal ultrasonography in the mid-luteal phase. The level of blood flow impedance in the uterine radial artery was measured as a resistance index with a pulsed Doppler. The cut-off value of the endometrial thickness and level of blood flow impedance were defined as <8 mm and ≥0.81 mm, respectively, based on the authors’ previous studies. Three patients had a normal-thickness endometrium (endometrial thickness ≥8 mm) and three patients had a thin endometrium (Thin; endometrial thickness <8 mm) (Table 1). The endometrial thickness of the normal-thickness endometrium group and the thin-endometrium group was 9.53±0.65 and 6.33±0.68 mm, respectively. The difference in the endometrial thickness was significant (P=0.0042). A high level of blood flow impedance in the uterine radial artery was confirmed only in the patients with a thin endometrium (0.86±0.04 vs 0.76±0.03; P=0.027). The differences between the groups in age (31.7±3.21 vs 31.3±5.13 years), menstrual cycles (3.00±4.58 and 28.3±1.52 days), and serum levels of estradiol (171.3±16.6 vs 182.1±72.1 pg/mL) and progesterone (15.4±2.3 vs 21.5±7.6 μg/mL) were not significant. The endometrial tissue was obtained during the mid-luteal phase of the menstrual cycle. Samples of endometrial curettings were washed with saline to remove the blood, immersed in liquid nitrogen, and stored at −80°C until RNA extraction.

2.2 | Transcriptome analysis

The total RNAs were isolated from the tissues by using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and they were reverse-transcribed by using a QuantiTect Reverse Transcription Kit (Qiagen, Valencia, CA, USA), according to the manufacturer’s protocol. The transcriptome analysis gene expression was analyzed by using a GeneChip Human Genome U133 Plus 2.0 Array (Affymetrix, Santa Clara, CA, USA) that contained 54 120 probes supporting 18 599 genes, as previously reported. Briefly, the target cDNA was prepared from 200 ng of total RNA with the Ambion WT Expression kit (Ambion, Austin, TX, USA) and the Affymetrix GeneChip WT Terminal Labeling kit (Affymetrix). Hybridization to the microarrays, washing, staining, and scanning were performed by using the GeneChip system (Affymetrix), which was composed of the Scanner 3000 7G Workstation Fluidics 450 (Affymetrix) and the Hybridization Oven 645 (Affymetrix). The scanned image data were processed by using a gene expression analysis with the Partek Genomics Suite 6.5 software program (Partech, Munster, Germany). Then, 2000 randomly selected genes were used...
for the hierarchical clustering analysis and principal component analysis (PCA). Those genes whose expressions in the thin and normal endometrium differed by at least a factor of 2 and that had a false discovery rate of <.05 were judged as showing a significant difference.

2.3 | Bioinformatics

A hierarchical clustering and a PCA were conducted in R v. 3.2.4.11 DAVID Bioinformatics Resources v. 6.7 (http://david.abcc.ncifcrf.gov/home.jsp) was used to determine whether the functional annotation of the differentially expressed genes was enriched for specific Gene Ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways.12 Statistical significance was assessed with a modified Fisher’s exact test. In the GO and KEGG analyses, \( P < .01 \) and \( P < .05 \), respectively, were considered to indicate significant enrichment. All the information from the GO and KEGG pathway analyses is shown in Tables S1-S4.

3 | RESULTS

3.1 | Comparison of the whole mRNA expression profiles of the normal and thin endometrial tissues

Figure 1 shows the mRNA expression profiles in the thin and normal endometrial tissues (n=3 for each). The hierarchical dendrogram clearly separated the thin and the control endometria. The PCA (Figure 2) also clearly separated the thin and the control endometria.

3.2 | Gene Ontology and Kyoto Encyclopedia of Genes and Genomes pathway analyses of the up-regulated genes in the thin endometrium

In the thin-endometrium group, 318 genes were up-regulated and 322 genes were down-regulated, compared to the control endometrium group (Tables S5 and S6). The up-regulated genes in the thin endometrium were related to immunity processes, such as the "response to external stimulus," "defense response," "leukocyte mediated immunity," "immune response," "immune effector process," and "regulation of immune system process" (Tables 2 and S1). These GO terms

**FIGURE 1** Heat map and hierarchical clustering of the mRNA expression profiles of patients with thin and normal (control) endometria. The mRNA expression profiles of the thin endometrium (Thin 1, Thin 2, and Thin 3) and the normal-thickness endometrium (Control 1, Control 2, and Control 3) were compared. The \( x \)-axis represents the samples and the \( y \)-axis represents the gene clusters. The heat map in hierarchical clustering analysis indicates the mRNA expression levels from low (red) to high (yellow)

**FIGURE 2** Principal component analysis of the mRNA expression profiles of patients with thin and normal (control) endometria. The mRNA expression profiles of the thin endometrium (Thin 1, Thin 2, and Thin 3) and the normal-thickness endometrium (Control 1, Control 2, and Control 3) were compared. The \( y \)-axis and the \( x \)-axis show the principal component numbers, respectively
| Category                  | Term                              | Count | P value     | Gene                                                                 |
|--------------------------|-----------------------------------|-------|-------------|----------------------------------------------------------------------|
| GOTERM_BP_FAT            | Response to external stimulus     | 70    | 1.16E-09    | ARSB, FGFB18, NRPI1, MMP7, FASLG, PMAIP1, FOS, CD96, MYOCD, APOD, HPSE, RNASE7, IFNG, SLC2A1, CALCR, CFI, LBP, MX1, ANGPT2, FGRG3B, F11, ZFP36, TRPM8, KIF5A, SOCS3, GNL, P1M1, FOSB, PLAUR, TNFAIP6, SSR2, THBD, AD, IL20RB, TREMI1, DP3F, PRF1, RBP1, AS1, CCR1, STAT1, CXCL2, GAST, TRDC, CCL4, IAK3, RAC2, INPP5F, ARNT2, BHLHE40, PTTPC, ST6GAL1, SLCA1, AVAV3, AIMPI1, CFB, LMCD1, NR4A1, NR4A3, COT1L1, PLCAB, SOD2, CORO1A, CD55, SLCA72, CXC13, CMTM7, CP, XCL1, HTR2A |
| GOTERM_BP_FAT            | Defense response                  | 56    | 3.70E-09    | KLRC2, IL19, PMAIP1, FOS, CD96, APOD, RNASE7, IFNG, VNN1, CALCR, CFI, LBP, MX1, KLRC1, FGRG3B, DPP4, ZFP36, EGR1, NFKB1Z, SOCS3, GNL, GZMA, CD84, TNFAIP6, OR2H2, CAMK4, AD, IL20RB, TREM1, KIR3D1L1, KIR2DL4, KIR2DL2, DP3F, PRF1, AS1, CCR1, CXCL2, STAT1, TRDC, CCL4, IAK3, RASGRP1, BLNK, PTTPC, AIMPI1, CFB, LMCD1, COT1L1, PLCAB, CD55, CORO1A, SLCA72, CXC13, C1RL1, XCL1 |
| GOTERM_BP_FAT            | Leukocyte mediated immunity       | 21    | 1.62E-07    | PTPRC, KLRC2, GZMA, TRDC, C15, NR4A3, CD84, CD96, CORO1A, CD55, RAC2, CAMK4, IL20RB, RASGRP1, IFNG, C1RL1, TREMI1, CFI, XCL1, KLRC1, KIR3D1L1 |
| GOTERM_BP_FAT            | Immune response                   | 50    | 1.99E-07    | PRF1, KLRC2, AS1, CCR1, IL19, CXCL2, FASLG, C15, TRDC, ZEB1, CCL4, CD96, IRAK3, FOS, RAC2, RNASE7, RASGRP1, IFNG, VNN1, CFI, LBP, MX1, FGRG3B, KLRC1, BLNK, EGR1, PTTPC, ST6GAL1, VAV3, CFB, SOCS3, GZMA, GZMB, NR4A3, PRKCB, CTSW, CD84, CD38, CORO1A, CD55, MYO10, RS1, IL20RB, AD, CAMK4, CXCL13, C1RL1, TREMI1, XCL1, KIR3D1L1 |
| GOTERM_BP_FAT            | Immune effector process           | 34    | 2.07E-07    | DP3F, PRF1, RBPI4, KLRC2, PMAIP1, TRDC, C15, CD96, IRAK3, RAC2, RASGRP1, IFNG, LBP, CFI, MX1, FGRG3B, KLRC1, BLNK, PTTPC, VAV3, AIMPI1, CFB, LMCD1, GZMB, NR4A3, CD84, MYO10, CD55, CORO1A, IL20RB, CAMK4, C1RL1, TREMI1, XCL1, KIR3D1L1 |
| GOTERM_BP_FAT            | Regulation of immune system process | 46   | 1.96E-06    | DP3F, RBPI4, RBPI1, CCR1, CXCL2, C15, TRDC, ZEB1, CCL4, FOS, IRAK3, CD96, CDKNA2A, RAC2, APOD, RASGRP1, IFNG, VNN1, CFI, CFI, LG13, FGRG3B, KLRC1, DPP4, ZFP36, PTTPC, VAV3, SOCS3, CFB, LMCD1, NR4A3, PRKCB, CD84, CD38, CD55, MYO10, CORO1A, CAMK4, IL20RB, SLCA72, CXC13, C1RL1, TREMI1, XCL1, KIR3D1L1, KIR3D2 |
| GOTERM_CC_FAT            | Extracellular space               | 47    | 2.70E-06    | RBPI4, FGFB18, NRPI1, CXCL2, IL19, MMP7, FASLG, GAST, C15, TRDC, SFN, CCL4, MTHFD2, APOD, IFNG, SLC2A1, LBP, CFI, ANGPT2, QSOX1, SRGN, F11, AIMPI1, CFB, GNL, HSPG2, LMCD1, IGFL5, COL25A1, CTSW, PROM1, MMP10, TNFAIP6, DKK1, THBD, AD, SERPINS5, CXC13, FABP3, C1RL1, CMTM7, VCAN, IGFBP1, CP, XCL1, TNFAIP2, HABP2 |
| GOTERM_BP_FAT            | Vasculature development           | 26    | 5.11E-06    | FGRF2, ZFAND5, FGFB18, NRPI1, LEPR, FASLG, APOD, MYOCID, HPSE, IFNG, RBOB4, CALCR, ANGPT2, THBS2, EGR1, VAV3, AIMPI1, SOCS3, GNL, HSPG2, NR4A1, PRKCB, AD, CXCL13, SIX1, HIF3A, TNFAIP2 |
| GOTERM_BP_FAT            | Leukocyte activation              | 29    | 7.06E-06    | PRF1, LEPR, ZEB1, TRDC, CDKN2A, RAC2, RASGRP1, IFNG, VNN1, LBP, DPP4, BLNK, EGR1, PTTPC, IL20RB, VAV3, IKZF1, DOCK8, NR4A3, PRKCB, CD84, CD38, CORO1A, CD55, IL20RB, CAMK4, SLCA72, CMTM7, XCL1 |
| GOTERM_BP_FAT            | Lymphocyte mediated immunity      | 16    | 1.17E-05    | PTTPC, KLRC2, GZMB, TRDC, C15, CD96, CORO1A, CD55, IL20RB, RASGRP1, IFNG, C1RL1, CFI, XCL1, KLRC1, KIR3D1L1 |
| GOTERM_BP_FAT            | Inflammatory response             | 26    | 1.19E-05    | ZFP36, NFKB1Z, AIMPI1, AS1, SOCS3, CFB, CCR1, IL19, CXCL2, CCL4, FOS, CD96, TNFAIP6, CD55, CAMK4, IL20RB, APOD, SLCA72, CXC13, RASGRP1, VNN1, LBP, CFI, CALCR, XCL1, BLNK |
| GOTERM_BP_FAT            | Leukocyte migration               | 19    | 1.42E-05    | VAV3, AIMPI1, CCR1, CXCL2, DOCK8, CCL4, MMP7, SLCA711, CD84, CORO1A, THBD, RAC2, APOD, CXCL13, IFNG, TREMI1, LBP, XCL1, ANGPT2 |

(Continues)
| Category                   | Term                                  | Count | P value  | Gene                                                                 |
|----------------------------|---------------------------------------|-------|----------|----------------------------------------------------------------------|
| **GOTERM_BP_FAT**          | Locomotion                            | 45    | 1.59E-05 | ARSB, ZFAND5, FGFI8, NRPI, CCR1, CXCL2, FERMT1, CCL4, MMP1, DNAH6, NPHP4, Rac2, APOD, IFNG, ROBO4, INPP5F, LBP, DEPDC1B, ANGPT2, DPP4, PTPRC, SLC8A1, ST6GAL1, SATB2, S100P, VAV3, AIMPI, KIF5A, NR4A1, DOCK7, DOCK8, NR4A3, SLC7A11, PLAUR, CD84, MMP10, **TNFAIP6**, CORO1A, THBD, CXCL13, S1X1, CMTM7, VCAN, TREM1, XCL1 |
| **GOTERM_BP_FAT**          | Cell activation                       | 32    | 1.61E-05 | PRF1, LEPR, ZEB1, TRDC, CDKN2A, Rac2, MYOCD, RASGRP1, IFNG, VNN1, LBP, DPP4, BLNK, EGR1, PTPRC, IL2RB, VAV3, KIF5F1, DOCK8, NR4A3, SLC7A11, PRKCB, CD84, CD38, CORO1A, CD55, THBD, IL20RB, CAMK4, SLC7A2, CMTM7, XCL1 |
| **GOTERM_BP_FAT**          | Regulation of response to external stimulus | 30    | 1.68E-05 | FGFI8, DPF3, NRPI, CCR1, CXCL2, CCL4, Rac2, APOD, HPSE, INPP5F, CALCRL, CFI, LBP, FGCR3B, ANGPT2, ZFP36, F11, ST6GAL1, CFB, SOCS3, LMCD1, PLAUR, **TNFAIP6**, CD55, THBD, IL20RB, CXCL13, SLC7A2, TREM1, XCL1 |
| **GOTERM_BP_FAT**          | Blood vessel development               | 24    | 1.89E-05 | EGR1, FGFRI, FGFI8, NRPI, VAV3, AIMPI, SOCS3, LEPR, HSPG2, FASLG, NR4A1, PRKCB, MYOCD, APOD, ADM, HPSE, S1X1, IFNG, ROBO4, HIF3A, CALCRL, THBS2, **TNFAIP2**, ANGPT2 |
| **GOTERM_BP_FAT**          | Cardiovascular system development      | 32    | 3.29E-05 | FGFR2, ZFAND5, FGFI8, RBPI, NRPI, LEPR, PDLIM3, FASLG, ZIC3, APOD, MYOCD, HPSE, IFNG, ROBO4, CALCRL, ANGPT2, THBS2, EGR1, SLC8A1, VAV3, AIMPI, SOCS3, HSPG2, NR4A1, PRKCB, SOD2, DKK1, ADM, CXCL13, S1X1, HIF3A, **TNFAIP2** |
| **GOTERM_BP_FAT**          | Circulatory system development         | 32    | 3.29E-05 | FGFR2, ZFAND5, FGFI8, RBPI, NRPI, LEPR, PDLIM3, FASLG, ZIC3, APOD, MYOCD, HPSE, IFNG, ROBO4, CALCRL, ANGPT2, THBS2, EGR1, SLC8A1, VAV3, AIMPI, SOCS3, HSPG2, NR4A1, PRKCB, SOD2, DKK1, ADM, CXCL13, S1X1, HIF3A, **TNFAIP2** |
| **GOTERM_BP_FAT**          | Lymphocyte activation                  | 25    | 3.54E-05 | PRF1, LEPR, TRDC, ZEB1, CDKN2A, Rac2, RASGRP1, IFNG, VNN1, DPP4, BLNK, EGR1, PTPRC, IL2RB, VAV3, KIF5F1, DOCK8, PRKCB, CD84, CD38, CORO1A, IL20RB, CAMK4, CMTM7, XCL1 |
| **GOTERM_BP_FAT**          | Blood vessel morphogenesis             | 21    | 4.88E-05 | FGFR2, FGFI8, NRPI, VAV3, AIMPI, LEPR, HSPG2, NR4A1, FASLG, PRKCB, MYOCD, APOD, ADM, HPSE, S1X1, ROBO4, HIF3A, CALCRL, THBS2, **TNFAIP2**, ANGPT2 |
| **GOTERM_BP_FAT**          | Angiogenesis                           | 19    | 4.88E-05 | FGFR2, FGFI8, NRPI, VAV3, AIMPI, LEPR, HSPG2, NR4A1, FASLG, PRKCB, APOD, ADM, HPSE, ROBO4, HIF3A, CALCRL, THBS2, **TNFAIP2**, ANGPT2 |
| **GOTERM_BP_FAT**          | Negative regulation of multicellular organisantal process | 33    | 6.30E-05 | RBPI, NRPI, CCR1, STATH, FASLG, CD96, IRAK3, CDKN2A, APOD, MYOCD, IFNG, INPP5F, LBP, CALCRL, ANGPT2, THBS2, SRGN, NR2F1, ZFP36, F11, ANXA4, PLAUR, PLAC8, CD84, CD38, DKK1, THBD, ADM, IL20RB, CXCL13, S1X1, ID4, XCL1 |
| **GOTERM_BP_FAT**          | Regulation of cell activation          | 21    | 7.32E-05 | PTPRC, VAV3, ZEB1, TRDC, NR4A3, CD84, CD38, CORO1A, CD55, THBD, CDKN2A, Rac2, CAMK4, IL20RB, SLC7A2, RASGRP1, IFNG, VNN1, LBP, XCL1, DPP4 |
| **GOTERM_BP_FAT**          | Cell motility                          | 39    | 7.52E-05 | ARSB, ZFAND5, FGFI8, NRPI, CCR1, CXCL2, FERMT1, CCL4, MMP1, DHA6, NPH4, Rac2, APOD, IFNG, ROBO4, LBP, DEPDC1B, ANGPT2, DPP4, PTPRC, SLC8A1, SATB2, S100P, VAV3, AIMPI, NR4A1, DOCK7, DOCK8, SLC7A11, CD84, MMP10, **TNFAIP6**, CORO1A, THBD, CXCL13, S1X1, VCAN, TREM1, XCL1 |
| **GOTERM_BP_FAT**          | Localization of cell                  | 39    | 7.52E-05 | ARSB, ZFAND5, FGFI8, NRPI, CCR1, CXCL2, FERMT1, CCL4, MMP1, DHA6, NPH4, Rac2, APOD, IFNG, ROBO4, LBP, DEPDC1B, ANGPT2, DPP4, PTPRC, SLC8A1, SATB2, S100P, VAV3, AIMPI, NR4A1, DOCK7, DOCK8, SLC7A11, CD84, MMP10, **TNFAIP6**, CORO1A, THBD, CXCL13, S1X1, VCAN, TREM1, XCL1 |
| **GOTERM_BP_FAT**          | Regulation of leukocyte activation     | 20    | 8.61E-05 | PTPRC, VAV3, ZEB1, TRDC, NR4A3, CD84, CD38, CORO1A, CD55, CDKN2A, Rac2, CAMK4, IL20RB, SLC7A2, RASGRP1, IFNG, VNN1, LBP, XCL1, DPP4 |

(Continues)
| Category         | Term                                                        | Count | P value   | Gene                                                                 |
|------------------|-------------------------------------------------------------|-------|-----------|----------------------------------------------------------------------|
| GOTERM_BP_FAT    | Regulation of immune effector process                        | 19    | 8.63E-05  | PTPRC, RBP4, DPF3, CBF, LMC5, NR4A1, CD64, CD96, IRAK3, CD55, RAC2, IL20RB, RASGRP1, IFNG, TREM1, CFI, LBP, XCL1, FCRGR3B |
| GOTERM_CC_FAT    | Extracellular region part                                    | 88    | 9.24E-05  | ARSB, FGF18, NRPI, THRB, FAM20A, IL19, MMP7, FASLG, AURKB, MMP1, MTHFD2, AP0D, HPSE, RNASE7, DMKN, SLC2A1, IFNG, ROBO4, VNN1, CFI, LBP, DPP6, ANGPT2, FCRGR3B, DPP4, F11, GSTT2B, GNLY, IGFL5, COL25A1, PADI1, CTSW, PLAU, PRKCB, CD84, MMP10, TNFAIP6, CD38, THBD, CAMK4, ADM, SERPINB5, CYBRD1, VCAN, SLC38A1, COL24A1, TNFAIP6, PPIA2, RBP4, ASS1, CXCL2, GAST, C15, SFN, TRDC, CCL4, LAMB3, RAC2, THBS2, QSOX1, SRGN, PTPRC, GPR155, ST6GAL1, VAV3, S100P, AIMP1, CBF, LMC51, HSPG2, COT11, ANXA4, SOD2, PROM1, CORO1A, CD55, DKK1, C10ORF116, CXCL13, FABP3, C1RL, CMTM7, H3F3B, IGFBP1, CP, XCL1, FABP5, HABP2 |
| GOTERM_BP_FAT    | Response to biotic stimulus                                  | 32    | 9.65E-05  | PRF1, DPF3, ASS1, CXCL2, STATH, FASLG, PMAIP1, TRDC, CCL4, FOS, CD96, IRAK3, RNASE7, IFNG, LBP, MX1, FCRGR3B, ZFP36, PTPRC, AIMP1, SOCS3, GNLY, LMC51, COT11, PLAC8, SOD2, CD55, THBD, ADM, CXCL13, TREM1, XCL1 |
| GOTERM_BP_FAT    | Anatomical structure formation involved in morphogenesis     | 35    | 9.69E-05  | FGF2, RBP4, FGF18, NRPI, FAM20A, ABLIM3, LEPR, BS59, FASLG, MTHFD1L, WDR74, LAMB3, AP0D, HPSE, ROBO4, CALCRL, LMO3, ANGPT2, THBS2, VAV3, AIMP1, HSPG2, NR4A1, NR4A3, PRKCB, PROM1, DUSP5, DKK1, ADM, CXCL13, ET52, SIX1, HIF3A, TNFAIP2, DUSP6 |
| GOTERM_BP_FAT    | Cell chemotaxis                                             | 14    | 1.00E-04  | FGF18, NRPI, VAV3, CCR1, CXCL2, NR4A1, CCL4, CORO1A, RAC2, CXCL13, IFNG, LBP, TREM1, XCL1 |
| GOTERM_BP_FAT    | Response to other organism                                  | 31    | 1.05E-04  | PRF1, DPF3, ASS1, CXCL2, STATH, FASLG, PMAIP1, TRDC, CCL4, FOS, CD96, IRAK3, RNASE7, IFNG, LBP, MX1, FCRGR3B, ZFP36, PTPRC, AIMP1, SOCS3, GNLY, LMC51, COT11, PLAC8, SOD2, THBD, ADM, CXCL13, TREM1, XCL1 |
| GOTERM_BP_FAT    | Response to external biotic stimulus                         | 31    | 1.05E-04  | PRF1, DPF3, ASS1, CXCL2, STATH, FASLG, PMAIP1, TRDC, CCL4, FOS, CD96, IRAK3, RNASE7, IFNG, LBP, MX1, FCRGR3B, ZFP36, PTPRC, AIMP1, SOCS3, GNLY, LMC51, COT11, PLAC8, SOD2, THBD, ADM, CXCL13, TREM1, XCL1 |
| GOTERM_BP_FAT    | Cell proliferation                                          | 49    | 1.29E-04  | FGF2, RBP4, FGF18, NRPI, FERMT1, FASLG, PMAIP1, ZEB1, AURKB, SFN, FAM83B, CDKN2A, AP0D, RAC2, MYOCD, HPSE, IFNG, MTCPL1, CALCRL, QSOX1, DPP4, EGR1, ZFP36, PTPRC, ST6GAL1, VAV3, TRNP1, AIMP1, PIM1, NR4A1, DOCK7, NR4A3, DOCK8, PLAC8, SOD2, CD38, SSTR2, CORO1A, CD55, IL20RB, ADM, SERPINB5, SIX1, FABP3, H3F3B, ID4, XCL1, EMP1, HTR2A |
| GOTERM_BP_FAT    | Extracellular region                                        | 100   | 1.29E-04  | ARSB, FGF18, NRPI, THRB, FAM20A, LEPR, IL19, MMP7, FASLG, AURKB, MMP1, MTHFD2, AP0D, HPSE, DMKN, RNASE7, SLC2A1, IFNG, ROBO4, VNN1, CFI, DPP6, GFOID1, FCRGR3B, ANGPT2, CSCP2A, DPP4, F11, GSTT2B, GZMA, GNLY, IGFL5, COL25A1, PADI1, PRKCB, PLAU, CTSD, CDS84, MMP10, CD38, TNFAIP6, PS9G, THBD, ADM, CAMK4, SERPINB5, CYBRD1, VCAN, SLC38A1, TREM1, COL24A1, TNFAIP6, PPIA2, FGF2, RBP4, ASS1, STATH, CXCL2, GAST, C15, SFN, TRDC, CCL4, FAM19A5, LAMB3, RAC2, GLIPR1, QSOX1, THBS2, SRGN, PTPRC, GPR155, ST6GAL1, S100P, VAV3, AIMP1, CBF, HSPG2, LMC51, GRIA3, COT11, ANXA4, SOD2, PROM1, CD55, CORO1A, C10ORF116, DKK1, CXCL13, FABP3, C1RL, CMTM7, H3F3B, CP, IGFBP1, XCL1, FABP5, HABP2 |
| GOTERM_BP_FAT    | Regulation of cell proliferation                             | 43    | 1.36E-04  | FGF2, RBP4, FGF18, NRPI, CXCL2, FASLG, SFN, PMAIP1, ZEB1, CDKN2A, RAC2, MYOCD, AP0D, HPSE, IFNG, CHST11, CALCRL, QSOX1, DPP4, ZFP36, EGR1, PTPRC, ST6GAL1, VAV3, AIMP1, TRNP1, NR4A1, NR4A3, PLAC8, SOD2, CD38, SSTR2, CD55, CORO1A, IL20RB, ADM, SERPINB5, CXCL13, SIX1, FABP3, ID4, XCL1, HTR2A |

(Continues)
| Category             | Term                                      | Count | P value | Gene                                                                 |
|----------------------|-------------------------------------------|-------|---------|----------------------------------------------------------------------|
| GOTERM_BP_FAT        | Movement of cell or subcellular component | 47    | 1.38E-04| ARSB, ZFAND5, Dnah10, FGF18, NRP1, CCR1, CXCL2, FERMT1, CXCL4, MMP1, DNAH6, NPHP4, Rac2, APOD, RASGRP1, IFNG, ROBO4, INPP5F, VNN1, LBP, DEPDC1B, ANGPT2, DPP4, PTPRC, SLCA81, SATB2, S100P, VAV3, AIMP1, KIF5A, NR4A3, DOCK7, DOCK8, NR4A3, SLC7A11, PLAUR, CD84, MMP10, TNAIP6, CORO1A, THBD, SERPINB5, CXCL13, SIX1, VCAN, TREM1, XCL1 |
| GOTERM_BP_FAT        | Serine-type peptidase activity            | 14    | 1.52E-04| F11, GZMA, CFB, MMP7, GZMB, C15, TMPRSS5, MMP1, MMP10, C1RL, C1R, DPP6, DPP4, HABP2 |
| GOTERM_BP_FAT        | Regulation of immune response             | 29    | 1.58E-04| TRDC, C15, FOS, CD96, IRAK3, Rac2, RASGRP1, IFNG, LBP, CFI, KLRD1, PTPRC, VAV3, SOCS3, CFB, NR4A3, PRKCB, CD84, CD38, CD55, MYO10, IL20RB, CXCL13, C1RL, TREM1, XCL1, KIR3DL2, KIR2DL4 |
| GOTERM_BP_FAT        | Cell migration                            | 35    | 1.68E-04| ARSB, ZFAND5, FGF18, NRP1, CCR1, CXCL2, FERMT1, CXCL4, MMP1, Rac2, APOD, IFNG, LBP, DEPDC1B, ANGPT2, DPP4, PTPRC, SLCA81, SATB2, S100P, VAV3, AIMP1, NR4A1, DOCK7, DOCK8, SLC7A11, CD84, TNAIP6, CORO1A, THBD, CXCL13, SIX1, VCAN, TREM1, XCL1 |
| GOTERM_BP_FAT        | Serine hydrolase activity                 | 14    | 1.70E-04| F11, GZMA, CFB, MMP7, GZMB, C15, TMPRSS5, MMP1, MMP10, C1RL, C1R, DPP6, DPP4, HABP2 |
| GOTERM_BP_FAT        | Cell adhesion                             | 45    | 1.76E-04| PPFIA2, ASS1, SNX5, CCR1, LEPR, FERMT1, CLDN10, SFN, ZEB1, CXCL4, CD96, LAMB3, NPHP4, CDKN2A, Rac2, APOD, HPSE, RASGRP1, IFNG, VNN1, THBS2, ANGPT2, DPP4, EGR1, PTPRC, ST6GAL1, S100P, VAV3, AIMP1, MAGI, IGFALS, DOCK8, NR4A3, SLC7A11, CD84, TNAIP6, MYO10, CD55, CORO1A, CAMK4, IL20RB, CXCL13, VCAN, XCL1, HABP2 |
| GOTERM_BP_FAT        | Biological adhesion                       | 45    | 1.91E-04| PPFIA2, ASS1, SNX5, CCR1, LEPR, FERMT1, CLDN10, SFN, ZEB1, CXCL4, CD96, LAMB3, NPHP4, CDKN2A, Rac2, APOD, HPSE, RASGRP1, IFNG, VNN1, THBS2, ANGPT2, DPP4, EGR1, PTPRC, ST6GAL1, S100P, VAV3, AIMP1, MAGI, IGFALS, DOCK8, NR4A3, SLC7A11, CD84, TNAIP6, MYO10, CD55, CORO1A, CAMK4, IL20RB, CXCL13, VCAN, XCL1, HABP2 |
| GOTERM_BP_FAT        | Negative regulation of response to stimulus | 39    | 1.91E-04| NKD2, NRP1, IL19, Tmem161A, FaslG, IRAK3, CD96, NPHP4, APOD, CHTST11, INPP5F, VNN1, CALCRL, ANGPT2, F11, ZFP36, EGR1, PTPRC, ST6GAL1, SOCS3, NR4A3, Rgs14, PRKCB, PLAUR, SOD2, CD84, DUSP5, TNAIP6, CD55, Rgs1, Dkk1, THBD, IL20RB, ADM, CXCL13, HELB, IGFBP1, XCL1, DUSP6 |
| GOTERM_BP_FAT        | Negative regulation of response to external stimulus | 14    | 1.97E-04| ZFP36, F11, ST6GAL1, NRP1, SOCS3, PLAUR, TNAIP6, THBD, IL20RB, APOD, CXCL13, INPP5F, CALCRL, ANGPT2 |
| GOTERM_BP_FAT        | Serine-type endopeptidase activity        | 13    | 2.08E-04| F11, MMP10, CFB, GZMA, C1RL, MMP7, GZMB, CFI, C15, DPP4, MMP1, TMPRSS5, HABP2 |
| GOTERM_BP_FAT        | Chemotaxis                                | 21    | 2.57E-04| FGF18, ST6GAL1, NRP1, VAV3, AIMP1, KIF5A, CCR1, CXCL2, NR4A1, NR4A3, CCL4, PLAUR, CORO1A, Rac2, CXCL13, IFNG, CMTM7, TREM1, LBP, XCL1, ANGPT2 |
| GOTERM_BP_FAT        | Neutrophil chemotaxis                     | 8     | 2.61E-04| VAV3, Rac2, CXCL2, IFNG, TREM1, LBP, XCL1, CCL4 |
| GOTERM_BP_FAT        | Taxis                                     | 21    | 2.62E-04| FGF18, ST6GAL1, NRP1, VAV3, AIMP1, KIF5A, CCR1, CXCL2, NR4A1, NR4A3, CCL4, PLAUR, CORO1A, Rac2, CXCL13, IFNG, CMTM7, TREM1, LBP, XCL1, ANGPT2 |
| GOTERM_BP_FAT        | Regulation of cell–cell adhesion          | 17    | 2.64E-04| PTPRC, ASS1, NR4A3, ZEB1, CORO1A, CD55, MYO10, CDKN2A, Rac2, CAMK4, IL20RB, CXCL13, RASGRP1, IFNG, VNN1, XCL1, DPP4 |
| GOTERM_BP_FAT        | Regulation of apoptotic process           | 39    | 2.88E-04| FGFR2, NRP1, IL19, Tmem161A, FASLG, AURKB, SFN, PMAIP1, CDKN2A, MYOCD, IFNG, CHTST11, ROBO4, VNN1, PHLD1A, ZFP36, EGR1, ST6GAL1, IL2RB, VAV3, SOCS3, GZMA, BCL2A1, PIM1, NR4A1, GZMB, DOCK8, NR4A3, ANXA4, PLAUR, PLAC8, SOD2, CD38, CORO1A, ADM, SIX1, LGALS14, TNAIP6, DUSP6 |

(Continues)
| Category                     | Term                                                                 | Count | P value     | Gene                                                                 |
|------------------------------|----------------------------------------------------------------------|-------|-------------|----------------------------------------------------------------------|
| **GOTERM_BP_FAT**            | Cell killing                                                        | 9     | 2.90E-04    | PTPRC, CORO1A, RASGRP1, GNYL, IFNG, GZMB, TREM1, XCL1, KIR3DL1       |
| **GOTERM_BP_FAT**            | Regulation of leukocyte cell–cell adhesion                           | 15    | 2.95E-04    | PTPRC, ASS1, NR4A3, ZEB1, CORO1A, CD55, CDKN2A, RAC2, CAMK4, IL20RB, RASGRP1, IFNG, VNN1, XCL1, DPP4 |
| **GOTERM_BP_FAT**            | Natural killer cell mediated immunity                                | 7     | 3.01E-04    | CD96, KLRC2, CORO1A, RASGRP1, GZMB, KLRD1, KIR3DL1                  |
| **GOTERM_BP_FAT**            | Regulation of programmed cell death                                 | 39    | 3.49E-04    | FGF2R, NRP1, IL19, TEMEM161A, FASLG, AURKB, SFN, PMAIP1, CDKN2A, MYOCYD, IFNG, CHST11, ROBO4, VNN1, PHLDAA1, ZFP36, EGR1, ST6GAL1, IL2RB, VAV3, SOC53, GZMA, BCL2A1, PIM1, NR4A1, GZMB, DOCK8, NR4A3, ANXA4, PLAU, PLAC8, SOD2, CD38, CORO1A, ADM, SIX1, LGALS14, TNFAIP8, DUSP6 |
| **GOTERM_BP_FAT**            | Cell death                                                          | 49    | 3.99E-04    | FGF2R, PRF1, NRP1, IL19, TEMEM161A, FASLG, PMAIP1, AURKB, SFN, CDKN2A, MYOCYD, IFNG, CHST11, ROBO4, VNN1, MX1, SRGN, PHLDAA1, EGR1, ZFP36, ST6GAL1, IL2RB, VAV3, MAGI1, AIMP1, SOC53, GZMA, BCL2A1, PIM1, NR4A1, GZMB, NR4A3, DOCK8, ANXA4, PLAC8, SOD2, PLAU, PRKCB, CD38, CORO1A, ADM, SIX1, LGALS14, TNFAIP8, HIF3A, EMP1, HTTR2A, DUSP6, PRODH |
| **GOTERM_BP_FAT**            | Regulation of T cell differentiation in thymus                       | 5     | 5.38E-04    | CDKN2A, CAMK4, RASGRP1, VNN1, ZEB1                                  |
| **GOTERM_BP_FAT**            | Regulation of thymocyte aggregation                                  | 5     | 5.38E-04    | CDKN2A, CAMK4, RASGRP1, VNN1, ZEB1                                  |
| **GOTERM_BP_FAT**            | Positive regulation of chemotaxis                                    | 9     | 5.69E-04    | FGF18, NRP1, RAC2, CXCL13, CCR1, CXCL2, LBP, XCL1, CCL4             |
| **GOTERM_BP_FAT**            | Regulation of chemotaxis                                            | 11    | 5.84E-04    | FGF18, ST6GAL1, NRP1, RAC2, CXCL13, CCR1, CXCL2, LBP, XCL1, ANGPT2, CCL4 |
| **GOTERM_BP_FAT**            | Neutrophil migration                                                 | 8     | 5.86E-04    | VAV3, RAC2, CXCL2, IFNG, TREM1, LBP, XCL1, CCL4                    |
| **GOTERM_BP_FAT**            | Humoral immune response                                              | 12    | 6.16E-04    | CD55, ST6GAL1, ADM, CFB, RNASE7, IFNG, C1RL, CFI, C15, TREM1, TRDC, BLNK |
| **GOTERM_BP_FAT**            | Leukocyte chemotaxis                                                 | 11    | 6.34E-04    | CORO1A, VAV3, RAC2, CXCL13, CCR1, CXCL2, IFNG, TREM1, LBP, XCL1, CCL4 |
| **GOTERM_BP_FAT**            | Regulation of cell death                                             | 40    | 6.46E-04    | FGF2R, NRP1, IL19, TEMEM161A, FASLG, AURKB, SFN, PMAIP1, CDKN2A, MYOCYD, IFNG, CHST11, ROBO4, VNN1, PHLDAA1, ZFP36, EGR1, ST6GAL1, IL2RB, VAV3, SOC53, GZMA, BCL2A1, PIM1, NR4A1, GZMB, DOCK8, NR4A3, ANXA4, PLAU, PLAC8, SOD2, CD38, CORO1A, ADM, SIX1, LGALS14, TNFAIP8, DUSP6, PRODH |
| **GOTERM_BP_FAT**            | Negative regulation of apoptotic process                             | 26    | 6.57E-04    | FGF2R, NRP1, IL19, TEMEM161A, FASLG, AURKB, SFN, MYOCYD, CHST11, VNN1, ST6GAL1, IL2RB, SOC53, BCL2A1, PIM1, NR4A1, NR4A3, DOCK8, ANXA4, SOD2, PLAC8, PLAU, CD38, CORO1A, SIX1, TNFAIP8 |
| **GOTERM_BP_FAT**            | Innate immune response                                               | 26    | 6.99E-04    | KLRC2, ASS1, TRDC, C15, CCL4, CD96, IRAK3, RASGRP1, RNASE7, IFNG, VNN1, CFI, LBP, MX1, KLRD1, EGR1, CFB, SOC53, GZMB, CD84, CORO1A, CD55, C1RL, TREM1, XCL1, KIR3DL1 |
| **GOTERM_BP_FAT**            | Glycosaminoglycan metabolic process                                  | 10    | 7.21E-04    | ARSB, CHSY3, HPSE, CHST6, B3GNT7, CHST11, PIM1, HSPG2, VCAN, H2S2T1 |
| **GOTERM_BP_FAT**            | Programmed cell death                                                | 46    | 7.29E-04    | FGF2R, PRF1, NRP1, IL19, TEMEM161A, FASLG, AURKB, SFN, PMAIP1, CDKN2A, MYOCYD, IFNG, CHST11, ROBO4, VNN1, MX1, SRGN, PHLDAA1, ZFP36, ST6GAL1, IL2RB, VAV3, AIMP1, SOC53, GZMA, BCL2A1, PIM1, NR4A1, GZMB, DOCK8, NR4A3, ANXA4, PRKCB, PLAU, PLAC8, SOD2, CD38, CORO1A, ADM, SIX1, LGALS14, TNFAIP8, HIF3A, DUSP6, PRODH |
| **GOTERM_BP_FAT**            | Apoptotic process                                                    | 44    | 7.55E-04    | FGF2R, PRF1, NRP1, IL19, TEMEM161A, FASLG, AURKB, SFN, PMAIP1, CDKN2A, MYOCYD, IFNG, CHST11, VNN1, MX1, SRGN, PHLDAA1, ZFP36, ST6GAL1, IL2RB, VAV3, AIMP1, SOC53, GZMA, BCL2A1, PIM1, NR4A1, GZMB, DOCK8, NR4A3, ANXA4, PRKCB, PLAU, PLAC8, SOD2, CD38, CORO1A, ADM, SIX1, LGALS14, TNFAIP8, HIF3A, DUSP6, PRODH |
(Continues)
| Category            | Term                                      | Count | P value  | Gene                                                                 |
|---------------------|-------------------------------------------|-------|----------|----------------------------------------------------------------------|
| GOTERM_BP_FAT       | Negative regulation of programmed cell death | 26    | 7.88E-04 | FGFR2, NR1P1, IL19, TMEM161A, FASLG, AURKB, SFN, MYOCD, CHST11, VNN1, ST6GAL1, IL2RB, SOCS3, BCL2A1, PIM1, NR4A1, NR4A3, DOCK8, ANXA4, SOD2, PLAC8, PLAUR, CD38, CORO1A, SIX1, TNFAIP8 |
| GOTERM_BP_FAT       | Granulocyte chemotaxis                    | 8     | 8.39E-04 | VAV3, RAC2, CXCL2, IFNG, TREM1, LBP, XCL1, CCL4                        |
| GOTERM_BP_FAT       | Cytokine production involved in immune response | 7     | 8.41E-04 | IRAK3, CD96, CD55, CAMK4, NR4A3, TREM1, XCL1                           |
| GOTERM_BP_FAT       | Positive regulation of immune system process | 28    | 8.58E-04 | RBP4, PTPRC, VAV3, CFB, CCR1, CXCL2, C1S, TRDC, NR4A3, CCL4, PRKCB, CD84, FOS, IRAK3, CD38, CD55, MYO10, CORO1A, RAC2, CXCL13, RASGRP1, IFNG, C1RL, VNN1, LBP, CFI, XCL1, DPP4 |
| GOTERM_BP_FAT       | Leukocyte apoptotic process               | 8     | 8.88E-04 | ST6GAL1, CDKN2A, LGALS14, IFNG, FASLG, NR4A3, DOCK8, AURKB             |
| GOTERM_BP_FAT       | Production of molecular mediator of immune response | 10 | .001012913 | PTPRC, IRAK3, CD96, RBP4, CD55, CAMK4, IFNG, NR4A3, TREM1, XCL1        |
| GOTERM_BP_FAT       | Regulation of mononuclear cell proliferation | 11    | .001040266 | PTPRC, CD38, CD55, ST6GAL1, CORO1A, VAV3, CDKN2A, RAC2, IL20RB, IFNG, XCL1 |
| GOTERM_BP_FAT       | Aminoglycan metabolic process             | 10    | .001061591 | ARSB, CHSY3, HPSE, CHST6, B3GNT7, CHST11, PIM1, HSPG2, VCAN, H5ST1    |
| GOTERM_BP_FAT       | Response to bacterium                     | 20    | .001085717 | ZFP36, ASS1, SOCS3, GNLY, CXCL2, StatH, FASLG, TRDC, SOD2, PLAC8, CD96, IRAK3, FOS, THBD, ADM, CXCL13, RNASE7, IFNG, TREM1, LBP |
| GOTERM_BP_FAT       | Leukocyte cell–cell adhesion              | 18    | .001187931 | EGR1, PTPRC, ASS1, LEPR, NR4A3, ZEB1, DOCK8, CORO1A, CD55, CDKN2A, RAC2, CAMK4, IL20RB, RASGRP1, IFNG, VNN1, XCL1, DPP4 |
| GOTERM_BP_FAT       | Glycosaminoglycan binding                 | 11    | .001232209 | FGFR2, F11, TNFAIP6, NRP1, CXCL13, RNASE7, MMP7, COL25A1, VCAN, THBS2, HABP2 |
| GOTERM_BP_FAT       | Leukocyte aggregation                     | 17    | .001364809 | EGR1, PTPRC, LEPR, NR4A3, ZEB1, DOCK8, CORO1A, CD55, CDKN2A, RAC2, CAMK4, IL20RB, RASGRP1, FNG, VNN1, XCL1, DPP4 |
| GOTERM_BP_FAT       | Regulation of lymphocyte activation       | 16    | .001403667 | PTPRC, VAV3, TRDC, ZEB1, CD38, CORO1A, CD55, CDKN2A, RAC2, CAMK4, IL20RB, RASGRP1, IFNG, VNN1, XCL1, DPP4 |
| GOTERM_BP_FAT       | Regulation of leukocyte proliferation     | 11    | .00143279 | PTPRC, CD38, CD55, ST6GAL1, CORO1A, VAV3, CDKN2A, RAC2, IL20RB, IFNG, XCL1 |
| GOTERM_BP_FAT       | Leukocyte mediated cytotoxicity           | 7     | .001521044 | PTPRC, CORO1A, RASGRP1, GZMB, TREM1, XCL1, KIR3DL1                   |
| GOTERM_BP_FAT       | Positive regulation of leukocyte chemotaxis | 7     | .001521044 | RAC2, CXCL13, CCR1, CXCL2, LBP, XCL1, CCL4                           |
| GOTERM_BP_FAT       | Response to lipopolysaccharide            | 14    | .001525141 | ZFP36, IRAK3, CD96, FOS, THBD, ADM, ASS1, SOCS3, CXCL13, CXCL2, IFNG, FASLG, LBP, SOD2 |
| GOTERM_BP_FAT       | Granulocyte migration                     | 8     | .001597708 | VAV3, RAC2, CXCL2, IFNG, TREM1, LBP, XCL1, CCL4                       |
| GOTERM_BP_FAT       | Response to lipid                         | 27    | .001721042 | RBP4, THR8, ASS1, RBP1, CXCL2, TMEM161A, FASLG, CD96, IRAK3, FOS, IFNG, LBP, NR2F1, ZFP36, SOCS3, PIM1, NR4A1, FOSB, NR4A3, SOD2, CD38, SSTR2, THBD, DKK1, ADM, CXCL13, FABP3 |
| GOTERM_BP_FAT       | Response to drug                          | 17    | .001755254 | SLC8A1, VAV3, ASS1, CYP2B6, CYP2C9, SOCS3, FOSB, CCL4, SOD2, CD38, FOS, APOD, CD69, IFNG, FABP3, HTRA2, DUSP6 |
| GOTERM_BP_FAT       | Regulation of cell adhesion               | 21    | .001839233 | PTPRC, ST6GAL1, VAV3, ASS1, ZEB1, NR4A3, CORO1A, CD55, MYO10, CDKN2A, RAC2, CAMK4, IL20RB, APOD, CXCL13, RASGRP1, IFNG, VNN1, XCL1, ANGPT2, DPP4 |
| GOTERM_BP_FAT       | Regulation of T cell activation           | 13    | .001997259 | PTPRC, ZEB1, CORO1A, CD55, CDKN2A, RAC2, IL20RB, CAMK4, RASGRP1, IFNG, VNN1, XCL1, DPP4 |

(Continues)
**Table 2 (Continued)**

| Category | Term                                      | Count | P value | Gene                  |
|----------|-------------------------------------------|-------|---------|-----------------------|
| GOTERM_BP_FAT | Negative regulation of cytokine production | 11    | 0.002001253 | CD84, ZFP36, IRAK3, CD96, APOD, IL20RB, IFNG, LBP, XCL1, ANXA4, SRGN |
| GOTERM_BP_FAT | Cellular defense response                  | 6     | 0.002050165 | PRF1, KLRC2, GNLY, LBP, Kir2DL4, Kir3DL2 |
| GOTERM_BP_FAT | Inflammatory apoptotic process             | 4     | 0.002205885 | ST6GAL1, CDKN2A, IFNG, FASLG |
| GOTERM_BP_FAT | Response to molecule of bacterial origin   | 14    | 0.00213254  | ZFP36, IRAK3, CD96, FOS, THBD, ADM, ASS1, SOCS3, CXCL13, CXCL2, IFNG, FASLG, LBP, SOD2 |
| GOTERM_BP_FAT | Negative regulation of cell death          | 26    | 0.00245476  | FGFR2, NRPI, IL19, TMEM161A, FASLG, AURKB, SFN, MYOCD, CHST11, VNN1, ST6GAL1, IL2RB, SOCS3, BCL2A1, PIM1, NR4A1, NR4A3, DOCK8, ANXA4, SOD2, PLAC8, PLAU, CD38, CORO1A, SIX1, TNFAIP8 |
| GOTERM_BP_FAT | Mononuclear cell proliferation             | 12    | 0.002460795 | PTPRC, CD38, CD55, ST6GAL1, CORO1A, VAV3, CDKN2A, RAC2, IL20RB, IFNG, DOCK8, XCL1 |
| GOTERM_BP_FAT | Response to wounding                       | 21    | 0.002508224 | ZFP36, F11, NRPI, VAV3, AIMP1, CCR1, DOCK8, SLC7A11, PRKCB, PLAU, SOD2, THBD, RAC2, APOD, ADM, HPSE, INPP5F, H3F3B, IGFBP1, PAPSS2, FABP5 |
| GOTERM_BP_FAT | Positive regulation of cell proliferation  | 25    | 0.002576078 | FGFR2, FGFI8, NRPI, FASLG, RAC2, MYOCD, HPSE, IFNG, CALCRL, DPP4, EGR1, PTPRC, ST6GAL1, VAV3, NR4A1, NR4A3, PLAC8, CD38, CORO1A, CD55, ADM, SIX1, ID4, XCL1, HTR2A |
| GOTERM_BP_FAT | Myeloid leukocyte migration                | 9     | 0.002633977 | VAV3, RAC2, CCR1, CXCL2, IFNG, TREM1, LBP, XCL1, CCL4 |
| GOTERM_BP_FAT | Positive regulation of leukocyte activation | 13    | 0.002679897 | PTPRC, CD38, CD55, CORO1A, VAV3, RASGRP1, IFNG, VNN1, LBP, NR4A3, TRDC, XCL1, DPP4 |
| GOTERM_BP_FAT | Response to extracellular stimulus         | 17    | 0.002734588 | ZFP36, ARSB, SLC8A1, ASS1, RBPI, SOCS3, PIM1, MMP7, GAST, PMAIP1, SOD2, FOS, SSTR2, MYOCD, ADM, SLC2A1, CP |
| GOTERM_BP_FAT | Multi–multicellular organism process       | 11    | 0.002740849 | CD38, FOS, CD55, PG9, THBD, ADM, MMP7, H3F3B, SLC38A1, FOSB, ANGPT2 |
| GOTERM_BP_FAT | Regulation of inflammatory response        | 13    | 0.002812705 | ZFP36, TNFAIP6, CD55, APOD, IL20RB, SOCS3, CBF, SLC7A2, CFI, CALCRL, LBP, XCL1, CCL4 |
| GOTERM_BP_FAT | Single organismal cell–cell adhesion       | 22    | 0.002886539 | EGR1, PTPRC, ASS1, LEPR, DOCK8, NR4A3, ZEB1, SLC7A11, NPH4, CORO1A, CD55, MYO10, CDKN2A, RAC2, CAMK4, IL20RB, CXCL13, RASGRP1, IFNG, VNN1, XCL1, DPP4 |
| GOTERM_CC_FAT | Cell surface                               | 24    | 0.002962228 | PPFA2, ARSB, FGFR2, PTPRC, IL2RB, NRPI, TRPM8, AIMP1, CCR1, MMP7, FASLG, TRDC, ANXA4, SLC7A11, PROM1, CD38, CD55, THBD, CD69, IFNG, LBP, DPP6, KLRE1, DPP4 |
| GOTERM_BP_FAT | T cell aggregation                         | 16    | 0.002988034 | EGR1, PTPRC, LEPR, ZEB1, DOCK8, CORO1A, CD55, CDKN2A, RAC2, CAMK4, IL20RB, RASGRP1, IFNG, VNN1, XCL1, DPP4 |
| GOTERM_BP_FAT | T cell activation                          | 16    | 0.002988034 | EGR1, PTPRC, LEPR, ZEB1, DOCK8, CORO1A, CD55, CDKN2A, RAC2, CAMK4, IL20RB, RASGRP1, IFNG, VNN1, XCL1, DPP4 |
| GOTERM_BP_FAT | Lymphocyte aggregation                     | 16    | 0.003046718 | EGR1, PTPRC, LEPR, ZEB1, DOCK8, CORO1A, CD55, CDKN2A, RAC2, CAMK4, IL20RB, RASGRP1, IFNG, VNN1, XCL1, DPP4 |
| GOTERM_BP_FAT | Cellular modified amino acid metabolic process | 9     | 0.003065973 | CHDH, MTHFD2, MTHFS, GSTT2B, ASS1, SLC4A4I1, MTHFD1I1, PRODH, SOD2 |
| GOTERM_BP_FAT | Negative regulation of protein serine/threonine kinase activity | 8     | 0.003199245 | DUSP5, IRAK3, CDKN2A, MYOCD, PKIB, SFN, RGS14, DUSP6 |
| GOTERM_BP_FAT | Positive regulation of cell migration      | 15    | 0.003220579 | PTPRC, TNFAIP6, FGFI8, SLC8A1, CORO1A, NRPI, RAC2, CXCL13, CCR1, CXCL2, IFNG, DOCK7, LBP, XCL1, CCL4 |
| GOTERM_BP_FAT | Aging                                      | 13    | 0.003269145 | ASS1, SOCS3, BCL2A1, MMP7, AURKB, SOD2, FOS, CDKN2A, APOD, ADM, IFGBP1, CP, HTR2A |
| GOTERM_BP_FAT | Positive regulation of cell activation     | 13    | 0.003376692 | PTPRC, CD38, CD55, CORO1A, VAV3, RASGRP1, IFNG, VNN1, LBP, NR4A3, TRDC, XCL1, DPP4 |
TABLE 2  (Continued)

| Category         | Term                                      | Count | P value       | Gene                                                                 |
|------------------|-------------------------------------------|-------|---------------|----------------------------------------------------------------------|
| GOTERM_BP_FAT    | Female pregnancy                          | 10    | .003499079    | CD38, FOS, PSF9, THBD, ADM, MMP7, H3F3B, SLC38A1, FOSB, ANGPT2       |
| GOTERM_BP_FAT    | Regulation of lymphocyte proliferation    | 10    | .003499079    | PTPRC, CD38, CD55, CORO1A, VAV3, CDKN2A, RAC2, IL20RB, IFNG, XCL1  |
| GOTERM_BP_FAT    | Leukocyte proliferation                   | 12    | .003703152    | PTPRC, CD38, CD55, ST6GAL1, CORO1A, VAV3, CDKN2A, RAC2, IL20RB, IFNG, DOCK8, XCL1 |
| GOTERM_BP_FAT    | Defense response to other organism        | 19    | .003727248    | PTPRC, PTPRC, DPF3, AIMP1, GNLY, STAT1, LMC1, TRDC, PMAIP1, COTL1, PLAC8, ADM, CXCL13, RNASE7, IFNG, TREM1, LBP, MX1, FCGR3B |
| GOTERM_BP_FAT    | Regulation of leukocyte chemotaxis        | 7     | .004046973    | RAC2, CXCL13, CCR1, CXCL2, LBP, XCL1, CCL4                            |
| GOTERM_BP_FAT    | Negative regulation of immune effector process | 7     | .004245428    | CD84, PTPRC, IRAK3, CD96, CD55, IL20RB, XCL1                         |
| GOTERM_BP_FAT    | Positive regulation of mononuclear cell proliferation | 8     | .00427214    | PTPRC, CD38, CD55, ST6GAL1, CORO1A, VAV3, IFNG, XCL1                |
| GOTERM_BP_FAT    | Positive regulation of cell motility      | 15    | .004330368    | PTPRC, TNFAIP6, FGF18, SLC8A1, CORO1A, NRPI, RAC2, CXCL13, CCR1, CXCL2, IFNG, DOCK7, LBP, XCL1, CCL4 |
| GOTERM_BP_FAT    | Positive regulation of cell–cell adhesion | 11    | .004408001    | PTPRC, CD55, MYO10, CORO1A, CXCL13, RASGRP1, IFNG, VNN1, NR4A3, XCL1, DPP4 |
| GOTERM_BP_FAT    | Response to cold                          | 5     | .00466463     | FOS, ADM, TRPM8, PLAC8, SOD2                                         |
| GOTERM_BP_FAT    | Positive regulation of neutrophil chemotaxis | 4     | .005125974    | RAC2, CXCL2, LBP, XCL1                                               |
| GOTERM_MF_FAT    | Aminopeptidase activity                   | 5     | .005195241    | F11, MASP1D, PHEX, DPP6, DPP4                                        |
| GOTERM_BP_FAT    | Positive regulation of leukocyte proliferation | 8     | .00539139    | PTPRC, CD38, CD55, ST6GAL1, CORO1A, VAV3, IFNG, XCL1                |
| GOTERM_BP_FAT    | Negative regulation of leukocyte mediated immunity | 5     | .005435985    | CD84, PTPRC, CD96, IL20RB, XCL1                                     |
| GOTERM_BP_FAT    | Positive regulation of cellular component movement | 15    | .005527572    | PTPRC, TNFAIP6, FGF18, SLC8A1, CORO1A, NRPI, RAC2, CXCL13, CCR1, CXCL2, IFNG, DOCK7, LBP, XCL1, CCL4 |
| GOTERM_BP_FAT    | Positive regulation of locomotion         | 15    | .005622113    | PTPRC, TNFAIP6, FGF18, SLC8A1, CORO1A, NRPI, RAC2, CXCL13, CCR1, CXCL2, IFNG, DOCK7, LBP, XCL1, CCL4 |
| GOTERM_BP_FAT    | Endothelial cell chemotaxis               | 4     | .005762154    | FGF18, NRPI, CXCL13, NR4A1                                           |
| GOTERM_BP_FAT    | Myeloid leukocyte mediated immunity       | 6     | .005944619    | CD84, RAC2, CAMK4, RASGRP1, NR4A3, TREM1                              |
| GOTERM_BP_FAT    | Renal system vasculature development      | 4     | .006443547    | EGR1, NRPI, IFNG, ANGPT2                                             |
| GOTERM_BP_FAT    | Kidney vasculature development            | 4     | .006443547    | EGR1, NRPI, IFNG, ANGPT2                                             |
| GOTERM_BP_FAT    | Single organism cell adhesion             | 22    | .006492015    | EGR1, PTPRC, ASS1, LEPR, DOCK8, NR4A3, ZEB1, SLC7A11, NPHP4, CORO1A, CD55, MYO10, CDKN2A, RAC2, CAMK4, IL20RB, CXCL13, IFNG, VNN1, XCL1, DPP4 |
| GOTERM_BP_FAT    | Regulation of locomotion                  | 22    | .006605694    | ARSB, PTPRC, FGF18, SLC8A1, ST6GAL1, NRPI, CCR1, CXCL2, DOCK7, CCL4, TNFAIP6, MMP10, CORO1A, RAC2, APOD, CXCL13, IFNG, ROBO4, INPP5F, LBP, XCL1, ANGPT2 |
| GOTERM_BP_FAT    | Glycosaminoglycan biosynthetic process    | 7     | .006645161    | CHSY3, CHST6, B3GNT7, CHST11, HSPG2, VCAN, HS2ST1                    |
| GOTERM_BP_FAT    | Cytokine production                       | 19    | .006800751    | ZFP36, EGR1, IL19, NR4A3, ANXA4A, CD84, CD96, IRAK3, CD55, CAMK4, IL20RB, APOD, HPSE, RASGRP1, IFNG, TREM1, LBP, XCL1, SRGN |

(Continues)
| Category | Term | Count | P value | Gene |
|----------|------|-------|---------|------|
| GOTERM_BP_FAT | Lymphocyte proliferation | 11 | .006835525 | PTPRC, CD38, CD55, CORO1A, VAV3, CDKN2A, RAC2, IL20RB, IFNG, DOCK8, XCL1 |
| GOTERM_BP_FAT | Aminoglycan biosynthetic process | 7 | .006929937 | CHSY3, CHST6, B3GNT7, CHST11, HSPG2, VCAN, HS2ST1 |
| GOTERM_BP_FAT | Mucopolysaccharide metabolic process | 7 | .006929937 | ARSB, CHSY3, CHST6, B3GNT7, CHST11, PIM1, VCAN |
| GOTERM_BP_FAT | Cellular response to chemical stimulus | 56 | .007101409 | FGF18, NRP1, THRB, LEPR, TMEM161A, FASLG, PMAIP1, ZEB1, FOS, MYOCID, CHST11, IFNG, VNN1, CALCRL, LBP, MX1, ANGPT2, CSF2RA, NR2F1, ZFP36, EGR1, SATB2, SOCS3, PIM1, FOSB, PRKCB, SSTR2, IL20RB, GUCY1B3, TREM1, PRODH, FGFR2, ASS1, CYP2B6, CCR1, CXCL2, CCL4, IRAK3, RAC2, CD69, UGT1A5, PTPRC, IL2RB, SLCA81, VAV3, CYP2C9, NR4A1, NR4A3, SOD2, CORO1A, DKK1, CXCL13, HIF3A, IGFBP1, XCL1, DUSP6 |
| GOTERM_BP_FAT | Positive regulation of granulocyte chemotaxis | 4 | .007170933 | RAC2, CXCL2, LBP, XCL1 |
| GOTERM_BP_FAT | Lymphocyte differentiation | 12 | .007197221 | EGR1, PTPRC, CDKN2A, CAMK4, IKZF1, LEPR, RASGRP1, IFNG, CMTM7, VNN1, ZEB1, BLNK |
| GOTERM_BP_FAT | Epithelial cell migration | 10 | .007524598 | ARSB, FGF18, NRP1, S100P, CXCL13, IFNG, FERMT1, NR4A1, ANGPT2, DPP4 |
| GOTERM_BP_FAT | Positive regulation of leukocyte migration | 7 | .00752586 | RAC2, CXCL13, CCR1, CXCL2, LBP, XCL1, CCL4 |
| GOTERM_BP_FAT | Regulation of neutrophil chemotaxis | 4 | .007945027 | RAC2, CXCL2, LBP, XCL1 |
| GOTERM_BP_FAT | Epithelium migration | 10 | .008151142 | ARSB, FGF18, NRP1, S100P, CXCL13, IFNG, FERMT1, NR4A1, ANGPT2, DPP4 |
| GOTERM_BP_FAT | Formation of primary germ layer | 7 | .008157921 | FGFR2, DUSP5, LAMB3, DKK1, ETS2, NR4A3, DUSP6 |
| GOTERM_BP_FAT | Response to hypoxia | 12 | .008231471 | EGR1, CD38, SLCA81, ADM, MYOCID, SOCS3, HIF3A, PMAIP1, ANGPT2, DPP4, PRKCB, SOD2 |
| GOTERM_BP_FAT | Regulation of cell migration | 20 | .008409748 | ARSB, PTPRC, FGF18, SLCA81, NRP1, CCR1, CXCL2, DOCK7, CCL4, TNFAIP6, MMP10, CORO1A, RAC2, APOD, CXCL13, IFNG, ROBO4, LBP, XCL1, ANGPT2 |
| GOTERM_BP_FAT | Regulation of cell motility | 21 | .008458009 | ARSB, PTPRC, FGF18, SLCA81, NRP1, CCR1, CXCL2, DOCK7, CCL4, TNFAIP6, MMP10, CORO1A, RAC2, APOD, CXCL13, IFNG, ROBO4, INPP5F, LBP, XCL1, ANGPT2 |
| GOTERM_BP_FAT | Regulation of leukocyte apoptotic process | 6 | .008502574 | ST6GAL1, CDKN2A, LGALS14, NR4A3, DOCK8, AURKB |
| GOTERM_BP_FAT | Regulation of response to stress | 33 | .008516958 | DPF3, TMEM161A, PMAIP1, CCL4, CD96, IRAK3, CDKN2A, APOD, HPSE, RASGRP1, IFNG, INPP5F, VNN1, LBP, CFI, CALCRL, FGR53B, ZFP36, F11, SOCS3, CFB, LMCD1, NR4A3, PLAU, SOD2, TNFAIP6, CD55, THBD, IL20RB, HELB, SLC7A2, TREM1, XCL1 |
| GOTERM_BP_FAT | Negative regulation of defense response | 8 | .008550667 | ZFP36, IRAK3, CD96, TNFAIP6, APOD, IL20RB, SOCS3, CALCRL |
| GOTERM_BP_FAT | Ameboidal-type cell migration | 12 | .008776187 | ARSB, ZFAND5, FGF18, SLCA81, NRP1, S100P, CXCL13, IFNG, FERMT1, NR4A1, ANGPT2, DPP4 |
| GOTERM_BP_FAT | Response to oxygen-containing compound | 36 | .008811937 | RBP4, ASS1, CXCL2, TMEM161A, FASLG, ZEB1, FOS, CD96, IRAK3, APOD, IFNG, LBP, CALCRL, ANGPT2, EGR1, ZFP36, SLCA81, ST6GAL1, SOCS3, PIM1, NR4A1, FOSB, NR4A3, PRKCB, SOD2, CD38, SSTR2, CD55, DKK1, THBD, ADM, CXCL13, FABP3, GUCY1B3, IGFBP1, HTRA2 |
| GOTERM_BP_FAT | Response to nutrient levels | 15 | .008839745 | ZFP36, ARSB, SLCA81, ASS1, RBP1, SOCS3, PIM1, MMP7, GAST, PMAIP1, SOD2, SSTR2, ADM, SLC2A1, CP |

(Continues)
| Category          | Term                                                                 | Count | P.value  | Gene                                                                 |
|-------------------|----------------------------------------------------------------------|-------|----------|----------------------------------------------------------------------|
| GOTERM_BP_FAT     | Regulation of T cell proliferation                                   | 8     | 0.008844981 | PTPRC, CD55, CORO1A, CDKN2A, RAC2, IL20RB, IFNG, XCL1               |
| GOTERM_BP_FAT     | Negative regulation of transcription from RNA polymerase II promoter | 21    | 0.09041599 | ZFP36, EGR1, FGFR2, SATB2, THRB, IKZF1, LMCD1, FASLG, FOSB, AURKB, NR4A3, ZEB1, DKK1, MYOCID, ET52, SIX1, IFNG, ID4, BHLHE40, S100A1, NR2F1 |
| GOTERM_BP_FAT     | Apoptotic mitochondrial changes                                       | 7     | 0.01976322 | CDKN2A, BCL2A1, GZMB, PMAIP1, SFN, SOD2, PLAUR                      |
| GOTERM_BP_FAT     | Regulation of leukocyte mediated immunity                             | 8     | 0.09445487 | CD84, PTPRC, CD96, RAC2, IL20RB, RASGRP1, IFNG, XCL1               |
| GOTERM_BP_FAT     | Tissue migration                                                     | 10    | 0.00936961 | ARSB, FGFR1, NR1P1, S100P, CXCL13, IFNG, FERMT1, NR4A1, ANGPT2, DPP4 |
| GOTERM_BP_FAT     | Positive regulation of neutrophil migration                          | 4     | 0.009635883 | RAC2, CXCL2, LBP, XCL1                                             |
| GOTERM_BP_FAT     | Urogenital system development                                        | 12    | 0.0975718  | FGFR2, PROM1, EGR1, RBP4, NR1P1, ASS1, MYOCID, SERPINB5, SIX1, IFNG, ID4, ANGPT2 |
| GOTERM_BP_FAT     | Negative regulation of protein kinase activity                       | 10    | 0.009782435 | DUSP5, PTPRC, IRAK3, CDKN2A, MYOCID, SOCS3, PKIB, SFN, RGS14, DUSP6 |
| GOTERM_BP_FAT     | Extracellular matrix disassembly                                     | 6     | 0.00980152  | MMP10, LAMB3, HSPG2, MMP7, DPP4, MMP1                                 |
| GOTERM_BP_FAT     | Fat cell differentiation                                             | 9     | 0.00988103  | ZFP36, LAMB3, BBS9, NR4A1, ID4, NR4A3, PLAC8, HTR2A, SOD2           |
| GOTERM_BP_FAT     | Embryonic skeletal system development                                 | 7     | 0.009903808 | FGFR2, RBP4, SATB2, SIX1, CHST11, ZEB1, MTHFD1L                    |

Bold denotes gene names which are picked up in the results and discussion.

included genes for interferon gamma (IFN-γ), cytotoxic T-lymphocyte proteinase 1 (GZMA) and 2 (GZMB), tumor necrosis factor ligand superfamily member 6 (FASLG), and tumor necrosis factor alpha (TNF-α)-induced genes, such as TNF-α-induced protein 2 (TNFAIP2) and 6 (TNFAIP6).

The KEGG pathways that were associated with these genes involved the pathways that were related to immunity, such as “natural killer cell mediated cytotoxicity,” “complement and coagulation cascades,” “antigen processing and presentation,” “Graft-versus-host disease,” and “allograft rejection” (Tables 3 and S2). These KEGG pathways also included IFN-γ, GZMB, and FASLG.

3.3 Gene Ontology and Kyoto Encyclopedia of Genes and Genomes pathway analyses for the down-regulated genes in the thin endometrium

The down-regulated genes in the thin endometrium were related to metabolic processes, such as “small molecule catabolic process,” “single-organism catabolic process,” “organic acid catabolic process,” and “carboxylic acid catabolic process” (Tables 4 and S3). The GO terms included genes for carnitine palmitoyltransferase I (CPT1), 3-hydroxy-3-methylglutaryl-coenzyme A (CoA) synthase 2 (HMGC2), and 3-oxoacid CoA-transferase 1 (OXCT1), which are known to play important roles in generating energy in cells and tissues.13-15

In addition, several of the down-regulated genes (peroxisome proliferator-activated receptor γ [PPAR-γ], xanthine dehydrogenase [XDH], carbonyl reductase 3 [C3], isocitrate dehydrogenase 1 [IDH1], and CPT1) fell under the GO term “oxidation-reduction process” (Tables 4 and S1). These genes have essential roles in the response to oxidative stress.15-25

Two KEGG pathways that have a strong association with the down-regulated genes are “butanoate metabolism” and “metabolic pathways” (Tables 5 and S2). These pathways include HMGC2, OXCT1, XDH, IDH1, and C3.

4 DISCUSSION

4.1 Up-regulated genes in the thin endometrium

Although a thin endometrium is known to be involved in implantation failure, the mechanism has not been elucidated. The authors recently found that a high level of blood flow impedance of the uterine radial artery underlies a thin endometrium.4 The present study investigated the cause of implantation failure in the thin endometrium by using genome-wide mRNA expression analysis. Hierarchical clustering and a PCA demonstrated that the thin endometrium and the control endometrium clearly had different mRNA expression profiles, suggesting that aberrant gene expression is involved in implantation failure in a thin endometrium.
The GO analyses showed that the up-regulated genes in the thin endometrium included a number of genes that are related to immunity. In fact, a KEGG pathway analysis indicated that a number of genes related to natural killer cell cytotoxicity are up-regulated in thin endometria, suggesting the presence of a cytotoxic condition. Aberrant immunological factors play roles in recurrent miscarriage and implantation failure.\textsuperscript{6,26,27} Interestingly, 56.6% of the patients who experienced embryo implantation failures showed local immune overactivation in the endometrium at the mid-luteal phase.\textsuperscript{6} An influx of immune cells and a switch of local immunity from the adaptive (Th1) type to the innate (Th2) type have been observed during the implantation window.\textsuperscript{6,28} The Th2 cytokines allow the development of local mechanisms that promote immunotrophism and also down-regulate the inflammation and cytotoxic pathways.\textsuperscript{6,29} This immune switch, from a Th1 pro-inflammatory environment to a Th2 anti-inflammatory environment, is fundamental to the establishment of local maternal tolerance and is crucial for implantation. In this period, uterine natural killer (uNK) cells, together with macrophages and dendritic cells, increase in the endometrium and have a significant role in innate (Th2) immunity.\textsuperscript{30} Unlike peripheral natural killer cells, the uNK cells are not cytotoxic and their main biological functions are to produce immunotrophic and angiogenic cytokines. The activation of adequate uNK cells is important for maternal tolerance during the implantation window.\textsuperscript{31-33} However, once the uNK cells are highly activated, a Th1 pro-inflammatory condition is induced, with the local production of IFN-\(\gamma\) and TNF-\(\alpha\).\textsuperscript{34,35} The IFN-\(\gamma\) and TNF-\(\alpha\) activate the uNK cells to become cytotoxic.\textsuperscript{34,35} In fact, the present study showed that the IFN-\(\gamma\), FASLG, GZMB, and TNF-\(\alpha\)-induced genes, such as TNFAIP2 and TNFAIP6, were up-regulated and that natural killer cell cytotoxicity was elevated in the thin endometrium. These results suggest that aberrant overactivation of the uNK cells and a cytotoxic/Th1 pro-inflammatory environment are present in a thin endometrium, which is associated with implantation failure. However, it is still unknown how impaired blood flow is associated with the aberrant immunity.

### TABLE 3  Kyoto Encyclopedia of Genes and Genomes pathway analysis for the genes that were up-regulated in the thin endometrium

| Term                                      | Count | \( P \) value | Gene                                                                 |
|-------------------------------------------|-------|---------------|----------------------------------------------------------------------|
| Natural killer cell-mediated cytotoxicity | 10    | 8.21E-04      | PRF1, VAV3, RAC2, IFNG, FASLG, GZMB, FCGR3B, KLRD1, KIR2DL4, PRKCB   |
| Complement and coagulation cascades       | 7     | 0.0270751     | F11, CD55, THBD, CFB, CFI, C15, PLAUR                                 |
| Transcriptional misregulation in cancer   | 10    | 0.00722048    | PROM1, NFKBIZ, IL2RB, UTY, SIX1, BCL2A1, GRIA3, GZMB, H3F3B, NR4A3   |
| Antigen processing and presentation       | 6     | 0.01912738    | KLR2C, IFNG, KLRD1, KIR3DL1, KIR2DL4, KIR3DL2                        |
| Jak-STAT signaling pathway                | 8     | 0.02846128    | IL2RB, IL20RB, SOCS3, LEPR, IL19, IFNG, PIM1, C5S2RA                |
| Graft-versus-host disease                 | 4     | 0.02903814    | PRF1, IFNG, FASLG, GZMB                                             |
| Allograft rejection                       | 4     | 0.0390192     | PRF1, IFNG, FASLG, GZMB                                             |
| Amphetamine addiction                    | 5     | 0.04526534    | FOS, CAMK4, GRIA3, FOSB, PRKCB                                      |
| p53 signaling pathway                     | 5     | 0.04740558    | CDKN2A, SERPINB5, RRM2, PMAIP1, SFN                                 |

The GO analyses also showed that the down-regulated genes in the thin endometrium included a number of genes related to energy synthesis in the cell. The deficiency of energy could be associated with cellular dysfunction in the endometrium, resulting in implantation failure.

### 4.2  Down-regulated genes in the thin endometrium

The GO analyses indicated that the down-regulated genes included a number of genes related to catabolic processes, which are essential in breaking down large molecules, such as polysaccharides, lipids, and proteins, into smaller units, such as monosaccharides, fatty acids, and amino acids. These small units are used to synthesize acetyl-CoA, which is needed to produce adenosine 5’-triphosphate in the citrate cycle. Acetyl-CoA is also used for the synthesis of ketone bodies, which can be an energy source. The KEGG pathway analysis showed that the genes related to butanoate metabolism were down-regulated in the thin endometrium. Genes, such as CPT1, HMGCS2, and OXCT1, are essential for generating acetyl-CoA and ketone bodies in butanoate metabolism.\textsuperscript{13-15} Butanoate is a substrate that is used to generate energy in both aerobic and anaerobic processes. The present findings suggest that energy synthesis in the cell is impaired in the thin endometrium. The deficiency of energy could be associated with cellular dysfunction in the endometrium, resulting in implantation failure.

The GO analyses also identified a number of genes related to oxidation-reduction processes. These genes included PPAR-\(\gamma\), XDH, CBR3, IDH1, and CPT1, which have essential roles in the cellular responses to oxidative stress. The activation of PPAR-\(\gamma\) is an important factor in the protection against oxidative stress in cells, such as vascular endothelial cells and cardiomyocytes.\textsuperscript{16,17,20,21,24,25} Xanthine dehydrogenase reduces age-related oxidative stress in tissues and immune cells.\textsuperscript{23} Carbonyl reductase 3 is regulated via NRF2-dependent signaling pathways and helps to alleviate oxidative stress.\textsuperscript{18} Isocitrate dehydrogenase 1 acts as an antioxidant in melanocytes,\textsuperscript{19} and when mutated, it sensitizes cells to oxidative stress.\textsuperscript{36} Carnitine palmitoyltransferase 1 is involved in mitochondrial beta-oxidation of long-chain fatty acids.\textsuperscript{15} The inhibition of CPT1 leads to the generation of reactive oxygen species.\textsuperscript{22} Oxidative stress in the endometrium has been associated with failures of embryo implantation and embryo development.\textsuperscript{37} The fact that these anti-oxidative
| Category               | Term                                          | Count | P value     | Gene                                                                 |
|-----------------------|-----------------------------------------------|-------|-------------|----------------------------------------------------------------------|
| GOTERM_BP_FAT         | Small molecule catabolic process              | 21    | 2.80E-07    | NUDT16, ALDH6A1, EC12, KYNU, SORD, BCKDHB, CYP26A1, ALDH3B2, HGD, CBR3, ACADL, CPTIA, OXCT1, FUT3, QPRT, GAD1, GPT2, PCCA, CROT, DCXR, XYLb |
| GOTERM_BP_FAT         | Single-organism catabolic process             | 33    | 1.44E-06    | XDH, KYNU, SORD, OXCT1, PDE1A, FUT3, IDH1, ENTPD3, PLCB1, GAD1, GPT2, NUDT16, EC12, ALDH6A1, HERPUD1, PLD6, BCKDHB, HGD, ALDH3B2, CYP26A1, COL25A1, CBR3, COL5A3, ACADL, CPTIA, PLA2G4A, COL1A2, ACE2, QPRT, PCCA, CROT, DCXR, XYLb |
| GOTERM_BP_FAT         | Organic acid catabolic process                | 16    | 1.58E-06    | ALDH6A1, EC12, KYNU, SORD, BCKDHB, CYP26A1, HGD, ACADL, CPTIA, QPRT, GAD1, GPT2, PCCA, CROT, DCXR, XYLb |
| GOTERM_BP_FAT         | Carboxylic acid catabolic process             | 14    | 8.69E-06    | ALDH6A1, EC12, SORD, HGD, CYP26A1, ACADL, CPTIA, QPRT, GAD1, GPT2, PCCA, CROT, DCXR, XYLb |
| GOTERM_BP_FAT         | Oxidation-reduction process                  | 33    | 3.78E-05    | XDH, TM7SF2, C15orf48, PAM, STEAP4, CYP2J2, SORD, OPRK1, PPARG, DUOX1, FMO5, IDH1, NFATC4, SC5D, HHIP, EC12, ALDH6A1, BCKDHB, HGD, ALDH3B2, CYP26A1, CYB5A, CBR3, ACADL, CPTIA, DHR57, IVY, ACSM1, SQLE, GNAT5, PHF8, CROT, DCXR |
| GOTERM_BP_FAT         | Secretion                                    | 36    | 4.37E-05    | XDH, COPA, PAM, NAAA, OPRK1, PML, POSTN, TPD52, TLR6, TRH, KCN53, CAS5P, NOV, WNK4, OXCT1, SYN2, SYBU, CREB3L1, CHRNA6, GAD1, MAP2K6, ABCA12, TRPM4, ACTN1, GAL, ISIS, NLRP2, PCLO, CPTIA, PLA2G4A, CHGA, STXBP6, SYTL4, GNAT5, CA2, CFPB |
| GOTERM_BP_FAT         | Carboxylic acid metabolic process             | 29    | 6.36E-05    | PAM, KYNU, CYP2J2, SORD, PPARG, AGMAT, IDH1, UGT8, SC5D, GAD1, GPT2, GGT1A1P, EC12, ALDH6A1, PDK4, BCKDHB, HGD, CYP26A1, CYB5A, ACADL, CPTIA, ACSM3, ACSM1, PLA2C4A, QPRT, PCCA, DCXR, CROT, XYLb |
| GOTERM_BP_FAT         | Oxoacid metabolic process                    | 29    | 7.06E-05    | PAM, KYNU, CYP2J2, SORD, PPARG, AGMAT, IDH1, UGT8, SC5D, GAD1, GPT2, GGT1A1P, EC12, ALDH6A1, PDK4, BCKDHB, HGD, CYP26A1, CYB5A, ACADL, CPTIA, ACSM3, ACSM1, PLA2G4A, QPRT, PCCA, DCXR, CROT, XYLb |
| GOTERM_BP_FAT         | Peptide transport                            | 15    | 9.83E-05    | TRPM4, SLC15A2, ISIS, GAL, TRH, PCLO, CPTIA, KCN53, NOV, OXCT1, TAP2, SYBU, SYTL4, GNAT5, CA2 |
| GOTERM_BP_FAT         | Transmembrane transport                      | 39    | 1.02E-04    | C15orf48, CALH1M, SLC39A14, SLC38A4, ATP1B1, MFSD3, SLC15A2, OPRK1, KCNIP4, KCN53, SLC24A4, MCOLN3, ANK3, TAP2, WNK4, SLC25A48, TTYH2, SLC39A8, CHRNA6, SLC43A1, ANO10, ABCA12, TRPM4, TRPM6, ABC13, CYB5A, GAL, ANKH, ABCG1, CPTIA, GJB2, SLC26A3, ATP6V0E2, ADAMTS8, CLIC5, KCN53, SLC7A1, CA2, SLC46A2 |
| GOTERM_BP_FAT         | Ion transport                                | 42    | 1.10E-04    | C15orf48, CALH1M, SLC39A14, STEAP4, SLC38A4, ATP1B1, MFSD3, SLC15A2, OPRK1, PPARG, PML, TRH, KCNIP4, SEC14L1, KCN53, SLC24A4, MCOLN3, ANK3, WNK4, TTYH2, SLC39A8, CHRNA6, SLC43A1, MAP2K6, ANO10, TRPM4, RAMP2, TRPM6, CYB5A, GAL, ANKH, CPTIA, SLC26A3, PLA2G4A, ATP6V0E2, ADAMTS8, PKP2, CLIC5, KCN53, SLC7A1, CA2, CROT |
| GOTERM_MF_FAT         | Cofactor binding                             | 15    | 1.22E-04    | XDH, TM7SF2, ALDH6A1, EC12, KYNU, SORD, DUOX1, CBR3, ACADL, FM05, SQLE, IDH1, HHIP, GAD1, GPT2 |
| GOTERM_BP_FAT         | Monocarboxylic acid metabolic process        | 22    | 1.29E-04    | GGT1A1P, PAM, EC12, KYNU, SORD, CYP2J2, BCKDHB, PPARG, PDK4, CYP26A1, ACADL, CPTIA, ACSM3, PLA2G4A, ACSM1, IDH1, UGT8, SC5D, PCCA, DCXR, CROT, XYLb |

(Continues)
| Category         | Term                                      | Count | P value  | Gene                                                                 |
|------------------|-------------------------------------------|-------|----------|----------------------------------------------------------------------|
| GONTERM_BP_FAT   | Organic acid metabolic process            | 30    | 1.40E-04 | PAM, KYNU, CYP2J2, SORD, PPARG, AGMAT, FOLR1, IDH1, UGT8, SCD5, GAD1, GPT2, GATA1P, EC2, ALDH6A1, PKD4, BCKDH2, HGD, CYP26A1, CYB5A, ACADL, CPT1A, ACSM3, ACSM31, PL2A2GA4, QPRT, PCCA, DCKX, CROT, XYLB |
| GONTERM_BP_FAT   | Nitrogen compound transport               | 26    | 1.66E-04 | CALHM1, SLC38A4, SLC15A2, OPRK1, TRH, SEC14L1, KCNS3, NOV, FOLR1, OXCT1, TAP2, SYBU, CHRNA6, SLC43A1, ABCA12, TRPM4, ISL1, GAL, PCLO, CPT1A, CHGA, SLC7A1, SYTL4, ACE2, GNAS, CA2 |
| GONTERM_BP_FAT   | Monocarboxylic acid catabolic process     | 9     | 1.86E-04 | EC12, SORD, CYP26A1, ACADL, PCCA, CROT, CPT1A, DCKX, XYLB            |
| GONTERM_BP_FAT   | Tissue morphogenesis                      | 23    | 2.03E-04 | FRAS1, COBL, NF2, TNC, PML, NTN4, SIX3, FZD5, ISL1, MAGED1, ACTG2, EYA1, EPHA7, KRAS, PKP2, FOLR1, WNK4, TSC2, TFAP2A, NFATC4, CA2, HHIP, PRKACB |
| GONTERM_BP_FAT   | Regulation of secretion by cell           | 25    | 2.20E-04 | PAM, OPRK1, PML, POSTN, TLR6, TRH, KCNS3, NOV, CASP5, WNK4, OXCT1, SYBU, CHRNA6, MAP2K6, TRPM4, GAL, ISL1, NLRP2, PCLO, CPT1A, CHGA, STXB6, SYTL4, GNAS, CPB2 |
| GONTERM_BP_FAT   | Epithelium development                    | 32    | 3.93E-04 | FRAS1, XDH, COBL, TNC, PPARG, PML, CERS3, MAGED1, KRAS, MCOLN3, FOLR1, WNK4, UPK1B, NFATC4, HHHIP, PRKACB, ABCA12, SMAD9, NF2, NTN4, SIX3, FZD5, GAL, CPT1A, EYA1, EPHA7, CLIC5, TSC2, TFAP2A, GNAS, CA2, LRP4 |
| GONTERM_BP_FAT   | Odontogenesis                             | 9     | 4.17E-04 | ASPN, PAM, NF2, SLC24A4, TNC, COL1A2, TFAP2A, CA2, LRP4              |
| GONTERM_BP_FAT   | Regulation of secretion by cell           | 23    | 4.36E-04 | TRPM4, PAM, OPRK1, PML, POSTN, TRH, TLR6, ISL1, GAL, PCLO, NLRP2, CPT1A, KCNS3, CASP5, NOV, CHGA, STXB6, OXCT1, SYBU, SYTL4, GNAS, CHRNA6, CPB2 |
| GONTERM_BP_FAT   | Chemical homeostasis                      | 31    | 8.72E-04 | STEAP4, SLC39A14, ATP1B1, PPARG, PML, CKB, PDE6A, SLC24A4, ANK3, TAP2, OXCT1, WNK4, SLC39A8, PRKACB, ABCA12, TRPM4, HERPUD1, PKD4, TRIM24, GJB6, ACADL, ABCG1, ACSM3, SLC26A3, ACSM1, PL2A2GA4, ATP6V0E2, PKP2, GNAS, CA2, CPB2 |
| GONTERM_BP_FAT   | Response to endogenous stimulus           | 42    | 8.80E-04 | ASPN, PAM, KYNU, SORD, OPRK1, TNC, PPARG, DUOX1, PML, POSTN, GN11, TRH, GREM2, PEA15, KRAS, FOLR1, GSN, OXCT1, IDH1, CHRNA6, PRKACB, RAMP2, SMAD9, STMN2, PKD4, TRIM24, GAL, ISL1, ABCG1, GJB2, SLC26A3, PL2A2GA4, ATP6V0E2, HMGC52, TSC2, COL1A2, GNAS, CA2, BMP1R1B, HADCA9, CROT, LRP4 |
| GONTERM_CC_FAT   | Lamellipodium                             | 11    | 8.99E-04 | ACTG2, SLC39A14, NF2, SORBS2, GSN, PLEKHH2, STMN2, NEDD9, IQGAP2, TSN1, CTNNB2 |
| GONTERM_BP_FAT   | Secretion by cell                         | 29    | .001130969 | PAM, NAAA, OPRK1, PML, POSTN, TLR6, TRH, KCNS3, CASP5, NOV, OXCT1, SYN2, SYBU, CREB3L1, CHRNA6, GAD1, ABCA12, TRPM4, ACTN1, ISL1, GAL, PCLO, NLRP2, CPT1A, CHGA, STXB6, SYTL4, GNAS, CPB2 |
| GONTERM_BP_FAT   | Organ morphogenesis                       | 29    | .001210502 | FRAS1, ASPN, PAM, TNC, PML, MAGED1, ACTG2, KRAS, SLC24A4, FOLR1, WNK4, HHIP, NF2, SIX3, NTN4, GJB6, FZD5, ISL1, CTNNB2, EYA1, PKP2, CLIC5, COL1A2, TFAP2A, GNAS, CA2, BMP1R1B, CPB2, LRP4 |
| GONTERM_BP_FAT   | Regulation of biomineral tissue development| 7     | .001363066 | ASPN, TRPM4, PL2A2GA4, TFAP2A, BMP1R1B, TMEM119, ANKH |

(Continues)
| Category       | Term                                         | Count | P value | Gene                          |
|----------------|----------------------------------------------|-------|---------|-------------------------------|
| GOTERM_BP_FAT  | Regulation of protein secretion              | 16    | .001462035 | TRPM4, PAM, PML, POSTN, TLR6, ISL1, TRH, NLRP2, CPT1A, KCNS3, NOV, CASP5, OXCT1, SYBU, SYTL4, GNAS |
| GOTERM_CC_FAT  | Proteinaceous extracellular matrix           | 16    | .001470486 | FRA51, ASPN, HAPLN1, TNC, OLFML2B, NTN4, POSTN, COL5A3, NOV, OGN, BGN, ADAMTS8, KAZALD1, CCEB1, COL1A2, TFPI2 |
| GOTERM_BP_FAT  | Nervous system development                   | 52    | .00156293 | ATL1, PPARG, POSTN, CKB, CASP5, CDDC141, OGN, PRMT1, MCOLN3, ANK3, GSN, PRKACB, HHIP, CDK5RAP2, PLCB1, ATOH7, TNK1, STMN2, SIX3, COL25A1, GAL, PCLO, CTNNM2A, EYA1, CLIC5, TFAP2A, PAM, COBL, FRYL, TNC, KRAS, FOLR1, OXCT1, NFATC4, NDRG2, UGT8, DCLK1, HAPLN1, SMAD9, NF2, NTN4, FZD5, ISL1, EPD7, HMGC52, TSC2, MAP2, MPPED2, HADC9, BMRP1B, PHF8, LRIP |
| GOTERM_BP_FAT  | Inorganic ion transmembrane transport        | 23    | .001683334 | CALHM1, C15orf48, TRPM4, SLC39A14, ATP1B1, TRPM6, OPRK1, CYB5A, GAL, ANKH, KCNIP4, KCNS3, SLC26A3, ATP6V0E2, ADAMTS8, SLC24A4, MCOLN3, ANK3, CLIC5, KCNN3, TTYH2, SLC39A8, ANO10 |
| GOTERM_BP_FAT  | Peptide hormone secretion                    | 12    | .001842318 | KCNS3, TRPM4, NOV, OXCT1, SYBU, SYTL4, GNAS, ISL1, TRH, GAL, PCLO, CPT1A |
| GOTERM_BP_FAT  | Regulation of insulin secretion              | 10    | .001898558 | KCNS3, TRPM4, NOV, OXCT1, SYBU, SYTL4, GNAS, ISL1, TRH, CPT1A |
| GOTERM_BP_FAT  | Regulation of peptide transport              | 11    | .002159806 | KCNS3, TRPM4, NOV, OXCT1, SYBU, SYTL4, GNAS, CA2, ISL1, TRH, CPT1A |
| GOTERM_BP_FAT  | Xylulose 5-phosphate metabolic process       | 3     | .002231113 | SORD, DCXR, XYL8 |
| GOTERM_BP_FAT  | Glucuronate catabolic process to xylulose 5-phosphate | 3     | .002231113 | SORD, DCXR, XYL8 |
| GOTERM_BP_FAT  | Xylulose 5-phosphate biosynthetic process    | 3     | .002231113 | SORD, DCXR, XYL8 |
| GOTERM_BP_FAT  | Glucuronate catabolic process                | 3     | .002231113 | SORD, DCXR, XYL8 |
| GOTERM_BP_FAT  | Cation transport                             | 28    | .002320182 | CALHM1, C15orf48, SLC38A4, SLC39A14, STEAP4, ATP1B1, MFSD3, SLC15A2, OPRK1, PML, SEC14L1, KCNIP4, KCNS3, MCOLN3, SLC24A4, ANK3, WNK4, SLC39A8, CHRNA6, ANO10, TRPM4, RAMP2, TRPM6, CYB5A, GAL, ATP6V0E2, PKP2, KCNN3 |
| GOTERM_BP_FAT  | Peptide secretion                            | 12    | .002431887 | KCNS3, TRPM4, NOV, OXCT1, SYBU, SYTL4, GNAS, ISL1, TRH, GAL, PCLO, CPT1A |
| GOTERM_BP_FAT  | Morphogenesis of an epithelium               | 18    | .002441073 | FRAS1, COBL, TNC, PML, NTN4, FZD5, MAGED1, EPHA7, EYA1, KRAS, FOLR1, WNK4, TSC2, TFAP2A, NFATC4, CA2, PRKACB, HHIP |
| GOTERM_BP_FAT  | Anion transport                              | 17    | .002448168 | SLC38A4, PPARG, TRH, ANKH, CPT1A, SLC26A3, PLA2G4A, ADAMTS8, CLIC5, TTYH2, SLC7A1, WNK4, CA2, SLC43A1, MAP2K6, CROT, ANO10 |
| GOTERM_BP_FAT  | Epithelial tube morphogenesis                | 13    | .002731156 | COBL, TNC, PML, MAGED1, EYA1, EPHA7, KRAS, FOLR1, WNK4, TSC2, NFATC4, PRKACB, HHIP |
| GOTERM_BP_FAT  | Signal release                               | 16    | .002766541 | TRPM4, NAAA, OPRK1, ISL1, GAL, TRH, PCLO, CPT1A, KCNS3, NOV, OXCT1, SYN2, SYBU, SYTL4, GNAS, GAD1 |
| GOTERM_BP_FAT  | Regulation of hormone secretion              | 12    | .002882484 | KCNS3, TRPM4, NOV, OPRK1, OXCT1, SYBU, SYTL4, GNAS, ISL1, TRH, GAL, CPT1A |

(Continues)
| Category       | Term                                      | Count | P value     | Gene                                                                 |
|----------------|-------------------------------------------|-------|-------------|----------------------------------------------------------------------|
| GOTERM_BP_FAT | Protein localization                      | 55    | .002908085 | COPA, ATP1B1, SLC15A2, PPARG, VPS37B, POSTN, SELENBP1, TLR6, KCNI4,  |
|                |                                           |       |             | APIS3, NOV, CAPS5, ANK3, GSN, WNK4, TTC21A, TRPM4, RAMP2, TNK,       |
|                |                                           |       |             | SIX3, GAL, NLRP2, PCLO, EYA1, HEPACAM, STXBPD, CLIC5, GNAS, FRAS1,  |
|                |                                           |       |             | PAM, PML, RABGAP1L, TRH, KCNS2, TMED3, OXCT1, TAP2, SYBU, UGT8,     |
|                |                                           |       |             | DCLK1, ABCA12, HERPD1, NF2, ITGA4, FZD5, ISL1, CPT1A, ABCG1, ATP6V0E2,|
|                |                                           |       |             | PKP2, KCNN3, TSC2, SYT4L4, SXN30, LRP4                               |
|                | Regulation of transport                    | 44    | .002936176 | CALHM1, PAM, ATP1B1, OLF4M4, OPRK1, PPARG, PML, VPS37B, RABGAP1L,  |
|                |                                           |       |             | POSTN, TLR6, TRH, KCNI4, KCNS3, CAPS5, NOV, PEAI5, ANK3, WNK4, OXCT1,|
|                |                                           |       |             | SYBU, CHRNA6, MAP2K6, SGI1, ABCA12, TRPM4, FZD5, GAL, ISL1, NLRP2,  |
|                |                                           |       |             | PCLO, ABG1, CPT1A, PL2G4A, CHGA, STXBPD, PKP2, CLIC5, KCNN3, TSC2,  |
|                |                                           |       |             | SYT4L4, GNAS, CA2, CBP2                                             |
|                | Hormone secretion                         | 13    | .003111804 | TRPM4, OPRK1, GAL, TRH, ISL1, PCLO, CPT1A, KCNS3, NOV, OXCT1, SYT4L4,|
|                | Protein secretion                         | 17    | .003213878 | TRPM4, PML, POSTN, TRH, TLR6, ISL1, GAL, NLRP2, PCLO, CPT1A, KCNS3,  |
|                | Morphogenesis of a branching structure     | 10    | .003228966 | MAGED1, EYA1, EPHA7, KRAS, TNC, PML, NTN4, NFATC4, HHP, FZD5         |
|                | Insulin secretion                         | 9     | .003253189 | TRPM4, NOV, OXCT1, SYBU, SYT4L4, ISL1, TRH, GAL, PCLO               |
|                | Monovalent inorganic cation transport      | 17    | .003456998 | C15ORF48, TRPM4, SLC38A4, ATP1B1, MFS3, SLC15A2, OPRK1, CYB5A, GAL,|
|                |                                           |       |             | KCNI4, KCNS3, ATP6V0E2, SLC24A4, PKP2, ANK3, KCNN3, WNK4             |
|                | Regulation of hormone levels              | 17    | .003534129 | TRPM4, OPRK1, DUOX1, CYP26A1, TRH, ISL1, GAL, PCLO, CPT1A, IYD,     |
|                | Sulfur compound binding                   | 11    | .003932387 | KCNS3, NOV, OXCT1, SYBU, SYT4L4, ACE2, GNAS                         |
|                | Hormone transport                         | 13    | .004094245 | TRPM4, OPRK1, GAL, TRH, ISL1, PCLO, CPT1A, KCNS3, NOV, OXCT1, SYT4L4,|
|                | Dorsal/ventral pattern formation           | 7     | .00420815  | SIX3, HHP, PRKACB, BMP1R1, FZD5, GREM2, LRP4                         |
|                | Perinuclear region of cytoplasm           | 22    | .004356611 | DYNC1I1, CAPN6, COBL, PAM, OLF4M4, TNK, NF2, STMN2, PPARG, TPDS21L,|
|                |                                           |       |             | FZD5, TPDS2, CHGA, PL2G4A, GSN, SORB52, TSC2, GNAS, CALN1, NDRG2,    |
|                |                                           |       |             | CDK5RAP2, PKP2, PRKACB                                             |
|                | Fatty acid metabolic process              | 14    | .0044624   | GGTA1P, EC2, PAM, CYP212, PPARG, PDK4, ACADL, CPT1A, ACSM3, PL2G4A, |
|                |                                           |       |             | ACSM1, SCDS5, PCCA, CROT                                            |
|                | Active transmembrane transporter activity  | 14    | .004482151 | SLC26A3, SLC38A4, ATP1B1, ATP6V0E2, SLC24A4, MFS3, SLC38A2, TAP2,   |
|                |                                           |       |             | SLC7A1, ABCC13, ANKH, ABCG1, ABCA12, SLC46A2                        |

(Continues)
| Category          | Term                                      | Count | P value  | Gene                                                                 |
|-------------------|-------------------------------------------|-------|----------|----------------------------------------------------------------------|
| GÖTERM_MF_FAT     | Ion binding                               | 88    | .004564204 | ASPN, STEAP4, ATP1B1, KNYU, CYP2J2, ATL1, ZFP42, ZNF530, PPARG, DUOX1, POSTN, TPD52, JTSN1, KCNIP4, SLC24A4, GSN, SERPINA5, CCB1, CALN1, CHRNA6, HHP, PRKACB, SCD5, PLCB1, GPT2, NUDT16, TRPM6, PLD6, CYP26A1, ACTN1, CYB5A, PCLQ, NME7, NEBL, ZNF233, EYA1, ADAMTS8, CA8, CAPN12, COL1A2, GNAS, ADAM18, CA2, ADAM12, PCCA, ZNF436, FRAS1, CAPS, XDH, PAM, SOR, YPEL4, PML, ST8SIA3, AGMAT, PDE6A, RNF125, KRAS, GALNT10, TCEA3, FOLR1, SORBS2, PDE1A, IDH1, GAD1, SMAD9, IKZF2, PDZRN4, VWCE, HGD, TRIM24, ITGA4, CSR2P2, ISL1, XPNPEP2, ACSM3, PAPOLA, ACSM1, PLA2G4A, ZIC4, SYT4, ACE2, MMPED2, HDAC9, BMP1R1B, PHF8, CPB2, LRP4 |
| GÖTERM_BP_FAT     | Regulation of bone mineralization          | 6     | .004761268 | TRPM4, PLA2G4A, TFAP2A, BMP1R1B, TMEM119, ANKH |
| GÖTERM_BP_FAT     | Extracellular matrix organization          | 13    | .005198597 | RAMP2, HAPLN1, BGN, GSN, KAZALD1, TNC, OLFML2B, COL1A2, CREB31L1, POSTN, ITGA4, COL5A3, CPB2 |
| GÖTERM_BP_FAT     | Celluar response to chemical stimulus      | 58    | .00526536  | ASPN, CYP2J2, PPARG, DUOX1, POSTN, NOV, ANK3, GSN, CCB1, CREB31L1, PRKACB, HHP, PLCB1, MAP2K6, IFNGR1, TRPM4, RAMP2, STMN2, CYP26A1, SERPINB9, SLC26A3, CHGA, BGN, COL1A2, TFAP2A, GNAS, CA2, XDH, OPRK1, TNC, PML, GNG11, TRH, GREM2, KRAS, FOLR1, OXT1, NFATC4, THPO, HERPD1, SMAD9, PDK4, ITGA4, TRIM24, GJB6, ISL1, CPT1A, GJB3, PLA2G4A, ACSM1, ATP6V0E2, GPR37, HMGC52, TSC2, HDAC9, BMP1R1B, CPB2, LRP4 |
| GÖTERM_BP_FAT     | Extracellular structure organization        | 13    | .00532233  | RAMP2, HAPLN1, BGN, GSN, KAZALD1, TNC, OLFML2B, COL1A2, CREB31L1, POSTN, ITGA4, COL5A3, CPB2 |
| GÖTERM_MF_FAT     | Glycosaminoglycan binding                  | 10    | .005386366 | NOV, OGN, HAPLN1, BGN, ADAMTS8, SERPINA5, COL25A1, POSTN, COL5A3, GREM2 |
| GÖTERM_BP_FAT     | Positive regulation of stress-activated MAPK cascade | 8     | .005588879 | XDH, PRMT1, TNK, OPRK1, TPD52L1, TLR6, FZD5, PLCB1 |
| GÖTERM_BP_FAT     | Regulation of peptide hormone secretion    | 10    | .005698819 | KCNS3, TRPM4, NOV, OXT1, SYBU, SYTL4, GNAS, ISL1, TRH, CPT1A |
| GÖTERM_BP_FAT     | Morphogenesis of a branching epithelium    | 9     | .005744299 | MAGED1, EYA1, KRAS, TNC, PML, NTN4, NFATC4, HHP, FZD5 |
| GÖTERM_BP_FAT     | Positive regulation of stress-activated protein kinase signaling cascade | 8     | .005806735 | XDH, PRMT1, TNK, OPRK1, TPD52L1, TLR6, FZD5, PLCB1 |
| GÖTERM_MF_FAT     | NADP binding                              | 5     | .006314099 | TM7SF2, FM05, DUOX1, IDH1, CB3R |
| GÖTERM_BP_FAT     | Ion transmembrane transport               | 26    | .006573948 | CALHM1, C15ORF48, SLC39A14, ATP1B1, OPRK1, KCNIP4, KCNS3, MCOLN3, SLC24A4, ANK3, TTYH2, WNK4, SLC39A8, CHRNA6, AN010, TRPM4, TRPM6, CYB5A, GAL, ANKH, CPT1A, SLC26A3, ATP6V0E2, ADAMTS8, KCNS3, CLIC5 |
| GÖTERM_BP_FAT     | Regulation of peptide secretion            | 10    | .006609351 | KCNS3, TRPM4, NOV, OXT1, SYBU, SYTL4, GNAS, ISL1, TRH, CPT1A |
| GÖTERM_BP_FAT     | Response to hormone                        | 25    | .00677892  | PAM, SORD, TNC, OPRK1, PPARG, GNG11, TRH, KRAS, OXT1, IDH1, PRKACB, RAMP2, PDK4, TRIM24, ISL1, GAL, ABCG1, GJB2, PLA2G4A, ATP6V0E2, HMGC52, TSC2, GNAS, CA2, HDAC9 |
| GÖTERM_BP_FAT     | Response to vitamin                        | 7     | .006968045 | PLA2G4A, KNYU, FOLR1, GSN, TNC, PPARG, POSTN |

(Continues)
TABLE 4 (Continued)

| Category         | Term                             | Count | P value  | Gene                                                                 |
|------------------|----------------------------------|-------|----------|----------------------------------------------------------------------|
| GOTERM_BP_FAT    | Homeostatic process              | 39    | .006995908 | STEAP4, SLC39A14, ATP1B1, OPR1K1, PPARG, PML, CKB, NOV, PDE6A, PRMT1, KRIAS, SLC24A4, ANK3, TAP2, WNK4, OXCT1, SYBU, SLC39A8, PRKACB, SGIP1, ABCA12, TRPM4, HERPUD1, HMBOX1, PDK4, TRIM24, GB6, ACADL, ABCG1, ACSM3, SLC26A3, PLA2G4A, ACSM1, ATP6V0E2, PKP2, GNAS, CA2, CPB2, SLC46A2 |
| GOTERM_BP_FAT    | Tube morphogenesis               | 13    | .00712882 | COBL, TNC, PML, MAGED1, EYA1, EPAH7, KRIAS, FOLR1, WNK4, TSC2, NFATC4, PRKACB, HHIP |
| GOTERM_BP_FAT    | Response to organic substance    | 61    | .007992486 | ASPN, KYNU, PPARG, DUOX1, POSTN, TLRE, GSN, CCB1, CREB3L1, CHRNA6, PRKACB, HHIP, PLCB1, MAP2K6, IFNGR1, TRPM4, RAMP2, STMN2, GAL, SLC26A3, BGN, SQRLE, COL1A2, TFA2PA, GNAS, CA2, CROT, XDH, PAM, SORD, OPR1K1, TNC, PML, GNG11, TRH, GREGM2, PEA15, KRIAS, FOLR1, OXCT1, IDH1, THPO, HERPUD1, SMDA9, PDK4, ITGA6, TRIM24, GB6, FZD5, ISL1, OPT1A, ABCG1, GB2, PLA2G4A, ATP6V0E2, HMGCS2, TSC2, HDAC9, BMP1R1B, CPB2, LRP4 |
| GOTERM_CC_FAT    | Cell leading edge                | 14    | .008364418 | COBL, SLC39A14, NF2, STMN2, NEDD9, IQGAP2, ACTN1, ITSN1, CTNNA2, ACTG2, GSN, SORBS2, PLEKHH2, GNAS |
| GOTERM_BP_FAT    | Regulation of peptidase activity | 14    | .008369752 | XDH, SERPINB9, HERPUD1, EPAH7, CARD16, GSN, SERPINAS5, PPARG, PML, CST1, NLRF2, TPII2, WDFDC2, P1I5 |
| GOTERM_BP_FAT    | Macromolecule localization       | 59    | .009247219 | COPA, ATP1B1, SLC15A2, PPARG, VPS37B, POSTN, SELENBP1, TLRE, KCNIP4, CASP5, NOV, APIS3, ANK3, GSN, SERPINAS5, WNK4, TTC21A, MAP2K6, TRPM4, RAMP2, TN1K, SIX3, GAL, NLRF2, PCLO, EYA1, HEPCAM, STXB6, CLIC5, GNAS, CROT, FRA51, PAM, PML, RABGAP1L1, TRH, KCN53, TMED3, TAP2, OXCT1, SYBU, UGT8, DCLK1, ABCA12, HERPUD1, NF2, ITGA6, FZD5, ISL1, OPT1A, ABCG1, PLA2G4A, ATP6V0E2, PKP2, KCNNS3, TSC2, SYT14, SNX30, LRP4 |
| GOTERM_BP_FAT    | Glucuronate metabolic process    | 4     | .009462291 | SORD, UGT8, DCKR, XYLb |
| GOTERM_BP_FAT    | Regulation of fatty acid oxidation | 4    | .009462291 | PDK4, PPARG, ACADL, CPT1A |
| GOTERM_BP_FAT    | Uronic acid metabolic process    | 4     | .009462291 | SORD, UGT8, DCKR, XYLb |
| GOTERM_BP_FAT    | Response to estrogen             | 6     | .009528125 | SERPINB9, OPR1K1, PPARG, TRIM24, CA2, GAL |
| GOTERM_BP_FAT    | Axon development                 | 15    | .009632965 | COBL, AT1L, TNC, NTN4, COL25A1, ISL1, CTNNA2, OGN, EPAH7, KRIAS, FOLR1, ANK3, BMP1R1B, LRP4, DCLK1 |
| GOTERM_BP_FAT    | Organonitrogen compound catabolic process | 13 | .009943818 | XDH, NUDT16, ALDH6A1, KYNU, BCKDHB, HGD, ACADL, OGN, BGN, PDE1A, ACE2, GAD1, GPT2 |

MAPK, mitogen-activated protein kinase; NADP, nicotinamide adenine dinucleotide phosphate. Bold denotes gene names which are picked up in the results and discussion.

stress genes were down-regulated in the thin endometrium suggests that a decreased response to oxidative stress is associated with implantation failure.

In conclusion, the present study revealed that the thin endometrium possesses an aberrant Th1-pro-inflammatory/Th2-anti-inflammatory balance and increased cytotoxic condition and that a protective response to oxidative stress is impaired. These aberrant molecular mechanisms in the thin endometrium might be associated with implantation failure. These findings could lead to better treatments for patients with implantation failure as a result of a thin endometrium.

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TABLE 5 Kyoto Encyclopedia of Genes and Genomes pathway analysis for the genes that were down-regulated in the thin endometrium

| Term                                      | Count | P value  | Gene                                      |
|-------------------------------------------|-------|----------|-------------------------------------------|
| Butanoate metabolism                      | 5     | .001114534 | ACSM3, ACSM1, **HMGC52**, **OXCT1**, GAD1 |
| Metabolic pathways                        | 34    | .005119787 | **TM7SF2**, **XDH**, KYNU, SORD, CYP2J2,  |
|                                          |       |          | CERS4, CERS3, AGMAT, CKB, GALNT10, FUT3,  |
|                                          |       |          | **IDH1**, UGT8, PLCB1, GAD1, GPT2, ALDH6A1, |
|                                          |       |          | BCKDHB, HGD, ALDH3B2, CYP26A1, **CBR3**,  |
|                                          |       |          | ACADL, NME7, ACSM3, PLA2G4A, ACSM1, ATP6V0E2,  |
|                                          |       |          | **HMGC52**, SQLE, QPRT, PCCA, DCXR, XYLB   |
| Thyroid hormone synthesis                 | 6     | .00715768 | ATP1B1, CREB3L1, GNAS, PRKACB, PLCB1, IYD |
| Valine, leucine, and isoleucine degradation| 5     | .00867887 | ALDH6A1, **HMGC52**, **OXCT1**, BCKDHB,   |
|                                          |       |          | PCCA                                      |
| Amoebiasis                                | 7     | .01007625 | SERPINB9, COL1A2, ACTN1, GNAS, PRKACB,   |
|                                          |       |          | COL5A3, PLCB1                             |
| Serotonergic synapse                      | 7     | .012474846| PLA2G4A, CYP2J2, Kras, GNG11, GNAS,      |
|                                          |       |          | PRKACB, PLCB1                             |
| Salivary secretion                        | 6     | .016558258| ATP1B1, CST1, GNAS, MUC7, PRKACB, PLCB1  |
| Protein digestion and absorption          | 6     | .018126807| ATP1B1, COL1A2, ACE2, COL5A3, CPB2,      |
|                                          |       |          | XPANPEP2                                  |
| GnRH signaling pathway                    | 6     | .020659132| PLA2G4A, Kras, GNAS, PRKACB, PLCB1, MAP2K6|
| Pancreatic secretion                      | 6     | .022469965| SLC26A3, ATP1B1, GNAS, CA2, PLCB1, CPB2  |
| Inflammatory mediator regulation of TRP   | 6     | .027439494| PLA2G4A, CYP2J2, GNAS, PRKACB, PLCB1,    |
| channels                                  |       |          | MAP2K6                                    |
| Glucagon signaling pathway                | 6     | .028510884| PRMT1, CREB3L1, GNAS, PRKACB, PLCB1, CPT1A|
| Melanogenesis                             | 6     | .029608533| Kras, CREB3L1, GNAS, PRKACB, FZD5, PLCB1  |
| Arrhythmogenic right ventricular          | 5     | .034477703| PKP2, SGCD, ACTN1, ITGA4, CTNNA2          |
| cardiomyopathy                            |       |          |                                           |
| Gastric acid secretion                    | 5     | .037626762| ATP1B1, GNAS, PRKACB, CA2, PLCB1          |
| Vasopressin-regulated water reabsorption  | 4     | .040391867| DYNC1I1, CREB3L1, GNAS, PRKACB           |
| Endocrine and other factor-regulated      | 4     | .04274525 | ATP1B1, GNAS, PRKACB, PLCB1               |
| calcium reabsorption                      |       |          |                                           |
| Cholinergic synapse                       | 6     | .043457379| Kras, CREB3L1, GNG11, PRKACB, CHRNA6, PLCB1|

GnRH, gonadotropin-releasing hormone; TRP, transient receptor potential. Bold denotes gene names which are picked up in the results and discussion.

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DISCLOSURES

Conflict of interest: The authors declare no conflict of interest. Human Rights: The study protocol was reviewed and approved by the Institutional Review Board of Yamaguchi University Graduate School of Medicine. Informed consent was obtained from the participants before the collection of any sample. All the experiments that involved the handling of human tissues were performed in accordance with the tenets of the Declaration of Helsinki.

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**SUPPORTING INFORMATION**

Additional Supporting Information may be found online in the supporting information tab for this article.

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