J. Dairy Sci. 105:6125–6143
https://doi.org/10.3168/jds.2022-21811

Subclinical endometritis differentially affects the transcriptomic profiles of endometrial glandular, luminal, and stromal cells of postpartum dairy cows

Gonçalo Pereira,[1,2]* Yongzhi Guo,[3]* Elisabete Silva,[1,2] Marta Filipa Silva,[1,2] Claudia Bevilacqua,[4] Gilles Charpigny,[5] Luís Lopes-da-Costa,[1,2]†‡ and Patrice Humblot[3]†
[1]Centre for Interdisciplinary Research in Animal Health (CIISA), Faculty of Veterinary Medicine, University of Lisbon, Avenida da Universidade Técnica, 1300-477 Lisboa, Portugal
[2]Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), 1300-477 Lisboa, Portugal
[3]Department of Clinical Sciences, Swedish University of Agricultural Sciences, SLU, PO Box 7054, 750 07 Uppsala, Sweden
[4]INRAE, AgroParisTech, GABI, Université Paris-Saclay, 78350 Jouy-en-Josas, France
[5]INRAE, ENVA, BREED, Université Paris-Saclay, 78350 Jouy-en-Josas, France

ABSTRACT

In postpartum dairy cows, subclinical endometritis (SCE) is characterized by persistent endometrial inflammation, which has profound detrimental effects on subsequent reproductive performance. To date, transcriptomic studies related to this condition were either based on biopsy-derived whole-endometrium tissue or endometrial swab or cytobrush samples, thus masking effects of disease on cell type–specific gene expression. This study tested the hypothesis that different endometrial health statuses are associated with distinct transcription profiles of endometrial stromal, glandular, and luminal epithelial cells. At 44 d postpartum (DPP), endometrial biopsies were taken from dairy cows (n = 24) classified as healthy, recovered from SCE, or affected by persistent SCE, according to endometrial cytology taken at 21 and 44 DPP. Stromal, glandular, and luminal epithelial cells were isolated from the whole-tissue biopsy by laser capture microdissection, and the cell-specific transcription profiles were determined by RNA sequencing. Differential gene expression was analyzed with DESeq2 (https://bioconductor.org/packages/release/bioc/html/DESeq2.html). Results demonstrated that global transcriptomic profiles and corresponding lists of differentially expressed genes between cows with different health statuses were distinct among cell types. Results also showed that although healthy and recovered cows presented similar endometrial clinically healthy phenotypes at 44 DPP, the prior presence of immune cells still affected the transcriptome of endometrial cells at this stage, delaying complete functional recovery. Recovery or persistence of inflammation was associated with gene expression patterns involved not only in immune function but also in tissue remodeling, cell adhesion, and uterine receptivity in a cell type–specific manner. Identifying these signatures may contribute to the development of novel diagnostic tools and therapeutic strategies. In addition, these results may help to define preventive measures or ways to stimulate recovery from endometrial inflammation, thus helping to restore the fertility of postpartum dairy cows.

Key words: endometrium, subclinical endometritis, laser capture microdissection, transcriptome

INTRODUCTION

In postpartum dairy cows, uterine involution, characterized by the elimination of bacterial contamination and regeneration of the endometrial tissue, is essential to restore uterine receptivity and establishment of pregnancy (Sheldon, 2004). However, due to impaired immune response, cows are not always successful in eliminating uterine pathogens quickly (Sheldon et al., 2006; LeBlanc, 2008). This is associated with a persistent inflammatory response in endometrial tissue, which is the source of endometritis (Raliou et al., 2019). Clinical endometritis is easily diagnosed; in contrast, despite being highly prevalent and exerting profound detrimental effects on subsequent reproductive performance (LeBlanc, 2008), subclinical endometritis (**SCE**) is often undiagnosed because of the absence of clinical signs (Sheldon et al., 2006). At present, such cases are identified by an increased proportion of PMN revealed by endometrial cytology or biopsy (Sheldon et al., 2006; Madoz et al., 2014; Bogado Pascottini et al., 2016). Subclinical endometritis is characterized by uncontrolled persistent inflammation associated with tissue damage, leading to the release of damage-associated molecular

Received January 13, 2022.
Accepted April 5, 2022.
*These authors contributed equally to this work.
†These authors contributed equally to this work.
‡Corresponding author: lcosta@fmv.ulisboa.pt

patterns, further stimulating inflammation and contributing to its persistence (Sheldon et al., 2019).

The analysis of the full transcriptome following exposure of bovine endometrial mixed epithelial and stromal cells (Oguejiofor et al., 2015) or isolated epithelial cells (Guo et al., 2019) to LPS showed that this virulence factor triggered the differential expression of a large number of genes, including critical genes for the establishment of pregnancy, such as those encoding proteins involved in the control of tissue remodeling, immune tolerance, uterine receptivity, and pregnancy establishment. When using the same in vitro model as Guo et al. (2019), changes in proteins related to the above pathways were also observed (Piras et al., 2017). In vivo, cows with SCE present increased local gene expression of proinflammatory cytokines characteristic of acute inflammatory processes (Kasimanickam et al., 2014; Fagundes et al., 2019; Pereira et al., 2020). In addition, cows affected by endometritis exhibit a large number of genes involved in immune response, cell adhesion, chemotaxis, apoptosis, and G-protein coupled receptor signaling pathways differentially expressed compared with healthy cows (Salilew-Wondim et al., 2016). These changes are also associated with increased expression of molecules involved in LPS signaling, tissue remodeling, and acute phase response (Raliou et al., 2019). Overall, these reports highlight that SCE not only triggers an inflammatory response but also alters the expression of networks of genes encoding proteins involved in the control of tissue remodeling, immune tolerance, and pregnancy establishment.

However, to date, gene expression studies aiming to analyze the effects of SCE from in vivo material were either based on biopsy-derived whole-endometrium tissue (Salilew-Wondim et al., 2016; Raliou et al., 2019) or endometrial swab or cytobrush samples (Kasimanickam et al., 2014; Fagundes et al., 2019), without discriminating between endometrial cell types. The bovine endometrium is a complex and heterogeneous tissue, and quantification of gene transcription from the whole endometrium may not reflect cell-specific transcription. In postpartum dairy cows, stromal (**ST**), glandular epithelial (**GE**), and luminal epithelial (**LE**) endometrial cells isolated by laser capture microdissection (**LCM**) exhibit very distinct molecular signatures (Chankeaw et al., 2021a), differentially influenced by progesterone (Pereira et al., 2022) and negative energy balance (Chankeaw et al., 2021b).

The present study was designed to test the hypothesis that SCE differentially affects gene transcription profiles of the 3 endometrial cell types (ST, GE, and LE). In addition, because former studies aiming at identifying differences between healthy and SCE cows included only 2 groups of animals for which the uterine health status was determined at a single time point, the transcriptomic profiles of the 3 cell types were compared between healthy cows, cows that apparently recovered from SCE, and cows with persistent SCE.

Differences between the transcriptomic profiles of these uterine health phenotypes provide key information to understand the mechanisms associated with the persistence of endometrial inflammation and subsequent consequences on the establishment of pregnancy.

MATERIALS AND METHODS

Ethics Statement

The project was approved by the Institutional Animal Care and Use Committee (reference CEIE no. 37/2019). All clinical procedures were conducted in compliance with the European Union legislation on the protection of animals used for scientific purposes (Directive 2010/63/EU).

Animals

Animals entering this study correspond to a subset of those included in a published study, where the experimental design, animal handling, and sampling procedures are described in detail (Pereira et al., 2020; Table 1). Briefly, sample collection was performed in a single dairy farm in Benavente, Portugal, where postpartum dairy cows (n = 24), without signs of puerperal disease and antibiotic or anti-inflammatory therapy, were submitted to a gynecological examination and endometrial cytology at 21 ± 0.4 and 44 ± 0.7 d postpartum (**DPP**), plus a uterine biopsy at 44 DPP. Based on genital tract evaluation and endometrial cytology, cows were classified as healthy (n = 6), recovered (n = 7), or having persistent SCE (persistent; n = 11) (Figure 1). This classification, initially based on cytology results, was confirmed by retrospective histological analysis (Figure 2).

Genital Tract Evaluation and Endometrial Cytology

The vaginal discharge was graded (Williams et al., 2005) following collection with a Metricheck device (EndoControl Sampler, Minitube), and animals with a vaginal discharge score ≥1 were excluded from further exploration. In addition, the genital tract was examined by rectal palpation and visualized by ultrasonography. Endometrial cytology was performed at the uterine body using the cytobrush technique, as described by Pereira et al. (2020), and the percentage of PMN was assessed by counting 400 cells. Cows were regarded as healthy when PMN percentage was <18% or <5% at 21

Table 1. Clinical characterization of cow groups: healthy, recovered, and persistent subclinical endometritis (SCE)

Characteristic	Healthy	Recovered	Persistent SCE
Number of cows	6	7	11
Milk yield (kg) by 44 DPP[1,2]	1,770 ± 92	1,743 ± 77	1,807 ± 91
BW loss (%) by 44 DPP[2]	4.2 ± 1.9	4.7 ± 1.7	3.9 ± 1.7
Lactation number[2]	2.0 ± 0.5	1.6 ± 0.2	2.5 ± 0.4
21 DPP			
PMN %[2]	6.3 ± 1.5	52.0 ± 6.4	60.7 ± 7.2
Range of PMN %	2.5–12.5	25.5–71	25–90
44 DPP			
PMN %[2]	1.3 ± 0.3	1.1 ± 0.4	13.5 ± 2.3
Range of PMN %	0.5–2.5	0–3.5	6–33

[1]Days postpartum.
[2]Mean ± SEM.

and 44 DPP, respectively. Cows from the healthy group had a healthy uterus at both examinations, whereas cows in the recovered group had a healthy uterus only at 44 DPP, and cows in the persistent group had PMN percentages higher than the above cut-offs at 21 and 44 DPP (Figure 2, Table 1).

Collection of Biopsies

As previously described, endometrial biopsies were collected using a Kevorkian–Younge instrument (Alcyon; Pereira et al., 2020). Following endometrial cytology, the biopsy instrument was guided into the first third of one uterine horn, where an endometrial sample of 0.5 to 1 cm^2, 3 to 5 mm thick, was recovered. The endometrial samples were immediately frozen in dry, ice-cold isopentane (2-methylbutane, Sigma Aldrich) for 60 s and embedded in a cryomold with optimal cutting temperature compound (Tissue-Tek OCT Compound, Sakura Finetek). Cryomolds were transferred to the laboratory on dry ice and kept at −80°C until tissue processing.

Endometrial Tissue Processing, Staining, Microdissection, and RNA Extraction

Endometrial tissue processing and staining procedures were performed as previously described (Chankeaw et al., 2021a,b; Pereira et al., 2022). Briefly, 8-µm-thick sections were cut from the tissue blocks on a cryostat (Cryotome FSE, Thermo Scientific) set at −20°C, mounted on glass slides at 4°C, and immersed for 60 s in 75% ethanol inside the cryostat chamber (−20°C). Then, at room temperature, slides were transferred to 75% ethanol for 20 s, stained with cresyl violet (1% in 50% ethanol for 25 s), and dehydrated by rinsing successively with 75% ethanol (30 s), 95% ethanol (2 × 1 min), 100% ethanol (2 × 1 min), and xylene (M-xylene, Sigma-Aldrich; 2 × 5 min), as described by Bevilacqua et al. (2010). Slides were then air-dried to remove xylene residues before microdissection. The endometrial cell types (LE, GE, and ST) were isolated from the whole-tissue sections using an Arcturus XT Laser Capture Microdissection System and software (Applied Biosystems), as recently described (Chankeaw

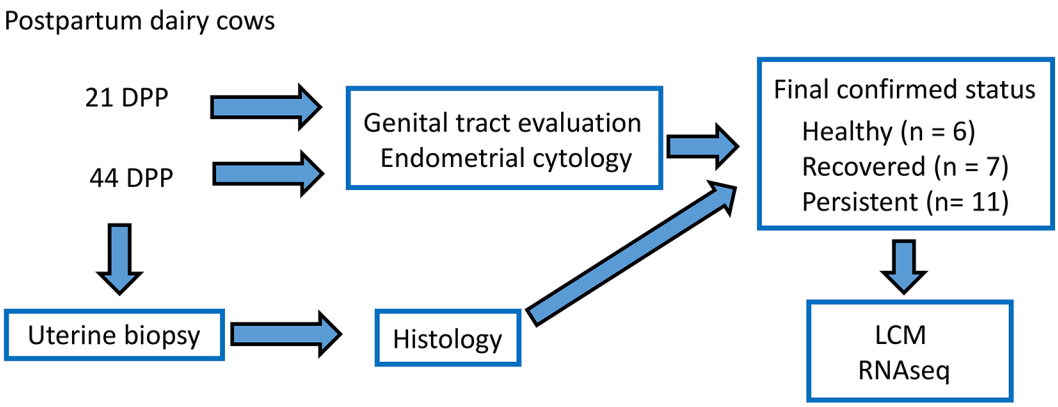

Figure 1. Workflow of the experimental design. DPP = days postpartum; LCM = laser capture microdissection; RNAseq = RNA sequencing; Persistent = cows with persistent subclinical endometritis.

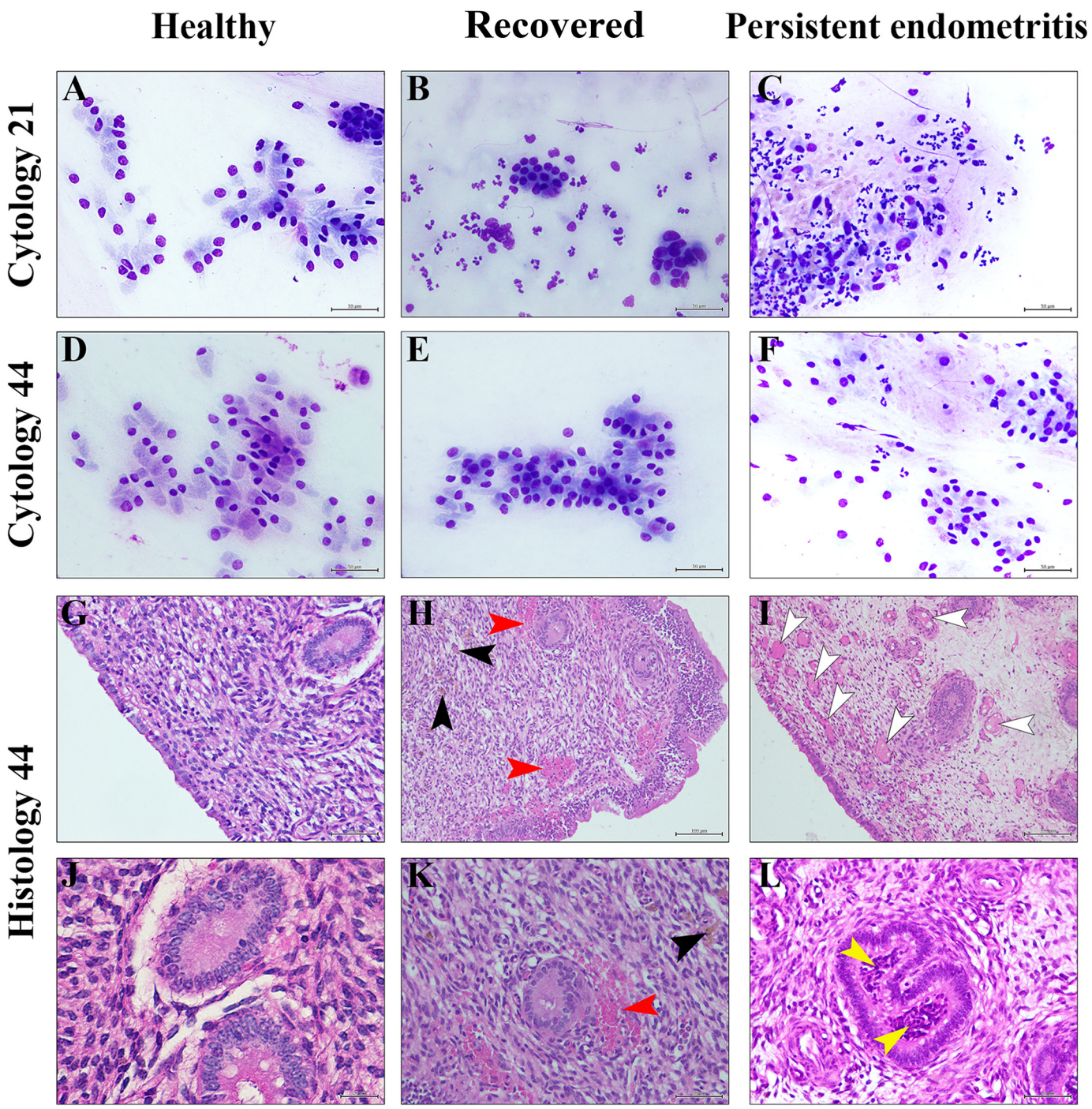

Figure 2. Representative photomicrographs of endometrial cytology (at 21 and 44 days postpartum, DPP) and biopsies (44 DPP) from cows in the healthy, recovered, and persistent subclinical endometritis sub-groups. Endometrium of recovered cows displayed areas of hemorrhage (red arrows, panels H and K) and infiltration of macrophages with hemosiderin (black arrows, panels H and K). The endometrium of persistent endometritis cows exhibited areas of vascular congestion (white arrows, panel I) and accumulation of inflammatory cells in the lumen of glands (yellow arrows, panel L). Scale bars = 100 μm (panels H, I), 50 μm (panels A, B, C, D, E, F, G, K, L), and 20 μm (panel J).

et al., 2021a,b; Pereira et al., 2022). Following capture, each LCM plastic cap (CapSure LCM macrocaps, Applied Biosystems) was examined at the quality control station and, if necessary, undesired cells were removed from the cap by a low-power UV laser. The full microdissection processing of each sample took less than 90

Table 2. Number of cows and respective RNA integrity number (RIN) values of samples used for sequencing from each cell type of cows in different sub-groups (total, n = 55)

Endometrial cell type	Cow sub-group[1]			RIN[2]
	H	R	PE	
Stromal	5	7	10	7.32 ± 0.15
Glandular epithelial	4	6	10	7.35 ± 0.15
Luminal epithelial	2	5	6	7.18 ± 0.22

[1]H = healthy; R = recovered; PE = persistent subclinical endometritis.
[2]Values reported as mean ± SEM.

min to preserve RNA integrity. After microdissection, total RNA from LE, GE, and ST cells was extracted using the PicoPure RNA Isolation Kit (Applied Biosystems) following the manufacturer's protocol, and total RNA was eluted in 15 μL of elution buffer. The RNA quantity and quality (RNA Integrity Number, RIN) were assessed with the Agilent Bioanalyzer 2100 system (Agilent Technologies) and the RNA 6000 pico Chip Kit (Agilent Technologies). Due to difficulty in harvesting enough RNA with eligible RIN values (≥7) for gene expression measurements, from the initial 72 samples from 24 cows, only 13 LE, 20 GE, and 22 ST samples were available for RNA sequencing (**RNAseq**; Table 2).

RNA Sequencing and Data Analysis

RNA sequencing libraries from the 55 samples were prepared and sequenced on the GenomEast Platform (IGBMC; http://genomeast.igbmc.fr/). Full-length cDNA was generated from 2.5 ng of total RNA using Clontech SMART-Seq v4 Ultra Low Input RNA Kit for Sequencing (Takara Bio Europe, Ozyme) according to the manufacturer's instructions, with 10 cycles of PCR for cDNA amplification by Seq-Amp polymerase. Then, 600 pg of preamplified cDNA was used as input for Tn5 transposon tagmentation using the Nextera XT DNA Library Preparation Kit (Illumina), followed by 12 cycles of library amplification. Following purification with Agencourt AMPure XP beads (Beckman-Coulter), the size and concentration of libraries were assessed by capillary electrophoresis. Sequencing was performed on an Illumina HiSeq 4000 with 100-bp paired-end reads. Image analysis and base calling were performed using RTA 2.7.3 and bcl2fastq 2.17.1.14 (both available at https://support.illumina.com). The sequencing depth of RNAseq libraries was in the range of 71 to 100 million reads per sample, and all samples had a quality score >30, meaning that the base call accuracy was 99.9% in at least 90% of the sequenced bases.

All steps of the gene-level exploratory analysis and differential transcription analysis were carried out as described previously (Pereira et al., 2022), using the RNAseq workflow described by Love et al. (2015; updated version: https://bioconductor.org/help/course-materials/2017/CSAMA/labs/2-tuesday/lab-03-rnaseq/rnaseqGene_CSAMA2017.html). The Salmon method (Patro et al., 2017) was used to quantify transcript abundance, and the Tximport method (Soneson et al., 2015; R package version 1.8.0, https://www.r-project.org/) was then used to import Salmon's transcript-level quantifications. The cDNA sequence database for *Bos taurus* was obtained from Ensembl (release-98; Bos_taurus.ARS-UCD1.2.cdna.all.fa) and used to build a reference index for the bovine transcriptome (Patro et al., 2017). Power analysis was performed using the method described by Bi and Liu (2016) and compiled in the R package ssizeRNA (version 1.3.2; https://CRAN.R-project.org/package=ssizeRNA). Calculated at a false discovery rate of 0.05, the power was 58, 83, and 93% to detect $\log_2$ fold changes of 1.5, 2, and 3, respectively.

Principal component analysis (**PCA**) was performed using DESeq2 (R package, version 1.26.0; https://bioconductor.org/packages/release/bioc/html/DESeq2.html) and FactoMineR (R package, version 1.4.1) using the variance stabilizing transformation output files from DESeq2. The downstream analysis of differentially expressed genes (**DEG**) was performed using DESeq2 with the corresponding statistical methods (Love et al., 2015), including tests for differential transcription by use of negative binomial generalized linear models. The following terms for main effects and interaction were added in the model: cell_type (LE, GE, ST) + cow_group (healthy, persistent, recovered) + cell_type: cow_group, with a false discovery rate-adjusted *P*-value of 0.05 (using the method of Benjamini and Hochberg, 1995) for the identification of DEG. Following the use of this model, the group effect was analyzed within each cell type, and corresponding lists of DEG were produced. Data were deposited in the National Center for Biotechnology Information Gene Expression Omnibus and are accessible through GEO Series accession number GSE192545 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE192545).

Gene Ontology and Exploration of DEG List

Significant Gene Ontology (**GO**) terms of the "GO-slim Biological Process" domain were found using the PANTHER classification system (Protein ANalysis THrough Evolutionary Relationships, version 16.0; http://pantherdb.org). Identified GO terms were summarized by removing redundant GO terms with dispensability at medium level, using REVIGO (http://revigo.irb.hr/; Supek et al., 2011). In a second step,

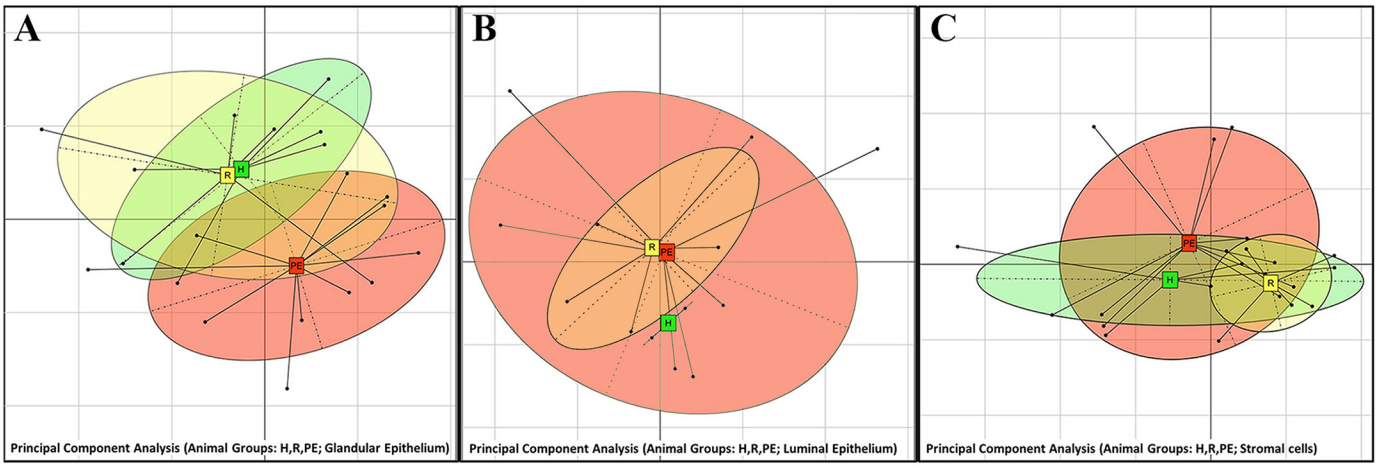

Figure 3. Principal component analysis of the 3 cell types among cows in the healthy (H), recovered (R), and persistent subclinical endometritis (PE) subgroups: (A) glandular epithelial cells, (B) luminal epithelial cells, and (C) stromal cells.

DEG encoding proteins involved in inflammatory status, tissue remodeling, cell adhesion, and IFN-mediated signaling were explored, considering significant differences when the adjusted P-value was ≤ 0.10.

Construction of Protein-Protein Interaction Networks

Protein-protein interaction (**PPI**) networks were assembled using the STRING database v11.0 (http://string-db.org; Szklarczyk et al., 2019), generated with "non/query protein only," and the sources of active interaction selected (Textmining, Experiments, Databases, Co-expression, Neighborhood, Gene Fusion, and Co-occurrence). Initially, only the highest confidence interactions (>0.9) were included, but if none were detected, high (>0.7) and medium (>0.4) confidence interactions were also considered. Then, networks were analyzed in Cytoscape version 3.8.2 (https://cytoscape.org/) and visualized by yFiles layout algorithms for the Cytoscape app.

RESULTS

Differences in Transcriptomic Patterns in Relation to the Health Status of the Endometrium

The PCA demonstrated that the 3 cell types exhibited distinct gene expression profiles in healthy, recovered, and persistent cows (Figure 3). Regarding GE cells, healthy and recovered cows express similar transcriptomic profiles, which were clearly distinct from that of persistent cows along the PCA horizontal and vertical axes. In contrast, LE cells of recovered cows exhibited a similar gene expression profile to that of persistent cows, and both were distinguishable from the profile of healthy cows along the vertical axis. In addition, there was large variation in profiles of persistent cows, as shown by the wide confidence ellipse for this group. The situation appeared more complex in ST cells, where persistent cows differed from healthy and recovered cows on the vertical axis, and persistent and healthy cows differed from recovered cows along the horizontal axis.

The number of DEG obtained through the 2 × 2 contrasts between the 3 types of uterine health status in each cell type (GE, LE, and ST) are shown in Table 3. Globally, when compared with healthy cows, recovered cows had a higher proportion of underexpressed genes in ST (82%), but not in GE (32%) or LE (45%) cells. In contrast, compared with healthy cows, persistent cows presented a large proportion of overexpressed genes in GE (69%), LE (77%), and ST (78%) cells. The same trend was observed when comparing persistent and recovered cows. The number of DEG common between the different comparisons (persistent vs. healthy, persistent vs. recovered, recovered vs. healthy), in each cell type group (GE, LE, ST) are presented in Supplemental Figure S1 (https://data.mendeley.com/datasets/wdn57mr477/2; Pereira et al., 2021a). In general, a low proportion of DEG were commonly affected in all 3 cell types by endometrial health status. These patterns highlight that in epithelial compartments (GE + LE), healthy cows display a fair proportion of DEG, which differ both in recovered and persistent cows, and that ST cells of persistent cows exhibit a set of DEG that are commonly affected compared with both recovered and healthy cows.

The full lists of over- and underexpressed genes in GE, LE, and ST and between healthy, recovered, and persistent cows are given in Supplemental File S1

Table 3. Differentially expressed genes either under- (<0) or overexpressed (>0) between cows with different uterine health statuses for each cell type

	Glandular epithelial			Luminal epithelial			Stromal		
Status[1]	<0	>0	Total	<0	>0	Total	<0	>0	Total
PE vs. H	60	134	194	19	65	84	54	197	251
PE vs. R	22	24	46	17	44	61	99	364	463
R vs. H	12	26	38	27	33	60	119	27	146

[1]PE = persistent subclinical endometritis; H = healthy; R = recovered.

(https://data.mendeley.com/datasets/x5kkf369kn/2; Pereira et al., 2021b). In most cases (95%), when genes were differentially expressed in common between 2 or 3 cell types, the gene expression was either increased or decreased in all cell types, only rarely varying in different directions.

Analysis of DEG Between Persistent and Healthy Cows

The majority of DEG resulting from the comparison between healthy and persistent cows were cell type–specific but 3 were common between GE and LE, 19 between GE and ST, and 7 between LE and ST; 2 were common among all 3 cell types (Figure 4A). In GE cells, 4 overrepresented GO terms were identified from the full list of DEG (Supplemental File S2; https://data.mendeley.com/datasets/922v7859tg/2; Pereira et al., 2021c). According to biological process classification, these DEG were related to immune effector processes (GO:0002252), immune system processes (GO:0002376), response to virus (GO:0009615), and response to stress (GO:0006950). No GO enrichment was identified from DEG of LE cells, whereas 22 enriched GO terms were revealed in the list of DEG of ST cells. These terms included response to virus (GO:0009615), muscle system process (GO:0003012), muscle contraction (GO:0006936), response to biotic stimulus (GO:0009607), defense response (GO:0006952), and other biological processes (Supplemental File S2; https://data.mendeley.com/datasets/922v7859tg/2; Pereira et al., 2021c).

Proteins encoded by the DEG between persistent and healthy cows were further explored through a PPI network analysis based on the STRING database. In GE cells, 6 modules were identified (Figure 4B), 1 linking underexpressed genes and 5 linking overexpressed genes. The module produced from underexpressed DEG included numerous genes encoding IFN-dependent or IFN-stimulated proteins. The modules from overexpressed DEG included genes encoding proteins related to NADH-ubiquinone oxidoreductase complex (NDUFS3, NDUFS5, NDUFA5, NDUFC2), ATP synthesis and defense against oxidative stress (SCO2, COX11), enzymes involved in amino acid metabolism (TST, CCBL1), mitochondrial ribosomal proteins (MRPS25, MRPS2), and immunoglobulins (IGHV1S20, ENSBTAP00000018855). In LE cells, only 1 module was identified from overexpressed genes encoding proteins involved in Eph/ephrin signaling (EPHA4, EFNA1) (Figure 4C). In ST cells, 9 modules were identified (Figure 4D), 1 linking underexpressed genes, 7 linking overexpressed genes, and 1 linking 1 underexpressed and 2 overexpressed genes. As in GE cells, the large module corresponding to underexpressed DEG included IFN-induced or IFN-stimulated genes. The modules with overexpressed genes encoded proteins of the keratin family (KRT14, KRT5), modulating cell adhesion and motility (TES, ENAH), smooth muscle cells and contractile function (ACTA2, MYH11, MYL9, PPP1R12A, ACTC1, ACTN2, TPM1, TPM2, TNNT1, TNNI1), enzymes of inositol phosphate metabolism (INPPL1, ITPKA), members of the R-spondin family of Wnt modulators (RSPO1, LGR6), and members of the heparan sulfate proteoglycans or involved in its biosynthesis (GPC5, HS3ST6). The module with under- and overexpressed genes encoded proteins involved in secretion events and vesicle trafficking (SYT2, SNAP25, SCGN).

Analysis of DEG Between Persistent and Recovered Cows

When comparing persistent and recovered cows, 3 DEG were found in common between GE and LE, 7 between GE and ST, and 4 between ST and LE; 2 were common among all 3 cell types (Figure 5A). In GE and LE cells, no GO enrichment was detected from DEG lists, whereas in ST cells, overrepresentation analysis revealed 1 enriched GO term composed of genes involved in ion homeostasis (GO:0050801; Supplemental File S2; https://data.mendeley.com/datasets/922v7859tg/2; Pereira et al., 2021c).

In GE cells, the PPI network analysis identified 2 modules (Figure 5B), 1 linking 1 overexpressed and 5 underexpressed genes encoding proteins related to mi-

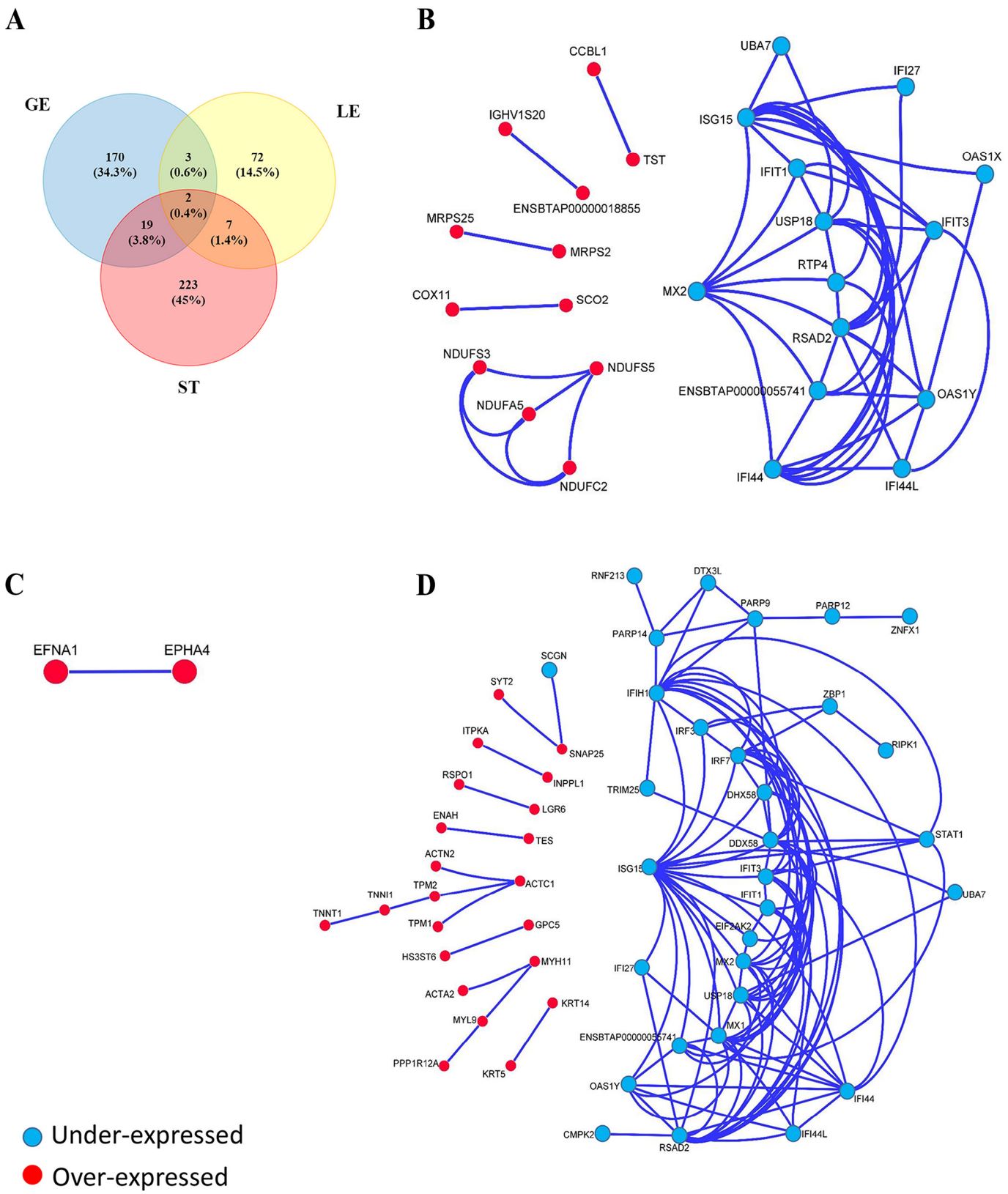

Figure 4. Differentially expressed genes (DEG) between cows with persistent subclinical endometritis and healthy cows. (A) Venn diagram with DEG identified in glandular epithelial (GE), luminal epithelial (LE), and stromal (ST) cells. (B, C, D) Protein-protein interaction network analysis of DEG identified in (B) GE cells, (C) LE cells, and (D) ST cells.

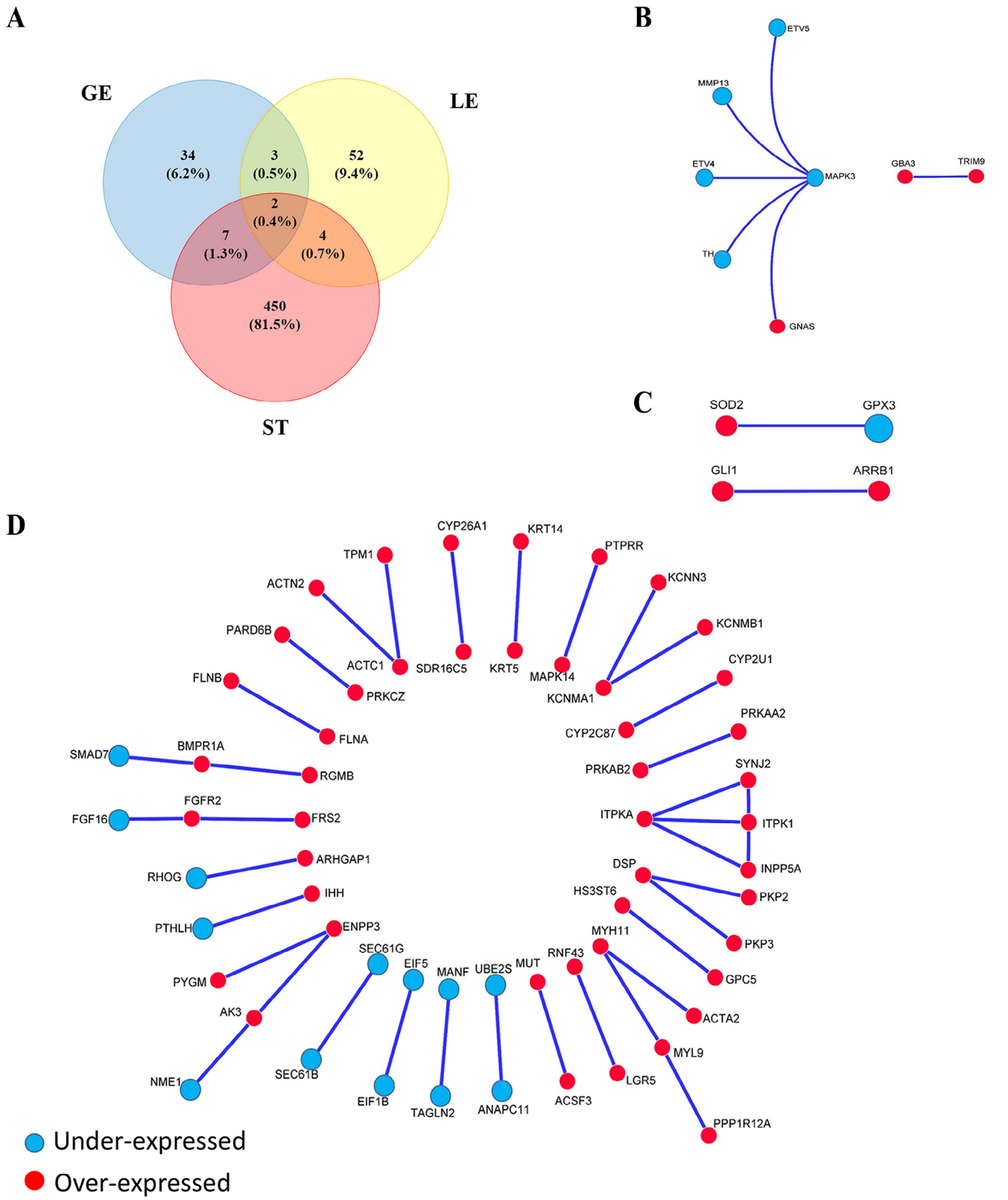

Figure 5. Differentially expressed genes (DEG) between cows with persistent subclinical endometritis and recovered cows. (A) Venn diagram with DEG identified in glandular epithelial (GE), luminal epithelial (LE), and stromal (ST) cells. (B, C, D) Protein-protein interaction network analysis of DEG identified in (B) GE cells, (C) LE cells, and (D) ST cells.

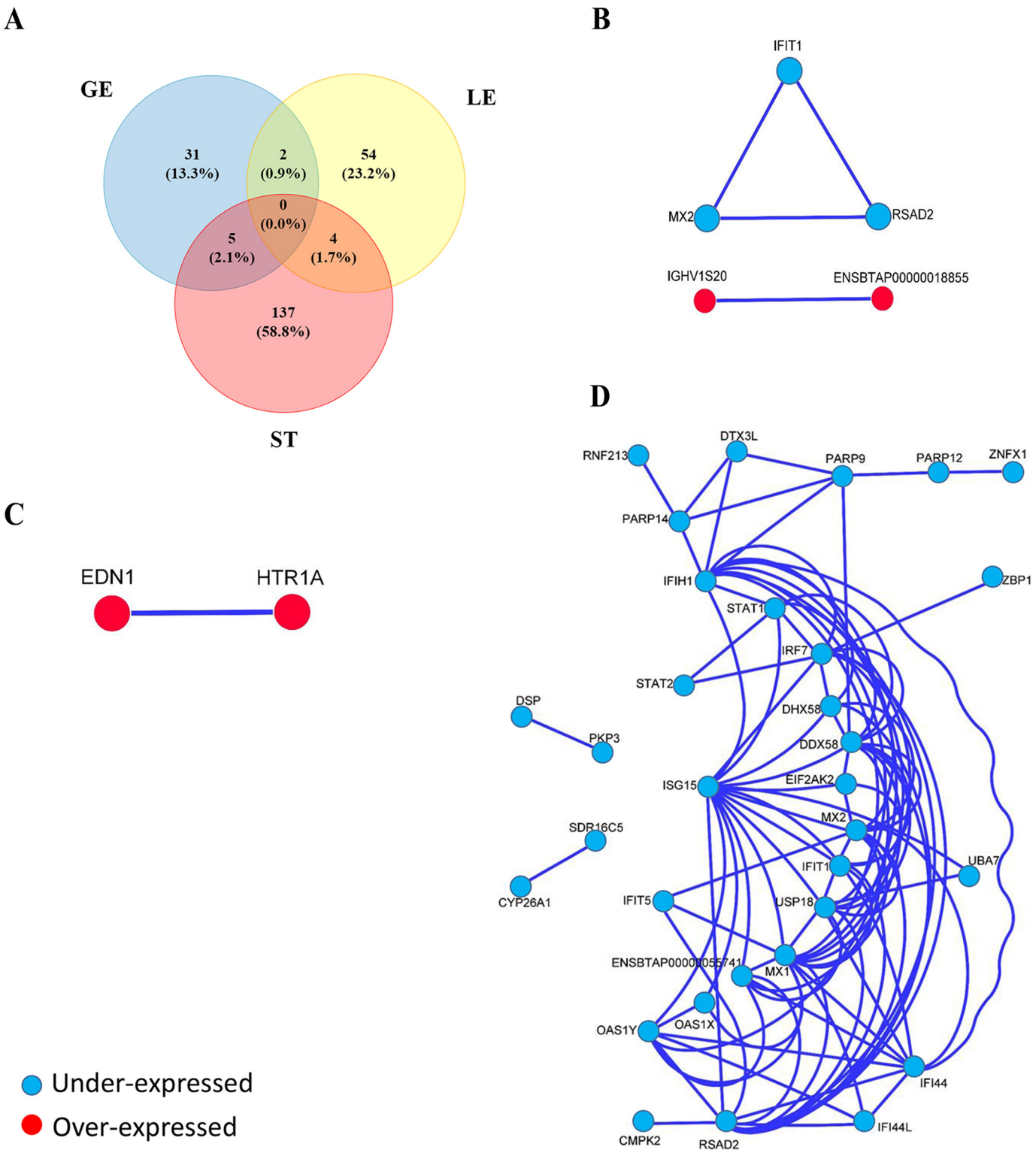

Figure 6. Differentially expressed genes (DEG) between recovered and healthy cows. (A) Venn diagram with DEG identified in glandular epithelial (GE), luminal epithelial (LE), and stromal (ST) cells. (B, C, D) Protein-protein interaction network analysis of DEG identified in (B) GE cells, (C) LE cells, and (D) ST cells.

togen-activated protein kinase signaling (MAPK3, TH, ETV4, MMP13, ETV5, GNAS) and 1 linking overexpressed genes encoding glycosides hydrolyzing enzyme (GBA3) and E3 ubiquitin ligase (TRIM9). In LE cells, 2 modules were identified (Figure 5C), 1 involving under- and overexpressed genes encoding enzymes involved in protection against oxidative damage (SOD2, GPX3), and 1 relating overexpressed genes encoding proteins associated with Hedgehog-GLI (HH-GLI) signaling (ARRB1, GLI1). In contrast, a large number of modules were identified from the DEG list of ST cells (Figure 5D). Four modules linked underexpressed genes encoding proteins related to translation initiation (EIF5, EIF1B), protein translocation (SEC61B, SEC61G), the ubiquitin-proteasome system (ANAPC11, UBE2S), and immune modulation (TAGLN2, MANF). Fifteen modules corresponded to overexpressed genes encoding proteins of epithelial cell apico-basolateral polarization (PARD6B, PRKCZ), retinoic acid (CYP26A1, SDR16C5), fatty acid (ACSF3, MUT) and inositol polyphosphate (INPP5A, ITPK1, ITPKA, SYNJ2) metabolism, mitogen-activated protein kinase signaling (MAPK14, PTPRR), of the filamin (FLNA, FLNB) and cytochrome P450 CYP2 (CYP2C87, CYP2U1) families, members of the R-spondin family of proteins (RNF43, LGR5), subunits of potassium calcium-activated channels (KCNMB1, KCNMA1, KCNN3), subunits of AMP-activated protein kinase (PRKAA2, PRKAB2) and desmosome-related proteins (PKP3, PKP2, DSP). Five modules comprised both under- and overexpressed genes encoding proteins of bone morphogenetic protein (RGMB, BMPR1A, SMAD7) and fibroblast growth factor (FGF) (FRS2, FGFR2, FGF16) signaling, enzymes involved in energy sensing, generation, and utilization (AK3, ENPP3, PYGM, NME1), and members of the Rho GTPase (ARHGAP1, RHOG) and Indian Hedgehog–PTH-related protein (Ihh-PTHrP) (PTHLH, IHH) pathways.

Analysis of DEG Between Recovered and Healthy Cows

The number of DEG emerging from the comparison between recovered and healthy cows was lower than for the comparisons that included persistent cows. However, similarly, the number of DEG per cell type was higher in ST than in GE and LE cells, and most DEG were also cell type–specific (Figure 6A), except for 2 that were common between GE and LE, 5 that were common between GE and ST, and 4 that were common between ST and LE.

Among DEG observed in GE and LE cells, no GO enrichment was identified. In ST samples, overrepresentation analysis revealed 17 enriched GO terms. The detected genes were related to response to virus (GO:0009615), defense response (GO:0006952), immune effector process (GO:0002252), immune system process (GO:0002376), positive regulation of cytokine production (GO:0001819), and other biological processes (Supplemental File S2; https://data.mendeley.com/datasets/922v7859tg/2; Pereira et al., 2021c).

Proteins encoded by DEG between recovered and healthy cows were also further explored through PPI network analysis. In GE cells, 2 modules were identified (Figure 6B), 1 involving underexpressed genes encoding IFN-dependent or IFN-stimulated proteins (RSAD2, IFIT1, MX2), and 1 connecting overexpressed genes encoding immunoglobulins (IGHV1S20, ENSBTAP00000018855). In LE cells, 1 module was identified that related overexpressed genes encoding proteins involved in smooth muscle contraction and vascular function (HTR1A, EDN1; Figure 6C). In ST cells, 3 modules connecting underexpressed genes were identified (Figure 6D), encoding desmosome-related proteins (PKP3, DSP), enzymes of retinoic acid metabolism (CYP26A1, SDR16C5), and several IFN-dependent or IFN-stimulated proteins.

Comparisons Between Groups of Cows Differing in Endometrial Health Status

The differences between the 3 endometrial health statuses were explored on a functional basis from DEG encoding proteins involved in inflammatory status (Supplemental File S3; https://data.mendeley.com/datasets/cpb5vznzhn/3; Pereira et al., 2021d), tissue remodeling, and cell adhesion (Supplemental File S4; https://data.mendeley.com/datasets/d36t3d438g/3; Pereira et al., 2021e) and genes related to IFN signaling. Regarding inflammatory status, GE and ST cells of persistent cows underexpressed a series of genes encoding receptors of inflammatory molecules compared with recovered and healthy cows (Figure 7A). Luminal epithelial cells of persistent cows overexpressed genes encoding chemokine ligand 21 (CCL21), compared with both healthy and recovered cows, and chemokine receptor 10 (CCR10) and chemokine ligand 19 (CCL19) compared with healthy and recovered cows, respectively. The LE cells of recovered cows underexpressed the gene encoding TGFBR1 compared with healthy cows.

When considering tissue remodeling and cell adhesion, DEG between persistent and recovered cows were identified almost exclusively in ST cells (Figure 7B), whereas differential expression with healthy cows was observed both in GE and LE cells. The only DEG between persistent and both recovered and healthy cows was *MMP13*, identified as such in GE cells.

Regarding IFN-dependent or IFN-stimulated genes, when compared with healthy cows, persistent and recovered cows showed striking underexpression of a large common set of genes belonging to this family in both GE and ST cells (Figures 3C, D and 5C, D).

DISCUSSION

Following a postpartum diagnosis of SCE, half of all cases develop inflammation without the presence of pathogens (Madoz et al., 2014; Bogado Pascottini and LeBlanc, 2020). This is regarded as unresolved inflammation following immune activation triggered by the initial bacterial assault (Brewer et al., 2020). The inflammatory response comprises the induction phase, associated with fast and robust immune activation, and the resolution phase, which is critical to limit the acute response and restore tissue homeostasis (Schett and Neurath, 2018). In early postpartum, a temporal switch from a proinflammatory gene expression profile to a regenerative profile has been identified in healthy cows (Foley et al., 2012). In contrast, in cows that develop SCE, this transition is arrested or delayed, leading to suboptimal restoration of homeostasis (Foley et al., 2015). Even when an apparent clinical cure of endometritis takes place, these cows still have reduced fertility (Sheldon et al., 2020).

The present study aimed to describe the transcriptomic profiles associated with different endometrial cell types in cows with differing health statuses. To confirm results from former studies showing that endometrial cell types present very different transcriptomic signatures (Chankeaw et al., 2021a; Pereira et al., 2022), LCM was successfully used here to isolate cell types and study their specific response to persistent inflammation of the endometrium or recovery. The relevance of the approach was demonstrated by the different transcriptomic profiles between groups of cows with contrasting health status, showing that the majority (>90%) of DEG between healthy, recovered, and persistent cows was cell type–specific.

Overall, this study showed that endometrial ST cells were the most affected by persistent or transient inflammation (persistent and recovered cows, respectively), as depicted by the higher number of DEG and enriched GO terms, compared with those in GE and LE cells. However, this finding was also influenced by the higher statistical power arising from the higher number of ST samples compared with GE and LE samples. In fact, the main limitation of the present study was the num-

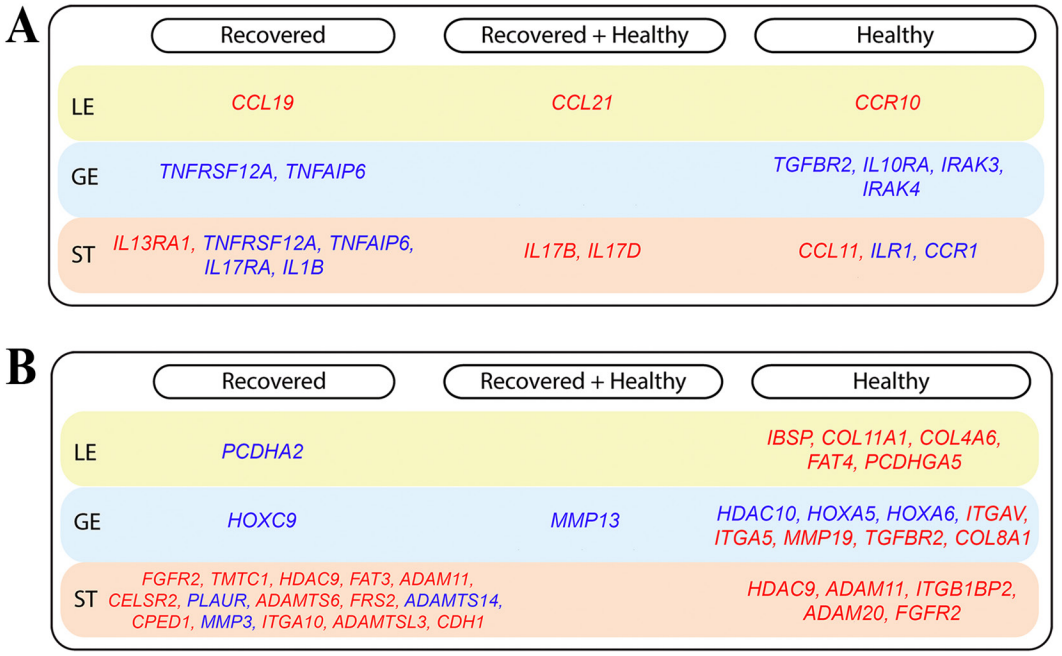

Figure 7. Differentially expressed genes (DEG) encoding proteins involved in inflammatory status (A), and tissue remodeling and cell adhesion (B). Each panel shows DEG observed between persistent subclinical endometritis cows and recovered cows only (left), between persistent and both recovered and healthy (middle), and between persistent and healthy cows only (right). Overexpressed genes are shown in red letters and underexpressed genes in blue letters, in the 3 types of cells: luminal epithelial (LE; yellow row), glandular epithelial (GE; blue row), and stromal (ST; orange row).

ber of LE samples with sufficient RNA for RNAseq. Because of this, the lists of DEG identified from LE samples, although accurate, are limited; they would be more complete with a higher number of samples of this cell type.

Interestingly, PCA revealed different gene expression patterns between cows with different health statuses in each cell type. Such differences were expected between the samples from cows from the extreme phenotypes healthy and persistent (Salilew-Wondim et al., 2016; Raliou et al., 2019). However, the present study clearly revealed that even though healthy and recovered cows had a similar "endometrial clinically healthy phenotype" (as shown by the low percentage of immune cells) at cytology and biopsy sampling, the prior presence of immune cells was associated with other mechanisms still affecting the transcriptome of endometrial cells at a later stage. This finding confirms an earlier report that past microbial exposure has long-term effects in the endometrial transcriptome of postpartum dairy cows (Moore et al., 2019). This is especially true for LE and ST cells, which showed very distinct transcriptomic profiles between healthy and recovered cows. Overall, differences in gene expression between healthy and recovered cows were less prominent in GE cells, although many individual genes were differentially expressed in response to former health status.

Due to the above findings and the vast number of individual DEGs in our model, we developed the discussion focusing on various functions related to immune response, cell adhesion, and tissue remodeling, and the subsequent establishment of pregnancy and how these respond to contrasting uterine health phenotypes.

Endometrial Health Status and Genes Related to Immune Response

In GE cells, both persistent and recovered cows underexpressed genes encoding transforming growth factor (TGF)-β receptors, and persistent cows also underexpressed *IL10RA*. TGF-β regulates the onset and resolution of inflammation, acting with IL10 to ensure a controlled inflammatory response through the regulation of T cells (Li and Flavell, 2008a,b). In epithelial cells, TGF-β and *IL10RA* prevent LPS-driven damage, eliciting anti-inflammatory and tolerogenic responses (Jarry et al., 2008; Mallikarjunappa et al., 2020). Moreover, an association between endometrial IL-10 mRNA and protein expression by uterine immune cells and conceptus survival was found in dairy heifers (Vasudevan et al., 2017). In GE cells, persistent cows also underexpressed the IRAK3- and IRAK4-coding genes, which regulate toll-like receptors (TLR) and IL-1 signaling (Jain et al., 2014; Singer et al., 2018), with IRAK3 promoting anti-inflammatory effects through a paradoxical "second wave" of nuclear factor (NF)-κB activation (Jain et al., 2014). Therefore, results from the present study showing the underexpression of genes encoding TGF-β receptors, *IL10RA*, IRAK3, and IRAK4 confirmed that crucial pathways in the regulation of innate immunity and inflammation are affected in GE cells of cows with persistent endometrial inflammation.

In LE cells, the overexpression of genes encoding CCR10 and CCL21 in persistent cows compared with healthy cows, and CCL21 and CCL19 compared with recovered cows, was consistent with the roles of these chemokines and receptors in the local recruitment of circulating immune cells (Xiong et al., 2012; Hjortø et al., 2016). In mucosal tissues, CCR10 and its ligands are involved in the homing of leukocytes, such as B cells, T cells, and eosinophils (Choi et al., 2016). CCL21 enhances mature dendritic cell receptor-mediated endocytosis (Kikuchi et al., 2005) and regulates T-cell migration and activation (Flanagan et al., 2004). Both CCL21 and CCL19 are involved in chemotaxis and activation of naive T cells and antigen-presenting dendritic cells (Marsland et al., 2005; Hjortø et al., 2016), and CCL21 can be involved in the regulation of ovarian function (Mellouk et al., 2019). Thus, the increased expression of *CCL21* in persistent cows supports the extensively explored link between endometrial inflammation and impaired ovarian function in postpartum dairy cows (Mateus et al., 2002; Sheldon et al., 2002). In LE cells, the comparison between persistent and healthy cows also revealed the overexpression of ephrin signaling genes (*EPHA4* and *EFNA1*). These genes, reported to be involved in proliferation, protection against endoplasmic reticulum stress, and inflammatory responses in bovine endometrial and mammary epithelial cells (Kang et al., 2018; Lim et al., 2019), were associated here with persistent inflammation.

In ST cells, one of the major changes observed in persistent cows compared with other groups was the overexpression of genes from the IL17 inflammatory cascade (*IL17B* and *IL17D*). These genes are associated with tissue regeneration but also with the recruitment of immune cells and the progression of inflammation (Bie et al., 2017; Liu et al., 2020). Our results are consistent with Foley et al. (2015), showing persistent gene overexpression of *IL17D* in cows with SCE. In addition, persistent cows overexpressed genes encoding members of the R-spondin family of proteins (RSPO1 and LGR6 compared with healthy cows; RNF43 and LGR5 compared with recovered cows), which are known for their capacity to amplify β-catenin/Wnt signaling, which may be related to incomplete tissue repair in these cows

(Nagano, 2019). LGR6 is known to promote tissue repair and regeneration when activated by maresin 1, a potent immune-resolving agent enhancing macrophage uptake of apoptotic PMN and limiting PMN infiltration (Chiang et al., 2019). Compared with recovered cows, persistent cows also overexpressed *CYP2C87* and *CYP2U1* (members of cytochrome P450, CYP2 family), which are involved in biosynthesis and inactivation of proinflammatory and proresolving lipid mediators (Divanovic et al., 2013), and *CYP26A1* and *SDR16C5*, which regulate the cellular level of retinoic acid (Stevison et al., 2015) and its biosynthesis (Wu et al., 2019), respectively. Retinoic acid is essential for the normal regeneration of mucosal barriers damaged by infection and for the function of neutrophils, macrophages, and natural killer cells (Stephensen, 2001). Taken together, our results showing the overexpression of genes involved in the resolution of inflammation and mucosal barrier regeneration, still present at 44 DPP, may indicate that the above processes remain active and delayed in persistent cows compared with recovered and healthy cows.

In ST cells, persistent cows transcribe less *IL1R1* (compared with healthy cows), which encodes the receptor for IL1-β and IL1-α and is responsible for eliciting a proinflammatory response (Dinarello, 2018). Persistent cows also underexpressed the *IL1B*, *TNFRSF12A*, and *TSG6* genes compared with recovered cows. This was unexpected because both IL1-β and TNF are proinflammatory cytokines (Lukens et al., 2012), with increased concentrations in cows with endometritis (Galvão et al., 2011). TNFRSF12A is upregulated in epithelial and natural killer cells in diseased tissues (Dohi and Burkly, 2012; Qi et al., 2016) and acts as a receptor of TWEAK (TNFSF12), eliciting signaling related to tissue remodeling (Dohi and Burkly 2012). The transient activation of TWEAK/TNFRSF12A pathway may be beneficial for tissue repair after acute injury, whereas excessive or sustained activation mediates pathological tissue damage and remodeling (Burkly, 2014). Therefore, the results from the present study suggest that persistent cows, in contrast to recovered cows, do not elicit a TNFRSF12A-dependent beneficial tissue repair program. Persistent cows also underexpressed *TSG6*, a member of the tumor necrosis factor superfamily upregulated upon exposure to inflammatory mediators (Day and Milner, 2019). TSG6 is released from neutrophils, mast cells, macrophages, and stromal cells, and it displays anti-inflammatory and tissue-protective properties (Day and Milner, 2019). These include inhibition of neutrophil migration, polarization of macrophages to an anti-inflammatory M2 phenotype, and modulation of endometrial matrix turnover and organization (Capp et al., 2014; Day and Milner, 2019). The present results lead to the hypothesis that underexpression of *TSG6* in ST cells of persistent cows may be a relevant marker for the persistence of endometrial inflammation or a putative therapeutic target.

Endometrial Health Status and Genes Related to Tissue Remodeling and Cell Adhesion

In GE cells, when compared with healthy cows, persistent cows underexpressed the *MMP19*, which encodes a basement membrane-degrading protease, MMP-19, involved in tissue remodeling, wound healing, and epithelial cell migration (Cui et al., 2017). However, in contrast to most matrix metallopeptidases, MMP-19 is also expressed in healthy epithelial cells, maintaining epithelial barrier function and regulating innate immune response, especially the influx of neutrophils (Brauer et al., 2016). As found in a mice model of epithelial barrier damage (Brauer et al., 2016), underexpression of *MMP19* in persistent cows suggests that adequate epithelial expression of MMP-19 is necessary for effective uterine involution and resolution of inflammation. Persistent cows also overexpressed *HDAC10* in GE cells. Members of the corresponding family of enzymes remove acetyl groups from lysine residues of histones, leading to transcriptionally silenced chromatin (Lawlor and Yang, 2019). Changes in gene expression of this group have been associated with alterations of endometrial remodeling as well as pathologies such as cancer, endometriosis, and infertility (Gujral et al., 2020). Downregulation of histone deacetylases coupled with TGF-β stimulation is favorable to epithelial barrier regeneration (Friedrich et al., 2019). In addition, genes of the integrin family (*ITGAV* and *ITGA5*) were also underexpressed in persistent cows. The ITGAV integrins regulate inflammation in epithelial cells through activation of TGF-β (Munger et al., 1999; Mu et al., 2002). Considering the above, the results of the present study (*HDAC10* overexpression coupled with *ITGAV* underexpression leading to lower expression of *TGFBR2* in GE cells of persistent cows) suggest that these changes may lead to delayed epithelial regeneration and alter immunotolerant mechanisms in persistent cows.

In ST cells, persistent cows underexpressed *MMP3* compared with recovered cows, and no difference was found with healthy cows. These results appear to contrast with those of the literature where endometrial mRNA and protein levels of MMP-3 were positively correlated with the severity of endometritis and the inflammatory response to LPS in an in vitro model of endometrial epithelial cells (Zhang et al., 2021). Differences may be related to differences in cell type or the postpartum stage.

A member of the FGF signaling pathway (*FGFR2*), which regulates tissue repair and repair-related angiogenesis through cell migration, proliferation, differentiation, and survival (Xie et al., 2020), was overexpressed in ST cells of persistent cows compared with recovered and healthy cows. An active role of FGFR2 was demonstrated in postpartum goats, where its high protein expression in fibroblasts was associated with epithelial and stromal cell regeneration (Sánchez et al., 2002). The results obtained here support the idea that *FGFR2* overexpression is related to delayed endometrial involution and that persistent cows are still under active repair processes.

Stromal cells of persistent cows also overexpressed genes encoding proteins associated with smooth muscle cells and contractile function (*ACTA2*, *MYH11*, *MYL9*, *PPP1R12A*, *ACTC1*, *ACTN2*, *TPM1*, *TPM2*, *TNNT1*, *TNNI1*). Most of these genes were also identified from enriched GO terms related to muscle system process, myofibroblasts, smooth muscle cells, and muscle contraction; expression of the corresponding encoded proteins is restricted to smooth muscle cells and myofibroblasts (Rockey et al., 2013; Queckbörner et al., 2021). The differentiation of fibroblasts into myofibroblasts and building of the extracellular matrix are critical components for wound healing and tissue repair (Rockey et al., 2013), contributing to cessation of hemorrhage, restoration of barrier integrity, and re-establishment of tissue function (Klingberg et al., 2013). As mentioned before for other sets of genes, the overexpression of these genes in persistent cows may reflect the ongoing process of endometrial tissue repair.

Finally, persistent cows overexpressed *GPC5* and *HS3ST6* in ST cells compared with recovered and healthy cows. Heparan sulfate is found on the surface of most cell types, and its expression is induced by LPS and TNF-α in endometrial and endothelial cells, respectively, where it is involved in the formation of perivascular chemokine gradients contributing to the transendothelial recruitment of leukocytes from the circulation to the site of inflammation (Oguejiofor et al., 2015; Collins and Troeberg, 2019). Moreover, heparan sulfate regulates angiogenesis by playing a proangiogenic role (Fuster and Wang, 2010) and, given that IL-1 regulates inflammation and angiogenesis (Healy et al., 2014), the overexpression of these heparan sulfate–related genes and the underexpression of *IL1R1* in ST cells of persistent cows may result in differential regulation of angiogenesis and tissue remodeling. In addition, underexpression of *IL1R1* may be detrimental for subsequent fertility of persistent cows, because this system is involved in the establishment of pregnancy in cattle (Correia-Álvarez et al., 2015).

Endometrial Health Status and Genes Related to Uterine Receptivity and Pregnancy Establishment

A large number of genes encoding regulators of IFN signaling were underexpressed in persistent and recovered cows compared with healthy cows in both GE and ST cells. Most of them were identified in enriched GO terms related to immune system or effector processes, virus response, and stress response. Interferons are regulators of the neutrophil-based inflammatory response (Glennon-Alty et al., 2021), and in vitro LPS stimulation of bovine endometrial cells induced the upregulation of IFN-stimulated genes (Oguejiofor et al., 2015; Guo et al., 2019). Type I IFN activates the innate immune response (Kovarik et al., 2016; Kopitar-Jerala, 2017) and, depending on the context, can either enhance or inhibit the immune response (Kovarik et al., 2016; Fox et al., 2020). When inflammation results from failure to repair and regenerate damaged tissues, rather than inefficient pathogen clearance, type I IFN is regarded as protective because it suppresses *IL1B* expression and neutrophil chemoattractants (Kovarik et al., 2016). In the present study, the underexpression of IFN-stimulated genes in persistent and recovered cows may lead to failure of the protective role of IFN signaling. This agrees with Iyer (2013), who defined a type I IFN, IL-27, and IL-10 gene program required to resolve an acute inflammatory response and protect against tissue injury.

Embryonic IFN-τ (IFNT), the pregnancy recognition signal in ruminants, induces the same IFN-stimulated genes as type I IFN (Schabmeyer et al., 2021). Most of these genes, such as *MX1*, *MX2*, *RSAD2*, *USP18*, *OAS1*, *RNF213*, and *ISG15*, have been found to be overexpressed in the bovine endometrium at the time of maternal recognition of pregnancy (Day 16) as a consequence of IFNT production by the conceptus (Forde et al., 2011). The enhanced expression of these IFN-stimulated genes is hypothesized to regulate uterine receptivity as well as conceptus elongation and implantation (Spencer et al., 2016). Therefore, underexpression of the corresponding set of genes in GE and ST cells of persistent and recovered cows may prove detrimental for the success of embryo–maternal crosstalk.

CONCLUSIONS

In conclusion, the transcriptomic signatures of the endometrial compartments were altered by the persistence of inflammation in a cell type–specific manner. Recovery or persistence of inflammation was associated with gene expression patterns involved not only in im-

mune function but also in tissue remodeling and uterine receptivity. Recovered and healthy cows were similar in expression of genes related to immunity and tissue remodeling, but strong differences remained, especially in stromal cells, for genes related to cell adhesion and IFN-dependent or IFN-stimulated genes. This revealed a lack of complete functional recovery at 44 DPP in cows presenting a high percentage of immune cells in endometrial cytology at an earlier stage, and may explain why cows apparently recovering from inflammation still present low fertility. Functional recovery occurred more quickly in glandular than in luminal and stromal cells. The obtained data set, including many differentially expressed genes poorly annotated, provides a base for future studies aiming to develop novel diagnostic and therapeutic targets to prevent the persistence of endometrial inflammation and restore postpartum fertility.

ACKNOWLEDGMENTS

Gonçalo Pereira is a PhD student supported by a grant from Fundação para a Ciência e Tecnologia (FCT, Lisboa, Portugal; SFRH/BD/130923/2017). Elisabete Silva is funded by FCT (DL 57/2016/CP1438/CT0001). This work was supported by FCT (Project UIDB/00276/2020 and PTDC/CVT-CVT/6932/2020), FORMAS (Stockholm, Sweden; grant no. 2015-00888), and LA/P/0059/2020 - AL4AnimalS (Lisboa, Portugal). The authors thank Barão & Barão Lda (Benavente, Portugal) for providing the facilities for this study and for their assistance with animal care. The authors have not stated any conflicts of interest.

REFERENCES

Benjamini, Y., and Y. Hochberg. 1995. Controlling the false discovery rate: A practical and powerful approach to multiple testing. J. R. Stat. Soc. B 57:289–300. https://doi.org/10.1111/j.2517-6161.1995.tb02031.x.

Bevilacqua, C., S. Makhzami, J. C. Helbling, P. Defrenaix, and P. Martin. 2010. Maintaining RNA integrity in a homogeneous population of mammary epithelial cells isolated by laser capture microdissection. BMC Cell Biol. 11:95. https://doi.org/10.1186/1471-2121-11-95.

Bi, R., and P. Liu. 2016. Sample size calculation while controlling false discovery rate for differential expression analysis with RNA-sequencing experiments. BMC Bioinformatics 17:146. https://doi.org/10.1186/s12859-016-0994-9.

Bie, Q., C. Jin, B. Zhang, and H. Dong. 2017. IL-17B: A new area of study in the IL-17 family. Mol. Immunol. 90:50–56. https://doi.org/10.1016/j.molimm.2017.07.004.

Bogado Pascottini, O., M. Hostens, P. Dini, J. Vandepitte, R. Ducatelle, and G. Opsomer. 2016. Comparison between cytology and histopathology to evaluate subclinical endometritis in dairy cows. Theriogenology 86:1550–1556. https://doi.org/10.1016/j.theriogenology.2016.05.014.

Bogado Pascottini, O., and S. J. LeBlanc. 2020. Modulation of immune function in the bovine uterus peripartum. Theriogenology 150:193–200. https://doi.org/10.1016/j.theriogenology.2020.01.042.

Brauer, R., J. Tureckova, I. Kanchev, M. Khoylou, J. Skarda, J. Prochazka, F. Spoutil, I. M. Beck, O. Zbodakova, P. Kasparek, V. Korinek, K. Chalupsky, T. Karhu, K. H. Herzig, M. Hajduch, M. Gregor, and R. Sedlacek. 2016. MMP-19 deficiency causes aggravation of colitis due to defects in innate immune cell function. Mucosal Immunol. 9:974–985. https://doi.org/10.1038/mi.2015.117.

Brewer, A., P. Cormican, J. J. Lim, A. Chapwanya, C. O'Farrelly, and K. G. Meade. 2020. Qualitative and quantitative differences in endometrial inflammatory gene expression precede the development of bovine uterine disease. Sci. Rep. 10:18275. https://doi.org/10.1038/s41598-020-75104-7.

Burkly, L. C. 2014. TWEAK/Fn14 axis: The current paradigm of tissue injury-inducible function in the midst of complexities. Semin. Immunol. 26:229–236. https://doi.org/10.1016/j.smim.2014.02.006.

Capp, E., C. M. Milner, J. Williams, L. Hauck, J. Jauckus, T. Strowitzki, and A. Germeyer. 2014. Modulation of tumor necrosis factor-stimulated gene-6 (TSG-6) expression in human endometrium. Arch. Gynecol. Obstet. 289:893–901. https://doi.org/10.1007/s00404-013-3080-9.

Chankeaw, W., S. Lignier, C. Richard, T. Ntallaris, M. Raliou, Y. Guo, D. Plassard, C. Bevilacqua, O. Sandra, G. Andersson, P. Humblot, and G. Charpigny. 2021a. Analysis of the transcriptome of bovine endometrial cells isolated by laser micro-dissection (1): Specific signatures of stromal, glandular and luminal epithelial cells. BMC Genomics 22:451. https://doi.org/10.1186/s12864-021-07712-0.

Chankeaw, W., S. Lignier, C. Richard, T. Ntallaris, M. Raliou, Y. Guo, D. Plassard, C. Bevilacqua, O. Sandra, G. Andersson, P. Humblot, and G. Charpigny. 2021b. Analysis of the transcriptome of bovine endometrial cells isolated by laser micro-dissection (2): Impacts of post-partum negative energy balance on stromal, glandular and luminal epithelial cells. BMC Genomics 22:450. https://doi.org/10.1186/s12864-021-07713-z.

Chiang, N., S. Libreros, P. C. Norris, X. de la Rosa, and C. N. Serhan. 2019. Maresin 1 activates LGR6 receptor promoting phagocyte immunoresolvent functions. J. Clin. Invest. 129:5294–5311. https://doi.org/10.1172/JCI129448.

Choi, Y., H. Seo, J. Han, I. Yoo, J. Kim, and H. Ka. 2016. Chemokine (C-C motif) ligand 28 and its receptor CCR10: Expression and function at the maternal-conceptus interface in pigs. Biol. Reprod. 95:84. https://doi.org/10.1095/biolreprod.116.141903.

Collins, L. E., and L. Troeberg. 2019. Heparan sulfate as a regulator of inflammation and immunity. J. Leukoc. Biol. 105:81–92. https://doi.org/10.1002/JLB.3RU0618-246R.

Correia-Álvarez, E., E. Gómez, D. Martín, S. Carrocera, S. Pérez, J. Otero, N. Peynot, C. Giraud-Delville, J. N. Caamaño, O. Sandra, V. Duranthon, and M. Muñoz. 2015. Expression and localization of interleukin 1 beta and interleukin 1 receptor (type I) in the bovine endometrium and embryo. J. Reprod. Immunol. 110:1–13. https://doi.org/10.1016/j.jri.2015.03.006.

Cui, N., M. Hu, and R. A. Khalil. 2017. Biochemical and biological attributes of matrix metalloproteinases. Prog. Mol. Biol. Transl. Sci. 147:1–73. https://doi.org/10.1016/bs.pmbts.2017.02.005.

Day, A. J., and C. M. Milner. 2019. TSG-6: A multifunctional protein with anti-inflammatory and tissue-protective properties. Matrix Biol. 78–79:60–83. https://doi.org/10.1016/j.matbio.2018.01.011.

Dinarello, C. A. 2018. Overview of the IL-1 family in innate inflammation and acquired immunity. Immunol. Rev. 281:8–27. https://doi.org/10.1111/imr.12621.

Divanovic, S., J. Dalli, L. F. Jorge-Nebert, L. M. Flick, M. Gálvez-Peralta, N. D. Boespflug, T. E. Stankiewicz, J. M. Fitzgerald, M. Somarathna, C. L. Karp, C. N. Serhan, and D. W. Nebert. 2013. Contributions of the three CYP1 monooxygenases to pro-inflammatory and inflammation-resolution lipid mediator pathways. J. Immunol. 191:3347–3357. https://doi.org/10.4049/jimmunol.1300699.

Dohi, T., and L. C. Burkly. 2012. The TWEAK/Fn14 pathway as an aggravating and perpetuating factor in inflammatory diseases: Focus on inflammatory bowel diseases. J. Leukoc. Biol. 92:265–279. https://doi.org/10.1189/jlb.0112042.

Fagundes, N. S., A. L. Rezende, P. B. Alvarenga, L. Q. Magalhães, R. M. Santos, S. A. Headley, M. J. B. Silva, M. E. Beletti, and J. P. E. Saut. 2019. Short communication: Proinflammatory gene expression relative to the collection technique of endometrial samples from cows with and without subclinical endometritis. J. Dairy Sci. 102:5511–5517. https://doi.org/10.3168/jds.2018-15212.

Flanagan, K., D. Moroziewicz, H. Kwak, H. Hörig, and H. L. Kaufman. 2004. The lymphoid chemokine CCL21 costimulates naive T cell expansion and Th1 polarisation of non-regulatory CD4+ T cells. Cell. Immunol. 231:75–84. https://doi.org/10.1016/j.cellimm.2004.12.006.

Foley, C., A. Chapwanya, J. J. Callanan, R. Whiston, R. Miranda-CasoLuengo, J. Lu, W. G. Meijer, D. J. Lynn, C. O' Farrelly, and K. G. Meade. 2015. Integrated analysis of the local and systemic changes preceding the development of post-partum cytological endometritis. BMC Genomics 16:811. https://doi.org/10.1186/s12864-015-1967-5.

Foley, C., A. Chapwanya, C. J. Creevey, F. Narciandi, D. Morris, E. M. Kenny, P. Cormican, J. J. Callanan, C. O'Farrelly, and K. G. Meade. 2012. Global endometrial transcriptomic profiling: Transient immune activation precedes tissue proliferation and repair in healthy beef cows. BMC Genomics 13:489. https://doi.org/10.1186/1471-2164-13-489.

Forde, N., F. Carter, T. E. Spencer, F. W. Bazer, O. Sandra, N. Mansouri-Attia, L. A. Okumu, P. A. McGettigan, J. P. Mehta, R. McBride, P. O'Gaora, J. F. Roche, and P. Lonergan. 2011. Conceptus-induced changes in the endometrial transcriptome: How soon does the cow know she is pregnant? Biol. Reprod. 85:144–156. https://doi.org/10.1095/biolreprod.110.090019.

Fox, L. E., M. C. Locke, and D. J. Lenschow. 2020. Context is key: Delineating the unique functions of IFNα and IFNβ in disease. Front. Immunol. 11:606874. https://doi.org/10.3389/fimmu.2020.606874.

Friedrich, M., L. Gerbeth, M. Gerling, R. Rosenthal, K. Steiger, C. Weidinger, J. Keye, H. Wu, F. Schmidt, W. Weichert, B. Siegmund, and R. Glauben. 2019. HDAC inhibitors promote intestinal epithelial regeneration via autocrine TGFβ1 signalling in inflammation. Mucosal Immunol. 12:656–667. https://doi.org/10.1038/s41385-019-0135-7.

Fuster, M. M., and L. Wang. 2010. Endothelial heparan sulfate in angiogenesis. Prog. Mol. Biol. Transl. Sci. 93:179–212. https://doi.org/10.1016/S1877-1173(10)93009-3.

Galvão, K. N., N. R. Santos, J. S. Galvão, and R. O. Gilbert. 2011. Association between endometritis and endometrial cytokine expression in postpartum Holstein cows. Theriogenology 76:290–299. https://doi.org/10.1016/j.theriogenology.2011.02.006.

Glennon-Alty, L., R. J. Moots, S. W. Edwards, and H. L. Wright. 2021. Type I interferon regulates cytokine-delayed neutrophil apoptosis, reactive oxygen species production and chemokine expression. Clin. Exp. Immunol. 203:151–159. https://doi.org/10.1111/cei.13525.

Gujral, P., V. Mahajan, A. C. Lissaman, and A. P. Ponnampalam. 2020. Histone acetylation and the role of histone deacetylases in normal cyclic endometrium. Reprod. Biol. Endocrinol. 18:84. https://doi.org/10.1186/s12958-020-00637-5.

Guo, Y., T. van Schaik, N. Jhamat, A. Niazi, M. Chanrot, G. Charpigny, J. F. Valarcher, E. Bongcam-Rudloff, G. Andersson, and P. Humblot. 2019. Differential gene expression in bovine endometrial epithelial cells after challenge with LPS; specific implications for genes involved in embryo maternal interactions. PLoS One 14:e0222081. https://doi.org/10.1371/journal.pone.0222081.

Healy, L. L., J. G. Cronin, and I. M. Sheldon. 2014. Endometrial cells sense and react to tissue damage during infection of the bovine endometrium via interleukin 1. Sci. Rep. 4:7060. https://doi.org/10.1038/srep07060.

Hjortø, G. M., O. Larsen, A. Steen, V. Daugvilaite, C. Berg, S. Fares, M. Hansen, S. Ali, and M. M. Rosenkilde. 2016. Differential CCR7 targeting in dendritic cells by three naturally occurring CC–chemokines. Front. Immunol. 7:568. https://doi.org/10.3389/fimmu.2016.00568.

Iyer, S. 2013. Characterizing type I interferon mediated inflammatory responses during acute and chronic infections. PhD Thesis. Molecular Biology Institute, University of California–Los Angeles. Accessed Oct. 8. 2021. https://escholarship.org/uc/item/769019n3..

Jain, A., S. Kaczanowska, and E. Davila. 2014. IL-1 receptor-associated kinase signaling and its role in inflammation, cancer progression, and therapy resistance. Front. Immunol. 5:553. https://doi.org/10.3389/fimmu.2014.00553.

Jarry, A., C. Bossard, C. Bou-Hanna, D. Masson, E. Espaze, M. G. Denis, and C. L. Laboisse. 2008. Mucosal IL-10 and TGF-beta play crucial roles in preventing LPS-driven, IFN-gamma-mediated epithelial damage in human colon explants. J. Clin. Invest. 118:1132–1142. https://doi.org/10.1172/JCI32140.

Kang, M., W. Jeong, H. Bae, W. Lim, F. W. Bazer, and G. Song. 2018. Bifunctional role of ephrin A1-Eph system in stimulating cell proliferation and protecting cells from cell death through the attenuation of ER stress and inflammatory responses in bovine mammary epithelial cells. J. Cell. Physiol. 233:2560–2571. https://doi.org/10.1002/jcp.26131.

Kasimanickam, R., V. Kasimanickam, and J. P. Kastelic. 2014. Mucin 1 and cytokines mRNA in endometrium of dairy cows with postpartum uterine disease or repeat breeding. Theriogenology 81:952–958.e2. https://doi.org/10.1016/j.theriogenology.2014.01.018.

Kikuchi, K., Y. Yanagawa, and K. Onoé. 2005. CCR7 ligand-enhanced phagocytosis of various antigens in mature dendritic cells-time course and antigen distribution different from phagocytosis in immature dendritic cells. Microbiol. Immunol. 49:535–544. https://doi.org/10.1111/j.1348-0421.2005.tb03759.x.

Klingberg, F., B. Hinz, and E. S. White. 2013. The myofibroblast matrix: Implications for tissue repair and fibrosis. J. Pathol. 229:298–309. https://doi.org/10.1002/path.4104.

Kopitar-Jerala, N. 2017. The role of interferons in inflammation and inflammasome activation. Front. Immunol. 8:873. https://doi.org/10.3389/fimmu.2017.00873.

Kovarik, P., V. Castiglia, M. Ivin, and F. Ebner. 2016. Type I interferons in bacterial infections: A balancing act. Front. Immunol. 7:652. https://doi.org/10.3389/fimmu.2016.00652.

Lawlor, L., and X. B. Yang. 2019. Harnessing the HDAC-histone deacetylase enzymes, inhibitors and how these can be utilized in tissue engineering. Int. J. Oral Sci. 11:20. https://doi.org/10.1038/s41368-019-0053-2.

LeBlanc, S. J. 2008. Post-partum uterine disease and dairy herd reproductive performance: A review. Vet. J. 176:102–114. https://doi.org/10.1016/j.tvjl.2007.12.019.

Li, M. O., and R. A. Flavell. 2008a. Contextual regulation of inflammation: A duet by transforming growth factor-beta and interleukin-10. Immunity 28:468–476. https://doi.org/10.1016/j.immuni.2008.03.003.

Li, M. O., and R. A. Flavell. 2008b. TGF-beta: A master of all T cell trades. Cell 134:392–404. https://doi.org/10.1016/j.cell.2008.07.025.

Lim, W., H. Bae, F. W. Bazer, and G. Song. 2019. Ephrin A1 promotes proliferation of bovine endometrial cells with abundant expression of proliferating cell nuclear antigen and cyclin D1 changing the cell population at each stage of the cell cycle. J. Cell. Physiol. 234:4864–4873. https://doi.org/10.1002/jcp.27275.

Liu, X., S. Sun, and D. Liu. 2020. IL-17D: A less studied cytokine of IL-17 family. Int. Arch. Allergy Immunol. 181:618–623. https://doi.org/10.1159/000508255.

Love, M. I., S. Anders, V. Kim, and W. Huber. 2015. RNA-Seq workflow: Gene-level exploratory analysis and differential expression. F1000Res. 4:1070. https://doi.org/10.12688/f1000research.7035.1.

Lukens, J. R., M. J. Barr, D. D. Chaplin, H. Chi, and T. D. Kanneganti. 2012. Inflammasome-derived IL-1β regulates the production of GM-CSF by CD4(+) T cells and γδ T cells. J. Immunol. 188:3107–3115. https://doi.org/10.4049/jimmunol.1103308.

Madoz, L. V., M. J. Giuliodori, A. L. Migliorisi, M. Jaureguiberry, and R. L. de la Sota. 2014. Endometrial cytology, biopsy, and bacteriology for the diagnosis of subclinical endometritis in grazing dairy cows. J. Dairy Sci. 97:195–201. https://doi.org/10.3168/jds.2013-6836.

Mallikarjunappa, S., U. K. Shandilya, A. Sharma, K. Lamers, N. Bissonnette, N. A. Karrow, and K. G. Meade. 2020. Functional analy-

sis of bovine interleukin-10 receptor alpha in response to *Mycobacterium avium* subsp. *paratuberculosis* lysate using CRISPR/Cas9. BMC Genet. 21:121. https://doi.org/10.1186/s12863-020-00925-4.

Marsland, B. J., P. Bättig, M. Bauer, C. Ruedl, U. Lässing, R. R. Beerli, K. Dietmeier, L. Ivanova, T. Pfister, L. Vogt, H. Nakano, C. Nembrini, P. Saudan, M. Kopf, and M. F. Bachmann. 2005. CCL19 and CCL21 induce a potent proinflammatory differentiation program in licensed dendritic cells. Immunity 22:493–505. https://doi.org/10.1016/j.immuni.2005.02.010.

Mateus, L., L. Lopes-da-Costa, F. Bernardo, and J. Robalo Silva. 2002. Influence of puerperal uterine infection on uterine involution and postpartum ovarian activity in dairy cows. Reprod. Domest. Anim. 37:31–35. https://doi.org/10.1046/j.1439-0531.2002.00317.x.

Mellouk, N., C. Rame, D. Naquin, Y. Jaszczyszyn, J. L. Touzé, E. Briant, D. Guillaume, T. Ntallaris, P. Humblot, and J. Dupont. 2019. Impact of the severity of negative energy balance on gene expression in the subcutaneous adipose tissue of periparturient primiparous Holstein dairy cows: Identification of potential novel metabolic signals for the reproductive system. PLoS One 14:e0222954. https://doi.org/10.1371/journal.pone.0222954.

Moore, S. G., A. C. Ericsson, S. K. Behura, W. R. Lamberson, T. J. Evans, M. S. McCabe, S. E. Poock, and C. Lucy. 2019. Concurrent and long-term associations between the endometrial microbiota and endometrial transcriptome in postpartum dairy cows. BMC Genomics 20:405. https://doi.org/10.1186/s12864-019-5797-8.

Mu, D., S. Cambier, L. Fjellbirkeland, J. L. Baron, J. S. Munger, H. Kawakatsu, D. Sheppard, V. C. Broaddus, and S. L. Nishimura. 2002. The integrin alpha(v)beta8 mediates epithelial homeostasis through MT1-MMP-dependent activation of TGF-beta1. J. Cell Biol. 157:493–507. https://doi.org/10.1083/jcb.200109100.

Munger, J. S., X. Huang, H. Kawakatsu, M. J. Griffiths, S. L. Dalton, J. Wu, J. F. Pittet, N. Kaminski, C. Garat, M. A. Matthay, D. B. Rifkin, and D. Sheppard. 1999. The integrin alpha v beta 6 binds and activates latent TGF beta 1: A mechanism for regulating pulmonary inflammation and fibrosis. Cell 96:319–328. https://doi.org/10.1016/S0092-8674(00)80545-0.

Nagano, K. 2019. R-spondin signaling as a pivotal regulator of tissue development and homeostasis. Jpn. Dent. Sci. Rev. 55:80–87. https://doi.org/10.1016/j.jdsr.2019.03.001.

Oguejiofor, C. F., Z. Cheng, A. Abudureyimu, A. A. Fouladi-Nashta, and D. C. Wathes. 2015. Global transcriptomic profiling of bovine endometrial immune response in vitro. I. Effect of lipopolysaccharide on innate immunity. Biol. Reprod. 93:100. https://doi.org/10.1095/biolreprod.115.128868.

Patro, R., G. Duggal, M. I. Love, R. A. Irizarry, and C. Kingsford. 2017. Salmon provides fast and bias-aware quantification of transcript expression. Nat. Methods 14:417–419. https://doi.org/10.1038/nmeth.4197.

Pereira, G., R. Bexiga, J. Chagas e Silva, E. Silva, C. Ramé, J. Dupont, Y. Guo, P. Humblot, and L. Lopes-da-Costa. 2020. Adipokines as biomarkers of postpartum subclinical endometritis in dairy cows. Reproduction 160:417–430. https://doi.org/10.1530/REP-20-0183.

Pereira, G., Y. Guo, E. Silva, C. Bevilacqua, G. Charpigny, L. Lopes-da-Costa, and P. Humblot. 2022. Progesterone differentially affects the transcriptomic profiles of cow endometrial cell types. BMC Genomics 23:82. https://doi.org/10.1186/s12864-022-08323-z.

Pereira, P., Y. Guo, E. Silva, M. Silva, C. Bevilacqua, G. Charpigny, L. Lopes-da-Costa, and P. Humblot. 2021a. JDS-LCM-Supplemental Figure S1. Mendeley Data V1. https://doi.org/10.17632/wdn57mr477.1

Pereira, P., Y. Guo, E. Silva, M. Silva, C. Bevilacqua, G. Charpigny, L. Lopes-da-Costa, and P. Humblot. 2021b. JDS-LCM-Supplemental File S1. Mendeley Data V1. https://doi.org/10.17632/x5kkf369kn.1.

Pereira, P., Y. Guo, E. Silva, M. Silva, C. Bevilacqua, G. Charpigny, L. Lopes-da-Costa, and P. Humblot. 2021c. JDS-LCM-Supplemental File S2. Mendeley Data V1. https://doi.org/10.17632/922v7859tg.1.

Pereira, P., Y. Guo, E. Silva, M. Silva, C. Bevilacqua, G. Charpigny, L. Lopes-da-Costa, and P. Humblot. 2021d. JDS-LCM-Supplemental File S3. Mendeley Data V1. https://doi.org/10.17632/cpb5vznzhn.1.

Pereira, P., Y. Guo, E. Silva, M. Silva, C. Bevilacqua, G. Charpigny, L. Lopes-da-Costa, and P. Humblot. 2021e. JDS-LCM-Supplemental File S4. Mendeley Data V1. https://doi.org/10.17632/d36t3d438g.1.

Piras, C., Y. Guo, A. Soggiu, M. Chanrot, V. Greco, A. Urbani, G. Charpigny, L. Bonizzi, P. Roncada, and P. Humblot. 2017. Changes in protein expression profiles in bovine endometrial epithelial cells exposed to *E. coli* LPS challenge. Mol. Biosyst. 13:392–405. https://doi.org/10.1039/C6MB00723F.

Qi, X., M. Lei, L. Qin, M. Xie, D. Zhao, and J. Wang. 2016. Endogenous TWEAK is critical for regulating the function of mouse uterine natural killer cells in an immunological model of pregnancy loss. Immunology 148:70–82. https://doi.org/10.1111/imm.12588.

Queckbörner, S., C. von Grothusen, N. R. Boggavarapu, R. M. Francis, L. C. Davies, and K. Gemzell-Danielsson. 2021. Stromal heterogeneity in the human proliferative endometrium—A single-cell RNA sequencing study. J. Pers. Med. 11:448. https://doi.org/10.3390/jpm11060448.

Raliou, M., D. Dembélé, A. Düvel, P. Bolifraud, J. Aubert, T. Mary-Huard, D. Rocha, F. Piumi, S. Mockly, M. Heppelmann, I. Dieuzy-Labaye, P. Zieger, D. G. E. Smith, H. J. Schuberth, I. M. Sheldon, and O. Sandra. 2019. Subclinical endometritis in dairy cattle is associated with distinct mRNA expression patterns in blood and endometrium. PLoS One 14:e0220244. https://doi.org/10.1371/journal.pone.0220244.

Rockey, D. C., N. Weymouth, and Z. Shi. 2013. Smooth muscle α actin (Acta2) and myofibroblast function during hepatic wound healing. PLoS One 8:e77166. https://doi.org/10.1371/journal.pone.0077166.

Salilew-Wondim, D., S. Ibrahim, S. Gebremedhn, D. Tesfaye, M. Heppelmann, H. Bollwein, C. Pfarrer, E. Tholen, C. Neuhoff, K. Schellander, and M. Hoelker. 2016. Clinical and subclinical endometritis induced alterations in bovine endometrial transcriptome and miRNome profile. BMC Genomics 17:218. https://doi.org/10.1186/s12864-016-2513-9.

Sánchez, M. A., P. García, S. Menéndez, B. Sánchez, M. González, and J. M. Flores. 2002. Fibroblastic growth factor receptor (FGF-R) expression during uterine involution in goat. Anim. Reprod. Sci. 69:25–35. https://doi.org/10.1016/S0378-4320(01)00169-5.

Schabmeyer, S. T., A. M. Kneidl, J. K. Schneider, S. Kirsch, Y. Zablotski, W. Petzl, F. Weber, H. Zerbe, and M. M. Meyerholz. 2021. Concentration-dependent type 1 interferon-induced regulation of mx1 and FABP3 in bovine endometrial explants. Animals (Basel) 11:262. https://doi.org/10.3390/ani11020262.

Schett, G., and M. F. Neurath. 2018. Resolution of chronic inflammatory disease: Universal and tissue-specific concepts. Nat. Commun. 9:3261. https://doi.org/10.1038/s41467-018-05800-6.

Sheldon, I. M. 2004. The postpartum uterus. Vet. Clin. North Am. Food Anim. Pract. 20:569–591. https://doi.org/10.1016/j.cvfa.2004.06.008.

Sheldon, I. M., J. G. Cronin, and J. J. Bromfield. 2019. Tolerance and innate immunity shape the development of postpartum uterine disease and the impact of endometritis in dairy cattle. Annu. Rev. Anim. Biosci. 7:361–384. https://doi.org/10.1146/annurev-animal-020518-115227.

Sheldon, I. M., G. S. Lewis, S. LeBlanc, and R. O. Gilbert. 2006. Defining postpartum uterine disease in cattle. Theriogenology 65:1516–1530. https://doi.org/10.1016/j.theriogenology.2005.08.021.

Sheldon, I. M., P. C. C. Molinari, T. J. R. Ormsby, and J. J. Bromfield. 2020. Preventing postpartum uterine disease in dairy cattle depends on avoiding, tolerating and resisting pathogenic bacteria. Theriogenology 150:158–165. https://doi.org/10.1016/j.theriogenology.2020.01.017.

Sheldon, I. M., D. E. Noakes, A. N. Rycroft, D. U. Pfeiffer, and H. Dobson. 2002. Influence of uterine bacterial contamination after parturition on ovarian dominant follicle selection and follicle growth and function in cattle. Reproduction 123:837–845. https://doi.org/10.1530/rep.0.1230837.

Singer, J. W., A. Fleischman, S. Al-Fayoumi, J. O. Mascarenhas, Q. Yu, and A. Agarwal. 2018. Inhibition of interleukin-1 receptor-associated kinase 1 (IRAK1) as a therapeutic strategy. Oncotarget 9:33416–33439. https://doi.org/10.18632/oncotarget.26058.

Soneson, C., M. I. Love, and M. D. Robinson. 2015. Differential analyses for RNA-seq: transcript-level estimates improve gene-level inferences. F1000Res. 4:1521. https://doi.org/10.12688/f1000research.7563.1.

Spencer, T. E., N. Forde, and P. Lonergan. 2016. The role of progesterone and conceptus-derived factors in uterine biology during early pregnancy in ruminants. J. Dairy Sci. 99:5941–5950. https://doi.org/10.3168/jds.2015-10070.

Stephensen, C. B. 2001. Vitamin A, infection, and immune function. Annu. Rev. Nutr. 21:167–192. https://doi.org/10.1146/annurev.nutr.21.1.167.

Stevison, F., J. Jing, S. Tripathy, and N. Isoherranen. 2015. Role of retinoic acid-metabolizing cytochrome P450s, CYP26, in inflammation and cancer. Adv. Pharmacol. 74:373–412. https://doi.org/10.1016/bs.apha.2015.04.006.

Supek, F., M. Bošnjak, N. Škunca, and T. Šmuc. 2011. REVIGO summarizes and visualizes long lists of gene ontology terms. PLoS One 6:e21800. https://doi.org/10.1371/journal.pone.0021800.

Szklarczyk, D., A. L. Gable, D. Lyon, A. Junge, S. Wyder, J. Huerta-Cepas, M. Simonovic, N. T. Doncheva, J. H. Morris, P. Bork, L. J. Jensen, and C. von Mering. 2019. STRING v11: Protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. Nucleic Acids Res. 47(D1):D607–D613. https://doi.org/10.1093/nar/gky1131.

Vasudevan, S., M. M. Kamat, S. S. Walusimbi, J. L. Pate, and T. L. Ott. 2017. Effects of early pregnancy on uterine lymphocytes and endometrial expression of immune-regulatory molecules in dairy heifers. Biol. Reprod. 97:104–118. https://doi.org/10.1093/biolre/iox061.

Williams, E. J., D. P. Fischer, D. U. Pfeiffer, G. C. England, D. E. Noakes, H. Dobson, and I. M. Sheldon. 2005. Clinical evaluation of postpartum vaginal mucus reflects uterine bacterial infection and the immune response in cattle. Theriogenology 63:102–117. https://doi.org/10.1016/j.theriogenology.2004.03.017.

Wu, L., O. V. Belyaeva, M. K. Adams, A. V. Klyuyeva, S. A. Lee, K. R. Goggans, R. A. Kesterson, K. M. Popov, and N. Y. Kedishvili. 2019. Mice lacking the epidermal retinol dehydrogenases SDR16C5 and SDR16C6 display accelerated hair growth and enlarged meibomian glands. J. Biol. Chem. 294:17060–17074. https://doi.org/10.1074/jbc.RA119.010835.

Xie, Y., N. Su, J. Yang, Q. Tan, S. Huang, M. Jin, Z. Ni, B. Zhang, D. Zhang, F. Luo, H. Chen, X. Sun, J. Q. Feng, H. Qi, and L. Chen. 2020. FGF/FGFR signaling in health and disease. Signal Transduct. Target. Ther. 5:181. https://doi.org/10.1038/s41392-020-00222-7.

Xiong, N., Y. Fu, S. Hu, M. Xia, and J. Yang. 2012. CCR10 and its ligands in regulation of epithelial immunity and diseases. Protein Cell 3:571–580. https://doi.org/10.1007/s13238-012-2927-3.

Zhang, S., D. Wang, and Z. Yan. 2021. Increasing of matrix metalloproteinase 3 in bovine endometritis. Theriogenology 175:83–88. https://doi.org/10.1016/j.theriogenology.2021.09.001.

ORCIDS

Gonçalo Pereira https://orcid.org/0000-0003-3533-6893
Yongzhi Guo https://orcid.org/0000-0002-5057-9095
Elisabete Silva https://orcid.org/0000-0002-2332-7642
Marta Filipa Silva https://orcid.org/0000-0002-0299-4825
Claudia Bevilacqua https://orcid.org/0000-0003-0423-4624
Gilles Charpigny https://orcid.org/0000-0003-3954-7663
Luís Lopes-da-Costa https://orcid.org/0000-0001-5165-3034