Received: 27 December 2022 | Revised: 18 April 2023 | Accepted: 29 April 2023

DOI: 10.1111/rda.14371

ORIGINAL ARTICLE

Reproduction in Domestic Animals WILEY

Transcriptomic analysis reveals gene expression changes in peripheral white blood cells of cows after embryo transfer: Implications for pregnancy tolerance

Jorge A. De los Santos[1] | João Paulo N. Andrade[1] | L. R. Cangiano[1] | Andres Iriarte[2] | Francisco Peñagaricano[1] | John J. Parrish[1]

[1]Department of Animal and Dairy Sciences, University of Wisconsin-Madison, Madison, Wisconsin, USA

[2]Departamento Desarrollo Biotecnológico, Facultad de Medicina, Universidad de la República, Montevideo, Uruguay

Correspondence
John J. Parrish, Department of Animal and Dairy Sciences, University of Wisconsin-Madison, Madison, Wisconsin, USA.
Email: jjparris@wisc.edu

Funding information
Agencia Nacional de Investigación e Innovación

Abstract

Most embryo losses occur in the first trimester of pregnancy in cows and include losses following embryo transfer. There is a resulting negative economic impact on cattle production systems when this occurs. Cellular and molecular mechanisms behind the maternal immune response to the growing embryo have not been fully characterized. The objective of this study was to examine the gene expression profiles of peripheral white blood cells (PWBCs) from pregnant cows 21 days after an embryo was transferred, and cows that were treated equally but lost the embryo. Specifically, we obtained and compared the transcriptome of PWBC from heifers that became pregnant at day 21 ($N = 5$) or failed to become pregnant after the embryo transfer ($N = 5$). Sequencing data can be accessed by Gene Expression Omnibus (GEO) with the accession number GSE210665. A total of 13,167 genes were evaluated for differential expression between groups. A total of 682 genes showed differential expression (p-value <.01), 302 genes were up-regulated while 380 were down-regulated due to pregnancy. The most significant genes were COL1A2, H2AC18, HTRA1, MMP14, CD5L, ADAMDEC1, MYO1A and RPL39, among others. Most of the significant genes are related to the up-regulation of inflammatory chemokine activity and immune defence response. Our findings extend the current knowledge that pregnancy alters the PWBC by promoting immune tolerance, cell chemotaxis, blood coagulation, angiogenesis, inflammatory response, cell adhesion and cytokine secretion. Our data suggest that pregnancy and ectoparasites could trigger poorly described genes in PWBC of cows, and a few previously escribed genes, such as IFI44. These results could shed light on the genes and mechanisms that promote tolerance to pregnancy and allow survival of the developing embryo.

KEYWORDS
bovine interferon-stimulated genes, early pregnancy diagnosis, immune regulation

1 | INTRODUCTION

Peripheral white blood cells (PWBCs) originate in the bone marrow and circulate in the blood including granulocytes, monocytes (mature macrophages) and lymphocytes (B and T cells and cytotoxic T cells are highly specialized). Typically, after receiving a biochemical signal, these cells in the blood increase the signalling by cytokine secretion or by bringing more cells to the source of signalling to start the healing process (Dean, 2005; Nicholson, 2016). These characteristics make the PWBC the most important cells in the immune response.

During the development of the embryo, three main characteristics define the maternal and fetus interaction. First, the anatomical separation from the mother prevents maternal immune cells from accessing fetal antigens. Second, trophoblast fetal cells do not express alloantigens. Third, immunological tolerance to fetal antigens is induced in the mother. The concept of immunological maternal tolerance was developed by Medawar in 1964 (Croy, 2014). It is currently unknown how the maternal immunological system responds to pregnancy, particularly at the early stages.

In cattle, the embryo attaches itself to the endometrium and this begins approximately 18 days post mating (King, 1993). The developing embryo begins to secrete specific hormones that signal to the cow's body that pregnancy has been established. During the attachment process, the cow's immune system plays an important role in either accepting or rejecting the embryo (Spencer et al., 2016). Understanding this process can help in improving reproductive success in cattle.

One important challenge is to understand how the maternal immune system responds differently to antigens and the growing fetus. Data from humans show that natural killer cells coordinate the placentation and allow the fetus to develop (Moffett et al., 2017). It has been proposed that the developmental adaptation during pregnancy promotes immune exclusion and prevents immune responses against the growing conceptus, by microchimerism. In other words, the early exchange of cells between the mother and fetus affects the immune tolerance of the mother to the fetus (Kinder et al., 2017).

Understanding the underlying molecular mechanisms that promote immune tolerance in pregnant cows would enable technical and scientific interventions to minimize pregnancy loss after embryo transfer or shorten the breeding period. Most embryo losses occur in the first 16 days after breeding, which has been linked to high cost in dairy production systems (Diskin et al., 2016; Wiltbank et al., 2016). Furthermore, the timing of breeding impacts total milk production and the maintenance costs of non-pregnant animals (Consentini et al., 2021). In cows, the conceptus secretes Interferon Tau (IFN-τ), pregnancy-associated glycoproteins and prostaglandins as key proteins affecting immune blood cell function during early pregnancy (Ott, 2019). IFN-τ affects the gene expression in PWBC, particularly in mononuclear leukocytes and polymorphonuclear granulocytes cells (Shirasuna et al., 2012). In ruminants, this cytokine is secreted by the conceptus that regulates transcriptional factors and modulates the maternal expression of a group of genes called interferon-stimulated genes (ISGs) (Ealy & Yang, 2009). For those reasons, identifying genes at early pregnancy and how the maternal immune system response is essential to fully understand the embryo development process.

Previously, two independent groups reported genes differentially expressed in cows during pregnancy. The first study reported a total of 220 differentially expressed genes in the blood of *Bos indicus* beef heifers on day 18 of pregnancy. A total of 200 genes were up-regulated and 20 down-regulated. The study discusses possible novel genes as early biomarkers of pregnancy in cows' blood as early as 18 days into pregnancy (Rocha et al., 2020). The second study reported a total of 1860 genes and 237 microRNAs differentially expressed in *Bos taurus* on the same day of artificial insemination. The authors argue that the co-expressed genes in PWBC and free circulating microRNA can be linked with fertility in heifers (Moorey et al., 2020).

Our main research question was whether pregnancy at day 21 after embryo transfer (i.e. 28 days of pregnancy) could affect PWBC gene expression. We hypothesized that the transcriptome of the PWBC would be different for pregnant cows in comparison with cows that lost the embryo.

2 | MATERIALS AND METHODS

2.1 | Ethics approval

Animal procedures were performed following the Guide for the Care and Use of Agricultural Animals and were approved by the Animal Care and Use Committee of the College of Agriculture and Life Sciences, University of Wisconsin protocol number A006314.

2.2 | Animals

Ten Holstein heifers enrolled in this study were between 1 and 2 years old. All the heifers were treated under the same sanitary and feeding conditions at a Wisconsin commercial dairy farm. The protocol (Figure 1) starts 8 days before the insertion of an intravaginal Controlled Internal Drug Release device (CIDR) with 1.38 g of progesterone (P4; Eazi-Breed CIDR, Zoetis). On day minus three (D−3) heifers were injected with 500 mg i.m. of cloprostenol (Estroplan®, Parnell Pharmaceuticals) and the CIDR was removed. To induce ovulation 3 days later (D 0), GnRH i.m. was administered (100 μg gonadorelin acetate; Gonabreed®, Parnell Pharmaceuticals). Five days after GnRH, the criteria to select recipients was the presence of at least one corpus luteum (CL) > 15 mm in diameter on at least one of the ovaries evaluated by ultrasonography. Then 7 days (D 7) after GnRH injection, one fresh in vitro fertilization embryo (cultured for 7 days previously from commercial production, unknown paternity, embryo quality for transfer was evaluated under IETS guidelines) was transferred to the uterine horn ipsilateral to the CL.

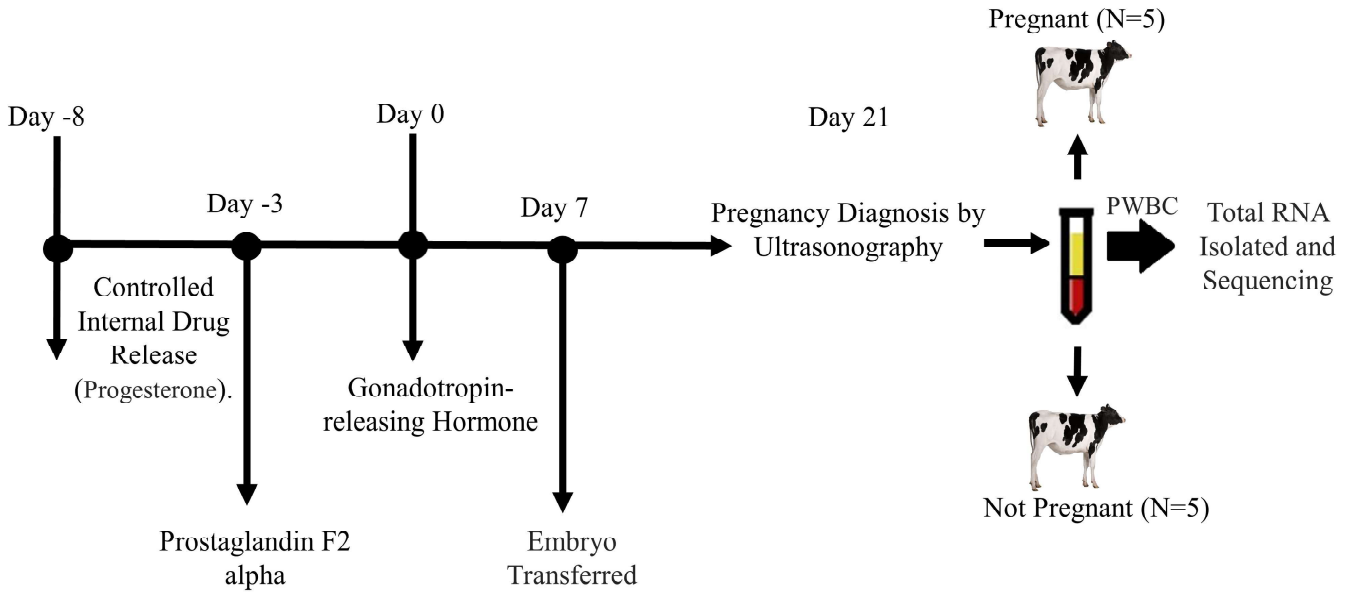

FIGURE 1 Experimental design. Controlled internal drug release with 1.38 g of progesterone was used between days −8 and −3. The prostaglandin F2 alpha hormone was injected on day −3. Day 0 gonadotropin-releasing hormone was injected. On day 7 an embryo was transferred and on day 21 after embryo transfer (day 28 of the protocol) pregnancy diagnosis was performed by rectal ultrasonography. Blood samples were from five pregnant and five not pregnant cows. PWB cells and total RNA were isolated before sequencing.

The pregnancy diagnosis was performed using a portable ultrasound (Easi-Scan; BFC Technologies USA Ltd. LLC) with a linear-array transducer (7.5-MHz) on day 21 following embryo transfer. This is equivalent to day 28 of pregnancy in an inseminated female. Heifers were considered pregnant by visualization of the embryonic vesicle with a viable embryo having a heartbeat.

Blood samples were collected from five pregnant and five non-pregnant heifers on day 28 after GnRH injection (day 21 after embryo transfer; Figure 1). Blood samples were collected in K2 EDTA Vacutainer tubes (Becton Dickinson) by tail venipuncture, using 18G needles (Becton Dickinson). Buffy coat layers for each sample were separated by centrifugation at 4°C for 15 min and then washed three successive times with hypertonic solution (saccharose 0.3 M, Tris–HCl pH = 8.0 10 mM, MgCl2 10 mM, Triton X 100 1%) to avoid red blood cells contamination. The resulting pellet was stored in a 1.5 mL conical tube at −80°C until RNA extraction was performed.

2.3 | RNA-seq analysis: RNA extraction, library preparation and sequencing

Total RNA was isolated and purified using TRIzol LS Reagent based on a protocol previously described (Chomczynski & Sacchi, 2006) and treated with DNAse to avoid DNA contamination. The resulting RNA yield was measured through a Nanodrop One (Thermo Fisher Scientific). The quality of RNA was evaluated using Agilent Bioanalyzer, with RNA integrity number values between 8 and 10. To avoid RNA ribosomal contamination, the total RNA extracted was subject to RNA depletion. Libraries from individual samples were sequenced with Illumina's HiSeq 2000 at Macrogen (Korea) using 100 bp paired end reads.

2.4 | RNA-seq analysis: Quality control and read mapping

The quality of the sequencing reads was evaluated before and after trimming using the software FastQC (version 0.11.7, Babraham Bioinformatics). Read trimming was performed using the software Trim Galore (version 0.4.4, Babraham Bioinformatics) with the following parameters: *--paired*, *--length* 50, *--clip_R1* 12 and *--clip_R2* 12. After processing, paired-end sequencing reads were mapped to the bovine reference genome ARS-UCD1.2 using the software Hisat2 (v2.1.0; Kim et al., 2015).

2.5 | RNA-seq analysis: Expression analysis

The number of reads that mapped to each annotated gene in the bovine annotation file (GTF file) was obtained using the python script *htseq-count* using the option *intersection-nonempty* (Anders et al., 2015). Differentially expressed genes between groups were detected using the *R* package edgeR (Robinson et al., 2010). This R package combines the use of the trimmed mean of M-values as a normalization method, an empirical Bayes approach for estimating tagwise negative binomial dispersion values and generalized linear models and likelihood ratio tests for detecting differentially expressed genes. All the analyses were performed using the default settings for all parameters.

2.6 | Gene-set enrichment analysis

The enrichment of Gene Ontology and InterPro terms with differentially expressed genes was analysed using a Fisher's exact test, a hypergeometric-based overrepresentation test commonly used to evaluate 2×2 contingency tables. Differentially expressed genes (*p*-value <.01) that had ENSEMBL annotations were tested against the background set of all expressed genes with ENSEMBL annotations. All these analyses were performed using the *R* package EnrichKit (https://github.com/liulihe954/EnrichKit).

3 | RESULTS

3.1 | Total RNA sequencing of peripheral white blood cells

We obtained and compared the transcriptome of PWBC from heifers that became pregnant (*N* = 5) or failed to become pregnant (*N* = 5) after embryo transfer. Figure 2 describes the overall results of the gene expression analysis. On average, 96% of the reads per sample were successfully mapped to the latest bovine reference genome ARS-UCD 1.2. Each sample generated between 32 to 43 million reads. Sequencing data can be accessed by GEO with the accession number GSE210665.

3.2 | Differentially expressed genes associated with pregnancy status

We compared the expression of 13,167 genes in PWBC from heifers that were pregnant and those that were not (as shown in Figure 2). The results indicated that 682 of the genes had significant differences in expression (with a $p < .01$), with 302 genes being up-regulated and 380 genes being down-regulated due to pregnancy (as indicated in Figure 3). The study identified key genes, including A4IF68, F1N720, NFKBIA, C1R, Q32PA1, G3N3S3, IFI44, LOC100297046, H2AC18, HTRA1, MMP14, CD5L, ADAMDEC1, MYO1A, RPL39, CD153, G3N2T2, Q2KIQ3, F1MWN4, E1BMV7 and C1QA (as shown in Figure 4). These genes correspond to

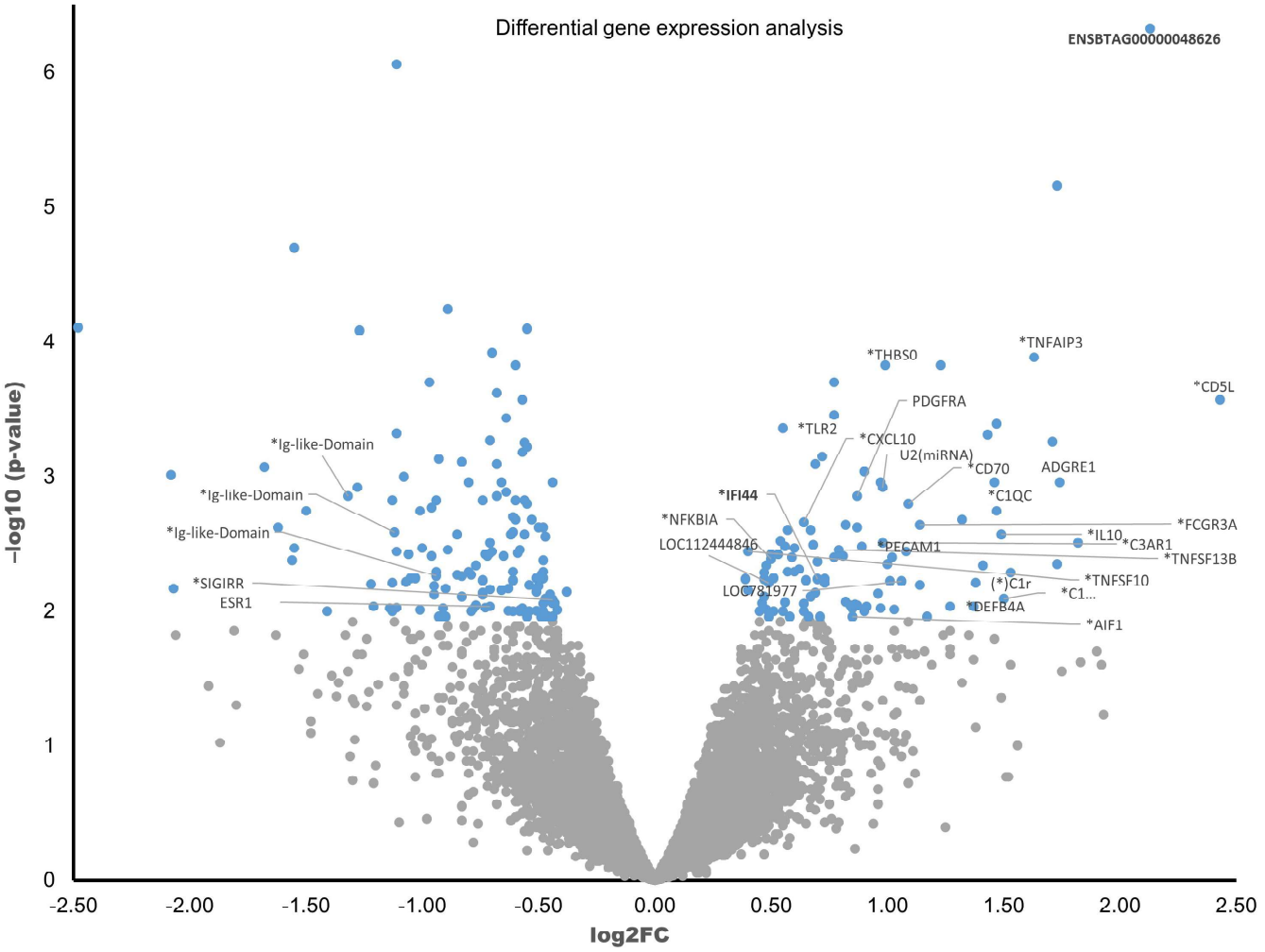

FIGURE 2 Volcano plot showing the results of the gene expression analysis. Blue dots represent differentially expressed genes (*p*-value < .01).

FIGURE 3 Gene-set terms significantly altered by pregnancy status. The y-axis displays the names of the gene sets. The size of the dots represents the significance of the enrichment ($-\log_{10}$ *p*-value, Fisher's exact test) and the *x*-axis represents the percentage of significant genes.

various proteins, including ID1 protein, Toll-like receptor 2, NF kappa B inhibitor alpha, CD59 glycoprotein, 40S ribosomal protein S15a, Interferon-induced protein 44, C-C motif chemokine, Histone H2A, Serine protease HTRA1, Matrix metalloproteinase-14, CD5 molecule like, ADAM-like decysin 1, Unconventional myosin-Ia, CD151 antigen, Ig-like domain-containing protein, WD repeat domain 54, SFI1 centrin binding protein, ADAM metallopeptidase with thrombospondin type 1 motif 10 and Choline kinase beta.

3.3 | Gene-set analysis: Evaluation of different gene ontology and InterPro terms

Gene-set enrichment analysis, also known as overrepresentation analysis, was performed to gain additional insight into the biological processes and molecular functions associated with the set of differentially expressed genes. Notably, the gene set that was significantly enriched (*p*-adj <.05) in response to pregnancy was associated with the immune system, cellular signalling, cell nuclear reorganization

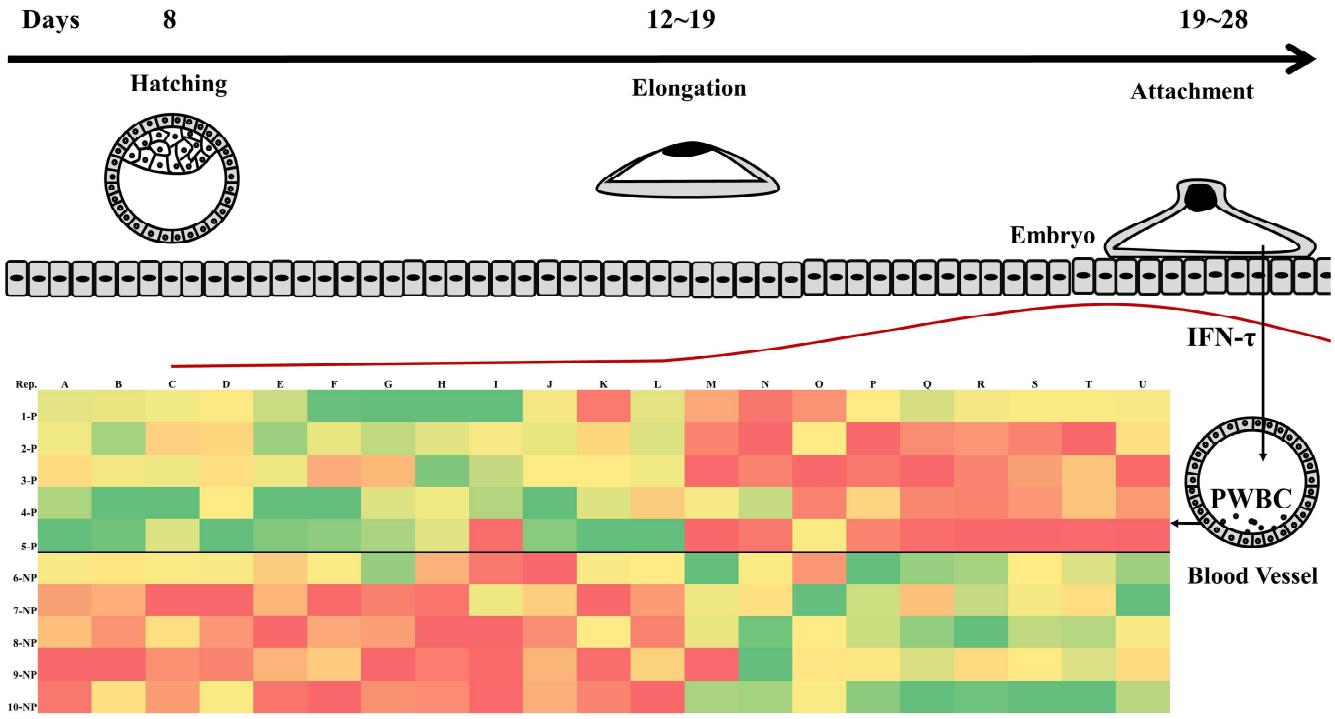

FIGURE 4 Timeline with the physiological and molecular events that impact the immune system during early pregnancy and embryo development. During the elongation of the conceptus, IFN-τ up-regulates the expression of interferon-stimulated genes, in peripheral white blood cells (PWBCs). The heatmap shows the level of expression for each cow and the most significant genes. In early pregnancy, the embryo secretes IFN-τ by trophoblast, which plays an important role in affecting the transcriptome of PWBC. Rep. repetition, P pregnant, NP not pregnant. Genes A- A4IF68, B-F1N720, C-NFKBIA, D-C1R, E-Q32PA1, F-G3N3S3, G-IFI44, H- LOC100297046, I-H2AC18, J-HTRA1, K-MMP14, L-CD5L, M-ADAMDEC1, N-MYO1A, O-RPL39, P-CD153, Q-G3N2T2, R-Q2KIQ3, S-F1MWN4, T- E1BMV7 and U-C1QA.

and cell adhesion (Figure 3). Some of the enriched terms are directly involved in immunological status, for instance, positive regulation of inflammatory (GO:0050729), chemokine activity (GO:0008009), defence response to protozoan (GO:0042832), immune system process (GO:0002376) and defence response (GO:0006952). The gene-set analysis revealed terms related to blood cellular signalling in blood for immune response and coagulation process. All these terms contain crucial genes that play key roles in the immune response of the mother and are probably involved in the allocation of the embryo.

4 | DISCUSSION

Our study reveals that immunoglobulin-like domain genes, which are part of the adaptive immune system, were differentially expressed genes between pregnant and non-pregnant individuals. The immunoglobulin-like domains are very common in surface proteins, particularly in leukocytes, and are related to low-affinity interaction between cells. It has been shown that these cellular membrane proteins play a key role in the immune system by regulating cell-to-cell recognition and cytoplasmic signalling (Barclay, 1999). In addition, our data show that the genes CXCL10, IL10-IF2A and C1QA were down-regulated, while MS4A7, NFKBIA, SIGIRR, FCGRIA and IFI44 were up-regulated. All these genes are associated with the immune response, and our study shows for the first time that they are also present in the PWBC of pregnant cows. In addition, we identified a novel gene ENSBTAG00000048626 as up-regulated in pregnant heifers. Notably, this gene has been recently reported as up-regulated in PWBC of *Brangus cattle* in response to ectoparasites (tick) infections (Valdivieso et al., 2022). This suggests a possible link between how pregnancy and ectoparasites trigger specific genes in the PWBC cows.

Interestingly, one of the differently expressed gene, IFI44, was previously reported as up-regulated in *Bos indicus* at day 18 of pregnancy (Rocha et al., 2020). We confirm herein that the gene is also up-regulated at day 28 of pregnancy in Holstein cows. The IFI44-knockout mice model shows an increase in respiratory syncytial virus infections and implies that the IFI44 gene is a crucial regulator of the immune system (Busse et al., 2020). Additionally, this gene was up-regulated in transcriptomic studies of CL biopsies at days 14 and 20 in non-lactating cattle and at days 18 and 21 of pregnancy in lactating Holstein cows (Hughes et al., 2022). The expression of the gene IFI44 could be a potential maternal blood biomarker of early pregnancy in cows.

The COL1A2 gene was up-regulated in PWBC of pregnant cows. Data from humans show that this gene is overexpressed in decidua at early attachment compared with blood CD14+ cells (Gustafsson et al., 2008). Data from cows show that *COL1A2* is a crucial ligand in the endometrium during conceptus elongation around day 15 (Hayashi et al., 2017). This gene may play a role as an immunomodulator and

tissue endometrial remodelling, during embryo attachment. Gene LOC100337213 is a G protein-coupled receptor and associated with cytoplasmic cellular signalling. Gene LOC100297044 encodes for a beta chemokine receptor, like ACKR2, a transmembrane G protein-coupled receptor. Recent research shows that ACKR2 is overexpressed in the placenta and presumably helps to maintain the fetus from inflammatory attacks and spontaneous abortion in humans (Gowhari et al., 2022). Gene H2AC18 encodes for a histone protein, and gene HTRA1 is a serine protease that may help to degrade proteins and maintain DNA packaged respectively. Gene MMP14 encodes a protein that is part of the matrix metalloproteinase family. It is shown that these proteins degrade the extracellular matrix that is vital for embryonic development and tissue remodelling (Zheng et al., 2021). Finally, the overexpressed CD5L typically expressed in macrophages and secreted in exosomes responds to inflamed tissues by lipid metabolism in humans, and it has been associated with arteriosclerosis (Wang et al., 2022). Therefore, the proper attachment of the embryo probably depends on the coordinated action of these genes that play a key role in embryonic development, including maintaining the fetus from inflammatory attacks, degrading proteins and remodelling tissue. Our gene-set analysis also supports the importance of processes such as remodelling tissue and immune response.

Genes MYO1A and RPL39, down-regulated in pregnant heifers, encode for a member of the myosin superfamily and a member of the ribosomal subunit, respectively. Interestingly, Histone H4 was also down-regulated due to pregnancy. Another down-regulated gene, ADAMDEC-1, is implicated in the regulation of the cellular activity of endothelial cells and trophoblasts. The development of mouse placenta has been associated with disintegrin and metalloprotease activities. In a recent study it has been shown that overexpression of ADAMDEC-1 in the placenta is associated with spontaneous abortions in mice (Kuniyoshi et al., 2021).

Studies in mice showed that PWBC with markers such as CD4(+) CD25(+) and regulatory T cells play a central role in immune tolerance at the attachment of the mouse embryo (Shima et al., 2010). Regulatory T cells are important to tolerate the fetus's antigens in early pregnancy. A disbalance in the number of these cells in decidua and the peripheral blood is associated with spontaneous abortions in humans (Yang et al., 2008). Therefore, the presence of an imbalanced number of these cells in the decidua and peripheral blood has been linked to spontaneous abortions in humans.

The ruminant's attachment is unique, and this may reflect the embryo's survival in different ways. First, the embryo attachment presents binuclear giant cells that secrete pregnancy-associated glycoproteins (PAGs), which impact the PWBC and CL survival (Spencer et al., 2004; Wooding, 1992). Second, the cow placenta secretes large amounts of hormones such as oestrogen (E1) and progesterone (P4). In addition, it has been shown that trophoblast secretes exosomes that are crucial for embryo attachment in dairy cows (Su et al., 2022). Finally, transcriptomic analysis of PWBC from beef heifers after embryo attachment was previously reported, but the biological interaction between embryo attachment and immune blood cell response is not clearly described in ruminants (Dickinson & Biase, 2018). IFN-τ is secreted by the embryonic trophoblast cells in early pregnancy (see Figure 4) and impacts the maternal PWBC-stimulating ISGs genes. It has been shown that ISGs promote the expression of interferon-stimulated protein 15 kDa (ISG15), myxovirus resistance protein 1 (MX1), myxovirus resistance protein 2 (MX2) and 20-50-oligoadenylate synthetase (OAS-1), on day 20 after artificial insemination in beef cattle (Pugliesi et al., 2014). Another study performed in *Bos indicus* shows that interferon-stimulated gene 15 (ISG15) increases expression in response to IFN-τ on days 16 and 18 (Soumya et al., 2017). It has been proposed that ISGs respond to IFN-τ up-regulate gene transcripts as ISG15, MX1, MX2 and OAS1 in neutrophil cells (Panda et al., 2020). One of the limitations of the study by Panda and colleagues is that the ultrasonography was performed on day 45 of pregnancy and blood samples were taken on days 0, 10, 18 and 36 after artificial insemination. Our study did not display the classic ISGs genes response at day 28 of pregnancy. This can be explained by the differences between artificial insemination and cows' breeding procedures or due to different experimental designs.

In vitro experiments have demonstrated that increased cell-free fetal DNA trigger sterile inflammation responses in humans (Kazemi et al., 2021). Furthermore, fetal-derived cells in humans reach the maternal tissues called feto-meternal microchimerism and impact maternal autoimmune disease outcomes during and after pregnancy (Nelson, 2003). In cows, we demonstrate for the first time that the embryo attachment modulates the gene expression in PWBC to protect itself. While also acknowledging the possibility that there may be other roles of these genes that have yet to be discovered. This opens an unexplored area of feto-maternal microchimerism and immune maternal response in domestic animals.

5 | CONCLUSIONS

Our study showed that most of the differentially expressed genes in PWBC due to pregnancy are related to immune tolerance, cell chemotaxis, blood coagulation, angiogenesis, inflammatory response, cell adhesion and cytokine secretion. These findings provide a basis for further research on the interaction between embryo attachment and immune cell response in ruminants. Furthermore, our study suggests that pregnancy and ectoparasites could trigger poorly described genes in PWBC cows. Finally, the gene IFI44 was identified as a potential maternal blood biomarker for early pregnancy in cows.

This unique study compares two groups of animals for the first time: one group that maintained their pregnancy and another that experienced embryo loss after embryo transfer. Further research is required to improve the limitations of our approach, both at the technical and experimental levels. At the technical level, correlating blood samples with pregnancy status during and pre-embryo attachment in different cattle breeds. At the experimental level, future studies should focus on measuring the expression level of these genes before embryo transfer. These additional studies will help to

validate the current findings and provide recommendations for enhancing fertility, improving animal welfare and eventually increasing farmer profits.

AUTHOR CONTRIBUTIONS

J.A. De los Santos designed and performed the experiment and wrote the manuscript. F. Peñagaricano designed the experiment, analysed the data. J.P.N. Andrade collected the samples. L. R. Cangiano and A. Iriarte helped with data interpretation and edited the manuscript. J.J. Parrish supervised the research and edited the final version of the manuscript.

ACKNOWLEDGEMENTS

This work was supported by Agencia Nacional de Investigación e Innovación (ANII) Uruguay, under the project HPI_X_2018_1_147272.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest and financial, personal or other relationships with other people or organizations.

DATA AVAILABILITY STATEMENT

All raw sequencing files and raw read counts produced in this work are deposited in the National Center for Biotechnology Information, Gene Expression Omnibus (GEO) database number GSE210665.

ORCID

Jorge A. De los Santos https://orcid.org/0000-0002-6659-4732
João Paulo N. Andrade https://orcid.org/0000-0003-2447-3710
L. R. Cangiano https://orcid.org/0000-0003-3552-5520
Andres Iriarte https://orcid.org/0000-0002-5862-0152
Francisco Peñagaricano https://orcid.org/0000-0001-6661-3991
John J. Parrish https://orcid.org/0000-0002-4085-556X

REFERENCES

Anders, S., Pyl, P. T., & Huber, W. (2015). HTSeq—A Python framework to work with high-throughput sequencing data. *Bioinformatics*, *31*(2), 166–169. https://doi.org/10.1093/bioinformatics/btu638

Barclay, A. N. (1999). Ig-like domains: Evolution from simple interaction molecules to sophisticated antigen recognition. *Proceedings of the National Academy of Sciences*, *96*(26), 14672–14674. https://doi.org/10.1073/pnas.96.26.14672

Busse, D. C., Habgood-Coote, D., Clare, S., Brandt, C., Bassano, I., Kaforou, M., Herberg, J., Levin, M., Eléouët, J.-F., Kellam, P., & Tregoning, J. S. (2020). Interferon-induced protein 44 and interferon-induced protein 44-like restrict replication of respiratory syncytial virus. *Journal of Virology*, *94*(18), e00297–e00220. https://doi.org/10.1128/JVI.00297-20

Chomczynski, P., & Sacchi, N. (2006). The single-step method of RNA isolation by acid guanidinium thiocyanate–phenol–chloroform extraction: Twenty-something years on. *Nature Protocols*, *1*(2), 581–585. https://doi.org/10.1038/nprot.2006.83

Consentini, C. E., Wiltbank, M. C., & Sartori, R. (2021). Factors that optimize reproductive efficiency in dairy herds with an emphasis on timed artificial insemination programs. *Animals: An Open Access Journal from MDPI*, *11*(2), 301. https://doi.org/10.3390/ani11020301

Croy, B. A. (2014). Reproductive immunology issue one: Cellular and molecular biology. *Cellular and Molecular Immunology*, *11*(5), 405–406. https://doi.org/10.1038/cmi.2014.64

Dean, L. (2005). Blood and the cells it contains. In: *Blood groups and red cell antigens* [Internet]. National Center for Biotechnology Information (US). https://www.ncbi.nlm.nih.gov/books/NBK2263/

Dickinson, S. E., & Biase, F. H. (2018). Transcriptome data of peripheral white blood cells from beef heifers collected at the time of artificial insemination. *Data in Brief*, *18*, 706–709. https://doi.org/10.1016/j.dib.2018.03.062

Diskin, M. G., Waters, S. M., Parr, M. H., & Kenny, D. A. (2016). Pregnancy losses in cattle: Potential for improvement. *Reproduction, Fertility, and Development*, *28*(1–2), 83–93. https://doi.org/10.1071/RD15366

Ealy, A. D., & Yang, Q. E. (2009). REVIEW ARTICLE: Control of interferon-tau expression during early pregnancy in ruminants. *American Journal of Reproductive Immunology*, *61*(2), 95–106. https://doi.org/10.1111/j.1600-0897.2008.00673.x

Gowhari, A., Jadidi-Niaragh, F., Mohammadi, H., Ebrahimzadeh, F., Oveisee, M., Jahanara, A., & Gholizadeh Navashenaq, J. (2022). The role of atypical chemokine receptor D6 (ACKR2) in physiological and pathological conditions; friend, foe, or both? *Frontiers in Immunology*, *13*, 861931. https://doi.org/10.3389/fimmu.2022.861931

Gustafsson, C., Mjösberg, J., Matussek, A., Geffers, R., Matthiesen, L., Berg, G., Sharma, S., Buer, J., & Ernerudh, J. (2008). Gene expression profiling of human decidual macrophages: Evidence for immunosuppressive phenotype. *PLoS One*, *3*(4), e2078. https://doi.org/10.1371/journal.pone.0002078

Hayashi, K.-G., Hosoe, M., Kizaki, K., Fujii, S., Kanahara, H., Takahashi, T., & Sakumoto, R. (2017). Differential gene expression profiling of endometrium during the mid-luteal phase of the estrous cycle between a repeat breeder (RB) and non-RB cows. *Reproductive Biology and Endocrinology*, *15*, 20. https://doi.org/10.1186/s12958-017-0237-6

Hughes, C. H. K., Mezera, M. A., Wiltbank, M. C., & Pate, J. L. (2022). Insights from two independent transcriptomic studies of the bovine corpus luteum during pregnancy. *Journal of Animal Science*, *100*(7), skac115. https://doi.org/10.1093/jas/skac115

Kazemi, N. Y., Fedyshyn, B., Yelsa, I., Fedyshyn, Y., Ruano, R., Markovic, S. N., Chakraborty, R., & Enninga, E. A. L. (2021). Increased cell-free fetal DNA release after apoptosis and sterile inflammation in human trophoblast cells. *American Journal of Reproductive Immunology*, *86*(5), e13483. https://doi.org/10.1111/aji.13483

Kim, D., Langmead, B., & Salzberg, S. L. (2015). HISAT: A fast spliced aligner with low memory requirements. *Nature Methods*, *12*(4), 357–360. https://doi.org/10.1038/nmeth.3317

Kinder, J. M., Stelzer, I. A., Arck, P. C., & Way, S. S. (2017). Immunological implications of pregnancy-induced microchimerism. *Nature Reviews Immunology*, *17*(8), 483–494. https://doi.org/10.1038/nri.2017.38

King, G. J. (Ed.). (1993). *Reproduction in domesticated animals. World animal science* (1st ed.). Elsevier. https://www.elsevier.com/books/reproduction-in-domesticated-animals/king/978-0-444-89530-1

Kuniyoshi, N., Imai, H., Kiso, Y., Nagaoka, O., & Kusakabe, K. T. (2021). Biological potentials for a family of disintegrin and metalloproteinase (ADAMDEC)-1 in mouse normal pregnancy. *The Journal of Veterinary Medical Science*, *83*(3), 512–521. https://doi.org/10.1292/jvms.20-0570

Moffett, A., Chazara, O., & Colucci, F. (2017). Maternal Allo-recognition of the fetus. *Fertility and Sterility*, *107*(6), 1269–1272. https://doi.org/10.1016/j.fertnstert.2017.05.001

Moorey, S. E., Walker, B. N., Elmore, M. F., Elmore, J. B., Rodning, S. P., & Biase, F. H. (2020). Rewiring of gene expression in circulating white blood cells is associated with pregnancy outcome in heifers (*Bos taurus*). *Scientific Reports*, *10*(1), 16786. https://doi.org/10.1038/s41598-020-73694-w

Nelson, J. L. (2003). Microchimerism in human health and disease. *Autoimmunity*, *36*(1), 5–9. https://doi.org/10.1080/0891693031000067304

Nicholson, L. B. (2016). The immune system. *Essays in Biochemistry*, *60*(3), 275–301. https://doi.org/10.1042/EBC20160017

Ott, T. L. (2019). Symposium review: Immunological detection of the bovine conceptus during early pregnancy*. *Journal of Dairy Science*, *102*(4), 3766–3777. https://doi.org/10.3168/jds.2018-15668

Panda, B. S. K., Mohapatra, S. K., Chaudhary, D., Alhussien, M. N., Kapila, R., & Dang, A. K. (2020). Proteomics and transcriptomics study reveals the utility of ISGs as novel molecules for early pregnancy diagnosis in dairy cows. *Journal of Reproductive Immunology*, *140*, 103148. https://doi.org/10.1016/j.jri.2020.103148

Pugliesi, G., Miagawa, B. T., Paiva, Y. N., França, M. R., Silva, L. A., & Binelli, M. (2014). Conceptus-induced changes in the gene expression of blood immune cells and the ultrasound-accessed luteal function in beef cattle: How early can we detect pregnancy?1. *Biology of Reproduction*, *91*(4), 95. https://doi.org/10.1095/biolreprod.114.121525

Robinson, M. D., McCarthy, D. J., & Smyth, G. K. (2010). edgeR: A Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics (Oxford, England)*, *26*(1), 139–140. https://doi.org/10.1093/bioinformatics/btp616

Rocha, C. C., da Silva Andrade, S. C., de Melo, G. D., Motta, I. G., Coutinho, L. L., Gonella-Diaza, A. M., Binelli, M., & Pugliesi, G. (2020). Early pregnancy-induced transcripts in peripheral blood immune cells in *Bos indicus* heifers. *Scientific Reports*, *10*(1), 13733. https://doi.org/10.1038/s41598-020-70616-8

Shima, T., Sasaki, Y., Itoh, M., Nakashima, A., Ishii, N., Sugamura, K., & Saito, S. (2010). Regulatory T cells are necessary for implantation and maintenance of early pregnancy but not late pregnancy in allogeneic mice. *Journal of Reproductive Immunology*, *85*(2), 121–129. https://doi.org/10.1016/j.jri.2010.02.006

Shirasuna, K., Matsumoto, H., Kobayashi, E., Nitta, A., Haneda, S., Matsui, M., Kawashima, C., Kida, K., Shimizu, T., & Miyamoto, A. (2012). Upregulation of interferon-stimulated genes and Interleukin-10 in peripheral blood immune cells during early pregnancy in dairy cows. *Journal of Reproduction and Development*, *58*(1), 84–90. https://doi.org/10.1262/jrd.11-094K

Soumya, N. P., Das, D. N., Jeyakumar, S., Mondal, S., Mor, A., & Mundhe, U. T. (2017). Differential expression of ISG 15 mRNA in peripheral blood mononuclear cells of nulliparous and multiparous pregnant versus non-pregnant *Bos indicus* cattle. *Reproduction in Domestic Animals = Zuchthygiene*, *52*(1), 97–106. https://doi.org/10.1111/rda.12815

Spencer, T. E., Forde, N., & Lonergan, P. (2016). The role of progesterone and conceptus-derived factors in uterine biology during early pregnancy in ruminants. *Journal of Dairy Science*, *99*(7), 5941–5950. https://doi.org/10.3168/jds.2015-10070

Spencer, T. E., Johnson, G. A., Bazer, F. W., & Burghardt, R. C. (2004). Implantation mechanisms: Insights from the sheep. *Reproduction (Cambridge, England)*, *128*(6), 657–668. https://doi.org/10.1530/rep.1.00398

Su, Y., Li, Q., Zhang, Q., Li, Z., Yao, X., Guo, Y., Xiao, L., Wang, X., & Ni, H. (2022). Exosomes derived from placental trophoblast cells regulate endometrial epithelial receptivity in dairy cows during pregnancy. *The Journal of Reproduction and Development*, *68*(1), 21–29. https://doi.org/10.1262/jrd.2021-077

Valdivieso, E. F., Ross, E. M., Raza, A., Naseem, M. N., Kamran, M., Hayes, B. J., Jonsson, N. N., James, P., & Tabor, A. E. (2022). Transcriptional changes in the peripheral blood leukocytes from Brangus cattle before and after tick challenge with *Rhipicephalus australis*. *BMC Genomics*, *23*, 454. https://doi.org/10.1186/s12864-022-08686-3

Wang, L., Liu, L., Qian, W., & Zheng, Z. (2022). CD5L secreted by macrophage on atherosclerosis progression based on lipid metabolism induced inflammatory damage. *Archivum Immunologiae et Therapiae Experimentalis*, *70*(1), 10. https://doi.org/10.1007/s00005-022-00643-y

Wiltbank, M. C., Baez, G. M., Garcia-Guerra, A., Toledo, M. Z., Monteiro, P. L. J., Melo, L. F., Ochoa, J. C., Santos, J. E. P., & Sartori, R. (2016). Pivotal periods for pregnancy loss during the first trimester of gestation in lactating dairy cows. *Theriogenology*, *86*(1), 239–253. https://doi.org/10.1016/j.theriogenology.2016.04.037

Wooding, F. B. P. (1992). The synepitheliochorial placenta of ruminants: Binucleate cell fusions and hormone production. *Placenta*, *13*(2), 101–113. https://doi.org/10.1016/0143-4004(92)90025-O

Yang, H., Qiu, L., Chen, G., Ye, Z., Lü, C., & Lin, Q. (2008). Proportional change of CD4+CD25+ regulatory T cells in decidua and peripheral blood in unexplained recurrent spontaneous abortion patients. *Fertility and Sterility*, *89*(3), 656–661. https://doi.org/10.1016/j.fertnstert.2007.03.037

Zheng, C.-L., Lu, Q., Zhang, N., Jing, P.-Y., Zhang, J.-P., Wang, W.-P., & Li, G.-Z. (2021). Comprehensive analysis of the immune and prognostic implication of MMP14 in lung cancer. *Disease Markers*, *2021*, 5917506. https://doi.org/10.1155/2021/5917506

How to cite this article: De los Santos, J. A., Andrade, J. P. N., Cangiano, L. R., Iriarte, A., Peñagaricano, F., & Parrish, J. J. (2023). Transcriptomic analysis reveals gene expression changes in peripheral white blood cells of cows after embryo transfer: Implications for pregnancy tolerance. *Reproduction in Domestic Animals*, *58*, 946–954. https://doi.org/10.1111/rda.14371