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Comparative Medicine Section

Using Comparative Transcriptomics and Histology to Identify Significant Differentially Expressed Genes Associated with Retained Placenta in Humans and Rhesus Macaques (*Macaca mulatta*)

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Retained placenta is an important reproductive complication that affects humans and nonhuman primates (NHPs). Accurate prediction of retained placenta in both species is a current challenge because the etiology is unknown, biomarkers are inadequate, and data are heterogeneous. Through a comparative approach, this study identifies 34 significantly differentially expressed genes associated with retained placenta shared between humans and NHPs. Pathway enrichment revealed upregulation in innate and adaptive immunity in addition to pathways related to hemostasis. Retained placentas in NHPs had higher histologic evidence of inflammation as compared with human samples. These cross-species transcriptional results can serve as an initial step to guide NHP refinement as a model system and inform retained placenta biomarker discovery in both humans and NHPs.

Abbreviations and Acronyms: BCR, B cell receptor; DEG, differentially expressed gene; FFPE, formalin-fixed, paraffin-embedded; H&E, hematoxylin and eosin; NHP, nonhuman primate; PLAUR, urokinase-type plasminogen activator receptor; PPH, postpartum hemorrhage; RNAseq, RNA sequencing.

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Introduction

Retained placenta significantly affects humans and NHPs. In humans, it is the second leading cause of postpartum hemorrhage (PPH) with up to 10% case mortality rates in resource-poor areas.[1,2] In certain NHP breeding colonies (baboons, cynomolgus macaques, and rhesus macaques), it is the most common reproductive complication requiring clinical admission.[3] Accurately predicting those at risk of retained placenta is a current challenge in human and veterinary medicine. Retained placenta is a retrospective diagnosis made only after delivery of the neonate. In humans, failure of the placenta to deliver within 30 to 60 min of neonatal delivery defines retained placenta.[4–8] In NHPs, placentas are typically delivered within 15 min after the neonate. However, night deliveries, heterogeneous clinical presentations, and placentophagy can complicate timely diagnosis.[3,9,10] Accurate prediction of retained placenta is currently needed to improve NHP reproductive management.[11] It may also help reduce morbidity and mortality associated with retained placenta in both humans and NHPs.[3,10–12]

Predicting retained placenta in humans and NHPs is challenging for 3 main reasons: data heterogeneity, inadequate biomarkers, and unknown etiology. Risk factor data vary widely because of study design methodology and quality, which limits identifying disease-specific factors via meta-analysis.[1,13] Thus, risk-scoring mechanisms have poor predictive capabilities and are not generalizable.[14,15] Inadequate biomarkers negate the ability to identify at-risk individuals before delivery.[4,16–20] Finally, poor pathophysiologic understanding affects the types of data collected and may omit mechanisms that are unknown a priori.[1,3,13,21–23] The proposed mechanisms, including poor contractility, placental hypoperfusion, and abnormal implantation, are currently broad and lack specificity.[1,2,13,22,24–28] Elucidating the etiology of retained placenta is crucial, as it can guide both biomarker discovery and reduce data heterogeneity.

High-throughput techniques such as RNA sequencing (RNAseq) efficiently provide mechanistic knowledge to identify biologic pathways and molecular mechanisms.[29–35] In dairy cattle with a retained placenta, RNAseq shows upregulation in immune modulation pathways (for example, mature B cell differentiation and positive lymphocyte migration) and downregulation in lipid metabolic pathways.[36] Proteins that function in local cell adhesion, cytoskeletal organization, and oxidative stress have also been identified in bovine-retained placentas with predictive markers linked to immunosuppression and inflammatory

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signals.[37,38] Ruminant placentation is different from humans, and thus translatability to human pathophysiology remains unclear. RNAseq of human cesarean hysterectomy samples has added to pathophysiologic understanding of placenta accreta spectrum (a retained placenta subtype) from an 'invasive trophoblast' to a decidual 'loss of boundary limits.'[39] To our knowledge, RNAseq has not been conducted in NHP retained placentas or in human non-accreta retained placentas. Comparative analysis of placental marker genes in humans and rhesus macaques (*Macaca mulatta*) using RNAseq shows similar expression patterns in most of these genes, although species-specific differences exist for certain obstetric conditions.[40]

Comparatively analyzing human and NHP retained placentas can efficiently advance pathophysiologic understanding of retained placenta. Aside from consistencies in placentation, NHPs and humans have many similarities in retained placenta pathogenesis, including incidence (0.5% to 4.8% in humans, 1% to 3.3% in NHPs), risk factors (stillbirth, cesarean, and premature deliveries), recurrence rates (13% to 25% in humans, 10% to 22% in NHPs), and spontaneously occurring disease.[1,3,10,11,13,41–44] We hypothesized that conducting a cross-species transcriptomic analysis in disease and control placentas will reveal mutually helpful mechanistic insights for human and veterinary medicine. In this study, we used bulk RNAseq to identify shared significant differentially expressed genes (DEGs) associated with retained placenta in humans and rhesus macaques (*Macaca mulatta)*. We performed a comparative histologic analysis of all samples using hematoxylin and eosin (H&E) staining to identify disease or species-specific patterns. Our primary goal was to identify disease-specific transcriptional differences associated with a retained placenta shared between human and NHP placentas.

Materials and Methods

Sample collection and clinical features. ***Human samples.*** De-identified formalin-fixed, paraffin-embedded (FFPE) human placental samples were selected from the Penn State Health Hershey Medical Center Pathology Department archives for analysis. All samples were obtained from singleton, live-birth vaginal deliveries submitted to pathology as part of routine obstetric care. All samples were taken from the same pathologic block in the central region of the placenta. Retained placenta samples were defined as placentas that had not separated by 30 min or more after neonatal delivery, underwent manual removal with or without additional curettage, and without histologic evidence of accreta from routine clinical pathologic assessment as confirmed by the pathology report. Limited clinical information, including additional obstetric diagnoses, the presence or absence of placental infection (for example, chorioamnionitis or endometritis), immediate PPH, placental delivery type, and gestational age, was also obtained from archived data. Eleven cases met retained placenta criteria and were included. Control cases were defined as placentas that had separated and delivered spontaneously without intervention within 30 min of neonatal delivery. An attempt was made to exclude samples with a pregnancy complication (for example, preeclampsia, fetal growth restriction, preterm birth, gestational diabetes) to control for comorbidities. Only one sample met the control criteria, as routine submission of normal placentas to pathology was not a standard clinical practice at the study institution.

NHP samples. FFPE rhesus macaque placental samples were provided by the Emory National Primate Research Center via their diagnostic necropsy archives. Due to the limited clinical information accompanying the archived samples, some data were missing. However, the available clinical information from the archives included a brief synopsis of the institutional pathology report for each sample. This report detailed the age of the dam at the time of necropsy, placental infection, PPH, and histologic findings. The diagnosis of retained placenta was made clinically (visual evidence of undelivered placenta, physical or ultrasound findings demonstrating retained placenta or placental tissue) and was confirmed based on pathologic evidence with some cases having associated sections of uterus if collected at necropsy. Control samples were also histologically evaluated. Three cases met the criteria for retained placenta, while 2 met the control criteria.

This study met the criteria for exempt research by the study institution's Institutional Review Board (study no. 00018948). Collection and provision of tissues through the Emory National Primate Research Centers Biospecimen Distribution Program were approved by the Emory University IACUC in accordance with the Animal Welfare Act and the U.S. Department of Health and Human Services *Guide for Care and Use of Laboratory Animals*.

RNA extraction, library generation, sequencing, and statistical analysis. RNA from FFPE tissue (5 to 10 μm) was isolated by using Quick-RNA FFPE kit from Zymo Research (Irvine, CA), following the manufacturer's instructions. The tissue samples were deparaffinized, digested by proteinase K, and de-crosslinked before the RNA purification using the membrane spin columns and ethanol, respectively. The RNA quality and concentration were determined using an Agilent Bioanalyzer 2100 instrument and RNA 6000 Pico kit (Agilent Technologies, Santa Clara, CA). This isolated RNA was stored at −80 °C until further use.

RNAseq libraries were generated using KAPA RNA HyperPrep kits with RiboErase (HMR) (Roche Sequencing Solutions, Inc., Pleasanton, CA), which targets and depletes rRNA using DNA probes, followed by treatment with RNase H and DNase to remove rRNA duplexed to DNA and original DNA probes, respectively. The unique index sequences (NEXTflex unique dual index barcodes; Bioo Scientific, Austin, TX) were incorporated in the adaptors for multiplexed high-throughput sequencing. The final product was assessed for size distribution and concentration using a Bioanalyzer high sensitivity DNA kit (Agilent Technologies, Santa Clara, CA). The libraries were pooled and diluted to 3 nM using 10 mM Tris-HCl (pH 8.5) and then denatured using the Illumina protocol. The denatured libraries were loaded onto an S1 flow cell on an Illumina NovaSeq 6000 (Illumina, San Diego, CA) and run for 2 × 50 cycles according to the manufacturer's instructions. De-multiplexed and adapter-trimmed sequencing reads were generated using Illumina bcl2fastq (released version 2.20.0.422), allowing no mismatches in the index read. BBDuk was used to trim/filter low-quality sequences using "qtrim=lr trimq=10 maq=10" option. Next, alignment of the filtered reads to the human (GRCh38) or rhesus macaque (Mmul_10) reference genome was done using HISAT2 (version 2.1.0) applying –no-mixed and –no-discordant options.[45] Read counts were calculated using high-throughput sequencing by supplementing Ensembl gene annotation (GRCh38.78 for human and Mmul_10.108 for rhesus macaque).[46] The BiomaRt R package was used to match orthologous genes between humans and rhesus macaques, and both datasets were consolidated by extracting only common genes between humans and rhesus macaques.[47]

The DESeq2 R package was used to identify DEGs between disease and control tissues, focusing on the common genes shared between humans and rhesus macaques while accounting for batch effect. Genes were considered significant if they had an adjusted P value <0.1, calculated by the Benjamini–Hochberg method to control the false discovery rate.[48]

Pathway enrichment analysis. Enrichment analysis was conducted to identify pathways enriched in the entire retained placenta-associated gene set, all significant DEGs, significant DEGs separated by expression pattern (upregulated or downregulated), and in the raw RNAseq data. The Enrichr platform was used to perform first-pass pathway inquiry for the complete (119 genes) and DEG set (34 genes) analyses.[49–51] The Enrichr platform contains access to multiple databases (for example, Gene Ontology, ChEA, KEGG, OMIM), allowing for both breadth and depth during the initial exploratory phase of pathway analysis. As a second-pass pathway analysis, the Reactome Knowledgebase (https://reactome.org), was used to analyze our raw RNAseq dataset.[52,53] The Reactome Knowledgebase was also used to separately analyze significant DEGs (34 genes) by expression pattern (upregulated or downregulated).

Histopathological evaluation. All FFPE samples (human and NHP) were prepared for histologic evaluation. A 5-µm section was taken from each paraffin block and stained using routine H&E stain before being coverslipped and scanned at 20× brightfield using an Aperio AT2 slide scanner (Leica Biosystems, Deer Park, IL). Tissues present on the slide (for example, placenta, uterus), placental layers (for example, decidua, trophoblastic shell, syncytial villi, chorionic membrane), microscopic diagnoses, and a semiquantitative inflammation score (0 to 3) were recorded for all samples by a board-certified veterinary pathologist (HA) who was not affiliated with the primary location from which the NHP samples came. The histologic analysis conducted for this study was independently performed by HA but not fully blinded, as HA had access to NHP necropsy and pathology records if needed. For NHP cases that had multiple samples corresponding to the same case, each sample was assessed for tissue types present and given a total score. The highest inflammation score was assigned to each case and used to calculate species averages. An inflammatory score of 0 indicated that the tissue was within normal limits, while a score of 1 correlated to mild inflammation, 2 to moderate inflammation, and 3 to severe inflammation.

Results

Sample characteristics. Of all the control samples (n = 3), human and NHP, none had PPH or infection. The average gestational age for all human samples was 36.5 wk (range 31 to 39 wk), and all NHP samples except one were from full-term deliveries. Available case demographic data are reported in Table 1. In aggregate, all placental layers were present in human

Table 1. Case demographics and clinical archived information

No.	Group	Species	Age	Gestational age	Tissue collection time	Euthanasia/cause of death	Case pathologic finding
1	C	R	4 y 10 mo	Early third trimester	4 h postmortem	Euthanized due to emaciation, chronic diarrhea, weight loss, hindlimb atrophy	Pathology: normal gravid uterus
2	C	R	14 y	Full term	Unknown	Not applicable	Pathology: normal placenta
3	C	H	32 y	39 wk 2 d	At delivery	Not applicable	Spontaneous labor Pathology: normal placenta
4	RP	R	11 y	Full term	32 h postmortem	Euthanized secondary to concern for poor prognosis after retained placenta management Clinical examination: lethargic animal concerning for hypovolemic shock and septicemia	Umbilical cord protruding from vulva and attached to infant Ultrasound: complete retained placenta D&C attempted but complicated by full uterine prolapse Bacteriology: *Acinetobacter lwoffii*, normal uterine microflora Pathology: moderate multifocal erosive suppurative endometritis, normal placenta
5	RP	R	19 y 2 mo	Full term	4 h postmortem	Euthanized due to neurologic deficits Clinical examination: lethargy and dehydration secondary to endometritis	Uterus enlarged and firm Ultrasound: mixed echogenic mass within uterus suggestive of retained placental fragments D&C performed with retrieval of placental tissue Bacteriology: *Staphylococcus aureus* isolated from heart, blood, and uterine swab Pathology: moderate multifocal suppurative endometritis
6	RP	R	4 y 11 mo	Full term	8 h postmortem	Clinical examination: animal found deceased postdelivery secondary to hemorrhage	On necropsy partial retained placenta attached at fundus Bacteriology: uterine swab with *Staphylococcus warneri* and *Aerococcus viridans*, but tissue section without infectious process Pathology: marked hemorrhage dissecting into myometrium
7	RP	H	35 y	37 wk 2 d	At delivery	Not applicable	Pathology: umbilical venous congestion, increased perivillous fibrin, focal intraplacental hematoma
8	RP	H	39 y	39 wk 2 d	At delivery	Not applicable	Pathology: mild distal villous hypoplasia
9	RP	H	32 y	35 wk 0 d	At delivery	Not applicable	Pathology: increased perivillous fibrin

(*continued*)

Table 1. (Continued)

No.	Group	Species	Age	Gestational age	Tissue collection time	Euthanasia/cause of death	Case pathologic finding
10	RP	H	30 y	39 wk 1 d	At delivery	Not applicable	Pathology: increased syncytial knots, mild distal villous hypoplasia, amnion hyperplasia
11	RP	H	35 y	37 wk 3 d	At delivery	Not applicable	Pathology: disrupted maternal surface 30%, accessory lobe
12	RP	H	37 y	39 wk 5 d	At delivery	Not applicable	Pathology: normal placenta
13	RP	H	20 y	39 wk 3 d	At delivery	Not applicable	Pathology: increased perivillous fibrin and calcifications
14	RP	H	32 y	33 wk 4 d	At delivery	Not applicable	Pathology: disrupted maternal surface
15	RP	H	32 y	31 wk 5 d	At delivery	Not applicable	Pathology: funisitis, amnion hyperplasia
16	RP	H	26 y	32 wk 3 d	At delivery	Not applicable	Pathology: focal maternal fibrinoid decidual vasculopathy with atherosclerosis and watershed infarction, peripheral increased syncytial knots, hemorrhage
17	RP	H	31 y	38 wk 2 d	At delivery	Not applicable	Pathology: normal placenta

C, control; D&C, dilation and curettage; H, human; R, rhesus; RP, retained placenta.

and NHP samples but all layers were not present in each individual case. Uterine tissue was present in NHP samples only (Table 2). Of all the retained placenta cases (n = 14), 50% had PPH (n = 7) and 21.4% had infection (n = 3). Species differences in these clinical characteristics were noted: a larger proportion of PPH cases were human (85.7%, n = 6), and a larger portion of infection cases were NHP (66.67%, n = 2). Human retained placenta samples had microscopic findings often associated with retained placentas, including villous hypoplasia and increased syncytial knots.[54] None of these features was observed in the controls. Representative images of the H&E histology are shown in Figure 1.

Comparative histology reveals higher average inflammation scores in NHP samples without significant vasculopathy in either human or NHP samples. H&E staining of all samples (human and NHP) showed species-specific differences in inflammation scores in the retained placentas. All NHP retained placenta cases (n = 3) had neutrophilic inflammation with an average inflammation score of 2. No human retained placenta cases (n = 11) had inflammation. No control samples (human or NHP) had inflammation. No significant vasculopathy was seen in human or NHP slides (Figure 1).

Cross-species transcriptomic analysis identified retained placenta-associated genes shared between human and NHP placentas. Bulk RNAseq identified a total of 119 shared genes

Table 2. Independent comparative histologic evaluation of all cases

No.	Group	Species	PPH	Infection	Tissue type	Tissue Layers Present					Inflammation score (0–3)	Inflammation type
						M	D	TS	SV	CM		
1	C	R	No	No	U+P	X	X	X	X	X	0	n/a
2	C	R	No	No	P		X	X	X	X	0	n/a
3	C	H	No	No	P		X	X	X	X	0	n/a
4	RP	R	No	Yes	U+P	X	X	X	X	X	2[a]	Neutrophilic
5	RP	R	No	Yes	U+P	X	X	X	Necrosis		3[a]	Neutrophilic
6	RP	R	Yes	No[b]	U+P	X	X	X	X	X	1[a]	Neutrophilic
7	RP	H	Yes	No	P		X	X	X	X	0	n/a
8	RP	H	Yes	No	P		X	X	X	X	0	n/a
9	RP	H	No	No	P				X	X	0	n/a
10	RP	H	No	No	P		X	X	X	X	0	n/a
11	RP	H	Yes	No	P		X	X	X	X	0	n/a
12	RP	H	No	No	P		X		X	X	0	n/a
13	RP	H	Yes	No	P		X		X	X	0	n/a
14	RP	H	No	No	P		X	X	X		0	n/a
15	RP	H	No	No	P		X	X	X		0	n/a
16	RP	H	Yes	No	P		X	X	X	X	0	n/a
17	RP	H	Yes	Yes	P		X	X	X	X	0	n/a

C, control; CM, chorionic membrane; D, decidua; H, human; M, myometrium P, placenta; R, rhesus; RP, retained placenta; SV, syncytial villi; TS, trophoblastic shell; U, uterus. Infection information was provided from clinical documentation either from a routine pathology report for human cases or a necropsy report for NHPs.

[a]Cases with 2 samples each, highest inflammation score reported in above table: case 4 details (score of 2 for sample with M+D; score of 0 for sample with D+TS+SV+CM), case 5 details: (score of 3 for sample with M+D; score of 2 for sample with D+TS), case 6 details: (score of 0 and 1 for first and second samples, both samples with all layers present).

[b]Case without clinical documentation of infection but inflammation seen on H&E.

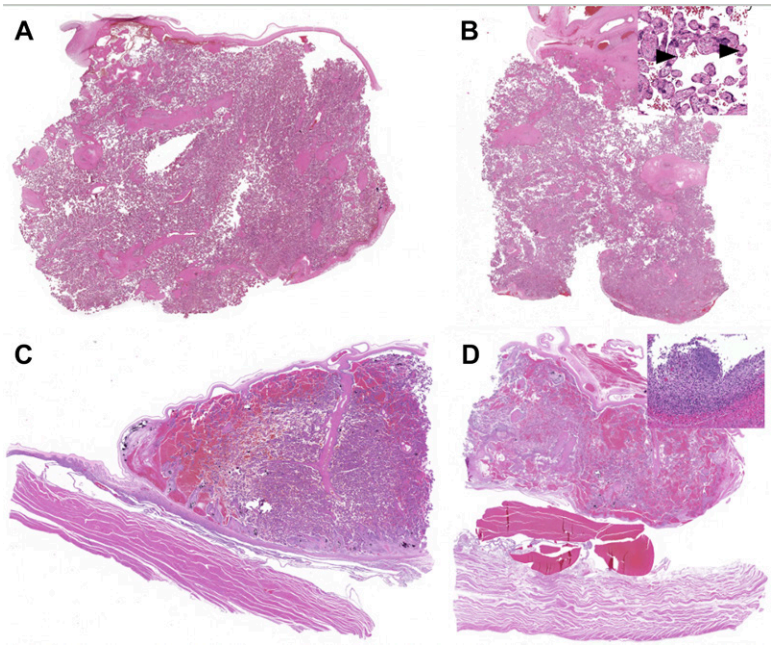

Figure 1. Representative H&E slides of controls and cases. (A) Third-trimester placenta from a 32-y-old woman. Placental layers include decidua, trophoblastic shell, villi, and chorionic membrane. No myometrium is present on the slide since it was an uncomplicated birth. 5× original magnification. (B) Retained placenta from a woman. 5× original magnification. Inset shows syncytial knots and villus hypoplasia, often observed in retained placentas. 200× original magnification. (C) Third-trimester control NHP placenta from a 4-y-old rhesus macaque, showing multiple intact layers, including the myometrium, decidua, trophoblastic shell, villi, and chorionic membrane. 6× original magnification. (D) Retained placenta from a NHP with areas of inflammation. 4× original magnification. Inset shows an area of suppurative inflammation with colonies of bacterial rods within the placental membranes. 200× original magnification. All images are of routine H&E stains.

between humans and NHPs that were associated with retained placenta. Due to the small sample size, RNAseq could not be clustered by clinical characteristics (infection or PPH). Of these 119 shared genes, 34 were significant DEGs in the disease state compared with the control. Of the 34 significant DEGs, 17 were upregulated and 17 were downregulated in disease compared with the control (Table 3).

Shared retained placenta-associated genes demonstrated biologically plausible pathway associations, especially in immune modulation. First-pass functional enrichment analysis using Enrichr of all 119 conserved retained placenta-associated genes demonstrated statistically significant pathways (adjusted *P* value <0.05) involved in immune modulation (for example, Fc receptor–mediated stimulatory signaling, positive regulation of leukocyte degranulation, cellular response to IL6, IL3-mediated signaling, inflammatory response), cell adhesion/extracellular matrix (regulation of cell adhesion, negative regulation of extracellular matrix organization, integrin-mediated signaling, regulation of homotypic cell–cell adhesion), positive regulation of cell differentiation, positive regulation of superoxide anion generation, extrinsic apoptotic signaling pathway, phagocytosis, and positive regulation of Notch signaling, when searching via the Gene Ontology biologic processes database within Enrichr.

Second-pass analysis using the Reactome Knowledgebase of the raw RNAseq dataset showed pathways significantly ($P \leq 0.05$) upregulated in the immune system (innate immune system, IL10/IL4/IL13 signaling, inflammasome), hemostasis, energy metabolism, and surfactant metabolism. Pathways that were significantly ($P \leq 0.05$) downregulated included generic transcription pathways (RNA polymerase II transcription, transcriptional regulation by E2F6, VENTX, and TP53), signal transduction (Notch and Rho GTPases), defective pyroptosis, cell cycle (that is, mitosis, cell cycle checkpoints), and cellular stress response (that is, senescence-associated secretory phenotype, regulation of HSF1-mediated heat shock response, response of EIF2AK4 to amino acid deficiency) (Figure 2).

Upregulated and downregulated significant DEGs showed unique pathway associations but demonstrated pathway overlap in the hemostasis category. First-pass Enrichr analysis of the 34 significant DEGs revealed associations with immune modulation (that is, immune response, B cell differentiation, complement signaling), spindle checkpoint regulation, positive regulation of vasculogenesis, fibroblast apoptosis, and endothelial cell differentiation; however, these did not demonstrate statistical significance via the Gene Ontology biologic process database ($P < 0.05$ [unadjusted *P* value]). Subsequent searches using additional available databases through Enricher, however, did demonstrate statistically significant pathway associations (adjusted *P* value <0.05). Cell types associated with enriched expression included placental myeloid and placental vascular endothelial cells (adjusted *P* value <0.05).

Second-pass pathway analysis using Reactome Knowledgebase of the 34 significant DEGs separated by expression pattern showed unique pathway associations but did overlap in the hemostasis pathway category. Pathways significantly ($P \leq 0.05$) associated with upregulated genes included adaptive immune signaling (B cell receptor [BCR] signaling, immunoregulatory interactions between a lymphoid and nonlymphoid cell), innate immune signaling (neutrophil degranulation, activation of C3 and C5), glutathione synthesis and recycling, diseases of metabolism, hemostasis (dissolution of fibrin clot), posttranslational protein modifications (*O*-linked glycosylation, attachment of glycosylphosphatidylinositol anchor to a urokinase-type plasminogen activator), signal regulatory protein family interactions, and integrin cell surface interactions (Figure 3). Pathways significantly ($P \leq 0.05$) associated with downregulated genes included hemostasis (Tie2), pyroptosis, apoptosis, bicarbonate

Table 3. Significant DEGs in retained compared with control placentas

Gene	Location	Type	Expression	Protein name	Molecular functions
ARHGEF26	3q25.2	Protein	Decreased	Rho guanine nucleotide exchange factor 26	Activates RhoG and plays a role in promoting micropinocytosis
CCDC68	18q21.2	Protein	Decreased	Coiled-coil domain containing protein 68	Involved in microtubule anchoring at centrosome and protein localization
CD22	19q13.12	Protein	Increased	B cell receptor CD22	Involved in B cell activation, negative regulation of B cell receptor signaling. and regulation of endocytosis
CFP	Xp11.23	Protein	Increased	Properdin	Positively regulates the alternative complement pathway, stabilizes C3- and C5-convertase enzyme, leading to membrane attack complex formation and cell lysis
DOK6	18q22.2	Protein	Decreased	Docking protein 6	Member of DOK family of intracellular adaptors that play a role in the RET signaling cascade
FPR3	19q13.41	Protein	Increased	*N*-formyl peptide receptor 3	Predicted to enable *N*-formyl peptide receptor activity, complement receptor activity, and positive regulation of cytosolic calcium concentration
GGT5	22q11.23	Protein	Increased	Glutathione hydrolase 5 proenzyme	Encoded enzyme converts leukotriene C4 to leukotriene D4
GRAMD1C	3q13.31	Protein	Decreased	Protein Aster-C	Predicted to enable cholesterol binding and transfer activity as well as cellular response to cholesterol
GRB14	2q24.3	Protein	Decreased	Growth factor receptor–bound protein 14	Encoded protein is a growth factor receptor–binding protein that interacts with insulin and insulin-like growth factor receptors likely with an inhibitory effect
HK3	5q35.2	Protein	Increased	Hexokinase-3	Hexokinases phosphorylate glucose to produce glucose-6-phosphate; hexokinase-3 may enhance myeloid cell survival by nonglycolytic functions
IER5L	9q34.11	Protein	Increased	Immediate early response gene 5–like protein	May modulate cell proliferation
ITGAD	16p11.2	Protein	Increased	Integrin α-D	Belongs to the β_2 integrin family of membrane glycoproteins encoding the α subunit of the cell surface heterodimer involved in activation and adhesion functions of leukocytes
LDB2	4p15.32	Protein	Decreased	LIM domain–binding protein 2	Functions as adapter molecules to allow assembly of transcriptional regulatory complexes
LFNG	7p22.3	Protein	Increased	β1,3-*N*-acetylglucosaminyltransferase lunatic fringe	Leads to elongation of *O*-linked fucose residues on Notch, altering Notch signaling
LRCH2	Xq23	Protein	Decreased	Leucine-rich repeat and calponin homology domain containing protein 2	Functions as a cytoskeletal scaffolding protein and has a domain that mediates interactions with actin filaments
LRRC36	16q22.1	Protein	Decreased	Leucine rich repeat–containing protein 36	Unknown
MUC5B	11p15.5	Protein	Increased	Mucin-5B	Encodes a member of the mucin family and is the major gel-forming mucin in mucus
MXRA5	Xp22.33	Protein	Decreased	Matrix-remodeling-associated protein 5	Encodes one of the matrix-remodeling-associated proteins and contains immunoglobulin-like C2-type domains related to perlecan
MYBPC1	12q23.2	Protein	Increased	Myosin-binding protein C, slow type	Plays an important role in muscle contraction by recruiting muscle-type creatine kinase to myosin filaments
NDC80	18p11.32	Protein	Decreased	Kinetochore protein NDC80 homolog	Encodes a component of the kinetochore complex and functions to organize and stabilize microtubule–kinetochore interactions
PALMD	1p21.2	Protein	Decreased	Palmdelphin	Predicted to be involved in regulation of cell shape and active in cytoplasm
PKDCC	2p21	Protein	Increased	Extracellular tyrosine protein kinase	Enables nonmembrane spanning protein tyrosine kinase activity and is located in the extracellular region

(*continued*)

Table 3. (Continued)

Gene	Location	Type	Expression	Protein name	Molecular functions
PLAUR	19q13.31	Protein	Increased	Urokinase plasminogen activator surface receptor	Encodes the receptor for urokinase plasminogen activator and likely influences processes related to cell surface plasminogen activation and localized degradation of the extracellular matrix
RELT	11q13.4	Protein	Increased	Receptor expressed in lymphoid tissue/TNF receptor superfamily member 19L	Encodes a member of the TNF receptor superfamily and may play a role in regulating immune response by stimulating T cell proliferation in the presence of CD3 signaling
SIGLEC5	19q13.41	Protein	Increased	Sialic acid binding immunoglobulin-like lectin 5	Encoded protein inhibits the activation of several immune cell types, and binding of GBS to this protein plays a role in GBS immune evasion
SIRPB1	20p13	Protein	Increased	Signal-regulatory protein β-1	Receptor-type transmembrane glycoproteins involved in the negative regulation of receptor tyrosine kinase–coupled signaling
SLC4A1	17q21.31	Protein	Decreased	Band 3 anion transport protein	Functions as a chloride/bicarbonate exchanger involved in carbon dioxide transport
SNORD114-7	14q32.31	snoRNA	Decreased	n/a	Unknown
TMEM100	17q22	Protein	Decreased	Transmembrane protein 100	Involved in BMP signaling pathway and may play a role in the development of inflammation
TMEM178A	2p22.1	Protein	Decreased	Transmembrane protein 178A	Predicted to be involved in regulation of cytosolic calcium ion concentration
TP63	3q28	Protein	Decreased	Tumor protein 63	Encodes a member of the p53 family of transcription factors
VAV1	19p13.3	Protein	Increased	Proto-oncogene VAV	Guanine nucleotide exchange factors for Rho family GTPases that activate pathways leading to actin cytoskeletal rearrangements and plays a role in T cell and B cell development and activation
VIPR2	7p36.3	Protein	Increased	Vasoactive intestinal polypeptide receptor 2	Encodes a receptor for vasoactive intestinal peptide, which is involved in smooth muscle relaxation, exocrine and endocrine secretion, and water and ion flux
ZGRF1	4q25	Protein	Decreased	Protein ZGRF1	May function in DNA binding through the GRF zinc finger domain

An alphabetical list of 34 significant DEGs shared between human and NHP samples is shown. Gene and protein information were obtained manually from the National Center for Biotechnology Information gene database and UniProt Knowledgebase database, respectively.

transporters, O_2 and CO_2 exchange in erythrocytes, activation of BH3-only proteins, transcriptional regulation by TP53, and RET signaling (Figure 4).

Discussion

Our comparative approach identified disease-specific transcriptional differences associated with retained placenta shared in humans and NHPs. Of these, 34 were significant DEGs. Consistent with the literature, we found pathways involved in immune modulation, oxidative stress, cell surface interactions, and metabolic dysfunction to name a few.[36–38] Our results provide an initial starting point for advanced mechanistic inquiry, further development of the NHP as a model system, and possibly for improved clinical management. Advancing translational research on placental retention is an important priority, but limitations in research models impede progress.[55–57] In vitro models have greatly advanced mechanistic knowledge, especially in implantation and early placentation.[40,58–66] These models, however, cannot simultaneously recapitulate the coordinated series of events and signals that occur between the retroplacental myometrium, trophoblast, decidua, and immune cells, at the maternal/fetal interface during placental separation.[58,59,62,66] Normal separation requires synchronization between retroplacental myometrial contractions and cessation of blood flow between the placenta and uterine wall.[2,26–28] At this time, in vivo systems are still necessary to analyze this interplay and identify clinically relevant insights. Retained placenta management is complex, including the need to balance pathophysiology risks (for example, PPH, infection) with management risks (timing and types of intervention).[1–8,12,24,67–75] NHPs are valuable animal models with financial and ethical considerations, but refining their use in retained placenta research efficiently addresses existing gaps in human and veterinary medicine.[76,77] One future direction for refinement would be to use gene editing techniques to systematically evaluate the function of the 34 DEGs and downstream effects. Such inquiries are not possible in human subjects. CRISPR/Cas9 technology has been used to model human disease phenotypes and trial pharmacologics in NHPs.[78,79] This technique has even demonstrated preclinical safety in editing proviral DNA in SIV-infected rhesus macaques.[80]

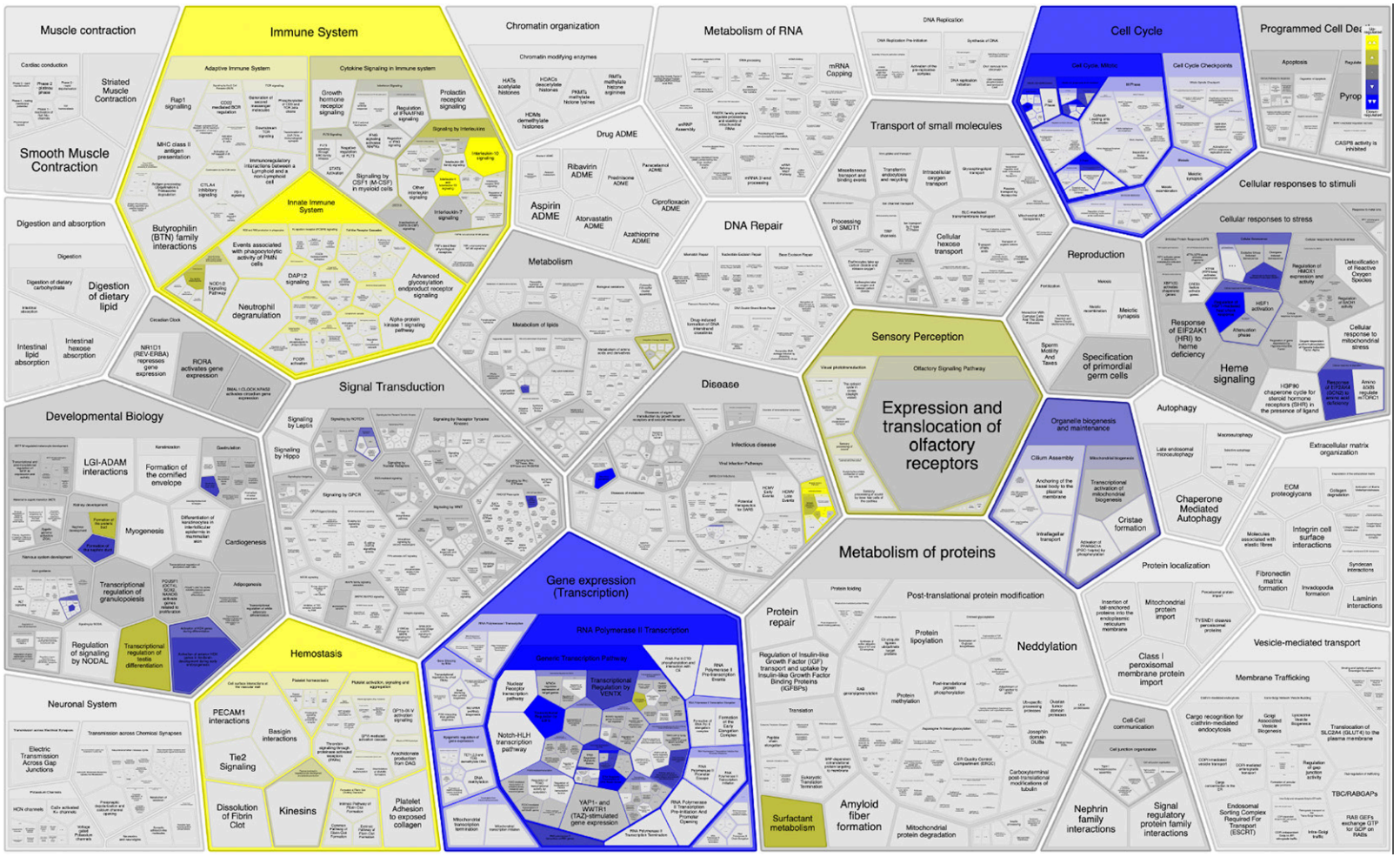

Figure 2. Voronoi visualization created using Reactome Knowledgebase[52,53] of overrepresented pathways for all shared retained placenta genes using the full RNA dataset. Yellow corresponds to pathways associated with upregulated expression, and blue corresponds to pathways associated with downregulated expression.

Vol 64, No 4
Journal of the American Association for Laboratory Animal Science
July 2025

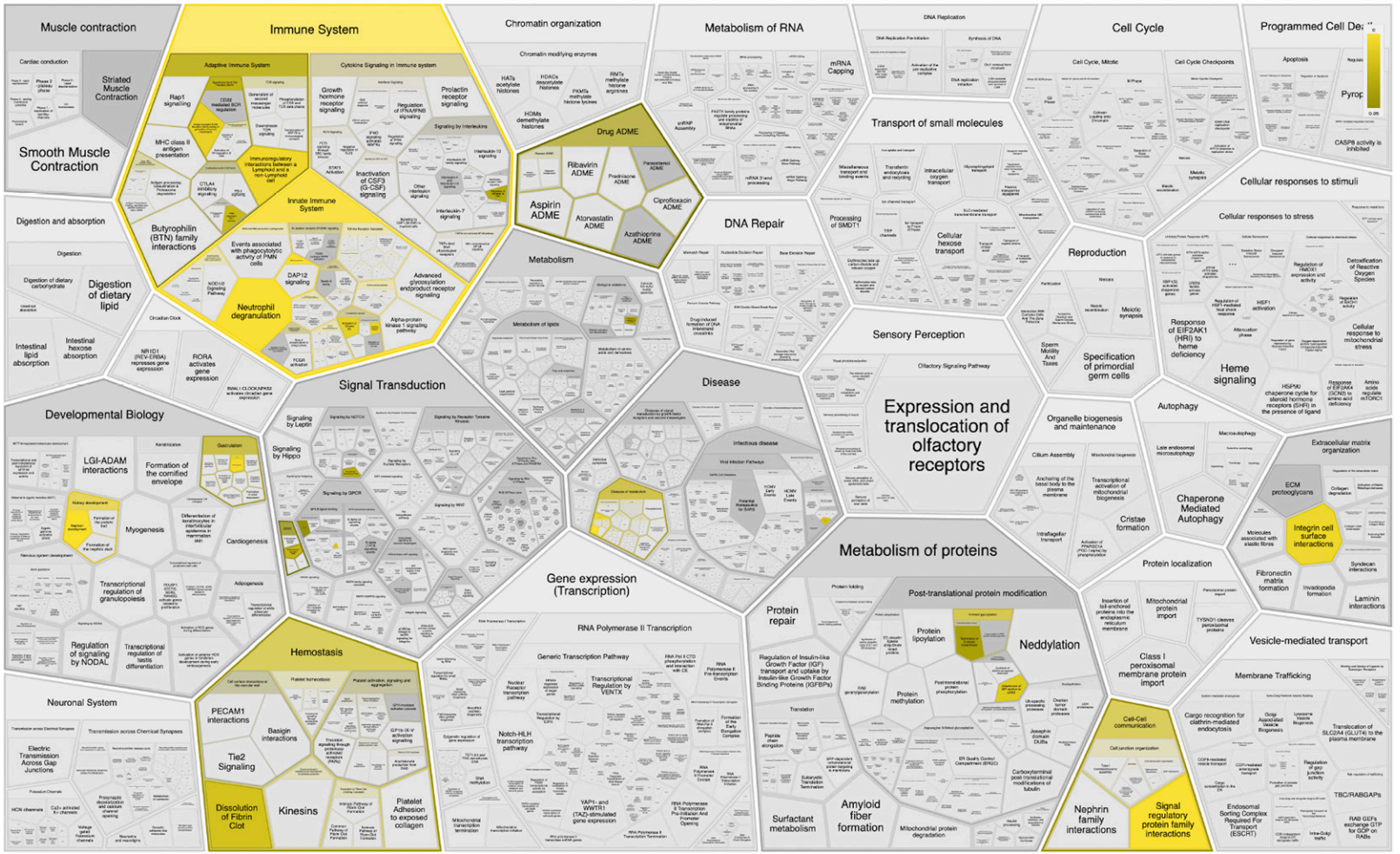

Figure 3. Voronoi visualization created using Reactome Knowledgebase[52,53] of overrepresented pathways for the 17 upregulated significant DEGs. Pathways in yellow are those that are overrepresented in gene set analysis. Gradation of yellow corresponds to *P* value of overlap significance. Note dissolution of fibrin clot pathway highlighted in yellow in the category of hemostasis (bottom left).

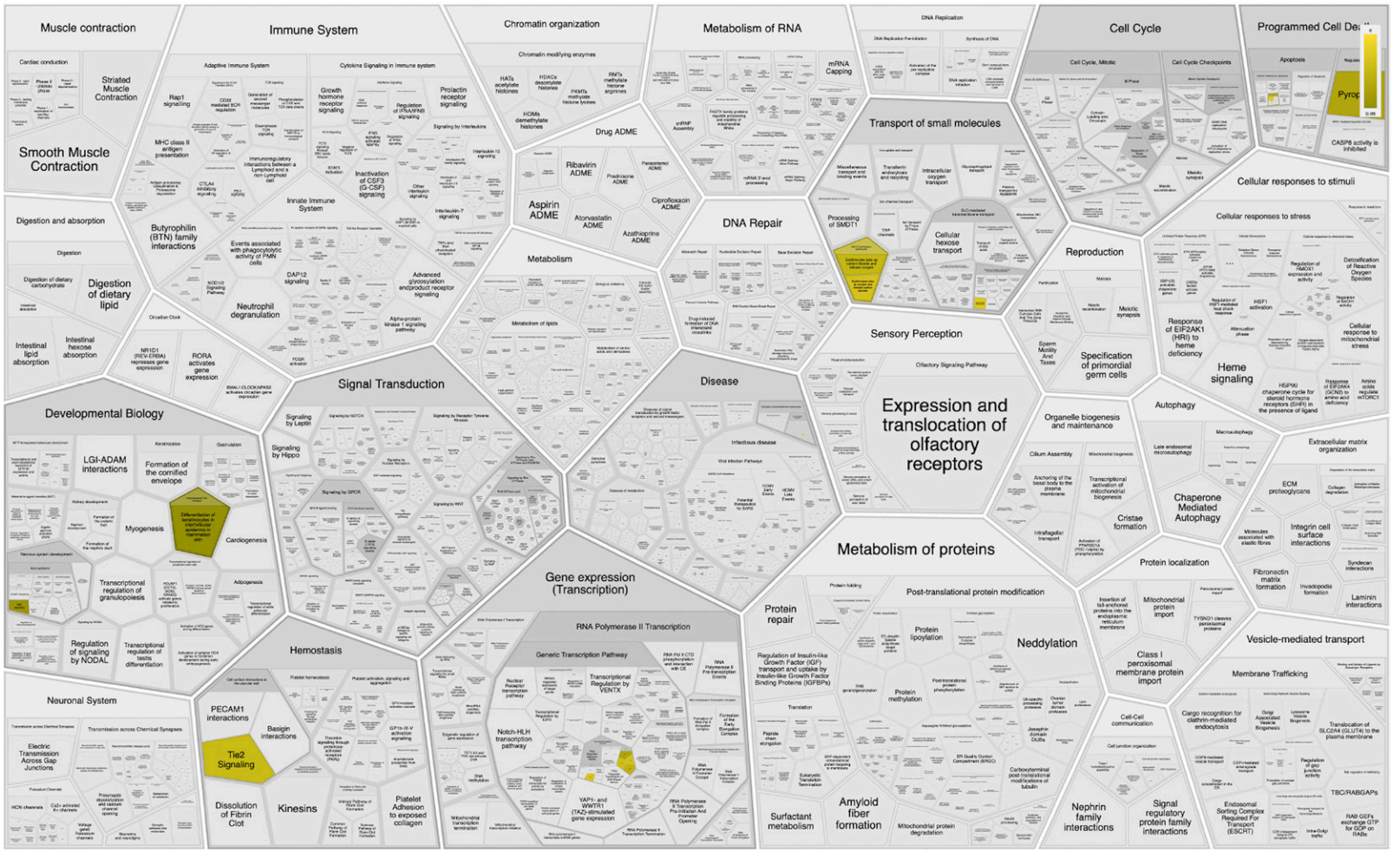

Figure 4. Voronoi visualization created using Reactome Knowledgebase[52,53] of overrepresented pathways for the 17 downregulated significant DEGs. Pathways in yellow are those that are overrepresented in gene set analysis. Gradation of yellow corresponds to *P* value of overlap significance. Note the Tie2 signaling pathway highlighted in yellow in the category of hemostasis (middle left).

Although there is still much more research to be done to understand the genes and molecular mechanisms that result in retained placenta, the future possibility of using CRISPR technology in an NHP model for biomarker discovery or disease prevention that benefits both humans and NHPs is a priority to pursue.

Independent histologic review in our study showed a higher average inflammation score in NHP (2) compared with human (0) samples (Table 2). Our human results are consistent with existing data showing no statistically significant differences in inflammation between cases and controls.[25] Our NHP results are more difficult to interpret given the limited literature on histologic analysis of retained placentas in NHPs. In one retrospective study of breeding colonies, 7 baboons had positive bacterial cultures, but no rhesus or cynomolgus macaques did. However, histologic evidence of inflammation was not reported.[3] Archived data on NHP fetal outcome indicated that cases 2, 4, 5, and 6 had live births. Case 1 was euthanized while pregnant due to worsening clinical status of the dam. All human cases had live births. Thus, it is unlikely that the differences in inflammation seen in our results were associated with stillbirth. Additional retained placenta risk factors such as uterine scarring/previous cesarean history were not possible to account for due to limitations in available NHP archived data. No human cases had a documented history of previous cesarean section. Data regarding preterm delivery were available for both NHP and human cases; however, preterm delivery did not seem to account for inflammation differences either. Only one NHP case was preterm (case 1 euthanized control); however, the inflammation score for this case was 0. Of the 4 human cases with preterm deliveries (3 spontaneous preterm labor, 1 with pre-labor rupture of membranes), all had inflammation scores of 0. Of the 2 other available clinical features that could be compared in NHPs and humans (infection and hemorrhage), it seems that PPH did not account for inflammation differences either since most PPH cases were in humans. The extent to which infection played a role to account for inflammation differences in our study is unclear. Of the NHP retained placenta cases, 66.67% ($n = 2/3$) were considered to have infection compared with 9.09% of the human retained placenta cases ($n = 1/11$). Infection in NHP samples could be ascertained from bacterial cultures, tissue sections, or both (Table 1). In contrast, bacterial cultures are not routinely obtained when infection is suspected or diagnosed in humans, and thus available information is limited to clinical documentation and/or placental pathology report. Of our 2 NHP retained placenta cases, both had documentation of suppurative endometritis on necropsy reports and culture information (case 4 with *Acinetobacter lwoffii* and case 5 with *Staphylococcus aureus*). Case 4, however, had archive data documenting a histologically normal placenta; no such documentation was available for case 5. NHP case 6 had documentation of a positive uterine swab with *Staphylococcus warneri* and *Aerococcus viridans* (Table 1), but cause of death in case 6 was suspected to result from hemorrhage and, thus, for the purposes of our study, we considered case 6 as 'no' for infection (Table 2). In the one human case considered as 'yes' for infection in our study (Table 2, case 17), diagnostic criteria for infection were met according to available case information. Interestingly, this case of infection was an endometritis after delivery of the placenta and not an infection during delivery (that is chorioamnionitis). As uterine tissue is not usually obtained in human deliveries (normal or retained), determining whether uterine infection was present or whether inflammation scores differed in myometrium compared with other tissue layers in the human cases was not possible. To evaluate for the tissue types that may have accounted for the increased inflammation seen in NHPs, inflammation scores for all NHP samples were analyzed. Of the 2 NHP controls, only 1 case had the presence of all tissue layers in the sample provided (case 1 euthanized while pregnant), and the other control had only placental tissue (case 2). All retained placenta NHP cases provided had 2 samples for each case (cases 4 to 6). In case 4, the inflammation score was 2 with the sample that had myometrium but 0 for the sample that did not have myometrium. In case 5, the inflammation score was 3 for the sample that had myometrium and 2 for the sample that did not have myometrium (necrosis of syncytial villi was seen). In case 6, both samples had all tissue layers present with scores of 0 and 1. Thus, whether infection accounted for the species-specific inflammation differences seen in our results is unclear because human samples did not have myometrial tissue or culture information. The higher inflammation in scores in NHPs may be due to the presence of uterine tissue in NHP samples. Future studies designed to histologically assess for inflammation differences in identified archived human samples that may contain myometrial tissue/fibers can help to determine whether location-specific differences in inflammation may also occur in humans, as they appear to be in our NHP samples. Furthering these analyses by using additional RNAseq techniques (for example, single cell, spatial) can help to identify cell-specific or location-specific transcriptional differences associated with retained placenta pathophysiology in humans and NHPs. Subsequent comparative approaches should consider obtaining uterine bacterial cultures routinely in retained placenta research protocols for both humans and NHPs so that consistency exists in available data between species. Additional studies with increased numbers of controls and retained placenta cases are needed for both humans and NHPs to determine the extent to which infection, inflammation, and associated clinical risk factors/features play a role in retained placenta pathogenesis.

Although we cannot determine the exact extent to which infection contributed to the upregulation of innate and adaptive immunity pathways seen in our analysis, infection should not be the only mechanism to consider. Innate and adaptive immunity upregulation could also suggest a possible role for immune modulation in placental separation. Immune cells are a key component of the pregnant uterus, and each cell type's functions during pregnancy have been an area of active research.[81] Innate immune cells function in host defense but also modulate adaptive immunity in maternal tolerance.[82] Innate pathways overexpressed in our data include neutrophil degranulation and activation of C3 and C5. Impaired neutrophil function is associated with retained placenta in dairy cattle,[83] and in humans, neutrophils can produce both pro- and anti-inflammatory cytokines.[84] Neutrophils that migrate to the myometrium produce proinflammatory signals that contribute to normal labor, but leukocyte degranulation in fetal membranes may lead to preterm labor or chorioamnionitis.[85–88] Trophoblasts can modulate innate immunity by regulating neutrophil function through the immunosuppressive and anti-inflammatory effects of vasoactive intestinal peptide (VIP).[88] Notably, the vasoactive intestinal peptide receptor 2 (VIPR2, DEG in this study) was found to be upregulated. Innate immune cells seem to mediate the labor process through proinflammatory signaling, although their exact function in placental separation is unclear.[89] Despite seeing greater histologic inflammation in our NHP samples compared with those from humans, the upregulation of neutrophil degranulation pathways was shared between the 2 species in our analysis. From our data, it is unclear what factors are associated with neutrophil degranulation upregulation or whether species-specific differences may exist. Additional

studies comparing the cytokine profile between retained and control placentas in humans and NHPs may provide additional insights.

Interestingly, upregulation of adaptive immunity seen in our data showed associations with anti-inflammatory signaling (BCR and IL10 signaling). Adaptive immunity is needed for maternal tolerance and is not significantly changed in normal labor but may lead to abnormal labor if dysregulated.[86,89] Adaptive immune pathways overexpressed in our results included BCR and IL10 signaling favoring an anti-inflammatory milieu (119-gene dataset). Our results showed upregulation of the gene CD22 (DEG in this study), which is a negative regulator of BCR signaling. In addition, CD22 may function to inhibit Toll-like receptor B cell activation to self-antigens.[90] If adaptive immunity does not significantly change during normal labor,[86] our data may suggest that upregulation in the adaptive pathways identified may be a mechanism that contributes to retained placenta pathogenesis. Consistent with our findings, earlier knowledge-driven biomarker molecular studies of placental separation revealed upregulation of an immune evasion protein, RCAS1/EBAG9 (receptor-binding cancer antigen expressed on SiSo cells), in retained placenta. RCAS1, expressed by extravillous cytotrophoblasts and uterine endometrial cells,[91,92] is a ligand for a receptor on peripheral lymphocytes inhibiting their growth and playing a role in maternal tolerance.[92,93] Our results did not specifically identify changes in RCAS1, but the alterations in adaptive immunity seen in our transcriptional data are consistent with those of earlier studies. We found strong associations in BCR signaling (OR86.0), β_2 integrin (DEG in this study) cell surface interactions (OR92.38), and TCR signaling (OR21.71). β_2 integrins and BCR signaling via CD22 function in IL10-mediated immune suppression,[90,94] and in vitro studies have demonstrated B cell upregulation of IL10 by trophoblasts.[95] These anti-inflammatory associations may play a larger role in human retained placenta pathogenesis given that all human samples had an inflammation score of 0. Areas of future exploration include immunohistochemical evaluation of our DEGs to further elucidate the interplay between innate and adaptive immune functions that contribute to retained placenta. Although more research is needed, our findings are consistent with and add to the limited knowledge that currently exists in this space.

Our transcriptional data also demonstrate the importance of hemostasis-related pathways with both upregulated and downregulated DEGs. Upregulation was enriched in coagulation (urokinase-type plasminogen activator–mediated signaling), and downregulation was enriched in angiogenesis (Tie2-mediated signaling). Pathways enriched in extracellular matrix disruption, coagulation cascade, and inflammation may relate to certain cohorts of patients who experience retained placenta.[96] Although we noted species-specific differences in the prevalence of PPH between humans and NHPs (85.7% compared with 14.3%, respectively), the upregulation in hemostasis pathways may be due to the variety of biologic functions in which they engage. The urokinase-type plasminogen activator receptor (PLAUR, a DEG in this study) is traditionally known to activate urokinase, but it also has nonproteolytic processes such as inflammation, tissue remodeling, and angiogenesis.[97] This system promotes trophoblast invasion, is modulated by natural killer cells,[98,99] and, in NHPs, also mediates trophoblast invasion and functions in angiogenesis.[100] In the placenta, urokinase plasminogen activator expression is seen in endothelial cells and macrophages with a role in perivillous fibrinoid deposit clearance.[101] Overexpression of PLAUR in cancer cells is associated with cell surface proteolysis and the ability to overcome basal membrane or extracellular matrix barriers.[102] Interestingly, it has been suggested that reduced urokinase receptor expression by villous trophoblasts at term may be a physiologic adaptation to reduce PPH risk.[103] The upregulation of PLAUR in our study may be due to the large presence of PPH in the retained placenta group (mostly in human samples). Due to small sample size, we were unable to cluster our transcriptional findings by PPH presence and cannot definitely state if PLAUR is associated with PPH in retained placenta pathogenesis. Downregulated genes associated with retained placenta were enriched in Tie2-mediated signaling. Tie2 pathways can function in trophoblast invasion, vascular development/stabilization/remodeling, and trophoblast differentiation in humans and NHPs.[104–106] In humans, Tie2/angiopoietin 1 and 2 are expressed in extravillous trophoblast and maternal vascular smooth muscle and endothelial cells.[107] Decreased Tie2 expression has been noted in placenta accreta specimens, and our data show that Tie2 may also be associated with the non-accreta retained placenta subtype.[105]

A strength of our study was that all placental samples were adequate for histologic evaluation and confirmed to be accreta absent, allowing for focus on the nonpathologically adherent retained placenta subtype. Another strength was the cross-species design of our study. Retained placenta naturally occurs in NHP breeding colonies, mimicking human disease incidence. Thus, we focused on identifying shared significant DEGs to use our results for future efforts to develop an animal model. Limitations of our work include a lack of generalizability to all retained placenta subtypes and an inadequate number of samples to identify species-specific or clinical characteristic pathway differences. Although we were able to achieve a minimum number of biologic replicates for controls ($n = 3$), the limited number of controls in our study may have missed additional shared DEGs associated with retained placenta in humans and NHPs. In addition, bulk RNAseq provides an average of transcriptional information and may not identify a tissue or cell-specific pathway contributing to disease. We were able to confirm that in aggregate, all placental layers were present despite individual cases missing some layers. In contrast, myometrial tissue was only present in NHPs, as samples were obtained from necropsy. Myometrium is not routinely obtained in human non-accreta cases during manual removal, and thus the lack of myometrium in our project was expected. We plan to address these limitations in future projects.

Conclusion

Retained placenta is a significant reproductive challenge in both humans and NHPs. Advancing pathophysiologic understanding of this condition is key to improving management in human and veterinary medicine. This study identified 34 significant DEGs associated with retained placenta shared between humans and NHPs. Functional enrichment emphasized the importance of innate and adaptive immunity, Tie2, and urokinase signaling pathways in the pathophysiology of retained placentas in humans and NHPs. Our data add valuable knowledge in refining NHPs for use as an animal model for retained placenta research in addition to highlighting the potential mechanisms that contribute to retained placenta pathogenesis.

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Conflict of Interest

The authors have no conflicts of interest to declare.

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References

1. Perlman NC, Carusi DA. Retained placenta after vaginal delivery: risk factors and management. *Int J Womens Health.* 2019;11:527–534.
2. Weeks AD. The retained placenta. *Afr Health Sci.* 2001;1(1):36–41.
3. Bauer C, Harrison T. Retrospective analysis of the incidence of retained placenta in 3 large colonies of NHP. *Comp Med.* 2016;66(2):143–149.
4. American College of Obstetricians and Gynecologists; Society for Maternal-Fetal Medicine. Obstetric care consensus no. 7: placenta accreta spectrum. *Obstet Gynecol.* 2018;132(6):e259–e275.
5. Committee on Practice Bulletins-Obstetrics. Practice bulletin no. 183: postpartum hemorrhage. *Obstet Gynecol.* 2017;130(4):e168–e186.
6. Mavrides E, Allard S, Chandraharan E, et al.; on behalf of the Royal College of Obstetricians and Gynaecologists. Prevention and management of postpartum haemorrhage. *BJOG.* 2016;124:e106–e149.
7. National Institute for Health and Care Excellence. 2022. Intrapartum care for healthy women and babies. [Cited October 2024]. Available at: https://www.ncbi.nlm.nih.gov/books/NBK555206/.
8. World Health Organization. 2009. WHO guidelines for the management of postpartum haemorrhage and retained placenta. [Cited October 2024]. Available at: https://iris.who.int/bitstream/handle/10665/44171/9789241598514_eng.pdf.
9. Hendrickx AG, Dukelow WR. Breeding. In: Bennett BT, Abee CR, Henrickson R, eds. *Nonhuman Primates in Biomedical Research. Biology and Management.* Academic Press; 1995:335–374.
10. Stockinger DE, Torrence AE, Hukkanen RR, Vogel KW, Hotchkiss CE, Ha JC. Risk factors for dystocia in pigtailed macaques (*Macaca nemestrina*). *Comp Med.* 2011;61(2):170–175.
11. Naiken S, Griffiths M, Edouard L, Padayatchy N. Factors influencing reproduction in captive-bred cynomolgus monkeys (*Macaca fascicularis*) from Mauritius. *Am J Primatol.* 2015;77(12):1290–1298.
12. Furukawa S, Fujisaki M, Maki Y, Oohashi M, Doi K, Sameshima H. Manual removal of placenta in women having unpredictable adherent placenta. *J Obstet Gynaecol Res.* 2019;45(1):141–147.
13. Favilli A, Tosto V, Ceccobelli M, et al. Risk factors for non-adherent retained placenta after vaginal delivery: a systematic review. *BMC Pregnancy Childbirth.* 2021;21(1):268.
14. Favilli A, Tosto V, Ceccobelli M, Bini V, Gerli S. Risk factors analysis and a scoring system proposal for the prediction of retained placenta after vaginal delivery. *Eur J Obstet Gynecol Reprod Biol.* 2018;228:180–185.
15. Wei X, Wei J, Wang S. Risk factors for postpartum hemorrhage in patients with retained placenta: building a predict model. *J Perinat Med.* 2022;50(5):601–607.
16. Desai N, Krantz D, Roman A, Fleischer A, Boulis S, Rochelson B. Elevated first trimester PAPP-A is associated with increased risk of placenta accreta. *Prenat Diagn.* 2014;34(2):159–162.
17. Ersoy AO, Oztas E, Ozler S, et al. Can venous ProBNP levels predict placenta accreta? *J Matern Fetal Neonatal Med.* 2016;29(24):4020–4024.
18. Kupferminc MJ, Tamura RK, Wigton TR, Glassenberg R, Socol ML. Placenta accreta is associated with elevated maternal serum alpha-fetoprotein. *Obstet Gynecol.* 1993;82(2):266–269.
19. Lyell DJ, Faucett AM, Baer RJ, et al. Maternal serum markers, characteristics and morbidly adherent placenta in women with previa. *J Perinatol.* 2015;35(8):570–574.
20. Zhou J, Li J, Yan P, et al. Maternal plasma levels of cell-free β-HCG mRNA as a prenatal diagnostic indicator of placenta accrete. *Placenta.* 2014;35(9):691–695.
21. Bronson E, Deem SL, Sanchez C, Murray S. Placental retention in a golden lion tamarin (*Leontopithecus rosalia*). *J Zoo Wildl Med.* 2005;36(4):716–718.
22. Greenbaum S, Wainstock T, Dukler D, Leron E, Erez O. Underlying mechanisms of retained placenta: evidence from a population based cohort study. *Eur J Obstet Gynecol Reprod Biol.* 2017;216:12–17.
23. Halbwax M, Mahamba CK, Ngalula A, André C. Placental retention in a bonobo (*Pan paniscus*). *J Med Primatol.* 2009;38(3):171–174.
24. Brandt ML. The mechanism and management of the third stage of labor. *Am J Obstet Gynecol.* 1933;25(5):662–667.
25. Endler M, Saltvedt S, Papadogiannakis N. Macroscopic and histological characteristics of retained placenta: a prospectively collected case-control study. *Placenta.* 2016;41:39–44.
26. Herman A, Weinraub Z, Bukovsky I, et al. Dynamic ultrasonographic imaging of the third stage of labor: new perspectives into third-stage mechanisms. *Am J Obstet Gynecol.* 1993;168(5):1496–1499.
27. Krapp M, Baschat AA, Hankeln M, Gembruch U. Gray scale and color doppler sonography in the third stage of labor for early detection of failed placental separation. *Ultrasound Obstet Gynecol.* 2000;15(2):138–142.
28. Mo A, Rogers MS. Sonographic examination of uteroplacental separation during the third stage of labor. *Ultrasound Obstet Gynecol.* 2008;31(4):427–431.
29. Camunas-Soler J, Gee EPS, Reddy M, et al. Predictive RNA profiles for early and very early spontaneous preterm birth. *Am J Obstet Gynecol.* 2022;227(1):72.e1–72.e16.
30. Chen Q, Shan D, Xie Y, et al. Single cell RNA sequencing research in maternal fetal interface. *Front Cell Dev Biol.* 2022;10:1079961.
31. Cox B, Leavey K, Nosi U, Wong F, Kingdom J. Placental transcriptome in development and pathology: expression, function, and methods of analysis. *Am J Obstet Gynecol.* 2015;213(4 Suppl):S138–S151.
32. Kaplan JM, Wong HR. Biomarker discovery and development in pediatric critical care medicine. *Pediatr Crit Care Med.* 2011;12(2):165–173.
33. Lei Y, Tang R, Xu J, et al. Applications of single-cell sequencing in cancer research: progress and perspectives. *J Hematol Oncol.* 2021;14(1):91.
34. Li X, Wang C. From bulk, single-cell to spatial RNA sequencing. *Int J Oral Sci.* 2021;13(1):36–36.
35. Liu Y, Fan X, Wang R, et al. Single-cell RNA-seq reveals the diversity of trophoblast subtypes and patterns of differentiation in the human placenta. *Cell Res.* 2018;28(8):819–832.
36. Moradi M, Zhandi M, Sharafi M, Akbari A, Atrabi MJ, Totonchi M. Gene expression profile of placentomes and clinical parameters in the cows with retained placenta. *BMC Genomics.* 2022;23(1):760.
37. Wawrzykowski J, Franczyk M, Hoedemaker M, Kankofer M. The comparison of protein map between retained and released bovine placenta. *Res Vet Sci.* 2019;125:45–51.
38. Amin YA, Hussein HA. Latest update on predictive indicators, risk factors and 'omic' technologies research of retained placenta in dairy cattle—a review. *Reprod Domest Anim.* 2022;57(7):687–700.
39. Afshar Y, Yin O, Jeong A, et al. Placenta accreta spectrum disorder at single-cell resolution: a loss of boundary limits in the decidua and endothelium. *Am J Obstet Gynecol.* 2024;230(4):443.e1–443.e18.
40. Rosenkrantz JL, Gaffney JE, Roberts VHJ, Carbone L, Chavez SL. Transcriptomic analysis of primate placentas and novel rhesus trophoblast cell lines informs investigations of human placentation. *BMC Biol.* 2021;19(1):127.
41. Coviello EM, Grantz KL, Huang C, Kelly TE, Landy HJ. Risk factors for retained placenta. *Am J Obstet Gynecol.* 2015;213(6):864.e1–864.e11.
42. Nikolajsen S, Løkkegaard ECL, Bergholt T. Reoccurrence of retained placenta at vaginal delivery: an observational study. *Acta Obstet Gynecol Scand.* 2013;92(4):421–425.
43. Rottenstreich M, Rotem R, Bergman M, Rottenstreich A, Grisaru-Granovsky S. Recurrence of retained placenta in multiple consecutive deliveries. *J Matern Fetal Neonatal Med.* 2021;34(21):3574–3579.

44. Ruiter L, Kazemier BM, Mol BWJ, Pajkrt E. Incidence and recurrence rate of postpartum hemorrhage and manual removal of the placenta: a longitudinal linked national cohort study in the Netherlands. *Eur J Obstet Gynecol Reprod Biol.* 2019;238:114–119.
45. Kim D, Langmead B, Salzberg SL. HISAT: a fast spliced aligner with low memory requirements. *Nat Methods.* 2015;12(4):357–360.
46. Anders S, Pyl PT, Huber W. HTSeq—a Python framework to work with high-throughput sequencing data. *Bioinformatics.* 2015;31(2):166–169.
47. Durinck S, Spellman PT, Birney E, Huber W. Mapping identifiers for the integration of genomic datasets with the R/Bioconductor package biomaRt. *Nat Protoc.* 2009;4(8):1184–1191.
48. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* 2014;15(12):550.
49. Chen EY, Tan CM, Kou Y, et al. Enrichr: interactive and collaborative HTML5 gene list enrichment analysis tool. *BMC Bioinformatics.* 2013;14:128.
50. Kuleshov MV, Jones MR, Rouillard AD, et al. Enrichr: a comprehensive gene set enrichment analysis web server 2016 update. *Nucleic Acids Res.* 2016;44(W1):W90–W97.
51. Xie Z, Bailey A, Kuleshov MV, et al. Gene set knowledge discovery with Enrichr. *Curr Protoc.* 2021;1(3):e90.
52. Griss J, Viteri G, Sidiropoulos K, Nguyen V, Fabregat A, Hermjakob H. ReactomeGSA—efficient multi-omics comparative pathway analysis. *Mol Cell Proteomics.* 2020;19(12):2115–2125.
53. Milacic M, Beavers D, Conley P, et al. The Reactome pathway knowledgebase 2024. *Nucleic Acids Res.* 2024;52(D1):D672–D678.
54. Loukeris K, Sela R, Baergen RN. Syncytial knots as a reflection of placental maturity: reference values for 20 to 40 weeks' gestational age. *Pediatr Dev Pathol.* 2010;13(4):305–309.
55. Bączkowska M, Zgliczyńska M, Faryna J, Przytuła E, Nowakowski B, Ciebiera M. Molecular changes on maternal-fetal interface in placental abruption—a systematic review. *Int J Mol Sci.* 2021;22(12):6612.
56. Burke SD, Zsengellér ZK, Karumanchi SA, Shainker SA. A mouse model of placenta accreta spectrum. *Placenta.* 2020;99:8–15.
57. Ma Y, Hu Y, Ma J. Animal models of the placenta accreta spectrum: current status and further perspectives. *Front Endocrinol (Lausanne).* 2023;14:1118168.
58. Abbas Y, Turco MY, Burton GJ, Moffett A. Investigation of human trophoblast invasion in vitro. *Hum Reprod Update.* 2020;26(4):501–513.
59. Bačenková D, Trebuňová M, Čížková D, et al. In vitro model of human trophoblast in early placentation. *Biomedicines.* 2022;10(4):904.
60. Burrows TD, King A, Loke YW. Trophoblast migration during human placental implantation. *Hum Reprod Update.* 1996;2(4):307–321.
61. Cherubini M, Haase K. A bioengineered model for studying vascular-pericyte interactions of the placenta. *Methods Mol Biol.* 2023;2608:409–423.
62. Lee B, Kim J. Integrating high-throughput approaches and in vitro human trophoblast models to decipher mechanisms underlying early human placenta development. *Front Cell Dev Biol.* 2021;9:673065.
63. Muzzio D, Foglia ML, Desimone MF, Zygmunt M. 3D in vitro models of early pregnancy: How to choose the right scaffolding material? *Curr Pharm Des.* 2017;23(24):3603–3613.
64. Park JY, Lim H, Qin J, Lee LP. Creating mini-pregnancy models in vitro with clinical perspectives. *EBioMedicine.* 2023;95:104780.
65. Weimar CHE, Post Uiterweer ED, Teklenburg G, Heijnen CJ, Macklon NS. In-vitro model systems for the study of human embryo-endometrium interactions. *Reprod Biomed Online.* 2013;27(5):461–476.
66. Zhou J, West RC, Ehlers EL, et al. Modeling human peri-implantation placental development and function. *Biol Reprod.* 2021;105(1):40–51.
67. Combs CA, Laros RK. Prolonged third stage of labor: morbidity and risk factors. *Obstet Gynecol.* 1991;77(6):863–867.
68. Dombrowski MP, Bottoms SF, Saleh AA, Hurd WW, Romero R. Third stage of labor: analysis of duration and clinical practice. *Am J Obstet Gynecol.* 1995;172(4 Pt 1):1279–1284.
69. Frolova AI, Stout MJ, Tuuli MG, López JD, Macones GA, Cahill AG. Duration of the third stage of labor and risk of postpartum hemorrhage. *Obstet Gynecol.* 2016;127(5):951–956.
70. Magann EF, Evans S, Chauhan SP, Lanneau G, Fisk AD, Morrison JC. The length of the third stage of labor and the risk of postpartum hemorrhage. *Obstet Gynecol.* 2005;105(2):290–293.
71. Moore LG, Wesolowski SR, Lorca RA, Murray AJ, Julian CG. Why is human uterine artery blood flow during pregnancy so high? *Am J Physiol Regul Integr Comp Physiol.* 2022;323(5):R694–R699.
72. Petraglia F, Florio P, Nappi C, Genazzani AR. Peptide signaling in human placenta and membranes: autocrine, paracrine, and endocrine mechanisms. *Endocr Rev.* 1996;17:156–186.
73. Soltani H, Hutchon DR, Poulose TA. Timing of prophylactic uterotonics for the third stage of labour after vaginal birth. *Cochrane Database Syst Rev.* 2010;(8):CD006173.
74. Ushida T, Matsuo S, Nakamura N, et al. Reassessing the duration of each stage of labor and their relation to postpartum hemorrhage in the current Japanese population. *J Obstet Gynaecol Res.* 2022;48(7):1760–1767.
75. Yeligulashvili LS. 1964. *Gestation and Parturition in Apes and Monkeys.* Moscow (Russia): State Publishing House for Medical Literature.
76. Carter AM. Animal models of human pregnancy and placentation: alternatives to the mouse. *Reproduction.* 2020;160(6): R129–R143.
77. Grigsby PL. Animal models to study placental development and function throughout normal and dysfunctional human pregnancy. *Semin Reprod Med.* 2016;34(1):11–16.
78. Kang Y, Chu C, Wang F, Niu Y. CRISPR/Cas9-mediated genome editing in nonhuman primates. *Dis Model Mech.* 2019;12(10):dmm039982.
79. Lin Y, Li J, Li C, et al. Application of CRISPR/Cas9 system in establishing large animal models. *Front Cell Dev Biol.* 2022;10: 919155.
80. Burdo TH, Chen C, Kaminski R, et al. Preclinical safety and biodistribution of CRISPR targeting SIV in non-human primates. *Gene Ther.* 2024;31(5-6):224–233.
81. Mor G, Abrahams VM. Immunology of pregnancy. In: Lockwood CL, Moore T, Copel J, Silver RM, Resnik R, Dugoff L, Louis J, eds. *Creasy and Resnik's Maternal-Fetal Medicine.* 9th ed. Elsevier; 2023:127–142.e8.
82. Weng J, Couture C, Girard S. Innate and adaptive immune systems in physiological and pathological pregnancy. *Biology (Basel).* 2023;12(3):402.
83. Kimura K, Goff JP, Kehrli ME, Reinhardt TA. Decreased neutrophil function as a cause of retained placenta in dairy cattle. *J Dairy Sci.* 2002;85(3):544–550.
84. Bert S, Ward EJ, Nadkarni S. Neutrophils in pregnancy: new insights into innate and adaptive immune regulation. *Immunology.* 2021;164(4):665–676.
85. Bollapragada S, Youssef R, Jordan F, Greer I, Norman J, Nelson S. Term labor is associated with a core inflammatory response in human fetal membranes, myometrium, and cervix. *Am J Obstet Gynecol.* 2009;200(1):104.e1–11.
86. Gomez-Lopez N, StLouis D, Lehr MA, Sanchez-Rodriguez EN, Arenas-Hernandez M. Immune cells in term and preterm labor. *Cell Mol Immunol.* 2014;11(6):571–581.
87. Motomura K, Romero R, Galaz J, et al. RNA sequencing reveals distinct immune responses in the chorioamniotic membranes of women with preterm labor and microbial or sterile intra-amniotic inflammation. *Infect Immun.* 2021;89(5):819.
88. Zhang C, Cao J, Xu M, Wu D, Li W, Chang Y. The role of neutrophils in chorioamnionitis. *Front Immunol.* 2023;14:1198831.
89. Huang J, Zhang W, Zhao Y, et al. Deciphering the intercellular communication network of peripartum decidua that orchestrates delivery. *Front Cell Dev Biol.* 2021;9:770621.
90. Clark EA, Giltiay NV. CD22: a regulator of innate and adaptive B cell responses and autoimmunity. *Front Immunol.* 2018;9:2235.
91. Sonoda K, Kaku T, Hirakawa T, et al. The clinical significance of tumor-associated antigen RCAS1 expression in the normal, hyperplastic, and malignant uterine endometrium. *Gynecol Oncol.* 2000;79(3):424–429.

92. Wicherek L, Dutsch M, Mak P, Klimek M, Skladzien J, Dubin A. Comparative analysis of RCAS1 level in neoplasms and placenta. *Acta Biochim Pol.* 2003;50(4):1187–1194.
93. Wicherek L, Klimek M, Dutsch-Wicherek M, Kolodziejski L, Skotniczny K. The molecular changes during placental detachment. *Eur J Obstet Gynecol Reprod Biol.* 2006;125(2):171–175.
94. Fagerholm SC, Guenther C, Llort Asens M, Savinko T, Uotila LM. Beta2-integrins and interacting proteins in leukocyte trafficking, immune suppression, and immunodeficiency disease. *Front Immunol.* 2019;10:254.
95. Benner M, Feyaerts D, García CC, et al. Clusters of tolerogenic B cells feature in the dynamic immunological landscape of the pregnant uterus. *Cell Rep.* 2020;32(13):108204.
96. Le Gallee M, Lee S, Spence AR, Czuzoj-Shulman N, Klam S, Abenhaim HA. Association between first-trimester bleeding and retained placenta requiring dilatation and curettage. *J Obstet Gynaecol Can.* 2021;43(4):463–468.
97. Breuss JM, Uhrin P. VEGF-initiated angiogenesis and the uPA/uPAR system. *Cell Adh Migr.* 2012;6(6):535–615.
98. Naruse K, Lash GE, Bulmer JN, et al. The urokinase plasminogen activator (uPA) system in uterine natural killer cells in the placental bed during early pregnancy. *Placenta.* 2009;30(5):398–404.
99. Renaud SJ, Macdonald-Goodfellow SK, Graham CH. Coordinated regulation of human trophoblast invasiveness by macrophages and interleukin 10. *Biol Reprod.* 2007;76(3):448–454.
100. Feng Q, Liu K, Liu YX, Byrne S, Ockleford CD. Plasminogen activators and inhibitors are transcribed during early macaque implantation. *Placenta.* 2001;22(2-3):186–199.
101. Pierleoni C, Castellucci M, Kaufmann P, Lund LR, Schnack Nielsen B. Urokinase receptor is up-regulated in endothelial cells and macrophages associated with fibrinoid deposits in the human placenta. *Placenta.* 2003;24(6):677–685.
102. Mondino A, Blasi F. uPA and uPAR in fibrinolysis, immunity and pathology. *Trends Immunol.* 2004;25(8):450–455.
103. Multhaupt HA, Mazar A, Cines DB, Warhol MJ, McCrae KR. Expression of urokinase receptors by human trophoblast. A histochemical and ultrastructural analysis. *Lab Invest.* 1994;71(3):392–400.
104. Dunk C, Shams M, Nijjar S, et al. Angiopoietin-1 and angiopoietin-2 activate trophoblast tie-2 to promote growth and migration during placental development. *Am J Pathol.* 2000;156(6):2185–2199.
105. Tseng J, Chou M. Differential expression of growth-, angiogenesis- and invasion-related factors in the development of placenta accreta. *Taiwan J Obstet Gynecol.* 2006;45(2):100–106.
106. Wulff C, Weigand M, Kreienberg R, Fraser HM. Angiogenesis during primate placentation in health and disease. *Reproduction.* 2003;126(5):569–577.
107. Wang Q, Lash GE. Angiopoietin 2 in placentation and tumor biology: the yin and yang of vascular biology. *Placenta.* 2017;56:73–78.