

Immune Regulation in Eutherian Pregnancy: Live Birth Coevolved with Novel Immune Genes and Gene Regulation

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Novel regulatory elements that enabled expression of pre-existing immune genes in reproductive tissues and novel immune genes with pregnancy-specific roles in eutherians have shaped the evolution of mammalian pregnancy by facilitating the emergence of novel mechanisms for immune regulation over its course. Trade-offs arising from conflicting fitness effects on reproduction and host defenses have further influenced the patterns of genetic variation of these genes. These three mechanisms (novel regulatory elements, novel immune genes, and trade-offs) played a pivotal role in refining the regulation of maternal immune systems during pregnancy in eutherians, likely facilitating the establishment of prolonged direct maternal–fetal contact in eutherians without causing immunological rejection of the genetically distinct fetus.

1. Introduction

Mammalian pregnancy entails the coexistence of a genetically distinct entity (i.e., the fetus) within the mother, and therefore, is an intrinsically immunological process. Marsupials and eutherians, the two lineages of mammals that give live birth, differ in several aspects of pregnancy, including the status of the maternal immune system.

Marsupial pregnancy is generally shorter than the eutherian one (mean of 25 days versus 131 days),^[1,2] and for the bulk of the gestational period, the embryo is separated from the maternal uterine epithelial cells by a thick but permeable eggshell (Figure 1a). During this period, there is no direct maternal–fetal contact, and the developing embryo receives nutrients from uterine secretions.^[3–5] This eggshell is only

breached shortly before labor, followed by apposition and attachment of the embryo and the formation of a short-lived yolk sac placenta (Figure 1a).^[5,6]

In contrast, eutherian pregnancy involves prolonged contact between the mother and the fetus, beginning at implantation that occurs early on in pregnancy (Figure 1b): the embryo is apposed to the uterine wall, which subsequently attaches, and in some species, invades, the uterus.^[7,8] This is followed by the formation of the placenta (Figure 1b), which serves nutritive and immune-modulatory roles for the remainder of the pregnancy. Conserved sets of inflamma-

tory markers and immune pathways are involved in both eutherian implantation and marsupial attachment and labor.^[5,9] However, in contrast to marsupials, eutherians experience major switches in the overall activities of the immune system in the uterus during pregnancy: the upregulation of inflammation in the uterus during implantation is followed by an extended anti-inflammatory period^[5,10] (Figure 1b), which likely stems from the need to protect the developing fetus from detrimental immune responses. Toward the end of pregnancy, the eutherian uterus experiences a spike in inflammation yet again, resulting in the initiation of labor.^[10,11] Eutherian mothers must sustain a genetically distinct fetus without inducing an immunological reaction and at the same time maintain optimal host defense against pathogens. Therefore, the successful establishment of pregnancy in eutherian mammals must have involved the evolution of novel mechanisms for immune modulation.^[5]


Examination of the molecular mechanisms that underlie the evolution of pregnancy in mammals, and more specifically of the emergence of novel mechanisms for immune modulation in eutherians, has identified three major evolutionary processes at work. First, extensive rewiring of regulatory networks involving pre-existing immune genes has enabled appropriate transcriptional responses to promote successful physiological changes to support pregnancy (Figure 2a–c). Second, novel immune genes that serve purposes specific to pregnancy in eutherian mammals have emerged (Figure 2d–f). Third, species-specific as well as population-level adaptations stemming from trade-offs between optimizing reproduction and host defense have likely resulted in diverse strategies of pregnancies within eutherian mammals.

In this review, we discuss the molecular and evolutionary mechanisms underlying these three processes in detail. We

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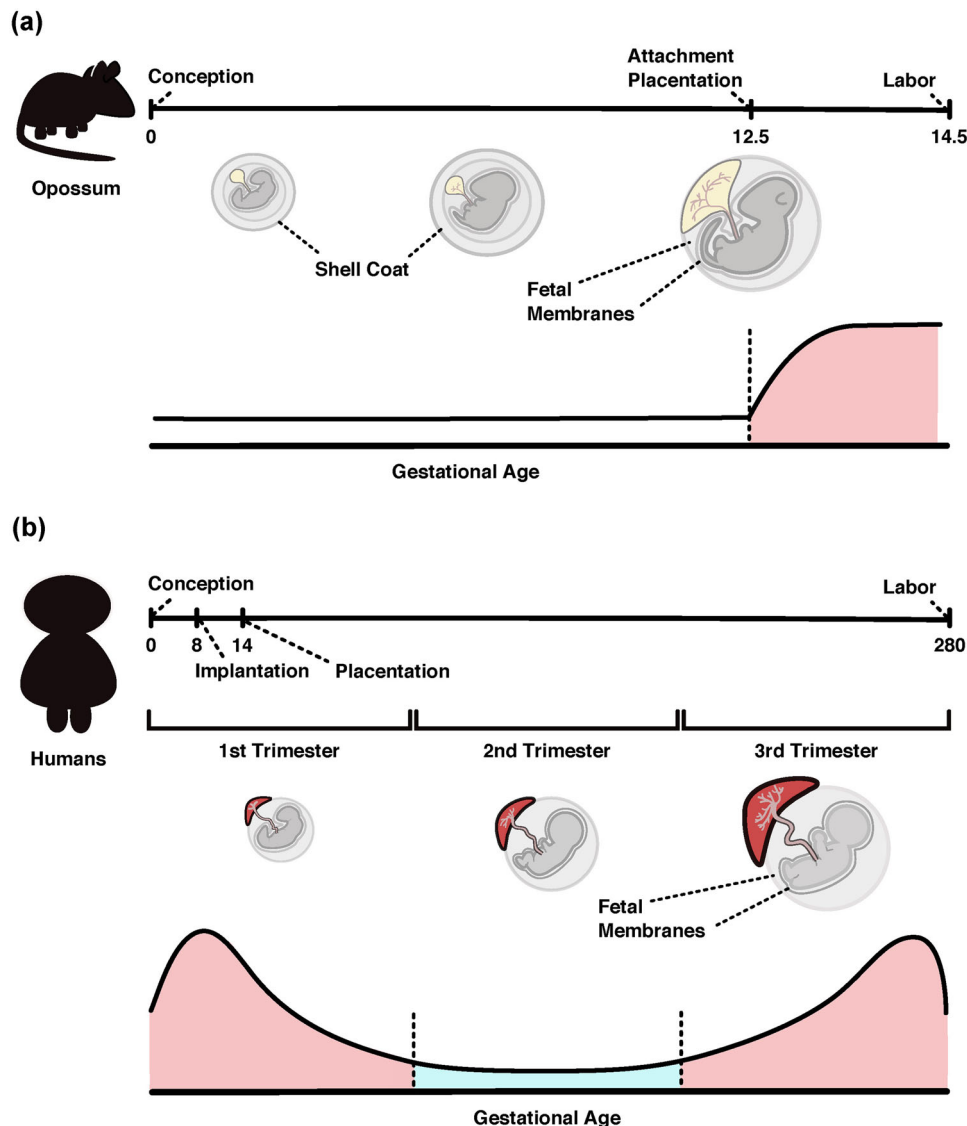
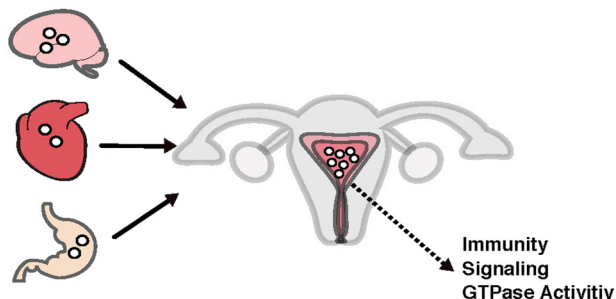


Figure 1. Comparison of pregnancy in eutherians and marsupials. A schematic illustration of the major differences in pregnancy between two lineages of mammals. The gestation lengths (shown in days) of the two lineages of mammals are not on the same scale. a) Marsupial pregnancy is relatively short (mean gestation length of 25 days) and for the majority of the gestational period, the fetus is separated from maternal tissues by a shell coat. This shell coat is only breached shortly before the initiation of labor and is accompanied by the formation of a transient yolk sac placenta. The formation of the placenta is associated with a spike in inflammation, which persists until labor. b) In contrast, eutherian mammals exhibit longer periods of pregnancy (mean gestation length of 131 days). Implantation and subsequent placentation occur relatively early on in pregnancy, establishing direct maternal–fetal contact that lasts for the remainder of the pregnancy. Eutherian pregnancy is further distinguished from marsupial pregnancy in the occurrence of immune modulation throughout the entire gestational period: after the proinflammatory (the portion of the graph shaded in salmon) state following implantation, the immune state switches to a prolonged anti-inflammatory state (the portion of the graph shaded in light blue), likely allowing optimal growth of the fetus. Toward the end of pregnancy, there is another surge in inflammation, which likely leads to the initiation of labor.

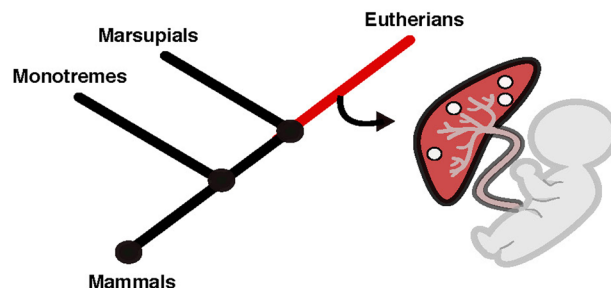
first outline the mechanisms underlying the evolutionary innovations that occurred with the emergence of eutherian mammals, namely, the rewiring of regulatory networks of pre-existing genes and the emergence of novel genes. We follow this up with a more focused discussion of the evolution of two immune gene families in particular: killer immunoglobulin-like receptors (KIRs) and sialic-acid binding immunoglobulin-like lectins (SIGLECs). We briefly

discuss the global processes that led to the emergence of these two gene families and conclude with the discussion of how differential advantages in terms of reproductive success and host defense activities have likely influenced their patterns of genetic variation in modern humans. Our review showcases how regulatory rewiring, the birth of novel genes, and reproduction versus immunity trade-offs have shaped the evolution of eutherian pregnancy.

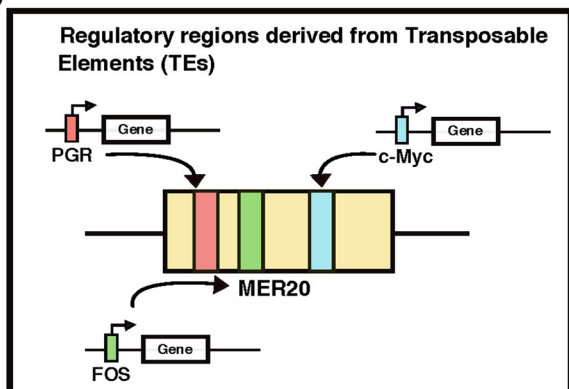
(a) Recruitment of expression of pre-existing genes into the endometrium



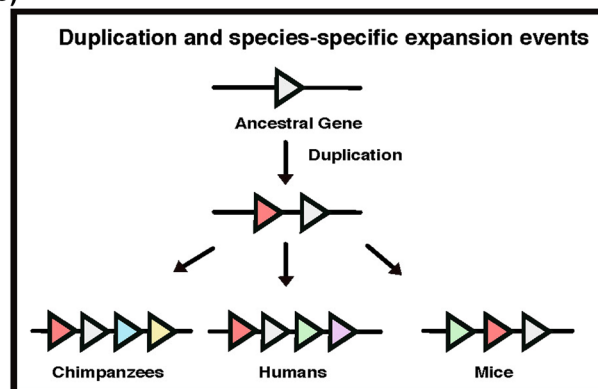
(d) Emergence of novel placenta-specific genes



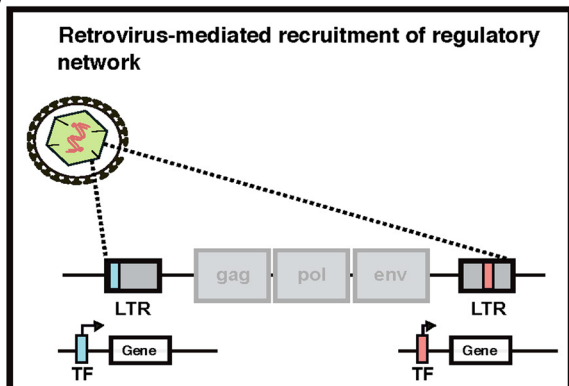
(b)



(e)



(c)



(f)

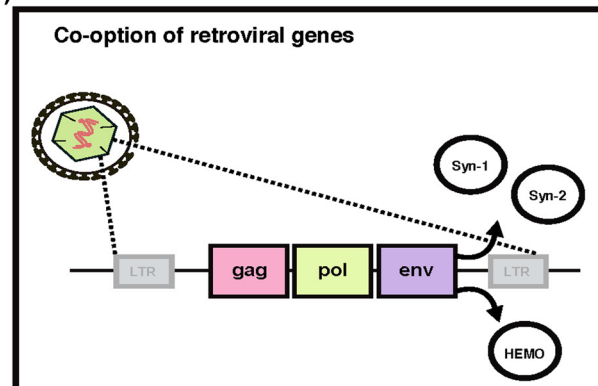


Figure 2. Molecular mechanisms underlying the evolution of eutherian pregnancy. A schematic illustration of the molecular mechanisms that have contributed to the evolution of eutherian pregnancy. a) Both pre-existing immunity genes with nonreproductive roles as well as novel immunity genes associated with pregnancy-specific processes contribute toward promoting optimal growth of the fetus. One mechanism by which pre-existing genes were co-opted to participate in reproductive processes was the recruitment of such genes into an expression within reproductive tissues. This could have been facilitated by the contribution of regulatory regions by b) TEs and c) retroviruses. In addition, e) gene duplication events, followed by subsequent species-specific expansions and f) co-option of retroviral genes contributed to the emergence of novel immunity genes involved in the pregnancy.

2. Novel Regulatory Elements Enabled Expression of Pre-Existing Genes in Eutherian Reproductive Tissues

Changes in gene regulation have majorly contributed to the evolution of animal form,^[12,13] including of the eutherian placenta.^[14,15] More recently, several studies have shown that

changes in gene regulation also underlie the extensive differences between marsupial and eutherian pregnancies. For example, eutherian decidualized stromal cells (DSCs) were likely formed by reprogramming of the pregnancy-related stress response in marsupial (gray short-tailed opossum) endometrial stromal fibroblasts (ESFs).^[16] Interestingly, when marsupial ESFs are stimulated with decidualization signals, such as progesterone and

cyclic adenosine monophosphate, several core regulatory genes known to be involved in eutherian decidualization are also upregulated. Some of these regulatory genes are transcription factors (TFs) with well-known roles in decidualization, such as *PGR*, *CEBPB*, *HOXA11*, and *STAT3*. However, some regulatory genes that are upregulated in human DSCs (i.e., *PRL* and *IGFBP1*) are eutherian-specific and are not found in marsupial ESFs, suggesting that the recruitment of new TFs contributed to the evolution of DSCs. Subsequent functional enrichment analyses revealed that genes upregulated in the opossum were enriched for stress responses and apoptosis, indicating that the decidualization signals trigger a different biological process in marsupials compared to eutherians. Interestingly, *FOXO1*, a TF that is upregulated in opossum ESFs upon stimulation with decidualization signals, regulates different sets of genes in human DSCs and opossum ESFs, suggesting that decidualization in eutherians evolved through co-option of the marsupial stress pathway by rewiring downstream targets genes of the highly conserved regulatory network.

In addition, striking differences exist in the reconstructed ancestral transcriptomes of ESFs of eutherian mammals and the transcriptome of opossum ESFs.^[17] For example, genes that gained uterine expression in eutherian mammals are enriched for functions involved in cell division and proliferation, while genes expressed in opossum ESFs but not in ancestral eutherians are enriched for inflammation, cell movement, and adhesion processes. Other examples include *SIGLEC5*, 6, and 14, which gained placental expression only in humans.^[18,19] While the mechanism by which *SIGLEC5* and *SIGLEC14* gained expression in the human placenta remains unknown, one study suggests that *SIGLEC6* likely gained human-specific placental expression via the loss of the GATA-binding site and gain of E-box and Oct-1 sites in its 5'-untranslated region.^[18] More recently, extensive differences have been found in species-specific TFs between mice and humans, possibly accounting for the large differences that exist between these two species in terms of placental development.^[20] For instance, *VGLL1*, a potential human-specific regulator of trophoblast differentiation of embryonic stem cells, is not detected in mouse placenta at any point in pregnancy.

More broadly, the evolution of eutherian pregnancy is associated with loss and gain of uterine expression of numerous genes.^[21] Genes that were recruited to be expressed in the uterus are often involved in the regulation of immune responses, metabolic processes, and cell divisions (Figure 2a) and this may stem from the need for the careful regulation of such processes in the uterine environment during the prolonged period of gestation. Below, we discuss two major mechanisms that have contributed to regulatory evolution within eutherian mammals, transposable elements (TEs) and endogenous retroviruses (ERVs) (Figure 2b,c).

2.1. Eutherian-Specific TEs Have Facilitated Appropriate Transcriptional Responses to Pregnancy Signals

The first major mechanism involves TEs (TEs are DNA sequences that can “jump” to new sites within genomes)

(Figure 2b). TE sequences are often found near genes involved in the pregnancy. For instance, a significant proportion of genes that undergo changes in gene expression upon decidualization are in proximity to *MER20*, an hAT-Charlie family DNA transposon.^[22] In addition, *MER20* sequences are able to bind to TFs important for hormone responsiveness and pregnancy, such as *PGR*, *FOXO1A*, and *HOXA11*, and other more general TFs such as *CTCF*, *p53*, and *p300*.^[22] In addition, depending on the combination of bound TFs, *MER20* sequences can act as either insulators or cis-regulatory elements (i.e., enhancers and repressors). Similarly, the proximal part of alternative prolactin (*PRL*) enhancer that results in extrapituitary expression occurs within *MER20* and *MER39*, another long terminal repeat (LTR) TE.^[23]

More broadly, locations of regulatory elements active in DSCs often overlap with ancient mammalian TEs (AncMam-TEs) and genes associated with regulatory regions derived from such TEs exhibit significant changes in expression levels upon decidualization,^[24] which is especially true for genes that have evolved novel uterine expression.^[21] In addition, these AncMam-TEs are enriched for binding sites for TFs with roles in pregnancy, such as those that mediate hormone responses (e.g., *PGR*, *NR4A1*, and *ERRA*) or have functions in endometrial cells or immune regulation (e.g., *ELK4*, *ARNT*, *c-Myc*, and *E2F1*).^[21] In short, TEs have provided novel regulatory elements in eutherian mammals to enable an appropriate transcriptional response to decidualization.

2.2. Retroviruses Have Provided Additional Novel Regulatory Elements for Pregnancy-Related Genes in Eutherians

The second major mechanism of regulatory evolution in the context of eutherian pregnancy involves ERVs, retroviruses that originally infected host germline cells but whose sequences have since been integrated into the host genomes and vertically inherited. A typical ERV consists of three core retroviral genes (*env*, *gag*, and *pol*), which are flanked by long LTRs.^[25] While ERV LTRs normally function to promote transcription of the viral genome, it has been hypothesized that they could also act as novel promoters or enhancers for nearby genes if they contain appropriate binding sites for trophoblast-specific TFs (Figure 2c).^[25] Such LTR-derived regulatory elements could potentially co-opt entire gene regulatory networks, resulting in extensive changes in the placental transcriptome. While less investigated than the co-option of ERV genes (which will be discussed in the following sections), some studies have uncovered instances of LTR-derived regulatory elements. For example, a recently discovered anthropoid primate-specific LTR-derived *THE1B* element regulates the expression of corticotropin-releasing hormone, a hormone that is involved in controlling gestation lengths in humans, and other placental genes.^[26] This *THE1B* element was also found to bind to *DLX3*, a TF that contributes to trophoblast differentiation. Another example involves a recently discovered novel trophoblast-specific enhancer that is required for the expression of *HLA-G* in human extravillous trophoblasts (EVTs), which lies within an LTR region associated with *ERV1*.^[27] Similar to the *THE1B* element described above, this enhancer exhibits binding

activities for CEBP (CEBPB) and GATA family TFs (GATA2 and GATA3) that are highly expressed in the placenta. Other examples of ERV-driven regulatory evolution include leptin,^[28] pleiotropin,^[29] and endothelin B receptor,^[30,31] which have gained placental expression via LTR-derived promoters.

While a large body of research on novel regulatory elements for pregnancy-associated genes has focused on TEs of the LTR class, it is interesting to note that non-LTR transposons (i.e., LINEs and SINEs) have also likely contributed novel regulatory elements to placenta-specific genes: for instance, placenta-specific hypomethylation of a SINE-derived alternative promoter has been shown to enable placenta-specific expression of *KCNH5*.^[32] In addition, another study found that L1PA2, a subfamily of the LINE-1 family, acts as a promoter for long noncoding RNAs with placenta-specific expression.^[33]

In summary, novel regulatory elements arising from LTRs of ERVs and other TEs could have facilitated the recruitment of gene expression in reproductive tissues, including the uterus and trophoblast, resulting in appropriate transcriptional responses to promote successful pregnancy.

3. Novel Genes with Roles in Pregnancy Have Emerged in Eutherian Mammals

As discussed above, eutherian pregnancy involves activities of both pre-existing genes that were originally involved in other biological processes (Figure 2a) as well as novel evolutionarily young genes (Figure 2d).^[14,15,34] For instance, genes highly expressed in the developing placenta of mice are enriched for ancient genes (i.e., genes sharing orthologs with eukaryotic organisms).^[34] These ancient genes are enriched for roles in growth and metabolic processes. In contrast, genes highly expressed in the mature placenta are often clade-specific and are enriched for functions associated with pregnancy, reproductive processes, and negative regulation of physiological and cellular processes.^[34] In addition, a large-scale classification of proteins on the basis of sequence similarity has identified numerous genes that have emerged in the stem lineage of eutherian mammals and have either been conserved in all species or lost across different subgroups of eutherian mammals.^[35] Functional enrichment analyses suggest that these genes are often involved in immune responses, development, and regulation of transcription. Two mechanisms, namely gene duplication (Figure 2e) and co-option of ERV genes (Figure 2f), could result in the emergence of novel genes in eutherians.

3.1. Gene Duplications Have Resulted in the Birth of Novel Gene Families in Eutherian Mammals

Several phylogenetic analyses suggest gene duplication, followed by species-specific expansion or contraction events, to have played a role in the emergence of several novel gene families (Figure 2e), including pregnancy-specific glycoproteins (PSGs), KIRs, and SIGLECs. Part of the carcinoembryonic antigen family, PSGs are the most abundantly secreted trophoblast-specific proteins detected in the maternal blood

during pregnancy and may play roles in modulating the maternal immune responses. These include inducing monocytes and dendritic cells to produce anti-inflammatory cytokines^[24,36,37] and promoting of alternative macrophage activation that results in the suppression of T-cell activation and proliferation.^[38] To date, PSGs have been found exclusively in hemochorial mammals (e.g., humans, rodents, and some primates) or mammals that possess a population of trophoblasts with invasive properties (e.g., horses).^[39] Phylogenetic studies have shown that PSGs have likely emerged from duplication of an ancestral *CECAM* (*CECAM1*) gene, followed by additional subsequent expansion events.^[39] For example, PSGs have undergone a major expansion in dry-nosed or haplorhine primates, a group that includes tarsiers, Old World monkeys, and New World monkeys, followed by independent expansion events in apes and rhesus macaques.^[40]

Another example involves KIRs, a family of genes that is part of the leukocyte receptor complex on chromosome 19 in humans.^[41] KIRs are expressed on uterine natural killer cells (uNKs) that come into contact with EVT cells invading into the decidua (Figure 3c).^[42] The interaction between uNK cells and EVT cells regulates the degree of trophoblast invasion into the decidua and subsequent remodeling of the spiral arteries.^[43,44] A subset of KIRs expressed on uNK cells binds to human leukocyte antigen (HLA) type C on the EVT cells (Figure 3c).^[45,46] This KIR–HLA-C interaction results in the activation of uNK cells, leading to the secretion of cytokines and angiogenic factors that facilitate placentation.^[47] Duplication event of an ancestral KIR gene that occurred approximately 140 million years ago resulted in two founder KIR genes (Figure 3b): *KIR3DX* for cattle and *KIR3DL* for simian-primates.^[48] During the past 58–40 million years of simian-primate evolution (during which features such as increased sizes of the brains [temporal lobes] and prolonged gestation emerged), KIR genes have expanded from the single *KIR3DL* gene.^[48,49] For instance, a subset of KIRs that can bind to major histocompatibility complex-C (MHC-C) (HLA-C in humans) first expanded in orangutans,^[50] followed by additional species-specific expansions in chimpanzees and humans.^[51] In contrast, KIRs have experienced extensive deletions and mutations in gibbons, resulting in contraction of the KIR locus.^[52]

A similar scenario unfolded in SIGLECs, immunoglobulin-like lectins expressed on immune cells that bind sialic acids ubiquitously found on host cells. Some genes belonging to the subgroup CD33-related (CD33r) SIGLECs exhibit expression in reproductive tissues.^[18,19] While the exact role that other CD33r SIGLECs play in pregnancy is less well known, one mechanism may involve regulation of the peripheral maternal immune system during pregnancy, as discussed in more detail below (Figure 4c). In mammals, the primordial CD33r SIGLECs cluster, which formed via tandem duplications of the ancient SIGLECs cluster, has been shown to have undergone a large-scale inverse gene duplication event approximately 180 million years ago (i.e., before the eutherian/marsupial split), followed by species-specific expansions and contraction events (Figure 4b).^[53] For example, while the early postduplication cluster underwent additional duplication events in primates and dogs, it was contracted in rodents.

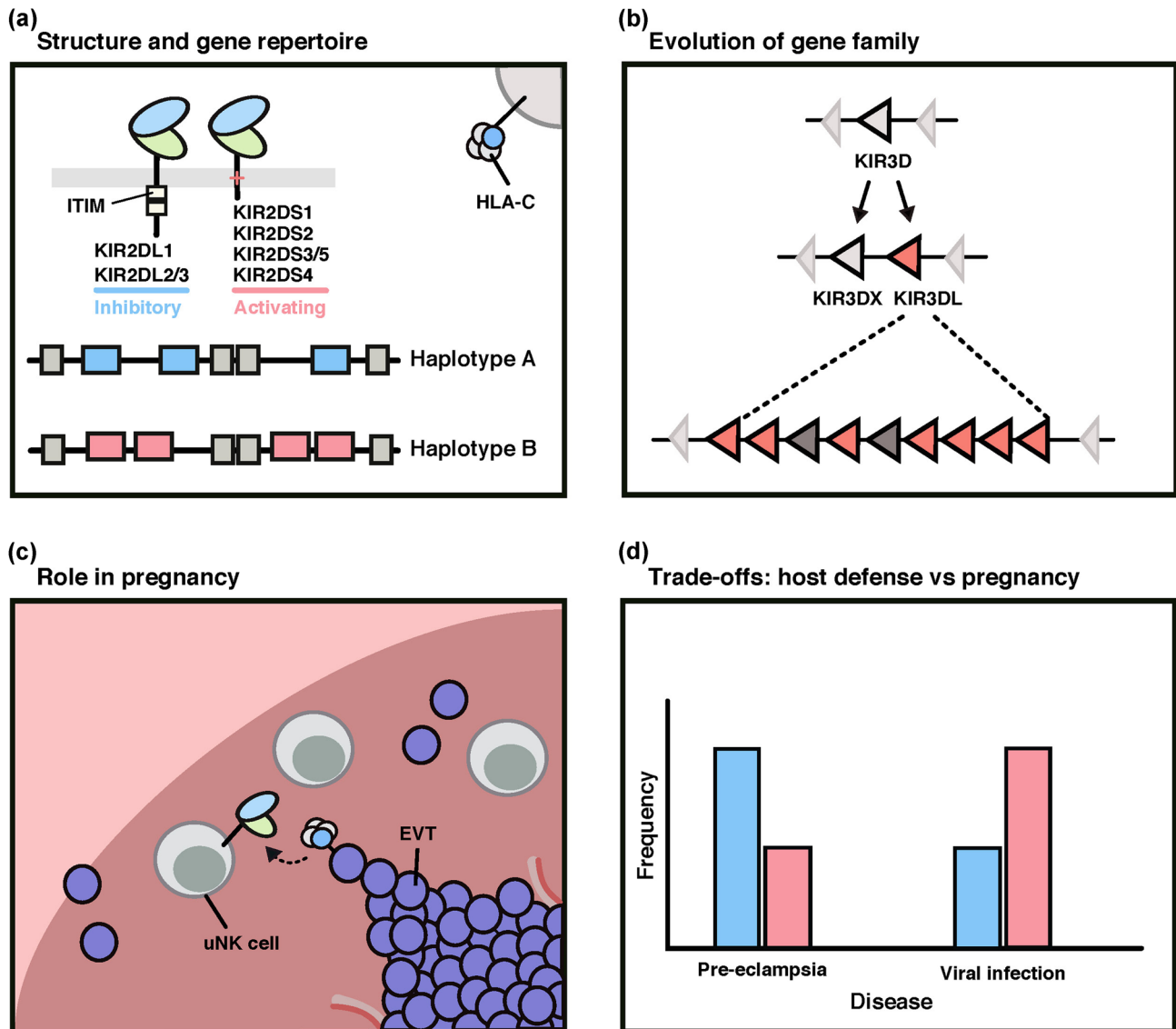


Figure 3. Examples of trade-offs between reproductive processes and host defense: KIRs. A summary of the key features of the KIR family that has been shown to play roles in both reproduction and host defense. This figure illustrates what is known about the a) general structure of the gene and repertoire of the KIR family, b) the evolutionary process by which this gene family has emerged, c) the known roles of KIRs in pregnancy, and d) trade-offs between pregnancy and immune activities. Inhibitory KIR genes and haplotypes (i.e., haplotype A) are indicated in light blue, while the activating KIRs and haplotypes (i.e., haplotype B) are shown in salmon color.

Other examples of pregnancy-related genes arising from gene duplications include hormones, such as growth hormones in primates,^[54–57] PRLs^[58] and *Rhox* genes in rodents,^[59,60] and chorionic gonadotropin subunit genes.^[61,62] Additional examples consist of the galectins, a highly conserved family of β -galactosidase-binding lectins expressed on immune cells.^[63,64] In all cases, there is considerable diversity in the repertoire of such gene families among species, resulting from species-specific expansion or contraction events that occurred after the major duplication event of a single ancestral gene. In this regard, it is interesting to note that Knox and Baker^[34] also found that the majority of the rodent-specific genes highly expressed in the mature placenta are members of three gene

families that experienced major rodent or mouse-specific expansions: PRLs, PSGs/CECAMs, and mouse PECs.

3.2. ERVs Have Contributed Novel Genes That Play Critical Roles in Eutherian Pregnancy

A rather unusual mechanism by which novel genes arose in eutherian mammals involves genes from ERVs (Figure 2f). While most ERV genes no longer code for functional proteins due to the accumulation of mutations and indels, some proteins have been retained to serve important functions for their hosts.

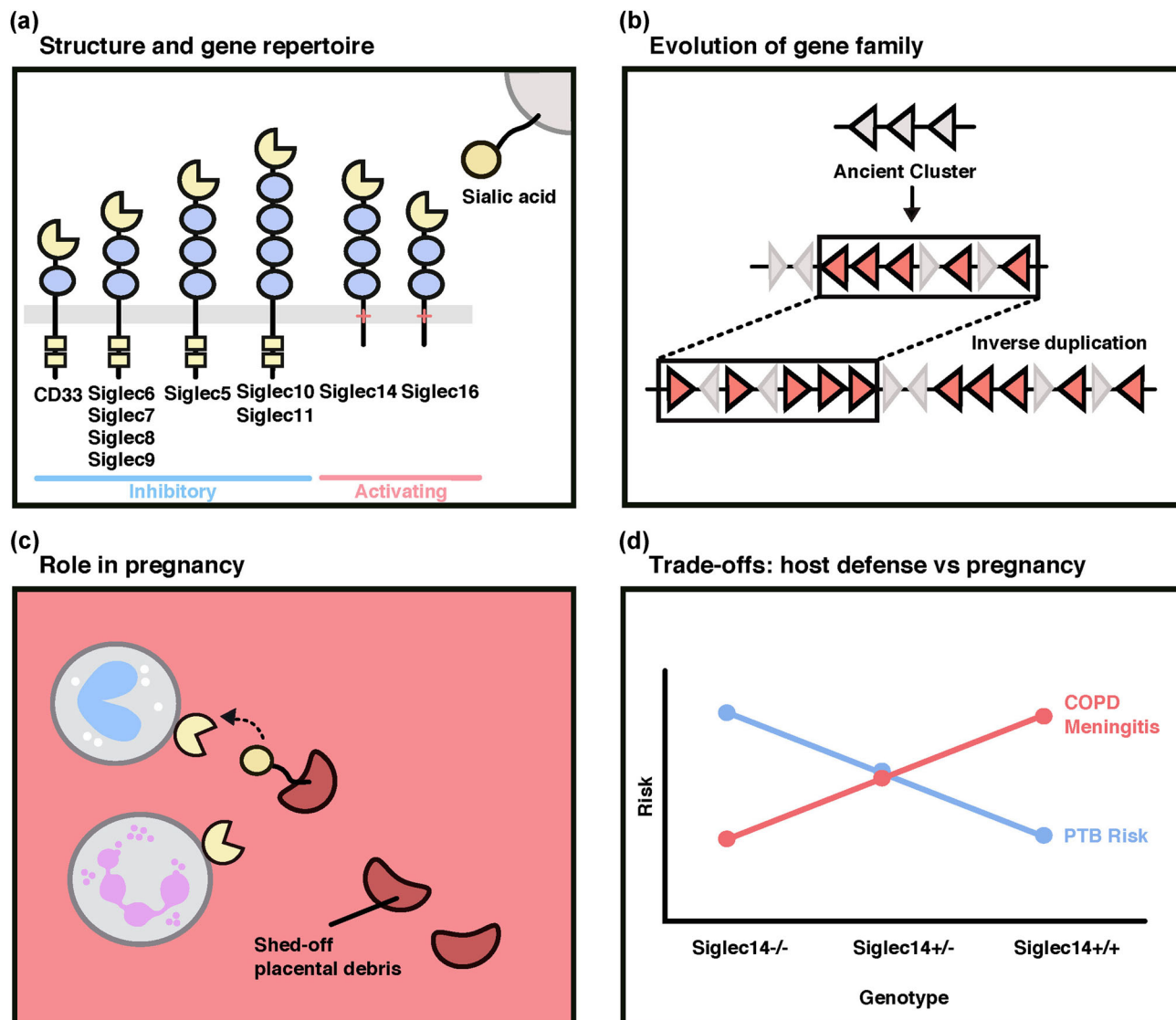


Figure 4. Examples of trade-offs between reproductive processes and host defense: SIGLECs. A summary of the key features of the SIGLECs family that has been shown to play roles in both reproduction and host defense. This figure illustrates what is known about the a) general structure of the gene and repertoire of this gene family, b) the evolutionary process by which this gene family has emerged, c) the predicted roles of CD33r SIGLECs in pregnancy (with SIGLECs expressed on monocytes and neutrophils), and d) trade-offs between pregnancy and immune activities.

Syncytin-1 (*Syn-1*) and syncytin-2 (*Syn-2*), envelope genes of human ERV-W (HERV-W)^[65] and HERV-FRD,^[66] respectively, are such genes. Early experimental studies established the direct role of these genes in mediating the fusion of the mononucleate cytotrophoblasts to form the multinucleate syncytiotrophoblasts (SYN), the outer layer of trophoblasts that comes into direct contact with maternal blood and is involved in the maternal–fetal exchange.^[65] Furthermore, reduced expression of syncytins is associated with adverse outcomes of pregnancy, such as pre-eclampsia in humans and embryonic lethality in mice.^[67,68] Furthermore, ERV *env* genes have been integrated and co-opted independently in multiple lineages throughout mammalian evolution.^[69] In addition to its involvement in the formation of the SYN, *Syn-1* may also play a role in suppressing detrimental antiviral immune responses, thereby

creating an immunologically tolerant environment for the fetus.^[70] One study discovered that *Syn-1*-treated immune cells from nonpregnant women exhibited reduced production of interferons (IFNs) (e.g., IFN- λ and IFN- α), whereas levels of the anti-inflammatory cytokines (e.g., interleukin-10 [IL-10]) were increased, and changes in the levels of such cytokines were similar to those seen in pregnant women without *Syn-1* treatment.^[70]

Apart from syncytins, genome-wide searches for ERV Env proteins have uncovered 45 Env-encoding open reading frames, one of them encoding an unusual Env protein, HEMO of the MER34 family, which is lacking several characteristics shared among other Env proteins.^[71] Unlike other Env proteins, HEMO does not possess fusogenic properties and is shed into the local and peripheral blood in pregnant women. While the

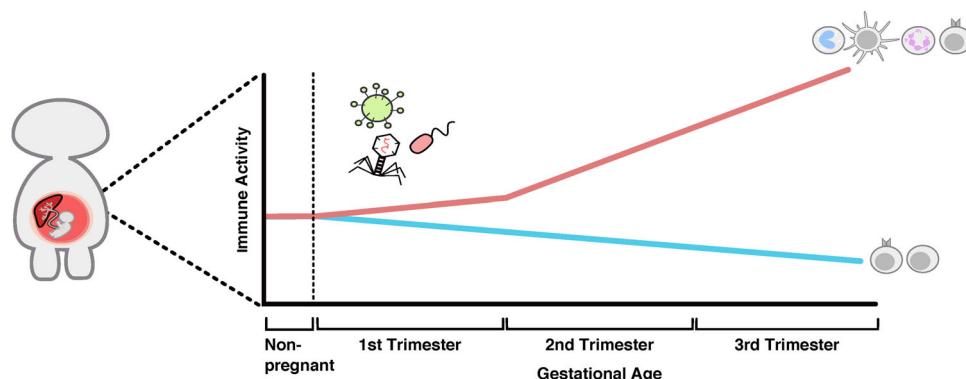


Figure 5. Changes in the maternal immune system that occur during pregnancy. Different components of the maternal immune system experience alternations in their activities over the course of gestational period: more specifically, activities of monocytes, dendritic cells, granulocytes, and regulatory T cells (T_{reg} cells; from left to right in the figure) increase (salmon-colored line) over the course of pregnancy, especially starting in the second trimester. In contrast, activities of $CD4^{+}$ and $CD8^{+}$ T cells, as well as natural killer cells (from left to right in the figure), decrease (teal-colored line) with gestational age.

exact role of HEMO in pregnancy is yet unknown, it is speculated that the shed HEMO proteins may act to sequester receptors that could be used by other retroviruses, thereby aiding host defense activities.^[71] While the exact roles in reproduction are yet unknown, there are other examples of ERV-derived genes (and proteins) expressed in the placenta. These include the transmembrane protein (resulting from cleavage of the Env in the Golgi) of HERV-K,^[72] the *env* gene of HERV-E.^[73] These ERV-derived elements are hypothesized to play immunosuppressive roles via their immunosuppressive domain.

In summary, the evolution of eutherian pregnancy was likely facilitated by both co-option of pre-existing ancient genes to promote early growth of the placenta and the emergence of novel evolutionarily younger (often species-specific) genes via gene duplication and/or ERV-co-option events, which act in later stages of placental development to regulate more pregnancy-specific processes.^[14,15]

4. Trade-Offs between Reproductive Processes and Host Defense Have Influenced the Patterns of Genetic Variation of Pregnancy-Associated Genes Within Humans

Prolonged direct maternal–fetal contact in eutherian pregnancy (and in some species, exposure of the fetus to the maternal circulation) requires careful regulation of maternal immune responses to prevent immunological rejection of the fetus. For instance, in humans, T-cell activities are strongly suppressed at the maternal–fetal interface by induction of apoptosis via Fas–FasL signaling, starvation of these cells via tryptophan depletion by indolamine 2,3-dioxygenase, and expression of PD-L1 on T cells.^[74] In addition, differentiation of regulatory T cells with anti-inflammatory properties,^[75] alternative activation of macrophages leading to suppression of cytotoxic T-cell activities,^[76] reduced production of costimulatory molecules, and increased secretion of Th2 cytokines by dendritic cells also occur at the maternal–fetal interface,^[37] creating an immunologically tolerant environment for the growth of the fetus.

Furthermore, in humans, starting from the second trimester onward, shed-off SYN microparticles (likely resulting from trophoblastic apoptosis that is involved in the continuous renewal of the SYN) can be detected in the maternal circulation.^[77] These floating microparticles come into close contact with maternal immune cells and the peripheral immune system is therefore modulated to downregulate detrimental cell-mediated immunity (Figure 5).^[75,78,79] Conversely, it has been shown that certain components of the innate immune system are activated to compensate for this relative suppression of cell-mediated immune activities: circulating innate immune cells exhibit activated phenotypes and are primed to produce proinflammatory cytokines upon stimulation (Figure 5).^[78,80] Therefore, the alert maternal innate immune components must be carefully regulated and loss of control (and subsequent excessive activation) has been linked to pregnancy complications.^[81,82] This refined immune regulation must occur while maintaining efficient host defense and it is believed that the resulting selective pressures on these processes can be conflicting. For this discussion, we will focus on two families of immunity-related genes, KIRs and SIGLECs (Figure 3), which play roles in both reproduction and host defense.

4.1. Conflicting Selective Pressures on Host Defense and Reproduction Have Influenced the Frequencies of KIR Haplotypes and Genes in Human Populations

KIR haplotypes can be divided into two functionally distinct groups, A and B, which are found only within humans. KIR A haplotypes are characterized by fixed gene content that mostly consists of inhibitory receptors with a strong binding affinity (Figure 3a).^[51] The inhibitory KIR A haplotype, due to insufficient placental growth resulting from suppressed uNK activities, is associated with lower birth weight and adverse pregnancy outcomes such as pre-eclampsia, especially in combination with fetal HLA-C with the C2 epitope of paternal origin (Figure 3d).^[83–86] Interestingly, KIR A haplotypes are

associated with more effective host defenses against acute viral infections such as Ebola and hepatitis than B haplotypes,^[87,88] likely due to the attenuated KIRs of the B haplotypes and increased polymorphism of the KIR A haplotypes (Figure 3d). In contrast, KIR B haplotypes are more variable in gene content and consist of less polymorphic and attenuated KIRs, some of which are activating.^[51] In contrast to KIR A haplotypes, the combination of maternal KIR B haplotype with fetal HLA-C1 is associated with the lowest risk for pre-eclampsia.^[84,86] KIR B haplotypes are also associated with KIRs that exhibit attenuated activities or even lost binding affinity for MHC-C ligands,^[51] and therefore, are likely to confer weaker host defense against pathogens (Figure 3d).

The frequencies of KIR haplotypes and of individual KIR genes vary among human populations; the same is true of the diversity and identity of profiles of KIR haplotypes.^[89] For example, there are more KIR haplotype profiles in Ugandans than in Europeans, homozygosity for the KIR A haplotype being the most common in Ugandans. In addition, the frequency of the HLA-C2 epitope is higher in Ugandans and other sub-Saharan Africans compared to elsewhere in the world, likely because of its protective role against pathogens, such as malaria. Furthermore, *KIR2DS5*, which is present in higher frequencies in Ugandans compared to Europeans, confers protection against pre-eclampsia only in Ugandans when present in the centromeric region of KIR B haplotypes.^[84,86] In contrast, *KIR2DS1*, which is the protective gene in Europeans and not in Ugandans,^[86] is present in higher frequencies in Europeans.^[89] More broadly, it has been suggested that within a population, the frequencies of the KIR haplotypes (and HLA-C epitopes) fluctuate over time in response to changing selective pressures.^[90] For example, emergence of a novel pathogen might result in higher frequencies of KIR A haplotypes (and HLA-C1 epitope), but following successful eradication of the pathogen, increased selective pressures on reproduction could result in an increase in the frequencies of KIR B haplotypes (and the HLA-C2 epitope).^[90] In this regard, it is interesting to note that the risk for pre-eclampsia is high among sub-Saharan Africans,^[91] which is likely due to the high frequencies of KIR A haplotypes and HLA-C2 epitope.^[89] In summary, patterns of genetic variation of KIRs within any population reflect the actions of selection on both the well-regulated extent of placentation and optimal host defense against pathogens.

4.2. Different Genotypes of the Activating CD33r SIGLECs Gene Confer Opposing Selective Advantages for Host Defense and Reproductive Processes

Some CD33r SIGLECs occur as paired receptors, that is, receptors with binding affinity to almost identical ligands, but with opposing signaling motifs (Figure 4a). SIGLEC5 and SIGLEC14 are such paired receptors: SIGLEC5, via the immunoreceptor tyrosine-based inhibitory motif on its cytoplasmic tail, acts as an inhibitory receptor, while SIGLEC14 associates with the immunoreceptor tyrosine-based activation motif-bearing adaptor DAP12 and therefore acts as an

activating receptor.^[92] Interestingly, some individuals lack a functional *SIGLEC14* gene.^[93] This is due to the fusion between *SIGLEC5* and *SIGLEC14* into a single gene (*SIGLEC14/5*) that is under the control of the *SIGLEC14* promoter but is functionally equivalent to *SIGLEC5*.^[93] Interestingly, this null allele in infants is associated with incidences of preterm birth only in the context of maternal Group B *Streptococcus* rectovaginal colonization, speculated to be associated with suppressed innate immune responses (Figure 4d).^[19] This possibly protective role of the wild-type *SIGLEC14* allele against preterm birth is in contrast with its association with exacerbation of disease symptoms (Figure 4d). For instance, patients with increased *SIGLEC14* allele dosage are more likely to experience exacerbation of chronic obstructive pulmonary disease symptoms, such as tightening of airways, increased mucus production, inflammation, and reduced amount of airflow, when infected with nontypeable *Haemophilus influenzae*.^[94] This is thought to be due to the strong proinflammatory immune reactions mounted by the *SIGLEC14* wild-type allele. Similarly, the *SIGLEC14* null allele is associated with increased protection against *Mycobacterium tuberculosis* in a Vietnamese patient cohort.^[95] While the exact nature of the protective effect against this disease is yet unclear, one possible mechanism may involve IL-2 because the *SIGLEC14* null allele is associated with increased secretion of proinflammatory IL-2 in response to BCG stimulation.^[95] As IL-2 is associated with Th1-type adaptive immune responses,^[96] this could partly explain the increased efficiency with which the causative pathogen is cleared from individuals with the *SIGLEC14* null allele. In this regard, it is interesting to note that *SIGLEC14* null allele frequency varies among different populations, with the frequencies being the highest among East and South Asians, followed by middle Eastern populations and sub-Saharan Africans, and lowest in Northern Europeans.^[93]

As pregnancy requires careful immune modulation both at the local and at the systemic level, it is possible that other immunity genes have experienced selective pressures arising from both reproductive processes and host defense activities. For instance, galectins are known to be involved in both modulation of immune responses during infection^[97,98] and suppression of detrimental immune responses at the maternal-fetal interface.^[99,100] More broadly, a recent study uncovered several precisely timed events of systemic immune modulations that occur over the course of a normal pregnancy.^[101] While studies comprehensively examining the recent evolution of genes involved in such immune pathways are still lacking, we hypothesize that the patterns of genetic variation of these genes would reflect selection acting on both reproductive success and efficient host defense. In conclusion, these examples suggest that selection on reproductive success and effective host defense often acts in opposing directions, and that the patterns of genetic variation in relevant genes reflect the action of selection on both in the context of environmental factors.

5. Conclusions and Outlook

The evolution of eutherian pregnancy entailed several innovations. These innovations were enabled by extensive rewiring of

regulatory networks to incorporate pre-existing genes into pregnancy and the emergence of novel genes with pregnancy-specific roles. The regulatory rewiring was facilitated by the contribution of regulatory regions by TEs that provided binding sites for TFs with critical roles in pregnancy. Gene duplications, followed by species-specific expansions, as well as co-option of ERV-derived genes, underlie the birth of novel eutherian genes. Interestingly, genes involved in immune regulation often gain expression within reproductive tissues^[21] and also undergo duplications within eutherians.^[35,39,53] This could partly be explained by the need for precise regulation of the maternal immune system due to the extended direct maternal-fetal contact. Importantly, the maternal immune system is not globally suppressed: distinct components of the immune system are activated at different stages of pregnancy^[10,78,80] to facilitate optimal fetal growth. This careful immune modulation must occur while retaining effective host defenses. Infectious diseases result in considerable mortality, and therefore, pathogens strongly influence human genetic variation among different populations.^[102] Therefore, the patterns of genetic variation of such genes also reflect adaptation to local pathogen threats.

More broadly, regulatory changes, in contrast to protein-coding changes, are more likely to be modular; for example, modifications of regulatory elements can alter gene expression in a particular context without affecting expression in others, whereas changes in the protein-coding parts of genes typically influence the protein's function in all contexts. The modular organization of regulatory elements can therefore facilitate phenotypic evolution while minimizing pleiotropic effects.^[12,13,103] Indeed, much like the rest of mammalian as well as recent human evolution, the emergence of eutherian pregnancy is associated with novel regulatory elements, which are often involved in modulating the transcriptional activities of genes involved in immune responses, metabolism, and other pregnancy-specific processes.^[21,22,27] However, studies investigating patterns of genetic variation of regulatory regions involved in pregnancy and reproductive tissues are lacking and a gene-centric paradigm still dominates our thinking of the evolution of eutherian pregnancy. We believe that our understanding of the evolution of eutherian pregnancy will be greatly enhanced by focusing on the evolution of regulatory regions involved and their roles in this unique and remarkable biological process.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

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