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The inflammation paradox in the evolution of mammalian pregnancy: turning a foe into a friend

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A widely discussed physiological puzzle of mammalian pregnancy is the immunological paradox, which asks: why is the semi-allogenic fetus not attacked by the mother's adaptive immune system? Here, we argue that an additional, and perhaps more fundamental paradox is the question: why is embryo implantation so similar to inflammation while inflammation is also the greatest threat to the continuation of pregnancy? Equally puzzling is the question of how this arose during evolution. We call this the *inflammation paradox*. We argue that acute endometrial inflammation was ancestrally a natural maternal reaction to the attaching blastocyst, a situation still observed in the opossum. Eutherian implantation arose through a transformation of the acute inflammation into a process essential for implantation by causing vascular permeability and matrix reorganization as well as by suppressing the effects deleterious to the fetus. We propose that this model allows us to understand the differences between 'good inflammation' and 'bad inflammation'. Further, it allows us to understand the influence of inflammation on the outcome of pregnancy and maternal health.

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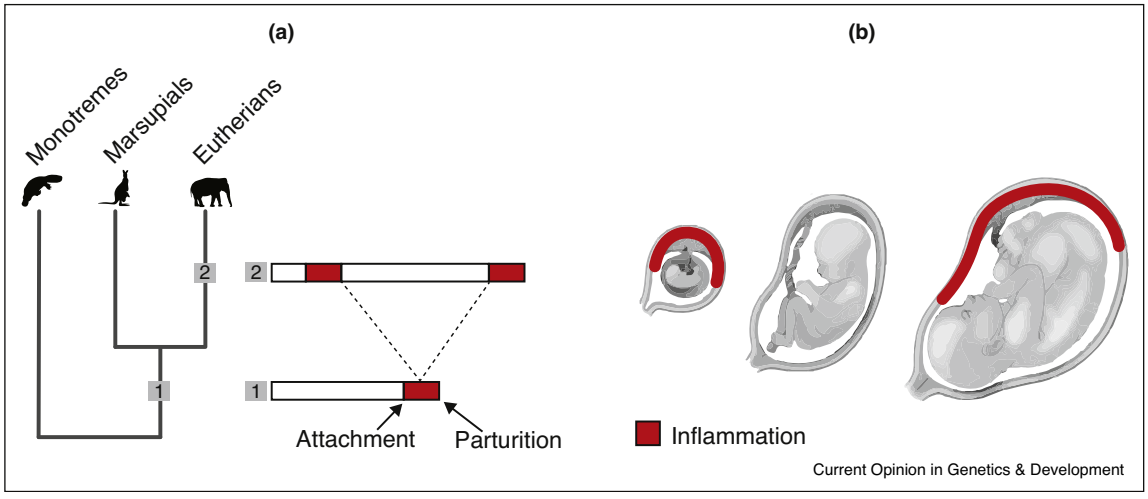
Mammalian pregnancy presents multiple biological *paradoxa*

Pregnancy, as it presents itself in humans, is a complex multistage process starting with fertilization followed by attachment and implantation of the blastocyst, the 'recognition' of pregnancy by the mother, the development, growth and maturation of the fetus including the placenta, and finally parturition. Not all of these processes happen in other animals that give birth to live offspring, i.e. are viviparous. In fact, what is commonly referred to as 'mammalian' pregnancy is quite unique and different from other forms of viviparity, for instance that in sharks or reptiles (see Figure 1a) [1–6]. More precisely, what is commonly referred to as 'mammalian' pregnancy is actually only found in the eutherian mammals (aka 'placental' mammals, even though marsupials also have a placenta). Eutherian pregnancy ancestrally involved a highly invasive conceptus (blastocyst, embryo or fetus) [7–9], where the fetus breaches the basal membrane of the uterine epithelium and further maintenance of pregnancy requires the 'acceptance' of the fetus by the mother, that is, the recognition of pregnancy. This process results in the creation of a *fetal–maternal unit*. To the best of our knowledge, this highly integrated form of pregnancy is limited to eutherian mammals (Figure 1a). Here we discuss a unique feature of this form of pregnancy, which we call '*the inflammation paradox*', namely that this form of pregnancy requires overruling the natural mechanisms responsible for the maintenance of tissue integrity. We will argue that this obstacle for the evolution of an extended pregnancy is different from and in addition to the well-recognized 'immunological paradox' proposed by Medawar in 1953 [10]. It has even been argued that the concept of the immunological paradox, which conceptualizes the fetus as a semi-allograft, has been misleading both for research and for clinical practice [11–13].

Gynecologists have long recognized that there is a puzzling relationship between inflammation and pregnancy. Human gestation can be roughly divided into three major phases: implantation, development and growth, and parturition [11] (Figure 1b). Signs of inflammation have been found during implantation and parturition but are normally absent during the middle phase of pregnancy [12]. On the other hand, inflammation, in particular inflammation of the fetal membranes, is understood as *the* major threat to the maintenance of pregnancy often leading to abortion or premature birth [14]. This paradox led some gynecologists to distinguish between 'good inflammation' and 'bad inflammation' (Gil Mor, personal communication). Here we want to briefly review the 'good inflammation' during implantation and parturition.

Figure 1



Inflammation in pregnancy. **(a)** Extended eutherian pregnancy (as seen in humans) evolved by the insertion of an anti-inflammatory phase in the attachment-induced inflammatory reaction that ancestrally directly led to parturition instead of a sustained fetal–maternal interface. Figure based on [27]. **(b)** Anti-inflammatory phase in human pregnancy is sandwiched between two inflammatory phases, those associated with implantation and parturition. Figure adapted from [12].

During implantation the endometrial lining of the uterus shows many signs of an inflammatory process. The pro-inflammatory Th1 type signals increase, most notably IL1, IL6, IL8, LIF, and TNF. In addition, the density of leukocytes also increases, including natural killer cells (NK), macrophages (Mph) and dendritic cells (DC) [15], but not neutrophils. These signs of inflammation are expected in species with invasive placentation, like humans. During implantation the blastocyst erodes the endometrial epithelium, invades the underlying endometrial stroma and partially destroys the blood vessels. The absence of neutrophils suggests that to some extent this inflammatory reaction has been curbed to prevent a full blown immune response, as neutrophils are the first immune cells recruited to the site of infection and typically amplify the inflammatory signal attracting other immune cells. The modified inflammatory response can be seen as the beginning of tissue stabilization necessary for the accommodation of the placenta.

There are several lines of evidence that parts of the inflammatory pathways are necessary for the establishment of pregnancy. The most direct evidence comes from experiments in mice where the dendritic cells (DC) have been depleted [16]. This treatment leads to implantation failure and resorption of the blastocysts. This outcome happens even in syngenic matings with impaired T-cell response and thus is not due to a dysregulation of the adaptive immune response. Another widely cited piece of evidence is that endometrial injury due to biopsy prior to *in vitro* fertilization treatment dramatically increases the chance of implantation [17], at the site of the endometrial scar. Inflammation prone parts of the uterus are preferred sites of implantation. These examples and additional evidence lead to the hypothesis that, the inflammatory reaction to tissue injury in the receptive uterus has been modified into the implantation cascade.

Inflammation was originally studied as part of the response to infection. While it is true that the adaptive immune system requires an inflammatory process from the so-called innate immune system to become activated, it is not true that infection is necessary to induce inflammation. A moment's reflection suffices to show that this is true. For instance, if someone sprains their ankle, they will suffer an inflammatory reaction, with the typical signs of swelling, redness and pain. Yet it is likely that their ankle did not contract an infection unless the injury also broke the skin. Hence, inflammation happens without infection. Increasingly inflammation is being seen as a general reaction to compromised tissue integrity, regardless of the reason [18,19]. This also explains the observation of inflammation in diabetes resulting from stress of adipocytes, and not due to infection [20].

Since implantation happens through destruction of maternal tissue, it is understandable that implantation of the human blastocyst activates, at least partially, the inflammatory pathway. While previous work has shown that the human implantation process is in fact necessary for the establishment of pregnancy, it must have been a major obstacle for the origin of the eutherian mode of pregnancy. Any form of tissue irritation, even from a mother's own fetus, is expected to elicit inflammation that would attack the irritant, regardless of the allogenic status of the fetus. Given that an inflammatory pathway is

Box 1 Diversity of mammalian reproduction and placentation

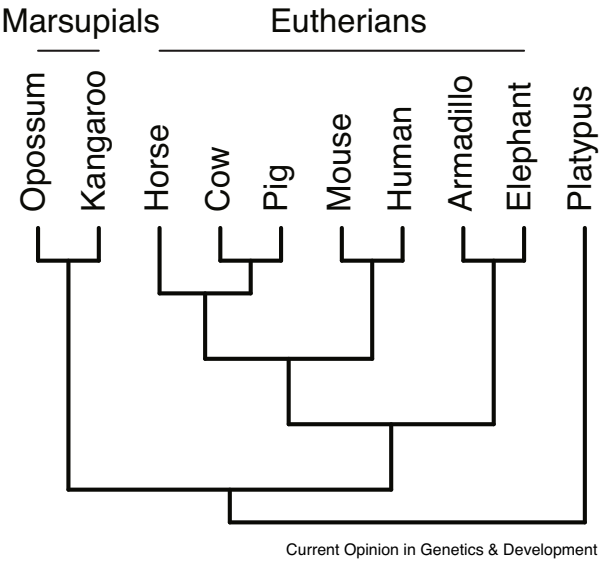
Pregnancy, in the specific sense of the term as used here, is *not* a shared feature of all mammals. To contextualize what we will say about the evolution of pregnancy in mammals we give a very brief overview of mammalian evolution and reproduction. Extant mammals fall in three major clades, monotremes, marsupials and eutherian mammals, where marsupials and eutherian mammals are more closely related to each other than each of them is to monotremes (Box Figure 1). Monotremes are egglaying mammals; they contain the definitive features of mammals (hair and mammary glands for producing milk), but still retain many 'reptiliomorph' features, both in their skeleton as well as their reproduction [73,74]. They include only five species and come in two kinds, platypus and echidna. Monotremes lay eggs and incubate them between 10 and 12 days until the hatching of very immature young, similar to the neonates of marsupials. The young are nourished by the milk of the mother that is secreted from a diffuse hair patch at her abdomen, that is, there are no distinct nipples.

Even though monotremes lay eggs, there is some evidence that development within the eggshell does not preclude maternal provisioning of the growing fetus within the egg. Comparisons of the volume of the unfertilized oocytes and the size of laid eggs show that nutrition has to pass from the mother through the eggshell to the fetus. That is to say that one aspect of the derived form of pregnancy, maternal provisioning to the fetus *in utero*, arose before invasive placentation and pregnancy evolved [1]. Thus, the evolution of pregnancy is not coincidental with the origin of maternal provisioning during fetal development and growth, but rather pregnancy is an elaboration of an oviparous condition where eggs were provided with additional nourishment in the uterus prior to oviposition.

Marsupials are a morphologically diverse group of animals that includes the new world opossums, and a diversity of forms found in Australia. Australian marsupials include charismatic species such as kangaroos, koalas, and wombats as well as lesser known species such as the dasyurids (carnivorous marsupials) and possums (which are phylogenetically distantly related to opossums). While for the most part, marsupial pregnancy is relatively consistent between major groups, there are some traits that are variable. The most extreme differences exist between the lineages that are distantly related, the opossums at one end and the macropodids (wallabys and kangaroos) at the other. In the case of opossums, females ovulate multiple eggs and this is induced by male pheromones. Gestation is very short, about two weeks, during which the fetus remains in the egg coat for up to 12 days. This is followed by a short period (2–3 days) of superficial placentation (attachment) and then parturition of highly immature neonates. The hormonal profile of the female during gestation is virtually indistinguishable from that during an estrus cycle without fertilization and thus there is no or at most a very limited recognition of pregnancy [75]. In contrast, in wallabies there is definite recognition of pregnancy, gestation is longer, 33–38 days, but gestation still ends with the birth of a very immature neonate that spends up to 15 months attached to the nipple of the mother during an extensive postnatal phase of development and growth. The wallaby type of gestation is clearly derived within the marsupials, given the position of macropods in the marsupial tree of life [76–78].

The female reproductive biology of eutherian mammals is also highly diverse, even more so than that of marsupials. The availability of well resolved phylogenetic trees for mammals has led to a consensus about the ancestral form of female reproductive biology. Phylogenetic evidence suggests that the ancestor of eutherian mammals had an invasive placenta, although there is still some question of the degree of invasiveness [7–9,79]. That implies two important conclusions for our argument: firstly, that non-invasive forms of placentation, as found in cows, horses, pigs and other mammals, have evolved secondarily from ancestors with invasive placentation, and are not homologous to the non-invasive form of placentation found in marsupials; secondly, evolution of invasive placentation in eutherians must have involved many biological innovations. It is these events that we discuss in this paper. These include: the recognition of pregnancy, the ability of the mother to tolerate the partial destruction of the inner uterine lining (the endometrium) and extended gestation. All of these innovations can be seen as a complex collection of traits which jointly establish the eutherian mammalian form of pregnancy.

Box Figure 1



Mammalian phylogeny. Phylogenetic relationship between mammalian species.

activated at implantation, how has tolerance to an invasive embryo evolved without an immediate destruction of the fetus? This is what we call the *Inflammation Paradox*. To approach this question, we first turn to the closest relatives of eutherian mammals, the marsupials, e.g. opossums, wallabies, kangaroos and others. See Box 1 for an overview of the evolution of mammalian pregnancy.

Pregnancy and inflammation in marsupials

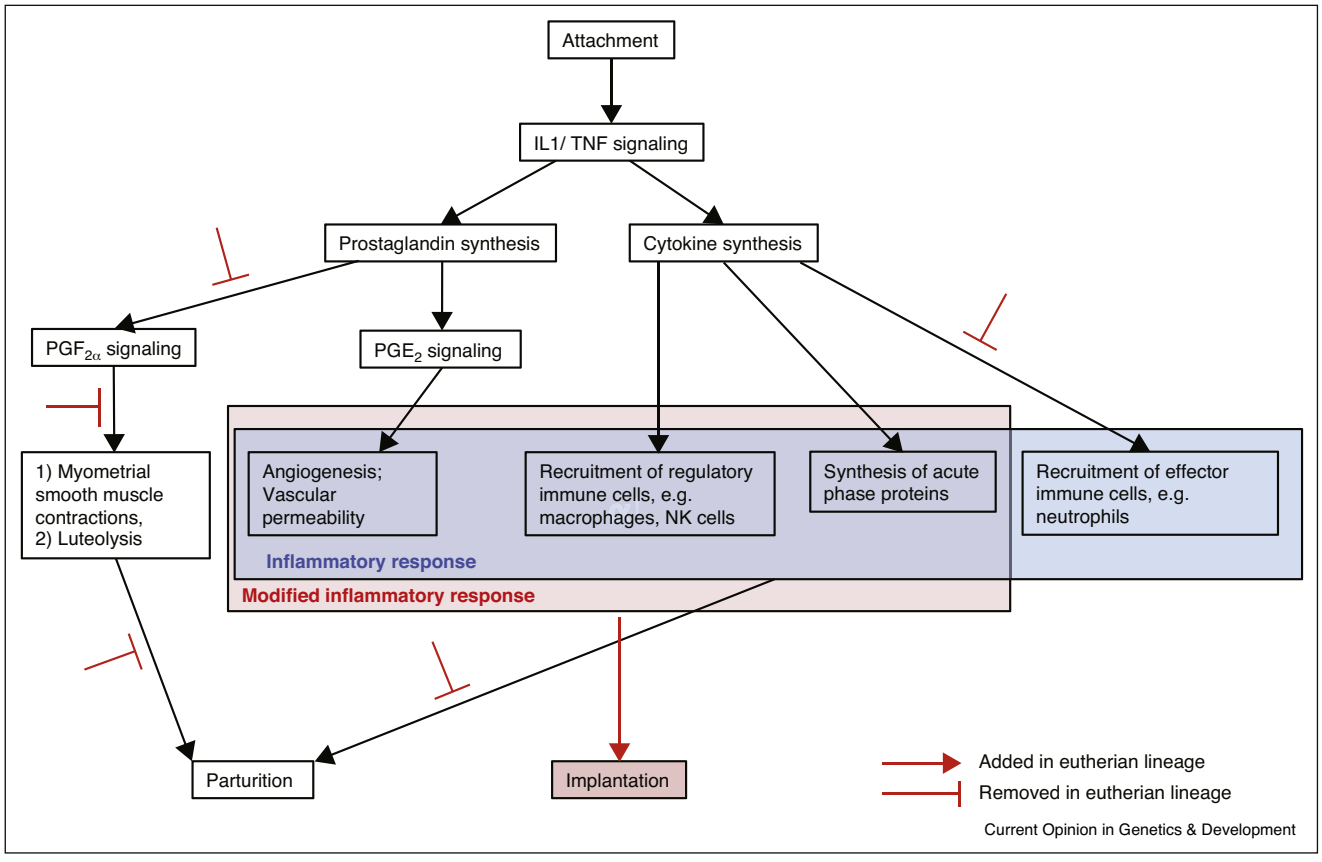
Even though marsupials are viviparous and placental, their form of placentation and gestation is very different from that of eutherian mammals and also varies among marsupials. Most research on marsupial reproduction has been done on opossums and wallaby, although there is also a considerable body of work on other marsupial species (for an overview see [21–24]). Opossums and wallabies represent two extremes among the marsupials, with opossum likely more ancestral with respect to the biology of the female reproductive tract while wallabies are more derived [25]. Opossums have a short gestation of 14.5 days post copulation, where the conceptus retains the

egg-coat for most of the time, up to 11.5–12 dpc ([26]; see also [27]). After that, the conceptus attaches to the uterine lining (endometrium) and is born two to three days later. This form of pregnancy is non-invasive, and has been called 'intra-cyclic' since it is shorter than the non-pregnant ovarian/estrus cycle and there is no evidence for maternal recognition of pregnancy [28]. In wallaby the gestation is longer, and there is definite recognition of pregnancy, clearly an independently evolved situation [29–31]. Below we briefly summarize what is known about the fetal–maternal interaction in the 'laboratory' opossum, *Monodelphis domestica*.

After fetal attachment the opossum uterine transcriptome is characterized by expression of immune related genes [27], including IL1A, IL6, TNF, PTGS2 (aka COX2), PTGES (present in trophoblast tissue), IL17A, and neutrophil elastase. This is consistent with the attachment resulting in acute inflammation followed by parturition. This progression from inflammatory attachment to parturition is quite different from the situation in eutherians including humans, where the implantation is followed by an extended anti-inflammatory or non-inflammatory period (Figure 1). In addition, there is evidence that the inflammation related prostaglandin Prostaglandin F2 alpha ($PGF_{2\alpha}$) is a key factor for regulating parturition in marsupials. $PGF_{2\alpha}$ is necessary and sufficient to induce parturition behavior in the wallaby, and is required for normal luteolysis [32,33]. Furthermore, $PGF_{2\alpha}$ is sufficient to induce parturition behavior in the grey short tailed opossum [34].

Given that in the opossum fetal attachment leads to acute inflammation, it is likely that the implantation cascade in humans and other eutherians evolved from a classical mucosal inflammation by suppressing effects deleterious to the fetus and the maintenance of the beneficial effects. The beneficial effects may include increase in vascular permeability and remodeling of the extracellular matrix. In the next section we will discuss the kind of evolutionary modifications the uterine inflammation underwent to enable extended pregnancy.

Figure 2



Model for the evolution of eutherian implantation from attachment-induced inflammation. Signaling pathways shown in this figure mediate the attachment-induced inflammatory reaction and subsequently parturition in opossum and presumably in the therian ancestor. Modules of a genuine inflammatory response activated at attachment are shown in blue box. Red arrows represent evolutionary changes in the eutherian lineage, which modify the inflammatory response (red box) in a way that leads to implantation rather than immediate parturition.

Evolution of eutherian implantation from attachment-associated inflammation

Here we propose a model (Figure 2) for the evolutionary origin of implantation from an ancestral inflammatory reaction to blastocyst attachment. We think that in the therian ancestor, pregnancy was similar to that still observed in the opossum as described above. In the eutherian lineage, the attachment-associated inflammation evolved into an implantation reaction by modification of two pathways: firstly, prevention of neutrophil infiltration, and secondly, downregulation of $PGF_{2\alpha}$ signaling to prevent luteolysis and myometrial contraction. These two modifications ensure that embryo attachment does not result in acute inflammation and the destruction of the conceptus. The parts of the ancestral inflammation retained in the implantation reaction are as follows: firstly, Prostaglandin E2 (PGE_2) signaling leading to vascular permeability, secondly, activation of regulatory immune cells such as macrophages and NK cells, and thirdly, production of acute phase proteins. These retained modules facilitate endometrial as well as vascular remodeling necessary for implantation. Below we review shared features between acute inflammation and implantation, and point out the differences between the two that support this model.

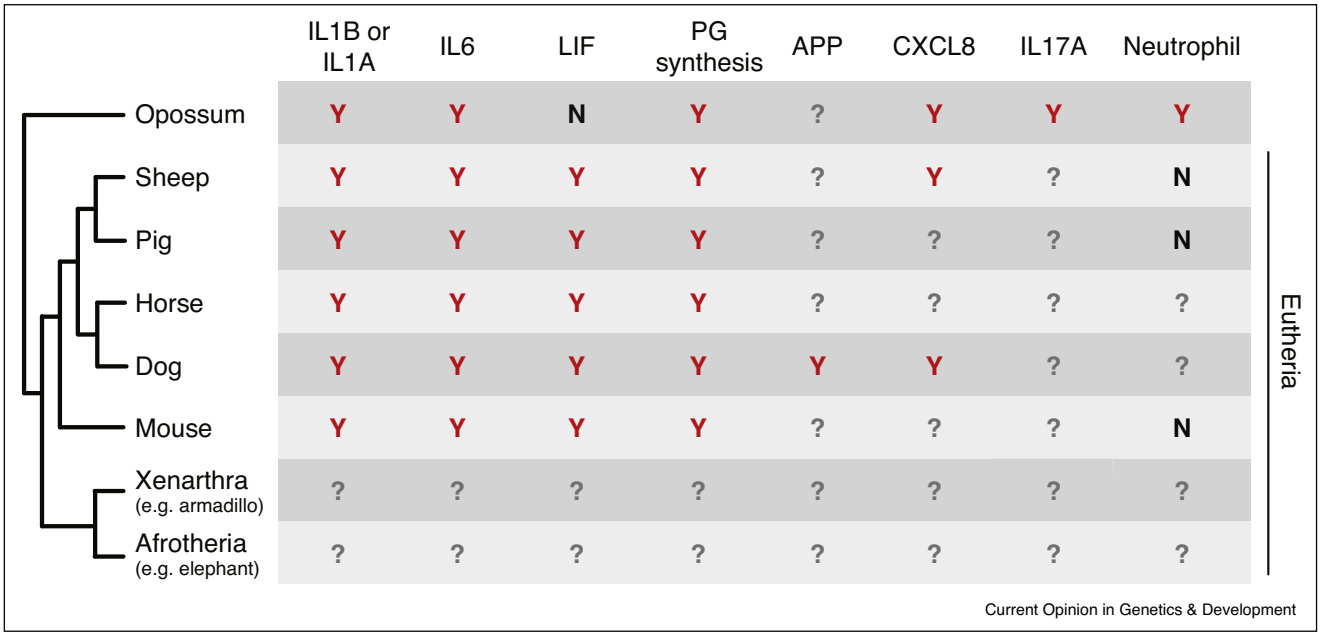
Shared features of eutherian implantation and inflammation

Interleukin-1 cytokines (IL1B and IL1A) are involved in the implantation process in nearly all eutherians [35,36]. These two cytokines signal via binding to the same receptor. Upon injury, IL1 is among the earliest cytokines produced. Secreted IL1 binds the receptor IL1R1 and activates NFκB signaling. The role of IL1B has been studied more extensively than that of IL1A. While there is variation among species in the tissue of origin, IL1B is typically produced by the blastocyst, and its receptor is present in the endometrial epithelium, where NFκB signaling leads to production of LIF, IL6, and PTGS2 (aka COX2). The fact that the blastocyst produces the inflammatory cytokine indicates that the inflammatory reaction is in the evolutionary interest of the fetus.

Similarly LIF, IL6, and PTGS2 are expressed at the fetal–maternal interface in all eutherians examined, and their expression is crucial for successful implantation. LIF and IL6 are multifunctional cytokines that signal to various cell-types in the endometrium to prepare for implantation, e.g. in decidualizing species, LIF is an important signal for the decidualization of endometrial stromal cells [37]. PTGS2 encodes a rate-limiting enzyme in the synthesis of prostaglandins.

The data about cytokine signaling in the fetal–maternal interface described above (summarized in Figure 3) are derived from well-studied species such as mouse, human, ruminants, and carnivores, belonging to the clade of Boreotheria within Eutheria. We are not aware of any studies investigating molecular agents of inflammation at

Figure 3



Mediators of inflammation at the implantation stage fetal–maternal interface in select therian mammals. Y, present/expressed; N, absent/not expressed; ?, not known; PG, prostaglandin; APP, acute phase proteins. References: opossum ([27], mRNA expression of neutrophil elastase used as a proxy for neutrophils), sheep [48,55–57], pig [35,42,55,58–60], horse [61–63], dog [64–66], mouse [44,46,67–72]. There are no reports on the expression or the role of these inflammation mediators in Xenarthra and Afrotheria.

the fetal–maternal interface in the most basally branching eutherian clades, Xenarthra (e.g. armadillo, sloth, anteater) and Afrotheria (e.g. elephant, hyrax, tenrec). However, given the invasive nature of fetal tissue [38,39] we might expect that the physiology of these lineages is consistent with that of other eutherian clades. Detailed molecular studies of the fetal–maternal interface in these species are needed to establish that inflammation is an ancestral feature of eutherian implantation.

Differences between implantation and inflammation

While the molecular and histological changes leading to implantation have compelling similarities to the classical inflammatory reaction, they do not represent acute inflammation but instead a modified tissue state that sets the stage for the establishment of the fetal–maternal interface. Implantation is different from inflammation in the following ways:

1) *Neutrophil infiltration:* Unlike acute inflammation, implantation is not associated with neutrophil infiltration. Neutrophils are one of the first cell-types recruited to the site of inflammation. Their non-selective secretion of digestive enzymes helps clear pathogens but also causes damage to the host tissue. During implantation, neutrophil infiltration is not observed.
   At least in eutherian mammals, copulation induces an acute inflammatory response in the female reproductive tract [40,41]. This provides a useful comparison for implantation since it occurs in the same tissue and is a genuine immune response to the threat of infection that could occur following copulation. Copulation-induced inflammation results in huge infiltration of neutrophils, macrophages, and dendritic cells into the endometrium. However, this response lasts for only about a day, with neutrophilia resolved within 24 hours [42–44]. Neutrophil density comparable to this is not observed in the uterus during implantation [42,45–47].
   Although inflammatory signaling occurs during implantation, the first line of inflammatory defense, that is, neutrophils, are prevented from entering the endometrium. The mechanisms by which neutrophil infiltration is prevented are unclear, but one likely mechanism is suppression of the cytokine signaling involved in neutrophil recruitment. In the context of pregnancy, CXCL8 (aka IL8) and IL17A are two such cytokines. CXCL8 is reported to be expressed at implantation stage in mouse, human, and sheep, but its role in neutrophil recruitment may be suppressed, perhaps by progesterone. In sheep, removal of corpus luteum (the main site of progesterone production) during pregnancy leads to neutrophil infiltration into the uterus [48]. We were not able to find any studies investigating the expression of IL17A specifically during implantation.
2) *Prostaglandin signaling:* Prostaglandins are produced during acute inflammation. Their activity leads to the cardinal signs of inflammation; vascular permeability causing redness and swelling, as well as pain and fever. The principal prostaglandins in the inflammatory response are PGE_2 and $PGF_{2\alpha}$. $PGF_{2\alpha}$ also induces contraction of myometrial smooth muscles during parturition, and luteolysis [49]. Both myometrial contractions and luteolysis would be detrimental to the maintenance of pregnancy. Accordingly, PGE_2 signaling is emphasized and $PGF_{2\alpha}$ signaling is reduced during eutherian implantation [49,50].

Based on these observations, we suggest that the evolution of extended eutherian type pregnancy consisted of two steps: 1) intra-uterine 'hatching' of the blastocyst and attachment to the uterus. Ancestrally, this led to an acute inflammatory reaction limiting the duration of gestation. This is the situation we still find in opossum. Nevertheless, the attachment-induced inflammation also had positive effects, most notably an increase in permeability of the maternal blood vessels, which is still a sign of implantation in eutherians [51,52]. This model is supported by the fast rate of fetal growth during the short attachment phase in the opossum. 2) To extend the gestation beyond that which can be sustained during an acute phase inflammation, two components of the inflammatory reaction needed to be modified: a) suppression of neutrophil infiltration, and b) suppression of $PGF_{2\alpha}$ signaling. Suppression of $PGF_{2\alpha}$ is a key event in the recognition of pregnancy, preventing both luteolysis and uterine contraction. The exact molecular mechanisms that enabled these changes are phylogenetically variable [49].

Conclusions and future directions

In this paper we argue that the biggest challenge in the evolution of extended pregnancy (as seen in eutherian mammals) was that of overruling the attachment-induced inflammation. Implantation leads to tissue destruction, which leads to the activation of the inflammatory response, which in turn, if not checked, would lead to the destruction of the conceptus. This problem is highlighted by the similarity between acute inflammation and implantation in humans and other eutherians. We identify two key differences between acute inflammation and implantation: firstly, the exclusion of neutrophil infiltration, and secondly, a decrease in the signaling of $PGF_{2\alpha}$. The first prevents the destruction of the conceptus at implantation, the second prevents luteolysis and uterine contraction. How this result was achieved in evolution is of yet unclear. We suggest that a more complete understanding of the species differences in the regulation of attachment and implantation in marsupials and basally branching eutherian lineages, like Afrotheria (e.g. elephants, hyrax) and Xenarthra (e.g. armadillo) will be critical to understand how the pro-gestational state was achieved in evolution and how

inflammatory pathways participate in the establishment of a successful pregnancy in women. Our model identifies specific modules of inflammatory reaction that were coopted into implantation process. Teasing apart the puzzling relationship between inflammation and implantation would facilitate further improvements in assisted reproductive techniques, for example, *in vitro* fertilization, for which implantation remains the rate-limiting step.

Furthermore, we argue that the inflammation paradox is a new way of understanding the role of the immune system in pregnancy, which may or may not be generalizable to independently evolved viviparous lineages. This paradigm should be tested in viviparous lizards, which have evolved viviparity using maternal and fetal tissues homologous to those in mammals [53,54].

Conflict of interest statement

Nothing declared.

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