Peer-reviewed version available at *Integrative and Comparative Biology* **2020**; doi:10.1093/icb/icaa030

The Primacy of Maternal Innovations to the Evolution of Embryo Implantation

Daniel J. Stadtmauer[1, 2] & Günter P. Wagner[1,2,3,4]
1. Department of Ecology and Evolutionary Biology
Yale University
2. Yale Systems Biology Institute
3. Department of Obstetrics, Gynecology and Reproductive Sciences
Yale University
4. Department of Obstetrics, Gynecology
Wayne State University

Daniel J. Stadtmauer <daniel.stadtmauer@yale.edu>
165 Prospect Street, New Haven, CT, USA
ORCID ID 0000-0001-6249-5924

Günter P. Wagner <gunter.wagner@yale.edu>
300 Heffernan Drive, West Haven, CT, USA
ORCID ID 0000-0002-3097-002X

Abstract

Embryo implantation is a hallmark of the female reproductive biology of eutherian (placental) mammals and does not exist in a sustainable form in any other vertebrate group. Implantation is the initial process that leads to a sustained fetal-maternal unit engendering a complex functional relationship between the mother and the embryo/fetus. The nature of this relationship is often portrayed as one of conflict between an aggressive embryo and a passive or defensive maternal organism. Recent progress in elucidating the evolutionary origin of eutherian pregnancy leads to a different picture. The emerging scenario suggests that the very initial stages in the evolution of embryo implantation require evolutionary changes to the maternal physiology, which modified an ancestral generic mucosal inflammation in response to the presence of the embryo into an active embedding process. This "female-first" evolutionary scenario also explains the role of endometrial receptivity in human pregnancy. On the marsupial side, where in most animals the fetal-maternal interaction is short and does not lead to a long term sustainable placentation, the relationship is mutual. In these mammals uterine inflammation is followed by parturition in short order. The inflammatory signaling pathways, however, are cooperative, *i.e.* they are performed by both the fetus and the mother and therefore we call this relationship "cooperative inflammation." Based on these discoveries we reconceive the narrative of the maternal-fetal relationship.

Keywords: pregnancy; implantation; invasion; conflict; evolutionary innovation; feminism

Peer-reviewed version available at *Integrative and Comparative Biology* **2020**; doi:10.1093/icb/icaa030

Introduction

Science is a human endeavor, and as such cannot be fully removed from other elements of society. Sexual reproduction, fundamental to the process of evolution by natural selection as our domain of life experiences it, is also deeply ingrained within human culture. Inevitable spillover of influence from culture can prejudice the ways in which scientific findings are interpreted and which kinds of questions are deemed worthy of pursuit. As culture evolves, as we look at old problems with fresh eyes, and as new experimental results come to light, the biological narratives in use change. Of the topics subsumed under the heading of reproduction, implantation biology in particular is currently undergoing a conceptual reassessment due to a greater appreciation of the mother's active role in this process and the fetal-maternal interface as an integrated unit of function and the pivotal role of maternal evolutionary changes to enable implantation and eventually the establishment of the placenta. In this essay we introduce such a view and discuss its implications for the evolutionary narrative of mammalian viviparity.

The pregnant mother and her fetus can be seen as distinct and opposing forces. Within such a framework, functions that involve both parties may be dominated by one or the other. Pregnancy is bookended by two key processes, the process of uterine implantation into the uterine stroma at the beginning and the initiation of parturition at the end. Driving questions within this line of inquiry therefore include whether the fetus or the mother is in control of the degree of placental invasion, and ultimately, whether the timing of parturition is under fetal or maternal control. Traditional narratives propose that the intrauterine embedding process in placental mammals is a fetal anti-maternal invasion that enables extended gestation, whereas the equivalent inflammatory stage in marsupial pregnancy is a maternal anti-fetal immune rejection that leads to immediate parturition. However, investigation of implantation in these two lineages suggests greater complexity, with the seemingly passive or defensive parties in each case having a more active role. Rather than merely changing attributions of control, we propose that the fetal-maternal interaction is functionally integrated and cooperative – although not necessarily harmonious.

Evolutionary History of Mammalian Viviparity

Viviparity, defined as development of offspring directly within the parent unseparated by the shell coat, has evolved over one hundred times in jawed vertebrates (gnathostomes), including chondrichthyans, teleost fish, amphibians, squamates, and mammals (Blackburn 2015). Viviparity most likely evolved only once in mammals, from an oviparous ancestral state like that of living monotremes, in the stem lineage of therian mammals (Lillegraven 1969; Marshall 1979) during the late Triassic or early Jurassic period (Madsen 2009). Viviparity was further elaborated in both of the living therian lineages, marsupials (metatherians) and placental (eutherian) mammals, in ways distinct from other lineages in which it independently arose. The maternal-fetal relationship established in the pregnancy of placental mammals, where extraembryonic fetal membranes come into direct contact with maternal blood and can embed into the uterine stroma, is unparalleled among other live-bearing gnathostomes in its intimacy and degree of physiological integration.

The question is how the mammalian viviparous condition was modified over the course of evolution to give rise to the major patterns we see today. The challenge is that the details of pregnancy are not easily discernible from the fossil record, nor are many intermediate phenotypes retained in the extant mammalian phylogeny. We do know of at least three important evolutionary events. The first was the origin of viviparity itself and maternal-fetal attachment (Griffith *et al.* 2017) and of the placental organ in the stem lineage of therian mammals. The mammalian placenta evolved as a novel apposition of previously existing tissues (Müller and Wagner 1991), the fetal extraembryonic membranes and uterine mucosa, by loss of the shell coat separating them (Griffith and Wagner 2017);

Peer-reviewed version available at *Integrative and Comparative Biology* **2020**; doi:10.1093/icb/icaa030

the placenta, so defined, can evolve easily in tandem with viviparity (Blackburn 1995). Second, placental mammals saw major maternal innovations in the origin of the decidua (Mess and Carter, 2006) and hemochorial placentation (Wildman *et al.* 2006; Elliot and Crespi 2009) in the stem lineage, and likely multiple origins of interstitial embryo implantation (Mess and Carter 2006) and extended gestation (Chavan *et al.* 2016) in the crown. In the marsupial lineage, alternative strategies were pursued (Renfree 1981). In contrast to the many eutherian lineages with extended gestation, marsupials, with the exclusion of the macropods such as kangaroos and wallabies, exhibit nearly universal short gestation, which is complemented by specialized lactation. Their implantation biology, rather than evolving deep interstitial implantation, became a cooperative inflammatory process linked to parturition (Stadtmauer and Wagner 2020).

There is a tendency to tell this 170 million year old story as the conquest of the fetus in achieving invasion into uterine territory, overcoming maternal immune defenses, or winning increased maternal investment. By dividing this particular history into a series of evolutionary steps or challenges that had to be overcome, we find that along the way the mother has been an active agent in the evolving physiology of pregnancy as has the fetus. We propose and attempt to exemplify the study of mammalian pregnancy from a perspective in which the mother has an active role in development and evolution, and demonstrate how this has and continues to inform an active research program on the evolution of decidual-placental interactions.

Did the Evolution of Pregnancy Violate Physiological Homeostasis?

Pregnancy has traditionally been seen as a deviation from homeostasis, and thus paradoxical. Organisms maintain their own stability and integrity through homeostatic processes (Cannon 1929). The immune system and inflammation likely evolved as an extension of homeostasis for perturbations too great to be handled by normal homeostatic processes (Medzhitov 2008; Kotas and Medzhitov 2015). Medawar's (1953) immunological paradox asks why the presence of paternally-derived alloantigen in the fetus does not elicit a graft rejection-like response in the mother. Indeed, the adaptive immune system, a shared derived character of gnathostomes, preceded the evolution of pregnancy in therian mammals. Therefore, explaining how pregnancy was superimposed on a physiological condition which had already evolved to reject foreign bodies has become a goal of reproductive immunobiology.

Inflammation due to compromised tissue integrity, executed by the innate immune system, is independent of and more immediate than immune rejection according to Medawar's theory which requires the activation of the adaptive immune system. For instance allograft rejection takes 19 days in the opossum (Stone *et al.* 1997) whereas the first viviparous mammals likely had much shorter post-attachment periods of gestation, on the order of several days (Zeller and Freyer 2001; Chavan *et al.* 2016). Injury-reduced inflammation, on the other hand, is induced within minutes to hours. The inflammation paradox (Chavan *et al.* 2017), a necessary addition to Medawar's scenario, asks why inflammatory processes are at times indispensable for successful pregnancy, but are also sufficient to elicit premature parturition if induced at the wrong time. This paradox is apparent on two levels. At the physiological level it results from the observation that embryo implantation and parturition are pro-inflammatory processes, while the growth phase of human pregnancy requires an anti-inflammatory regime at the fetal-maternal interface (Mor *et al.* 2011). At the evolutionary level, the paradox arises from the fact that the embryo compromises the tissue integrity of the endometrium and as such should elicit an inflammatory response, which should lead to immunological destruction of the embryo (Chavan *et al.* 2017). From these considerations, it follows that the evolution of pregnancy first required an evolutionary change in the maternal physiology that attenuates the inflammation in response to embryo attachment (Chavan *et al.* 2017), only after which could gestation be extended to the point that the adaptive immune response became relevant.

Peer-reviewed version available at *Integrative and Comparative Biology* **2020**; doi:10.1093/icb/icaa030

Traditional views of pregnancy see the physiology of pregnancy as a deviation from homeostasis, the correction of which must actively be maintained by the fetus and imposed upon the mother via the placenta. In this view, however, pregnancy is an evolutionarily unstable state. Instead, as suggested by recent evolutionary research (*e.g.* Griffith *et al.* 2017), pregnancy can be thought of as an alternate homeostatic state, where the system regulated is the fetal-maternal unit and does not consist in a manipulation of the mother by the fetus (Nuña de la Rosa *et al.* 2019). Inflammation upon embryo attachment and at parturition is the product of tight regulation, both positive and negative (Pavličev *et al.* 2017). The key feature of this view is not suppression of a defensive processes, as such, but rather a shift in homeostatic set points. Inflammation, as a defense against potential survival threats of injury and infection, carries higher "physiological priority" than normal homeostatic mechanisms and therefore can override them (Kotas and Medzhitov 2015). The maintenance of homeostatic variables such as vascular permeability, angiogenesis, blood pressure, and plasma protein delivery outside of their normal homeostatic range is standard in acute inflammation, and these same variables are central to the establishment of an integrated maternal-fetal physiology. Cooption of inflammatory processes is therefore an intriguing potential way how the shift into a physiological state conducive to extended pregnancy was achieved. This evolutionary perspective suggests that the maternal contribution to the evolution of implantation was more than a destructive tendency that had to be turned off; it was also a constructive force harnessed in the evolution of a novel physiological state.

Is Implantation a Tissue Biological Paradox?

Solution of the inflammation paradox was a necessary prerequisite for the evolution of embryo implantation, the process in placental mammals in which the fetus becomes embedded within the endometrium. Embryo implantation leads to a radical deviation from the oviparous maternal-fetal tissue topology. Ancestral amniote reproduction entails internal conception and the passage of zygotes down the Müllerian-derived ducts (Lombardi 1998), the vestment of the zygote in various eggshells and shell coats (Frankenberg and Renfree 2018), and eventual release from the body. Extended gestation in mammals involves not merely an arrest of the flow of contents through the reproductive tract, but an intricate integration of fetal and maternal tissues in a process termed implantation – or more evocatively nidation, from the Latin *nidus* meaning "nest". Embryo implantation is traditionally divided into five stages: *shedding* of the blastocyst coat (zona pellucida), *orientation* of the blastocyst, *apposition* of the fetal and the uterine epithelia, physical *attachment* of fetal tissues to the uterus, and *invasion* of the trophoblast into the uterus (Spencer *et al.* 2004). Not all viviparous species exhibit all of these stages: For instance, while independent origins of viviparity in squamates abound, in many cases the implantation process only proceeds to apposition, at which point the placenta is held in place by myometrial tension rather than tissue attachment (Stewart and Blackburn 2015). In marsupials such as the opossum, attachment does occur (Griffith *et al.* 2017), but invasion does not or is limited.

Eutherian pregnancy is anomalous from a tissue biological perspective. Successful implantation depends upon properties of the endometrium as well as of the blastocyst. The reproductive tract is a mucosal surface: mucus secreted at the apical side of the epithelium makes it nonadhesive, prevents water loss, and creates a barrier against potential pathogens or irritants. In order for implantation to occur, the apical side of the uterine epithelium must instead become adhesive. As a consequence, the temporal window of implantation is limited to when defenses such as mucin can be actively removed (Carson *et al.* 1998). In areas where mucin is removed, trophoblast can displace luminal epithelium but pauses at the basal lamina (Schlafke *et al.* 1985). Disintegration of the basal lamina is required for embedding to progress, and in the mouse this disintegration is aided by the maternal cells of the decidua (Blankenship and Given 1992). On the blastocyst side, too, intrinsic changes must occur to allow implantation; this is evident because mouse blastocysts before zona hatching are unable to successfully attach (Paria *et al.* 1993).

Peer-reviewed version available at *Integrative and Comparative Biology* **2020**; doi:10.1093/icb/icaa030

Implantation is a developmental process where later stages mechanistically depend upon the completion of earlier stages (*e.g.* the blastocyst cannot attach if it has not yet hatched from the zona). For this reason, it can be reasonably expected that the evolution of the stages of implantation recapitulated their order in ontogeny to some degree (Riedl 1978:218; Wagner 2014:320-321). It follows that the process of endometrial receptivity during apposition and attachment, or in more active terms maternal facilitation of implantation, precedes trophoblast invasion in evolution as in development. Not every intermediate stage of this theoretical trajectory is represented among living mammals, but notably, while marsupials and placental mammals share all stages up to "attachment," the final stage of "invasion" occurs only in placental mammals. We propose that an effective way to understand the complex fetal-maternal interaction of implantation is by tracing the steps by which it was put together over the course of evolution.

The Final Stage of Implantation, Invasion or Embedding, Was a Maternal Innovation

Grosser (1909; 1927) recognized three primary types of maternal-fetal interfaces, differentiated according to the tissue layers between maternal and fetal blood supplies. In epitheliochorial placentation, the epithelium of the chorion apposes to the uterine epithelium directly; in endotheliochorial placentation, the uterine luminal epithelium is eroded and the stroma and endothelium come into contact with the chorion; in hemochorial placentation, the endothelium of maternal blood vessels is breached or replaced with extravillous trophoblast, and fetal tissue comes into direct contact with maternal blood. These three conditions have been ordered in terms of increasing "invasiveness" of fetal into maternal tissues. The development of a maternal-fetal interface of one of the latter two types requires a stage to the implantation process called either embedding, interstitial implantation, or invasion. The evolution of this final stage, fetal and maternal contributions to its development, and connotations of the terminology used to describe it are all deserving of analysis.

Human pregnancy is unusual in its degree of fetal-maternal integration, with deep interstitial implantation and arterial remodeling. Among the primates, strepsirrhines such as the lemurs and lorises have epitheliochorial placentae whereas their sister group the haplorrhines which include humans, other apes, and tarsiers have hemochorial placentation. This pattern, plus the uncertain phylogenetic position of "insectivoran" mammals with epitheliochorial placentation, contributed to an anthropocentric narrative of the evolution of placentation as a gradual progression from primitive, non-invasive lineages to the advanced, highly invasive human condition (*e.g.* Huxley 1880; Hill 1932). Hill (1932) divided the history of primate placentation into four "stages" of evolution, the lemuroid, tarsioid, pithecoid, and anthropoid stages, referring each to a supposedly more derived form of placentation. In his description of the most highly invasive form of interstitial implantation in hominins, Hill (1932; emphasis added) wrote, "in this way the Primate germ reaches the *acme of its endeavour* to maintain itself in the uterus and to obtain an adequate supply of nutriment at the earliest possible moment." Implicit in this model is the judgment that noninvasive epitheliochorial placentation is crude and inefficient, and that more invasive placentation is a fetal adaptation to increase the efficiency of nutrient transfer.

Phylogenetic systematics promised to be a more rigorous way to test the theory that the human condition is derived, and early cladistic analyses of placentation supported epitheliochorial placentation as the ancestral condition for placental mammals (Luckett 1974; 1975; 1976). However, after molecular data revised the topology of the mammalian tree of life, and with increased phylogenetic scope and sample size, subsequent analyses have shown that the most recent common ancestor of placental mammals most likely had a hemochorial placenta (Wildman *et al.* 2006; Mess and Carter 2006; Elliot and Crespi 2009), the opposite of the expected polarity. Importantly, this does not mean that hemochorial placentation is not evolutionarily derived with respect to the first placental type to evolve in therian mammals (their sister group, the marsupials, likely ancestrally had noninvasive placentation:

Peer-reviewed version available at *Integrative and Comparative Biology* **2020**; doi:10.1093/icb/icaa030

Zeller and Freyer 2001), but rather that it first originated in the stem eutherian lineage. This timing coincides with the origin of the decidua (Mess and Carter 2006), and raises the question of whether the origin of the decidua enabled hemochorial placentation or was an accommodation to limit it. In other words, this new scenario suggests a need to shift focus from the fetus to the mother. Before discussing investigations of fetal versus maternal contributions, it is necessary to trace the history of the invasion concept itself.

Warfare has long been used as an analogy for the embedding of trophoblast within the uterus, and persists to the present day (*e.g.* Ashary *et al.* 2018). Unlike some androcentric depictions of reproduction which date to antiquity, the concept of the fetus as a militant invader into the maternal tissues seems to have arisen in the late 1890s (Haig 2010): it has been suggested that this concept arose as a consequence of early depictions of implantation-stage human embryos (Peters 1899), comparative biology of other hemochorial species, appreciation of the invasive nature of trophoblast-derived cancers or choriocarcinomas, and a militaristic ethos of the time (Haig 2010). The term “invasion” connotes territory intrusion, with the maternal tissue – namely the endometrium – being a passive substrate, or having the primary function of resistance to fetal aggression (Fothergill 1899). The migration of fetal cells, particularly extravillous trophoblast, into the maternal tissue is likened to an incursion into enemy territory, not unlike depictions of conception that romanticize the sperm (Martin 1991; Holt and Fazeli 2016). In one of the first evolutionary hypotheses for the origin of the decidual stromal cell, Fothergill (1899) posited, “The fertilized ovum, in arranging for its own nutrition, is known to make an attack on the maternal structures. The decidual cell, it is suggested, has been evolved as a protection, its function being to prevent injurious invasion of the uterine wall by the fœtal elements of the placenta.” If pregnancy is war then the fetal-maternal interface or *Umlagerungszone* [rearrangement zone] (Peters 1899), with turnover of cells “perishing in the struggle” (Fothergill 1899), is the front or “fighting line” (Johnstone 1914:258). In this framework, the position of the fetal-maternal interface was presumed to be the outcome of a struggle between the fetal tissues, with an inherent propensity to invade, and the maternal tissues, with an inherent propensity to resist invasion. This left little room to ask whether maternal tissues actively encapsulate fetal tissues or invite trophoblast cells to enter the endometrium.

The relative contributions of fetal and maternal tissues to implantation have been investigated by experimentally induced ectopic pregnancy. Transplantation of guinea pig (Loeb 1914), mouse (Billington 1965), or rat (Jollie 1961) embryos into non-uterine tissues led in several cases (though at varying success rates) to implantation and invasion into the recipient tissue. These experiments demonstrated an inherent potential to implant or embed within tissue in the embryos of these hemochorial species. Notably, even in the pig, a species with epitheliochorial placentation (secondarily derived, however, from the hemochorial state ancestral to placental mammals), trophoblast invasion was observed when fetuses were transplanted to ectopic sites in the uterine myometrium or deeper in the mucosa (Samuel and Perry 1972). This suggests that the superficial epitheliochorial state is actively maintained by uterine-specific maternal factors, rather than loss or lack of invasive potential by the embryo (Pijnenborg *et al.* 1985; Martin 2008).

On the other hand, *ex vivo* or *in vitro* assays that measure cell migration under controlled conditions suggest that the decidua has a substantial role in orchestrating trophoblast invasion, both through positive regulation and negative regulation (Sharma *et al.* 2016). The results from these studies have been illuminating, but perhaps because of confounding factors, sometimes contradictory.

Due to the intractability of experimental studies of invasion itself *in vivo*, much inference has relied upon or proxies of invasive capability. A long-studied proxy for invasive or merely destructive capability of cells is been protease secretion, as proteolytic degradation of extracellular matrix is a cardinal element of cell migration and tissue remodeling. In a series of elegant experiments, Gräfenberg (1910; summarized in Haig 2010) found that first-trimester placental homogenate had proteolytic activity that was lacking at later stages and was neutralized by addition of decidual tissue homogenate.

Peer-reviewed version available at *Integrative and Comparative Biology* **2020**; doi:10.1093/icb/icaa030

Production of matrix metalloproteinases is indeed elevated in human extravillous trophoblast (Godbole *et al.* 2011; Menkhorst *et al.* 2012), and decidual stromal cells in contrast have been shown to produce tissue inhibitors of matrix metalloproteinases that have an antagonistic effect (Lala and Graham 1990; Burrows *et al.* 1996). Furthermore, decidual cells secrete an array of growth factors and signaling molecules, both invasion-promoting and invasion-opposing (Sharma *et al.* 2016), that influence the protease expression of fetal trophoblast (Menkhorst *et al.* 2012), and other key invasion factors. The complexity of the decidual secretome, with apparently contradictory signals as in other fetal-maternal interactions of pregnancy, is expected in a system where the outcome is tightly controlled (Pavličev *et al.* 2017). It also may reflect a history of many compensatory or back-and-forth changes of small effect in the evolution of degree of embedding.

Matrix-like gel invasion assays demonstrate that endometrial stromal fibroblasts decidualized *in vitro* have increased motility themselves and capacity to encapsulate trophoblast cells (Gellersen *et al.* 2010). These findings suggest that the decidua not only secretes signals that promote trophoblast invasion, but by movement and by decidual metalloproteinase secretion in response to extravillous trophoblast signals, the decidua moves to enclose the trophoblast during implantation. Supernatants from decidual cell suspensions have also been shown to increase invasion ability of extravillous trophoblast cells in these assays (Lash *et al.* 2010).

The results of these experiments show that in addition to legitimate activity of the fetus, the maternal tissue has an active role in orchestrating the implantation process that is obscured by the invasion narrative. To highlight the active role of the decidua in the implantation process, a return to use of the more neutral term of "embedding" rather than "invasion" for the process of interstitial implantation has been suggested (Macklon and Brosens 2014). The use of the term embedding was even antecedent to the rise of invasion terminology (*e.g.* Hart 1893). Furthermore, at the conceptual level, recognition that embedding is facilitated by both the mother and the fetus is important. Therefore, in addition to the concept of "invasiveness" or "aggression" (which could use substitution) as a trait of the fetus, an equivalent metric of the maternal tissue's activity in actively encapsulating the trophoblast and facilitating its migration is needed as well. We have for the time being referred to this concept as "invasibility," and demonstrated that it can be studied as a quantitative trait resulting from biological properties of the uterine stroma (Kshitiz *et al.* 2019). Evolutionary changes in placentation can result from changes to either or both attributes, and many of the evolutionary transitions in degree of implantation within placental mammals may be best studied from the maternal point of view.

Placentocentrism

The placenta clearly has an active role in implantation in species with well established placentae, but the decidua's contribution is not negligible either as summarized above. A placentocentric view of the evolution of implantation is one where the major innovations, such as hemochorial placentation in therian mammals, are the consequence of modifications to trophoblast or placental biology, such as increased invasive potential. Under the adaptive immune model, Medawar (1953) argued that the most likely explanation for the origin of viviparity in mammals was establishment of the placenta, in particular the trophoblast, as a physical barrier between mother and fetus; this was later incorporated by Lillegraven (1975) into a historical narrative of mammal evolution that attributed the short gestation of marsupials to failure to solve the immunological paradox of pregnancy. Despite bearing the name Placentalia, crown eutherian mammals inherit the placental organ from our most recent common ancestor with marsupials, the therian common ancestor. While the origin of the placenta was certainly an event upon which other events in pregnancy evolution were contingent, this timing means that its origin cannot be the sole explanation of extended gestation or differences between marsupial and eutherian pregnancy (Taylor and Padykula 1978).

Peer-reviewed version available at *Integrative and Comparative Biology* **2020**; doi:10.1093/icb/icaa030

Difference of opinion over the relative importance of the placenta versus the decidua in therian evolution has to do with which is seen as driver versus enabler of evolutionary innovation (Donoghue 2005; Martin and Wagner 2019). From a developmental evolutionary point of view, the main flaw in a placentocentric model of the evolution of eutherian mammals is the fact, discussed above, that the first trait to be modified in the evolution of a sustained maternal-fetal interface is the maternal inflammatory reaction to the presence of the embryo. This would not be possible without first a maternal mechanism to attenuate and regulate the inflammation response after apposition and perhaps attachment. Most likely the key innovation that made this possible was the origin of the decidual stromal cell (Wagner *et al.* 2014; Chavan *et al.* 2016; Chavan *et al.* 2017), *i.e.* a maternal innovation. Only after the inflammation barrier was overcome can elaboration of the placenta into a derived organ (Griffith and Wagner 2017) proceed, and also only after gestation is extended does adaptive immune rejection of the fetus become an issue. It follows that the role of the placenta is downstream of maternal innovations, both in terms of driving evolution as well as in terms of the physiological process of embedding.

It has not escaped our notice that an appropriate experiment to test whether fetal or maternal innovations contributed to the evolutionary origin of interstitial embedding would be to investigate the potential of the marsupial embryo to invade tissues in an ectopic pregnancy. To our knowledge, such an experiment has not been reported. The model outlined in this section leads to the prediction that the marsupial embryo would be invasive into non-uterine tissues. Such a finding would support the notion that the evolution of hemochorial placentation in the eutherian stem lineage involved maternal innovations to more effectively embed the fetus within the uterus, rather than increased aggression of the embryo.

The Marsupial Homolog of Implantation Is Also a Cooperative Process

Functional integration of fetal and maternal tissues is not restricted to the eutherian lineage. In marsupials such as the opossum, the process of implantation proceeds to the attachment stage, at which point an inflammatory cascade unfolds at the fetal-maternal interface (Griffith *et al.* 2017). Unlike in eutherians, where implantation is only the beginning of pregnancy, parturition follows soon after attachment (Hansen *et al.* 2017). If the fetus and mother are viewed as two distinct physiological individuals, such an event resembles a host-versus-pathogen immune response. It has been proposed that such a maternal anti-fetal immune response was a major constraint in marsupial evolution that explains their almost complete lack of extended gestation (Moors 1974; Lillegraven 1975; Lillegraven *et al.* 1987).

Evaluation of the opossum fetal-maternal interactions from a perspective of maternal immune attack upon the fetus reveals that several of this model's predictions are not met. First, in a normal inflammatory reaction, the host (in this case the mother) is the one that senses a perturbation in the form of an intruder or damage, produces inflammatory mediators, and ultimately attempts to remove the perturbation. The intruder presumably benefits by evading detection or suppressing host defense. Production of prostaglandin E_2, a key inflammatory mediator ubiquitous in pregnancy, results from two synthetic steps. While the enzyme cyclooxygenase-2 which catalyzes production of the intermediate prostaglandin H_2 is localized to the maternal endometrium, the enzyme catalyzing the second step of conversion of this intermediate into prostaglandin E_2, prostaglandin E synthase, is highly expressed in the fetal trophoblast (Griffith *et al.* 2017). Interleukin-17, an inflammatory cytokine involved in mucosal defense, also appears to be transcribed in the trophoblast (Stadtmauer and Wagner 2020). While not yet comprehensive, these observations indicate that the fetal-maternal relationship in the opossum is not merely that of a host rejection of a pathogen, as the fetus has an active contribution to the pro-inflammatory state that develops upon attachment: this situation has been termed cooperative inflammation (Stadtmauer and Wagner 2020).

Peer-reviewed version available at *Integrative and Comparative Biology* **2020**; doi:10.1093/icb/icaa030

It is important to note that such a pattern does not contradict the homology proposed between the attachment-induced inflammatory process and the eutherian processes of embryo implantation (Griffith *et al.* 2017). Developmental processes can be traced across evolution (Gilbert and Bolker 2001) despite redistribution to different body parts (Baum and Donoghue 2002), and like other characters maintain identity despite changes in state. What it does suggest is that while host-pathogen-like conflict may characterize the very initial response to viviparity, it is not a fitting model for the pregnancy of living marsupials, which appears to be derived and physiologically cooperative.

Evolutionary Narratives of Conflict

Parent-offspring conflict (Trivers 1974) is a prediction following from the principles of kin selection: if a mother is only 50% related to her offspring, selection on the offspring will favor a greater level of maternal investment than is optimal to provide from the perspective of the mother's genes. This framework has been productively applied to human pregnancy (Haig 1993). Pregnancy includes as many as three distinct genetic individuals – the non-inherited maternal genome, inherited maternal genome, and inherited paternal genome – each of which may have different optimal levels of investment in current versus future reproduction (Haig 1993; Haig 2019). The conflict theory makes clear that the conflict is between genes and not between organisms, as discussed above regarding host-pathogen organismal conflict, although the language of conflict can make this distinction difficult to maintain in practice. The different evolutionary interests of these three genomes can be realized during mammalian pregnancy through genetic imprinting, the parent-of-origin-specific expression of alleles (Moore and Haig 1991).

Genetic conflict is biochemically mediated by signaling molecules falling into the category of coercion (Diggle *et al.* 2007) – those whose expression evolved due to selection upon the sender but do not benefit the receiver to respond. Manipulation of and interference with growth factor pathways would fall into this category. If conflict is ubiquitous, one may be justified in questioning the honesty of any signaling interaction in pregnancy. Haig (1993) writes, "Relations between a mother and her fetus are not subject to the intricate homeostatic mechanisms that are characteristic of interactions between different tissues of the same body because messages cannot be trusted. Both parties often have an incentive to send misleading information." Our reconstruction of the early evolution of viviparity in mammals as a modified homeostatic state suggests that this assumption may be most applicable only to the pregnancy of eutherians with the state of interstitial embryo implantation.

Parent-offspring is most salient with respect to traits that affect nutrient transfer and allocation. The total maternal investment before weaning is estimated to be comparable between monotremes, marsupials, and placental mammals, with the primary difference being whether resources are apportioned before or after parturition (Renfree *et al.* 2009). In marsupials, the majority of resource provisioning is postnatal during lactation, a derived specialization (Hayssen *et al.* 1985). While any form of nutrient allocation is potentially subject to parent-offspring conflict, physiological conflict between uterus and placenta is only possible prenatally (Haig 1993). Furthermore, imprinted genomic regions of eutherians contain greater numbers of imprinted genes than marsupials, suggesting a greater role of this correlate of conflict in the eutherian lineage (Renfree *et al.* 2009). It is therefore likely that with respect to the relationship between maternal and fetal gestational tissues in the evolution of implantation, cooperation and physiological integration were prominent before the evolution of extended gestation in placental mammals raised the stakes of nutrient allocation.

Both cooperation and conflict can be thought of as two extremes of a continuum (Hayssen and Orr 2017), and are not necessarily mutually exclusive competing hypotheses: maternal-fetal interactions consist of interactions that fall into both categories (Haig 2010). We argue that cooperation, defined here as physiological integration of maternal and fetal tissues behaving as if they *are* "different tissues of the same body" (Haig 1993), preceded conflict as a major force in the evolution of

Peer-reviewed version available at *Integrative and Comparative Biology* **2020**; doi:10.1093/icb/icaa030

mammalian viviparity. Conflict in its strongest form became much more relevant to eutherian evolution with the evolution of interstitial implantation and extended gestation. Conflict theory seems best not as an explanation for the evolutionary origin of viviparity or embryo implantation, but its subsequent modification in eutherian mammals, as originally proposed (Haig 1993).

Neither Eternal Harmony, Nor Unending Conflict: It's Physiology Evolving!

In conclusion, if the evolutionary historical scenario outlined above is correct, then yes, the therian blastocyst (early embryo) has destructive capabilities that affect the maternal tissue, and yes, one has to expect that the initial response of the maternal organism was a defensive inflammatory reaction. But as the maternal-fetal relationship evolved, the functional role of maternal and fetal factors changed. The maternal organism evolved the decidua, which was and is essential for the sustainability of a tight physiological integration between fetus and mother (Chavan *et al.* 2016; Chavan *et al.* 2017). In marsupials, both fetus and mother jointly drive an inflammatory process with the objective to effect birth (Stadtmauer and Wagner 2020). Any static model of conflict or harmony is unlikely to capture the highly dynamic physiological feedback that underlies the emergence of the maternal-fetal unit (Nuña de la Rosa *et al.* 2019).

A divide between two approaches to socially conscious research has been likened through a gardening analogy to weeding and planting (Segerstrålle 2000). Both processes are pertinent to the development of a discipline. Criticism of unfounded bias is valuable, in line with the notion of debunking as a positive science (Gould 1981), but on the other hand alternative explanations to those under criticism are ultimately needed as well. Having identified inconsistencies in the invasion narrative, the above has been our attempt at planting a new explanatory framework for understanding the maternal-fetal relationship in implantation. The potentials for research into female perspectives on reproduction is rich: there is much more planting to be done.

Bibliography

Ashary N, Tiwari A, Modi D 2018. Embryo implantation: war in times of love. Endocrinol 159:1188-1198.

Baum DA, Donoghue MJ 2002. Transference of function, heterotopy and the evolution of plant development. In: Cronk Q, Bateman R, Hawkins J, editors. Developmental Genetics and Plant Evolution. Taylor and Francis. pp. 52-69.

Billington WD 1965. The invasiveness of transplanted mouse trophoblast and the influence of immunological factors. J Reprod Fertil 10:343-352.

Blackburn DG 1995. Saltationist and punctuated equilibrium models for the evolution of viviparity and placentation. J Theor Biol 174:199-216.

Blackburn DG 2015. Evolution of vertebrate viviparity and specializations for fetal nutrition: a quantitative and qualitative analysis. J Morphol 276:961-990.

Blankenship TN, Given RL 1992. Penetration of the uterine epithelial basement membrane during blastocyst implantation in the mouse. Anat Rec 233:196-204.

Burrows TD, King A, Loke YW 1996. Trophoblast migration during human placental implantation. Hum Reprod Update 2:307-321.

Cannon WB 1929. Organization for physiological homeostasis. Physiol Rev 9(3):399-431.

Carson DD, DeSouza MM, Regisford EGC 1998. Mucin and proteoglycan functions in embryo implantation. BioEssays 20:577-583.

Chavan AR, Bhullar BAS, Wagner GP 2016. What was the ancestral function of decidual stromal cells? A model for the evolution of eutherian pregnancy. Placenta 40:40-51.

Peer-reviewed version available at *Integrative and Comparative Biology* **2020**; doi:10.1093/icb/icaa030

Chavan AR, Griffith OW, Wagner GP 2017. The inflammation paradox in the evolution of mammalian pregnancy: turning a foe into a friend. Curr Opin Genet Dev 47:24-32.

Diggle SP, Gardner A, West SA, Griffin AS 2007. Evolutionary theory of bacterial quorum sensing: when is a signal not a signal? Phil Trans R Soc B 362:1241-1249.

Donoghue MJ 2005. Key innovations, convergence, and success: macroevolutionary lessons from plant phylogeny. Paleobiology 31(2):77-93.

Elliot MG, Crespi BJ 2009. Phylogenetic evidence for early hemochorial placentation in Eutheria. Placenta 30:949-967.

Fothergill WE 1899. The function of the decidual cell. Edinb Med J 5:265-273.

Frankenberg S, Renfree MB 2018. Conceptus coats of marsupials and monotremes. Curr Top Dev Biol 130:357-377.

Gellersen B, Reimann K, Samalecos A, Aupers S, Bamberger AM 2010. Invasiveness of human endometrial stromal cells is promoted by decidualization and by trophoblast-derived signals. Human Reprod 25(4):862-873.

Gilbert SF, Bolker JA 2001. Homologies of process and modular elements of embryonic construction. J Exp Zool Mol Dev Evol 291:1-12.

Godbole G, Suman P, Gupta SK, Modi D 2011. Decidualized endometrial stromal cell derived factors promote trophoblast invasion. Fertil Steril 95(4):1278-1283.

Gould SJ 1981. The mismeasure of man. W.W. Norton & Company.

Gräfenberg E 1910. Belträge zur Physiologie der Eienbettung. Z Geburtsh Gynäk 65:1-35.

Griffith OW, Chavan AR, Protopapas S, Maziarz J, Romero R, Wagner GP 2017. Embryo implantation evolved from an ancestral inflammatory attachment reaction. Proc Natl Acad Sci USA 2017:E6566–E6575.

Griffith OW, Wagner GP 2017. The placenta as a model for understanding the origin and evolution of vertebrate organs. Nat Ecol Evol 1:0072.

Grosser O 1909. Vergleichende Anatomie und Entwicklungsgeschichte der Eihäute und der Placenta: Mit Besonderer Berücksichtigung des Menschen. Vienna: Wilhelm Braumüller.

Grosser O 1927. Frühentwicklung, Eihautbildung und Placentation des Menschen und der Säugetiere. München: J.F. Bergmann.

Haig D 1993. Genetic conflicts in human pregnancy. Q Rev Biol 68(4):495-532.

Haig D 2010. Fertile soil or no man's land: cooperation and conflict in the placental bed. In: Pijnenborg R, Brosesns I, Romero R, editors. Placental Bed Disorders: Basic Science and Its Translation to Obstetrics. Cambridge University Press. pp. 165-173.

Haig D 2019. Cooperation and conflict in human pregnancy. Curr Biol 29:R455-R458.

Hansen VL, Faber LS, Salehpoor AA, Miller RD 2017. A pronounced uterine pro-inflammatory response at parturition is an ancient feature in mammals. Proc R Soc B 284:20171694.

Hart B 1893. Placenta prævia. Trans Edinb Obstet Soc 18:25-35.

Hayssen V, Lacy RC, Parker PJ 1985. Metatherian reproduction: transitional or transcending? Am Nat 126(5):617-632.

Hayssen V, Orr TJ 2017. Reproduction in mammals: the female perspective. Baltimore: Johns Hopkins University Press.

Hill JP 1932. Croonian lecture: the developmental history of the primates. Philos Trans R Soc B 221:474-482.

Holt WV, Fazeli A 2016. Sperm selection in the female mammalian reproductive tract. Focus on the oviduct: hypotheses, mechanisms, and new opportunities. Theriogenology 85:105-112.

Huxley TH 1880. On the application of the laws of evolution to the arrangement of the Vertebrata, and more particularly of the Mammalia. Proc Zool Soc Lond 43:649-662.

Peer-reviewed version available at *Integrative and Comparative Biology* **2020**; doi:10.1093/icb/icaa030

Johnstone RW 1914. Contribution to the study of the early human ovum based upon the investigation of I. A very early ovum embedded in the uterus and II. A very early ovum embedded in the infundibulum of the tube. J Obstet Gynaecol Br Emp 25:231-276.

Jollie WP 1961. The incidence of experimentally produced abdominal implantations in the rat. Anat Rec 141(2):159-167.

Kotas ME, Medzhitov R 2015. Homeostasis, inflammation, and disease susceptibility. Cell 160:816-827.

Kshitiz, Afzal J, Maziarz JD, Hamidzadeh A, Liang C, Erkenbrack EM, Nam H, Haeger J-D, Pfarrer C, Hoang T, Ott T, Spencer T, Pavličev M, Antczak DF, Levchenko A, Wagner GP 2019. Evolution of placental invasion and cancer metastasis are causally linked. Nat Ecol Evol 3:1743-1753.

Lala PK, Graham CH 1990. Mechanisms of trophoblast invasiveness and their control: the role of proteases and protease inhibitors. Cancer Metastasis Rev 9:369-379.

Lash GE, Otun HA, Innes BA, Percival K, Searle RF, Robson SC, Bulmer JN 2010. Regulation of extravillous trophoblast invasion by uterine natural killer cells is dependent on gestational age. Human Reprod 25(5):1137-1145.

Lillegraven JA 1969. Latest Cretaceous mammals of upper part of Edmonton Formation of Alberta, Canada, and a review of marsupial-placenta dichotomy of mammalian evolution. Paleont Contr 50:1-122.

Lillegraven JA 1975. Biological considerations of the marsupial-placental dichotomoy. Evolution 29:707–722.

Lillegraven JA, Thompson SD, McNab BK, Patton JL 1987. The origin of eutherian mammals. Biol J Linn Soc 32:281-336.

Loeb L 1914. The experimental production of an early stage of extra-uterine pregnancy. Proc Soc Exp Biol NY 11:103-106.

Lombardi J 1998. Comparative vertebrate reproduction. Norwell: Kluwer Academic Publishers.

Luckett WP 1974. Comparative development and evolution of the placenta in primates. Contrib Primatol 3:142-234.

Luckett WP 1975. Ontogeny of the fetal membranes and placenta. In: Luckett WP, Szalay FS, editors, Phylogeny of the Primates: A Multidisciplinary Approach. Plenum Press. pp. 157-182.

Luckett WP 1976. Cladistic relationships between primate higher categories: evidence of the fetal membranes and placenta. Folia Primatol 25:245-276.

Macklon NS, Brosens JJ 2014. The human endometrium as a sensor of embryo quality. Biol Reprod 91(4):98.

Medawar PB 1953. Some immunological and endocrinological problems raised by the evolution of viviparity in vertebrates. Symp Soc Exp Biol 44:320338.

Medzhitov R 2008. Origin and physiological roles of inflammation. Nature 454:428-435.

Madsen O 2009. Mammals (Mammalia). In: Kumar SB, editor. The Timetree of Life. Oxford University Press. pp. 459–461.

Marshall LG 1979. Evolution of metatherian and eutherian (mammalian) characters: a review based on cladistic methodology. Zool J Linn Soc 66:369-410.

Martin E 1991. The egg and the sperm: How science has constructed a romance based on stereotypical male-female roles. Signs 16(3):485-501.

Martin JF, Wagner GP 2019. The origin of platelets enabled the evolution of eutherian placentation. Biol Lett 15:20190374.

Martin RD 2008. Evolution of placentation in primates: implications of mammalian phylogeny. Evol Biol 35:125-145.

Peer-reviewed version available at *Integrative and Comparative Biology* **2020**; doi:10.1093/icb/icaa030

Menkhorst EM, Lane N, Winship AL, Li P, Yap J, Meehan K, Rainczuk A, Stephens A, Dimitriadis E 2012. Decidual-secreted factors alter invasive trophoblast membrane and secreted proteins implying a role for decidual cell regulation of placentation. PloS One 7(2):e31418.

Mess A, Carter AM 2006. Evolutionary transformations of fetal membrane characters in Eutheria with special reference to Afrotheria. J Exp Zool Part B 306(2):140–163.

Moore T, Haig D 1991. Genomic imprinting in mammalian development: a parental tug-of-war. Trends Genet 7(2):45-49.

Moors PJ 1974. The fœtal-maternal relationship and its significance in marsupial reproduction: a unifying hypothesis. Aust Mammal 1:263-266.

Mor G, Cardenas I, Abrahams V, Guller S 2011. Inflammation and pregnancy: the role of the immune system at the implantation site. Ann NY Acad Sci 1221:80-87.

Müller GB, Wagner GP 1991. Novelty in evolution: restructuring the concept. Annu Rev Ecol Syst 22:229-256.

Nuña de la Rosa L, Pavličev M, Exteberria A 2019. Rethinking the individuality of pregnancy: eutherian pregnancy as an evolved relational novelty. Preprints 2019:2019100127.

Paria BC, Huet-Hudson YM, Dey SK 1993. Blastocyst's state of activity determines the "window" of implantation in the receptive mouse uterus. Proc Natl Acad Sci USA 90:10159-10162.

Pavličev M, Wagner GP, Chavan AR, Owens K, Maziarz J, Dunn-Fletcher C, Kallapur SG, Muglia L, Jones H 2017. Single-cell transcriptomics of the human placenta: inferring the cell communication network of the maternal-fetal interface. Genome Res 27:349–361.

Peters H 1899. Ueber die Einbettung des menschlichen Eies und das früheste bisher bekannte menschliche Placentationsstadium. Franz Deuticke.

Renfree MB 1981. Marsupials: alternative mammals. Nature 293:100-101.

Renfree MB, Hore TA, Shaw G, Graves JAM, Pask AJ 2009. Evolution of genomic imprinting: insights from marsupials and monotremes. Annu Rev Genomics Hum Genet 10:241-262.

Riedl R 1978. Order in living organisms: a systems analysis of evolution. Translated by R.P.S. Jefferies. New York: John Wiley & Sons.

Samuel CA, Perry JS 1972. The ultrastructure of pig trophoblast transplanted to an ectopic site in the uterine wall. J Anat 113(1):139-149.

Schlafke S, Welsh AO, Enders AC 1985. Penetration of the basal lamina of the uterine luminal epithelium during implantation in the rat. Anat Rec 212:47-56.

Segerstråle U 2000. Defenders of the truth: the battle for science in the sociobiology debate and beyond. Oxford University Press.

Sharma S, Godbole G, Modi D 2016. Decidual control of trophoblast invasion. Am J Reprod Immunol 75:341-350.

Spencer TE, Johnson GA, Bazer FW, Burghardt RC 2004. Implantation mechanisms: insights from the sheep. Reproduction 128(6):657–668.

Stewart JR, Blackburn DG 2015. Viviparity and placentation in lizards. In: Rheubert JL, Siegel DS, Trauth SE, editors. Reproductive Biology and Phylogeny of Lizards and Tuatara. CRC Press. pp. 448-563.

Stone WH, Manis GS, Hoffman ES, Saphire DG, Hubbard GB, VandeBerg JL 1997. Fate of allogeneic skin transplantations in a marsupial (*Monodelphis domestica*). Lab Anim Sci 47(3):283-287.

Stadtmauer DJ, Wagner GP 2020. Cooperative inflammation: the recruitment of inflammatory signaling in marsupial and eutherian pregnancy. J Reprod Immunol 137:102626.

Taylor JM, Padykula HA 1978. Marsupial trophoblast and mammalian evolution. Nature 271:588.

Trivers RL 1974. Parent-offspring conflict. Amer Zool 14:249-264.

Wagner GP 2014. Homology, genes and evolutionary innovation. Princeton University Press.

Peer-reviewed version available at *Integrative and Comparative Biology* **2020**; doi:10.1093/icb/icaa030

Wagner GP, Erkenbrack EM, Love AC 2019. Stress-induced evolutionary innovation: a mechanism for the origin of cell types. BioEssays 41:1800188.

Wagner GP, Kin K, Muglia L, Pavličev M 2014. Evolution of mammalian pregnancy and the origin of the decidual stromal cell. Int J Dev Biol 58:117-126.

Wagner GP, Müller GB 2002. Evolutionary innovations overcome ancestral constraints: a re-examination of character evolution in male sepsid flies (Diptera: Sepsidae). Evol Dev 4(1):1-6.

Wildman DE, Chen C, Erez O, Grossman LI, Goodman M, Romero R 2006. Evolution of the mammalian placenta revealed by phylogenetic analysis. Proc Natl Acad Sci USA 103(9):3203-3208.

Zeller, U., Freyer, C., 2001. Early ontogeny and placentation of the grey short-tailed opossum, *Monodelphis domestica* (Didelphidae: Marsupialia): contribution to the reconstruction of the marsupial morphotype. J Zoolog Syst Evol Res 39:137–158.