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Fetal microchimerism and maternal health: A review and evolutionary analysis of cooperation and conflict beyond the womb

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The presence of fetal cells has been associated with both positive and negative effects on maternal health. These paradoxical effects may be due to the fact that maternal and offspring fitness interests are aligned in certain domains and conflicting in others, which may have led to the evolution of fetal microchimeric phenotypes that can manipulate maternal tissues. We use cooperation and conflict theory to generate testable predictions about domains in which fetal microchimerism may enhance maternal health and those in which it may be detrimental. This framework suggests that fetal cells may function both to contribute to maternal somatic maintenance (e.g. wound healing) and to manipulate maternal physiology to enhance resource transmission to offspring (e.g. enhancing milk production). In this review, we use an evolutionary framework to make testable predictions about the role of fetal microchimerism in lactation, thyroid function, autoimmune disease, cancer and maternal emotional, and psychological health.

Keywords:

attachment; autoimmune disease; cancer; inflammation; lactation; maternal-fetal conflict; parent-offspring conflict

Introduction

The function of fetal cells in maternal tissues is unknown

Maternal-offspring interactions are often viewed as solely cooperative, both parties having an interest in the other's survival and well-being. However, parent-offspring interactions are characterized by both cooperation – because shared genes lead to fitness benefits from maternal investment in offspring [1] – and also conflict – because parents and offspring do not share all genes and future maternal reproduction can be negatively impacted by too much maternal investment in current offspring [2]. In the context of pregnancy, resource conflict has led to the evolution of fetal manipulation of maternal systems to increase resource transfer via the placenta, and the evolution of maternal countermeasures to limit resource flow [3, 4]. This conflict is instantiated through a number of mechanisms including genomic imprinting and placental hormone production [3, 5]. The escalation of conflict between fetal and maternal systems over resource allocation has been proposed as an explanation for several pathological conditions of pregnancy including gestational diabetes and preeclampsia [3, 5]. Here, we extend the concept of maternal-offspring conflict beyond the womb to interactions between fetal cells and maternal tissues throughout the body both during and after pregnancy.

During pregnancy, there is a bi-directional flow of fetal and maternal cells (Fig. 1). The transfer of these cells is asymmetrical, with more fetal cells being transferred to the mother than vice versa [6]. Fetal cells increase in frequency in the maternal body with increasing gestational age [7, 8] and

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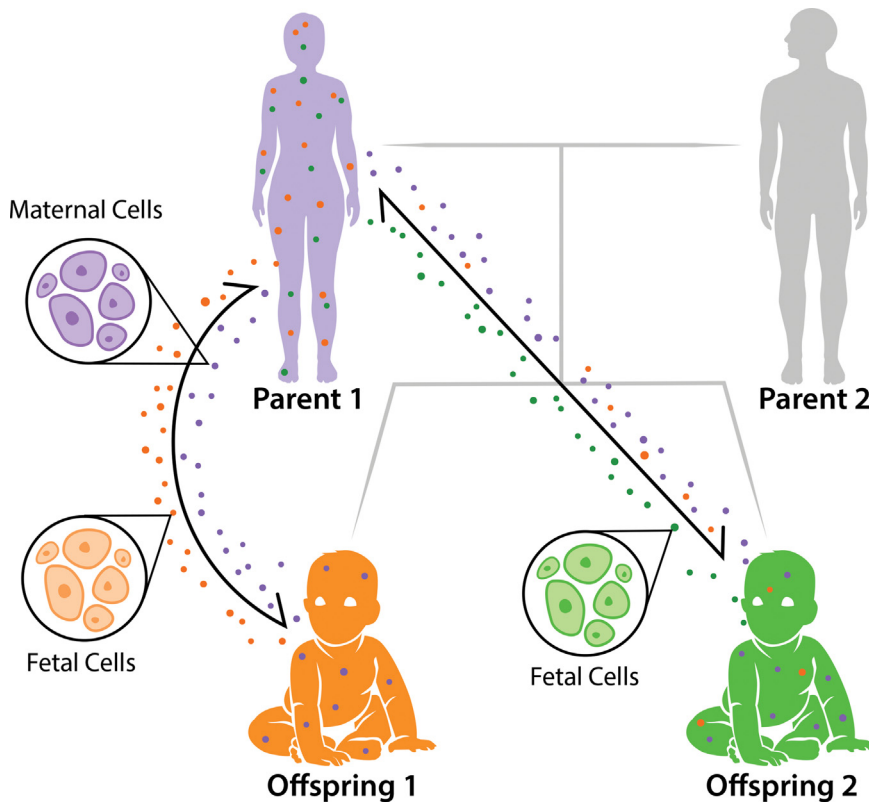


Figure 1. Pedigree of microchimerism. Microchimerism is a bidirectional exchange of fetal and maternal cells during pregnancy. During pregnancy, fetal cells (represented as orange and green circles) traffic into the maternal body, increasing in quantity throughout the gestational period. Likewise, each fetus inherits maternally derived cells (represented as purple circles). It has been predicted that younger siblings could also obtain older siblings' cells [15], as depicted with offspring 1 cells (orange) circulating within the younger sibling's body (offspring 2).

have been identified in maternal blood and tissues for decades following birth [9, 10]. After parturition, the maternal immune system actively clears some but not all fetal cells from the maternal blood through inducing apoptosis [11]. Maternal microchimerism (the transfer of maternal cells to the fetus) is commonly found in fetal tissues as well [12, 13], and also persist for decades after birth [14]. Microchimerism is not thought to be limited to just the bi-directional exchange of mother and fetal cells: cells from older siblings and even maternal grandmother cells might also be transferred to the fetus [15].

The current state of literature on fetal microchimerism and maternal health presents a paradoxical picture, with some papers concluding that fetal microchimerism contributes to poor health outcomes in certain cases and improved outcomes in others. For example, fetal cells have been proposed to play a role in maternal wound healing [16, 17], but have also been associated with pregnancy complications including, atypical fetal karyotype (e.g. Down Syndrome), pre-eclampsia, miscarriage, and premature birth [18–22]. Fetal cells have also been identified at multiple tumor sites, including breast, cervical, and thyroid cancers, as well as melanomas (reviewed in [23]). Here we review these contradictory findings and describe how the cooperation and conflict framework can help

elucidate both the proximate and evolutionary function of fetal cells in maternal tissues (Table 1).

Microchimerism is common among placental mammals

Considering a broader phylogenetic perspective, microchimerism is special case of chimerism, defined as organism being made up of more than one genetically distinct individual. Although large-scale chimerism is relatively rare among organisms [24], microchimerism is common in placental mammals. Placentas exhibit a high degree of diversity in physiology and morphology [25], and some of this placental diversity has been proposed to be the evolutionary consequence of ongoing conflict between mother and offspring over resource transfer [4]. One important dimension on which placentas vary is the depth of invasion of fetal trophoblast cells. Humans have the most invasive placental type, hemochorial (reviewed in [26]). Placental invasiveness has been proposed to enhance both nutrient transfer from mother to fetus and may also allow more exchange of both fetal and maternal cells [26]. Microchimerism has been detected in all placental types (hemochorial, epithelial, endothelial), including primates [14], dogs [27], cows [28],

and mice [8, 29]. Similar to fetal trophoblast cells invading maternal endometrium during placentation, fetal cells circulating in the maternal body may be able to colonize other tissues and persist in the maternal body after parturition.

Considering the evolutionary and phylogenetic origins of fetal cells suggests that they may have a similar function to the placenta. Just as the placenta's physiology is designed to transfer resources from the maternal body to the offspring in the womb [3, 5], the physiology of fetal cells in maternal tissues may enhance resource transfer to the offspring after parturition through, for example enhancing lactation or heat transfer. This resource transfer may be mutually beneficially for both maternal and offspring fitness interests, or fetal manipulation may push maternal tissues beyond the maternal optimum [2] leading to conflict over resource allocation. Fetal cells also may offer important benefits to maternal tissues for wound healing or somatic maintenance due to their stem-like nature.

In this paper we use an evolutionary approach to identify the domains in which we should expect cooperation or conflict between fetal cells and maternal tissues. We then use this framework to make predictions about the conditions under which the presence of fetal cells should have positive or negative effects on maternal health. We review the current

Table 1. Causes of microchimerism

Levels of explanation	Definition	Explanations for fetal microchimerism
Proximate	The immediate cause of the pathology	Placentation allows for the transfer of small numbers of cells between the fetus and the mother
Developmental	How the pathology arose as a result of events during an individual's life	Evidence suggests that fetal cell microchimerism begins before the placental is completely formed, likely beginning with the initiation of placentation itself [26, 111]
Evolutionary	How natural selection and other mechanisms of evolution (drift, migration) have left the body vulnerable to the pathology	Maternal-fetal genomic conflict, through genetic imprinting may have allowed for selection of higher proportions of fetal cell microchimerism
Phylogenetic	When, in evolutionary history, did the vulnerability to the pathology arise?	Microchimerism has thus far only been detected in eutherian mammals [14, 27–29], suggesting it arose at least in the common ancestor of eutherian mammals, approximately 93 million years ago [112]

Here, we describe the proximate, developmental, evolutionary, and phylogenetic explanations of microchimerism.

state of the literature on the effects of microchimerism on maternal health, identifying the tissues in which fetal microchimerism has been found and describing the functions of each of these tissues. The evolutionary and functional framework for fetal microchimerism that we present here explains some of the contradictory findings in the literature and identifies gaps in our understanding that can help guide future work.

Cooperation and conflict occur between fetal cells and maternal tissues

There are three main hypotheses about the role of fetal cells in maternal health in the existing literature: (1) fetal cells are deleterious, contributing to an inflammatory response that can cause maternal tissue damage; (2) fetal cells are protective, with fetal progenitor cells helping to repair and maintain maternal tissues or (3) fetal cells are simply bystanders, having no causal effect on maternal health [30]. These hypotheses may not be mutually exclusive. The cooperation and conflict approach makes a number of predictions about the conditions under which fetal cells may have positive or negative effects on maternal health.

Cooperation for offspring survival and maternal somatic maintenance

Parental investment is an important part of the human life history strategy [31] for survival and subsequent success of offspring. This begins in the womb and extends long after birth. Humans have very high levels of postpartum parental investment compared to other species, and have clear adaptations for parental investment which include lactation but also extended food sharing [32] both of which are facilitated by emotional attachment and bonding [33]. Both parent and offspring benefit from each others' survival, with maternal fitness interests being aligned with those of the offspring because of direct fitness benefits (i.e. enhancing

reproductive success of the parent) and offspring interests being aligned with mother's interests because of both indirect and direct benefits [5]. We predict that microchimerism should be associated with enhanced maternal health where there is potential for the fetus to enhance maternal health at low or no cost to itself, such as in the transmission of fetal stem cells that might provide a benefit for somatic maintenance (i.e. maternal tissue repair or replenishing stem cell niches).

Conflict arises over optimal allocation of resources to offspring

Offspring interests often favor higher levels of resource transmission than what is optimal for maternal fitness, likely leading to selection on fetal microchimeric cell phenotypes to manipulate maternal tissues to increase the level of resource transfer to offspring. One approach to understanding the function of fetal cells in maternal tissues is to consider parallels with the function of the placenta during pregnancy. During pregnancy, fetal cells may operate as an extension of the placenta, helping to coordinate manipulation of maternal resource transfer to the offspring across maternal tissues. After pregnancy, fetal cells may play important roles in continued maternal investment in the offspring through manipulation of lactation, thermoregulation, and attachment systems. The framework we propose here predicts that fetal cells should be more common in tissues that are the site of resource transfers (e.g. the breast, thyroid, and brain; Fig. 2), and that in these tissues, the presence of fetal cells may be associated with poorer health outcomes for the mother.

Maternal countermeasure and the escalation of conflict

Maternal-fetal conflict not only predicts the evolution of fetal adaptations to enhance resource flow to offspring, but also maternal countermeasures to limit that flow [3]. Extending this framework beyond the placenta suggests that the maternal systems should have adaptations to limit the

Mother-Offspring Tug-of-War

	Offspring's Interest	Mother's Interest
Brain	Upregulation of neuroendocrine systems underlying parental investment and bonding	Modulation of systems underlying parental investment to optimize allocation of resources over present and future offspring
Thyroid	Upregulation of heat production	Regulation of heat production at a level that optimizes allocation of resources over present and future offspring
Breast	Increase milk production	Modulate milk production to optimize allocation of resources over present and future offspring
Immune	Induced maternal tolerance. Evade detection and destruction by maternal immune system	Tolerate semi-allogeneic fetal material. Eliminate fetal cells that upregulate resource transfer to levels not consistent with optimized allocation of resources over present and future offspring

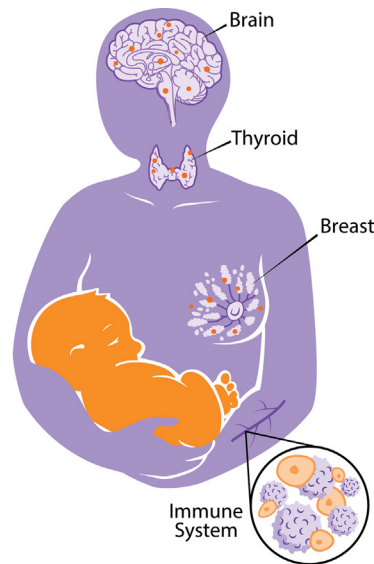


Figure 2. Mother-offspring tug-of-war. Cooperation and conflict theory can elucidate the paradoxical role fetal cell microchimerism plays in maternal health. We predict that tissues involved in resource allocation, such as the brain, thyroid and breast would likely be reservoirs for fetal cells (depicted as orange circles in maternal tissues). Additionally, the maternal immune system is likely to play an active role in fetal-maternal interactions. Within these tissues, the outcomes of these fetal-maternal negotiations (mother-offspring tug-of-war) are important in maternal health and wellbeing.

capacity of fetal cells to manipulate maternal tissues to the mother's fitness detriment. One likely candidate mechanism for maternal countermeasures is the targeting and elimination of fetal cells by maternal immune cells. In mice, it has been shown that fetal cells can be eliminated by the maternal immune system [34]. Limiting fetal cells may lead to a more maternally optimal resource transfer level to the offspring. This leads to the prediction that the capacity of the maternal immune system to limit the abundance of fetal cells may have positive impacts on maternal health. However, if fetal cells have the capacity to alter their phenotype and become more aggressive in response to maternal countermeasures, this could lead to costly escalation of conflict with potentially negative consequences for both parties.

Fetal cells may be able to respond to maternal countermeasures through increasing proliferation rates, producing signaling factors that manipulate maternal tissues more intensely, or even targeting the destruction of maternal immune cells. One study demonstrated that invading trophoblast cells can induce apoptosis in maternal T-cells [35], suggesting that fetal cells may have sophisticated countermeasures to maternal responses. This presents a critical question: does conflict between maternal tissues and fetal cells escalate, de-escalate or reach some sort of détente? The maternal-fetal dyad is a complex and dynamic system and there may be substantial individual differences with regard to this trajectory. The cooperation and conflict framework suggests that it is in the situations of escalating conflict that the greatest likelihood of negative outcomes for maternal health, similar to the negative health outcomes (e.g. gestational diabetes and preeclampsia) that have been attributed to escalating maternal-fetal conflict during pregnancy [3, 5].

It is important to point out here that cooperation and conflict between maternal and offspring interests does not imply intention on the part of either party, but it is instead a consequence of selection that can be instantiated through a variety of mechanisms. Genomic imprinting is one likely candidate, as it allows for phenotypic plasticity and it has

already been implicated in maternal-fetal conflict in the womb via imprinting of maternal and paternal genes in the placenta [36].

Contradictory effects of fetal cells on maternal health

Fetal cells are associated with wound healing/response to injury

Some fetal cells have stem-like properties [9, 37, 38] that may allow them to provide maternal benefits. The replenishing of stem cell niches may enhance maternal survival through counteracting some of the negative effects of stem cell loss and damage due to aging [39]. It is in the fitness interest of the offspring to enhance maternal survival and contribute to maintenance of the maternal body. This predicts that fetal cells may take over stem cell niches, which may be associated with greater survival and reduced aging of mothers. Additionally, fetal microchimeric phenotypes may be under selective pressure to contribute to maternal health through enhancing wound healing. This predicts that fetal cells should be found at the sites of wounds and that their presence should be associated with better outcomes for maternal health.

Several studies suggest that fetal cells may play a role in maternal wound healing. Murine injury models have tracked fetal cells actively migrating to the site of injury in the maternal body [17, 40–42]. Two of these injury models report clustering of fetal cells in relation to maternal blood vessels at sites of inflammation, suggesting participation in maternal angiogenesis [17, 40]. Additionally, in humans, fetal cells were identified in healed cesarean section scars and expressed markers of cytokeratin and collagen [16], suggesting that fetal cells actively participate in maternal wound healing.

Also, consistent with the hypothesis that fetal cells contribute to ongoing maternal somatic maintenance, fetal cells have been identified in many healthy tissues in human mothers [10, 43–45] and rodent mothers [41, 42, 46, 47].

Further, across tissues in the maternal body (in humans and mice), there is evidence that fetal cells differentiate into multiple cell types, including endothelial cells [16], neurons [47], smooth muscle cells, and cardiomyocytes [10, 48]. Mesenchymal cells of fetal origin have consistently been detected in the bone marrow of women who had given birth to at least one son [37], and in healthy women, fetal cells were present in numerous maternal immune cell populations, including T cells, B cells, and natural killer cells [49] as well as T cells and B cells in mouse models [38]. These studies suggest that fetal cells are at least passively contributing to increased somatic maintenance in mothers and are likely actively contributing to wound healing.

Fetal cells are found at sites of maternal-offspring resource allocation

In addition to the positive role that fetal cells may play in maternal wound healing and somatic maintenance, they may also play a negative role (from the perspective of maternal fitness interests) by manipulating resource transfer to offspring above the maternal optimum. Below we expand upon our predictions and then review the current literature regarding the presence of fetal cells in tissues involved in resource allocation and the association of these cells with maternal health or disease. Most human studies that we report below tested for the presence or absence of male DNA/male cells in blood or tissue from female patients who have or have not previously given birth to a son. We summarize these results below and are also shown in Table 2.

Fetal cells are found in the breast

Mother's milk provides calories, nutrients, and immunological protection for offspring [50, 51]; however, lactation is costly for the mother. This means that there can be conflict over maternal milk supply, with offspring interests favoring higher milk supply than what is optimal for the mother [5]. If fetal cells are able to migrate to the breast and up-regulate milk production either through producing factors that manipulate maternal mammary glands or by differentiating into mammary gland themselves, this could benefit offspring fitness. This predicts that the presence of fetal cells in the breast should be associated with higher levels of milk production or enhanced quality of mother's milk and could be associated with negative health outcomes for the mother in some cases [52].

Fetal cells are found frequently in normal breast tissue of women postpartum. In healthy women, male DNA was detected in the mammary glands of more than half of women sampled [53]. However, existing research presents a complicated picture of fetal cells role in diseases of the breast. Fetal cells have been found less frequently in the blood and tissue of women with breast cancer compared to healthy controls [54–57], suggesting that more fetal cells may actually be associated with better health of the mother. However, a murine model of breast cancer found that high-grade tumors harbored significantly more fetal cells than low-grade tumors [58] and fetal cells have also been found in the tumor

stroma of human females [59]. Additionally, the association of fetal cells with breast cancer may be subtype-specific, as higher levels of fetal cells were reported in the tissue of women diagnosed with HER-2 subtype of breast cancer, while lower levels of fetal cells were associated with estrogen receptor/progesterone receptor positive tissue [53].

It is also possible that fetal microchimerism, through its long co-evolutionary history with the maternal body (Table 1), may now play a role in normal breast physiology. The maternal mammary gland harbors a population of stem cells that contribute to normal breast development and can be transferred to the fetus during lactation [60]. Fetal cells with stem-like properties located in the mammary gland could be responding to the same maternal signals. Mouse fetal fibroblast cells have been shown to differentiate into mammary epithelioid cells when exposed to lactation hormones (insulin, progesterone, and oxytocin) in vitro [61] and a functional mammary gland has been generated from a single stem cell in an pregnancy mouse model [62]. This suggests that fetal progenitor cells can play a role in maternal milk supply. Interestingly, women who reported to have an oversupply of milk during lactation were more likely to develop breast cancer [52], while reports are inconsistent between insufficient milk supply and breast cancer [63]. Together these findings suggest that fetal microchimeric cells may play a role in breast physiology and milk supply, but the effects on maternal health are not yet clear.

Fetal cells are found in the thyroid

The thyroid is important for thermoregulation and metabolism [64]. Production of heat is metabolically costly for both the mother and fetus, meaning that the interests of the mother and offspring are not always aligned. Offspring benefit from maternal heat generation and transmission [65]. Fetal cells in the maternal thyroid could enhance offspring fitness by manipulating maternal thermoregulation after birth. This predicts that fetal cell presence in the thyroid should be associated with higher maternal body temperature. Presence of fetal cells in the thyroid might also contribute to thyroid cancer risk or susceptibility to other thyroid diseases if these cells are proliferating or producing factors that may induce abnormal thyroid physiology.

While fetal cells are found in healthy thyroid tissue postpartum [66], current research also suggests an association with thyroid diseases. Murine models of thyroiditis find a higher frequency of fetal cell microchimerism in the thyroid during and after pregnancy [67]. Fetal cells have been found more frequently in the blood and thyroid tissue of women with Hashimoto's thyroiditis [44, 68–70], Graves' disease [68–71], and cancer [44, 66], compared to healthy controls. These results suggest that fetal cell presence in the thyroid is associated with maternal disease rather than health.

Fetal cells are found in the brain

Maternal attachment and bonding are important for the health of the infant [72]. "Maternal hormones" such as oxytocin and prolactin are released in the brain and play important roles in "letdown" contractions in breastfeeding [73], maternal milk

Table 2. Overview of microchimerism in maternal health and disease

Tissue	Disease/Function	Sample	Species	Fetal cell association with maternal health or disease	Findings
Brain	Alzheimer's disease	Brain	Human	Health	Fetal cells found less frequently in tissue of patients compared to healthy controls [75]
	Parkinson's disease	Brain	Mouse	Disease	Fetal cells found more frequently in disease tissue compared to healthy controls initially, but not after long-term observation [47]
Breast	Cancer	Blood/Breast	Human	Health	Fetal cells less frequent in blood and tissue of patients compared to healthy controls [54–57]
	Cancer	Breast	Human	Health	Lower levels of fetal cells in ER/PR-positive breast cancer tissue compared to healthy controls [53]
	Cancer	Breast	Human	Disease	Higher levels of fetal cells in HER-2 breast cancer tissue compared to healthy controls [53]
	Cancer	Breast	Human	Disease	Presence of fetal-derived cells in tumor stroma [58]
	Cancer	Breast	Mouse	Disease	Fetal cells are present in murine breast carcinomas. High-grade tumors contain more fetal cells [59]
Thyroid	Cancer	Blood/Thyroid	Human	Health	Fetal cells/male DNA found more frequently in the blood of healthy controls compared to patients. Found no significant difference in tissue samples [66]
	Cancer	Thyroid	Human	Disease	Fetal cells found more frequently in the disease tissue of patients compared to healthy tissue [44, 66]
	Hashimoto's thyroiditis	Blood/Thyroid	Human	Disease	Fetal cells/male DNA found more frequently in patients compared to healthy controls [44, 68–70, 113]
	Graves' disease	Blood/Thyroid	Human	Disease	Fetal cells/male DNA found more frequently in patients compared to healthy controls [69–71]
	Thyroiditis	Thyroid	Mouse	Disease	Fetal cells found more frequently in patients compared to healthy controls [67]
Immune system	Systemic sclerosis	Blood/Skin lesion	Human	Disease	Fetal cells/male DNA found more frequently in patients than healthy controls [49, 79, 80]
	Systemic sclerosis	Blood	Human	No association	No difference in frequency of male DNA between patients and controls [87, 88]
	Sjögren syndrome	Blood/Salivary gland	Human	Disease	Male DNA was higher in tissue but not in blood in patients compared to healthy controls [81]
	SLE	Blood	Human	Disease	Male DNA/fetal cells higher in patients than healthy controls [82, 83]
	SLE	Multiple tissues	Human	Disease	Fetal cells/male DNA found more frequently in damaged tissue compared to healthy tissue [114]
	SLE	Blood	Human	No association	No difference in frequency or quantity of male DNA between patients and controls [89]
	Rheumatoid arthritis	Nodule	Human	Disease	Male DNA detected in rheumatoid nodules [86]
Rheumatoid arthritis	Blood	Human	Disease	Prevalence of fetal cells/male DNA higher in patients than healthy controls [83, 84]	
Rheumatoid arthritis	Blood	Human	No association	No difference in male DNA between patients and controls [90]	

(Continued)

Table 2. (Continued)

Tissue	Disease/Function	Sample	Species	Fetal cell association with maternal health or disease	Findings
Lungs	Cancer	Lung/Thymus	Human	Disease	Fetal cells found more frequent in diseased tissue compared to healthy tissue from same patient [92]
Heart	Injury model	Heart	Mouse	Health	Fetal cells home to injured maternal hearts and differentiate into endothelial cells, smooth muscle cells, and cardiomyocytes [48]
	Cardiomyopathy	Heart	Human	Disease	Fetal cells were found in found in patients, but not healthy controls [10]
Liver/Kidney/Spleen	Injury model	Injured tissue	Mouse	Health	Fetal cells home to injured tissues [41, 42]
Reproductive tissues	Cancer	Cervical tissue	Human	Disease	Male fetal cells found more frequently in tissue of patients compared to controls [115]
	Cancer	Endometrial tissues	Human	No association	Male DNA found in both benign and diseased tissue [116]
	Endometriosis	Endometrial tissues	Human	No association	Male DNA were not observed in disease or healthy tissue [117]
Colon	Cancer	Blood	Human	Disease	Fetal cells found more frequently in the blood of patients compared to healthy controls [56]
Skin	Caesarean section	Skin	Human	Health	Fetal cells identified in some healed maternal CS scars and expressed cytokeratin, and collagen I, III, and TGF- β 3 in healed maternal scar [16]
	Injury model	Skin	Mouse	Health	Fetal cells more frequent in maternal inflamed tissues and participate in maternal angiogenesis and inflammation [17, 40]
	Melanoma	Skin	Human/Mouse	Disease	Fetal cells detected more frequently in melanoma compared to benign or healthy tissue [118]
	PEP	Skin	Human	Disease	Male DNA detected in tissue of patients with no detection in healthy controls [85]

SLE, systemic lupus erythematosus; PEP, polymorphic eruptions of pregnancy; CS, cesarean section.

A summary of the current literature on fetal cell microchimerism and the proposed association with maternal health and disease.

supply, as well as maternal calm and interest in the offspring [74]. The present framework suggests that selection may have favored fetal microchimeric phenotypes that can manipulate maternal brain function to enhance maternal resource transfer and attachment to offspring.

Fetal cells in the mouse maternal brain have been found to be able to integrate into maternal brain circuitry and express appropriate immunocytochemical markers for brain tissue [47]. More generally, the totipotent nature of fetal cells [9, 37, 43] suggest that they have the capacity to differentiate into cells that could participate in the neural circuitry and chemical communication taking place in the brain, possibilities that should be tested in future research. At the moment, several studies have found male DNA in the human and mouse maternal brain [75, 47], but the function of these cells, if any, is unknown. However, one of these studies found fetal cell DNA in multiple regions of the maternal brain, and in one case, decades after the woman had given birth to a son [75], suggesting that fetal microchimerism in the human maternal brain may be pervasive and long-lasting.

Fetal microchimerism in the brain is not clearly associated with either maternal health or disease. Male DNA has been detected more frequently in disease tissues compared to healthy controls in a mouse model of Parkinson's disease [47]. However, fetal cells were found to be less common in the brains of women diagnosed with Alzheimer's disease compared to healthy controls [75]. These findings suggest that fetal cells might have important effects on brain function, though the underlying mechanisms are not yet understood.

If fetal cell manipulation of the maternal brain induces maternally suboptimal levels of resource transmission to offspring, maternal countermeasures such as immune targeting of fetal cells in the brain might also be expected, which could raise levels of inflammation in the brain. This raises the intriguing possibility that conflict between fetal cells in the brain and maternal immune cells counteracting those effects could be a partial explanation for several empirical findings, including the observation that post-partum depression [76] is associated with inflammation, and also with higher parity and shorter interbirth interval [77]. However, there are many other potential explanations for these associations and future research is needed to determine whether fetal microchimerism plays a role in maternal emotional health. The possibility the fetal microchimerism may play a role in maternal mental health has elsewhere been suggested [78], though not within the evolutionary framework proposed here.

Fetal cells are found at autoimmune disease sites

The prospect of an escalating conflict between fetal cells and maternal tissues, especially in the context of maternal immune targeting of fetal cells, may contribute to maternal disease after pregnancy. In situations where maternal-fetal cell conflict escalates, we predict co-localization of fetal cells and maternal immune cells, but co-localization could also be the result of cooperation between maternal immune cells and fetal cells for maternal wound healing. This makes it important to disentangle the predictions and effects from each of these potential roles of fetal cells. If fetal cells enhance maternal wound healing, co-localization of immune cells and fetal cells would simply be a

byproduct of the fact that both may be recruited to sites of injury. A characterization of the functional effects of fetal cells and immune cells on each other and on the maternal tissue would allow for the testing of the specific hypotheses that derive from the conflict escalation hypothesis.

Fetal cells have been found at the sites of inflammation and are associated with many autoimmune diseases. Fetal cells have been found more frequently in blood and/or tissue samples in women with systemic sclerosis [49, 79, 80], Sjögren's syndrome [81], systemic lupus erythematosus (SLE) [82, 83], and rheumatoid arthritis (RA) [83, 84]. Further, fetal cells have been detected at sites of inflammation in the maternal body, including skin lesions of women with polymorphic eruptions of pregnancy (PEP) [85], rheumatoid nodules of patients with RA [86], and in the goiter of a patient with thyroid disease [68]. In contrast, a few studies report no association between fetal cells and the diseases systemic sclerosis [87, 88], SLE [89], and RA [90], when compared to healthy controls. It is unknown whether these findings are due to active fetal cell migration from the blood into diseased tissue or whether the presence of fetal cells contributes to the disease. It is also not known whether the co-localization of fetal and maternal immune cells contributes to autoimmune disease.

Fetal cells are also found in maternal tissues not associated with resource conflict

During pregnancy, fetal cells enter the maternal body through the placenta and travel through the maternal vessels, being found frequently in the blood, and maternal lung [8, 29]. It is generally accepted that the presence of fetal cells in the lung is due to high fetal cell traffic, as the blood from the placenta must first pass through the mother's pulmonary circulation [30, 91]. However, despite the fact that fetal cells in the lung are likely just a byproduct of circulatory physiology, the presence of fetal cells in the lung does show an association with cancer: one study reported significantly more fetal cells in the lung and thymus tissue compared to the bone marrow and high levels of male DNA in the diseased lung compared to healthy bone marrow from the same individual [92].

Pregnancy is associated with changes in autoimmune disease presence and susceptibility

Pregnancy is associated with onset of some autoimmune diseases and cancer

Fetal cells enter the maternal tissues during pregnancy, possibly enhancing susceptibility to some diseases such as autoimmune diseases and cancer. Many studies have found an association of pregnancy with diseases of the breast and thyroid. While pregnancy is protective against long-term breast cancer risk, there is a transient increased risk of breast cancer in years immediately following pregnancy [93], suggesting a possible role for fetal cells. Thyroid disease is common in pregnancy, affecting 2–3% of women [94] and there is an increased incidence of Hashimoto's thyroiditis or Graves' disease

postpartum [95]. Women are at a higher risk than men for developing autoimmune diseases [96] and the risk of autoimmune disease in parous women is significantly higher after the first year postpartum [97]. However, these findings are inconsistent, as some studies report no link between parity and subsequent autoimmune disease [98]. Several pregnancy complications are associated with higher detectable fetal cells circulating in the maternal blood, including preeclampsia, abnormal karyotype and miscarriages [19–22].

Pregnancy alters symptoms of autoimmune diseases

Little research has focused whether fetal cells may contribute to pathogenesis or amelioration of symptoms of existing autoimmune diseases during pregnancy. During gestation, the maternal immune system develops a tolerance to the fetus, and suppression of the maternal response is lifted postpartum [99]. Tolerance to the fetus may help explain why some autoimmune disease symptoms are lessened during pregnancy in some women. For example, between 43 and 75% of patients with RA exhibit amelioration of some or all of their RA symptoms during pregnancy (reviewed in [100]). Further, in RA patients, symptoms tend to only show improvement during pregnancy, but exacerbation of symptoms postpartum [100]. Similarly, the rate of relapse declines with pregnancy in women diagnosed with multiple sclerosis (MS), with the lowest rate in the third trimester. Relapse rates return to prepregnancy levels postpartum [101]. However, in other autoimmune diseases, pregnancy is associated with no change symptoms or an exacerbation of symptoms [102, 103]. The present framework suggests that one contributor to autoimmune disease may be the maternal immune response to the presence and action of fetal cells that manipulate maternal tissues. Given that maternal tolerance of the fetus (and, therefore, also fetal cells) is lifted postpartum, it may be that the presence of high quantities of fetal cells (or fetal cells that are actively manipulating maternal tissues) leads to an intense maternal immune response, possibility increasing autoimmune disease symptoms. Together this suggests that fetal cells could be involved in autoimmune disease symptom changes, and should be incorporated into future studies of the role of pregnancy on autoimmune disease pathology.

Future research should use more sensitive methods for detecting fetal cells and determining their function

Major challenges for future work on fetal microchimerism and maternal health include: (1) distinguishing fetal alleles from maternal alleles; (2) detecting extremely low levels of fetal cells; (3) identifying the functional role of fetal cells in maternal tissues; and (4) measuring the interactions between fetal cells and maternal immune cells. Initial studies of fetal microchimerism relied on detecting the presence or absence of Y-linked alleles in tissue samples from the mother, specifically

evidence of the sex-determining region of the Y (SRY). Another common technique is to detect variable tandem repeat numbers in mothers versus offspring in microsatellite loci (short tandem repeats) within the Human Leukocyte Antigen (HLA) A and B [30], or detecting differences in unshared HLA-DRB1 alleles [104]. Common methods for distinguishing fetal from maternal alleles at a single locus include flow cytometry, fluorescence immunoprecipitation of the gene or through classical or quantitative PCR [30, 105]. PCR is more accurate than immunofluorescence for detecting microchimeric cells [42], but is not as sensitive as flow cytometry, with PCR detecting microchimeric events at about 0.2% frequency (~198 cells out of 1×10^5 host cells), while flow cytometry could detect microchimeric events at 0.05% frequency (~48 cells out of 1×10^5 host cells) [105]. Detection of low-frequency microchimeric alleles may also suffer from false positives due to contamination of samples with extraneous DNA, as may be common in clinical laboratory equipment [106].

To increase detection of fetal alleles, in each new pregnancy, the genotype of the mother and father could be used to develop multiple sensitive genomic loci for detection, either using traditional assays, or next-generation whole genome sequencing. This may also avoid confounding contemporary microchimerism with events from previous pregnancies. Testing multiple loci will likely also improve the ability to detect extremely low levels of fetal cell microchimerism. Low coverage whole genome sequencing of maternal plasma DNA is already used for sensitive non-invasive prenatal testing [107], and could additionally be utilized to detect fetal cell microchimerism both during and after pregnancy. Methods for detecting fetal cell microchimerism should also include testing of multiple tissues. Although blood is the most commonly and easily assayed tissue, and cell free fetal DNA is routinely detected in maternal blood during pregnancy [107], most fetal microchimerism in the blood is removed postpartum [11], and clinically relevant fetal microchimeric events may lie in the tissue related to the pathology of interest. Detecting microchimeric cells is one hurdle, but detection alone is not enough. Future studies will also need to consider the function of these cells, perhaps by flow sorting microchimeric cells (e.g. [108]) from tissues and assaying the transcriptome and proteome. Perhaps more important that understanding the function of the microchimeric cells alone will be how they interact with maternal tissues. For example, initial studies that have identified that cells of fetal origin (specifically extravillous trophoblasts) do modulate the maternal immune system by interacting with maternal leukocytes [109, 110], could be replicated in the breast, thyroid, and brain.

Conclusions and outlook

Microchimerism is common in humans and has been found across eutherian mammals of all placental types. Fetal cells have many parallels with the placenta and can perhaps be considered a far-reaching extension of the placenta “beyond the womb,” into maternal tissues during pregnancy and lasting long after parturition. The cooperation and conflict framework that we propose here can help to organize and explain some of the contradictory findings in the literature

Table 3. Testable predictions about microchimerism

Predictions	Rationale	Testing
Fetal microchimeric cells in the breast increases maternal milk production and quality	It is in the offspring's fitness interest to increase maternal milk production and quality	Assay microchimerism in maternal blood and breast milk and quantify the amount of milk produced at routine time points (e.g. 1, 2, 4, and 10 weeks postpartum)
Fetal cell microchimerism in the thyroid increases maternal body temperature	Offspring benefit from increased maternal heat production as they do not have to engage in costly heat production [65]	Quantify fetal cell abundance in blood and measure maternal body temperature prepartum, once each trimester, and postpartum
Attachment and bonding mechanism are enhanced by fetal cells	Offspring are likely to receive more resources as a result of increased maternal attachment and bonding with positive fitness consequences [73]	Measure abundance of circulating fetal cells and oxytocin prepartum (control), third trimester, and for three month intervals postpartum. Flow sort fetal cells and assay transcript abundance of genes regulating oxytocin and prolactin production
Microchimeric fetal cells contribute to the development and/or progression of cancers	Fetal microchimeric cells have some characteristics of cancer [119], including evading the maternal immune system [11] and inducing angiogenesis [40]. These cancer-like properties could lead to greater cancer vulnerabilities, specifically at the initial stages of tumor development, when escaping destruction from the immune system and growth may be most important	Investigate transcription of clones within the tumor and possible microchimeric tumor cells using single-cell sequencing from multiple positions in the tumor, including the center, and surrounding healthy tissue and compare across patients to distinguish between fetal and other microchimeric events (men, women who have never had a pregnancy, and women who have had at least one pregnancy)
Fetal microchimeric cells in the immune system increase autoimmune disease susceptibility	It would be in the fetal interest to evade detection and destruction by the maternal immune system, which may have variable response by HLA haplotypes [7]. An over-reactive response from the maternal immune system to small numbers of fetal cells, or a comparable response to over-proliferating fetal cells, may increase autoimmune disease susceptibility, or symptoms of an existing autoimmune disease. Alternatively, a reduced maternal response is expected to lead to amelioration of autoimmune disease symptoms	In patients with preexisting autoimmune disease, a family history of autoimmune disease, and no susceptibility to autoimmune disease, measure abundance of fetal cells and measure autoimmune disease symptoms, C-reactive protein levels, and abundance of T regulatory cells pre-partum, each trimester, and postpartum. Assay HLA profiles of maternal and fetal cells by sequencing the cell free DNA. Characterize antigen expressed on fetal cells
Fetal cell quantity in the maternal body contributes to signaling pregnancy success, completion, and/or termination	(1) Fetal cells may be important in conception and retention of the fetus. The maternal body must suppress immune function for a successful placentation and gestation. Fetal cells could play a role in tolerance for successful pregnancy by homing to thymus prior to placentation and stimulation T regulatory cells (2) Fetal cells increase in maternal blood until onset of labor [18, 8], this could be one component important for triggering labor, even pre-term labor, either through maternal immune response, or oxytocin signaling	Prospectively collect a cohort planning a pregnancy, without pre-existing diseases. Measure abundance of T-regulatory cells for 3 month intervals until conception, then continue to measure T-regulatory abundance as well as assay abundance and antigen composition of microchimeric cells in the blood at 6, 12, 20, 28, 36, 40 weeks, and 3 months postpartum

Based on our cooperation and conflict framework, we propose testable predictions, rationale, and methods for testing predictions for the potential role of fetal cells in maternal health.

regarding the effects of fetal cells on maternal health. However, many important questions remain. A casual role of fetal cells (and maternal immune response to fetal cells) in maternal health or disease has not yet been definitely shown. Improved methods for distinguishing maternal and fetal alleles and detecting low levels of microchimerism in the blood and maternal tissues will help to advance our

understanding of the role of fetal microchimerism in maternal health and disease. Our framework makes many predictions (Fig. 2 and Table 3) that can be tested in future work, with potentially important implications for our understanding of health and disease pathology, including lactation science, thyroid diseases, autoimmune diseases, cancer and even maternal emotional, and psychological health postpartum.

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