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The Origin of Platelets enabled the evolution of Eutherian Placentation

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Abstract

Invasive placentation with extended pregnancy is a shared derived characteristic unique to eutherian mammals which possess a highly effective system of haemostasis, platelets. These are found in all mammals but no other group of animals. We propose that platelets and megakaryocytes (large polyploid nucleated bone marrow cells that produce platelets) evolved from an ancestral 2N thrombocyte by polyploidization and that the possession of platelets enabled the evolution of invasive placentation. This could explain why invasive placentation is limited to mammals.

Key Words

Evolution, placenta, platelet, megakaryocyte, mammal.

Introduction

Mammals have many unique traits, two of which are: the megakaryocyte/platelet system (MK/P) and invasive (endothelio- and haemochorial) placentation. MK/P is not found in birds or reptiles [1]. Haemochorial placentation is only found in eutherian mammals [2-5] but not in marsupials and monotremes (Figure 1). We propose that haemochorial placentation required MK/P for its evolution, thus explaining this nested distribution.

Giving birth to live neonates (viviparity) rather than laying eggs is widespread. It has evolved many times in fishes, frogs, salamanders, lizards, snakes and mammals[6,7]. Among vertebrates, viviparous lineages are only absent from the cyclostomes and the archosaurs including birds. Probably viviparity has evolved more than 100 times in lizards and snakes alone[8-10]. Viviparity and placentation are also found in some invertebrates[11].

Surprisingly only eutherian mammals have evolved invasive, haemochorial placentation even though many lineages have evolved various complex forms of placentation [8,12,13]. Although viviparity is simple to evolve[14], the evolution of haemochorial placentation is limited to animals with MK/P. We suggest that MK/P was an 'exaptation' *sensu* Gould and Vrba[15]: a trait that has a biological role in an organism, not originating for that function but acquiring its role by transfer of function. We argue that the preceding evolution of platelets was the exaptation necessary for the origin of invasive placentation.

The evolution of mammalian reproduction

There are four types of reproduction in mammals: egg-laying in monotremes, short embryo attachment in marsupials, deep placentation in ancestral placental mammals, and reversion to non-invasive placentation as in horses and bovines[3-5]. The most ancestral form of mammalian reproduction is found in monotremes, egg-laying mammals, (Platypus and the Echidna) [16,17], which already have some degree of oviparous matrotrophy through the eggshell[18]. Marsupial

reproduction is characterized by a relatively long period of egg retention with "hatching" from the egg within the uterus, then a brief period of attachment to the uterine mucosa; a step towards placental mammals. In non-macropod marsupials embryo attachment is very brief producing immature neonates[19,20]. There is longer gestation in macropods [21]. Molecular phylogeny studies of mammals [3-5]suggest that the ancestral fetal-maternal interface in eutherians was haemo- or at least endotheliochorial.

In marsupials, the very brief embryo attachment involves uterine inflammation followed by parturition[22,23]. In eutherian mammals, embryo implantation also involves inflammatory activation[24], followed by an anti-inflammatory state. Hence the key event in the evolution of placental pregnancy was the ability to suppress the implantation related inflammation allowing deep implantation with destruction of maternal blood vessels creating the hemochorial fetal-maternal interface [22,25]. This progression towards deeply invasive placentation in eutherians was only possible in animals that could handle the challenging hemostatic consequences of hemochorial implantation.

The evolution and function of megakaryocytes and platelets

Platelets are small enucleate secretory cells, produced from megakaryocytes[26]. They aggregate to occlude a site of bleeding, to initiate thrombus formation and secrete growth factors to repair blood vessels. Platelets have similar function and structure in all mammals including monotremes[27]. For haemostasis reptiles and birds rely on the aggregation of circulating nucleated cells called thrombocytes[28] which are less efficient than platelets[29 30]. Thrombocyte like cells occur in arthropods: coagulocytes in insects[31] and amoebocytes in the limulus crab[32].

The physical and biological conditions of the pulmonary circulation support platelet production from megakaryocytes that have travelled in the venous circulation from the bone marrow. [33-36] Platelets are produced by physical fragmentation of megakaryocyte cytoplasm in the pulmonary circulation[37]. Megakaryocytes undergo true endomitosis: increase in nuclear DNA content within an intact nuclear membrane [38]. The unique step in the change from a 2N thrombocyte to a large polyploid megakaryocyte would have been a late failure of cytokinesis giving incomplete mitosis aborted in anaphase, then repeated up to 128N [38]. There is selective gene expression in higher ploidy cells [39,40].

Fragmentation of the polyploid nucleated cell to platelets would have given reproductive advantage due to enhanced haemostasis after attack or injury. MK/P was a quantitative haemostatic advance as small size gave a large increase both in cellular surface area and speed of granule secretion. A further, qualitative, advantage over 2N thrombocytes is that in response to bleeding megakaryocytes can increase their DNA content rapidly, up to 128N, producing even more active platelets with increased receptor density, more organelles per unit cellular volume, and increased capacity to produce pro thrombotic proteins and to reduce bleeding time [41 42 43 44 45 46 47 48 49]. Platelet granules contain about a hundred cargo proteins produced by the megakaryocyte. Platelet secreted proteins that are known to promote tumor growth (analogous to fetal growth) are VEGF, PDGF, EGF and TGF beta.

The Role of Platelets in Eutherian Reproduction

In eutherian pregnancy fertilization is associated with mild thrombocytopenia in mice [50] and women [51,52], due to the secretion of embryo derived platelet activating factor (ePAF) [53] which also induces early pregnancy factor (EPF). Pretreatment of mice with PAF leaves them unresponsive to ePAF and is associated with reduced implantation rate [54]. Platelets are a major storage compartment of serotonin (5HT). Maternal 5HT is essential for early development of the mouse embryo[55,56]. 5HT in early gestation is entirely supplied by maternal platelets[57]. This is surprising, given a pre-neuronal role of 5HT in embryo development in the frog *Xenopus*[58] and sea urchins[59] which lack placentas.

After extra villous trophoblasts (EVTs) lose proliferative activity they migrate towards uterine spiral arteries [60]. EVTs express the chemokine receptor CCR1[61]. Platelets secrete MIPI-1alpha and MCP-3 which are CCR1 ligands[62]. Probably these agents play a role in EVT migration and infiltration of the maternal arteries. Also, platelet alpha granule secreted EGF, VEGF and PDGF enhance trophoblast invasion[63,64] and encourage trophoblasts to infiltrate arteries[65].

Safe disconnection of the placenta from the uterus is essential for the survival of the mother. Contraction of the myometrium and endometrium are as important as is cellular haemostasis. Haemostatic balance tilts towards hypercoagulability during human pregnancy[66]. Evidence that platelets are important comes from human mothers with Bernard Soulier syndrome and Glanzmann's thrombasthenia, conditions manifesting a platelet dysfunction. Either primary or secondary hemorrhage occurs in 73% of pregnancies in patients with Bernard Soulier syndrome [67], and in 50% of mothers giving birth with Glanzmann's thrombasthenia[68]. KO experiments in mice show that maternal platelet defect is compatible with successful pregnancy [69].

The role of platelets in *postpartum* haemostasis alone is sufficient to support their role in the evolution of eutherian pregnancy. Other roles are rather specific to a sub-set of species and are thus likely derived, e.g. extra-villous trophoblasts are a cell type limited to hominids. Fetal dependency on maternal 5HT in early development also has to be a derived condition, given that amphibian and sea urchin embryos can supply their own 5HT. A process with potential generality is platelet activation by embryo derived PAF and its role in early implantation. The role of platelets in implantation, however, is likely part of the inflammatory nature of implantation[24], which probably evolved from an inflammatory attachment reaction in the stem lineage of therians, i.e. before the most recent common ancestor of marsupials and eutherians[22,25].

An evolutionary scenario

The evolution of haemochorial, invasive placentation faced at least two obstacles: inflammation caused by embryo-attachment to the uterine lining, and later, haemostasis. In marsupials, with the noted exception of Macropods [see above], fetal attachment to the uterine lining is followed quickly by various signs of inflammation, including neutrophil infiltration and parturition. In contrast, in eutherians the attachment/implantation of the fetus is followed by an anti-inflammatory phase that allowed the extension of pregnancy beyond the limits of the length of the estrus cycle[22]. The fact that inflammatory processes are involved in both marsupial and eutherian mammals, though with different outcomes, is correlated with the 'generic' aggressiveness of the therian blastocysts. In eutherians it leads to implantation. Even in marsupials without implantation the fetus is quite aggressive in attacking the luminal epithelium (LE) of the uterus ; in the gray short tailed opossum, *Monodelphis domestica*, at the end of gestation, cytoplasmic extensions of trophoblast cells can be seen to penetrate between the epithelial cells and breach the basal membrane of the LE[70,71] (Wagner pers. obs.), also in the Philander opossum[72], and bandicoots (Peramelidae)[73]. Differences in the invasiveness of the trophoblast between marsupials and eutherians are not differences in the fetus but rather in the way the maternal organism handles the situation. In marsupials, the partial invasion leads to expulsion (parturition) and in eutherians the inflammatory reaction is attenuated and pregnancy extended.

The situation in reptiles is not as clear. In most cases of placental viviparous lizards the placenta does not erode the luminal epithelium but is in apposition with the luminal epithelium and is held in place by uterine muscle contraction[74]. The lack of invasiveness could be explained by a lower aggressiveness of the fetus, as demonstrated in the case of an ectopic pregnancy in the southern grass skink (*Pseudemoia entrecasteauxii*[75]), which is a placentotrophic lizard. Any form of invasiveness is extremely rare in lizards given the large number of viviparous lizards. In one , the African skink *Trachylepis ivensi* (Scincidae), a rare example of lizard 'invasion' does not lead to the establishment of a haemochorial placenta[76]. It is unclear whether this less invasive form may have been a way of lizards evolving a sustainable fetal maternal relationship.

As soon as the mother had evolved a way of suppressing and managing the foetally induced inflammation another problem arose, haemostasis. Haemochorial implantation leads to the partial destruction of the maternal blood vessels in the endometrium and thus raises the question of how the bleeding is limited to the area of placentation. This second problem arises at parturition, where the fetal-maternal interface is dissociated, leaving, in many species, a broad exposed lesion in the uterus. Fast and reliable haemostasis at the wound is essential for the survival of the mother. Mammalian neonates rely on lactation for survival and maternal demise thus also leads to neonatal demise. We argue that the fact that mammals have a much more effective system for haemostasis than other vertebrates (the MK/P system) may have been a *key exaptation* for the evolutionary establishment of haemochorial placentation.

Eutherians vary greatly in how the haemochorial interface is organized which may lead to different needs for haemostasis at parturition. One extreme example is that of the nine-banded armadillo, *Dasypus novemcintus,* whose placenta is technically hemochorial, in that the villi of the placenta are in direct contact with maternal blood [77,78]. However, this is achieved in a

minimally invasive way. Single villi penetrate the endometrium and grow towards preformed maternal blood spaces and only expand and ramify once they have reached the varicosities (Figure 2A). Hence haemostasis during implantation and gestation is a minimal concern for armadillos, given that they have a well contained space preformed into which placental extensions reach. Never the less, even the armadillo has to face the danger of a major hemorrhage at parturition (Figure 2B). Another example is the massive postpartum bleeding in the African elephant, an animal with endotheliochorial placentation[79] and possibly also the dugong, also an afrotherian mammal[80] and the manatee[81]. Hence, we think the most important reason why haemochorial placenta is limited to eutherian mammals is that parturition of a haemochorial placenta leads to profuse bleeding in the uterus that needs to be arrested.

Conclusion

Deeply invasive haemochorial placentation is limited to the eutherian mammals. This is surprising given the large number of non-mammalian animals that have evolved viviparity and placentation. As well as the role of platelets in implantation we argue that hemochorial placentation is limited to a clade of mammals, because mammals are the only vertebrate group that has evolved a highly effective and unique system of haemostasis: platelets. The effectiveness of haemostasis is essential at parturition where even minimally invasive placentae can hemorrhage.

All neonatal mammals, regardless of how developed they are at birth, rely on maternal lactation for their initial growth and survival after birth, and thus the survival of the mother is critical. Consequently, the evolution of invasive placentation is most likely to succeed in a lineage that has already a highly effective system of haemostasis before the origin of deep placentation. From the standpoint of evolutionary theory, platelets are an *exaptation, sensu* Gould and Vrba[15], for the evolution of haemochorial placenta, i.e. a trait that has an important role but which evolved for another purpose and prior to taking over this role. Platelets could be called a *permissive exaptation* as it may have permitted the evolution of a novel trait, haemochorial placentation, rather than acquiring a new function itself.

Platelet production from megakaryocytes is an important area for research in thrombosis. The ideas presented here may help stimulate new research into the powerful thrombotic forces associated with evolution of the placenta but which also cause thrombosis of human arteries [41,82].

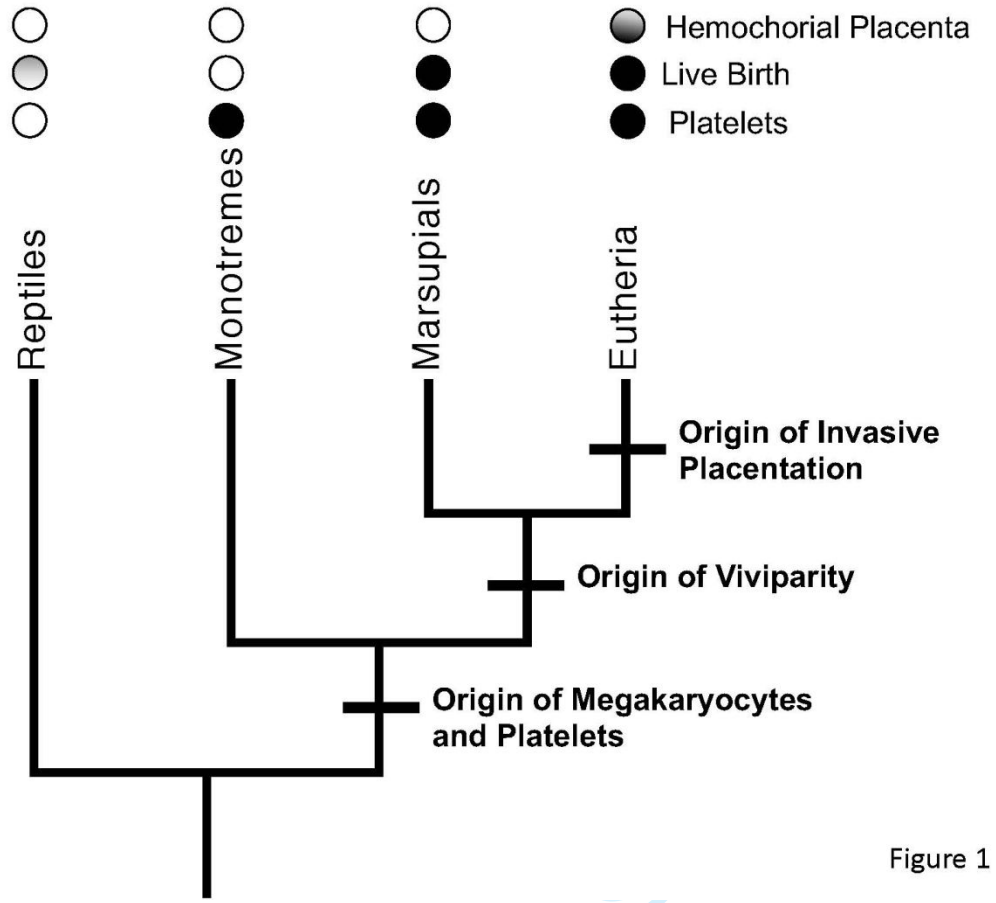
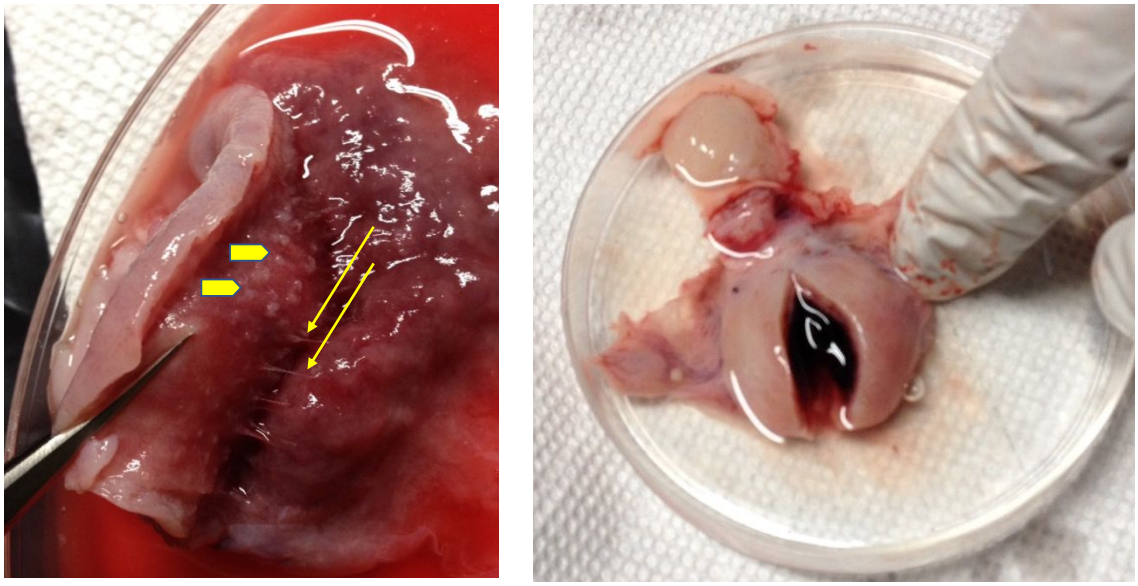


Figure 1

Figure 1: phylogenetic relationships of the major clades of mammals and the taxonomic distribution of haemostatic and reproductive characters. Platelets and megakaryocytes are found in all three clades of mammals but not in reptiles. Therians, i.e. eutherians and marsupials, share viviparity. In reptiles the mode of reproduction is variable. Only eutherians have hemochorial placenta. This condition is ancestral in eutherians, but there are some derived groups that have re-evolved non-invasive, epitheliochorial placentation: dot is shaded, with darker shading at the bottom, indicating ancestral condition.



A B

Figure 2: the need for haemostasis in a minimally invasive hemochorial animal *Dasypus novemcintus*, which belongs to the eutherian clade most distantly related to humans. A) the minimally invasive placenta of armadillo in third month gestation. The thin threads indicated by yellow arrows are the projections of the placenta entering the endometrium to the left. Arrow heads indicate penetration. The invasion though hemochorial is minimally destructive. B) postpartum uterus of armadillo, showing copious coagulated blood in the uterine cavity, indicating the need for effective haemostasis.

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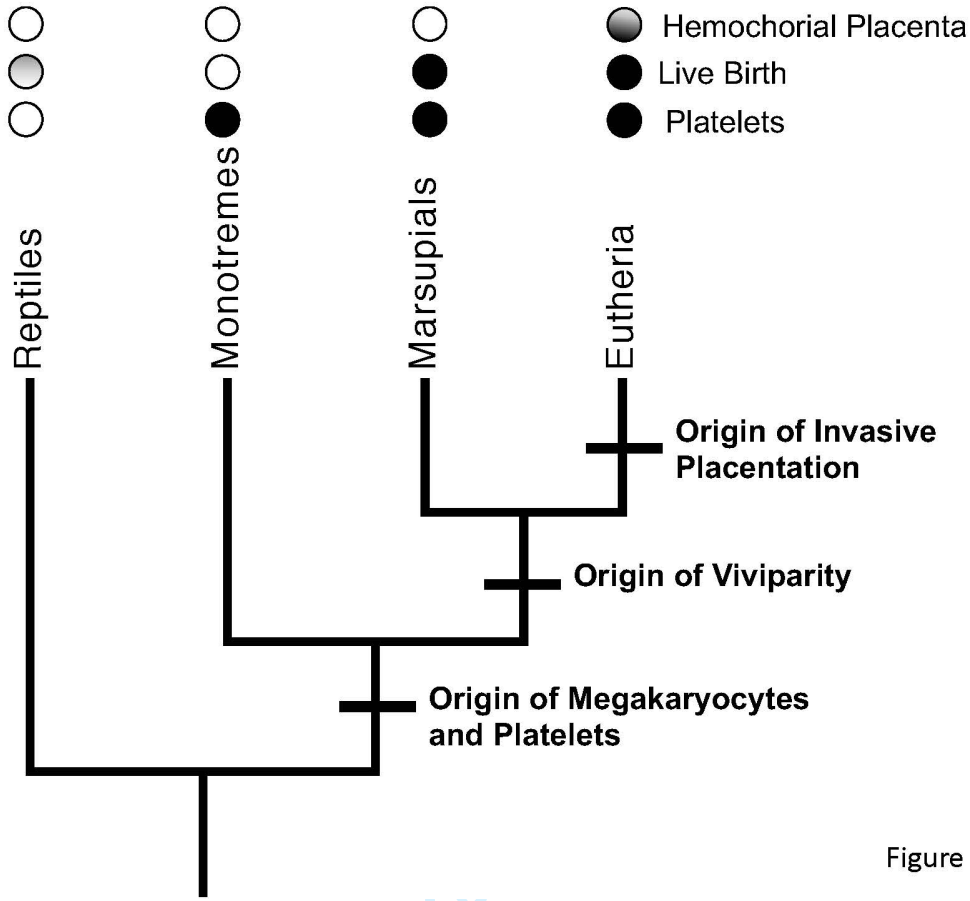
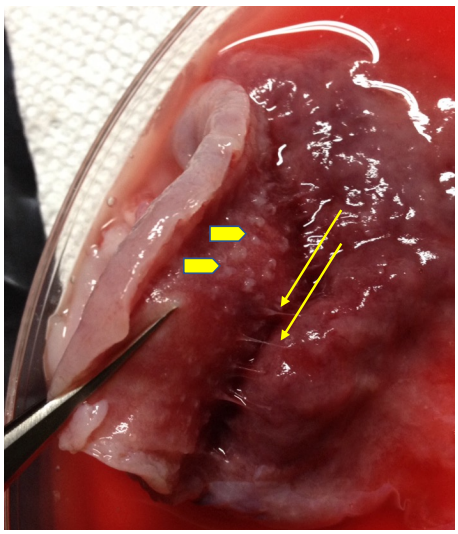
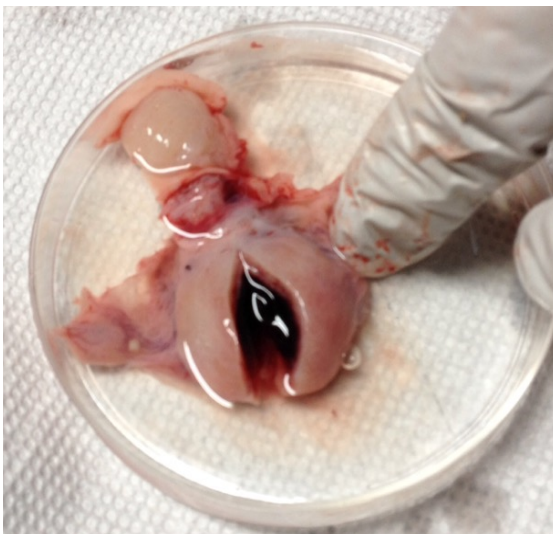


Figure 1



A



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