

THE ORIGIN OF HEMATOPOIESIS AND VASCULOGENESIS IN THE YOLK SAC AND THE ONSET OF INTRAEMBRYONIC BLOOD FLOW AND CARDIAC FUNCTION IN MAMMALIANS

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SUMMARY

The vascular system in embryos is essential in establishing the maternal-fetal exchange through the placenta. The purpose of this review is to gather comparative data to yield a better understanding of the mechanisms involved in embryonic hematopoiesis and vasculogenesis, in addition to data on maternal-fetal nutrients exchange, the extra embryonic yolk sac erythrocytes stream, the circulation development in the yolk sac and embryo, and the onset of cardiac function. In conclusion, the cardiovascular system is



fundamental for embryonic life in the uterus to replace yolk sac nutritional reserves. This review allows further studies on embryonic formation of the cardiovascular system.

Key words: circulation, embryo, hematopoiesis, yolk sac.

ABSTRACT

The cardiovascular system of mammals is the first functional system to develop in the embryo due to its importance in replacing short-lived nutrition through diffusion in the yolk sac. The formation of a vascular plexus is necessary to establish the maternal-fetal exchange in the embryo through the placenta. The purpose of this review is to gather comparative data that can yield a better understanding of the mechanisms involved in embryonic hematopoiesis and vasculogenesis originated in the yolk sac, as well as data related to maternal-fetal gas and nutrients exchange, to the extra embryonic yolk sac erythrocytes stream into the embryo itself, to the formation of circulation in the yolk sac and embryo and the onset of cardiac function. We conclude that the existence of a cardiovascular system is essential for life in the early post-implantation embryos in the uterus of placental animals. The review also points to further studies of the mechanisms involved in the embryonic formation of the cardiovascular system.

Key words: circulation, embryo, hematopoiesis, yolk sac.

INTRODUCTION

The production of cellular and figurative elements in the blood tissue is called hematopoiesis. The hematopoietic activity generates several cell types, divided into lymphocyte lineages (T and B lymphocytes, natural killer and dendritic cells) and an erythromyeloid lineage (macrophages, eosinophiles, neutrophiles, mast, erythrocytes, etc.) from the hematopoietic stem cells (HSC) (Weissman et al., 2001; Junqueira; Carneiro, 2004; Sadler, 2005). In the developing mammalian embryo, hematopoiesis and early vascular structures originate in the form of blood islands in the yolk sac, derived from the migration of mesodermal cells during gastrulation (Palis e Youder, 2001; Choi et al., 1998; Choi, 2002; McGrath e Palis, 2005). The yolk sac external cells



differentiate into endothelial cells, while the internal cells give rise to primitive blood (Choi et al., 1998; Choi, 2002).

The close spatio-temporal timing in the generation of blood cells and endothelial cells in yolk sac blood islands has suggested a common precursor for these cells named "hemangioblast" (Choi et al., 1998; Palis; Youder, 2001; Baron, 2003; Araújo et al., 2005; McGrath; Palis, 2005; Hyttel, 2010; Xu e Cleaver, 2011). The hemangioblasts derive from fibroblast growth factor (FGF-2) induction on the yolk sac blood islands (Evans, 1997; Palis; Youder, 2001; Baron, 2003; Sadler, 2005). Primitive hematopoietic stem cells arise in the yolk sac, while definitive stem cells arise in the mesoderm surrounding the aorta, the AGM (aorta/gonad/mesonephros) region, and these cells will seed the fetal liver, which becomes the main fetal hematopoietic organ. Subsequently, liver stem cells will seed the bone marrow, which will form "definitive" blood (McGrath; Palis, 2005; Sadler, 2005).

After a primary vascular layer has been established by vasculogenesis, additional vasculature is regulated by VEGF, which stimulates endothelial cell proliferation where new vessels will be formed by angiogenesis (McGrath et al., 2003; Sadler, 2005). The primitive blood and endothelial cells originated in the mammalian yolk sac blood islands increase in number, thus establishing a yolk sac vascular plexus. These cells, subsequently, enter the embryo proper to initiate circulation (Ji et al., 2003; MC Grath e Palis, 2005).

Hematopoietic development mechanisms, angiogenesis, vasculogenesis and initial circulation are involved in several pathological processes, such as fetal maldevelopment and cancer, as well as in research on cellular therapy through pluripotent cells generated in the yolk sac at the onset of mammal embryogenesis. This literary review is targeted at organizing data which could provide a better understanding of mechanisms involved in embryony hematopoiesis, vasculogenesis, angiogenesis and circulation, involving yolk sac derived progenitors, as well as connecting the extraembryonic yolk sac erythrocyte circulation into the embryo proper to the onset of heartbeat.



Yolk sac formation

The yolk sac is a foetal membrane found in all embryos of vertebrates and it is particularly developed in fish, reptiles and birds (Galdos et al., 2010). In mammals it appears as a balloon-shaped structure connected to the embryo's midgut region. Its main function is to store nutrients, to synthesize protein, as well as to carry out phagocytic activity and substance transfer. In placental mammals these functions are reduced, since nutrition occurs through the placenta, which is also responsible for production of blood and endothelial cells (Choi et al., 1998; Palis; Youder, 2001; Baron, 2003; McGrath; Palis, 2005; Vejlsted, 2010).

In mammals, during gastrulation, three germinal layers (ectoderm, mesoderm and endoderm) are established in the embryo from an anterior bilayer disc formed by the hypoblast and the epiblast (Choi et al., 1998; Palis; Youder, 2001; Baron, 2003; McGrath; Palis, 2005; Vejlsted, 2010). Formation of the yolk sac involves the endoderm, which covers the embryo's ventral surface and forms the yolk sac roof, and the paraxial mesoderm, which is divided into a somatic or parietal mesoderm layer, which covers the amnion, and the splanchnic or visceral mesoderm layer, which covers the yolk sac. Together these layers cover the newly formed intraembryonic coeloms, continuous to the extraembryonic layer, and initiate the somite formation (Sadler, 2005; Moore; Persaud, 2008; Vejlsted, 2010).

Blood and blood vessel formation and circulation initiation in mammal embryogenesis

The cardiovascular system in humans, in domestic animals and other vertebrates consists of: heart, arteries, capillaries, venules and veins, as well as blood forming organs (hematopoietic organs), in addition to a lymphatic subsystem composed by lymphoid organs, lymphatic ducts and trunks, and lymphonodes (Di dio, 2002; Dyce et al., 2010; Storer et al., 1989; Hildebrand, 1995). This system is considered an integrator system, together with the nervous and endocrine systems, since it direct or indirectly involves all organs (Storer et al., 1989; Di dio, 2002). Hematopoietic and cardiovascular systems in domestic animals and other vertebrates, such as mice, and in humans are the first ones to originate during embryogenesis (Dyce et al., 2010; Hildebrand, 1995; Ji et



al., 2003; McGrath et al., 2003; Moore; Persaud, 2008; Hyttel, 2010; Xu; Cleaver, 2011). Formation of the cardiovascular system in humans and in domestic animals is owed to the need of oxygen and nutrient transfer by blood vessels from the mother circulation to the embryo, through the placenta, when initial yolk sac nutrient storage diminishes (Moore; Persaud, 2008; Hyttel, 2010).

Blood is the liquid contained in a closed compartment of the circulatory system, which keeps it in a one-way, regular movement (Junqueira; Carneiro, 2004). It is formed by a great volume of fluid matrix (plasma) and several different cell kinds, including RBCs (red blood cells) or erythrocytes, which, in mammals, are enucleated and contain great amount of hemoglobin; platelets, which are disc-shaped, enucleated and contain blood coagulation factors; and leukocytes, which are spherical and colorless, in suspension in the blood, and participate in the body's cellular and immunocellular defense (Banks, 1991; Junqueira; Carneiro, 2004; Hyttel, 2010). Main functions of blood in mammals are: O₂, CO₂ transportation, hormone regulation and nutrient transportation, metabolic waste excretion, formation of buffer systems, body fluid volume maintenance, thermo regulation of the body and protection against microorganisms and other metabolic waste (Banks, 1991; Hildebrand, 1995; Junqueira; Carneiro, 2004; Hyttel, 2010).

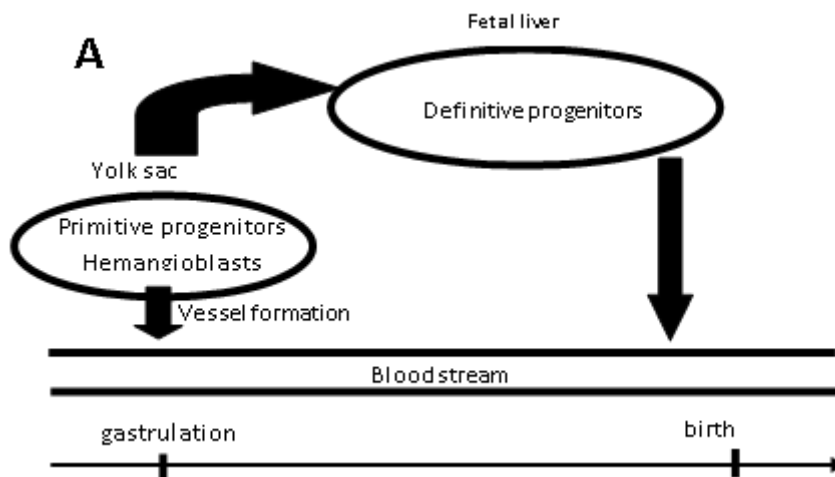
Mammalian embryo hematopoiesis initiates extraembryonically in the yolk sac, thus ensuring fetal survival until intraembryonic stem cells and cells from possible extraembryonic sites may develop the liver, which will provide continuous synthesis throughout fetal and postnatal life, a function subsequently fulfilled by the bone marrow (Godin; Cumano, 2002; McGrath; Palis, 2005; Baron, 2003). Primitive hematopoiesis in humans, mice and domestic animals occurs in the yolk sac during gastrulation and results in the primary production of large nucleated erythroblasts, endothelial cells which will form blood vessel walls, as well as megakaryocytes and primitive macrophages (Xu et al., 2001; Baron, 2003; Moore; Persaud, 2008; Sadler, 2005; Vejlsted, 2010; Mc Grath; Palis, 2005). (**Fig. 1**) On the other hand, definitive hematopoiesis has its origins in the mesoderm surrounding the aorta, the AGM (aorta/gonad/mesonephros) region (McGrath; Palis, 2005; Sadler, 2005; Hyttel, 2010).



Studies suggest granulocyte/monocyte progenitors and megakaryocyte/erythrocyte progenitors (Weissman et al., 2001). (**Fig. 1**)

Endothelial and hematopoietic cells emerge in spatio-temporal associations in yolk sac blood islands and share the expression of a number of genes, which include Flk11, CD34, Scl/tal-1, Flt1, Gata-2, Cbfa2/Runx/AML1 e Pecam1 (BARON, 2003), which, after being stimulated by the fibroblast growth fact (FGF-2), originate a common mesoderm progenitor: the hemangioblast (Conway et al., 2001; Palis et al., 2001; Palis; Youder, 2001; Choi, 2002; Baron, 2003; Li et al., 2005; Sadler, 2005; Hyttel, 2010). (**Fig. 2**) The hemangioblasts in the center of blood islands form hematopoietic stem cells which are the precursors of all hematopoietic cells, while peripheral hemangioblasts differentiate into angioblasts, which are the precursors of blood vessels (Choi, 2002; Baron, 2003). These angioblasts proliferate and are induced to form endothelial cells by the vascular endothelial growth factor (VEGF), secreted by circulating mesodermic cells, which regulate the coalescence of endothelial cells in the first primitive blood vessels (McGrath et al., 2003; Baron, 2003; Sadler, 2005; Damico, 2007).

After a primary vascular layer has been established by vasculogenesis, additional vasculature is regulated by VEGF which stimulates endothelial cell proliferation in the sites where new vessels will be formed by angiogenesis (McGrath et al., 2003; Sadler, 2005).



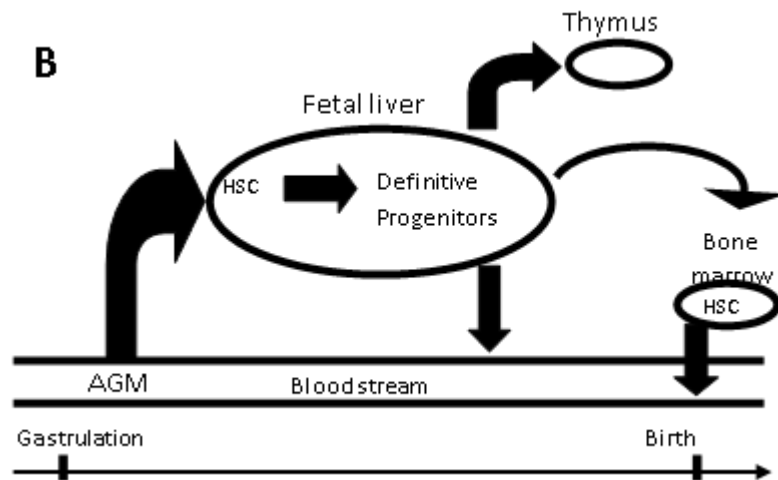


Figure 1 – Diagram illustrating primitive and definitive circulation. In A: demonstration of the origin of primitive hematopoietic and endothelial progenitors (hemangioblasts) in the yolk sac during gastrulation and subsequent construction of blood vessels, in addition to migration of progenitors to the fetal liver where the formation of definitive hematopoietic progenitors occurs and join the embryo’s blood stream before birth. In B: demonstration of the origin of hematopoietic stem cells (HSC) in the AGM region, from where they migrate to the liver which continues to generate definitive progenitors which populate the thymus and join the embryo’s circulation; and hematopoietic stem cells produced in the embryo’s bone marrow before joining the blood stream after birth. Adpt (McGrath; Palis, 2005).

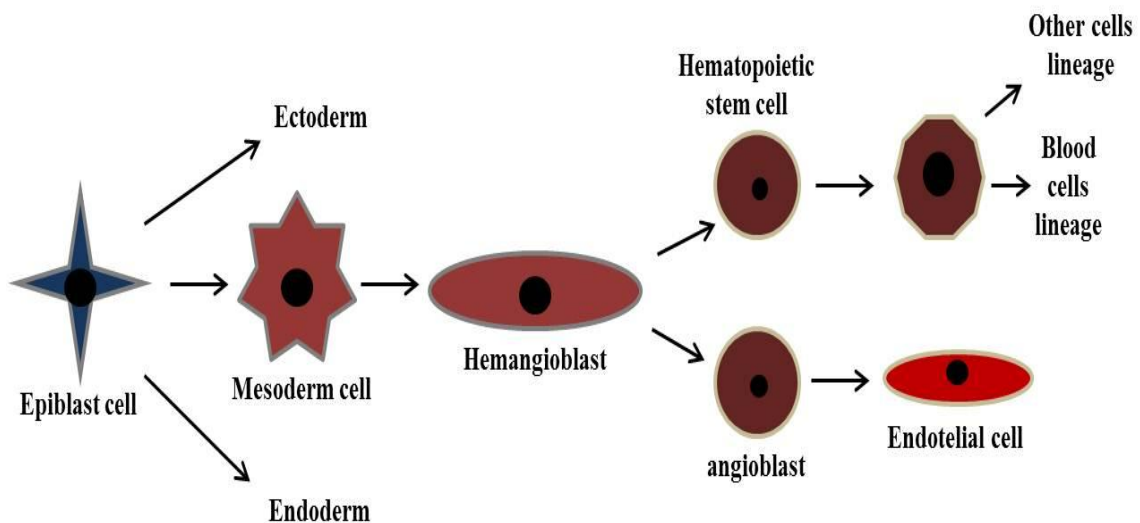


Figure 2 – Diagram illustrating the differentiation of epiblast cells which originate mesoderm cells, from where the hemangioblast arises. The hemangioblast, in turn, originates hematopoietic stem cells, which differentiate into several blood cell lineages, and the angioblast, which originates endothelial cells.

Vasculature maturation and remodelling are regulated by other growth factors, such as platelet-derived growth factor (PDGF) and transforming growth factor (TGF-beta), until the adult pattern is established (Sadler, 2005). Once the heart has begun to function as a pump, primitive erythroblasts start to circulate from the vitelline vessels of the yolk sac to the embryo and back, before the establishment of a vascular network committed to the embryo proper (Baron, 2003). The timing of the initial erythroblast migration from the yolk sac to the embryo proper is tightly coordinated with the onset of heartbeat in mice, which occurs after the aorta and a heart tube are formed, followed by an increased complexity of the vascular and hematopoietic system (Ji et al., 2003; McGrath et al., 2003). Larina et al. (2008) demonstrated by Doppler that in mice embryos of 9.5 days the circulation from the yolk sac to the embryo's dorsal aorta has already occurred. Ji et al. (2003) observed that before the onset of heartbeat in mice embryos no intraembryonic erythroblast was identified. However, upon the onset of heartbeat, scattered erythroblasts were observed within the head region and the presumed embryo's aorta (along the axis of the embryo). One concludes that the initial erythroblast migration inside the embryo proper is tightly coordinated with the onset of heartbeat, suggesting that the maturation of cardiovascular function and density of erythrocyte occur together.

Short after blood island develop in the mammal yolk sac, primitive erythroblasts enter the newly formed vasculature of the embryo proper. They continue to undergo mitotic divisions for several days and later on these primitive erythroblasts differentiate within the blood stream, gradually increasing the hemoglobin rate and losing basophilic activity. These erythroblasts continue to divide until they reach a hemoglobin level four times higher than found in adults (Palis; Youder, 2001). All hematopoietic activity occurs in the mammalian yolk sac until circulation is initiated and multilineage progenitors are detected in the blood stream and in the fetal liver, after its development.



The definitive hematopoietic cell progenitors emerging from the yolk sac migrate through the blood stream and the fetal liver to initiate the first phase of intraembryonic hematopoiesis. Studies show that the commitment to hematopoietic destinations initiates at early gastrulation. These studies also show that the yolk sac is the only primitive site of erythropoiesis and that it is the prime source of primitive and definitive progenitor hematopoietic cells during embryony development (McGrath; Palis, 2005).

VEGF participation in the formation of blood vessels

The mammalian embryo's vascular plexus originates through vasculogenesis, which is the formation of new vessels through angioblast proliferation stimulus. Angioblasts are precursory cells from the endothelium, originating from the splanchnic mesoderm, through angiogenesis (Yoshida, 2005).

VEGF (vascular endothelial growth factor) is of great importance among the pre-angiogenic factors. Its isolation was obtained in 1983, and it is used as a potent factor for the induction of vascular permeability increase, its potency being 10,000fold higher than histamine (SENGER et al. 1983). During angiogenesis, VEGF direct or indirectly stimulates endothelial cell mitosis (Leiser et al., 1989; Ferrara, 2004). VEGF acts by increasing cellular expression of metalloproteinases, thus degrading the extracellular matrix and enabling the penetration of new vessels in the tissue (Lamoreaux et. al. 1998; Hiratsuka et. al. 2002). Other important actions of VEGF are its inflammatory function, its neuroprotective and remodeling function, and its vascular stabilization function (Sakurai et. al. 2003; Benjamin et al. 1998).

Physiological angiogenesis occurs during embryogenesis, during tissue growth and during the reproductive cycle, while pathological angiogenesis is characterized by inefficient or excessive (as in tumors) neovascularization (Conway et al., 2001; Yoshida, 2005). There are numerous factors involved in these processes, giving clear evidence that the regulating mechanisms are complex, involving stimulating agents, inhibitors and modulators, including growth factors (GF), which are involved in almost all processes and are considered the most important factors (Rissanen et al., 2001; Yoshida, 2005).



CONCLUSION

Primitive and definitive hematopoieses are necessary to initiate circulation from the extraembryonic environment to the intraembryonic environment and these mechanisms stimulate the onset of circulation and heartbeat. Endothelial and hematopoietic cells originate from a common progenitor, the hemangioblast. Vasculature formation is stimulated by growth factors, mainly VEGF.

Nevertheless, further studies are required to elucidate the importance and the mechanisms involved in primitive and definitive hematopoiesis, such as clarifying how the erythrocyte flow begins and the formation of the extra and intraembryonic plexus, since these data are scarce in available literature.

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