Hemodynamics During Development and Postnatal Life

9

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Contents

Abstract

Unlike other embryonic organs, the developing heart must support, through its proper function, the developing embryo from the time diffusion becomes limiting. Hemodynamics is thus not only a key to remodeling of the developing vasculature but also a powerful stimulus for cardiac growth and differentiation. In this chapter are discussed prenatal models of hemodynamic perturbation that help clarifying the role of blood flow in embryogenesis.

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9.1   Introduction

The role of hemodynamics during embryonic development has been recognized for a long time [1], for example, its importance in molding the endocardial cushions into cardiac septa. Most of the experimental work was performed in the chick embryonic model, mostly because of its simplicity and the feasibility of experimental manipulations. However, other models, such as developing zebrafish, have been used to study the earliest phases of circulatory system formation, as the larvae are able to survive without a functional cardiovascular system for up to 1 week, allowing one to study otherwise lethal phenotypes. To better approximate the human situation, several groups have developed various mammalian models to answer specific questions. The lamb model has been most frequently used, mainly because of the specific ability of sheep uterus to tolerate surgery without induction of premature labor. We organize this chapter according to various animal models, ending with the examples from clinical cases in humans.

9.2   Studies in Developing Zebrafish

Three-dimensional studies of the tubular zebrafish heart enabled detailed visualization during different phases of the cardiac cycle [2] and changed our idea about the "peristaltic" contractions of the cardiac tube into a more appropriate model of a suction pump. Perturbation of the heart at such early stages via implantation of microbeads [3] altered normal blood flow through the heart and resulted in hearts with an extra third chamber, diminished looping, and abnormal valve formation, the last also known from human examples of congenital heart disease with blood flow alterations. This underscores the critical role of hemodynamics in cardiovascular morphogenesis.

Silent heart phenotype in zebrafish results in electrically normally active hearts, which however lack contraction because of a mutation in cardiac troponin T [4]. In this model, lack of endocardial cushion formation was observed, confirming the role of hemodynamic stress on the process of endocardial endothelial to mesenchymal transition. Since this hypothesis was strengthened by pharmacological inhibition of cardiac contractions, the authors postulated that abnormal function is translated into abnormal cardiac morphogenesis and could be an explanation for some forms of human cardiac congenital anomalies.

9.3   Chick Embryonic Model

The chick embryo has frequently been used to perform hemodynamic interventions, as both the visualization of the beating heart by videomicroscopy and more sophisticated functional evaluation (pressure monitoring, ultrasound biomicroscopy and Doppler, optical coherence tomography) are relatively straightforward. Hogers et al. introduced a venous clip model that mimics placental infarction and observed

resulting changes in intracardiac blood flow patterns [5]. These led frequently to abnormal morphogenesis translating in incomplete ventricular septation (VSD), valve anomalies, and pharyngeal arch artery malformations.

Hemodynamically induced shear stress is correlated with blood flow. To test the hypothesis that shear stress is also involved in cardiac development, Hogers et al. investigated the expression patterns of hemodynamically induced genes endothelin 1 (ET1), nitric oxide synthase 3 (NOS3), and Kruppel-like factor 2 (KLF2) at the mRNA level in a series of developmental stages of the chicken embryo. The authors found areas with mutually exclusive expression of ET1 and KLF2/NOS3, with KLF2 being expressed in the regions of highest shear stress. Visualization of stresses and strains in two altered hemodynamic models was performed by Buffinton et al. [6]. These investigators showed among other things the importance of realistic tissue geometry for determining the strains in mathematical models. The highest levels were found at the tips of ventricular trabeculae; ignoring this geometry and modeling the ventricle as a thick-walled shell, one would underestimate the values by up to two orders of magnitude.

Constriction of the outflow tract (conotruncal banding) was used as a model of increased pressure load of the embryonic ventricle [7]. The procedure itself is rather simple, as it is performed through a still avascular chest wall (Fig. 9.1). It has, in addition to increasing pressure load, pronounced effects on cardiac morphogenesis, producing lethality and defects in survivors [7, 8]. These include a VSD, with either double outlet right ventricle or persistent truncus arteriosus. This could be due, apart from considering mechanical effects of the suture, to hypoplasia of the conotruncal ridges as a result of altered intracardiac bloodstream patterns [9]. There are also pronounced effects on ventricular morphology. There is a change in shape due to dilation with increased proportion of the compact myocardium and spiraling of the trabeculae in the left ventricle. This is an interesting feature, since it resembles the definitive orientation of trabeculation in this location, and is similar to the course of muscle fibers in the compact layer [10]. This spiraling likely reflects adaptation to gradually increasing functional demands [11]. Increased thickness of the compact myocardium occurs within 48 h [9] and together with anomalies in the development of the coronary arteries [12] could be responsible for a decline in survival occurring around day 8 when the coronary circulation normally becomes functional. Interestingly, the growth of capillaries matches that of the myocytes, so the ratio is kept constant. Increased pressure loading is at this stage also a powerful stimulus for active myocardial growth based on cell proliferation [7, 13, 14].

The hemodynamically induced changes in myocardial architecture in this model also could be a substrate of altered electrical pathways. These were investigated as well using optical mapping on isolated hearts [15, 16]. It was found that increased pressure loading accelerated maturation of the ventricular conduction system. At the molecular level, these changes were paralleled by upregulation of conduction system differentiation markers endothelin-converting enzyme 1 (ECE1) and connexin 40 (GJA4).

Another popular model with a long history is left atrial clipping [17, 18] or ligation (LAL [9]) that mimics human hypoplastic left heart syndrome (HLHS). The
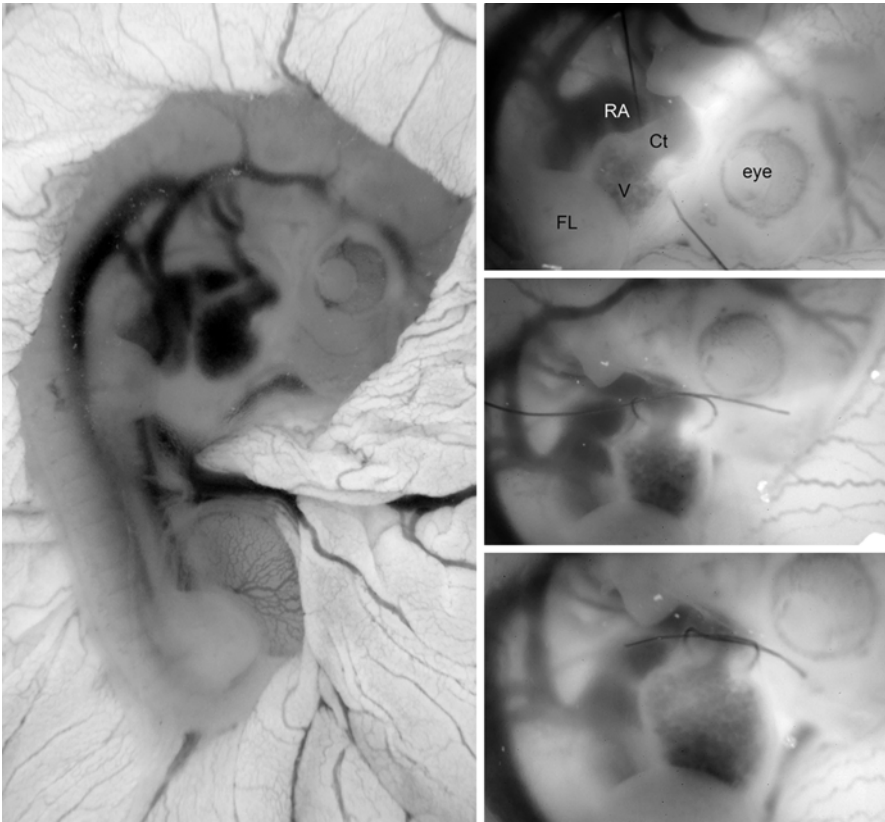
Fig. 9.1 Pressure overload model in the chick embryo. The embryo at day 4 of incubation (*left panel*) is exposed by amniotomy, and through the developing chest wall, a piece of 10-0 nylon thread is passed under the outflow tract (*top right*), tied in an overhand knot (*middle*), and cropped (*bottom*) to prevent piercing of the fragile surrounding vessels by its sharp ends. *Ct* conotruncus, *FL* fore limb bud, *RA* right atrium, *V* ventricle

phenotype of LAL hearts (Fig. 9.2) shows considerable variability from almost normal to extreme involution of the left ventricle with apex-forming right ventricle [19]. Occasional occurrence of VSD in about 25 % of the survivors is similar to results obtained in the left vitelline venous clip model [5] and likewise could be attributed to changes of intracardiac bloodstream patterns [20]. In severe cases, the right atrioventricular valve loses its typical muscular flap-like morphology and resembles a bicuspid fibrous valve. This suggests that hemodynamic stress is an important determinant of morphology of the developing valvular structures and influences differentiation of the connective tissue. In addition, there is a molecular phenotype [21] suggestive of heart failure, and a delay of about 2 days in expression of myocardial differentiation genes (contractile protein isoforms, energetic metabolism enzymes) was detected by microarray analysis in both ventricles. Adaptation of the right ventricle to gradually increasing volume load is progressive. First, there

is dilatation with in extreme cases alterations of trabecular orientation from radial to circumferential [9]. Second, there is an increased proliferation within the trabeculae [14] followed by eventual thickening of the compact myocardium, a finding that could be regarded as an acceleration of the normal course of development [22]. These changes in myocardial architecture and proliferative activity could be reversed by subsequent prenatal interventions. Surgical clipping of the right atrial appendage [20] normalized the hemodynamics and rapidly resulted in increased myocyte proliferation in the left ventricle and a tendency toward normalization of reduced left ventricular myocardial volume. This observation demonstrates the feasibility of fetal interventions aimed at mitigating the severity of a number of human congenital cardiac malformations [23, 24].

9.4 Prenatal Lamb Model

However simple and self-contained the chick model is, its relevance for human pathology has sometimes been questioned, and fetal mammalian models were thus developed. The lamb is the standard large animal model for investigating intrauterine development of the cardiopulmonary system and testing the effects of various prenatal interventions. In this model it is possible to reproduce typical anatomical and pathophysiological lesions and develop of procedures for their repair. Pioneering surgical studies were performed and summarized by Rudolph [25]. Early investigations demonstrated the suitability of this model for examination of both the pathophysiological consequences of cardiac lesions and the technical methods of intrauterine surgery [26]. With prenatal identification of cardiac lesions, intrauterine cardiothoracic surgery is possible [27, 28]. While a study comparing the effects of early vs. late pulmonary stenosis repair did not show any difference in transverse myocyte diameter even in non-repaired hearts [27], a study comparing the effects of pulmonary stenosis and atresia [28] showed an increase in myocyte width dependent on the level of overload and myocardial weight increase, suggesting a mix of both hypertrophy and hyperplasia. There are thus multiple factors, such as differences in methodological approach, the species used, timing of surgery, and the level of the overload, which influence the nature of myocardial response to prenatal hemodynamic challenge. It appears that even the adult heart, traditionally, and according to the latest evidence, rather incorrectly [29] regarded as a postmitotic organ, is capable of considerable proliferation in response to a long-standing pressure overload [30].

Both the pulmonary artery and aorta were banded in work by McAuliffe and Robbins [31]. This study revealed that only one dominant isoform of cardiac troponin is expressed during fetal and adult development and that expression levels do not change in response to pressure overload. Thus, changes in contractile protein isoform expression ("fetal gene program") known from adult models of heart overload [32] could not be recapitulated here. This model was used to study the changes in calcium-handling proteins, which could be interpreted as acceleration of the normal course of developmental [33]. At the cellular level, hypertrophy of cardiomyocytes was followed by hyperplasia and decreased proportion of binucleated cells.

This is an interesting observation, showing differences from the embryonic chick model as well as the ability of the fetal mammalian heart to behave similarly to the adult heart. As in the case of the embryonic chick pressure overload model, Flanagan and colleagues [34] found that capillary density was maintained in the overloaded group and that the functional flow parameters and coronary resistance were also within the normal range. This shows that the fetal heart adapts its structure in response to pressure challenge (within limits) in a coordinated manner and that the myocardium thus created is normal and well perfused.

Fishman et al. [35] created a prenatal sheep model of the hypoplastic left heart syndrome by obstructing the left ventricular inflow or outflow with a balloon catheter. With the inflow obstruction, within a week, the left to right ventricular weight ratio decreased to 70 % of control, and the mean chamber volume ratio decreased to less than 50 %. The outflow obstruction resulted in less pronounced left ventricular output decrease. Over the long term, the left to right ventricular weight ratio decreased even further, and the left ventricular chamber was nearly obliterated, simulating very severe congenital aortic stenosis. These experiments confirmed the existence of two possible hemodynamic pathways of pathogenesis of hypoplastic left heart syndrome: either through decreased preload or increased afterload of the left ventricle, as seen in fetal mitral or aortic stenosis, respectively.

9.5   Fetal Studies in Rodents

9.5.1   Guinea Pig

An elegant fetal surgical study in small animal models was performed in guinea pigs [36]. Fetal guinea pigs in the third trimester were accessed by hysterotomy, and their ascending aorta was constricted to 50 % of its original diameter. The heart to body weight ratio and left ventricular wall thickness increased significantly in the banded group. There was also a significant increase in percentage of marker of proliferation Ki-67-positive (i.e., proliferating) cells in both ventricles with no changes in apoptosis, suggesting the existence of an adaptation mechanism similar to that observed in the other prenatal models.

9.5.2   Mouse

Mouse embryo is not a frequent subject of hemodynamic studies, mainly because of its small size, the presence of multiple embryos in the same dam making individual longitudinal studies difficult, and general constraints of mammalian model. However, there is wealth of information that could be learned due to availability of transgenic animals mimicking sometimes human cardiac malformations. Basic study of mouse embryonic cardiovascular function and dimensions was performed by Keller et al. [37], replicating similar studies performed previously in chick and rat embryos. Experimental pacing study in murine embryos demonstrated feasibility of

Doppler measurements of flows and velocities in this model and confirmed deleterious effects of tachycardia on embryonic cardiac performance [38]. Later studies by this group revealed patterns of response of the embryonic heart rate to hypoxia, which varied according to developmental stage. The initial response to maternal hypoxia was bradycardia, followed at reoxygenation by either overshoot prior to return to baseline (paralleling the maternal response), continued bradycardia, or simple return to normal rate [39]. Pharmacological study of effects of moderate doses of caffeine [40] showed a slight but significant deleterious effect on embryonic growth and cardiac performance, possibly mediated via adenosine receptor blockade.

Phoon and colleagues investigated with ultrasound biomicroscopy mice with NFATc1 deletion [41]. These mice lack the outflow valves and die rather suddenly at mid-gestation. It was shown that the decline is indeed rapid, with bradycardia preceding the embryonic demise while the myocardial contractile function remained preserved despite regurgitant blood flow propagating back to the placental circulation.

9.5.3 Rat

Rat embryos were also used as models for pharmacological testing (recently reviewed by Sedmera et al. [42] from the perspective of fetal arrhythmias). Of course, significant arrhythmias often result in hemodynamic instability and could lead to fetal complications. Another recent report from rat model was investigating the pathogenesis of preeclampsia induced by inflammatory reaction after lipopolysaccharide injection [43].

9.6 Fetal Hemodynamics in Humans

The ultimate goal of studies performed in various animal models (Fig. 9.3) is to gain better understanding into working of prenatal human circulation, especially at stages which are not easily studied in depth (particularly the first trimester).

As mentioned above, the fetal hemodynamics is dependent upon normal and regular heart rate [42]. Alterations in hemodynamics are believed to be one of the causes for cardiac malformations such as hypoplastic left heart syndrome [19] or valvar atresias (Fig. 9.2). Abnormalities in cardiac performance can bear influence on all the systems in the developing fetus, including the central nervous system [44]. Experimental interventions aimed at normalizing hemodynamics and preventing irreversible changes include balloon aortic or pulmonary valvuloplasty [24, 45]. However, a recent American Heart Association statement [46] lists these treatment options as experimental, as not all of them might translate into desirable outcome despite technical success [47]. This highlights the need of further basic as well as translational research into mechanisms of interactions between the structure and function of the developing heart.
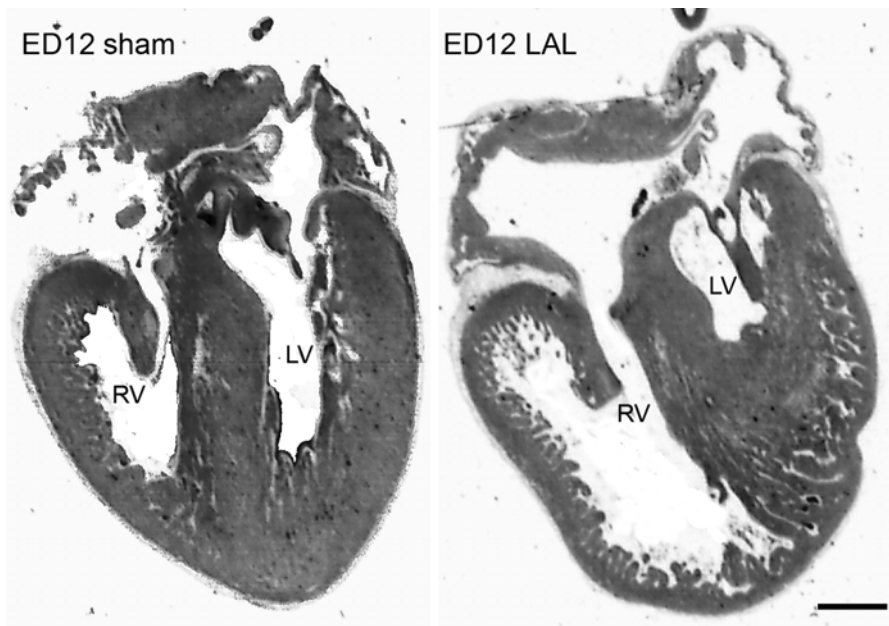
Fig. 9.2 Phenotype mimicking human hypoplastic left heart syndrome induced in the embryonic day 4 chick embryo by partial ligation of the left atrial appendage (*LAL*). Note the apex-forming right ventricle in the ligated embryo. Scale bar 1 mm
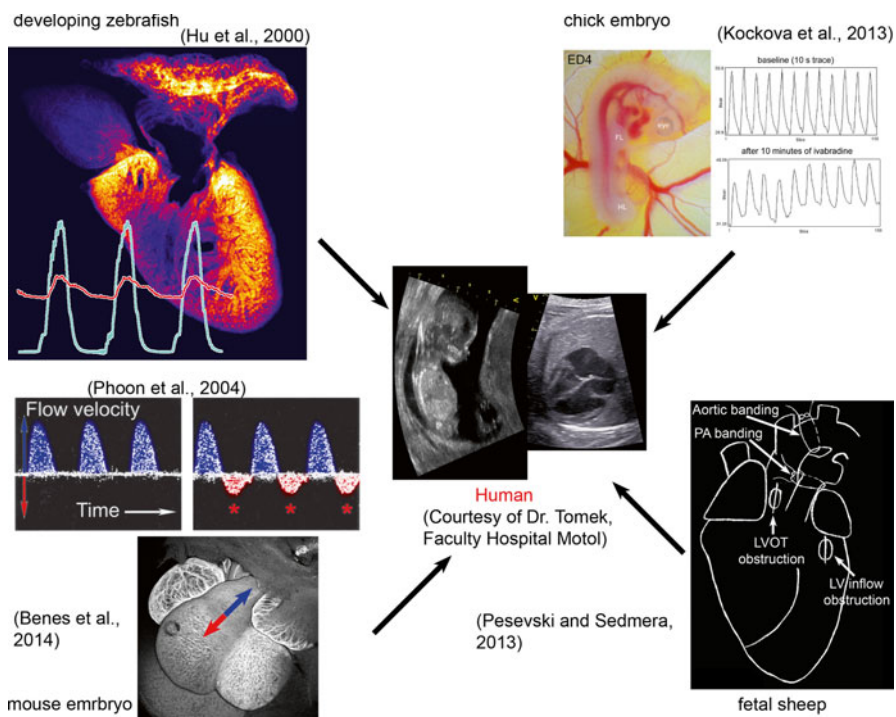

Fig. 9.3 Overview of various hemodynamic models discussed in this chapter [49–51]

9.7   Postnatal Hemodynamics

Abrupt changes in circulation occurring after birth have been the subject of numerous studies, and there is considerable variation among species in, e.g., mechanisms of closure of the foramen ovale (cell proliferation closing multiple foramina in birds or flap-like valve sealing the double septum in mammals). The neonatal heart temporarily retains some of the characteristics of the fetal heart, notably the proliferative activity that is used to compensate altered functional demands [48]. As we have recently reviewed this issue in depth [33], we may refer the readers to this book.

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