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Discussion

Factors in ventricular and atrioventricular valve growth: An embryologist's perspective

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Growth of the prenatal heart is predominantly based on adding new cells (hyperplasia), in contrast to the postnatal heart, where further increase of ventricular mass is due almost entirely to increase in myocyte size (hypertrophy) soon after birth. The key epigenetic factors regulating cardiac growth are the hemodynamics (volume/preload and pressure/afterload), whose gradual increase reflects the changing demands of the growing embryo and fetus. While genes may play a significant part in the etiology of congenital heart disease, hemodynamic perturbations also can lead to predictable changes in morphogenesis and produce cardiac lesions. At present, developmental perturbations seem to produce many of the common congenital defects, while genetic abnormalities are clearly linked to the many chromosomal syndromes that have been well described.

The aim of this article is to provide an overview of our knowledge of the growth and development of ventricles and valves gained from experimental fetal animal models. Most hemodynamic perturbation procedures by this author have been performed in the chick model. The incubation period of the chick embryo is 21 days and the tubular heart starts to beat on the second day of incubation. The chambers start to differentiate by the third day, which includes the development of the atrioventricular and outflow tract cushions. Ventricular and outflow tract septation is completed by day 8, i.e., after one third of the incubation period. Consequently, the developmental events proceed over a relatively short period of time, allowing the effects of hemodynamic perturbations to be directly studied.

Most hemodynamic data were collected by a combination of direct videomicroscopy, invasive ventricular servo-null pressure measurements and aortic and atrioventricular Doppler flow waveforms between days 2 and 6 when the heart grows exponentially [1,2], while data from later stages were only collected recently with the advent of ultrasound biomicroscopy [3,4].

The chick experimental model of hypoplastic left heart syndrome has been produced by a reduction in left ventricular filling as confirmed by decreased transmitral flow [5]. These hemodynamic changes also had direct sub-cellular consequences and decreased myocardial proliferation [6,7] producing a reduction in ventricular mass [8]. Similar results were also reported in fetal lambs [9]. Proper ventricular filling (preload) is thus a requirement for normal ventricular growth. If the myocardium is without additional significant pathology such as fibroelastosis, restoration of normal loading conditions in the early post-septation chick fetal heart can result in the normalization of growth [3], a paradigm that can be exploited in curative surgical approaches. From the biological point of view, the sooner such interventions are performed the better, in order to both limit the development of potentially irreversible secondary changes and to take advantage of the period when hyperplasia is still the dominate growth response. In humans, cell proliferation is believed to be active until approximately six months after birth [10,11].

Increased pressure loading is also a powerful stimulus for embryonic ventricular cell division. Hyperplasia of cardiomyocytes was demonstrated after conotruncal banding in the chick embryo [12], and the remodeling of embryonic myocardial architecture was also profound in this model (Fig. 1). During the period prior to the establishment of coronary circulation, ventricular myocardial mass increases mainly by the process of trabeculation to avoid myocardial ischemia due to

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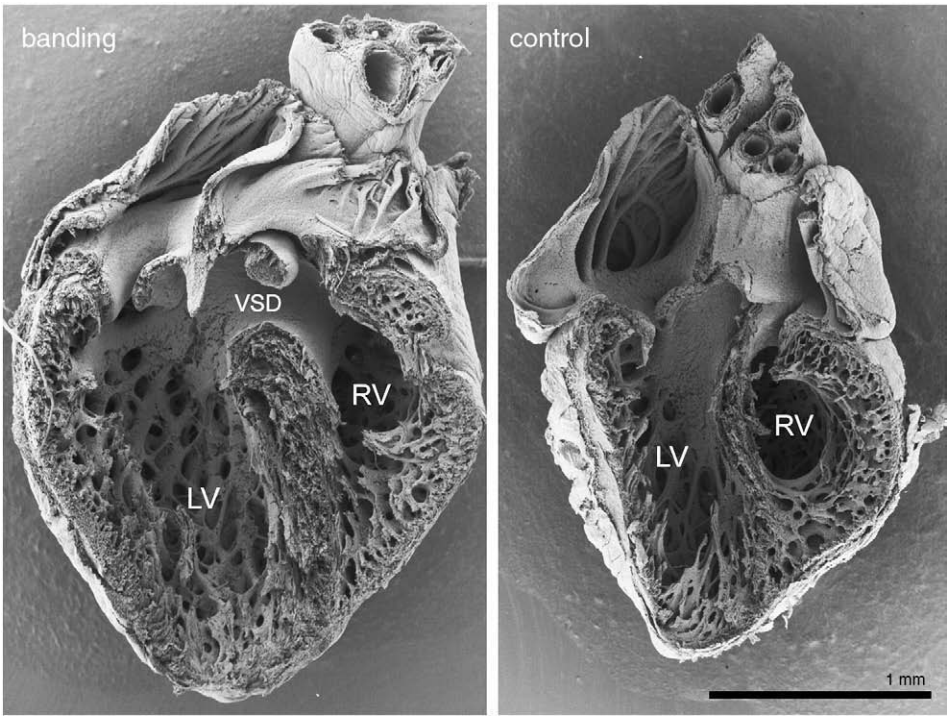

Fig. 1. Increased myocardial growth in experimental embryonic pressure overload in the chick involves a thickening of the compact myocardium and coarsening of the ventricular trabeculae. Scanning electron micrographs of four-chamber views of banded (at ED4, left) and control (right) ED8 hearts show also a ventricular septal defect (VSD) due to impaired cardiac septation due to outflow tract ligature. Anterior halves are shown; LV, left ventricle, RV, right ventricle.

exceeding diffusion limits. Ventricular trabeculae can form up to 80% of the ventricular myocardial volume in the pre-septation chick heart [13] as well as in corresponding stages in human [14]. Later, compaction of the trabeculae occurs and increases dramatically the proportion of the compact myocardium, which enables the ventricular chambers to generate higher pressure, and correlates in time with the completion of ventricular septation and establishment of coronary perfusion [15]. Further increase in the thickness of the compact myocardium is accompanied by the gradual organization of myocytes into a typical multi-layered spiral system [16,17].

In the embryonic pressure overload model, the changes observed could be interpreted as an acceleration of this normal developmental sequence. Within one day of aortic constriction, an increase occurs in the proportion and thickness of the compact myocardium [8], and there is a precocious spiraling of the trabeculae. At later (post-septation) stages, there is also an accelerated development of the spiral layers in the compact myocardium [18]. Because there are also frequent anomalies of the coronary arteries and the constant aortic constriction becomes increasingly more severe with further embryonic development, long-term survival, however, is problematic [19]. In fetal mammalian models of left ventricular pressure overload induced by aortic constriction, hyperplastic growth was also described [20,21]. There are also fetal mammalian models of right ventricular pressure overload [22,23]; which have shown both cellular hyperplasia [22], as well as some degree of myocyte hypertrophy [23].

In summary, normal (or enhanced) prenatal ventricular growth is dependent on both sufficient preload (see previous paragraph) as well as adequate afterload. Therefore, curative surgical approaches for PAIVS should take this into account, and aim to establish sufficient flow across the atrioventricular valves to promote both adequate valve growth as well as cavitary expansion. Afterload should also be normalized by relieving outflow obstruction that otherwise would lead to thick-walled, small cavity ventricles and later fibroelastosis, further decreasing inflow capacity [24]. This is exemplified by the successful growth of hypoplastic structures in a staged approach [25] that ultimately enabled anatomical repair with normal circulation.

Although the transition from hyperplastic to hypertrophic growth after birth occurs rather rapidly, there are some differences between the two ventricles [26,27]. While left ventricular wall thickness continues to increase after birth by a combination of hyperplasia then by myocyte hypertrophy, right ventricular compact layer thickness does not change significantly in the early postnatal period, because its pressure loading is actually reduced and replaced by volume loading. This transition is also accompanied by a brief period of increased myocyte apoptosis [26]. Nevertheless, any corrective surgery performed still in the hyperplastic phase would seem to have greater potential to induce more physiological compensatory remodeling based upon myocyte (and vasculature) proliferation [28]. The principles in the development of both the left and right ventricles are similar if the ultimate differences in pressure and volume ventricles are taken into account.

Changes in ventricular morphology are also accompanied by a transition of their activation pattern, as a functional ventricular conduction system develops. Accelerated ventricular growth is accompanied by a similar rapid maturation of bundle branch function, while decreased left ventricular growth is paralleled by a dysfunction of the left bundle branch [29]. Similarly, coronary vasculogenesis keeps pace with myocardial growth [19], and although the molecular mechanisms regulating these processes are likely linked in some way, it has been shown that they are controlled by genetically separable programs [30,31].

Myocardial growth is also dependent upon paracrine and autocrine signaling through numerous growth factors produced by myocytes themselves as well as by the epicardium. Among these, PDGF, FGF2 and IGF are the best studied [32–35], and recent studies have shown that their addition can induce myocyte proliferation independent of mechanical loading [36,37]. Conversely, growth factor down regulates myocardial proliferation in experimental models [7,36,38]. Systemic therapeutic inhibition of growth factor signaling cascades is currently exploited in some anti cancer therapies, but usage to treat localized stimulation of tissue growth has so far been limited [39–41].

Most developmental research has studied the left ventricle because of its importance and relative ease of model development. In addition, the precise imaging and estimation of right ventricular function is difficult due to its more complex geometry in comparison to the left ventricle. Three-dimensional echo brings promise of more accurate imaging of right ventricular volumes and contraction patterns, which are notoriously difficult to obtain from 2D images. Biplane averaging is necessary to obtain at least approximate volumes in B-mode echocardiograms.

Apart from the adequate development of the myocardium, heart pumping depends upon functioning valves. Indeed, prognosis of children with congenital heart disease with less than four (at least potentially) functional valves is much worse than if all are useable (absence of a functional pulmonary valve, however, is much better tolerated). A corrective approach is made difficult because no artificial valves available today are capable of the matching growth of babies and small children, necessitating their upsizing and replacement. Valve growth and maturation is, not surprisingly, dependent upon blood flow through its orifice, and deviation from the norm (either too much or too little) results in abnormal development. For example, decreased flow across the mitral orifice in experimental left heart hypoplasia can result in overgrowth of cardiac cushions that can produce, together with asymmetric division of the common atrioventricular canal, mitral atresia [42]. Conversely, increased flow across the right atrioventricular orifice results in dysmorphogenesis of the right atrioventricular valve [13] that often presents with regurgitation possibly due, at least in part to, annular dilation [3, and our unpublished data], which resembles the clinical observations often reported in this conditions [43].

Valvar morphogenesis is a complex process involving interplay of many factors and has been reviewed recently [44–46]. Cardiac valves form from the cardiac cushions and have a specific morphology governed by the anatomical location. The processes involved in their formation include endocardial to mesenchymal transformation [47], with the sources of cushion mesenchyme including neural crest cells [48], epicardially derived cells [49] as well as blood borne cells [50]. Their maturation is in part directed by blood flow [8], and involves differentiation of the fibroblasts with polarized synthesis of the extracellular matrix [51].

Semilunar valve development is dependent on adequate flow. A decrease in the aortic valve diameter together with increased pulmonary valve orifice is evident in experimental hypoplastic left heart syndrome (Fig. 2). On the other hand, it is well recognized that the fetal development of aortic atresia can lead to hypoplastic left heart syndrome with fibroelastosis. Because this is a developmental event, there have been successful attempts aimed at correcting this prenatally [24,52,53], which has shown considerable plasticity of valves during the fetal period. There are few data available, however, it seems likely the right ventricle in settings of pulmonary atresia will behave similarly [54].

The potential for postnatal right ventricular development (and catch-up growth) could be even better because of its larger functional reserve and pace of differentiation which generally lags a few steps behind the left ventricle [15]. To test this hypothesis, an animal model of this condition would be highly desirable; however, creation of an animal model of pulmonary atresia with intact interventricular septum presents a significant technical challenge. If intervention is aimed at stages prior to septation, it is likely that there will develop a "compensatory" ventricular septal defect to provide an outlet from the right ventricle, which at these stages of serial chamber connection forms the outflow portion of the embryonic heart. This was elegantly demonstrated by Rychter and Rychterova [42] who used silver microclip on atrial appendages to create a chick model of left or

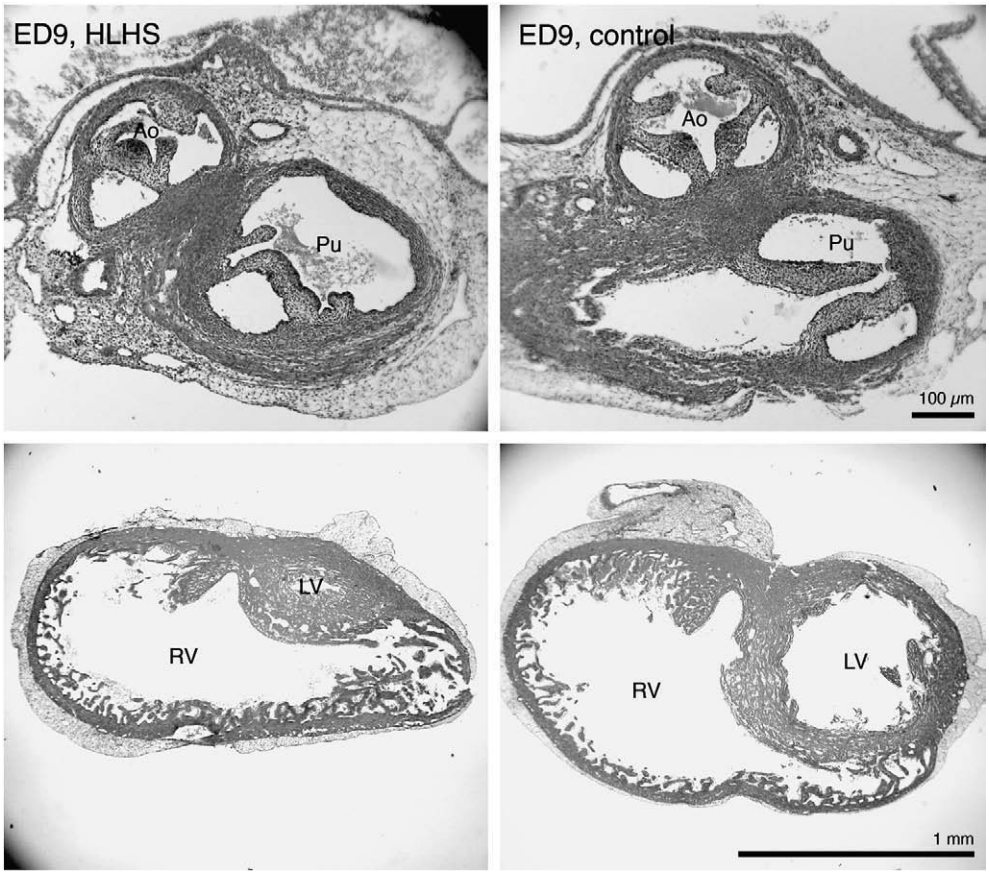

Fig. 2. Alteration of blood flow across the aortic and pulmonary orifice results in changes in their diameter. Histological sections from the chick experimental model of HLHS show also abnormal orientation of the enlarged pulmonary valve; however, changes at the ventricular level are much more dramatic at this early stage (ED9). Ao, aortic valve, LV, left ventricle, Pu, pulmonary valve, RV, right ventricle.

right ventricular hypoplasia. While it was relatively easy to induce a chick phenocopy of hypoplastic left heart syndrome by left atrial clipping that shunts blood from left to right heart structures, a similar intervention on the right atrium resulted in right ventricular hypoplasia only rarely, because in 75% of cases a ventricular septal defect allowed some blood to enter into the right ventricle. For the same reason, conotruncal banding will also lead to a ventricular septal defect (as is found in double outlet right ventricle or persistent truncus arteriosus) and is not a satisfactory model for PA–IVS either. While surgical interventions on the fetal (post-septation) chicks are technically quite challenging [3], it would be possible to perform a separate ligation of the pulmonary artery in the second third of incubation. Abrupt hemodynamic changes however, are poorly tolerated by the fetal and neonatal heart [11], so rather than being a survival model this intervention would be likely only useful to measure the acute reaction of the right ventricle to pressure overload.

Despite the difficulty in creating a model of PAIVS, the information gained from the models of left ventricular hyoplasia have considerable relevance. Surgical management with infants with PA–IVS should thus be dictated chiefly by clinical experience, although the procedures themselves should take lessons from the basic biological principles learned from experimental animal models.

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