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Early trabeculation and closure of the interventricular foramen in staged human embryos

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Internal differentiation of the ventricles was studied in staged serially sectioned human embryos of developmental stages 13–19 (postovulatory days 32–46). At stage 13 the trabeculation of both ventricles was advanced and the muscular part of the interventricular septum well marked. Dorsal and ventral endocardial cushions were fused and the atrioventricular canal was divided into two parts. In embryos at stage 18 the membranous interventricular septum was developing and the interventricular foramen was obliterated. At stage 19 the membranous part of the interventricular septum was becoming more cellular in structure. (Folia Morphol 2008; 67: 13–18)

Key words: human embryology, embryonic period, heart development, differentiation of ventricles

INTRODUCTION

During intrauterine development the heart is the first organ to begin mechanical function and this is initiated before structural organogenesis is complete. This raises the possibility that its early mechanical function affects its own morphogenesis [17]. Although morphogenesis of the heart varies between species the general steps during its development are common. These steps involve the following: determination and formation of cardiomyocytes, formation of the heart tube, looping, growth of the chambers, formation of the endocardial cushions, valvulogenesis, and septation.

Valvular and septal defects are among the most common and most deleterious of all cardiac malformations [15]. The endocardial cushions play a central role in cardiac septation and valve formation [13, 24]. In the development of the myocardium and endocardial cushions an important role is played in turn by the neural crest cells, which migrate from the cardiac neural crest region. Ablation of this region results in myocardial dysfunction and septal malformation [10, 20, 32].

Trabeculation and septation are interlinked events. Trabeculation has been implicated in enhancing contractility, ventricular septation and intraventricular conduction and in helping to direct blood flow before septation [7, 8, 11, 16–18].

Although there has been considerable research into human embryonic [2, 3, 5, 9, 16, 21–23, 25] and foetal hearts [1], as well as those of animals [12, 14, 29], there are still controversies as to the development of the interventricular septum and the exact timing of the differentiation of the ventricles, and differing conclusions have been reached regarding the time of closure of the interventricular foramen [5, 9, 16, 23].

Understanding of the mechanics of the development itself will provide the information required for the proper interpretation of postnatal morphology [2–4]. The aim

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of the present study is to establish the exact time of closure of the interventricular septum and the formation of the trabeculae in staged human embryos.

MATERIAL AND METHODS

Twelve human embryos ranging in age from 32 to 46 days were studied using a light microscope. The embryos belonged to the collection of the Department of Anatomy of the Medical University of Poznań. The embryos studied were from stage 13 to stage 19 (Table 1). They were sectioned serially in the sagittal, frontal, and horizontal planes and stained according to routine histological methods and with silver. From each embryo histological sections relevant to this study were selected and photographed.

Table 1. Developmental stage, age in postovulatory days, and plane of section of the embryos examined

Catalogue No.	Stage	Age [days]	Section
B171	13	32	Frontal
B218	13	32	Sagittal
B174	13	32	Horizontal
B272	14	33	Sagittal
B195	14	33	Sagittal
B115	15	36	Frontal
PJK21	15	36	Sagittal
PJK8	16	39	Horizontal
PJK2	17	41	Horizontal
BŁ4	18	44	Sagittal
B66	19	46	Horizontal
B173	19	46	Horizontal

RESULTS

In the embryos at stage 13 the dorsal and ventral endocardial cushions were fused and the atrioventricular canal was divided into right and left atrioventricular canals. The right and left atrioventricular sulci were evident and the interventricular sulcus was deep. The muscular part of the interventricular septum was well marked (Fig. 1). Each ventricle consisted of the atrioventricular inlet, the trabeculated part and the outlet, which was directed to the bulbus cordis, in which the bulbar ridges are evident (Fig. 2).

The dorsal atrioventricular cushion descended downward to the ventricular wall (Fig. 3), supplying evidence that the dorsal endocardial cushion contributes to the formation of the interventricular septum. In the peripheral part of the ventricular wall the compact myocardium was formed (Fig. 2).

The interventricular flange partially overlapped the interventricular septum and the atrioventricular canal was located to the left. The outflow tract, like the bulbus cordis, originated from the right ventricle. The bulbus continued as a truncus arteriosus. The

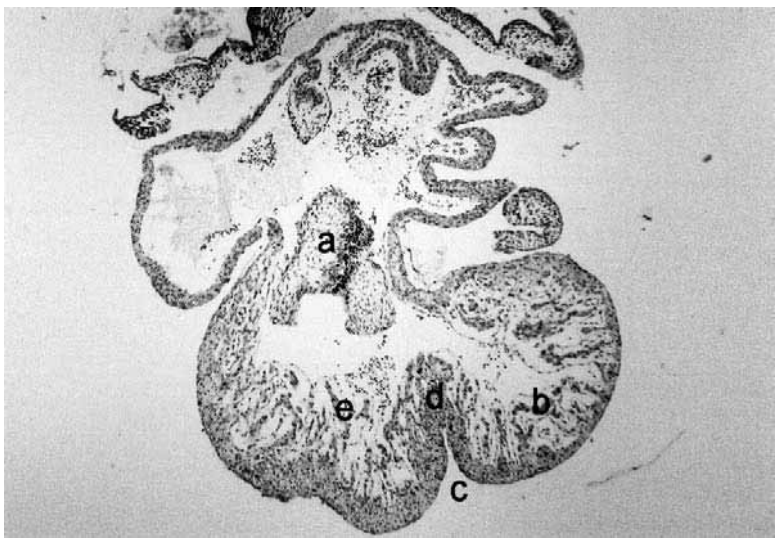

Figure 1. Frontal section of an embryo at stage 13. Cresyl violate, × 50; a — endocardial cushion, b — left ventricle, c — interventricular sulcus, d — interventricular septum (muscular part), e — right ventricle.

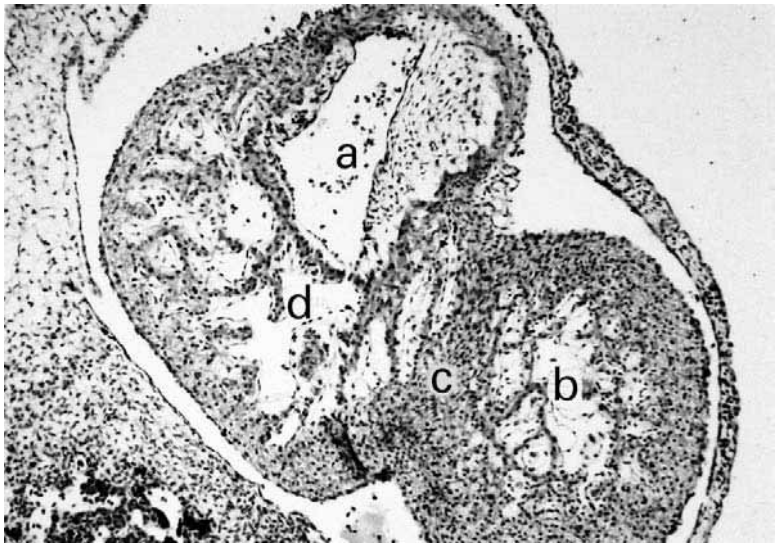

Figure 2. Horizontal section through the ventricles in an embryo at stage 13. Haematoxylin and eosin, × 100; a — bulbus cordis with ridges, b — left ventricle, c — interventricular septum, d — right ventricle.

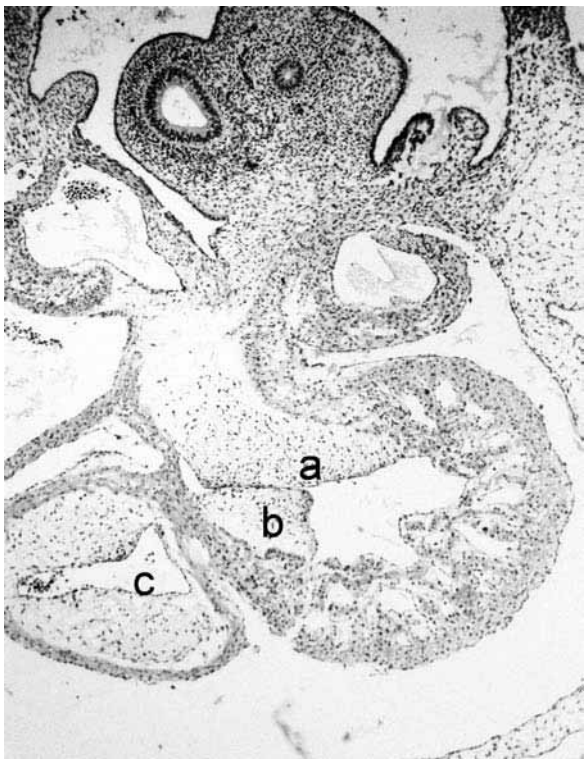

Figure 3. Horizontal section of an embryo at stage 13. Toluidine blue, × 50; a — dorsal endocardial cushion, b — ventral endocardial cushion, c — bulbus cordis.

Figure 4. Oblique horizontal section of the heart in an embryo at stage 14. Cresyl violate, × 50; a — endocardial cushion, b — interventricular flange, c — muscular interventricular septum; d — trabeculae in the right ventricle.

conotruncal ridges were fused and the outflow tract divided, forming two blood streams (Fig. 4, 5).

In addition, during stages 14 and 15 the interventricular septum was elevated and the expansion of the left ventricle and the growth of the left atrium were observed. The primary interventricular foramen was shifted to the left in the direction of the aortic outflow.

In embryos at stages 16 and 17 the interventricular flange was oriented to the left of the interven-

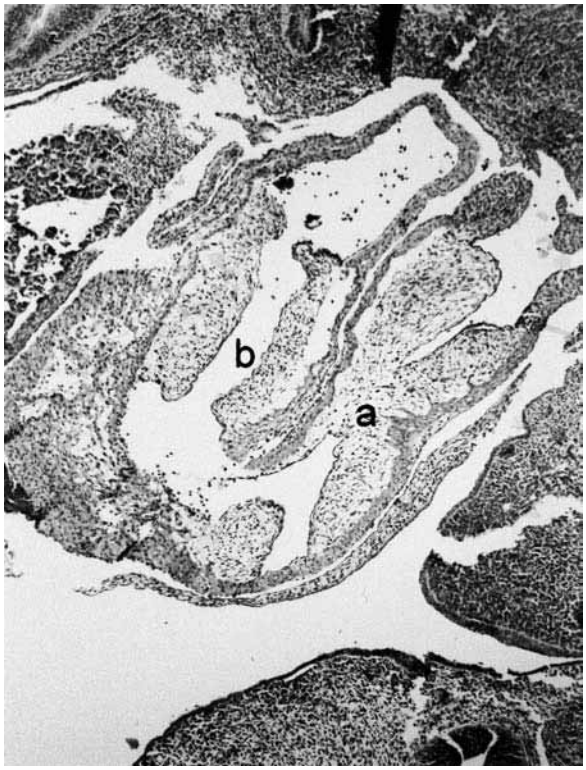

Figure 5. Sagittal section of an embryo at stage 14. Haematoxylin and eosin, × 50; a — fused conotruncal ridges, b — right atrioventricular canal.

tricular septum (Fig. 6) and the atrioventricular cushions were to the right of this flange. The auricules were enlarged and the secondary interventricular foramen was between the elevated muscular interventricular septum and the endocardial cushions.

There was also a counter-clockwise shift of the right and left ventricular outflow tracts from the horizontal to the sagittal axis.

In embryos at stage 18 the membranous interventricular septum developed from the cushion material and, together with the muscular septum, completed the separation of the right and left ventricles (Fig. 7).

In embryos at stage 19 the site of fusion of the membranous and muscular interventricular septum was an area of more cellular structure. Additionally, the aortic and pulmonary blood streams were completely divided (Fig. 8).

DISCUSSION

Trabeculation is evident in the early phase of the development of the heart. It first becomes evident along the inner myocardial layers near the maximum or greater curvature of the looped primitive ventricle [7, 8, 19]. The pattern of primitive trabecular ridges runs dorsoventrally (circumferential to the heart tube) and appears similar in different species [28]. Forces produced by the peristaltic contractile pattern stimulate patterned trabecular morphogenesis [6]. The primary mechanical consequences of ventricular trabeculation are more uniform transmural stress distribution and increased intramyocardial blood flow. Trabeculations are also important in co-ordinating intraventricular conduction. Patterns of trabeculation specific for the morphologically left, as opposed to the right, ventricles become apparent at the beginning of ventricular septation, and the

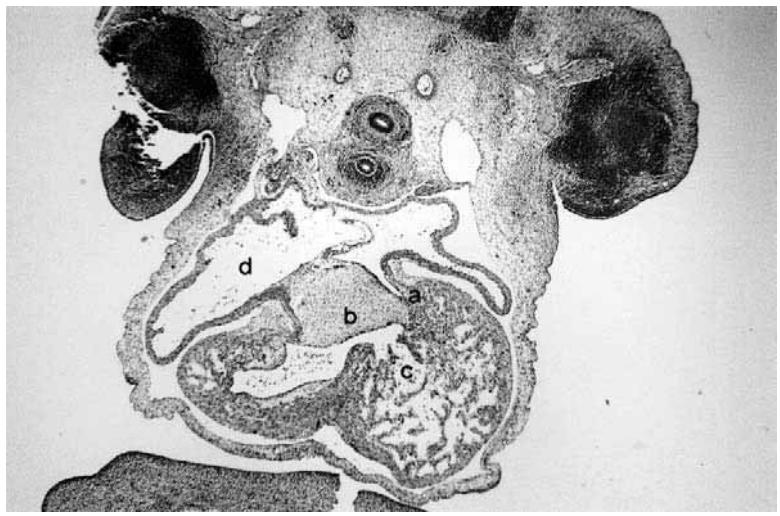

Figure 6. Oblique frontal section of an embryo at stage 16. Haematoxylin and eosin, × 50; a — interventricular flange, b — endocardial cushion, c — left ventricle, d — right atrium.

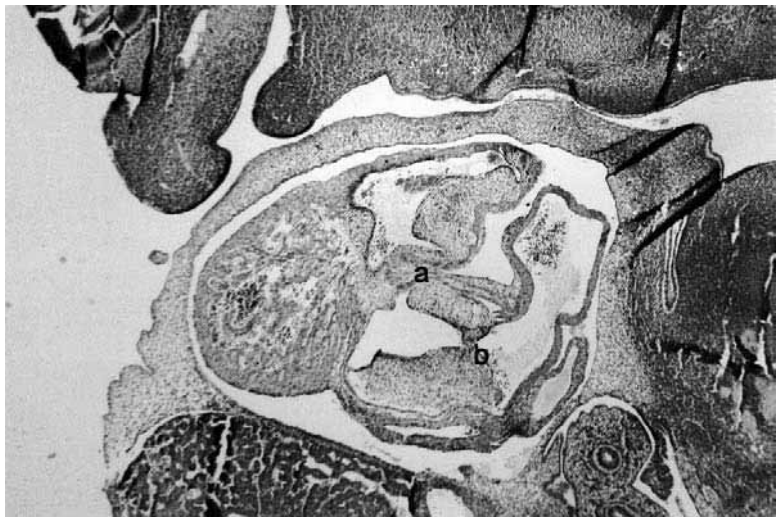

Figure 7. Sagittal section of an embryo at stage 18. Haematoxylin and eosin, × 50; a — membranous interventricular septum, b — right atrioventricular canal.

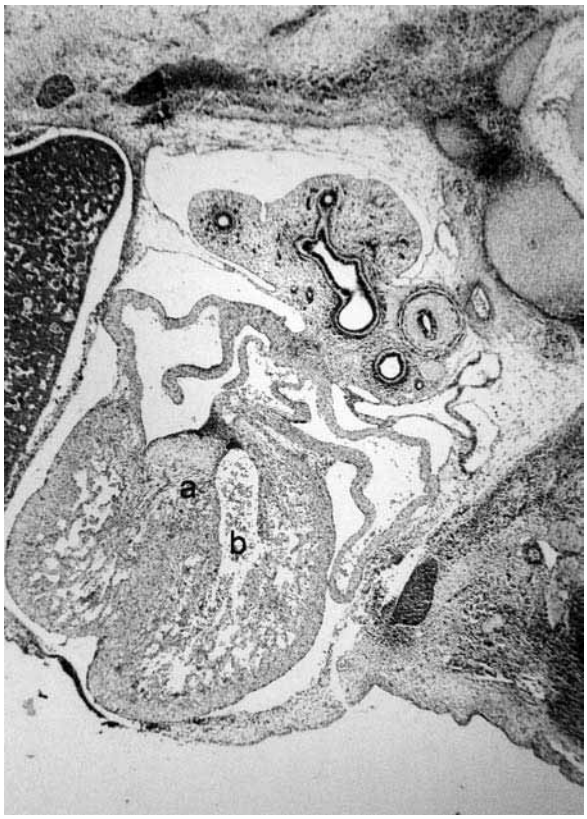

Figure 8. Oblique horizontal section of an embryo at stage 19. Haematoxylin and eosin, x 50; a — complete interventricular septum, b — left ventricle.

differences are more pronounced in birds than in mammals. The trabeculations in the left ventricle are generally thicker than those in the right ventricle [28, 33, 34].

In the present study the ventricular trabeculation was found in all embryos at stage 13 (the beginning of the 5th week). This is in accordance with descriptions of O'Rahilly and Müller [26]. It has to be pointed out that the trabeculations in the left ventricle are thicker and the centripetal growth of the trabeculations is seen.

The muscular part of the interventricular septum in human embryos begins to develop early in the 5th week (stages 11 and 12) [26]. In our study this part of the interventricular septum was well marked in embryos at stage 13. Genes Hand1 and Hand2 play an essential role in the formation of the interventricular septum and trabeculation [30].

Two opposed hypotheses have been proposed for the development of the interventricular septum [14]. Several authors [26, 27, 31] suggest that the muscular part of the interventricular septum is formed passively by apposition of the expanding ventricular walls. More recent studies have shown, however, that there is a multi-step model of interventricular septum development [14]. An initial passive growth phase delineates the apical/medial aspect of the interventricular septum, including a contribution by cardiomyocytes derived from the inner curvature of the heart. The second step is active ingrowth of the left and right derived cardiomyocytes. The third wave of septal growth involves the dorsoventral proliferation of cells within the septum.

The membranous part of the interventricular septum results from fusion of the subendocardial tissue from the right and left bulbar ridges and the dorsal endocardial cushion. The contribution of all

sources to the membranous septum was demonstrated in the present study. It was also shown that the interventricular foramen is obliterated at the end of the 6th week (stage 18).

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