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THREE-DIMENSIONAL RECONSTRUCTION DURING DIASTOLE AS A NEW TOOL IN THE UNDERSTANDING OF MORPHOLOGY OF EMBRYO HEART

Summary. Aim of our research was to investigate quantitative value of parameters of chicken heart on 15 day of incubation during diastole. This work was achieved by the use of serial histological sections of the embryonic heart, which were traced and put into 3 Dimensional reconstructive program, where we estimated additional stereometric values, which were used for further statistical analysis. Diastole was modeled with the use of KCl solution. It was received a 3 dimensional computer model of the chicken embryo heart during diastole, which was composed of models of separated units (left ventricle cavity, right ventricle cavity, atrium cavity, myocardium of both ventricles, subepicardium). In current work we marked a certain group of parameters for further analysis on different stages in embryonic development during systole and diastole.
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Гудлетт Т. Трехмерная реконструкция во время диастолы как новый инструмент в понимании морфологии эмбрионального сердца.
Резюме. Цель нашего исследования состояла в количественной оценке стереометрических и морфометрических параметров сердца куриного эмбриона на 15 сутки инкубации во время диастолы. В работе были использованы цифровые изображения серии последовательных гистологических срезов эмбрионального сердца, на которых обводились контуры отдельных структур. Полученные данные импортировались в программу для реконструкции трехмерных объектов, где измерялись дополнительные стереометрические параметры, которые использовались для дальнейшего статистического анализа. Моделирование диастолы осуществляли при помощи раствора KCl. В ходе работы была получена трехмерная компьютерная модель сердца куриного эмбриона во время диастолы, которая состояла из моделей отделенных составных частей (полостей левого и правого желудочков, предсердий, миокарда обоих желудочков, субэпикарда). В работе была выделена группа параметров для дальнейшего анализа на различных стадиях эмбрионального развития во время систолы и диастолы.
Ключевые слова: куриное сердце, диастола, трехмерное компьютерное моделирование, стереометрические параметры.

Introduction

Most cardiovascular malformations have their origins during early morphogenesis. Some forms of adult-onset cardiovascular diseases also arise during embryonic development. Innovations in embryo reconstruction not only facilitate medical education, they also serve as new tools for scientific investigation of cardiogenesis and congenital heart disease.

Besides established methods for analysis, i.e. morphological studies, cardiac morphometry, histology , scanning electron microscopy, immunhistochemistry, new imaging techniques, namely videocinephotography and time-lapse studies with a digital high-speed video camera, confocal and scanning electron microscopy, optical coherence tomography, magnetic resonance microscopy, micro-computed tomography, have become routine for 3D reconstruction of the heart (Yelbuz T.M. et al., 2002; Zhang X. et al., 2003; Männer J. et al., 2008; Davis A. et al., 2009).

These approaches facilitates understanding of architecture of embryonic heart and gives us the ability to estimate the quantitative amount of a wide spectrum of geometrical parameters of chambers and structure of the wall of the heart. They also serve as new tools for scientific investigation of cardiogenesis and congenital heart disease. But such methods do not yet provide anything like resolution achieved by histology and are therefore of limited use for studies of morphological detail. Also almost all of these studies and the new techniques are performed on larger objects of investigation, and cannot be applied for objects during embryogenesis to successfully show the fully accurate picture and performance of the heart during diastole. But in recent years such study on embryos was performed (Yelbuz T.M. et al., 2002) with the help of optical coherence tomography. They compared three-dimensional data with the plane parameters from histological sections and generated volumetric reconstructions of the early chicken heart during diastole.

As for magnetic resonance microscopy, this

method was used since 1986 for the same purpose (Zhang X. et al., 2003). But it has two main problems; one of them – is necessity to perfuse blood vessels, which is difficult to achieve on early stages of development, secondarily – is the inability to visualize non-luminal structures, for example, the wall of left ventricle etc.

Thus, the past few years we have seen the increasing popularity of the use of different methods of 3D reconstruction. One additional method that gives a good presentation of quantitative measurements of embryonic heart is a 3D computer modeling. The advantage of this method is the ability to reconstruct objects of that size, which can be distinguished by different methods of microscopy. It is also less costly and at the same time provides accurate information about objects of investigation, that's why it is widely used now in research work of different fields of embryogenesis.

In one of recent works in this field, the method of 3D computer modeling was used to illustrate the contribution of different sources to atrial septum and myocardium (Anderson R.H. et al., 2006; Snarr S.B. et al., 2007). Because the developing heart and its precursors rapidly transform in a spatially complex fashion, molecular data can only be interpreted in a proper three dimensional context. Because of this in a set of research works, a pattern of distribution of different molecules and processes (proliferation) was investigated on the surface of 3D computer models of an embryonic heart (Ruijter J.M. et al., 2004; van den Berg G. et al., 2009). This work showed 3D pattern of proliferation of cardiomyocytes with consideration of contribution of two heart forming fields in myocardium formation on different stages of embryonic heart development. This data was proved with the use of study of expression of connexin 40 (van den Berg G., Moorman A.F.M., 2009).

Also this method of reconstruction is also successfully used in research works involving an adult heart. In one of such works the spatial arrangement of cardiac myofibrils was shown.

Although 3D computer modeling method is frequently used for visualizing spatial interrelationships between embryonic structures, only in a little number of research works quantitative parameters of studying objects are measured.

Diastolic cardiac function is measured by muscular relaxation, redistribution of calcium, synchronization, myocardial structure, fibrosis, etc. The ability to study the chicken heart in early stages of development during diastole requires very precise and careful observation. Stopping the chicken heart at diastole with the use of buffer solution of KCl can be achieved with positive results for further assessment and 3D reconstruction. Some of this has been done as early as 1892 (Pickering J.W., 1892).

This valuable information can be fully appreciated with work performed on a study of heart morphogenesis in diastole during early embryogenesis and interpreted only through an adequate method of 3D visualization.

Purpose

The aim of our research was to investigate quantitative value of parameters of chicken heart on 15 day of incubation (HH stage 41) during diastole.

Materials and methods

As a material for the research chicken embryos of Cobb 500 cross have served; eggs were incubated at temperature 39,4˚C, relative humidity of 80 %. Rotation of eggs was carried out with an interval of 8 hours. A stage of development was defined according to V.Hamburger, H.Hamilton (1951), HH, taking into account recommendations of B.J.Martinsen (2005). Material was fixed in a Bouin's solution, dehydrated in graded ethanol, impregnated with chloroform, embedded in a paraplast. Serial sections (15 mm) were focused in horizontal plane. For reconstruction we used every 11th histological slice of embryo heart. Sections were stained with haematoxylin of Geydengieden.

Diastole was modeled with the help of KCl solution as it was previously described in the works by T.M.Yelbuz (2002), X.Zhang et al. (2003).

For the creation of computer models we used Photoshop CS2 software (preparation of photos), Amira for microscopy 5.0 (creation and alignment of contours), 3ds max 8.0 (definitive processing and visualisation). Reconstruction was performed according to recommendations of I.B.Твердохліб (2007).

Results and discussion

In our research 3 dimensional computer model of the chicken embryo heart during diastole was received. It comprised of models of separated heart compartments (left ventricle (LV) cavity, right ventricle (RV) cavity, atrium cavity, myocardium of both ventricles, subepicardium). For the convenience of orientation along the main axis of the heart LV and RV cavities were modeled with the cavities of the main vessels (aorta and pulmonary vein, respectively). Only using 3D modeling methods we can precisely calculate the volume, surface area, width and height of the investigated structures (tab. 1). Analyzing the models, it was revealed that the volume of LV cavity exceeds the volume of RV cavity by 41,63%, while the surface area of the RV cavity is only 15,48% larger then LV cavity. This tells why the ratio of volume to surface area in a left cavity is larger then in right cavity (by 1,64 times).

By visualizing the model, we can explain the prevalence of surface area of RV cavity to LV cavity due to irregular form of RV cavity which has multiple marginal blinded protrusions and because of the prevalence of the RV cavity width to LV cavity (96,94%), while their height is almost equivalent to each other (the difference is 9,52%). Also a RV cavity is C-shaped and covers a LVC (Fig. 1 H, 2 G) by almost 180□. The LV cavity is more correctly formed, approximating to cone-shape.

Table 1

Quantitative characteristics of individual compartments of the chicken heart in diastole, incubation day 15 (HH stage 41)

Name	Volume, $\times 10^9 mkm^3$	Surface Area, $\times 10^7 mkm^2$	Height, mkm	Width, mkm
LV cavity	6,10	2,84	3 450	2 196
RV cavity	4,31	3,28	3 150	4 325
Atrium cavity	3,91	3,04	2 250	442
Myocardium	30,41	11,80	4 350	5 215
Subepicardium	9,84	8,96	-	-

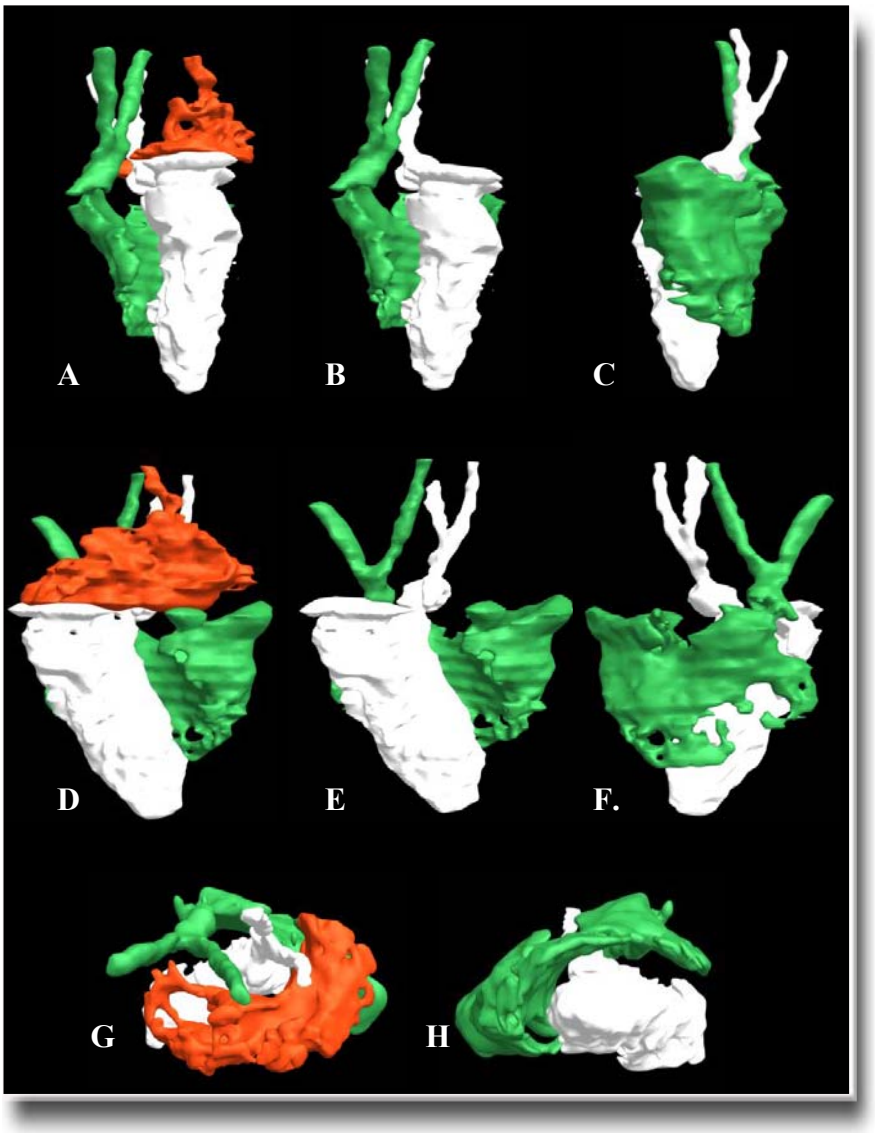

Fig. 1. Chicken embryo heart on 15 day of incubation, HH stage 41. Views: A, B – righ side, C - left side, D, E – posterior; F – front, G – top, H – bottom. Information concerning morphological reconstruction of the developing chicken embryo heart 41 stage during diastole was projected to give quantitative 3D reconstruction to show the cavity of the right ventricle and pulmonary artery marked by green color compared to the cavity of the left ventricle and pulmonary artery which are colored white. As the atrium lies on top of the ventricles (red color) it gives us a perfect view of how ventricles are in relationship to the whole embryonic heart.

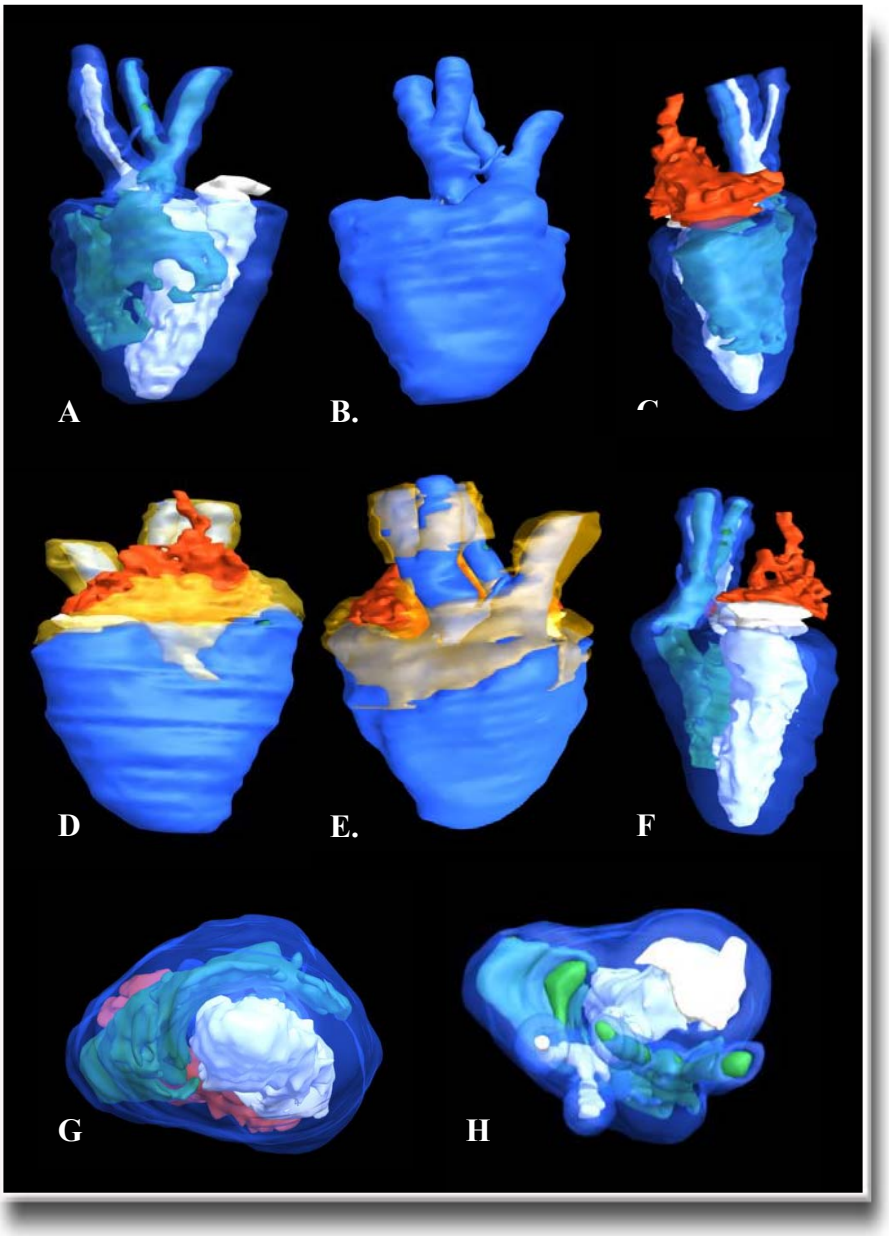

Fig. 2. Chicken embryo heart on 15 day of incubation, HH stage 41. Views: A, B, E – front, C - right side, D – posterior, F – left side, G – bottom, H - top. Myocardium - the transparent blue color (50%) which surrounds the right and left cavity gives us dimensional possibility to observe some parameters in relationship to the cavities. Further analyzing the models we can not miss the transparent yellow (50%) - subepicardium which additionally gives us a perfect representation of how it makes the pass during the developing stages of the formation of the hear

.

Analyzing the parameters of atrium cavity we estimated that the ratio of volume to surface area is 12,86 what is comparable to the same ratio of right ventricle cavity (13,13). This observation could be explained by the presence of apertures in the form of atrial cavity which are visible on fig. 1 (A, G), 2 (F).

For measuring the thickness of heart walls (LV RV and interventricular septum) we used histological sections which were performed on the level just below atrioventricular valves. We noticed that the thickness of LV wall was 537,62±13,85 mkm, RV – 404,96±17,62 mkm, and interventricular septum 640,62±11,32 mkm.

Using data from table 1 and morphometric measurements, ratio of thickness of LV, RV walls and interventricular septum to volume of myocardium of ventricles was calculated, and has made $1{,}77\times10^{-7}$, $1{,}33\times10^{-7}$, $2{,}11\times10^{-7}$, respectively. By subtracting the surface area of both ventricle cavities from the general surface area of myocardium we can figure out the external surface area of myocardium of ventricles ($56{,}84\times10^{6}$ mkm^2), which can be considered almost equivalent to the surface area of epicardium.

In the creating of model of the subepicardial space, was exposed only that part of this compartment, which has been filled with mesenhyme. As it could be seen from fig. 2 (D, E) this part of the

subepicardium extends along the basic grooves of a myocardium: atrioventricular and interventricular.

Comparing the thickness of LV wall to RV wall it was estimated that the difference was 32,76% in favor of LV. It was interesting to note that this value is comparable to difference of their volumes (41,63%).

As for the thickness of interventricular septum, it exceeded the value of the similar parameters of left and right ventricles walls by 19,16% and 58,19% respectively.

During further analyzing, the ratio of cavity volume to thickness of wall for both ventricles was calculated. It represents the next values: $8{,}81\times10^{-6}$ and $1{,}35\times10^{-6}$ for the left and right respectively.

Conclusions

In our work, models of different compartments of the heart of a chicken embryo in diastole have been created, and their quantitative and comparative analyses were performed; the group of parameters for further analysis on different stages of embryonic development has been determined.

Perspectives of further research

In our future research we would like to morphometrically analyze the same parameters not only during diastole but during systole on different stages of development of chicken embryo.

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Гудлетт Т. Тривимірна реконструкція під час діастоли як новий інструмент в розумінні морфології ембріонального серця.

Резюме. Мета нашого дослідження полягала в кількісній оцінці стереометричних та морфометричних параметрів серця курячого ембріона на 15 добі інкубації під час діастоли. У роботі були використані цифрові зображення серії послідовних гістологічних зрізів ембріонального серця, на яких обводилися контури окремих структур. Отримані дані імпортувалися в програму для реконструкції тривимірних об'єктів, де визначалися додаткові стереометричні параметри, які використовувалися для подальшого

статистичного аналізу. Моделювання діастоли проводили за допомогою розчину KCl. В ході роботи була отримана тривимірна комп'ютерна модель серця курячого ембріона під час діастоли, яка складалася з моделей окремих складових частин (порожнин лівого і правого шлуночків, передсердя, міокарда обох шлуночків, субепікарда). У роботі була виділена група параметрів для подальшого аналізу на різних стадіях ембріонального розвитку під час систоли та діастоли.

Ключові слова: куряче серце, діастола, тривимірне комп'ютерне моделювання, стереометричні параметри.