

**T. Goodlett**

Dnipropetrovsk State  
Medical Academy

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## THE VOLUMETRIC ANALYSIS OF CARDIAC CHAMBERS AND THREE-DIMENSIONAL CARDIAC RECONSTRUCTION DURING CHICKEN EMBRYO CARDIOGENESIS

*This study was done within the research "Analysis of normal and abnormal histogenesis tissue components of the cardiovascular system of humans and experimental animals" (state registration number 0105U007837).*

**Summary.** Aim of this study was to quantitatively examine chicken heart's parameters at the stage HH 29 and HH 35 (Hamburger and Hamilton, 1951). It was achieved by using three-dimensional (3D) reconstruction software to align multiple histological sections of the embryonic heart into the stack of images to make a model. We went further in the analysis done by David Sedmera et al., by estimating all the stereometric values (surface area, volume, width and height of ventricles, atrium, and myocardium), which we used for further statistical analysis. Obtained 3D computer models of the chicken embryo's heart in diastole was composed of the separate models of the main heart units (left ventricle cavity, right ventricle cavity, atrium cavity, myocardium of both ventricles). This study there is a certain group of parameters marked out for further analysis on through different stages of embryonic hearts development in diastole.

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**Гудлетт Т. Вольметричний аналіз серцевих камер і тривимірна реконструкція серця під час кардіогенезу у курячих ембріонів.**

**Резюме.** Мета дослідження полягала в кількісній оцінці параметрів серця курячих ембріонів на HH 29 HH 35 стадіях інкубації під час діастолі. У роботі були використані серії послідовних гістологічних зрізів ембріонального серця, контури яких імпортовані в програму для реконструкції тривимірних об'єктів, де була проведена оцінка додаткових стереометричних параметрів, які використовувалися для подальшого аналізу. У ході роботи були отримані тривимірні комп'ютерні моделі серця курячих ембріонів під час діастолі, які складалася з моделей окремих складових частин серця (порожнин лівого і правого шлуночків, передсердь, міокарда обох шлуночків і передсердь). У роботі була виділена група параметрів для подальшого аналізу на різних стадіях ембріонального розвитку серця у діастолі.

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

### Introduction

Approximately 0.4-1% of all live born infants suffer from congenital heart diseases (Pierpont M.E. et al., 2007). Innovations in embryo reconstruction not only facilitate medical education, they also serve as new tools for scientific studies of cardiogenesis and congenital heart diseases. During the cardiogenesis, sizes of the heart's chambers change significantly, but only few studies have attempted to quantify it.

Studies by P. Keller et al. (1996) have attempted to quantify ventricular volumes at different developmental stages showing their increase in size along with the embryo's grow, but their relation to the atria and outflow tract was not assessed. Many details of cardiac morphogenesis are being uncovered only recently, in part because of the complexity of the geometry taking place in development. Human heart has four chambers by week 8, which is

the time the embryo can be visualized by ultrasound scan and, therefore, is too late for detailed morphogenic study (Zimmer M. et al., 1994; Fong E. et al., 2004). Thus, embryonic animal models and 3D serial reconstruction of the histological sections has dramatically improved understanding of heart development by combining its geometry with the expression of cell and/or matrix proteins (Moorman A. et al., 2000; Groenendijk G. et al., 2005). While the exterior walls of the heart are generally smooth and have curvatures of large radii, the interior "lumens" of the heart are way more complex, with variety of trabecular, septal, and valvular geometries. Few studies to date have attempted to profile the changing geometry of the internal surface of heart's chambers. Several studies have used different imaging modalities to explore developing hearts using 3D technique to identify morphogenic defects (Smith L., 2001; Weningrand J., Mohun T., 2002; Schneider K. et

al., 2003; Soufan N. et al., 2004), but none of these studies focused on quantifying the 3D geometry of different segments and chambers.

Our objectives, therefore, were to compare and visualize heart lumen using serial section reconstruction and 3D computer remodeling of each developing segment and chamber at HH 29 and HH 35 stage of development. The right ventricle (RV) acquires a "coarse" trabeculation pattern, with deep pitting of the ventricular wall, while left ventricular (LV) trabeculation becomes finer. These walls become relatively smooth in comparison, although closer inspection reveals many, more shallow pits in the ventricular wall. LV trabecular number decreased over developmental stage concomitant with an increase in trabecular thickness, while spacing remained unchanged. RV trabecular number usually maintained between HH23 and HH30, but decreased by HH36, but in this case trabecular spacing increased (Butcher J. et al., 2007).

In chicken at stages HH 28 - 29, the interventricular septum has grown towards the atrioventricular cushions and has started to fuse, leaving a small gap called a primary interventricular connection (interventricular tunnel or canal) between the left and right ventricle (De la Cruz et al., 1983; Waldo O. et al., 1998). From day 6 to day 8, which corresponds to HH 29 and HH 35, there is an 80% increase in cell density in the ventricular myocardial compact zone (Pennisi B. et al., 2003) when ventricular septation completes (Waldo V. et al., 1998). In chickens in comparison to humans the portion of the interventricular septum that separates the inlet of the right ventricle from the left ventricular infundibulum is not formed by connective tissue as it is in humans, but still corresponds to the membranous region of the human interventricular septum (De la Cruz et al., 1983).

Chick hearts at HH 30, are visualized with a completed interventricular septum and separate outflow tracts. Both leaflets of the left AV valve have condensed and elongated further, while the septal leaflet of the right AV valve appears to be absorbed into the septum. The development of the avian right AV muscular flap valve is also clearly visible at HH 35 - HH 36, the development of the atrioventricular valves are mostly complete. The tendinous chord connections between already thin fibrous left AV leaflets and ventricular myocardium can be clearly seen in 3D computer modeling as well as the complexities of the leaflet shapes. The interventricular septum has increased its thickness, and the right septal cushion has almost completely disappeared. The pectinate muscles have developed in both the left and right atria, and the right AV flap valve is fully formed (Butcher J. et al., 2007).

This approach facilitates understanding of architecture of embryonic heart and gives us the ability to estimate the quantitative amount of a wide spectrum of geometrical parameters that describe

heart chambers and its wall structure. They also serve as new tool to study cardiogenesis and congenital heart diseases.

Thus, in the past few years we have seen increase of popularity of different 3D reconstruction methods. Additional method to present quantitative measurements of embryonic heart is 3D computer modeling. Main benefit that explains wide use of this method in different embryogenesis studies is the ability to reconstruct objects of small size, and at the same time provides precise information about objects of study. 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 quantitatively evaluate chicken heart's parameters on 6 and 8 days of incubation (which corresponds to HH 29 and HH 35) in modeled diastole.

#### **Materials and methods**

This study was performed on chicken embryos of Cobb 500 cross; eggs were incubated at temperature 39,4°C and relative humidity of 80%. Rotation of eggs was carried out with an interval of 8 hours. Developmental stage was defined according to V. Hamburger, H. Hamilton (1951), with regards to the recommendations of B.J. Martinsen (2005). Isolated chicken heart was fixed in a Bouin's solution, dehydrated in graded ethanol, impregnated with chloroform and embedded in paraplast. Serial sections, 10 µm each, were performed in horizontal plane. Sections were stained with haematoxylin of Geydengieden. Diastole was modeled by placing whole organ in 1,8% solution of KCl, as previously described in recent studies (Yelbuz T.M. et al., 2002, Zhang X. et al., 2003).

Using a digital camera attached to a microscope, with a normal bright-field illumination, images of stained histological sections were digitized. The obtained digitized images of the serial sections were saved in JPEG format and then transferred to a desktop computer. To create 3D models, Adobe Photoshop software was used for the image processing, Approximately 30-35 sections per heart were imaged. The images were then imported into AMIRA 5.0 software to be aligned and composed into a stack of images. Same software allows to generate luminal heart volume by using a cubic spline interpolation between each section (creation and alignment of contours), then imported into 3D MAX 2011 (definitive processing and visualisation). The 3D data was obtained in 3D MAX 2011. 3D reconstruction was performed according to recommendations of I.V. Tverdokhleba (2007).

#### **Results and discussion**

Our study reveals 3D model of the chicken embryo heart in diastole. Embryonic cardiac morphogenesis is a complex 3D process that occurs ra-

pidly. Several techniques have been and are routinely used to observe this developmental process, each with their own advantages and limitations. It is comprised of separate models of heart compartments (left ventricle cavity, right ventricle cavity, atrium cavity, myocardium of both ventricles). Quantitative data for individual compartments of the chicken heart in diastole are shown in Table 1 for incubation day 6 (HH stage 29) and Table 2 for incubation day 8 (HH stage 35).

Analyzing the chicken heart models on 6<sup>th</sup> and 8<sup>th</sup> days of incubation (HH 29 and HH 35) showed that the volume of LV cavity at HH 35 exceeds its value compared to HH 29 by 151,06 %, while the surface area of the LV at the same stages is 141,96 % greater. This explains decrease of the surface/volume ratio in the LV during that developmental stages, which 1.03 times lower at HH 35 compared to HH 29 (Fig. 1 A; Fig. 2 A).

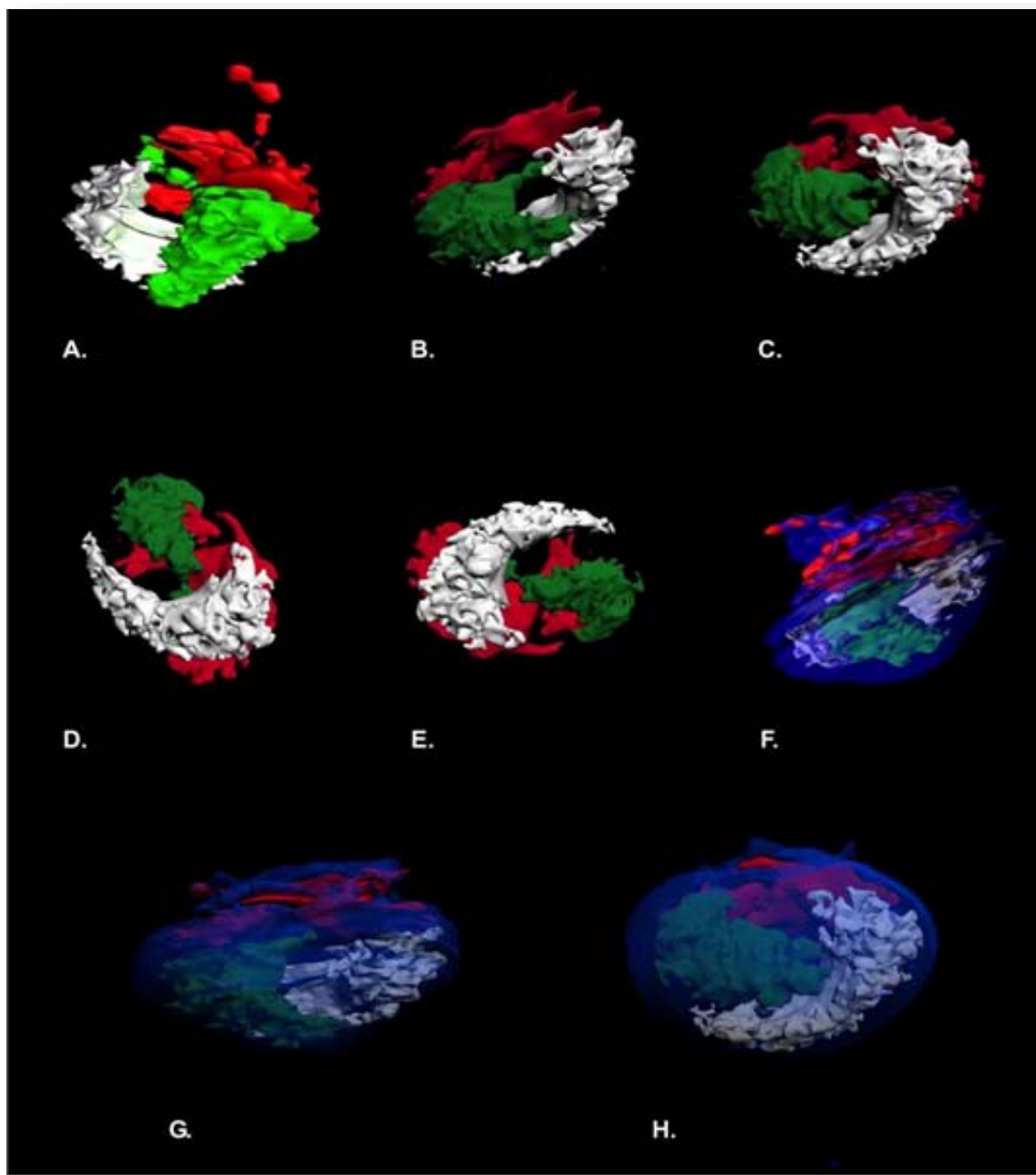


Fig. 1. 3D reconstruction of chicken embryo's heart at HH stage 29 (6<sup>th</sup> day of incubation), performed from the set of serial histological sections. A- side view; B, C, D, E – Bottom view; F, G, H – Myocardial layer, surrounding RV and LV have been added (transparent blue color), giving to the reader dimensional possibility to observe parameters in relation to the cavities. White color represents RV cavity, green – LV cavity, red – atrium.

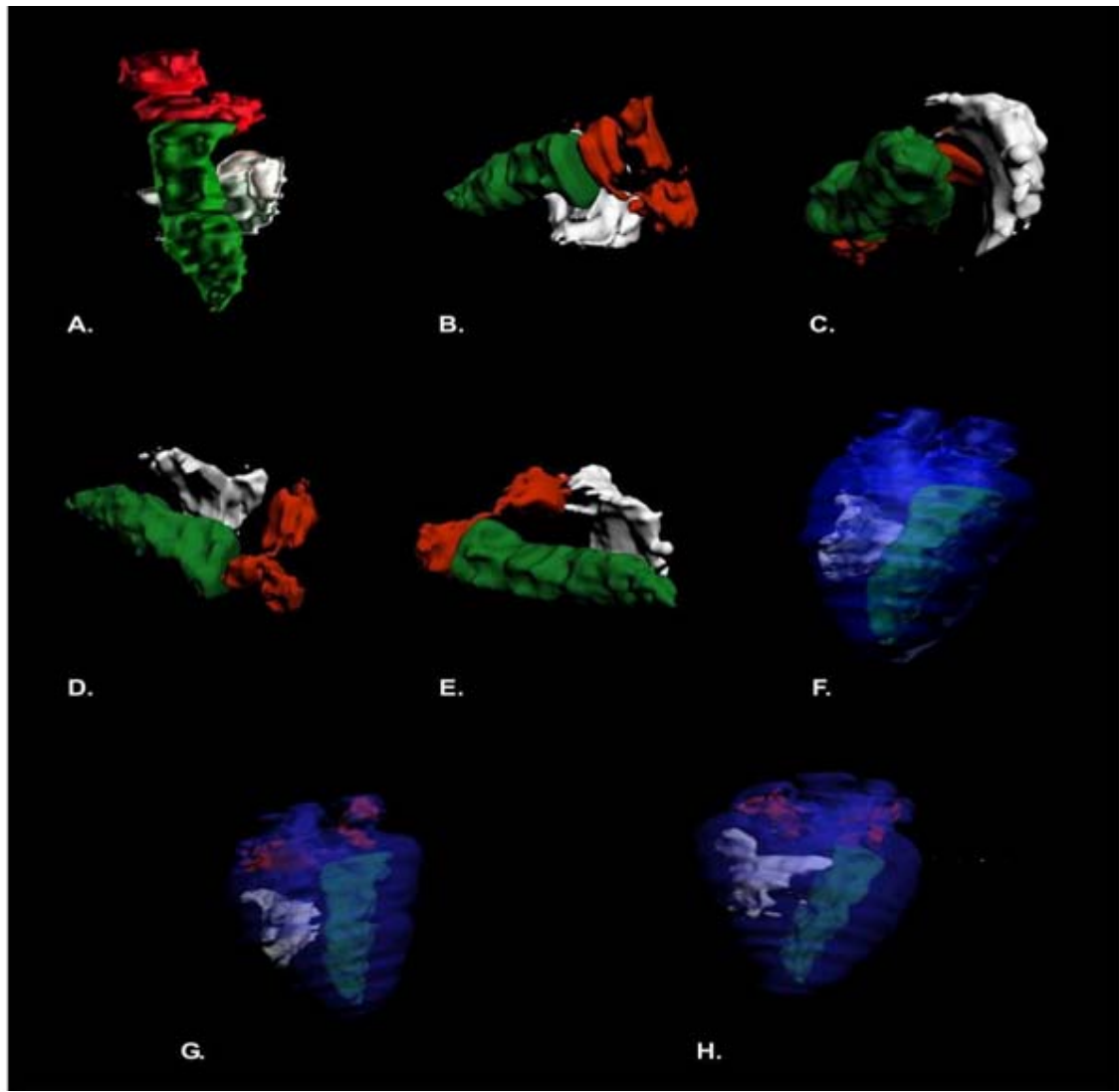


Fig. 2. Chicken embryo heart 8<sup>th</sup> day of incubation , HH stage 35. The 3D reconstruction of 2D serial histological sections. Views: A – Bottom; B – top; C, E – Bottom; D – Side; G, H – Top. Myocardial layer, surrounding RV and LV have been added (transparent blue color), giving to the reader dimensional possibility to observe parameters in relation to the cavities. White color represents RV cavity, green – LV cavity, red – atrium.

Table 1

Quantitative characteristics of individual compartments of the chicken heart in diastole, incubation day 6 (HH stage 29)

Name	Volume, $\times 10^9 \mu\text{m}^3$	Surface Area, $\times 10^7 \mu\text{m}^2$	Height, $\mu\text{m}$	Width, $\mu\text{m}$
LV cavity	0,94	1,12	1185	1217
RV cavity	0,72	2,05	1213	1325
Atrium cavity	1,15	2,67	1474	754
Myocardium	2,86	6,55	1618	1743

Evaluation of data from table 1 and table 2 reveals that the volume of RV cavity at the stage of HH 35 exceeds the volume of the one from HH 29 by 275 %, while its surface area at HH 35 is only 29,75 % larger than at HH 29. On 6<sup>th</sup> day of development, which corresponds to HH 29, surface area to volume ration in RV is 184%. On the 8<sup>th</sup> day (HH

35) it does not show significant changes, increasing only by 1,50%. Visualizing the models explains the prevalence of surface area of RV at HH 29 to the one from HH 35 due to irregular form of its cavity, which has multiple marginal blinded protrusions and prevalence in RV's width by (34.71%), while their height is almost equivalent to each other (46%). Ini-

tial structural similarity in morphology between the two chambers and cavities (LV and RV) diverge significantly by HH 35 (Fig. 1; Fig. 2). The right ventricle acquires a “coarse” trabeculation pattern, with

deep pitting of the ventricular wall, while left ventricular trabeculation becomes more fine with decrease of RV’s surface volume by HH 35 (Fig. 1 D, E; Fig. 2 A, C).

Table 2  
Quantitative characteristics of individual compartments of the chicken heart in diastole, incubation day 8 (HH stage 35)

Name	Volume, $\times 10^9 \mu\text{m}^3$	Surface Area, $\times 10^7 \mu\text{m}^2$	Height, $\mu\text{m}$	Width, $\mu\text{m}$
LV cavity	2,36	2,71	1565	1366
RV cavity	2,70	2,66	1771	1785
Atrium cavity	2,55	3,07	1630	762
Myocardium	6,11	8,16	2132	2317

The volumetric analysis of the models, reveals the prevalence of surface area of LV (HH 35) to LV (HH 29) due to irregular form of LV cavity which has multiple marginal blinded protrusions and because of the prevalence of the LV cavity width (12,24%), while their heights do not change. The prevalence of surface to volume is due to increase of height of LV 2.61 times and width of RV compared to LV (almost 1,32 times) (Figure 1 A; Figure 2 A).

It was revealed that the volume of myocardium at the stage of HH 35 exceeds the volume of myocardium at HH 29 by 113,6 %, while the surface area of the myocardium (HH 35) is 24,58 % larger than myocardium of the heart at HH 29. Therefore, surface / volume ratio in the myocardium at HH 29 is smaller than at HH 35 (4.62 times). While the width and the height ratio only 1,12 (Figure 1 G, H; Figure 2 G, H).

Analysis of the same parameters for the atriums at that developmental stages shows that the surface / volume ratio is (2,32) which is comparable to the same ratio of the RV cavity (2,89), LV ratio (1,03),

myocardium ratio (4,62), while the width and the height ratio (9,97). Parameters of ratio of surface to volume at (HH 35 and HH 29) atriums two times larger than myocardium, while the ratio of LV twice smaller than atriums. Between HH 29 and HH 35(6th-8th day) the total volume of the heart increases over 2 orders of magnitude (Figure 1 F; Figure 2 H).

#### Conclusion

In this study, models of different compartments (right ventricle, left ventricle, atrium, and myocardium) of the HH 29 and HH 35 chicken embryo’s heart in diastole have been created, the quantitative and comparative analysis 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 through the different stages of chicken embryo’s development.

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#### **Гудлетт Т. Волюметрический анализ сердечных камер и трехмерная реконструкция сердца во время кардиоге́неза у куриных эмбрионов.**

**Резюме.** Цель исследования состояла в количественной оценке параметров сердца куриных эмбрионов на HH 29 и HH 35 стадиях инкубации во время диастолы. В работе использованы серии последовательных гистологических срезов эмбрионального сердца, контуры которых были импортированы в программу для реконструкции трехмерных объектов, где была произведена оценка дополнительных стереометрических параметров, использованных для дальнейшего анализа. В ходе работы были получены трехмерные компьютерные модели сердца куриных эмбрионов во время диастолы, составленные из моделей отдельных составных частей сердца (полостей левого и правого желудочков, предсердий, миокарда обоих желудочков и предсердий). В работе была выделена группа параметров для дальнейшего анализа на различных стадиях эмбрионального развития сердца в диастоле.

**Ключевые слова:** куриное сердце, диастола, трехмерное компьютерное моделирование, стереометрические параметры.