

Histodevelopmental study of Heart development in Local Domestic Rabbit (*Oryctolagus Cuniculus*): prenatal study

Esraa Abdul Khaliq Zegyer ¹, Department of Pathological Analyses, College of Science, Wasit University.

Ahmed Mahdi Al-Badri², Department of Biology, College of Science, Wasit University

Bassim Abdullah Jassim³, College of Veterinary Medicine, AL-Muthanna University

Abstract

The study designed to investigate the histological modification of heart tube during the development stage in local domestic rabbit's embryo. The experimental animals were distributed into four groups each group consist of one male Rabbit and three female Rabbits. The pregnant female in all groups were sacrificed at the end of each period. The result showed the early stage of heart development at (4, 6, 8 and 10) days of gestation period, the first embryonic evidence of embryo was the neural tube creation belong the longitudinal axes of embryo. The result noted the primary divisions of neural tube occurs in cephalic part of embryo. Current result noted at six day of development time showed prominent cardiogenic plate located in limited region called intra embryonic cavity. The micrograph at eight day of heart development showed the heart tube have prominent wide ends, while at ten day noted establishment of primitive heart regions which included the truncus arteriosus, bulbo cordis, atrium, ventricle and sinus venosus.

Key words: Rabbit, Heart, Embryo, Cardiogenic, Development.

Introduction

Embryonic development is a series of changes undergone by the newly-formed individual; zygote and which culminate in production of an adult organism capable of reproduction. The development does not terminate at birth but is continuous process spanning the prenatal and postnatal periods [1]. The embryonic development in the vertebrates has deferent mechanism in development according to Importance the organ in early embryonic life. The heart which consider the first evidence in development which very important in early time of gestation period of animal [2]. The cells that composed of heart differentiate from intermediate mesoderm as a first cells aggregation to cardiac establishment [3].

The heart genesis begins from primary tissue called mesoderm after insemination. The mesoderm stratum is one of the three principal germ strata that initially differentiate during development and communally gives rise to entirely subsequent body tissues and organs. The heart initiates to develop adjacent the foreword of the embryo in a section identified as the cardiogenic zone. The heart tube, as primary structure in fetus which have a slight further than the originators cells that consist of left chamber (ventricle), while other precursor cells to other heart regions addition continuously. Both arterial and venous ends of the cardiac tubular structure which appear as growth outside the cardiac tube [4]. The histological development of the cardiac is a difficult mechanism. The hearts play important role in oxygen and nutrient rich of blood supply, the development of heart very important to deferent requirement, in deferent stages of development to avoid the deferent congenital heart malformations [5].

Aim of study:

To determine the early stage of the development of the heart compared to the age of fetus.

Material and methods**Experimental animals:**

The project was carrying out sixteen adult domestic rabbits which included twelve adult female rabbits and four adult male rabbits. The experimental animals which divided into four groups (A, B, C and D) each group consist of three adult female rabbits and one adult male rabbit in laboratory wood cages. The study applied in animal house in Biology Department in Science college – Al Muthanna University. Each pregnant female was scarified to obtained on the embryo after (4, 6, 8, and 10) days of pregnant periods.

Sample collection:

The early embryonic evidence of embryo which obtained from pregnant rabbits after each time of pregnant period which included (4, 6, 8, and 10). The tissue samples of embryo were washed with normal saline, the samples kept in labeled container for each age of experimental groups.

Histological technique:

The samples were passing through the deferent stapes of histological preparations, [6] which included:

- 1- **Fixation** with 10 % of formalin for 48 hours.
- 2- **Washing** with tap water for one hour.

- 3- **Dehydration** by ethanol alcohol for deferent concentrations.
- 4- **Clearing** by xylen.
- 5- **Infiltration** with paraffin wax.
- 6- **Embedding** and blocking.
- 7- **Cutting** by rotary microtome as thin sections with 5 μm in thickness.
- 8- **Staining** with H&E.

Result and Discussion

At four days of embryonic development:

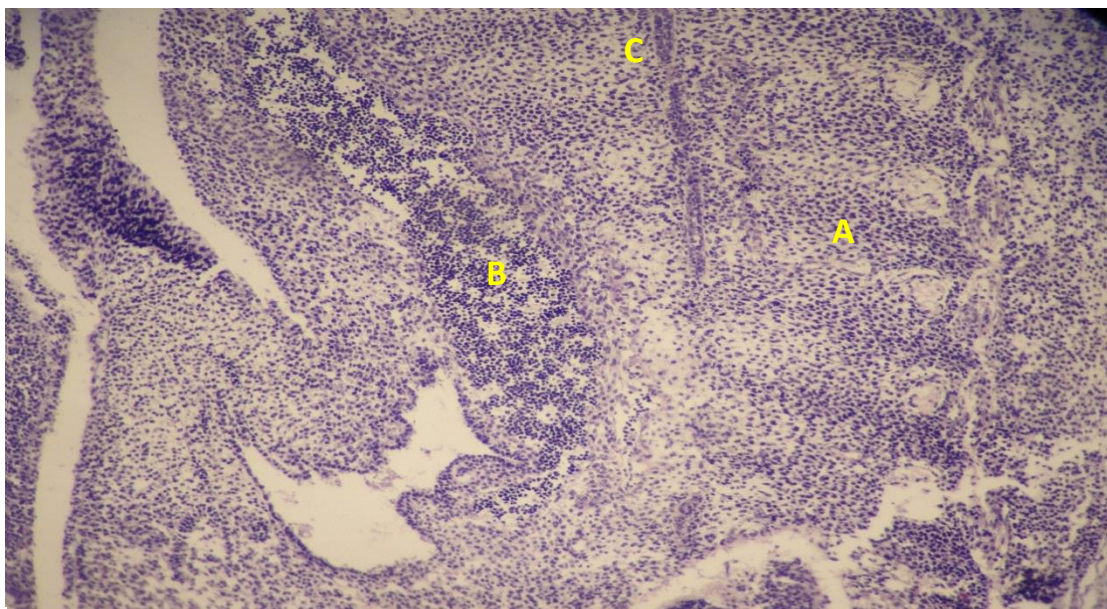
The tissue section of embryo showed the embryonic evidence during the fourth day of gestation period which noted the prominent neural tube formation belong the longitudinal axes of embryo. The result noted the brain divisions in cephalic end of embryo. The tissue sections of embryo at four days of gestation period showed clear mesodermal layer under the neural tube formation (Fig. 1).



(Figure 1): Micrograph of rabbit embryo at four day of gestation period noted: A-Neural tube. B- Brain divisions. C- Somite formation. (1000 X, H&E Stain)

The current result was the first evidence of neural tube formation at the same time of heart tube formation because the embryo need to blood supply this result was agreement with [7], which said

that the currently next its closing, therewith the neural tube turn into embedded in a compacted mesenchyme stratum that create the fibrous sheath (meninx primitive) (Fig. 1). This cover of the forebrain (forward neural plate) originates from the neural crest of the extra caudally that posterior diencephalic and mesencephalic fragments.



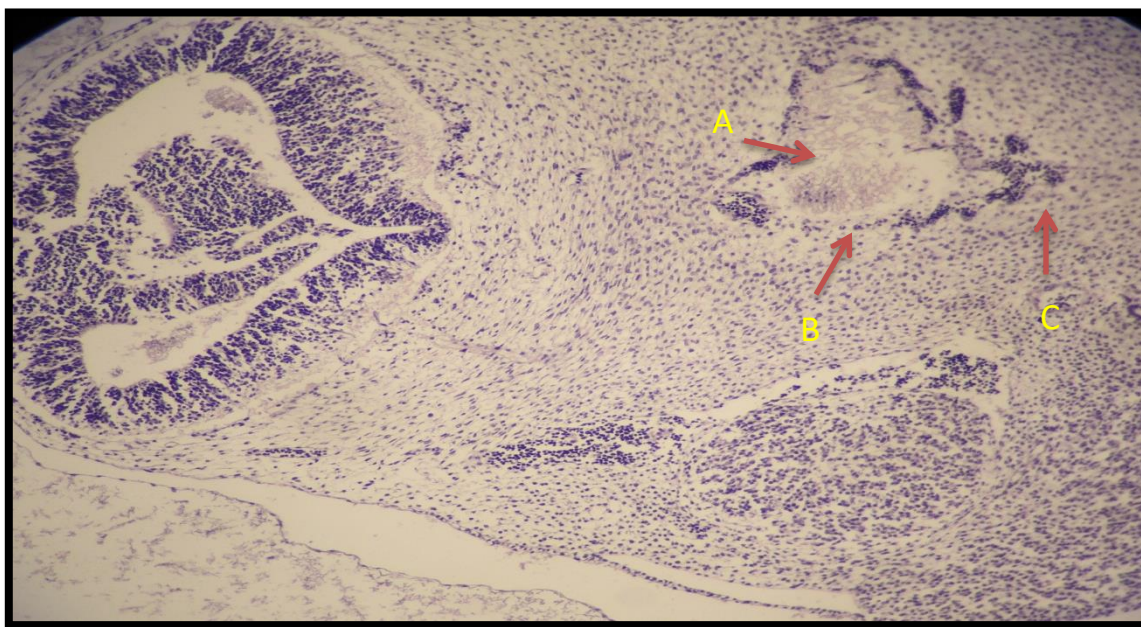
(Figure 2): Micrograph of rabbit embryo at four day of gestation period noted: A- Somite . B- Cardiogenic region. C- Somite formation. (400 X, H&E Stain)

The tissue sections of embryo at fourth day of pregnant period appeared the several somite formation which arranged on both side of neural tube formation belong the longitudinal axes of embryo, the result showed the prominent differentiated of mesoderm layer in rabbit embryo (Fig.2).

These results were the first evidence of embryonic development and establishment of cardiogenic region these results were similar to [2] which said that the embryonic development in the vertebrates has deferent mechanism in development according to importance the organ in early embryonic life. The heart which consider the first evidence in development which very important in early time of gestation period of animal. So, our results were confirmed with that noted the cells that composed of heart differentiate from intermediate mesoderm as a first cells aggregation to cardiac establishment.

At Six days of embryonic development:

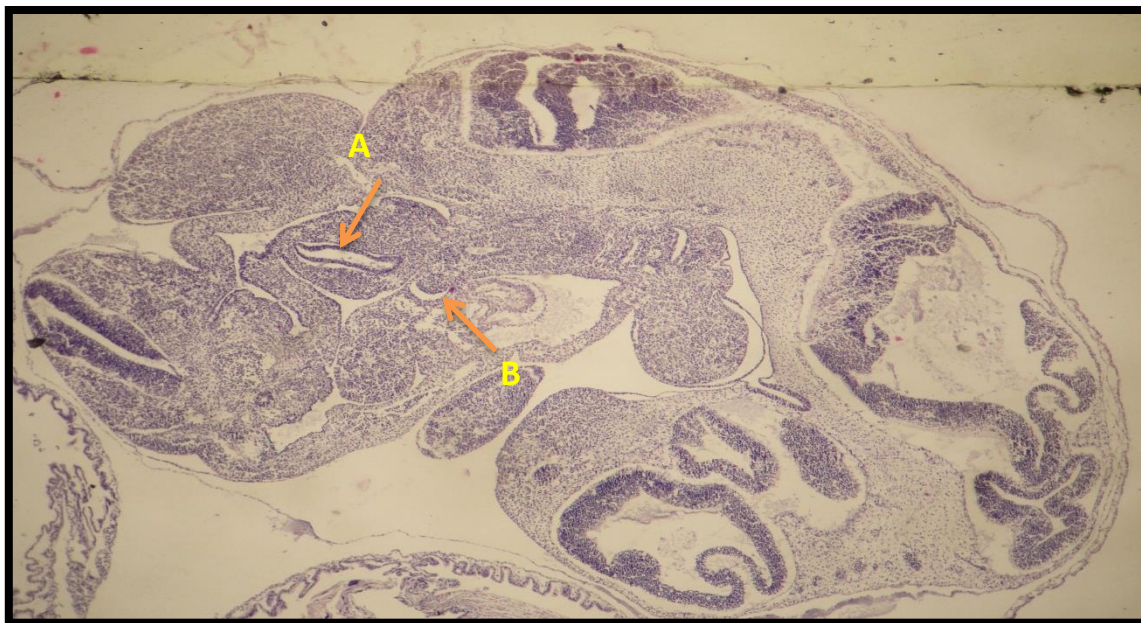
The histological result of heart development in rabbit embryo at six day of development time showed prominent cardiogenic plate located in limited region called intraembryonic cavity. The intraembryonic cavity which consider a specific area of heart development, which located nearly from early pharyngeal region of embryo. The tissue section noted prominent borders of the cardiogenic plate (Fig. 3). The cardiogenic plate which have cellular aggregations in deferent locations of cardiogenic plate these results were agreement with [4] which noted the starting of heart development tack place in the cardiogenic zone, which approaching to the head of embryo. On the other hand, the heart tube as primary structure in fetus which have a slight more than the originators cells that consist of left chamber (ventricle), while other precursor cells to other heart regions addition continuously.



(Figure 3): Micrograph of rabbit embryo at six day of gestation period noted: A- Intra- embryonic cavity. B- Cardiogenic plate. C- Cellular aggregation. (X1000, H&E stain).

At eight days of embryonic development:

The tissue section at seven day of gestation period noted irregular short tubular structure curved as horse shoe. The result found the tubular structure have prominent wall beside of lung bud and posterior vena cava. The micrograph of embryonic development of heart showed the heart tube has prominent wide ends (Fig. 4).



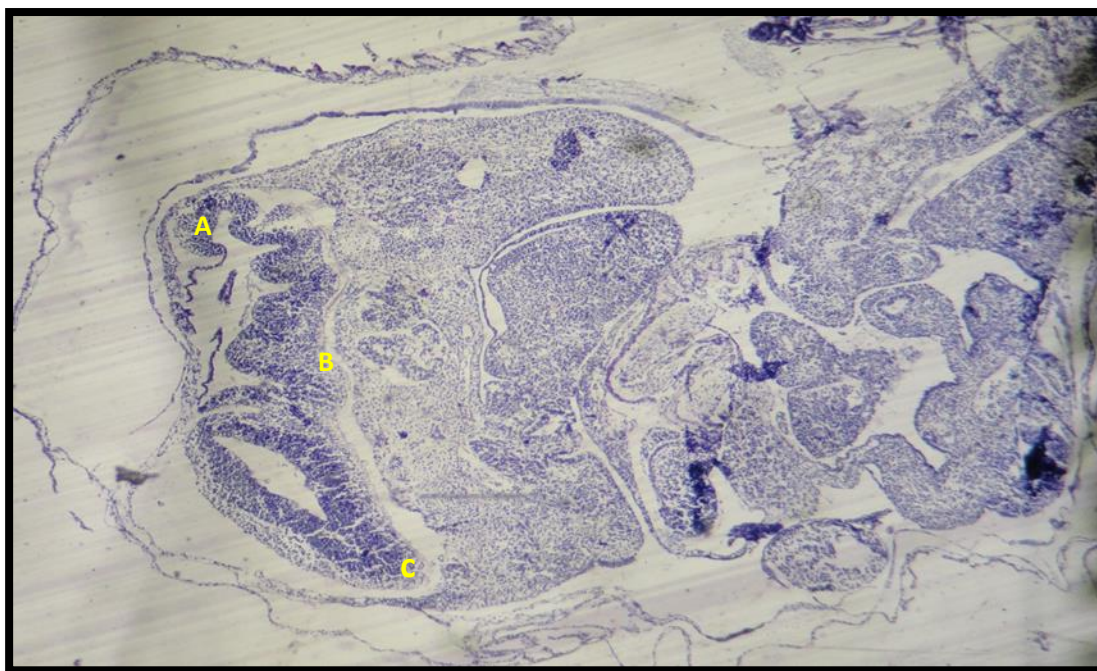
(Figure 4): Micrograph of rabbit embryo at eight day of gestation period noted: A- Irregular short tubular structure heart tube. B- Vena cava. (100X H&E stain).

This result was similar to [8] which said that unlike furthestmost other body systems, the main role of cardiovascular system is critical for embryonic survival and as a consequence, the heart has to beat for the sake providing blood circulation previously its whole morphogenesis. The heart functions may possibly have effects by its morphology, this notification was similar to [9] who mentioned the primary cardiac contractions inception at the straight tube period. This result was agreement with [10] that said the cardiac originators are establish presently after stage of gastrulation inside the mesodermal constituent of the splanchnopleural sheet of the anterior-most horizontal plate. This region is termed the cardiogenic area, as well as it formed by way of initial gastrulating embryonic mesoderm section. In addition, the cardiogenic area is individual and crescent-shaped in the embryo of mouse while it appeared as bilaterally paired in embryos human and birds.

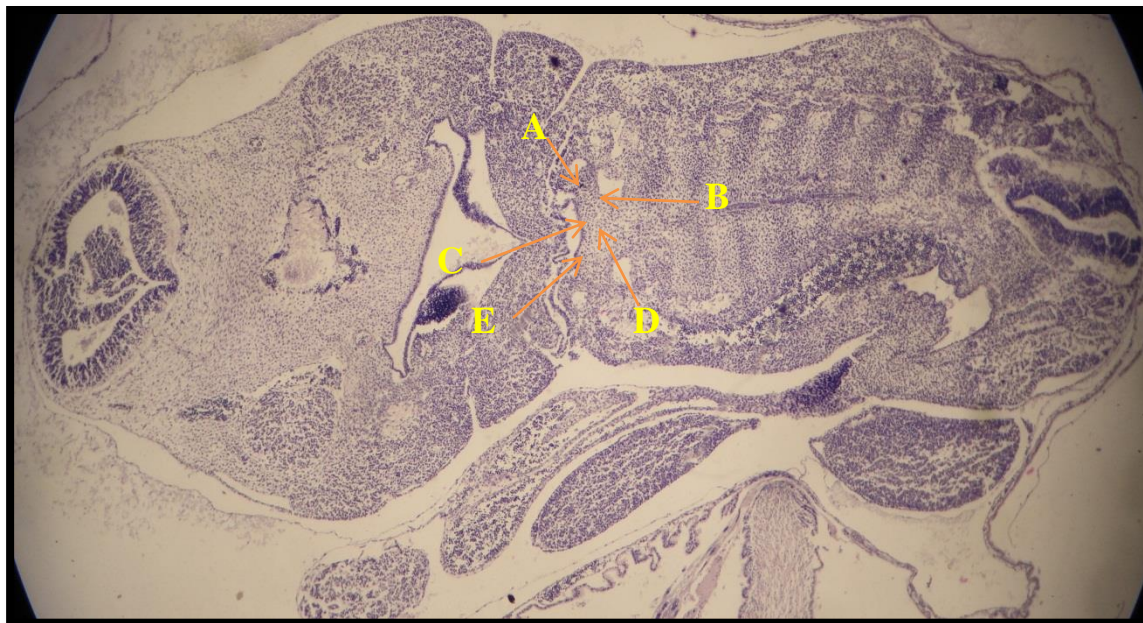
At ten days of embryonic development:

The histological result of cardiac development in local domestic rabbit at eight day of development time noted prominent heart tube structure with prominent primitive heart regions. The tissue section showed five primitive regions of heart which included branched end of heart tube called truncus arteriosus, under the first region showed bulging region of heart tube called bulbu

cordis, the third region of heart tube was as wide dilation of heart tube called ventricle, while the forth region which located under the ventricle was narrower than the ventricle which called atrium, the terminal end of heart tube was division into main branches which called sinus venosus (Fig.5). The tissue findings of rabbit fetuses at eight day of gestation period noted the five primitive region of heart tube were surrounded by prominent cellular aggregations which increased the thickness of heart tube wall (Fig. 6). This histological modification in the heart tube may be to preparation for the heart tube convergence.



(Figure 5): Micrograph of rabbit embryo at eight day of gestation period noted: A- Primitive heart tube. B- Vena cava. C- Stomodium (400X PAS stain).



(Figure 6): Micrograph of rabbit embryo at ten day of gestation period noted: A- Primitive five regions of heart tube. B-. truncus arteriosus C-.bulbus cordis. D- Ventricle. E-Atrium, F-Sinus venosus. (100X H&E stain)

These results were confirmed with [11, 12, 13] which they noted that almost immediately after the creation of looping, all morphological differentiation of myocardium layer which extends along the cardiac tube come to be apparent. These events include loss of cardiac jelly in both atria and ventricles chambers, corresponding with the process of trabeculae progress. Mostly the morphological variances are accompanied via alterations in gene expression design.

The result of present work was similar to [14] who noted the heart chamber creation thus takes place via hyper proliferation of isolated areas leading to local expanding of the linear tube of cardiac walls. As well as, these calculable studies at cellular determination also indicated a role for shape alterations in addition to cell size in through chamber formation.

References

- [1] Hyttel, P.; Sinowatz, F.; Vejlsted, M. and Betteridge, K. (2010): Domestic animal embryology. Saunders Elsevier. New-york, London and Sydney. P: 1-24.
- [2] Srivastava, D. and Olson, E.N. (2000): A genetic blueprint for cardiac development. Nature. 407, 221–226.

- [3] **Yutzey, K.E. and Kirby, M.L.** (2002) Wherefore heart thou? Embryonic origins of cardiogenic mesoderm. *Dev Dyn.* 223, 307–320.
- [4] **Marc Sylva Maurice J.B. van den Hoff Antoon F.M. Moorman.**(2014): Development of the human heart. *American Journal of Medical Genetics.* Volume164, Issue6 June 2014 Pages 1347-1371.
- [5] **Benjamin Kloesel, MD, MSBS, James A. DiNardo, MD, and Simon C. Body,** (2016): Cardiac embryology and molecular mechanism of congenital heart disease. *International Anesthesia Research Society .Volume 123 . Number 3.*
- [6] **Suvarna, K. S.; Layton, C. & Bancroft, J. D.** (2018). *Bancroft's theory and practice of histological techniques E-Book.* Elsevier Health Sciences.
- [7] **Charles Raybaud, MD** (2010): Normal and Abnormal Embryology and Development of the Intracranial Vascular System. *Neurosurg Clin N Am* 21. 399–426
- [8] **Srivastava, D. and Olson, E.N.** (2000): A genetic blueprint for cardiac development. *Nature.* 407, 221–226.
- [9] **Sedmera, D.** (2005): Form follows function: developmental and physiological view on ventricular myocardial architecture. *Eur J Cardiothoracic Surg*;28:526 –528.
- [10] **Chen F, De Diego C, Chang MG, McHarg JL, John S, Klitzner TS** (2010): Atrioventricular conduction and arrhythmias at the initiation of beating in embryonic mouse hearts. *Dev Dyn*;239:1941 –1949.
- [11] **Kenzo Ivanovitch, ID , Isaac Esteban ID and Miguel Torres** (2017): Growth and Morphogenesis during Early Heart Development in Amniotes. *J. Cardiovasc. Dev. Dis.* 4, 20; doi:1.
- [12] **Aanhaanen, WT, Mommersteeg MT, Norden J, Wakker V, de Gier-de Vries C, Anderson** (2010): Developmental origin, growth, and three-dimensional architecture of the atrioventricular conduction axis of the mouse heart. *Circ Res*;107: 728 –736.
- [13] **Christoffels, VM.** (2003): Cardiac chamber formation: development, genes, and evolution. *Physiol Rev*;83:1223 –1267.
- [14] **Soufan, A.T.; van den Berg, G.; Ruijter, J.M.; de Boer, P.A.; van den Hoff, M.J.; Moorman, A.F.** (2006): Regionalized sequence of myocardial cell growth and proliferation characterizes early chamber formation. *Circ. Res.*, 99, 545–552.