Histological observation on cardiogenesis during second trimester of domestic rabbit's fetuses (*Oryctolagus Cuniculus*)

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دراسة نسجية لعملية تكوين القلب في الثلث الثاني من الحمل في اجنة الارانب المحلية

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المستخلص

صممت الدراسة الحالية لمعرفة الصفات التطورية النسجية للقلب في اجنة الارانب المحلية والتي تضمنت تحديد وقت بداية النبض ودراسة التكوين النسجي لمكونات القلب .اجريت الدراسة على خمسة وثلاثين جنين ارنب وزعت على سبع مجاميع طبقا للاعمار الاتية: (9, 10, 12, 14, 16, 18,20) يوم حمل. الدراسة اظهرت ان التطور والتمايز للقلب يبدا مابين اليوم التاسع والعاشر من الحمل والذي فيه الجدار الناعم للانبوب القلبي يمتلك غطاء عضلة القلب الخارجي, الجيلاتين القلبي مع وجود البطانة القلبية من خلال المقاطع المتسلسلة لكل مرحلة عمرية. اظهر التركيب النسجي لكل مرحلة عمرية بان القلب يمر خلال مراحل شكلية متتابعة مختلفة خلال الحياة الجنينية ممثلة بتكوين الانبوب القلبي, التواء القلب, تكوين تجاويف القلب وتكوين حواجز القلب. الغرض من الدراسة هو لتوفير وصف نسيجي كامل لمراحل تكوين القلب في اجنة الارانب المحلية .

Abstract

The present work was designed to investigate the histological developmental characteristics of heart in the local rabbit fetuses. It which included the detection of timing of heart beat onset and studied morphohistogenesis of heart components. The study was performed on 35 rabbit fetuses distributed at seven groups according to the following ages: (9, 10, 12, 14, 16, 18, 20) days gestational age. The results of the current study showed that differentiation and formation of the heart began between 9^{th} and 10^{th} day of pregnancy in which the smooth-walled heart loop had an outer myocardial mantle, cardiac jelly and present of endocardium by histological study for serial section in each stage. The histological structures for each age showed that the heart passed through different sequential morphological stages in embryonic life represented by heart tube, looping heart, chamber formation and cardiac septation. The purpose of our study was to provide a more complete histological description of the stages of heart formation in rabbits during prenatal development.

Introduction

Domestic rabbits in Iraq are descended from the European rabbit, Oryctolagus cuniculus, which are characterized by the presence of a second small pair of upper incisors or peg teeth (1). Among the laboratory animals, the rabbits have been used as an experimental model for research (2), it is a means of research in the field of biology, physiology, medicine, toxicology, pharmacology, surgery and used as animal husbandry (3, 4).

The cardiovascular system in mammals consists of the heart and blood vessels. Heart formation is initiated in vertebrate embryos soon after gastrulation when the three embryonic germ layers, ectoderm, endoderm and mesoderm, are established in the primitive streak. The heart is derived from the mesoderm and is the first definitive organ to form in development (5).The primitive heart, the single median structure formed by the fusion of paired rudiments, is carried ventral to the foregut by the reversal process reshaping the head end of the embryo (6). Because there is no sufficient studies about histogenesis of the heart in rabbit fetuses, so we suggest studying the developmental and the timing of first appearance of the components of the heart at intrauterine life because it has importance in scientific research.

Materials and Methods

A total of 35 rabbit fetuses collected from uteri of the local breed pregnant does were used in this experiment. The does were mated with a buck for 1 to 2 hours and were then marked and kept in individual cages, five fetuses prepared for every stage beginning from (9,10, 12, 14, 16, 18, 20) days by which gestation occurred (Figure 1). The crown-rump lengths (CRL) were measured for all fetuses (CRL is the measurement from the vertex of the skull to the midpoint between the apices of the buttocks for prenatal only (7). The CRLs at each stage are summarized in table (1). The body weight was recorded for each prenatal fetus by using sensitive balance. The mean weight at each stage is summarized in table (2). Samples were taken directly after sacrificing animals. They were fixed in 10% formalin for 72 hours. After fixation, the specimens were washed in tap water for 3-4 hours to remove the formalin solution & transferred to the following steps:

Dehydration in ascending concentration of ethyl alcohol, clearing in zylol & embedding in paraffin wax and finally sectioning by the rotary microtome and the sections were stained with hematoxylin and eosin (8). A computerized program, the Statistical Package for Social Sciences (SPSS) version 15 was used to calculate the statistics (9). Finally, the study results were recorded at different histological sections by light microscope and (MEM1300) digital eyepiece for microscope installation instructions

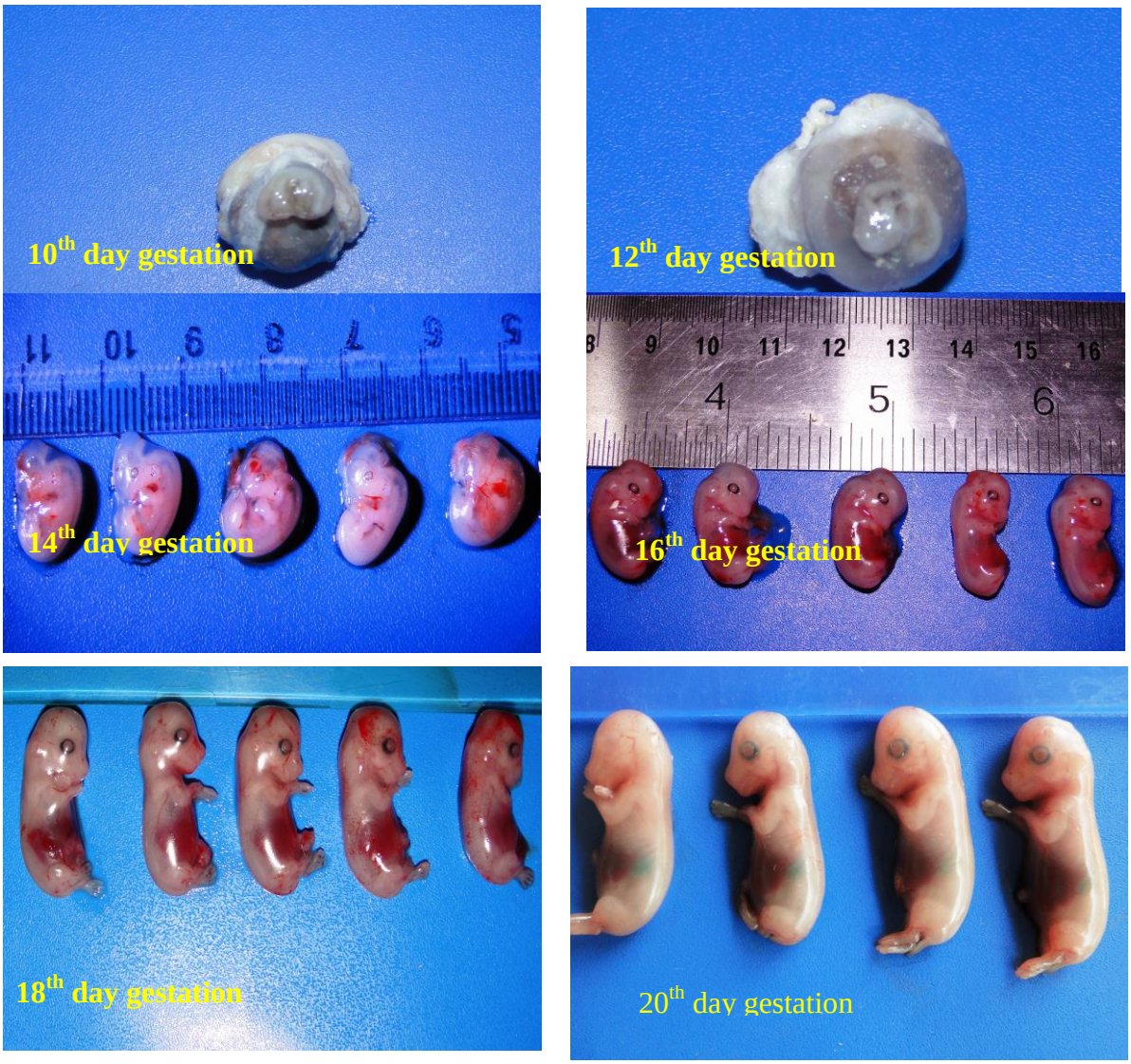


Figure (1): photograph illustrated the fetuses of rabbit in different stages of gestation period.

Results and discussion

The development of the rabbit heart pulsation starts during the 9th and 10th day of embryonic life, despite lacking valves and conduction system and this dos not agree with (10,11) who mentioned that the human embryonic heart starts its pumping action around the twenty-first day of development and approximately E7.25 in mouse. The precardiac mesoderm forms a primitive tubular heart, which starts beating at E8 in mouse (12) while cardic contractions begin at 18-19 days of gestation in dog and 35-38 hr of incubation in the chick (13) and the rhythmic pulsations within the ovine embryonic vesicle were first detected at Day18 or 19 after mating (14). The differences in onset of heart formation between species were due to variation in the length of gestation period between the different species including mice 20 days, rats 21 days, ovine 150 days, bovine 280 days, chicken 21 days, dogs 65 days and humans 266 days.

at 10th day of gestation, the fetal heart in rabbits appears as pair of endothelial tubes located ventral to hind brain and pharynx then become located progressively farther caudally as a result of growth of dorsal head and body structures that subsequently fuse to form a single heart tube that bulges into the pericardial cavity and is attached to the dorsal wall by a fold of tissue, the dorsal mesoderm (figure2,3). This is a derivative of foregut splanchnoplueric mesoderm. Eventually, the heart tube suspended in the pericardial cavity anchored cranially by the dorsal aorta and caudally by the vitelloumbilical veins. The heart tube at this stage becomes invested in a layer of myocardium as cardiac wall and a layer of a cellular matrix, the cardiac jelly, separates the myocardium and the endothelial heart tube. Thus, the heart is at this stage is composed of myocardial walls and the lumen being lined throughout by a layer of endocardial jelly with absent of epicardial layer and coronary blood vessels(figure 2,3,4). This is similar to that in human mentioned that embryonic heart muscle is a two-layered epithelium that not only lacks an epicardial covering but, additionally, lacks coronary blood vessels in early stages of heart development (15). Blood supply of heart during this period comes exclusively from the heart lumen whose endocardial lining is connected with the myocardium by a layer of cell-free extracellular matrix, called the cardiac jelly and this agrees with (15). In thepresent stage, also seen a development of pulmonary vein as endothelial evagination into the dorsal mesoderm(figure4) and this is similar to that in mammals(6,13,16)

at 12th day of gestation, the next stage of cardiogenesis is the transition from a straight tube to a looped heart. During looping of the heart, the primitive heart tube shows a series of regional dilations or primary cardiac chamber which are dubbed outflow tract, embryonic ventricle, embryonic atria, bulbus cordis and sinus venosus. The pervious compartments typically indicated as segments of the heart tube, represent key structures in the heart developing and heart located posterior to telencephalon and ventrally to tongue and thyroid anlage (figure 5).

The pervious result is not corresponding with (17) in mouse whom they mentioned that looping stage of heart starts at 8.5 days post-coitum and (18) who mentioned that the looping stage at HH11 in chicken while in human at 23 day of gestation according to (19). The heart tube undergoes bending and torsion due to narrow space inside the embryonic pericardial cavity and this is similar to that mentioned by (20)

The first signs of trabeculation could be found at 12th day of gestation as finger-like projections in the inner surface of the cardiac loop and the ventricular wall started to have a sponge-like appearance. The arrangement of the primitive trabeculation, which was the first morphological sign of ventricular differentiation and the tiny trabeculae, covere by a markedly thinner layer of a cellular jelly are formed in the ventricular wall and the distance between the endothelial cardiac tube and the myocardial mantle is very great throughout the whole extent of the heart anlagen (figure.5). This disagrees with (21) in chicken who mentioned that the first signs of trabeculation could be found at stage 16 as oval pits in the inner surface of the apical part of the cardiac loop.

At 14th day of gestation, By the stage of looping, the primary heart tube within the pericardial cavity can be divided into atria and ventricular components. The atrial and ventricular components are separated by the atrioventricular canal, which has significant length. The heart at this stage consists of left and right atrium and ventricle(figure.6). At the present stage, we see the evolution of both cushion of endocardium canal which gives arise to

aterioventricular valve and atrial septation when a crest of tissue grows from the posterior wall of the common atrium from a location cranial to the pulmonary vein orifice into the lumen which form the septum primum . The two limbs of this septum extend toward the endocardial cushions in the atrioventricular canal forming ostium-primum by the free edge of this septum .With further development, extensions of endocardial cushions grow along the edge of the septum primum, which later is closed by the non-muscular interatrium septum. Endocardial cushions, the precursors of the tricuspid and mitral valves are forming in the atrioventricular septum (figure 6) . On the other hand, our results were different from the facts of (22) who observed that in human heart, septation occurs between 4 and 7 weeks of development. These differences due to variation in pregnancy periods length among the animals, physiological and functional differences ..

Also, the beginning of the ventricular septation can be seen at these stages when the floor at the midline of the primitive ventricle produces the interventricular septum, separating the chamber in two portions gradually and grows from down towards the endocardial cushion represented anlagen of evaluation of this spetum (figure 7).

In the ventricle, sees not only an increase of volume in the myocardium, but also its further differentiation and finally the appearance of trabeculaei at the base of the common ventricular cavity as projecting elevations which started to change to long, thick bundles called trabeculae carneae, which gradually become more and more undermined, until finally, surrounded on all sides by the closely apposed endocardium, they traverse the ventricle more or less free (figure 6,7).

The endocardium consists of a layer of endothelial cells with large nuclei, while the myocardial mantle is composed of several layers of cells, which have more of a syncytial character, at least their boundaries are distinguishable only rarely and sporadically while third layer, epicardial are thin delicate epithelium layer on outer surface of heart. This disagrees with (23) who mentioned that in mouse embryos of 9.0 - 10.0 d.p.c the epicardial cells start to migrate from the pro-epicardial organ over the complete outer layer of the developing heart

At 16th day of gestation, The chambers and vessels are now aligned as in the adult heart and become fully integrated. The muscular inter-atrial and inter-ventricular septae fuse with the non-muscular atrioventricular septum, which is derived from the endocardial cushions of the atrioventricular canal, therefore completing the separation of the chambers and the well development of tricuspid valve (figure 8). Our findings demonstrate (in accordance with (24) and (25) in higher vertebrates) that the morphogenesis of intra-ventricular septum, intra-atrial septum and the development of myocardial architecture of the ventricles goes through several steps. Based on our current results and in agreement with pervious Scientists, we suggest three phases because, although in each one different events take place, only one or two basic processes (cell adhesion, cell proliferation, cell differentiation) are relevant.

The heart composed of three distinguished layers endocardium , myocardium and pericardium and abundant trabeculations were present throughout both ventricular cavities (figure 9). The current study shows that trabeculae change in orientation and patterning and this coincides with the described results reported by (26) in chicken , (16) in human and (13) in domestic mammals. This change in morphological characteristics of ventricular trabeculation plays a critical role in the contractile function of the heart during embryonic stage.

At 18th and 20th day of gestation, the atrio-ventricular and semilunar valves are well developed . The coronary vessels which surrounded the heart are visible. The left ventricle seems to have a thicker wall than the right and gerenally, the ventricle wall more thickness than the atrium (figure 10, 11) The heart in this stage consists of three well development cardic layares: pericardium, myocardium and endocardium. The pericardium is a fibroserous sac surrounds the heart and consists of fibrous layer and serous where later composed of parietal layer which lines fibrous layer and visceral layer which is also called the epicardium of the heart (figure 11). It is a single layer of mesothelium that envelops the heart and it is in continuity with the fibrous pericardium at the pericardial reflections at the great vessels. Between the two mesothelium of parietal and visceral layers, there is a potential space containing fluid that allows the surfaces to glide over one another(Figure 10,11,12). Myocardial blood supply via coronary blood vessels gradually replaces blood supply via myocardial sinusoids during the late embryonic and early fetal periods. This is accompanied by expansion of the outer compact layer of the ventricular myocardium and reduction of the inner spongy layer of the ventricular myocardium.

Table (1): shows crown rump length of embryo in different ages

parameter / Period	crown-rump length /mm
9	6.481±0.214 A
10	8.306 ± 0.125 A
12	10.381 ± 0.088 AB
14	12..008±0.216 B
16	18.350±0.244 C
18	25.981±0.209 D
20	34.576±0.279 E

Table (2): shows body weight of embryo in different ages

parameter / period	Weight of fetuses / gm
9	0.0400±0.003 A
10	0.0600 ± 0.002 A
12	0.1375 ± 0.004 A
14	0.283±0.020 A
16	0.537±0.027 A
18	1.107±0.053 A
20	3.156±0.077 B

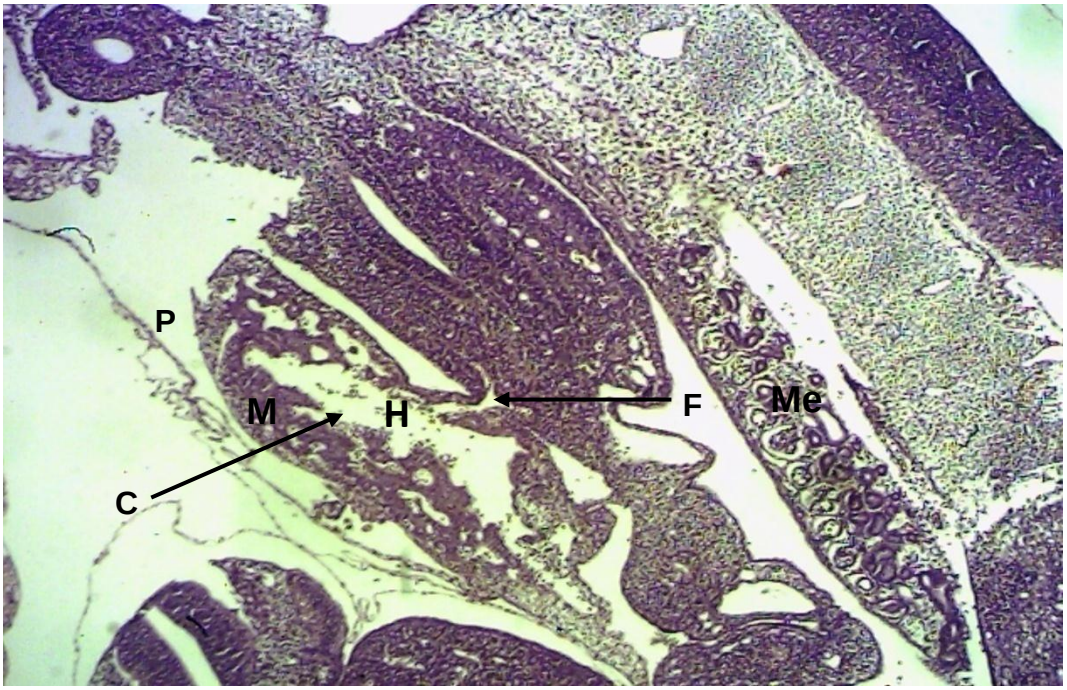


Figure (2): Parasagittal-section at 9th day of gestation in rabbit showing heart formation H- Heart tube P- pericardial cavity M-myocardium primordium C- cardiac jelly F- Fold of mesoderum. Me-Mesonephros (H&E. 4X)

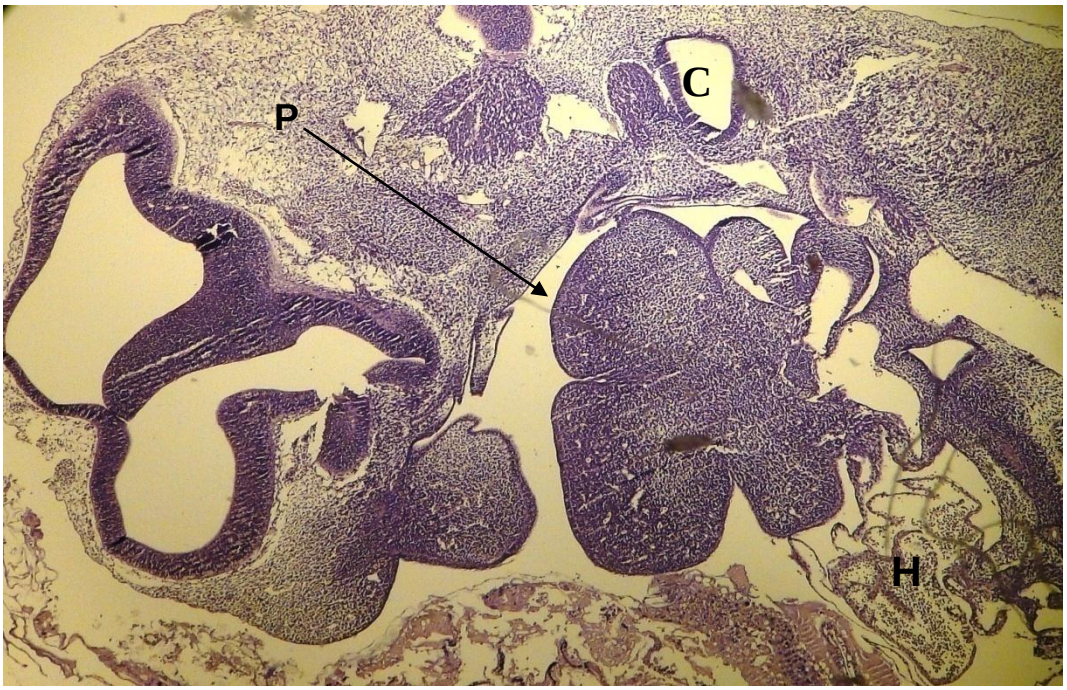


Figure (3) :Parasagittal-section at 10th day of gestation in rabbit showing H- Heart region P- pharynx C-hind brain (H&E. 4X)

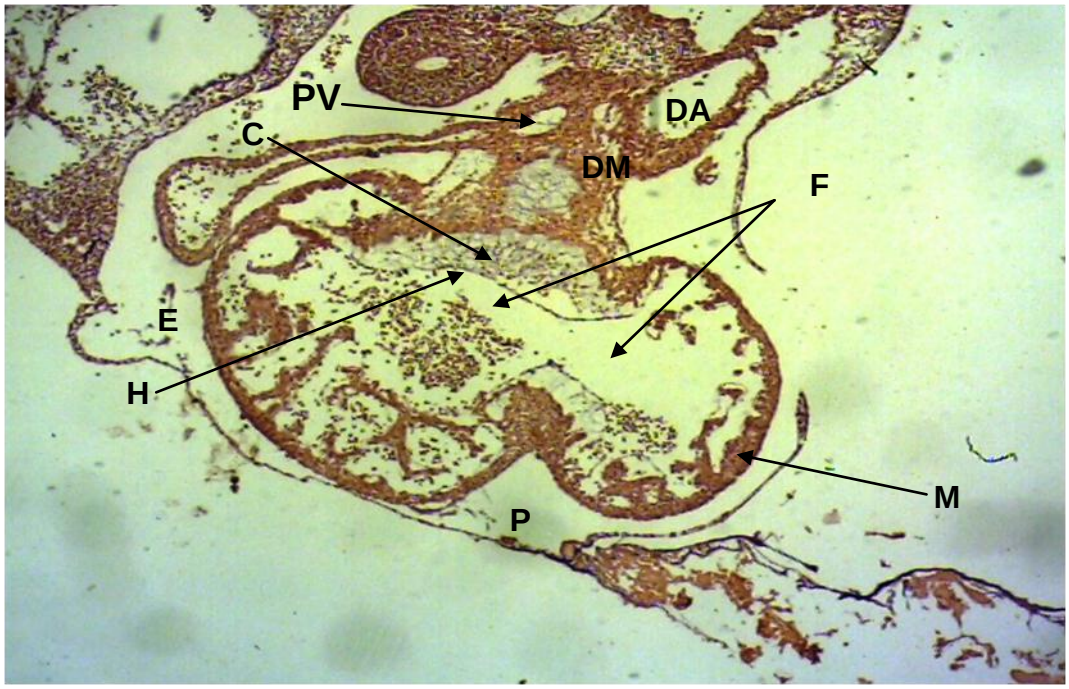


Figure (4): Cross-section of rabbit embryo at 10th day of gestation showing F- Fusion of pair endothelial tube C- cardiac jelly H- heart tube endothelial M- myocardial anlage P- pericardial cavity DM- dorsal mesoderum DA- dorsal aorta PV- Pulmonary vein (H&E.X 10)

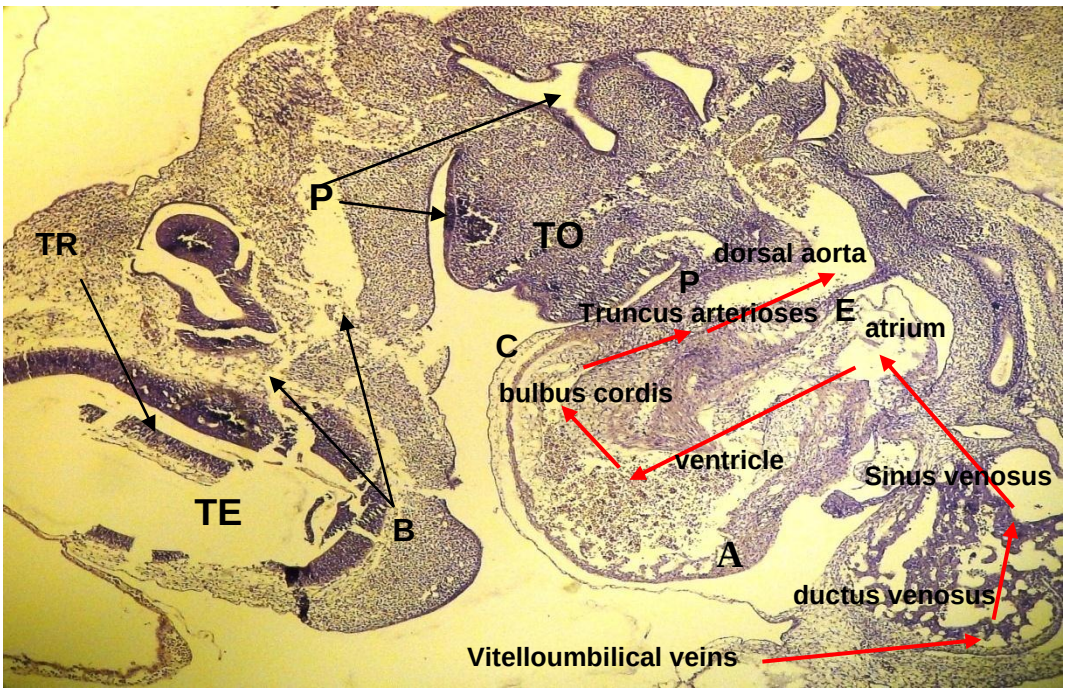
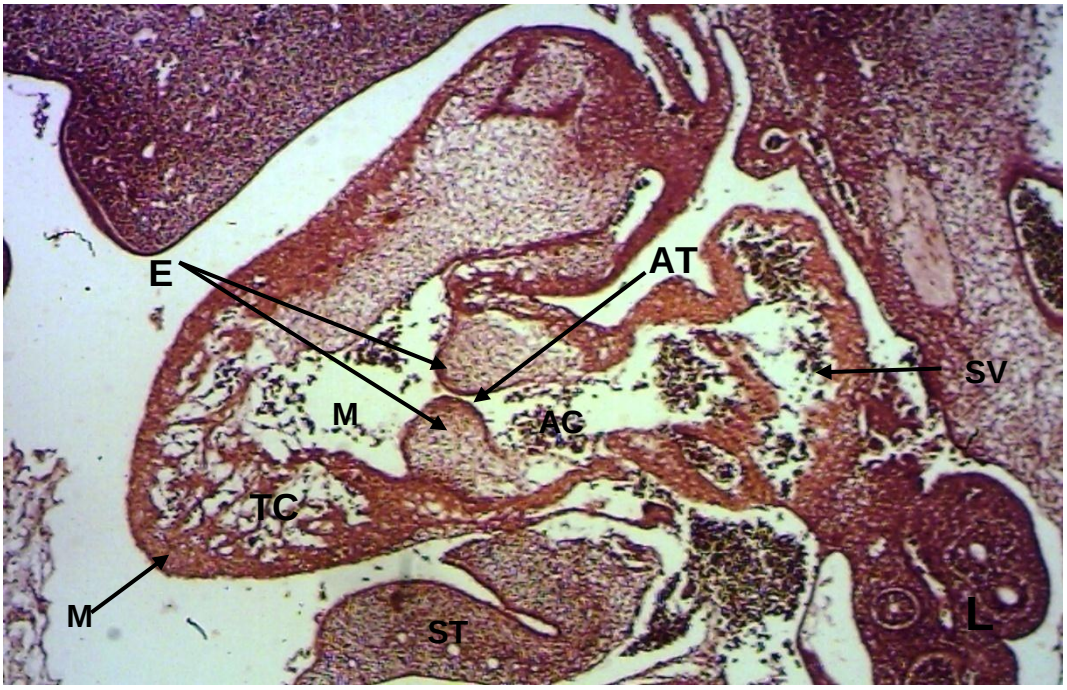


Figure (5): Parasagittal-section of rabbit embryo at 12th day of gestation showing TR- treabeaulaeof ventricles TE- telencephalon TO- tongue P- pharynx E- pericardial cavity F- atrium (H&E.X 10)



Figure(6): Parasagittal-section at 14th day of gestation in rabbit showing AC- aterial chamber SV- sinus venous L- lung primordium ST- septum transversum TC- trabeculae carneae of heart ventricle M- myocardial ventricular wall M- primitive ventricular chamber E- endocardial cushions AT- aterioventricular canal (H&E. 4X)

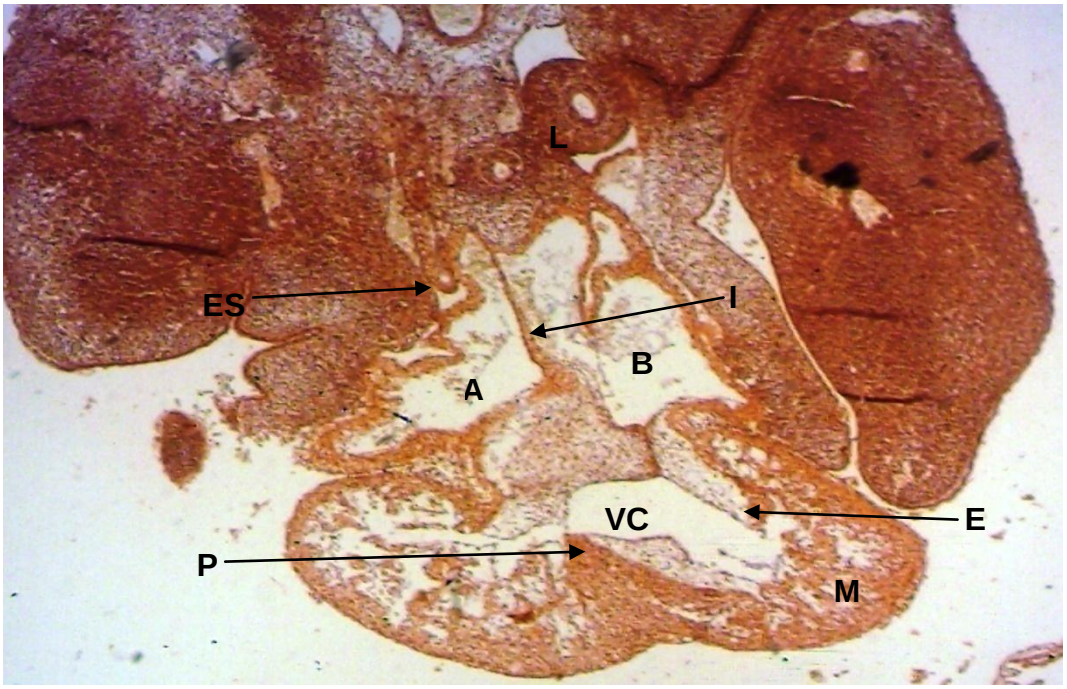


Figure (7): Cross-section of rabbit embryo at 14th day of gestation showing I- interaterial septum A- atrium E- endocardium M- myocardial ventricular wall VC- ventricular chamber P- primordium of interventricular septum ES- esophagus L- lung bud (H&E.X4)

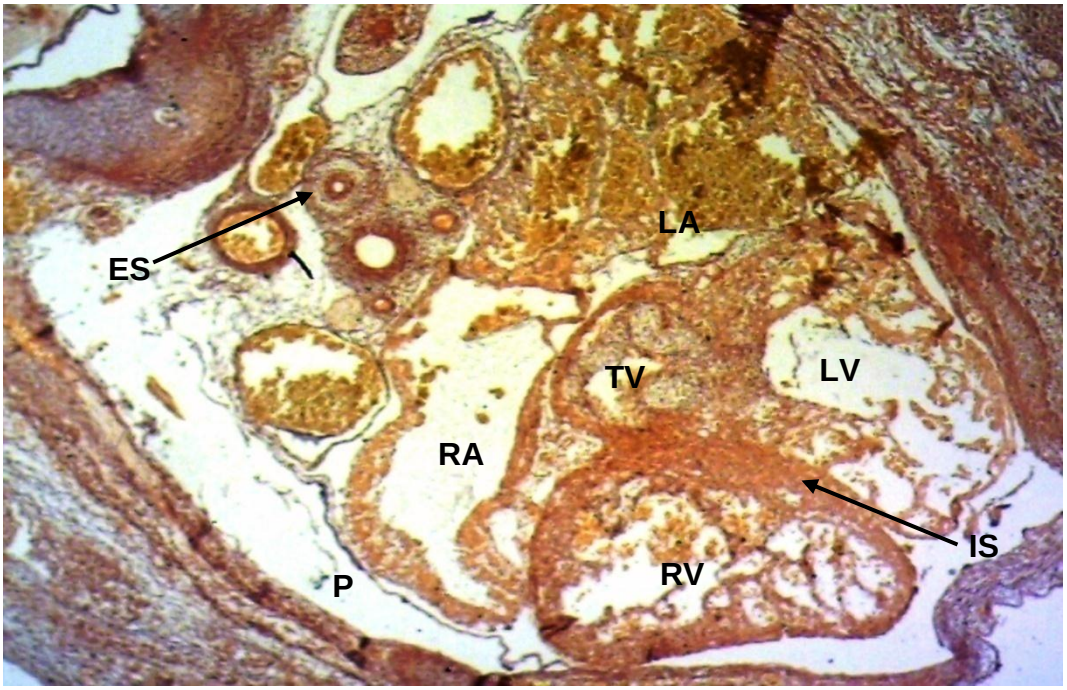


Figure (8): Cross-section of rabbit embryo at 16th day of gestation showing LA- left atrium LV- left ventricle IS- interventricular septum RV- right ventricle TV- tricuspid valve RA- right atrium P- pericardial cavity ES- esophagus (H&E.X4)

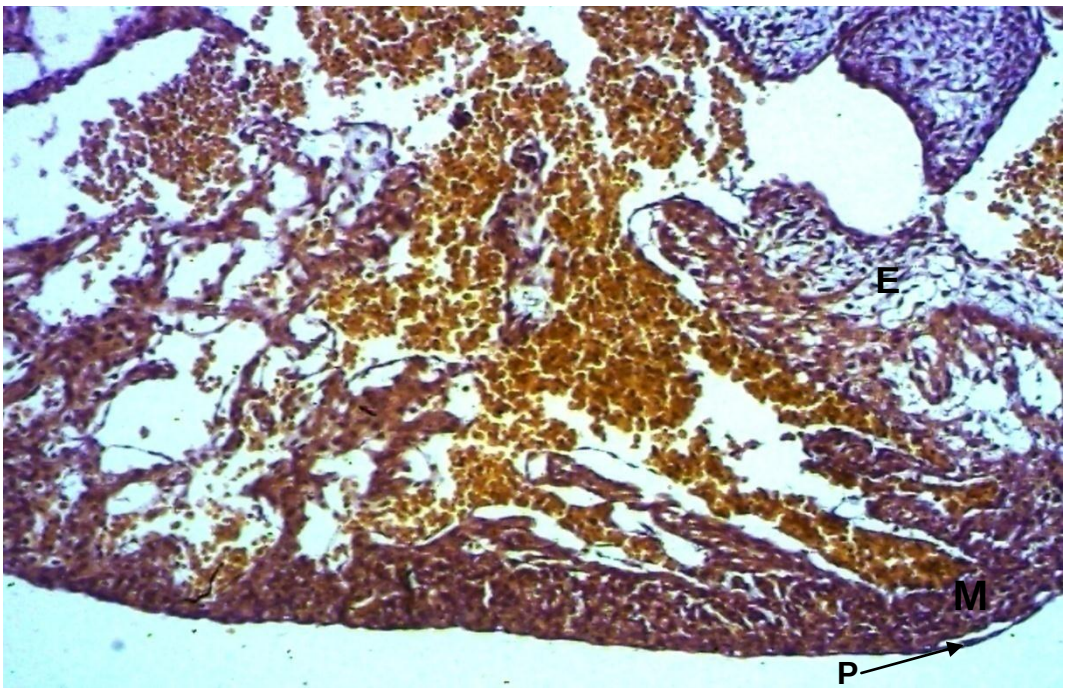


Figure (9): Parasagittal-section at 16th day of gestation in rabbit showing E- endocardial M- myocardium P- pericardium (H&E.10X)

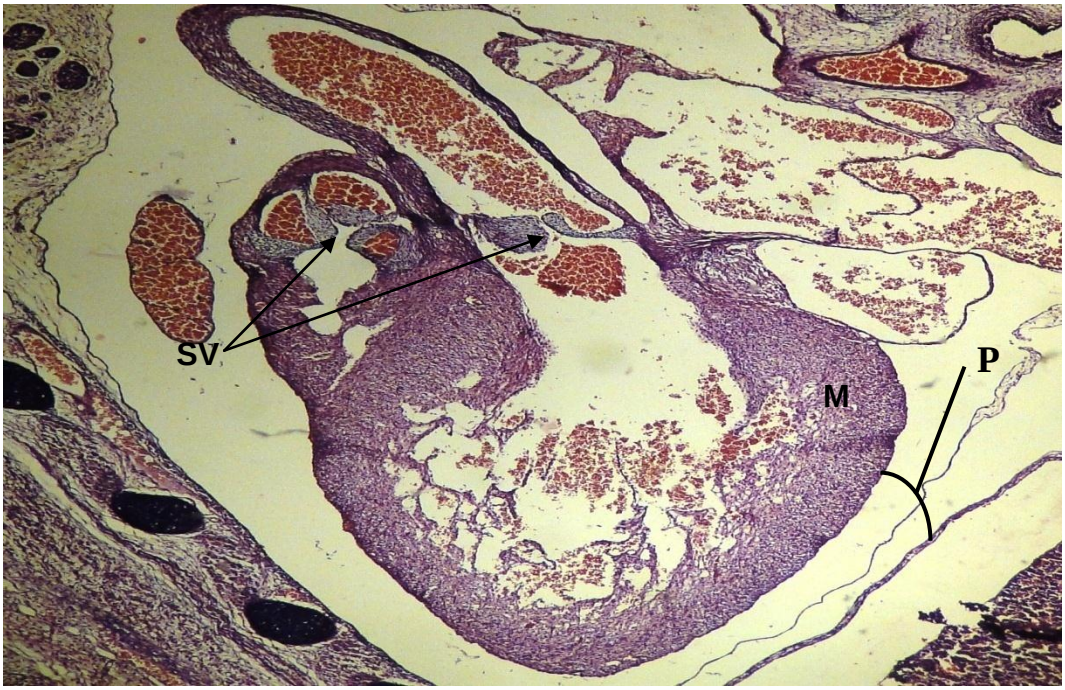


Figure (10): Parasagittal-section at 18th day of gestation in rabbit showing SV- semilunar valve M- myocardium P- pericardium (H&E.10X)

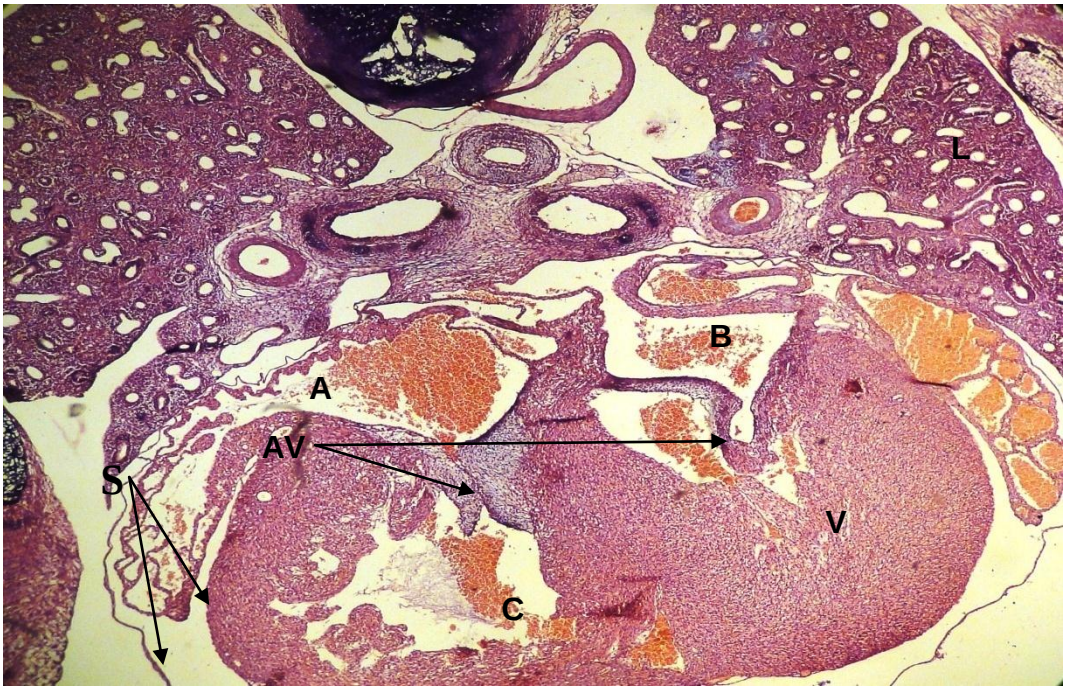
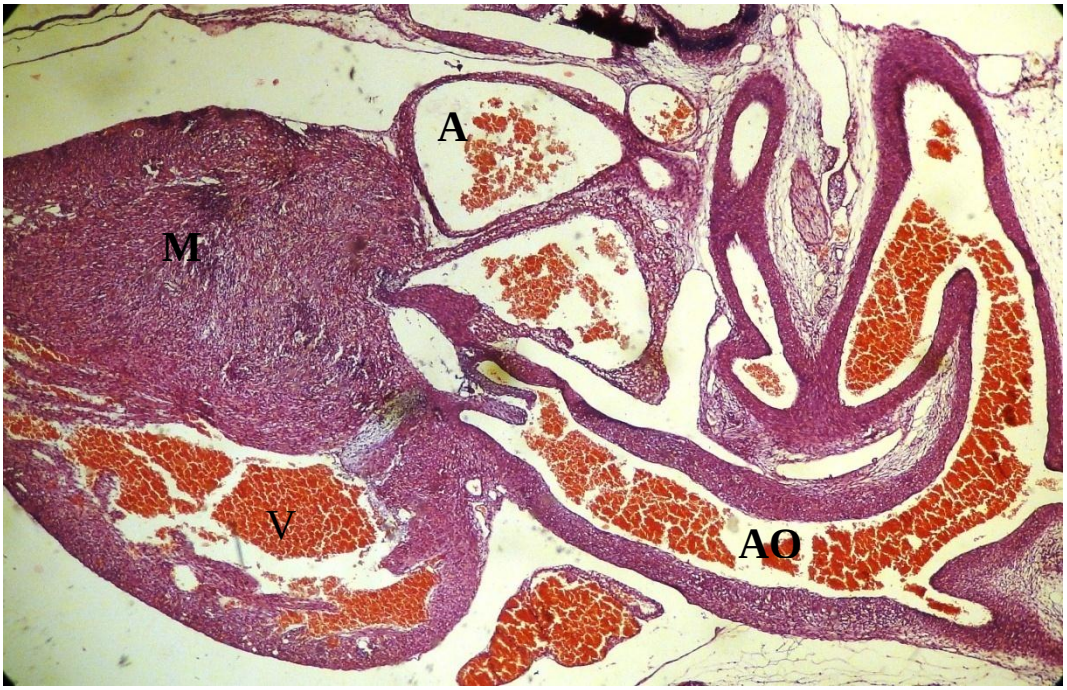


Figure (11) :Grosse-section at 20th day of gestation in rabbit showing AV- aterioventricular valve A- atrium V- ventricle L- lung S- serous layers of pericardium (H&E.4X)



Figure(12) :parasagittal-section at 20th day of gestation in rabbit showing A- atrium M- myocardium AO- aorta V- ventricle (H&E.4X)

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