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## Gross morphological changes in the prenatal development of heart in sheep (*Ovis aries*)

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### Abstract

The study on “Prenatal development of Heart in Sheep (*Ovis aries*)” was conducted in 30 sheep prenatal specimens (embryos and fetuses) ranging from 22 days to 146 days. The specimens were divided into three age groups viz., Group I (1-50 days), Group II (51-100 days) and Group III (101-152 days). The prenatal heart was well developed grossly at 53 days with sinus venarum, pectinate muscles, foramen ovale, chordae tendineae, papillary muscles and moderator bands. Chordae tendineae showed secondary and tertiary branching at 67 days.

**Keywords:** Gross morphology, heart, prenatal sheep (*Ovis aries*)

### 1. Introduction

The sheep (*Ovis aries*) is the first among the animals that has been used as a mammalian model of development with gestation period of 145-152 days. The circulatory system is the first functional unit in the developing embryo and the heart is the first functional organ (Gilbert and Barresi, 2016) [6]. The heart of sheep is similar to human heart in structure and function and is situated in the lower portion of the thoracic cavity projecting more to the left side. Prenatal development of heart has been studied in pig (Sathyamoorthy and Ramesh, 2008) [15], buffalo (Gupta 2014) [7], goat (Gupta, 2018) [8] and human (Nowak *et al.*, 2011) [13]. However, no attention is paid towards the prenatal development of heart of sheep in the literature cited. Hence, documentation of normal embryonic and foetal development of heart is necessary to understand consequences of harmful factors influencing at various stages of development. Therefore, the present study has been undertaken to fulfill the lacuna in the scientific literature in different age groups of prenatal sheep.

### 2. Materials and Methods

The study on “Prenatal development of Heart in Sheep (*Ovis aries*)” was conducted on 30 sheep prenatal specimens ranging from 22 days (0.9 cm CRL) to 146 days (42.3 cm CRL) in the Department of Veterinary Anatomy, College of Veterinary Science, Tirupati.

In the present study, the prenatal specimens (embryos and fetuses) of unknown age and irrespective of the sex were collected from pregnant uteri of sheep slaughtered at various slaughter houses located in and around Tirupati. The earliest aged specimens were collected by using magnifying lens and flushing of uterine horns. The CRL was measured from the most anterior part of the crown to the base of the tail (Chandrasekhara Rao and Jagapathi Ramayya, 2013) [2]. The accurate age estimation of the embryos and fetuses was determined on the basis of their CRL. Up to 3cm CRL, age of the embryos was identified as per the tabulation postulated by Bryden *et al.*, 1972 [1] and for later ages i.e beyond 3.0 cm CRL the age was estimated by using the formula  $X = 2.74 Y + 30.15$ , where X was the age of foetus and Y was the crown rump length of foetus in cm (Hejazi *et al.*, 2011) [10]. The specimens were divided into three age groups viz., Group I (1- 50 days), Group II (51-100 days) and Group III (101-152 days). In Group II and Group III i.e., beyond 50 days, the hearts of fetuses were collected and were subjected for gross morphological study.

### 3. Results and Discussion

#### 3.1 External Morphology

In group I, the heart size was very small. It was very difficult to dissect and distinguish the gross morphological features. So, the specimens were used only for histomorphological study. In all the fetuses of group II, the heart was narrow cone shaped and located in the

mediastinum more towards the left side of the midline in the cranio-ventral part of the thoracic cavity in front of the diaphragm. The heart was extended from 2nd intercostal space to 5th intercostal space (Fig.1). But in cattle foetuses of 15 – 25 weeks age, Gurbuz *et al.*, (2019) [9] reported that the heart was placed on the left side of the middle mediastinum from 3rd to 5th intercostal space, which agreed almost the present observation in sheep foetuses.

Whereas, in group III, the position of foetal heart was slightly shifted towards caudal side when compared to group II and it was extended from 3rd to 6th intercostal space (Fig.2). Similar change in the position of the heart was also noticed by Gupta (2014) [7] in buffalo foetuses. The change in the position of heart with the progression of age of the foetuses may be due to rapid growth of cervical region in the foetal period.

The foetal heart in sheep was surrounded by fibroserous pericardium and it was tough, semi-transparent in group II. In group III, pericardium became more fibrous and tougher than that of group II. In group III, the sternopericardial ligament was well developed, more fibrous and broader towards the heart. In contrary to this, in 15-25 weeks old cattle foetuses, apex of the heart was found to be connected to the diaphragm by phrenicopericardiac ligament by Gurbuz *et al.*, (2019) [9].

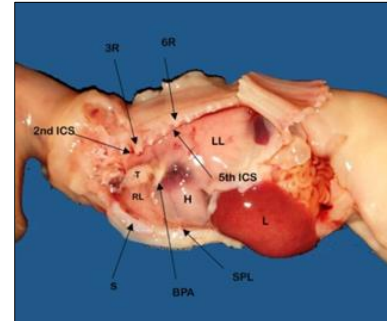
The base of heart extended from 2nd intercostal space to 5th intercostal space, whereas the apex was situated dorsal to sternum at the level of 6th rib in group II. In group III, the base of heart was shifted slightly to the level of 3rd intercostal space on the cranial side but the apex of heart was laid in same position i.e. at 6th rib close to the diaphragm. Whereas, the position of the apex of heart was reported at sixth chondrosternal joint in front of the diaphragm in goat foetuses of by Gupta (2018) [8].

In both group II and III foetal hearts of present study, the conus arteriosus was very much enlarged and prominent and it was situated at the level of 3rd rib. In group II, All the four chambers of heart were established and well developed. The coronary and longitudinal grooves were formed externally at 83 days. The intermediate groove was not visible clearly. Whereas, in group III foetal hearts, the coronary and longitudinal grooves were prominent well-marked and became deeper at 130 days. Intermediate groove was also clearly noticed first on the wall of the left ventricle in oblique direction at 130 days. Similarly, Gupta (2014) [7] reported the development of left, right and intermediate grooves from 32 – 116 days in buffalo and he noticed that they became prominent from 121-298 days.

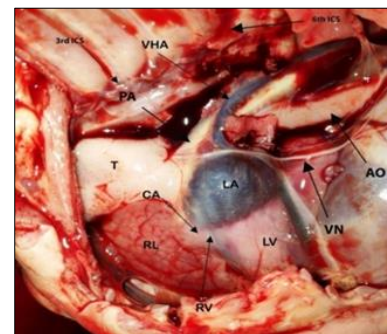
The left side of the developing heart i.e., part of the left and right ventricle as well as left atrium in both group II and III were in direct contact with left thoracic wall at the deep notch formed between apical lobe of right lung, cardiac and diaphragmatic lobes of left lung (Fig.3), whereas on the right side it was surrounded completely by the different lobes of right lung except at the level of right atrium and base of the right ventricle (Fig.4).

In both group II and group III, the cranial border of the heart was more convex. The cranial border was formed partly by right ventricle and it was covered completely by the apical lobe of the right lung. The caudal border was slightly convex above and nearly straight below (Fig.5) and formed completely by the left ventricle. Similarly, the caudal border of heart was reported to be shorter, thicker and slightly concave or nearly straight by Malik *et al.*, (1972) [12] in adult buffalo.

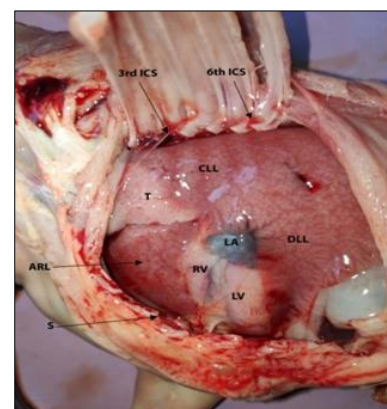
In group II, both right and left atrial walls were very thin and transparent but appeared dark brown in colour due to the presence of blood clots inside their cavities. Left atrium was larger in size, but paler when compared to the right atrium in group II. Whereas in group III, size of both the right and left atria was increased and they were well developed. The anterior and posterior venacavae were formed and appeared transparent and opened into right atrium.



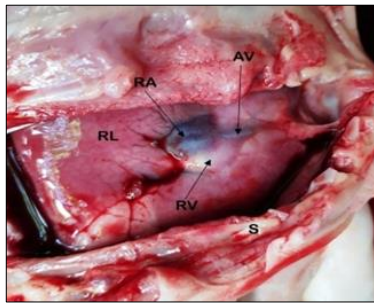
**Fig 1:** Photograph showing different parts of heart and its relation with other organs in thoracic cavity of 68 days foetus in sheep. L – Liver; 2nd ICS – 2nd Intercostal space; H – Heart; 5th ICS – 5th Intercostal space; LL – Left lung; 3R – 3rd Rib; RL – Right lung; 6R – 6th Rib; T – Thymus; SPL – Sternopericardial ligament; BPA – Base of pulmonary artery; S – Sternum



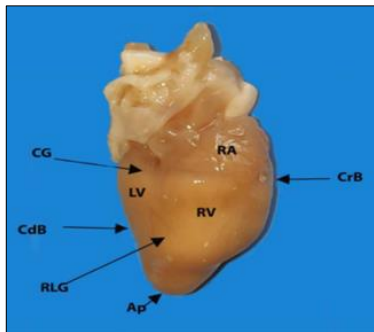
**Fig 2:** Photograph showing left side of heart and its relation with other organs in thoracic cavity at 107 days of sheep foetus. 3rd ICS – 3rd Intercostal space; LV – Left ventricle; 6th ICS – 6th Intercostal space; AO – Aorta; T – Thymus; VN – Vagus nerve; RL – Right lung; PA – Pulmonary artery; LA – Left atrium; RV – Right Ventricle; CA – Conus arteriosus; VHA – Vena hemiazygos



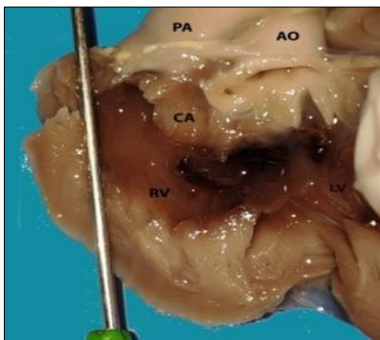
**Fig 3:** Photograph showing left side of heart and its relation with lungs in 135 days sheep foetus. LA – Left atrium; DLL – Diaphragmatic lobe of left lung; LV – Left ventricle; CLL – Cardiac lobe of left lung; RV – Right ventricle; 3rd ICS – 3rd Intercostal space; ARL – Apical lobe of right lung; 6th ICS – 6th Intercostal space; T – Thorax; S – Sternum



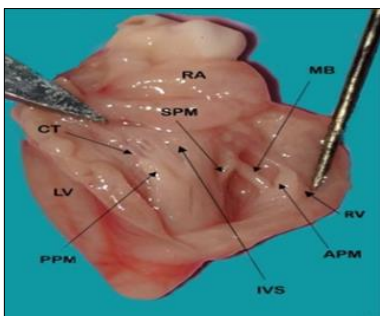
**Fig 4:** Photograph showing right side of heart and its relation with different lobes of lungs in thoracic cavity in 130 days sheep foetus. RA – Right atrium; AV – Anterior venacava; RV – Right ventricle; S – Sternum; RL – Right lung



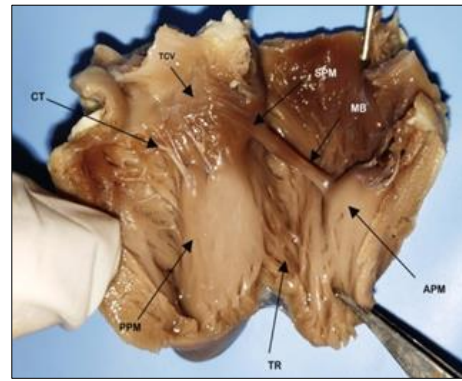
**Fig 5:** Photograph showing different chambers, borders and surfaces of sheep heart of 83 days foetus. LV – Left ventricle; RLG – Right longitudinal groove; RV – Right ventricle; CrB – Cranial Border; RA – Right atrium; CdB – Caudal Border; CG – Coronary groove; Ap – Apex of heart



**Fig 6:** Photograph showing the opening of pulmonary artery and aorta in heart of 83 days sheep foetus. RV – Right ventricle; LV – Left ventricle; CA – Conus arteriosus; PA – Pulmonary artery; AO – Aorta



**Fig 7:** Photograph showing internal structures of right ventricle of heart in 83 days foetus of sheep. IVS – Interventricular septum; PPM – Posterior Papillary muscle; MB – Moderator band; CT – Chordae tendineae; APM – Anterior Papillary muscle; RA – Right atrium; SPM – Septal Papillary muscle; LV – Left Ventricle; RV – Right ventricle



**Fig 8:** Photograph showing interior of right ventricle of heart in 141 days sheep foetus. TCV – Tricuspid valve; APM – Anterior Papillary muscle; CT – Chordae tendineae; SPM – Septal Papillary muscle; MB – Moderator band; PPM – Posterior Papillary muscle; TR – Trabeculae

### 3.2 Internal morphology

All the four chambers of heart i.e. atria and ventricles were well developed from 53 day onwards i.e., in group II. At 53 days Sinus venarum and auricle of right atrium were formed. The pectinate muscles appeared in both atria. The number of musculi pectinati were increased and extended across the atrial cavity with advancement of age in group II. Various internal structures in all the all the chambers of the foetal heart were developed and distinct from 67 day onwards. In all the ages group III, the musculi pectinati were well developed and increased in size and terminated on crista terminalis. Similar findings were also reported by Gupta (2014) [7] during the foetal life of buffalo

In group II, interatrial septum was membranous and incompletely developed at 53, 56 and 64 days. The openings of coronary sinus, anterior and posterior venacavae into right atrium as well as pulmonary veins into left atrium were well established at 53, 56 and 64 days. Similarly, Gupta (2014) [8] also reported the anterior and posterior venacavae and coronary sinus openings into the sinus venarum of the right atrium in buffalo foetal heart.

The interatrial septum was membranous and contained small and rounded foramen ovale at its base at 53 days. Similarly, Ottaway (1944) [14] also found the position of foramen ovale in interatrial wall in bovines and equines. He reported that it was more oval in shape and laid immediately to the left of caudal to crista dividens in the right atrium. The foramen ovale was found to be in the form of short tunnel in carnivores by Macdonald and Johnstone (1995) [11].

The right and left atrioventricular orifices were guarded with tricuspid and bicuspid valves respectively. The cusps of bicuspid valve were thin and membranous, while cusps of tricuspid valve were thicker and more fibrous than that of bicuspid valve and they were bulged at their free ends. Interventricular septal wall was formed first and directed obliquely towards the right ventricle at 53, 56 and 64 days. In both group II and III, left atrioventricular orifice was appeared smaller than the right atrioventricular orifice.

In all the ages of group III, cusps of tricuspid valve were formed, of which one cusp was situated between atrioventricular opening and conus arteriosus, one on the septal wall, and the third one was related to the lateral wall of ventricle (Fig.6). The cusps of bicuspid valve appeared larger and thicker than those of tricuspid valve which may be due to increased fibrous component.

In group II, the internal wall of ventricles was irregular and

showed muscular ridges of different sizes. Primitive muscular ridges of both right and left ventricles were developed. In group III, muscular ridges became prominent in left ventricle than that of right ventricle. Chordae tendineae were transparent, but fewer in number and appeared first in group II at 53 days. However, Gupta (2014) [7] reported the formation of chordae tendineae first at 68 day aged foetus in buffalo. In the present study, the chordae tendineae were larger in size and they were extended between the bulged apices of cusps of atrioventricular valve and apices of papillary muscles. At 67 and 73 days, chordae tendineae showed secondary and tertiary branches. Further at 83, 86, 87 and 98 days, the chordae tendineae arised from apices of papillary muscles were increased in number (Fig.7). In all the ages of group III, chordae tendineae were thin and long in left ventricle, whereas in right ventricle they were short and thick. Right ventricle showed three papillary muscles at 53 days viz., anterior, posterior and septal papillary muscles. Anterior and posterior (larger) papillary muscles were situated on the ventricular wall, whereas septal (smaller) papillary muscle was placed on the interventricular septum. Whereas in group II, left ventricle showed only two papillary muscles i.e., antero-lateral and postero-medial papillary muscles from 53 days onwards. In contrary to this, Gupta (2014) [7] reported the first appearance of papillary muscles at 68 days aged foetus in buffalo.

The trabeculae carnae were well developed in left ventricle than that of right ventricle. In both the ventricles, trabeculae carnae were more prominent in all ages of group III than that of group II.

Moderator band was noticed first in the right ventricle at 53

days. It was rod like, unbranched and observed across the lumen in the oblique direction from the base of the septal papillary muscle to base of anterior papillary muscle. Contrary to the above findings, Gupta (2018) [8] reported the formation of moderator band in both right and left ventricle of prenatal goat. The moderator band appeared flattened ribbon-like band with thick central portion and tapered ends from 83 days. But in all ages of group III, moderator band was thicker and cylindrical shaped in right ventricle (Fig.8).

The aortic opening was guarded by semilunar valve with three cusps and it was noticed clearly at 53 days. However, in 67 and 73 day foetal hearts, thickness of wall of aorta was increased but its lumen was narrower when compared to previous ages. An increased thickness of the aortic wall may be attributed to initiation in the functioning of systemic circulation.

There was an increase in average thickness of right and left atrial walls, right and left ventricular walls, interatrial septum and interventricular septum of foetal hearts gradually from group II to group III (Table.1). Increase in thickness of various above parameters was due to rapid increase in proliferation of myocardial cells during the development.

There was a significant increase in all the external and internal biometric parameters was noticed from group II to group III which include weight of heart, length of heart, width of heart, circumference of heart, length of left and right ventricles, length of left and right atria, length of cranial and caudal borders, thickness of left and right atrial walls, thicknesses of left and right ventricle walls, thicknesses of interatrial and interventricular septa (Table.1).

**Table 1:** Various external and internal biometric parameters of foetal heart in sheep

S.No	Parameter	Group - II	Group - III
1.	Average weight of heart (g)	2.133 ± 0.584	10.694 ± 1.182
2.	Average length of heart (mm)	19.00 ± 2.078	42.464 ± 1.179
3.	Average width of heart (mm)	14.865 ± 1.470	33.273 ± 1.197
4.	Average circumference of heart (mm)	35.722 ± 3.430	75.164 ± 2.481
5.	Average length of left ventricle (mm)	13.944 ± 1.663	30.082 ± 0.853
6.	Average length of right ventricle (mm)	10.788 ± 1.289	23.836 ± 0.620
7.	Average length of right atrium (mm)	4.374 ± 0.360	8.587 ± 0.378
8.	Average length of left atrium (mm)	6.268 ± 0.447	10.955 ± 0.412
9.	Average length of cranial border (mm)	15.907 ± 1.776	33.322 ± 1.420
10.	Average length of caudal border (mm)	13.398 ± 1.419	25.542 ± 0.889
11.	Average thickness of right atrium wall (mm)	0.554 ± 0.029	0.922 ± 0.026
12.	Average thickness of left atrium wall (mm)	0.617 ± 0.037	1.265 ± 0.064
13.	Average thickness of left ventricle wall (mm)	2.155 ± 0.145	3.959 ± 0.227
14.	Average thickness of right ventricle wall (mm)	1.308 ± 0.140	3.133 ± 0.220
15.	Average thickness of interatrial septum (mm)	0.558 ± 0.066	1.098 ± 0.035
16.	Average thickness of interventricular septum (mm)	2.003 ± 0.145	3.550 ± 0.093

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