HISTOLOGICAL, HISTOCHEMICAL AND MICROMETRIC STUDIES ON THE EMBRYONIC GALL BLADDER OF GADDI SHEEP IN DIFFERENT PRENATAL AGE GROUPS

RAZVI, R., RAJPUT, R. AND SHARMA, R.

Department of Veterinary Anatomy, Dr. G. C. Negi College of Veterinary and Animal Science, CSK.HP.KV-Palampur176061, Himachal Pradesh.
E. mail: doctorrahika356@gmail.com, Cell: 9622284455

Received: November 5, 2016; Accepted: November 20, 2016

Abstract: The objective in the present study was to explore the histological, histochemical and micrometric changes of developing gall bladder during the fetal life of Gaddi sheep. A total number of 40 Gaddi sheep foetii samples were collected irrespective of sex and without any external anomaly or pathology. The age estimation of each foetus was done and accordingly the foetii were grouped as; group-I (1-60days), group-II (61-90days) group-III (91-120days) and group-IV (121 –full term). The gall bladder from each foetii were excised hygienically. Gall bladder appeared at CRL 6.0 cm (46 days of gestational age). At CRL 11.0 cm (60 days of gestational age) the tunica mucosa, tunica submucosa, tunica muscularis and tunica serosa layers were clearly distinguishable. The lamina muscularis was absent in prenatal gall bladder in all stages of gestational age. The thickness of tunica muscularis, tunica serosa and luminal diameter of gall bladder also increased significantly with the advancement of gestational age. The non-ciliated simple columnar epithelium cells were covered by the discontinuous to continuous striated border of microvilli at CRL 24.5 cm (97 days of gestation) which became continuous at CRL 42.5 cm (146 days of gestational age). Strong reaction for proteins was observed in all layers of gall bladder at CRL 32.0 cm (117 days of gestational age). Strong PAS positive reaction was observed by tunica mucosa layer of gall bladder while the rest of its layers showed moderate reaction for PAS near full term. The reaction for proteins was mild at early stages development of gall bladder, which became strongest at CRL 32.0 (117 days of gestational age) in all layers of gall bladder.

Key words: Gall bladder, Gaddi sheep

INTRODUCTION

Gall bladder is important part of biliary system. It is a hollow pear-shaped sac which acts as a reservoir and concentrates the bile. In living body it is slate blue in colour, lies in a non-peritoneal fossa on the inferior surface of the right lobe of the liver [1]. The wall of the gallbladder consists of (from inner to outer), i) a highly folded mucosa with a simple columnar epithelium & lamina propria, ii) a thin fibromuscular layer, iii) perimuscular connective tissue layer and iv) an external adventitia or serosa [2].

Gallbladder diseases are diagnosed clinically and confirmed by various non-invasive as well as invasive procedures and wall thickness is the most important indicator to diagnose such diseases. Therefore full knowledge about histomorphological and micrometric features of thickness of the gallbladder wall has hard fast implication for the investigation, diagnosis and further management.
procedure. The present study was performed to find histomorphological, histochemical and micrometric feature and age related changes of the developing gallbladder wall in Gaddi sheep.

**MATERIAL AND METHODS**

All procedures were conducted in accordance with guide lines for animal experimentation of the faculty of veterinary science, department of Veterinary Anatomy, Dr.G.C.Negi, College of Veterinary and Animal Science, CSK.HP.KV. Palampur, Himachal Pradesh (India).

Sample collection and age estimation: Gravid uteri of Gaddi sheep were collected from and around local slaughter houses of Palampur region of Himachal Pradesh. Uteri then immediately opened to obtain fetus, which were washed properly and age of fetus was calculated. CRL (crown rump length) of foetii were recorded for age estimation by using the following given by Hijazi [3]:

\[ Y = 2.74X + 30.15 \]  
Where, \( Y \) = age of fetus in day,  
\( X \) = Crown Rump Length in cm

The foetii were grouped as; Group-I (1-60 days), Goup-II (61-90 days) Group-III (91-120 days) and Group-IV (121 –full term). Each group contained ten samples. Liver was then excised to expose the gall bladder. The gall bladder so exposed was detached from liver.

Histological and histochemical studies of foetal gall bladder: The tissue samples were taken from respective gall bladder samples and were immediately fixed in neutral buffered formalin for 48-72 hours for light microscopy. After fixation the samples were processed following alcohol-benzene schedule and paraffin embedding was done. The sections of 5-6μm were cut and mounted onto glass slides and then utilized for routine and special histological and histochemical staining techniques [4]. The stains done were; Haematoxylin and Eosin stain (H&E), Gomori’s method, Masson’s Trichrome, Periodic Acid Schiff’s stain, Bromophenol blue and Alcian Blue.

Micrometry: The detailed micrometric data was collected on gall bladder in different age groups of prenatal Gaddi sheep with the help of calibrated ocular micrometer. Measurements like thickness of mucosa layer, submucosa layer, muscular layer and serosa layer and luminal diameter of gall bladder were measured in each group.

Statistical analysis: All gross and micrometric data of embryonic gall bladder were analyzed by using Univariate ANOVA at 5% level of significance. Multiple comparison tests were used to compare the difference between the groups. All analysis was done by using SPSS-17. Statistical calculations (mean ± standard error) were recorded according to the standard statistical procedures recommended by Snedecor and Cochran [5].

**RESULTS**

Histological and histochemical studies: Group-I (1-60 days of age): The gall bladder appeared at CRL 6.0 cm (46 days of gestation). Histologically, different layers of wall of gall bladder were not properly developed at this stage (Figure 1). The mucosal layer was at developing stage without any formation of mucosal villi. Muscularis layer was not also differentiated till this stage. The layers of gall bladder were more developed at CRL 8.5 cm (53

---

Figures 1 to 8 are histological preparation of different stages of gall bladder development of Gaddi sheep stained with different stains

- **Fig. 1:** Photomicrograph showing structure of gall bladder at CRL 6.0 cm (46 days of gestation). 400X.
- **Fig. 2:** Photomicrograph showing different layers of gall bladder at CRL 11.0 cm (60 days of gestation). 400X.
- **Fig. 3:** Photomicrograph showing different layers of gall bladder at CRL 19.8 cm (84 days of gestation). 200X.
- **Fig. 4:** Photomicrograph showing the reticular fibres (arrow) in different layers of gall bladder at CRL 21.4 cm (88 days of gestation). Gomori’s stain, 200X.
- **Fig. 5:** Photomicrograph showing the invaginations (arrows) of the surface epithelium at CRL 28.5 cm (108 days of gestation). 200X.
- **Fig. 6:** Photomicrograph showing lamina epithelial and lamina propria layer of tunica mucosa at CRL 33.5 cm (121 days of gestation). 400X.
- **Fig. 7:** Photomicrograph showing continuous striated border (arrows) of tunica mucosa layer at CRL 33.5 cm (121 days of gestation). 200X.
- **Fig. 8:** Photomicrograph showing the collagen fibers (arrow) in tunica muscularis layer at CRL 42.5 cm (146 days of gestation). Masson’s trichrome, 200X.

TS = Tunica serosa, TM = Tunica mucosa, TSU = Tunica submucosa, LE = L. Epithelia, LP = propria.
days of gestation) whereas at CRL 11.0 cm (60 days of gestation) these layers became distinguishable. The tunica mucosa, tunica submucosa, tunica muscularis and tunica serosa layers were clearly distinguishable at this stage than the previous stages (Figure 2). Formation of mucosal villi also started at this stage. Moderate PAS and alcian blue positive reaction was observed in all layers of gall bladder at this stage.

Group-II (61-90 days of age): In this group the layers of gall bladder were better developed than the previous stages. The tunica mucosa of the gall bladder was mainly lined by non-ciliated simple columnar epithelium at CRL 19.8 cm (84 days of gestation). However, in some regions it varied from tall columnar to stratified columnar epithelium. These cells were covered by a discontinuous to discontinuous striated border of microvilli. The gall bladder musculature comprised of irregularly arranged muscular fibers and these fibers were discontinuous (Figure 3). The distribution of reticular fibres was more near luminal border than other layers of gall bladder (Figure 4) at CRL 21.4 cm (88 days of gestation). The invaginations of the surface epithelium were observed to have grown down deep than the previous stages at CRL 24.5 cm (97 days of gestation). The luminal wall of gall bladder had continuous undulated string like pattern of reticular fibers. Moderate PAS positive reaction was exhibited by all layers of gall bladder at CRL 31.3 cm (115 days of gestation). The collagen fibers were observed in tunica muscularis layers while these fibers were not seen in other layers of gall bladder at CRL 32.0 cm (117 days of gestation). These fibers were well developed and thick at this stage than the previous stages. The strong reactions for proteins was observed in all layers of gall bladder at CRL 32.0 cm (117 days of gestation) except in tunica serosa where the reaction for proteins was moderate. Mild reaction for acidic mucopolysaccharides was observed in tunica submucosa and tunica muscularis layer of gall bladder at CRL 22.0 cm (90 days of gestation).

Group-III (91-120 days of age): The tunica mucosal layer was better developed than the previous stages. The non-ciliated simple columnar epithelial cells were covered by the discontinuous to continuous striated border of moccovilli at CRL 24.5 cm (97 days of gestation). The invaginations of the surface epithelium were observed to have grown down deep than the previous stages at CRL 28.5 cm (108 days of gestation) (Figure 5). In this group the distribution of reticular fibres was more pronounced than the previous stages. The quantity of these fibers was more in tunica muscularis layer than other layers of gall bladder at CRL 31.3 cm (115 days of gestation). The luminal diameter of gall bladder had continuous undulated string like pattern of reticular fibers. Moderate PAS positive reaction was exhibited by all layers of gall bladder at CRL 31.3 cm (115 days of gestation). The collagen fibers were observed in tunica muscularis layers while these fibers were not seen in other layers of gall bladder at CRL 32.0 cm (117 days of gestation). These fibers were well developed and thick at this stage than the previous stages. The strong reactions for proteins was observed in all layers of gall bladder at CRL 32.0 cm (117 days of gestation) except in tunica serosa where the reaction for proteins was moderate. Mild reaction for acidic mucopolysaccharides was observed in tunica submucosa and tunica muscularis layer as in group-II.

Group-IV (120- full term): The invaginations of the

<table>
<thead>
<tr>
<th>Parameters (µm)</th>
<th>Group-I (1-60 days)</th>
<th>Group-II (61-90 days)</th>
<th>Group-III (91-120 days)</th>
<th>Group-IV (120- full term)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thickness of tunica mucosa</td>
<td>19.14±2.12 (10.10-18.00)</td>
<td>57.69±4.10 (32.12-54.12)</td>
<td>231.57±2.42 (64.0-221.12)</td>
<td>113.72±9.28 (60.10-105.11)</td>
</tr>
<tr>
<td>Thickness of tunica sub-mucosa</td>
<td>92.81±9.19 (61.10-89.11)</td>
<td>139.73±10.10 (92.10-121.13)</td>
<td>204.46±12.14 (145.00-199.0)</td>
<td>188.96±10.11 (120.00-178.12)</td>
</tr>
<tr>
<td>Thickness of Tunica muscularis layer</td>
<td>5.13±2.12 (2.12-5.00)</td>
<td>14.33±4.28 (7.10-12.10)</td>
<td>94.71±6.12 (74.15-95.10)</td>
<td>165.44±9.12 (95.10-155.14)</td>
</tr>
<tr>
<td>Thickness of tunica serosa layer</td>
<td>32.32±4.01 (21.12-30.00)</td>
<td>68.55±7.28 (37.10-65.14)</td>
<td>219.13±7.07 (98.14-221.0)</td>
<td>228.63±9.12 (98.00-225.10)</td>
</tr>
<tr>
<td>Luminal diameter of gall bladder</td>
<td>123.81±12.11 (97.16-120.00)</td>
<td>324.82±19.10 (194.12-321.00)</td>
<td>754.15±19.04 (416.10-750.00)</td>
<td>965.11±21.02 (749.14-950.00)</td>
</tr>
</tbody>
</table>

Table 1: Micrometrical parameters (Mean ± S.E) of different layers of gall bladder in different age groups of gestation in Gaddi sheep foetii. Values having different superscripts in each row differ significantly (P<0.05). Values within parenthesis denote the range of parameter.
surface epithelium were observed to have grown deeper than group-III. The tunica mucosa layer of gall bladder was well developed and composed of lamina epithelialis and lamina propria layer (Figures 6 and 7) at CRL 33.5 cm (121 days of gestation). The lamina muscularis mucosa was absent in gall bladder in all stages of gestation. Tunica mucosa had the continuous striated border of microvilli over the non-ciliated simple columnar epithelial cells at CRL 38.2 cm (135 days of gestation). Same structure was observed near full term. The distribution of reticular fibres was much denser in all layers of gall bladder at CRL 42.5 (146 days of gestation) than the previous stages. Strong PAS positive reaction was observed by tunica mucosa while in all other layers of gall bladder the moderate PAS positive reaction was observed at CRL 40.00 cm (139 days of gestation). Same pattern was observed near full term also. The collagen fibers in tunica muscularis layer was well developed at CRL 42.5 cm (146 days of gestation) (Figure 8). Strong reaction for acidic mucopolysaccharides was observed in tunica mucosa layer near the full term.

Micrometry of gall bladder: Thickness of tunica mucosa: The thickness of the tunica mucosa increased significantly from group-I to III than it showed a significant decrease in group-IV (Table 1). In group-I its minimum (10.10 µm) thickness was recorded at CRL 6.0 cm (46 days of gestation) and maximum (18.00 µm) at CRL 9.7 cm (57 days of gestation). For group-II, the highest (32.12 µm) and lowest (54.12 µm) values for thickness of tunica mucosa were recorded at CRL 14.8 cm (70 days of gestation) and at CRL 21.4 cm (88 days of gestation) respectively. In group-III its minimum (74.15 µm) value was observed at CRL 24.5 cm (97 days of gestation) and maximum (95.10 µm) value at CRL 31.3 cm (115 days of gestation). For group-IV its maximum (155.14 µm) and minimum (7.10 µm) values for thickness of tunica mucosa were recorded at CRL 33.5 cm (121 days of gestation) and at CRL 21.4 cm (88 days of gestation) respectively. In group-IV its minimum (74.15 µm) value was observed at CRL 24.5 cm (97 days of gestation) and maximum (95.10 µm) value at CRL 31.3 cm (115 days of gestation). For group-IV, the maximum (155.14 µm) and minimum (95.10 µm) values for thickness of tunica muscularis were recorded at CRL 33.5 cm (121 days of gestation) and at CRL 42.5 cm (146 days of gestation) respectively.

Thickness of tunica muscularis layer: The thickness of tunica muscularis layer increased significantly from group-I to IV (Table 1). In group-I its minimum (2.12 µm) thickness was recorded at CRL 8.5 cm (53 days of gestation) and maximum (5.00 µm) at CRL 11.0 cm (60 days of gestation). For group-II the maximum (12.10 µm) and minimum (7.10 µm) values for thickness of tunica muscularis were recorded at CRL 14.8 cm (70 days of gestation) and at CRL 21.4 cm (88 days of gestation) respectively. In group-III its minimum (74.15 µm) value was observed at CRL 24.5 cm (97 days of gestation) and maximum (95.10 µm) value at CRL 31.3 cm (115 days of gestation). For group-IV, the maximum (155.14 µm) and minimum (95.10 µm) values for thickness of tunica muscularis were recorded at CRL 33.5 cm (121 days of gestation) and at CRL 42.5 cm (146 days of gestation) respectively.

Thickness of tunica serosa layer: The thickness of tunica serosa layer increased significantly from group-I to IV (Table 1). The maximum (65.14 µm) and minimum (21.12 µm) values for the thickness of tunica serosa in group-I were recorded at CRL 6.0 cm (46 days of gestation) and 11.0 cm (60 days of gestation) respectively. For group-II, its highest (121.13 µm) value was observed at CRL 14.8 cm (70 days of gestation) and lowest (92.10 µm) value at CRL 22.0 cm (90 days of gestation). In group-III, the maximum (199.00 µm) and minimum (145.00 µm) values for the thickness of tunica sub-mucosa were recorded at CRL 24.5 cm (97 days of gestation) and 32.0 cm (117 days of gestation) respectively. For group-IV, its highest (178.12 µm) value was observed at CRL 33.5 cm (121 days of gestation) and lowest (120.00 µm) at CRL 42.5 cm (146 days of gestation).

Thickness of tunica sub-mucosa: The thickness of tunica sub-mucosa showed similar pattern like that of tunica mucosa. Its thickness increased significantly from group-I to III than it showed a significant decreased in group-IV (Table 1). The maximum (89.11 µm) and minimum (61.10 µm) values for the thickness of tunica sub-mucosa in group-I were recorded at CRL 8.5 cm (53 days of gestation) and 11.0 cm (60 days of gestation) respectively. For group-II, its highest (121.13 µm) value was observed at CRL 14.8 cm (70 days of gestation) and lowest (92.10 µm) value at CRL 22.0 cm (90 days of gestation). In group-III, the maximum (199.00 µm) and minimum (145.00 µm) values for the thickness of tunica sub-mucosa were recorded at CRL 24.5 cm (97 days of gestation) and 32.0 cm (117 days of gestation) respectively. For group-IV, its highest (178.12 µm) value was observed at CRL 33.5 cm (121 days of gestation) and lowest (120.00 µm) at CRL 42.5 cm (146 days of gestation).
Luminal diameter of gall bladder: The luminal diameter of gall bladder showed a significant increase from group-I to IV (Table 1). The maximum (120.0 µm) and minimum (97.16 µm) values for the luminal diameter of gall bladder in group-I were recorded at CRL 6.00 cm (46 days of gestation) and 11.0 cm (60 days of gestation) respectively. For group-II, its maximum (312.00 µm) value was observed at CRL 14.8 cm (70 days of gestation) and minimum (194.12 µm) value at CRL 22.0 cm (90 days of gestation). In group-III, the maximum (750.00 µm) and minimum (416.10 µm) values for the luminal diameter of gall bladder were recorded at CRL 24.5 cm (97 days of gestation) and 32.0 cm (117 days of gestation) respectively. For group-IV, its maximum (950.00 µm) value was observed at CRL 33.5 cm (121 days of gestation) and minimum (749.14 µm) at CRL 42.5 cm (146 days of gestation).

DISCUSSION

The results of our study provide normative data for growth of the fetal gall bladder throughout gestation. No literature as such was available on the embryonic gall bladder in ruminants. In conclusion, these results provide normative data of the fetal gall bladder of Gaddi sheep in various dimensions and across gestational ages, which can be used to assess variations from the norm. In addition, these data offer the potential for prenatal diagnosis of additional extrahepatic lesions. There were changes in histomorphology, histochemical and micrometric studies of the of the gallbladder wall in relation to age in this study. To establish a standard data similar study with larger sample size in different age groups and using more advance methods including both sexes are recommended.

REFERENCES