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Gross morphometrical studies on spleen at various age groups of prenatal sheep (*Ovis aries*)

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Abstract
A total number of eighteen sheep foetuses were divided into three groups *viz.* early gestational age (1-50 days), mid gestational age (51-100 days) and late gestational age (101-till birth) with six foetuses in each age group. The spleen of sheep was first observed at 50 days of gestation in the present study. The spleen appeared reddish brown in color and acquired the shape of oyster shell at 56 days of gestation. The surfaces of the spleen were clearly demarcated at 56 days of gestation. The borders were thin and anterior border of spleen was positioned at 10th rib whereas the posterior border was extended beyond 13th rib at 93 days of gestation. The biometric parameters such as length, width and weight of the spleen was progressively increased with the age advanced in all prenatal age groups.

Keywords: sheep, prenatal, spleen, gross morphology, biometry

1. Introduction
Spleen is the largest organ in the lymphatic system, which performs hemtopoietic and immunologic function (Elizabeth and Fredric, 2011, Gupta *et al.* 2017) [4, 6]. This organ demands central attention by several investigators in different mammals for understanding its immunological role (Rahman *et al.* 2016) [11]. The size of the spleen changes time to time even within a given individual as well as from species to species depending on various developmental factors (Fails and Magee, 2018) [5]. The comprehensive analysis are essential on the development of spleen especially at early gestational ages to comprehend the gestational changes. To the best of our knowledge, meagre reports on the development of sheep spleen is available. Hence, present study was designed to observe the gross morphometrical features of the spleen in sheep at various gestational ages.

2. Materials and Methods
The present study was conducted on the development of the spleen from 18 healthy foetuses of the sheep of either sex of non-descriptive sheep. The foetuses of the sheep were collected from the Chennai slaughter house, Chennai. The approximate age of the prenatal age groups was estimated by obtaining the crown rump length (CRL) and substituting in the formula (Noakes *et al*., 2009) [10], A= 2.1(B+17), Where 'A' is the developmental age of foetus in days and 'B' is crown rump length in centimetres. The CRL was measured from the anterior part of the crown to the base of the tail as described by Rao and Ramayya (2013) [12]. The spleen was obtained immediately after slaughter and fixed in 10% neutral buffered formalin (NBF). Total body weight of the foetus was measured by using digital mono pan balance. The length and width of the lymphoid organs of different age groups were measured by using the digital Vernier caliper. The weight of the spleen was taken with the help of digital electronic balance.

3. Results and Discussion
3.1 Early gestational age
At 50 days of gestation, the spleen of sheep was first grossly appeared as small white rounded mass in left side of abdominal cavity near developing rumen (Fig. 1a). Gupta *et al.* (2017) [6] observed the splenic primordium at 46 days of gestation in goat foetuses. Nishant *et al.* (2018) [9] noticed the splenic primordium in the form of white thickening on the dorso medial surface towards the cranial end of the stomach tube until 50 days of gestation. The spleen of sheep was located in between the 12th and 13th rib and its parietal and visceral surfaces were not distinct at 50 days of gestation. These observations were in consistence with Gupta *et al.* (2017) [6] and Nishant *et al.* (2018) [9].

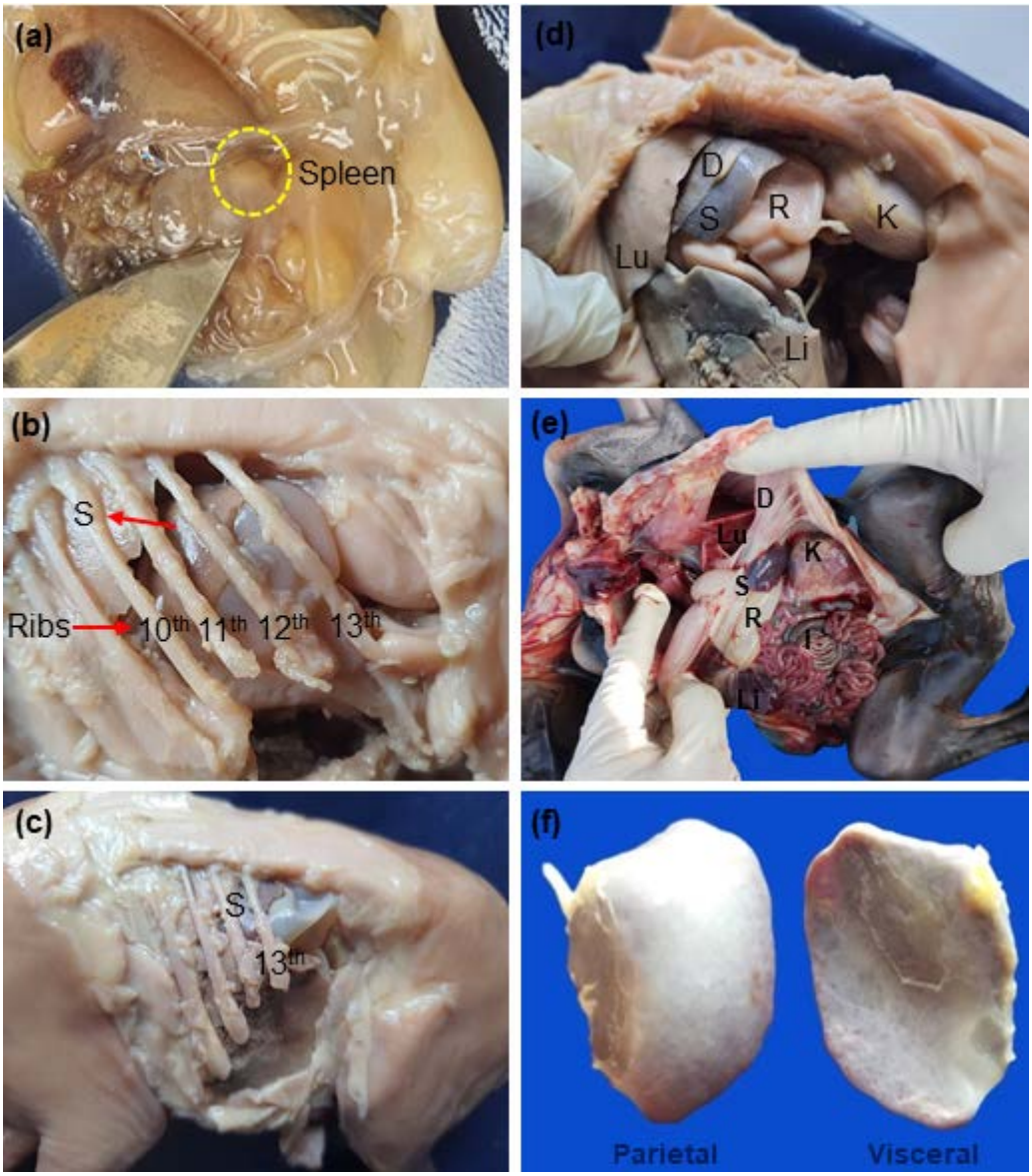

Fig 1: Spleen of sheep at 50 days of gestation (a), Gross photograph showing various organs at 76 days (b), 93 days (c), 103 days (d), 126 days of gestation (e) and Surfaces of spleen at 110 days of gestation (f). S- Spleen, R-Rumen, D-Diaphragm, K-Kidney, Lu-Lung, Li-Liver, I-Intestines

Table 1: The morphometric values of spleen at various days of gestation in prenatal sheep.

Group	CRL	Days of Gestation	Total Foetus Weight (g)	Spleen			
				Length (mm)	Width (mm)	Weight (g)	Splenic Index (%)
I	6.8	50	13	4.13	3.48	0.014	0.028
II	9.5	56	23	5.41	3.70	0.021	0.036
	11.5	60	46	7.66	4.88	0.037	0.062
	19	76	176	11.97	9.49	0.176	0.232
	21.5	81	310	14.72	10.65	0.235	0.290
	23	84	422	16.75	11.13	0.292	0.348
	27.2	93	642	21.98	13.87	0.787	0.846
III	32.2	103	793	26.3	17.66	1.295	1.257
	35.5	110	1065	27.4	18.77	1.995	1.814
	38.5	116	1239	29.92	21.1	2.188	1.886
	43	126	1760	31.21	22.51	2.898	2.300
	47	134	2081	33.74	23.28	3.413	2.547
	51	143	2573	34.98	25.4	3.871	2.707

3.2 Mid gestational age

At 56 days of gestation, the spleen appeared reddish brown in color and acquired the shape of oyster shell. Mehta *et al.* (2016) [8] reported the roughly triangle shape spleen in all age groups of Chotanagpuri sheep. According to Alhaji *et al.* (2019) [1], camel spleen was dark brown in color with semilunar shape. Malik *et al.* (2001) [7] reported irregular triangle shape spleen in prenatal age groups of goat. According to Gupta *et al.* (2017) [6] the color of the goat spleen was creamish in early stage which become reddish

with age advanced. Chaurasia *et al.* (2018) [3] reported reddish brown color spleen at 58 days of gestation and the shape of the spleen was quadrilateral in all age groups of Surati goat. This variation might be due to breed and species difference. The spleen of sheep foetus was located on the left side of abdominal cavity adherent to the developing rumen under the diaphragm and extended from posterior border of 10th to 13th rib. The spleen showed convex parietal surface and concave visceral surfaces at 56 days of gestation.

At 76 days of gestation, the visceral surface was concave and positioned on the dorsal sac of the rumen. The parietal surface was more convex than previous stage. The thick cranial border was attached to the rumen near hilus and the free ventral border was thin. The thickness of the dorsal border was less than cranial border. The caudal border was thin and slightly concave. The spleen was extended from posterior border of 10th intercostal space to 13th rib (Fig.1b). Mehta *et al.* (2016) [8] noticed that the spleen of Chotanagpuri sheep was in the range of 10-13th ribs in all age groups. According to Gupta *et al.* (2017) [6] the foetal goat spleen which was positioned in between 11th rib to 13th rib had nearly straight cranial border and the caudal border was little concave. At 84 days of gestation, the color of spleen became reddish brown and its concave visceral surface was positioned on the dorsal sac of the rumen. The parietal surface was more convex and related to the diaphragm and ribs.

At 93 days of gestation, the color of spleen appeared dark reddish brown. The visceral and parietal surfaces found more concave and convex, respectively. The anterior border was thick and positioned at 10th rib whereas the posterior border was extended beyond 13th rib (Fig. 1c).

The biometric parameters such as length, width and weight of the spleen in mid gestational age were recorded and has been given in Table 1. Bello *et al.* (2016) [2] also noticed the gradual increment of the foetal weight from 3.00±0.12 kg to 18.00±3.05 kg with age advanced from 1st trimester to 3rd trimester in camel. The length, width and weight of the spleen was increased gradually with the age advanced (Fig. 2). The mean length, width and weight of the spleen in mid gestational age were 13.08±0.74 mm, 8.95±0.32 mm and 0.258±0.12 g, respectively. Malik *et al.* (2001) [7] and Chaurasia *et al.* (2018) [3] were reported similar observations in goat foetuses. In the present study, the growth rate of the spleen length and width was found to be maximum during mid gestational age.

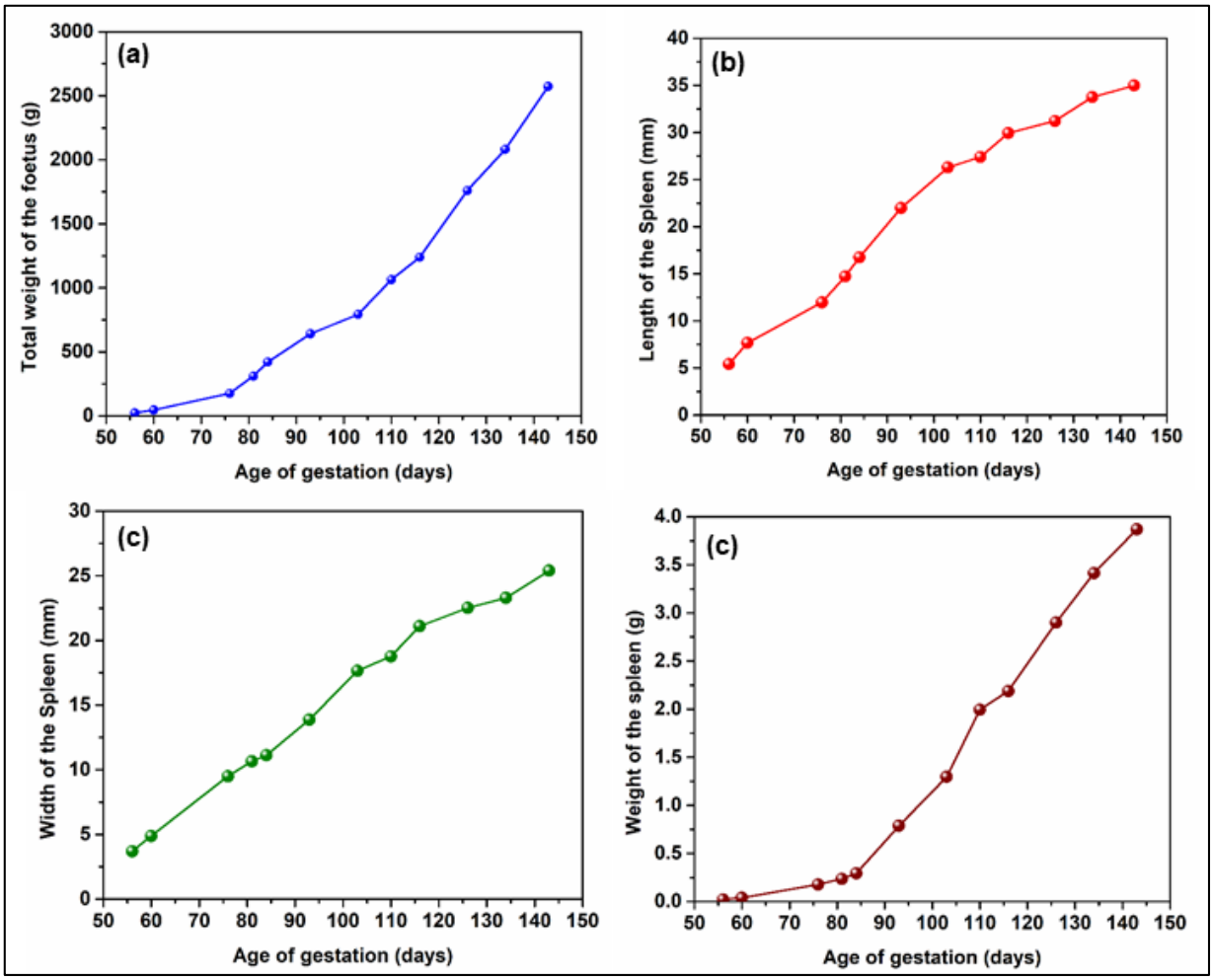

Fig 2: the variation of total body weight (a), length (b), width (c) and weight of the spleen (d) with advancement of age during mid and late gestational period.

3.3 Late gestational age

From 103 days of gestation, the color of foetal spleen was dark reddish brown and appeared as oyster shell shape with distinct borders and surfaces. The parietal surface was enclosed with the diaphragm except on the dorsal half of the caudal border (Fig. 1e). These observations were in agreement with the Nishant *et al.* (2018) [9] in goat foetuses. At 110 days of gestation, all borders were distinctively appeared with sharp edges in which cranial border was relatively thicker than rest of three borders (Fig. 1f). At 126 days of gestation,

the color of the spleen was much darker than previous age (Fig, 1e). The spleen of sheep was relatively larger and more developed than 110 days old foetus but the location of the spleen was almost similar to previous group. At 143 days of gestation, the caudal part of the surface was nearly flat but cranial part of the visceral surface was extremely concave. The dorsal border was prolonged caudally up to maximum distance beyond 13th rib.

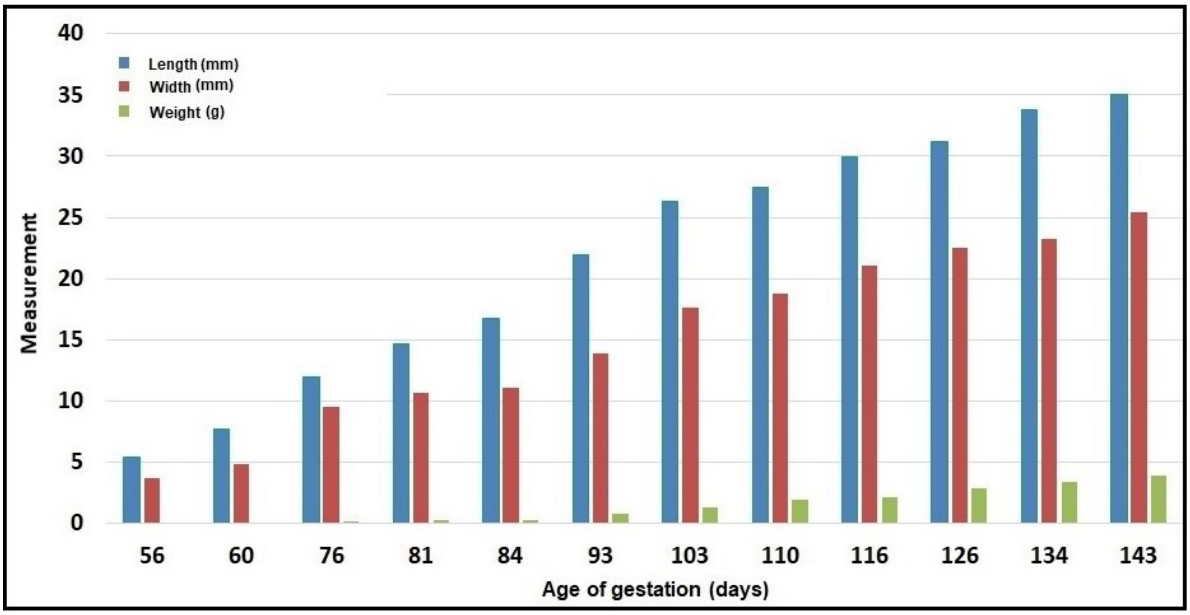

Fig 3: The difference between length, width and weight of the spleen at various ages of gestation.

The biometric parameters such as length, width and weight in late gestational age were recorded and has been given in Table 1. The length, width and weight of the spleen increased gradually with the age advanced (Fig. 2). The mean length, width and weight of the spleen in late gestational age were 30.59±1.12 mm, 21.45±0.98 mm and 2.61±1.05 g, respectively. The growth rate of the spleen weight was found to be maximum during late gestational age. These results were in consistence with the earlier report of Srivani *et al.* (2020) [13] in human, Gupta *et al.* (2017) [6] in goat. The relative weight of the spleen was increased gradually with age advanced during all prenatal age groups. In the present study, all biometric parameters such as length, width and weight of the spleen were progressively increased in all age groups during prenatal period (Fig. 3).

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