MICROMETRICAL OBSERVATIONS ON THE SPINAL CORD OF GOAT FOETUSES*

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Abstract

Micrometrical studies during the prenatal development of spinal cord in goat revealed that the cervical enlargement had the maximum height at all ages, with maximum gray matter and dorsal horn height except at fifth month, where it was at the lumbosacral enlargement. The maximum ventral horn height was also seen at the enlargements. Sparing a few exceptions, the percentage contribution of gray matter height, dorsal horn height and ventral horn height to the total spinal cord height showed a decreasing trend during gestation owing to a corresponding increase in the ventral funiculus. The gray matter height expressed as percentage of spinal cord height, was maximum at the lumbosacral enlargement. The greatest values for total width of spinal cord and distance from central canal to spinal cord left margin varied between cervical and lumbosacral enlargements at different stages of gestation. The total width of spinal cord was greater than the height during gestation except in the thoracic region during second month. Dorsal and ventral horns were wider at enlargements. The percentage of gray matter width decreased after third month of gestation indicating a comparative increase in the growth of lateral funiculus during later stages of gestation. Vertical gray matter percentage decreased after second month indicating an early growth of ventral funiculus.

Key words: *Micrometry, foetus, goat, prenatal, spinal cord*

The spinal cord proper in mammals consists of peripheral white matter surrounding the central gray matter. The size and shape of the spinal cord and relative amount of gray and white matter vary at different levels with the variation in the size of the nerve roots. In general, there is an increase in the white matter from caudal to cranial levels of the cord with the cervical segments containing the largest number of fibres in mammals. The general microscopic structure of spinal cord in animals has been described by several workers. But since a detailed study on the prenatal micrometry in goat is very limited, this study was conducted to elucidate the same at various stages of prenatal life in goat foetuses.

Materials and Methods

The study was conducted on 52 goat foetuses of different ages. The age was calculated using the formula derived by Singh *et al.* (1979), for goat foetuses, $W^{1/3} = 0.096$ (t-30), where, W = body weight in g and t = age of the foetus in days. The foetuses were grouped into five age groups corresponding to five months of gestation. The material was fixed in 10 per cent neutral buffered formalin. Embryos and small foetuses upto 50 days of gestation were fixed as such for 48 to 96 h. In embryos from 50 days to 90 days of age, the vertebral column with spinal cord *in situ* was separated, freed of its musculature and immersed in the fixative for 48 h. The spinal cord within the vertebral column was processed

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after cutting into region-wise pieces and serial sections were made. In foetuses above 90 days of age, the spinal cord was exposed by laminectomy, paramedian section and by clipping off the pedicles of the vertebrae. Then the spinal cord along with meninges, spinal nerve roots and dorsal root ganglia was dissected out, cut into pieces of two to three segments each and processed. Standard procedures were adopted for histological techniques and the serial sections were stained using Ehrlich's haematoxylin and eosin staining, Holzer's method for glial fibres, Sevier-Munger method for neural tissues, Van Gieson's method for collagen, Holmes silver nitrate method for axis cylinders and myelin sheaths, Aldehyde-Thionine-PAS method for central nervous system and phosphotungstic acid haematoxylin (PTAH) method for CNS tissue (Luna, 1968). Micrometrical data were recorded using an ocular micrometer and analysed statistically following Snedecor and Cochran (1985).

Results and Discussion

Micrometrical observations revealed that the maximum height and width of the neural tube was recorded at the regions corresponding to the anterior limb buds (the cervical enlargement) followed by the region of posterior limb buds (lumbosacral enlargement) during the first month. The minimum height and width of the neural tube were recorded at the coccygeal region (Table 1). During first month, height of neural tube exceeded its width at the lower aspect at cervical, thoracic, sacral and coccygeal regions. Basal plate height was maximum at the cervical enlargement, but the maximum alar plate height was at thoracic region. Neural tube

Table 1. Micro metrical data at the first month of gestation (Mean ± S. E.), µ m

Parameter	Regions						
	Cervical	Cervical enlargement	Thoracic	Lumbar	Lumbosacral enlargement	Sacral	Coccygeal
Neural tube height	587.500± 53.021	654.375± 54.963	551.667± 35.688	523.125± 33.713	597.500± 40.327	363.333± 11.118	210.000± 15.732
Roof plate height	22.500± 3.354	20.625± 2.745	20.000± 2.795	18.813± 2.894	15.000± 0.001	13.333± 1.667	13.500± 1.300
Floor plate height	70.000± 9.220	80.625± 4.858	61.722± 8.110	63.750± 4.701	91.667± 4.773	47.500± 10.680	25.500± 3.824
Lumen height	466.667± 33.133	486.875± 29.760	459.222± 30.022	436.875± 23.622	455.000± 25.593	317.500± 11.726	165.000± 10.062
Alar plate height	232.500± 21.823	228.750± 16.949	255.000± 17.678	243.750± 20.975	252.500± 21.360	176.667± 24.080	80. 342± 2.697
Basal plate height	280.000± 41.473	318.750± 33.391	235.000± 19.843	245.625± 16.020	290.000± 25.000	181.667± 27.588	122.367± 8.127
Neural tube width - upper	475.000± 17.607	534.375± 28.053	391.667± 21.570	468.750± 16.223	482.500± 55.644	240.000± 13.693	132.000± 9.950
Neural tube width - lower	532.500± 24.418	697.500± 61.651	453.889± 36.570	575.625± 45.966	655.000± 93.327	318.333± 10.240	87.000± 17.348
Lumen width- upper	95.000± 3.162	84.375± 2.745	66.667± 6.180	75.000± 6.708	77.500± 2.500	65.000± 3.536	52.500± 7.500
Lumen width- lower	90.000± 33.317	80.625± 12.006	22.500± 2.165	43.125± 9.997	55.000± 11.402	45.000± 6.124	15.000± 0.500
Ependymal thickness - alar plate	110.000± 6.325	103.125± 14.013	76.111± 7.158	95.625± 7.986	125.500± 20.736	65.000± 2.500	63.000± 7.348
Ependymal thickness - basal plate	57.500 ± 8.139	50.625± 7.986	51.667± 6.180	39.375± 8.475	55.000± 11.402	43.333± 3.909	63.000± 7.348
Mantle layer thickness –alar plate	57.500± 19.429	26.250± 3.750	38.333±6.18 0	39.375± 8.475	30.000± 5.477	15.000± 0.000	*
Mantle layer thickness –basal plate	157.500± 29.686	238.125± 34.537	153.333± 16.729	208.125± 45.747	220.000± 51.769	83.333± 7.546	*
Marginal layer thickness –alar plate	42.500± 10.368	75.000± 13.595	35.000± 3.536	46.875± 5.256	75.000± 22.249	17.500± 1.250	21.000± 6.452
Marginal layer thickness –basal plate	27.500± 2.500	30.000± 5.669	31.667± 4.640	33.750± 6.797	32.500± 9.014	16.667± 1.667	21.000± 6.452
Basal plate width	220.833± 32.975	324.375± 32.561	216.667± 17.159	259.750± 21.523	312.500± 47.971	141.667± 5.652	42.000± 2.000
Alar plate width	182.500± 22.389	219.375± 25.971	155.000± 15.612	185.625± 15.510	195.000± 38.536	104.167± 6.305	33.000± 3.000

**not measurable*

Table 2. Height of precoccygeal spinal cord structures at different regions (Mean ± S. E.)

Group	Parameters	Regions					
		Cervical	Cervical enlargement	Thoracic	Lumbar	Lumbosacral enlargement	Sacral
II	Spinal cord	1262.500± 65.431	1502.500± 32.755	1416.667± 30.405	1387.167± 35.133	1472.500± 35.148	1035.000± 26.833
	Total gray matter	956.833 ± 68.406	1265.500± 41.242	1090.333± 33.758	1097.500± 37.665	1237.500± 56.140	925.000± 44.72
	Dorsal horn	448.333± 19.944	649.667± 40.985	595.833± 39.829	584.167± 28.030	592.750± 54.622	492.500± 1.118
	Ventral horn	508.500± 49.068	615.833± 22.413	494.500± 21.694	513.333± 14.472	644.750± 39.72	432.500± 0.224
III	Spinal cord	1540.000± 100.822	1713.833± 61.528	1462.500± 64.791	1440.000± 50.133	1556.250± 32.620	1245.000± 8.944
	Total gray matter	1133.833± 55.965	1380.833± 50.580	1173.330± 61.409	1180.666± 68.999	1320.000± 64.161	990.000± 3.130
	Dorsal horn	580.000± 32.965	722.500± 46.704	608.333± 43.982	591.000± 30.985	660.000± 31.885	540.000± 1.342
	Ventral horn	553.833± 31.247	658.333± 27.070	565.000± 38.665	589.667± 46.816	660.000± 40.620	450.000± 1.789
IV	Spinal cord	2633.333± 85.612	2755.500± 98.678	1995.000± 94.393	1900.000± 68.932	2325.000± 81.009	1575.000± 31.305
	Total gray matter	1854.667± 114.051	2116.000± 100.225	1305.000± 108.695	1390.000± 26.158	1881.500± 32.898	1220.000± 17.889
	Dorsal horn	908.667± 43.186	1041.000± 64.815	618.833± 52.626	625.000± 61.339	937.500± 48.412	615.000± 18.371
	Ventral horn	946.000± 72.202	1075.000± 62.798	686.667± 67.484	765.000± 19.664	944.000± 31.451	610.000± 15.652
V	Spinal cord	3809.167± 177.696	3858.333± 189.040	3285.000± 282.134	3257.500± 162.018	3487.500± 89.268	2017.500± 7.826
	Total gray matter	2231.667± 171.205	2383.333± 123.603	1908.333± 175.209	1927.583± 137.038	2625.000± 45.415	1320.000± 8.944
	Dorsal horn	1064.167± 149.100	1270.833± 118.395	860.500± 112.540	979.250± 104.286	1278.750± 62.262	690.000± 40.249
	Ventral horn	1167.500± 43.565	1112.500± 49.054	1047.833± 83.153	948.333± 82.519	1346.250± 29.607	630.000± 13.416

was widest at the cervical enlargement. The width of alar and basal plates was also maximum in this region followed by the lumbosacral enlargement.

The region-wise average of height and width of precoccygeal spinal cord from second to fifth month of gestation are presented in tables 2 and 3 respectively. The total width of spinal cord was greater than the height along the entire length from second to fifth month of gestation. This partially concurs with the observation in goat foetuses by Taluja *et al.* (1990) who also found that the same was true except in the cervical region in the first trimester of gestation.

The greatest values for total width of spinal cord and distance from central canal to spinal cord left margin varied between cervical and lumbosacral enlargements at different stages of gestation (Table 3). But during fourth and fifth month, the cervical enlargement was wider than lumbosacral enlargement, indicating the dominance of the total width at the cervical enlargement during later stages of gestation. The greater width at the enlargements might be due to the increase in

Table 3. Width of precoccygeal spinal cord structures at different regions (Mean ± S. E)

Group	Parameters	Regions					
		Cervical	Cervical enlargement	Thoracic	Lumbar	Lumbosacral enlargement	Sacral
II	Spinal cord	1623.833± 108.559	1683.500 ± 83.060	1450.333± 41.138	1401.667± 49.694	1845.000± 31.225	1260.000± 26.833
	Central canal to sp cord left margin	771.917± 47.026	809.250± 40.589	692.917± 19.616	664.583± 20.881	875.625± 15.424	611.250± 15.093
	Central canal to gray matter left margin	463.750± 26.571	571.000± 50.824	451.333± 11.809	530.000± 23.345	668.875± 49.139	433.500± 14.982
	Dorsal horn	389.833± 33.708	481.333± 71.551	479.333± 17.772	518.167± 35.743	645.250± 32.369	460.000± 26.833
	Ventral horn	353.667± 41.024	587.167± 37.652	407.333± 14.591	555.500± 40.697	690.000± 43.301	407.000± 3.130
	Gray matter width %	57.253	67.855	62.217	75.624	72.507	68.810
III	Spinal cord	1657.500± 40.204	1852.500± 62.179	1581.667± 170.777	1671.000± 29.029	1926.500± 48.016	1560.000± 13.416
	Central canal to sp cord left margin	783.750± 52.328	868.250± 31.783	744.333± 36.479	784.500± 14.335	918.750± 24.136	761.000± 6.708
	Central canal to gray matter left margin	543.500± 36.170	670.000± 33.072	539.667± 18.458	574.333± 57.543	772.500± 52.856	536.250± 38.572
	Dorsal horn	610.333± 45.702	678.167± 17.350	548.333± 37.454	562.500± 14.068	738.750± 21.891	540.000± 13.416
	Ventral horn	496.333± 33.162	664.167± 35.810	511.667± 34.609	610.000± 67.688	767.000± 115.808	517.000± 10.062
	Gray matter width %	65.581	72.335	68.240	68.741	80.197	68.750
IV	Spinal cord	2775.333± 150.449	3430.000± 188.368	2300.000± 83.236	2480.000± 22.888	2925.000± 15.615	2010.000± 0.894
	Central canal to sp cord left margin	1345.167± 75.716	1657.500± 94.503	1110.000± 41.573	1190.000± 17.491	1413.750± 14.857	967.500± 6.485
	Central canal to gray matter left margin	790.000± 59.554	945.000± 42.922	708.333± 32.186	710.000± 22.586	990.000± 51.797	674.500± 31.081
	Dorsal horn	721.000± 43.955	812.667± 30.883	583.667± 39.823	688.000± 24.798	906.500± 24.185	625.000± 4.472
	Ventral horn	697.667± 51.004	921.000± 60.781	553.333± 53.324	651.000± 28.676	952.500± 101.438	530.000± 13.416
	Gray matter width %	56.930	55.102	61.594	57.258	67.692	67.114
V	Spinal cord	4973.667± 510.863	5375.000± 338.071	4196.000± 437.500	4260.000± 394.293	5215.000± 341.309	2085.000 ±6.708
	Central canal to sp cord left margin	2392.875± 246.801	2594.792± 395.089	2032.792± 212.047	2055.625± 176.939	2523.125± 168.407	993.750± 1.677
	Central canal to gray matter left margin	1131.250± 104.757	1254.583± 57.505	805.417± 68.253	990.833± 70.112	1398.750± 63.383	690.000± 6.708
	Dorsal horn	911.667± 85.468	900.333± 58.999	754.500± 42.844	795.833± 58.341	1125.250± 57.055	842.500± 41.367
	Ventral horn	850.000± 69.270	1493.750± 111.418	627.250± 32.424	814.667± 71.996	1501.250± 120.509	785.750± 38.349
	Gray matter width %	45.490	46.682	38.390	46.518	53.643	66.187

size of the nerve roots and the relative quantum of gray matter.

The cervical enlargement had the maximum height throughout the gestation. It was followed by the lumbosacral enlargement for the major part of gestation (Table 2). Taluja *et al.* (1990) also found that the height of spinal cord was maximum at cervical enlargement in goat foetuses. This increased size was attributed to the increased number of nerve fibres originating for supply towards the limbs.

The gray matter height and dorsal horn height were also maximum at the cervical enlargement during greater part of gestation,

Table 4. Average gray matter height percentage and contribution of dorsal horn and ventral horn height to gray matter and precoccygeal spinal cord height (µm)

Group	Parameters	Regions					
		Cervical	Cervical enlargement	Thoracic	Lumbar	Lumbosacral enlargement	Sacral
II	Gray matter	75.789	84.226	76.965	79.118	84.041	89.372
	Dorsal horn to spinal cord	35.511	43.239	42.057	42.112	40.255	47.585
	Dorsal horn to gray matter	46.856	51.337	54.646	53.227	47.899	53.243
	Ventral horn to spinal cord	40.277	40.987	34.906	37.008	43.786	41.787
	Ventral horn to gray matter	53.144	48.663	45.353	46.772	52.101	46.757
III	Gray matter	73.626	80.570	80.228	81.991	84.819	79.518
	Dorsal horn to spinal cord	37.664	42.157	41.5955	41.042	42.410	43.370
	Dorsal horn to gray matter	51.154	52.324	51.847	49.463	50.000	54.540
	Ventral horn to spinal cord	35.963	38.413	38.632	40.919	42.410	36.145
	Ventral horn to gray matter	48.846	46.677	48.153	49.351	50.000	45.454
IV	Gray matter	70.430	76.806	65.414	73.158	80.925	77.777
	Dorsal horn to spinal cord	34.506	37.786	30.994	32.895	40.323	39.048
	Dorsal horn to gray matter	48.994	49.197	47.382	44.964	49.827	50.410
	Ventral horn to spinal cord	35.924	39.020	34.419	40.263	40.603	38.730
	Ventral horn to gray matter	51.006	50.803	52.618	55.036	50.173	50.000
V	Gray matter	58.587	61.771	58.092	59.174	75.269	65.428
	Dorsal horn to spinal cord	27.937	32.937	26.195	30.061	36.667	34.201
	Dorsal horn to gray matter	47.685	53.321	45.092	50.802	48.714	52.273
	Ventral horn to spinal cord	30.649	28.834	31.898	29.112	38.602	31.227
	Ventral horn to gray matter	52.315	46.678	54.908	49.198	51.285	47.727

but by the fifth month, it was at lumbosacral enlargement. The maximum ventral horn height was also recorded at the enlargements with the lumbosacral enlargement predominating during the major part of the gestation. The minimum values for spinal cord height, gray matter height and dorsal and ventral horn height among the regions in the precoccygeal cord were at the sacral region in all age groups, since all these values decreased towards the caudal end of spinal cord (Table 2). All the regions showed a progressive increase in height during the entire gestation. Dorsal and ventral horns were widest at lumbosacral enlargement followed by cervical enlargement in all age groups from second to fifth month of gestation. It indicated that eventhough cervical enlargement was having greater width than lumbosacral enlargement in the last two months of gestation, its increased width was mostly due to increased amount of white matter at cranial levels of the spinal cord.

In the present study, sparing a few exceptions at thoracic, lumbar and lumbosacral enlargement regions between second and third month, the percentage contribution of gray matter height to the total spinal cord height showed a gradual decrease as the age advanced (Table 4). This indicated a corresponding increase in the white matter towards the end of gestation due to the development of fibre tracts. In dogs also, the mean ratio of gray matter diameter to spinal cord diameter showed a decreasing trend as the age advanced from 24 (Engel and Draper, 1982 a) to 44 days of gestation (Engel and Draper, 1982b).

The percentage contribution of gray matter height to the spinal cord height (vertical gray matter percentage) started to decrease from the second month (Table 4). This indicated an early growth of the ventral funiculus indirectly, because the contribution from the dorsal funiculus at the dorsal aspect was always less. The percentage of gray matter width (transverse gray matter percentage) decreased only after third month of gestation in all the regions indicating a comparative increase in the growth of lateral funiculus at later stages of gestation when compared to that of the ventral funiculus (Table 3). Jenkins (1978) opined that variations in the gray and white matter relationship at different levels of

Table 5. Percentage increase in height of precoccygeal spinal cord between age groups (µm)

Parameters and period (month)	Regions					
	Cervical	Cervical enlargement	Thoracic	Lumbar	Lumbosacral enlargement	Sacral
Spinal cord height						
2 – 3	21.980	14.065	3.235	3.809	5.688	20.290
3 – 4	70.996	60.751	36.410	31.944	49.398	26.506
4 – 5	44.652	40.048	64.662	71.447	50.000	28.095
2 – 5	201.238	156.794	131.882	134.831	136.842	94.928
Gray matter height						
2 – 3	18.499	9.114	7.612	7.578	6.667	7.027
3 – 4	63.575	53.241	11.222	17.730	42.538	23.232
4 – 5	20.327	12.634	46.232	38.675	39.516	8.197
2 – 5	133.235	88.331	75.023	75.634	112.121	42.703
Dorsal horn height						
2 – 3	29.368	11.211	1.091	1.170	11.345	9.645
3 – 4	56.667	44.083	2.656	5.753	4.205	13.889
4 – 5	17.113	22.078	39.164	56.680	36.400	12.195
2 – 5	137.361	95.613	44.420	67.632	115.732	40.102
Ventral horn height						
2 – 3	8.915	6.901	14.257	14.870	2.365	4.046
3 – 4	70.810	63.291	21.534	29.734	43.030	35.556
4 – 5	23.414	3.488	52.597	23.965	42.611	3.279
2 – 5	129.597	80.650	111.897	84.740	108.802	45.665

the cord occurred because of the variation in the size and number of fibres forming the spinal nerves, which attached to the cord at different levels. He also found that the ventral funiculus primarily contained descending phylogenetically older tracts in mammals. Hence, its differentiation happened earlier than other funiculi in the present study.

The gray matter height expressed as percentage of spinal cord height was maximum at lumbosacral enlargement from third to fifth month (Table. 4) indicating a dominance of gray matter at the lumbosacral enlargement, which in turn is related to the increased need for use of the hindlimbs by goat, which fits well into their feeding habits. The goats put their whole weight on their hind limbs, resting their forelimbs on the shrubs while they nibble the leaves.

The dorsal horn and ventral horn height expressed as percentage of gray matter total height did not show any particular trend. But during major part of gestation, the ventral horn height expressed as percentage of gray matter total height was more than that of dorsal horn at lumbosacral enlargement indicating the dominance of the motor component. This may be due to the fact that the hind limbs are more involved in the motor activity of forward propulsion of the body, which makes them to be more powerful than the forelimbs (Nickel *et. al*, 1986). Instead, at the cervical enlargement, the dorsal horn contribution was more than that by ventral horn during the major part of gestation, probably due to a comparative increase in the sensory component and a lesser motor involvement in the movement of the body since the forelimbs are concerned with only the support of the body, which is propelled from behind.

Sparing exceptions at the cervical region and lumbosacral enlargement region in third month, the percentage contribution of dorsal horn to spinal cord height also showed a decreasing trend. The maximum value was recorded at the sacral region upto third month,

Table 6. Percentage increase in width of precoccygeal spinal cord between age groups (µm)

Parameters and period (month)	Regions					
	Cervical	Cervical enlargement	Thoracic	Lumbar	Lumbosacral enlargement	Sacral
Spinal cord width						
2 – 3	2.073	10.039	9.018	19.215	4.417	23.810
3 – 4	67.441	85.155	45.416	48.414	51.830	28.846
4 – 5	79.210	56.706	82.435	71.774	78.290	3.731
2 – 5	206.292	219.275	189.213	203.924	182.656	65.476
Central canal to spinal cord left margin						
2 – 3	1.533	7.291	7.276	18.044	4.925	24.499
3 – 4	71.632	90.901	49.327	51.689	53.878	27.135
4 – 5	77.887	56.549	83.134	72.742	78.470	2.713
2 – 5	209.991	220.640	193.367	209.311	188.151	62.577
Central canal to gray matter left margin						
2 – 3	17.197	17.338	19.572	8.365	15.492	23.702
3 – 4	45.354	41.045	31.254	23.622	28.155	25.781
4 – 5	43.196	32.760	13.706	39.554	41.288	2.300
2 – 5	143.935	119.717	78.453	86.950	109.120	59.170
Dorsal horn width						
2 – 3	56.563	40.894	14.395	8.556	14.491	17.391
3 – 4	18.132	19.833	6.444	22.311	22.707	15.741
4 – 5	6.935	10.787	29.270	15.673	24.131	34.800
2 – 5	97.771	87.050	57.406	53.586	74.390	83.152
Ventral horn width						
2 – 3	40.480	13.114	25.614	9.811	11.159	27.150
3 – 4	40.423	38.670	8.143	6.721	24.185	2.416
4 – 5	21.835	62.188	13.359	25.141	57.612	48.255
2 – 5	140.339	154.400	53.089	46.655	117.573	93.059

but for the last two months, it was at the lumbosacral enlargement (Table 4).

The highest percentage contribution of ventral horn height to spinal cord height was in lumbosacral enlargement throughout the gestation (Table 4). Similar to the percentage height of dorsal horns, all regions showed a decreasing trend in the ventral horn height expressed as percentage of spinal cord height also, except the thoracic and lumbar regions in third month and cervical enlargement and sacral regions in the fourth month.

A greater rate of growth in the height of spinal cord, gray matter, dorsal horn and ventral horn were seen during the latter half of gestation in all regions (Table 5). This partially agrees with the observation in goat foetuses by Taluja *et al.* (1990) who found a greater rate of increase for the height and other vertical distances in latter half of gestation in all regions except at L5 segment. In the present study, the spinal cord width at all regions showed greater percentage of increase between third and fifth month except the sacral region, which showed a decreasing growth rate towards the end of gestation indicating the dedifferentiation towards the caudal end (Table 6).

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