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# **RESEARCH ARTICLE**

### **PROGRESS OF MYELINATION IN THE SPINAL CORD OF FOETAL GOAT**

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# **INTRODUCTION**

Myelin is an electrically-insulating dielectric material that forms a layer, the myelin sheath, surrounding the axons of many neurons, and that is essential for proper functioning of the nervous system. It is an outgrowth of glial cells, viz. the Schwann cells supply the myelin for peripheral neurons, whereas oligodendrocytes supply it to those of the central nervous system. Myelin made by different cell types varies in chemical composition and configuration, but performs the same function. Myelin is composed of about 80% lipid and about 20% protein (Ghooray and Martin, 1993). Even though the oligodendrocytes, as astrocytes, are able to respond to changes in the cellular and extracellular environment, possibly in relation to a glial network, the mutations affecting the most abundant protein in the central nervous myelin, the proteolipid protein, lead to dysmyelinating diseases in animals and human, viz. jimpy mutation and Pelizaeus-Merzbacher disease or spastic paraplegia, respectively (Nicole and Danielle, 2001). Since the information about the onset of myelination in the caprine spinal cord is sparse, this study was undertaken to elucidate the gradual changes which occur during myelin formation in foetal goat.

### **MATERIAL 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 of the foetus in g, t = Age of the foetus in

#### ABSTRACT

Onset of myelination in the spinal cord in goat was studied using 52 foetuses. Oligodendrocytes became distinguishable by 40 days. By the third month of gestation, the tracts bordering the ventral median fissure showed clear presence of lipids indicating the onset of myelination. The oligodendrocytes started to curve around axons to form myelin sheath by 142 days showing that the fibre tracts of spinal cord became differentiated towards the end of gestation in goat. This study revealed that the fibre tracts of the spinal cord in goat became myelinated towards the end of gestation, to make the new-born kid well suited to be classified as a 'mature' young one.

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days. The foetuses were grouped into five age groups corresponding to five months of gestation. The fixative used was 10 per cent neutral buffered formalin (NBF). Embryos and small foetuses upto 50 days of age were fixed as such. In foetuses from 50 days to 90 days of age, the spinal cord within the vertebral column was processed after cutting into regionwise pieces and serial sections were made. In foetuses above 90 days of age, the spinal cord was exposed by laminectomy, dissected out from the vertebral canal and cut into several pieces of two to three segments each and processed. The specimens were washed, dehydrated and embedded in high melting paraffin (MP 58-60°C) and serial sections of 5µm thickness were cut. Routine and special histological techniques for central nervous system were employed (Luna, 1968). The micrometrical data were recorded using an ocular micrometer.

### RESULTS

During the first month of gestation by 24 days, the primordium, viz. the neural tube presented three layers: inner ependymal, middle mantle and outer marginal layers. The ependymal layer was stratified with dividing neuroepithelial cells. Mantle layer was densely packed with developing neuroblasts and glioblasts. Glioblasts, the precursors of glial cells were small with round nuclei and exceeded the neuroblasts in number (Fig. 1). Marginal layer was predominated by the processes of neuroblasts of the mantle presented only laver and verv few glioblasts. Oligodendrocytes were first distinguished in the marginal layer by 40 days in second month with a size of 3.6 to 7.2  $\mu$ m,



1. Neuroblasts 2. Glioblasts Fig. 1. L.S. of neural tube wall at 24 days. H & E x 400.



 Astrocyte, 2. Oligodendrocyte, 3. Neuroblasts 4. Blood vessel Fig. 2. Spinal cord at 48 days. H & E x 400



Neurons, 2. Piamater, 3. Oligodendrocyte 4. Astrocyte
Fig. 3. Spinal cord at 62 days. H & E x 400

rounded nucleus and scanty cytoplasm (Fig. 2). As age advanced, these cells occupied both the gray and white matter with progressive increase in density. In third month, by 62 days, oligodendrocytes occurred as three types, viz. perineuronal satellites in gray matter (Fig. 3), interfascicular cells in white matter and juxtavascular cells near blood vessels. The progress in myelination during foetal life was subjected to variation between regions and groups. The lipids were detected for the first time in the white matter during the third month of gestation in the present study (Fig. 4), even though a well defined myelin sheath was observed around



1. Tracts near the ventral median fissure, Ventral median fissure 3. Piamater Fig. 4. C.S. of first cervical segment (81 days). Oil red 'O' x 100



Fig. 5. C.S. of first thoracic segment showing myelin sheath (M) in white matter at 142 days. Sevier Munger Method x 400



1.Gray matter, 2. White matter, 3. Piamater 4. Blood vessel Fig. 6. C.S. of first cervical segment (102 days). Oil red 'O' x 100

the axons only towards the end of gestation (Fig. 5). By the third month of gestation, the tracts in the ventral funiculus showed lipids (Fig. 6). Oligodendrocytes, which accounted for the formation of myelin, were numerous by the beginning of the fifth month. They started to curve around axons to form myelin sheath by 142 days of gestation (Fig. 7) and attained a size of 11.4 to  $15.2 \mu$ m. From the onset of myelin formation, their number also increased, and hence they could be seen in abundance. Towards the end of gestation, concentric layers of myelin (Fig. 8) also could be observed around axons in this study. The processes of multipolar neurons extending through



1. Oligodendrocyte 2. Neuron 3. Axon with myelin sheath

Fig. 7. White matter at third lumbar segment (142 days). H & E x 400



1. Axon, 2. Myelin sheath



the white matter could be well demonstrated towards the end of gestation as both bundles and tracts. In addition to the oligodendrocytes forming myelin sheaths, other oligodendrocytes with definite cell boundaries without forming the myelin sheath were also seen associated with nerve tracts as interfascicular cells and free cells in the white matter. So the white matter became a mixture of myelinated and nonmyelinated axons, blood vessels and neuroglia towards the end of gestation.

#### DISCUSSION

Oligodendrocytes were first distinguished in the marginal layer by 40 days. With advancing age, by 62 days, these cells occurred as perineuronal satellites, interfascicular cells and juxtavascular cells. The location of oligodendrocytes associated with nerve tracts was analogous to that of the neurilemmal cells in the peripheral nervous system (Jenkins, 1978). The lipids were detected for the first time in the white matter during the third month, eventhough a well defined myelin sheath was observed around the axons only towards the end of gestation in the present study. Jenkins (1978) also reported that before any fibres at all had acquired a medullary sheath, the myelin substance was already present in nervous tissues. Since myelin functions on nerve fibres similar to an insulator on an electric conductor, the myelinated fibres become the largest and most rapidly conducting fibres in the

body with the rate of nerve impulse conduction directly proportional to the thickness of myelin sheath. The onset of myelination in the present study corresponded to that in other species, viz. in  $17^{th}$  week of gestation in cattle (Kostyra, 1958), between  $36^{th}$  and  $60^{th}$  days in pigs (Majstruk, 1967), between  $40^{th}$  and  $45^{th}$  days after mating in cat (Nilsson and Berthold, 1988) and by middle of the foetal life in man (Sadler, 2004). By the third month of gestation, the tracts in the ventral funiculus showed lipids indicating that the phylogenetically older tracts undergo myelination first than those of the other funiculi, as confirmed also in rabbit by 24 days (Sturrock, 1982).

An early myelination in the ventral funiculus which contains the reticulospinal tract and vestibulospinal tracts concerned with the extensor mechanisms and the maintenance of postural tone, accounts make the new-born kid a 'mature young one' capable of performing all the reflex activities at birth. This confirmed the opinion of Rizzo (2006) that a nerve fibre becomes functionally active and able to participate in reflex acticvity only when it is myelinated. There is a linear progression of chemical growth, viz. accumulation of DNA, protein and lipids in the spinal cord as reported by Patterson et al. (1971) in sheep. Considering the lipid accumulation, the cord differed from the remainder of the central nervous system in growing more after the period studied (i.e. 50-day-oldfoetus to five-week-old lambs) than before it. The present study confirms this observation as the amount of lipids, indicated by an intense reaction, increased as the age advanced. Oligodendrocytes, were numerous with larger size and increased formation of myelin sheath by fifth month. They curved around axons to form concentric layers of myelin sheath towards the end of gestation in this study. According to Jenkins (1978), oligodendrocytes formed myelin sheath around more than one axon (even upto 50 myelinated axons) at a time and also performed nutritive function. Chemically myelin is a lipoprotein consisting of thin concentric lamellae of protein alternating with layers of lipids in mammals (Jenkins, 1978). Since the formation of myelin sheath influences the speed of conduction along nerve cell processes, an early myelination in the new born kid may contribute to its faster sensory perception and better developed motor activities.

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