

HISTOARCHITECTURE OF MENISCI OF STIFLE JOINT IN PRE AND POSTNATAL STAGES OF BUFFALO (*BUBALUS BUBALIS*)

Bharath Kumar M.L.^{1,*}, Pramod Kumar D.² and Rajendranath N.³

ABSTRACT

Menisci is a fibrocartiliginous structure made of dense regular connective tissue and organized into different layers as age advanced along with cellularity of chondrocytes which made it fragile in older age group of animals and hence laminitic. Group 1 specimens had more of short wavy collagen bundles which reorganized into bundles in other groups. Group 3 had more of thick bundles and in Group 4 chondrocytes of dense cellularity was observed.

Keywords: *Bubalus bubalis*, menisci, stifle joint, pre-natal, post-natal, buffalo, chondrocytes, fibrocartilage

INTRODUCTION

Stifle joint a composite, complicated special joint constituted by a special structures like patellar tendons, menisci etc. having an outstanding role in locomotion of animals. Menisci, C shaped fibrocartilage gives an additional support between the two incongruent articular surfaces of tibia and

femur.

Wear and tear of Menisci are the most common problems in human populations for which surgical intervention is needed. Most commonly, overuse, age, and traumatic injuries cause structural damage to the menisci and stifle that may limit its function. Amongst animals especially in buffalo which has major share of GDP of Indian economy has least anatomical study regarding menisci. Hence thorough knowledge of histoarchitecture of menisci from prenatal to postnatal stages of buffalo is aimed in this present study.

MATERIALS AND METHODS

Intact stifle joint specimens of twenty four apparently healthy buffaloes and fetuses were procured. Aging of the post natal specimens was done by carefully noting the dentition pattern of the slaughtered animals as per FAO (1994). In case of prenatal specimens the CVRL (Curved Crown Rump length) of the foetus was measured and the approximate age was estimated by employing the formula given by Soliman (1975) *i.e.*, $Y = 73.544 \pm 2.256 X$ (if CVRL is > 20 cm). The

¹Department of Veterinary Anatomy, Bangalore, Karnataka Veterinary Animal and Fisheries Science University, Bidar, India, *E-mail: bharathkumarmml@gmail.com

²Department of Veterinary Anatomy, College of Veterinary Sciences, Rajendranagar, Sri PV Naramsimha Rao Telangana State University for Veterinary, Animal and Fishery Science, Hyderabad, India

³Department of Veterinary Anatomy, College of Veterinary Sciences, Korutla, Sri PV Naramsimha Rao Telangana State University for Veterinary, Animal and Fishery Science, Hyderabad, India

collected specimens were divided into four groups. Group 1 (prenatal) Group 2 (0 to 3 years) Group 3 (3 to 6 years) Group 4 (> 6 years). The menisci tissue pieces were collected and fixed in 10% NBF (Singh and Sulochana, 1997) for routine paraffin processing technique and cryosection with sections of 6 to 8 μm and subjected to the staining methods like H and E staining, Van Gieson's technique for collagen fibers (Luna, 1970) and Masson's Trichrome for metachromasia of cartilage matrix and collagen fiber differentiation (Singh and Sulochana, 1997).

RESULTS AND DISCUSSION

The menisci of stifle joint of buffalo in prenatal stages (Group 1) were primarily dense connective tissue made up of collagen fibres. In early stage fetuses, longitudinal sections of menisci revealed collagen fibres in cross sections close to the inner edge (Figure 1) along with short wavy fibres. Its outer edge was covered by liberal amounts of loose connective tissue (LCT) with plenty of blood vessels (Figure 2). The bulk part of the meniscus consisted of wavy bundles of collagen arranged loosely in circular profile and inter laced with fibrocytes and small thin capillaries. Outer LCT contained large veins, numerous small arterioles and capillaries surrounded by fibroblasts (Figure 2).

In late prenatal stage the menisci were well developed with bundles of tightly packed wavy collagen fibres along with fibrocytes (Figure 3). In cross sections thin connective tissue lined the surface below which densely stained nuclei were seen. Connective tissue septae made up longitudinal collagen fibres extended into the interior of the meniscus partitioning the collagen fibres into

bundles. Within the bundles cross sections of collagen fibres were observed intermingled with elliptical dense nuclei of fibrocytes (Figure 4).

In post natal Groups 2 and 3 basic micro-architecture of menisci was similar with prenatal groups except that the collagen fibres were thicker, matured and arranged in tightly packed thick bundles (Figure 5). In longitudinal sections connective tissue divided the wavy collagen fibers into multiple short bundles which were abruptly cut off and populated with small chondrocytes and fibrocytes (Figure 6). The latter cells located between the collagen bundles had elongated cytoplasmic processes. Chondrocytes were observed in wide expanse of connective tissue within the meniscus in Groups 3 and 4 (Figure 7 and 8). In last group menisci thick longitudinal bundles of collagen fibres divided the cross sectional profiles of collagen (Figure 8). These observations are in accordance with the histological features of menisci described by Hifny *et al.*, (2012a) in adult buffalo, Cheriveret *et al.* (2009) in humans and domestic animals and Kawamura *et al.* (2003); McDermott *et al.* (2010); Sun *et al.* (2012) in humans.

Meniscular fibrocartilage described by Shaw and Martin (1962) comprised dense bundles of collagenous fibers oriented predominantly along their long axis with interlacing. They observed that free margin of meniscus had hyaline cartilage with obvious presence of fibers but elsewhere cartilage was seen in small groups between fibers which decreased towards outer margin. They referred ossification areas as ossicles or lunulae which were uncommon in man and higher orders, but was seen in rodents and guinea pigs. Such lunulae were absent in this study.

Histological features described in ovine menisci by Meller *et al.* (2009) revealed three zones *viz.*, red-red zone, intermediate or red-white

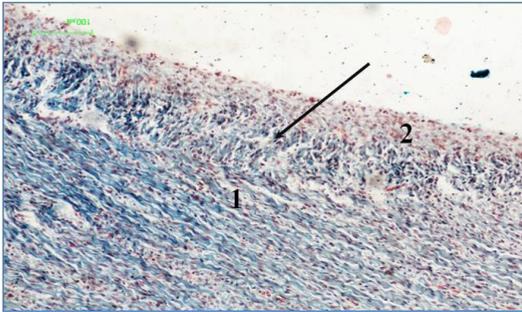


Figure 1. LS of menisci of Group 1 specimen aged 189 days (CVRL 51.2 cm) showing superficial part (1) with short wavy collagen fibers in cross (2) Mason's Trichrome 10X.

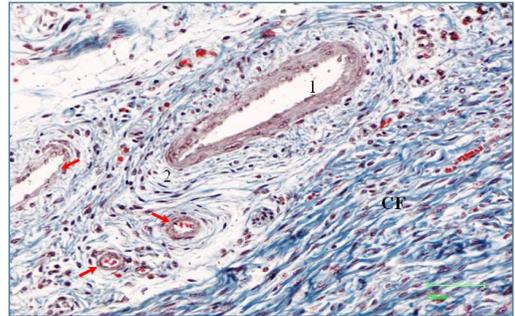


Figure 2. LS of outer surface of menisci of Group 1 aged 189 days (CVRL 51.2 cm) showing well developed connective tissue, blood vessels and capillaries. Mason's trichrome 10X.

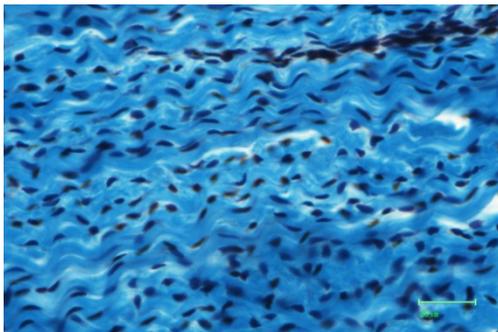


Figure 3. LS of menisci of late prenatal specimen aged 224 days (CVRL 67.6 cm) showing packed wavy collagen fibers. Mason's Trichrome 20X.

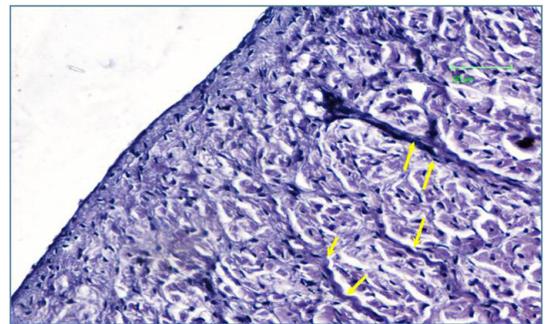


Figure 4. TS of menisci of Group 2 showing bundles of collagen fibers separated by well-developed connective tissue H and E 5X.

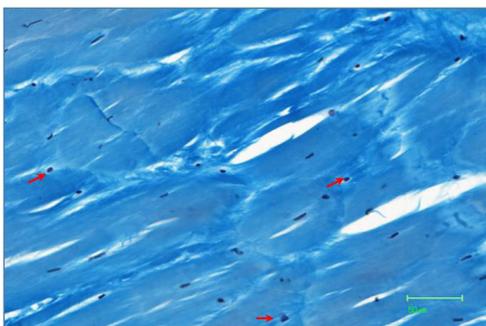


Figure 5. LS of menisci of Group 3 showing collagen fibers in multiple short bundles populated with fibrocytes and chondrocytes Mason's Trichrome 20X

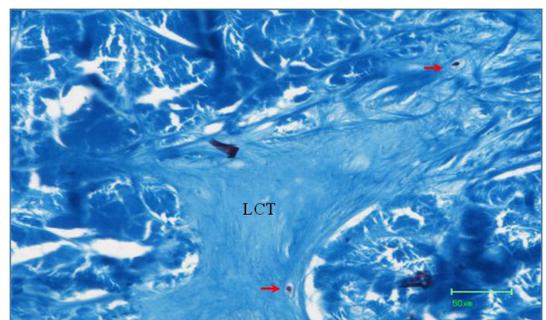


Figure 6. TS of menisci of Group 4 showing wide connective tissue in between bundles filled with chondrocytes. Mason's Trichrome 20X.

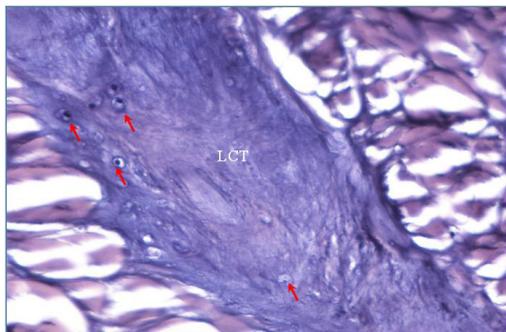


Figure 7. TS of menisci of Group 4 showing wide connective tissue in between bundles filled with chondrocytes. H and E 20X.

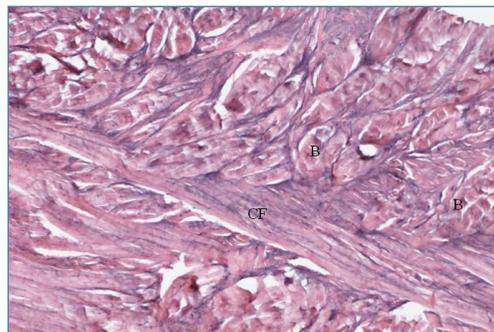


Figure 8. TS of menisci of Group 4 showing thick longitudinal bundle traversing cross section bundles of collagen. H and E 10X.

zone and the inner white-white zone of which former was rich in blood vessels in the periphery with circumferential fibre bundles and little ground substance which continued the intermediate or red-white zone with fewer blood vessels, increased ground substance and radial and circumferentially arranged collagen bundles. Innermost white-white zone devoid of blood vessels featured few fibres and loosely scattered cells embedded in extra cellular matrix composed mainly of ground substance. They noticed that blood vessel density was highest in one week old sheep which decreased with age. Difference between age groups was not much with respect to cellularity, fiber arrangement and cell morphology except for a slight decrease in cellularity from 1st to 18th week-old sheep.

The above description is in complete agreement with the findings in this study where in early stage fetal menisci revealed liberal amounts of loose connective tissue, few fibres and plenty of blood vessels in outer edge. Bulk part of the meniscus consisted of wavy bundles of collagen arranged in circular profile and inter laced with fibrocytes and small thin capillaries. Inner edge of menisci consisted short wavy collagen fibres

in cross sections with many fibroblasts. Outer and inner edges and bulk of menisci correspond to red-red zone, white-white zone and intermediate or red-white zone respectively reported by Meller *et al.* (2009).

CONCLUSION

Menisci was made up of dense collagen fibers of connective tissue with gradual increase in their size as age advanced. Menisci can be recognized into three distinct regions and chondrocytes population was more in aged specimens compared to more chondroblasts in Group 1 specimens implying fragile and impervious nature which may be the root cause in lameness of the animals.

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