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Age-Related Scanning Electron Microscopic Studies on the Articular Cartilage of Femoral Head in Buffaloes (*Bubalus bubalis)*

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ABSTRACT

Femoral head articular cartilages of 24 apparently healthy buffaloes irrespective of their breed, sex and nutritional status were procured from local slaughter houses in and around Hyderabad. Collected specimens were divided into four groups *viz.*, Group I (Prenatal stage)and three post-natal stages such as Group II (neonatal), Group III (young adult) and Group IV (aged). Scanning electron microscopic features of articular surface of femoral head articular cartilage in Group-I revealed a continuous surface without any break with longitudinal folds and numerous tightly packed chondrocytes, which were unidirectional and protruding outwards, separated by fine grooves. At much higher magnification, uniformly dispersed chondrocytes protruded as hemispherical, irregular or spindle-shaped elongations. In Group-II and III specimens articular cartilage surface of femoral head was smoother with few erythrocytes. Protrusions of chondrocytes were seen along with numerous minute debris of synovial fluid secretions. At the junction of articular cartilage and sub-chondral bone, cartilage matrix was smoother and osseous part comprised collagen fibres. Femoral head articular cartilage surface in specimens of Group III and IV revealed an uneven outline with deeply located cells and remnants of synovial fluid. Few cells were surrounded by a furrow caused by collapse of pericellular matrix. These findings demonstrate a progressive transition from a highly organized and proliferative chondrocytic arrangement in prenatal cartilage to smoother, functionally adapted surfaces in postnatal stages, followed by structural deterioration in aged animals. This study confirmed that the femoral head articular cartilage of buffaloes undergoes progressive degeneration as the age advances.

Key words: Buffalo, femoral head, articular cartilage, SEM

INTRODUCTION

Articular cartilage (AC) is a thin layer of connective tissue covering articulating ends of bones in synovial joints in mammals, birds and reptiles. In mammals, it consists of numerous chondrocytes, which make up approximately 2 to 5 % of the total volume of extracellular matrix (ECM) which is saturated with fluid. The interface with the bone is the so-called 'tide-mark' or chondro-osseous junction, which is an impermeable discrete band of mineralized cartilage (Van Turnhout, 2010).

Articular cartilage of femoral head plays a vital role in joint function, providing a smooth surface for movement and distributing loads to prevent bone damage.Age-dependent degeneration of the femoral head in human hip joints has been reported by Shanmugapriya *et al.* (2025), who highlighted a possible association between age-related degenerative changes and senile degenerative joint disease. Articular cartilage (AC) plays a central role in joint health and in conditions such as osteoarthritis it undergoes characteristic changes involving repair and remodeling (Taylor *et al.,* 2011). In the literature reviewed so far, many researchers have studied about articular cartilages of different joints of bovines and other animals. Most of their research was oriented towards humans and little work has been done on femoral head articular cartilage of animals, especially in buffaloes. Hip joint in large animals like buffaloes is an important joint involved in propulsive action in locomotion and also in weight bearing. Healthy AC is a key to increase productive health of a large animal, which is domesticated and used for various draft and other purposes in India. Keeping in view the clinical importance of articular cartilage in mammalian joints and also the use of buffaloes for agricultural purpose in India, the present investigation on articular cartilage of femoral head of buffalo hip joint is taken up to understand its microarchitecture, which is essential for effective

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treatment of age-related degenerative problems and diseases.

MATERIALS AND METHODS

Intact hip joint specimens of 24 apparently healthy buffaloes irrespective of their breed, sex and nutritional status were procured from GHMC Modern Abbattoir, Chengicherla and local slaughter houses in and around Hyderabad. Collected specimens were divided into four groups *viz.*, Group I (Prenatal stage) as per the formula of Soliman (1975) and three post-natal stagesas per their approximate age based on dentition pattern by FAO (1994) such as Group II (neonatal), Group III (young adult) and Group IV (aged). Samples were collected from both hind limbs by separating femur from joints on either side with intact femoral head and its articular cartilage. Ageing of prenatal specimens was determined by measuring the CVRL (Curved crown- rump length) of the foetus and its approximate age was estimated by the formula of Soliman (1975) *i.e.*, Y (Age of the foetus) =28.66 ± 4.496 X (where X is CVRL) , (if CVRL is d" 20cm) and Y (Age of the foetus) = 73.544 ± 2.256 X (if CVRL is e" 20cm). For post-natal specimens the dentition pattern of the animals before slaughter was carefully noted and approximate age assessed as per (Food and Agriculture Organization, 1994).

Details of the specimens collected along with grouping are shown in table 1. Soon after collection they were first cleaned with wet cloth to remove blood stains and they were packed neatly in polythene bags and kept in ice box for immediate transportation to the laboratory. Articular cartilage from femoral heads of all four groups was collected for ultra-structural studies and were transferred to vials and fixed in 2.5% Gluteraldehyde (EM grade) in 0.05 M phosphate buffer (pH 7.2) for 24 hrs at 40° C and post fixed in 2% aqueous Osmium tetroxide in same buffer for 2 hr. Post fixation, samples were dehydrated in series of graded alcohol and dried to critical point drying with CPD unit (EM Science). Dried samples were mounted over stubs with double- sided conductivity tape and coated by a thin layer of gold metal using an automated sputter coater (JEOL JFC-1600) for about 3 min (Bozzola and Russel, 1999). After the above procedure the samples were scanned in Ruska Lab under Scanning Electron Microscope (Model: JOEL-JSM 5600, JAPAN) at various magnifications and the SEM features were noted and photographed accordingly.

Table 1: Details of buffalo hip joint specimens collected

Sl. No.	Group No.	Animal details	No. of specimens	CVRL* (in cm)	Dentition** pattern	Approx. age (days) or years
1	I	Early prenatal (5 Nos.)	1	15.6	Not applicable	98
			1	16.6	- do -	103
			1	18.8	- do -	112
			1	19.2	- do -	114
			1	19.8	- do -	117
		Mid prenatal (1 No.)	1	25.3	- do -	131
2	II	Neonatal /Young	6	Not applicable	Deciduous teeth, central pair of incisors erupted	0 – 36 m or 0 - 3 yrs
3	III	Young Adult	6	-do-	Second pair of incisors or corners / lateral incisors erupted	3 – 6 yrs
4	IV	Aged / old	6	-do-	Full mouth, worn out teeth	6 yrs& =

Note: * CVRL – curved crown-rump length; m- months, yrs- years

RESULTS AND DISCUSSION

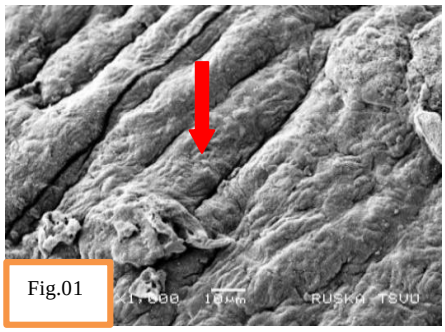
Scanning electron microscopic (SEM) features of articular cartilage (AC) of femoral head in Group-I of this study revealed a continuous surface without any break with longitudinal folds and numerous tightly packed chondrocytes which were unidirectional and protruding outwards, separated by fine grooves. Basically, the articular surface was even and regular with folds(Figs. 1, 2). At much higher magnification, uniformly dispersed chondrocytes protruded as hemispherical, irregular or spindle-shaped elongations. Their size ranged from 6.01 µm to 8.39 µm (Fig. 3).

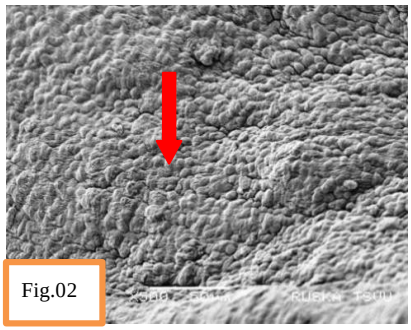
These findings are in concurrence with SEM studies of prenatal human femoral heads by Horky (1991a), who mentioned that observations revealed great changes on surface cartilage of human femoral heads in the period between 8th and 11th week after fertilization. He stated that up to 8th week of development, AC surface was uneven with numerous hemispherical or spindle-shaped elongated ridges and shallower or deeper grooves. The author stated that in 11th week of development, small groups of spindle- shaped ridges of chondroblasts appeared, whereas in greater magnification under SEM, cartilage surface of femoral heads showed tiny processes which gave a granular appearance. Horky (1991b) further stated that prenatal changes in human femoral head AC between 19 to 38 weeks under SEM showed spindle shaped elevations. From 36th week of development up to parturition the surface of AC under SEM was much less rough than in previous period with either solitary or pairs of low prominences which were chondroblasts. In higher magnifications grooves bordering prominences, separated from surrounding corrugated surface were seen.

Similarly, SEM studies of AC of femoral heads in Group-I showed a regular surface without any breaks, but studded with numerous chondroblasts projecting outwards on folded grooves.In Group-II and III specimens, AC surface of femoral head under SEM was smoother with few erythrocytes on surface. Mild protrusion of chondrocytes was seen along with numerous minute debris of synovial fluid secretions (Figs. 4, 5). At the junction of AC and sub-chondral bone, cartilage matrix was smoother and osseous part comprised collagen fibres (Fig. 6).

Above findings are in correlation with SEM features described by Horky (1980), who stated that joint cartilage surface of young human adults showed variations depending upon its load. In regions of less load, it showed slight undulation with almost parallelly arranged low ridges. In the region of more load, the author observed deep furrows with ridge- like protuberances. The author stated that in old persons ground amorphous substance decreased, which partly uncovered the superficial fibrils of cartilage. Similarly femoral head AC surface in specimens of Group III and IV revealed an uneven outline with deeply located cells and remnants of synovial fluid. Few cells were surrounded by a furrow caused by collapse of pericellular matrix (Fig. 7). In group IV,cross sectional SEMAC surface of femur showed finely cracked up longitudinal fissures with empty lacunae(Fig. 8). Chondrocytes were few and appeared as ridged projections on AC surfacewith wide intercellular spaces and extracellular matrix (Fig. 9).

Scanning electron microscopic features of femoral head AC were described by several authors in other animals such as Gardner and Woodward (1969) in guinea pigs, who reported that it was covered by broad, shallow dimples of 200-400 ¼m diameter except at the area adjacent to ligamentum teres. At higher magnification they noticed scattered deposits of altered synovial fluid. Similarly, Yan *et al*.(2014) cited that under SEM proximal tibial cartilage of guinea pigs showed normal cartilage with dense surface in one-month-old animals which by three months showed rough surface, ulcerations in 6-month-old animals, followed by collagen fiber degeneration into bundles and cracks in 9-month-old. Beyond this,it progressed to a severe state in 12-month-old animals. Horky and Tichy (2004) studied the AC of hip and humeral joints of male dogs by SEM and stated that both joints had similar structure. In young dogs the surface layer chondrocytes were spindle shaped and protruded above the level of surrounding extracellular matrix whereas in older dogs it had a different appearance. Few chondrocytes were surrounded by furrows caused by collapse of pericellular matrix. Similar features were appreciable in aged specimens of Group-IV in this study. These findings demonstrate a progressive transition from a highly organized and proliferative chondrocytic arrangement in prenatal cartilage to smoother, functionally adapted surfaces in postnatal stages, followed by structural deterioration in aged animals. This study confirmed that the femoral head articular cartilage of buffaloes undergoes progressive degeneration as the age advances.





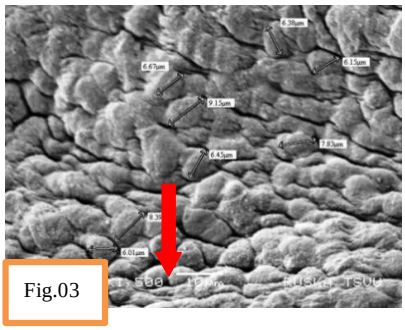
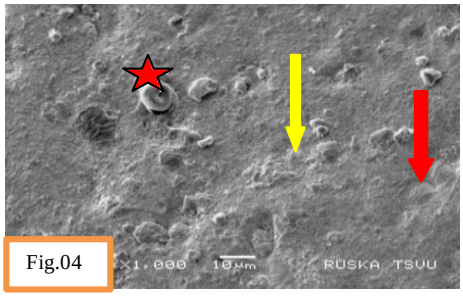
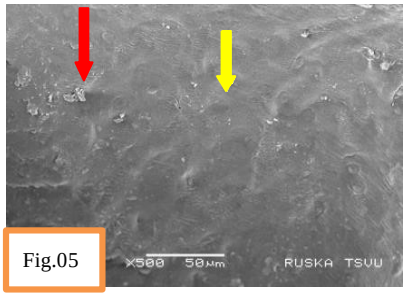


Fig.1 Scanning electron micrograph of femoral AC surface showing longitudinal folds (red arrow) in prenatal group aged 3months (Group- I). X 1000 **Fig.2** Scanning electron micrograph of femoral head AC showing tightly packed protruding chondrocytes in prenatal group aged 4 months (Group-I). X 300 **Fig.3** Scanning electron micrograph of femoral head AC showing semispherical, irregular or spindle shaped elongations in prenatal group aged 4 months (Group-I). X1500





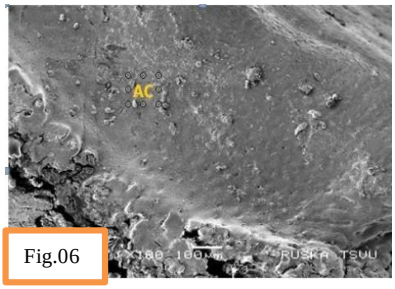
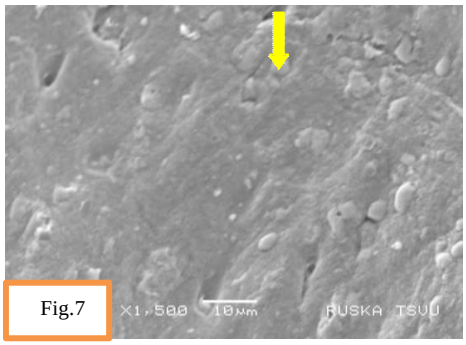
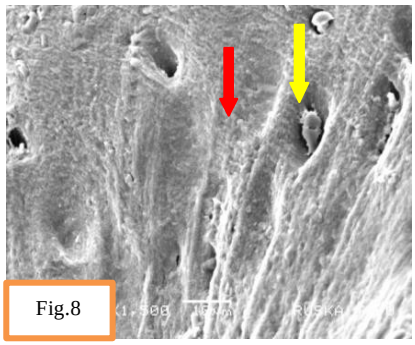


Fig.4 Scanning electron micrograph of femoral head AC showing protruding chondrocytes (red arrow) few erythrocyte (*) and secretions (yellow arrow) in 2.5-year-old buffalo (Group-II). X1000 **Fig.5** Scanning electron micrograph of femoral head AC showing deeply located chondrocytes (yellow arrow) and remnants of synovial secretions (red arrow) in 4.5-year-old buffalo (Group-III) X500 **Fig.6** Scanningelectron micrograph of femoral head AC showing junction of articular cartilage (AC) and subchondral bone in 2.5-year-old buffalo (Group- II). X100





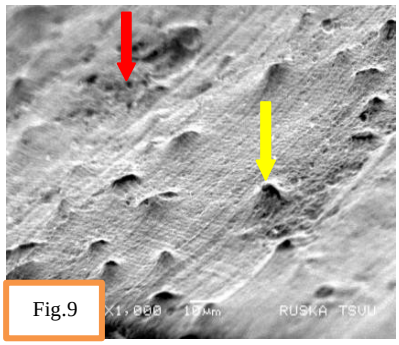


Fig.7 Scanning electron micrograph of femoral head AC showing protruded chondrocytes (yellow arrow) in 4.5-year-old buffalo (Group- III) X1500 **Fig.8** Cross sectional - Scanning electron micrograph of femoral head AC showing chondrocytes (yellow arrow) between the furrows (red arrow) in 5.5-year-old buffalo (Group-III) X1500 **Fig.9** Scanning electron micrograph of surface of femoral head AC showingridged chondrocytic projections (yellow arrow) and signs of wear and tear(red arrow) in 8years-old buffalo (Group-IV). X1000

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