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# The Role of Synovial Fluid Morphology in Joint Lubrication and Function

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#### ABSTRACT

Synovial joint lubrication is crucial for smooth movement and joint health. This study investigated the ultrastructural properties of synovial fluid in indigenous cattle to differentiate its characteristics from other species, aiding in improved prognosis and diagnosis of joint disorders. This study was conducted in Madhya Pradesh, India, and involved synovial fluid collection from the knee joints of two groups: young calves (6 months to 1 year) and adult indigenous cattle (4-5 years). Scanning electron microscopy (SEM) was used for detailed analysis. The observations revealed an intricate cross-linked network of filamentous structures and extracellular vesicles within the synovial fluid. These elements indicate a dual role in lubrication, combining both boundary and hydrodynamic mechanisms. However, the dominant presence of the filamentous network and unilamellar vesicles suggests that boundary lubrication may be the primary mechanism facilitating joint movement.

**Keywords:** Indigenous cattle, Extracellular vesicles, Synovial fluid, Scanning electron microscopy

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### Introduction

Synovial joints have long been a focus of research, particularly in humans, due to their susceptibility to various forms of arthritis that impact mobility. However, with shifts in livestock management practices—such as the adoption of intensive housing systems driven by limited grazing lands and urban expansion—joint-related conditions are becoming increasingly prevalent in animals as well. These issues not only affect their overall lifespan but also reduce productivity [1, 2].

Lubrication in synovial joints plays a vital role in ensuring smooth movement and preventing wear between articulating surfaces. It relies on both the structural characteristics of these surfaces and the composition of synovial fluid. Numerous theoretical and experimental studies have aimed to unravel the complex mechanisms behind synovial fluid lubrication in healthy joints and its role in joint degeneration [3]. Despite this research, a complete understanding of these processes remains elusive [4].

Several models have been proposed to explain how lubrication functions in synovial joints, with boundary lubrication and hydrostatic lubrication emerging as key theories that describe the interaction between joint components [5].

Researchers have noted that boundary lubrication plays a crucial role in reducing stick-slip motion, making it essential for smooth movement under high loads. In contrast, hydrodynamic fluid film lubrication happens when motion and deformation create pressure, driving the viscous lubricant between two surfaces.

## Marian et al.,

The pursuit of a single explanation for synovial joint lubrication has likely hindered a comprehensive understanding of how natural joint mechanics function. It is now believed that multiple lubrication modes may coexist within a single species [6]. Synovial fluid behaves as a pseudo-plastic (shear-thinning) fluid under both pathological and normal conditions. It also exhibits elasticity and generates a normal-force effect [7]. Low joint friction is typically associated with a full lubricant film that completely separates surfaces, whereas increased friction results from thin boundary films, where localized contact occurs between surface asperities [8].

Earlier, it was thought that hyaluronic acid viscosity was the primary factor influencing synovial fluid's lubrication properties. However, research by Tadmor *et al.* [9] suggests that hyaluronic acid alone does not provide exceptionally low friction. Instead, its association with phospholipids enhances its lubricating function, making it a more effective lubricant [10]. The lubrication capability of biochemical compounds depends not only on their concentration but also on how they interact at an ultrastructural level. Understanding the three-dimensional morphology of synovial fluid is therefore critical, and scanning electron microscopy (SEM) provides a powerful tool for detailed exploration of its structure [11].

Although various physicochemical techniques have been developed to characterize synovial fluid, the specific ultrastructural interactions across different species remain poorly understood. Some species have shown the presence of multilamellar vesicles and gel-type structures [12, 13], but how these patterns vary across species is still under study.

Given the critical structural and physiological roles of synovial fluid, the current research aimed to investigate its surface ultrastructure in indigenous cattle. The goal was to assess how it differs from other species, ultimately enhancing the prognosis and diagnosis of joint diseases in this particular species.

### **Materials and Methods**

The study took place in Madhya Pradesh, India, focusing on the examination of synovial fluid obtained from the knee joints of two distinct groups of Indigenous cattle: young calves (group I, 6 months to 1 year old) and adults (group II, 4-5 years old). For preservation, 200  $\mu$ l of synovial fluid was stored in 500  $\mu$ l of 2.5% glutaraldehyde solution with 0.05 M phosphate buffer, maintained at 4 °C for 6-8 hours. The fluid was then centrifuged at 8000 rpm for 10 minutes, yielding two sample sets. One set was filtered onto 0.22  $\mu$ m Millipore filter paper, while the other was placed on a cover slip and diluted with distilled water. After air-drying for two days, both samples were coated with gold particles. Scanning electron microscope imaging was performed using the Zeiss Evo 18 at SAIF, AIIMS, Delhi. Ethical approval for the procedure was given by the institutional ethics committee.

## **Results and Discussion**

Scanning electron micrograph of knee joint synovial fluid (group II, coverslip) displaying the cross-shaped stringlike network of an acid protein complex (arrow), synoviocyte B (arrowhead), and irregularly shaped cells (**Figure 1**).



**Figure 1.** Scanning electron micrograph of knee joint synovial fluid (group II, coverslip) displaying the crossshaped string-like network of an acid protein complex (arrow), synoviocyte B (arrowhead), and irregularly shaped cells (\*).

The same structure found in the synovial fluid of humans was previously noted by Walker *et al.* [14], with similar findings reported by Seller *et al.* [8]. Pasquali-Ronchetti *et al.* [15] also examined the interaction between hyaluronic acid of varying molecular weights and phospholipid vesicles. Their study indicated that the high molecular weight of hyaluronic acid contributes to the forming of sheet-like structures. They also suggested that temperature influences aggregation, with higher temperatures potentially affecting the melting process of lipid vesicles. Thus, the notable sheet-like structures observed in this study could be attributed to the tropical climate of the region.

The second type of extracellular vesicle found in bovine species exhibited a lamellated structure with a grey center surrounded by an electron-lucent periphery. While unilamellar vesicles were predominantly present, occasional oligolamellar vesicles were also observed (**Figure 2**).



Figure 2. Scanning electron micrograph of knee joint synovial fluid (group II, coverslip) showing electrondense layered vesicles (arrowhead).

Allen and Drauglis [16] examined boundary lubrication, noting that mono-lamellar films are ideal for boundary lubrication because of their ability to adhere to surfaces. However, multilamellar films also provide resistance to high shear forces, which allows them to endure greater pressure. Variations between species are likely linked to the movement type and the load applied.

Extracellular vesicles, which carry various compounds, have been suggested as potential markers of joint diseases' early detection [17]. Research by Matei *et al.* [12] and Ben-Trad *et al.* [13] found multilamellar vesicles in the synovial fluid of equine, human, and rat species, as well as in dogs and humans. Pasquali-Ronchetti *et al.* [15] proposed that low molecular weight hyaluronic acid keeps phospholipids in a vesicular state, implying that in healthy animals, the molecular weight of hyaluronic acid varies and maintains proper lubrication. However, disruptions in this balance can occur during disease. The differences in electron density at the core and edges of vesicles observed in the study are consistent with Sava *et al.* findings [4], where they noted that natural synovial fluid forms a gel structure, with hyaluronic acid and proteins forming the core and phospholipids surrounding it.

#### Conclusion

In conclusion, the identification of both cross-stringy networks and vesicular structures in the synovial fluid of cattle provides evidence for the involvement of both boundary and hydrodynamic lubrication mechanisms. The predominant presence of the cross-stringy network suggests that boundary lubrication plays a key role, likely due to the cattle's substantial weight of the body and slow, steady movement. The impact of dehydration and the warmer temperatures typical of tropical regions also cannot be overlooked. Future research is needed to explore how weight of body and temperature variations across different climates influence these mechanisms, to develop more effective treatments for arthritis.

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