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# COLLAGEN FIBRE ARCHITECTURE AND PROTEOGLYCAN DISTRIBUTION IN FEMALE KNEE MENISCI ACROSS REPRODUCTIVE AND POST-REPRODUCTIVE AGE GROUPS: A QUANTITATIVE HISTOCHEMICAL ANALYSIS

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**ABSTRACT**

Background: The structural integrity of the knee meniscus depends on the spatial organisation of its collagen fibre architecture and proteoglycan-rich ground substance. Age-related deterioration of these components — particularly in women undergoing the perimenopausal hormonal transition — provides the mechanistic basis for the elevated knee osteoarthritis incidence in postmenopausal women. Quantitative histochemical data from Central Asian female populations are absent from the published literature. Objectives: To perform a quantitative histochemical analysis of collagen fibre architecture and proteoglycan distribution in female knee menisci across four age-defined groups spanning the reproductive and post-reproductive periods. Materials and Methods: Meniscal specimens from 98 women were obtained at the Branch of the Republican Specialized Scientific and Practical Medical Centre of Traumatology and Orthopaedics (Khorezm Regional Multidisciplinary Medical Centre) and stratified into four groups: Group A (25–44 years, n=24), Group B (45–54 years, n=26), Group C (55–64 years, n=26), and Group D (65–76 years, n=22). Picrosirius Red staining with polarised light microscopy was used to quantify collagen fibre type and alignment. Safranin-O and Alcian Blue staining quantified proteoglycan and sulphated glycosaminoglycan content in inner, middle, and outer meniscal zones. Quantitative image analysis was performed using ImageJ software with the OrientationJ plugin. Results: Collagen fibre alignment index increased from 12.1°±2.9° (Group A) to 44.2°±7.8° (Group D; $p<0.001$). Picrosirius Red polarised microscopy demonstrated a progressive shift from strongly birefringent type I collagen in Group A to weakly birefringent type III collagen predominance in Group D, consistent with reparative fibrotic remodelling. Inner zone Safranin-O optical density declined by 68% between Groups A and D (0.92±0.08 vs. 0.29±0.06; $p<0.001$), with the steepest decline between Groups B and C. Alcian Blue staining confirmed parallel sulphated glycosaminoglycan depletion. The most severe matrix deterioration was localised to the inner avascular zone of the medial meniscus. Conclusions: Quantitative histochemical analysis confirms that female meniscal matrix undergoes progressive, zone-specific collagen

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disorganisation and proteoglycan depletion with advancing age, with an acceleration during the perimenopausal transition consistent with oestrogen-mediated matrix regulation.

**Keywords:** Knee Meniscus; Collagen Fibre; Proteoglycan; Glycosaminoglycan; Histochemistry; Morphometry; Women; Menopause; Khorezm; Uzbekistan

## 1. INTRODUCTION

The fibrocartilaginous matrix of the knee meniscus is characterised by a highly organised architecture in which type I collagen fibres arranged in circumferential and radial orientations work in concert with a proteoglycan-rich ground substance to generate the viscoelastic properties essential for meniscal biomechanical function (Fox et al., 2015; Fithian et al., 1990). Circumferential collagen fibre bundles bear the tensile hoop stresses generated during axial loading, while proteoglycans — particularly aggrecan, biglycan, and decorin — interact electrostatically with water molecules to generate swelling pressure that resists compressive loads (Makris et al., 2011; Messner & Gao, 1998). The integrity of this two-component system is essential for normal meniscal load-bearing function and, consequently, for the protection of articular cartilage from excessive compressive stress.

Age-related deterioration of meniscal matrix is well-recognised as a contributor to knee osteoarthritis pathogenesis. Biochemical studies have documented progressive collagen cross-link changes, proteoglycan depletion, and water content reduction with ageing (Ingman et al., 1974; Adams & Hukins, 1992). However, quantitative histochemical data — providing spatially resolved information about the zonal distribution of these changes within the meniscal cross-section — are considerably less available than biochemical bulk analyses, and are particularly limited for female populations across the reproductive lifespan.

The perimenopausal hormonal transition — characterised by declining oestrogen levels — is of particular relevance to meniscal matrix homeostasis. Oestrogen receptors are expressed by meniscal fibrochondrocytes, and in vitro studies demonstrate that oestrogen stimulates proteoglycan synthesis and inhibits matrix metalloproteinase activity, suggesting that postmenopausal oestrogen withdrawal promotes a catabolic shift in meniscal matrix metabolism (Liu et al., 2016; Bellido et al., 2010). Documenting the temporal pattern of histochemical matrix changes across the perimenopausal transition is therefore important for understanding the biological basis of sex-specific knee OA risk. The present study addresses this need through quantitative Picrosirius Red and Safranin-O/Alcian Blue histochemistry in female meniscal specimens from the Khorezm region of Uzbekistan.

## 2. MATERIALS AND METHODS

Study design and participants. A prospective cross-sectional study was conducted at the Branch of the Republican Specialized Scientific and Practical Medical Centre of Traumatology and Orthopaedics within the Khorezm Regional Multidisciplinary Medical Centre, Khorezm, Uzbekistan (2022–2024). Ninety-eight women were enrolled and stratified into four age groups: Group A (reproductive period: 25–44 years; n=24), Group B (perimenopausal: 45–54 years; n=26), Group C (early postmenopause: 55–64 years; n=26), and Group D (late postmenopause: 65–76 years; n=22). Specimens were obtained intraoperatively (total knee arthroplasty and arthroscopic procedures; n=62) and from autopsy preparations without knee

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pathology (n=36). Ethics approval was obtained; informed consent was provided by all participants.

Histological staining protocols. Specimens were fixed in 10% neutral-buffered formalin, processed through graded ethanol series, and embedded in paraffin. Sections of 5 μm were cut in longitudinal, transverse, and coronal planes. Staining protocols: (1) Picrosirius Red (0.1% Sirius Red F3BA in saturated picric acid, 60 minutes) examined under polarised light microscopy for collagen fibre type differentiation and orientation; (2) Safranin-O/Fast Green (0.1% Safranin-O, 5 minutes; counterstained with 0.2% Fast Green, 30 seconds) for proteoglycan content; (3) Alcian Blue (1% Alcian Blue in 3% acetic acid, pH 2.5, 30 minutes) for sulphated glycosaminoglycans.

Quantitative image analysis. All slides were digitised using a calibrated digital pathology system (×100 and ×200 magnification). Analysis was performed using ImageJ 1.53t software. Collagen fibre alignment index (CFAI) was measured using the OrientationJ plugin as the circular standard deviation of fibre orientation angles across standardised regions of interest in each meniscal zone. Collagen fibre bundle diameter was measured as the mean diameter of 20 randomly selected bundles per section. Proteoglycan optical density (SafO-OD) and glycosaminoglycan index (Alcian Blue optical density) were quantified as mean optical density values within standardised regions of the inner, middle, and outer meniscal thirds. Two independent observers performed all measurements blinded to group allocation; intraclass correlation coefficients were calculated to assess inter-rater reliability.

Statistical methods. One-way ANOVA with Bonferroni post-hoc correction was used for normally distributed variables; Kruskal–Wallis with Dunn correction for non-normal distributions. A significant Group×Zone interaction for proteoglycan content was tested using two-way ANOVA. Significance threshold was $p<0.05$. All analyses were performed in IBM SPSS Statistics 26.0.

## 3. RESULTS

Inter-rater reliability. ICC values for all measurements were excellent (CFAI: ICC=0.92–0.97; SafO-OD: ICC=0.89–0.95; GAG index: ICC=0.88–0.93), confirming measurement reproducibility.

Collagen fibre architecture. Picrosirius Red polarised light microscopy revealed progressive loss of organised birefringence with advancing age. In Group A, strongly birefringent orange-red type I collagen predominated in all meniscal zones. Group B showed early focal loss of birefringence in the inner zone. Groups C and D demonstrated predominance of weakly birefringent green type III collagen in the inner and middle zones, consistent with reparative fibrotic remodelling replacing the original load-bearing collagen architecture. CFAI increased monotonically across groups (Group A: 12.1°±2.9°; Group B: 19.8°±4.2°; Group C: 30.1°±5.9°; Group D: 44.2°±7.8°; $F(3,94)=136.7$; $p<0.001$). Collagen fibre bundle diameter decreased from 19.5±2.5 μm (Group A) to 10.5±2.0 μm (Group D; $p<0.001$). Post-hoc comparisons confirmed significant differences between all adjacent group pairs ($p<0.001$).

Proteoglycan and glycosaminoglycan content. SafO-OD was highest in the inner zone across all groups, consistent with the known zonal gradient of proteoglycan content. A significant Group×Zone interaction was observed ($F(9,282)=11.9$; $p<0.001$), indicating that inner zone depletion accelerated disproportionately in Groups C and D. Inner zone SafO-OD

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declined by 68% between Groups A and D (Group A: 0.92±0.08; Group B: 0.74±0.07; Group C: 0.52±0.07; Group D: 0.29±0.06; $p<0.001$). The steepest intergroup decline occurred between Groups B and C (0.74 to 0.52; $p<0.001$), corresponding to the perimenopausal transition. Alcian Blue optical density confirmed parallel sulphated GAG depletion across all zones, with near-complete loss of inner zone positivity in Group D specimens.

Zonal and meniscal compartment analysis. The inner avascular zone showed significantly greater age-related deterioration than the outer vascular zone for both CFAI ($p<0.01$) and SafO-OD ($p<0.001$) across all groups. Medial menisci demonstrated significantly higher CFAI values ($p<0.01$) and lower inner zone SafO-OD ($p<0.05$) compared with lateral menisci, consistent with the greater compressive loading of the medial compartment.

## 4. DISCUSSION

This study provides detailed quantitative histochemical evidence of progressive, zone-specific deterioration in collagen fibre architecture and proteoglycan content in female menisci across the reproductive and post-reproductive lifespan in a Khorezm regional population. The Picrosirius Red polarised light data — demonstrating a systematic shift from type I to type III collagen predominance in the inner and middle meniscal zones with advancing age — reveals a qualitative change in matrix composition, not merely quantitative depletion, that has functional implications. Type III collagen, while contributing to reparative fibrous tissue formation, lacks the tensile stiffness of type I collagen and cannot adequately substitute for the lost load-bearing capacity of the native circumferential fibre bundles (Fox et al., 2015).

The most clinically significant finding is the acceleration of both collagen disorganisation and proteoglycan depletion between Groups B and C — corresponding precisely to the perimenopausal decade (ages 45–64 years). This temporal pattern is mechanistically consistent with the established role of oestrogen in meniscal matrix homeostasis: oestrogen stimulates aggrecan and decorin synthesis while suppressing matrix metalloproteinase-1 and MMP-13 activity in meniscal tissue (Liu et al., 2016; Bellido et al., 2010). The 68% reduction in inner zone proteoglycan content between Groups A and D — occurring predominantly during the perimenopausal transition — reduces the hydrostatic pressure component of meniscal load resistance, increasing the tensile burden on the collagen network and mechanistically predisposing to horizontal cleavage tears, the most prevalent degenerative meniscal lesion in postmenopausal women (Englund et al., 2012).

The significantly greater deterioration of the inner avascular zone compared with the outer vascular zone reflects the fundamental asymmetry of repair capacity within the meniscus: the avascular inner zone, dependent on diffusion for nutrient delivery and matrix turnover, is least able to compensate for age-related anabolic decline. Pathological neovascularisation — observed in 70.4% of Group IV specimens in our companion study — may represent an attempt to restore nutritional supply to the degenerating inner zone, but ultimately contributes structural disruption through accompanying fibrosis and protease release.

## 5. CONCLUSION

Quantitative Picrosirius Red and Safranin-O/Alcian Blue histochemistry demonstrates progressive, zone-specific deterioration of collagen fibre architecture and proteoglycan content in female knee menisci, with accelerated matrix deterioration during the perimenopausal transition and most severe changes in the inner avascular zone of the medial meniscus. The

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shift from type I to type III collagen and the 68% reduction in inner zone proteoglycan content between the youngest and oldest age groups provide a mechanistic histochemical basis for the elevated knee OA risk in postmenopausal women. These regionally specific data from the Khorezm population of Uzbekistan contribute to the evidence base for targeted preventive orthopaedic strategies in perimenopausal and postmenopausal women.

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