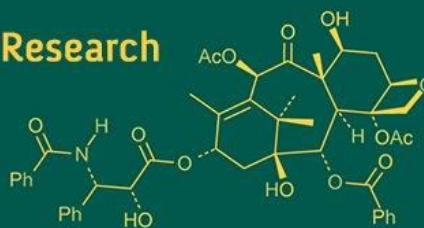


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Postnatal developmental changes in the glomerular filtration barrier of Indian domestic pig (*Sus scrofa domestica*)

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Abstract

This study explored the ultrastructural changes in the renal corpuscle (RC) of postnatal porcine kidneys using transmission electron microscopy (TEM). Conducted at the Department of Veterinary Anatomy, College of Veterinary Science, Rajendranagar, Hyderabad, the research utilized 18 pairs of fresh kidney samples from local slaughterhouses, categorized into three age groups: Group I (0-4 weeks), Group II (4-10 weeks), and Group III (>10 weeks). The glomerular filtration barrier (GFB) consists of glomerular endothelium, the glomerular basement membrane (GBM), and podocytes. In Group I, the GBM appeared as a dual-layered structure with distinct endothelial and epithelial lamina densa. In Groups II and III, these layers merged into a single GBM, shared by endothelial cells internally and podocytes externally. Filtration slits between podocyte foot processes were connected by slit diaphragms. The endothelial layer exhibited limited fenestrations in Group I, which increased in number with age. These fenestrations interfaced with the mesangium, supporting the GFB's structural integrity.

Keywords: Renal corpuscle, glomerulus, filtration barrier, podocytes, glomerular basement membrane

Introduction

The glomerular filtration barrier (GFB) serves as a critical interface in the kidney, selectively filtering blood to allow passage of small to medium-sized solutes while restricting macromolecules (Menon *et al.*, 2012) [11]. Comprising the capillary endothelium, glomerular basement membrane (GBM), and podocyte layer, the GFB ensures efficient filtration and structural stability (Stevens *et al.*, 1992) [15]. Alterations or deficiencies in slit diaphragm proteins can impair renal function. The porcine kidney, due to its anatomical and functional similarities to the human kidney, is a valuable model for experimental and therapeutic studies (Gomez *et al.*, 1999) [6]. As reports regarding the postnatal changes in the porcine kidney is limited, this study aimed to characterize age-related developmental changes in the GFB of postnatal Indian domestic pigs, focusing on ultrastructural transformations.

Materials and Methods

The study was conducted at the Department of Veterinary Anatomy, College of Veterinary Science, Rajendranagar, Hyderabad, India. Fresh kidney samples (18 pairs) from pigs were collected from slaughterhouses around Hyderabad and grouped by age: Group I (0-4 weeks), Group II (4-10 weeks), and Group III (>10 weeks). Tissue samples (1 mm³) were fixed in 2.5% electron microscopy-grade glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) at 4 °C for 24 hours, following protocols established at Ruska Labs, College of Veterinary Science, Hyderabad.

'TEM' working procedure

Kidney samples (0.5 × 0.5 mm) from each group were preserved in 2.5% glutaraldehyde (Sigma-Aldrich, USA; 0.1 M phosphate buffer, pH 7.3) at 4 °C for 24 hours. Samples were processed for TEM analysis as described by Lakshman (2017) [10]. After washing in buffer, tissues were post-fixed in 1% aqueous osmium tetroxide (Sigma-Aldrich, USA), dehydrated through increasing acetone concentrations, and embedded in Spurr's resin (Spurr, 1969; SPI

Supplies Araldite 6005 Embedding Kit, USA) [14]. Samples were incubated at 70°C for 24 hours in a universal incubator (NSW-151) for complete polymerization. Blocks were manually trimmed, and semithin sections (700-800 nm) were cut using an ultra-microtome (Leica Ultra-cut UCT-GAD/E-100, Germany), stained with 1% toluidine blue, and examined under a light microscope (Olympus-AX 70, USA) to identify target areas. Ultrathin sections (50 nm) were mounted on copper grids (SPI Supplies, USA), air-dried overnight, and stained with saturated uranyl acetate and 1% Reynold's lead citrate (Bozzola and Russell, 1998) [3]. Grids were observed under TEM at Ruska Labs, Department of Veterinary Pathology, following standard protocols. Microphotographs were captured and analyzed at various magnifications.

The study was approved by the Institutional Animal Ethics Committee (No. 39/24/IAEC-Pig/C.V.Sc/Hyd, dated 12.06.2021).

Results

The GFB comprised of endothelial cells, glomerular basement membrane (GBM) and podocytes. In Group I (0-4 weeks), the GBM appeared as a loose, amorphous dual layer, with one layer adjacent to podocytes and another to the endothelium, separated by mesenchymal matrix (Fig. 1). Fenestrations in the endothelial layer were sparse (Fig. 2). Podocytes displayed flat cytoplasmic extensions and foot processes. In Groups II (4-10 weeks) and III (>10 weeks), the GFB exhibited a mature trilaminar structure, with a compact GBM flanked by podocyte foot processes and fenestrated endothelium (Fig. 3). The GBM consisted of lamina rara interna, lamina densa, and lamina rara externa (Fig. 3). It appeared as a thick, moderately electron-dense structure. Tertiary podocyte processes formed small foot pedicels, and filtration slits were bridged by slit diaphragms (Fig. 4). Electron-dense granules, likely juxtaglomerular (JG) granules, were observed within the mesangial matrix (Fig. 5).

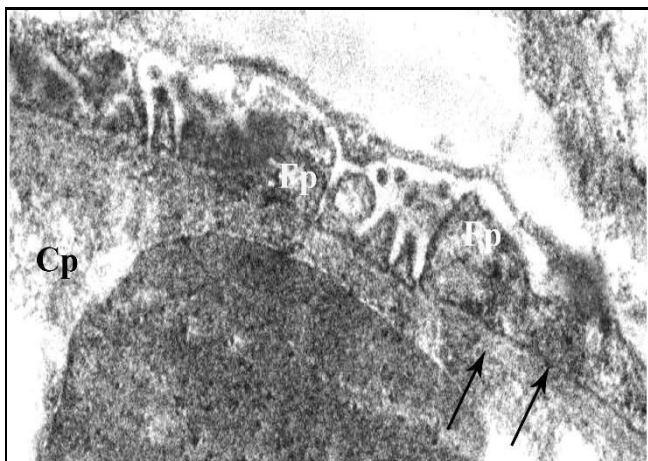


Fig 1: TEM image of a 7 PND glomerulus (Group I) showing podocyte foot processes (Fp) and dual basement membranes (arrow). Capillary (Cp). TEM 6000x

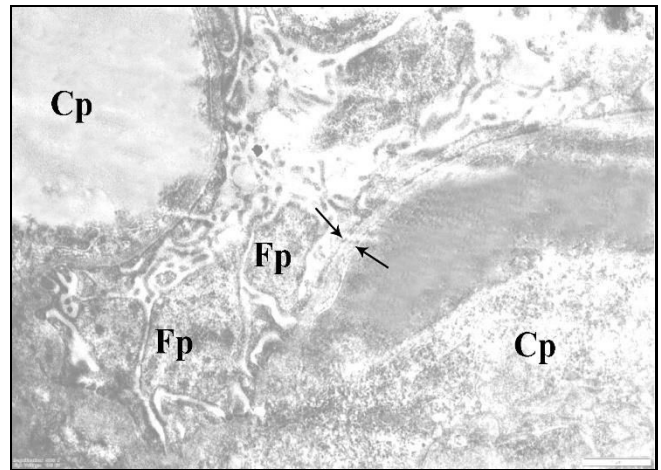


Fig 2: TEM image of a 7 PND glomerulus (Group I) displaying podocyte foot processes (Fp) and dual basement membranes (arrow) TEM 3000x.

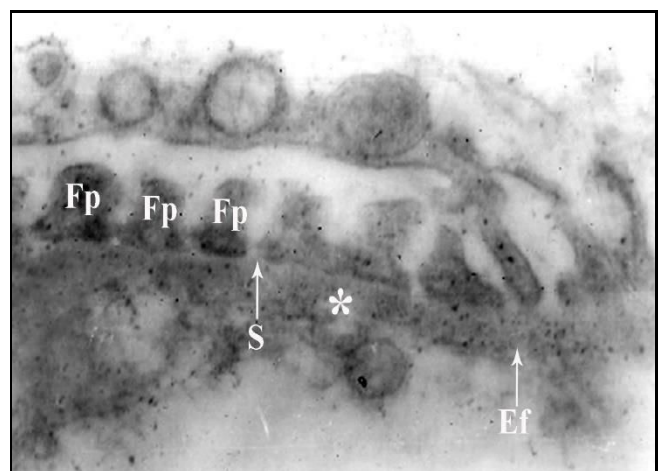


Fig 3: TEM image of a 152 PND glomerulus (Group III) showing the GFB(*) with foot processes (Fp), endothelial fenestrae (Ef), and slit diaphragm (S). TEM 11580x.

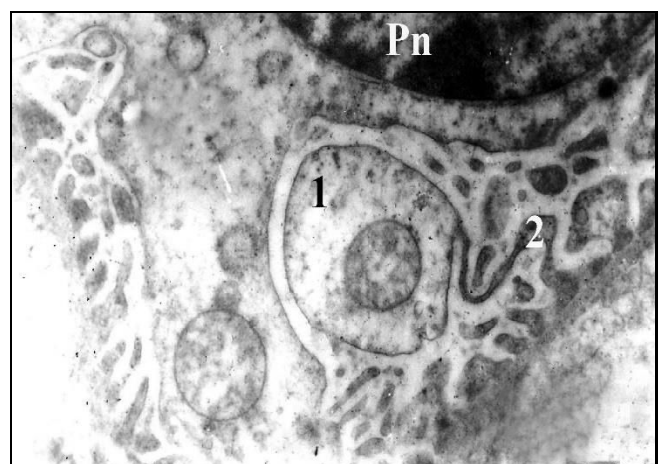


Fig 4: TEM image of a 6 PND glomerulus (Group I) showing podocyte nucleus (Pn), primary (1), secondary (2), and tertiary (3) processes. TEM 5790x.

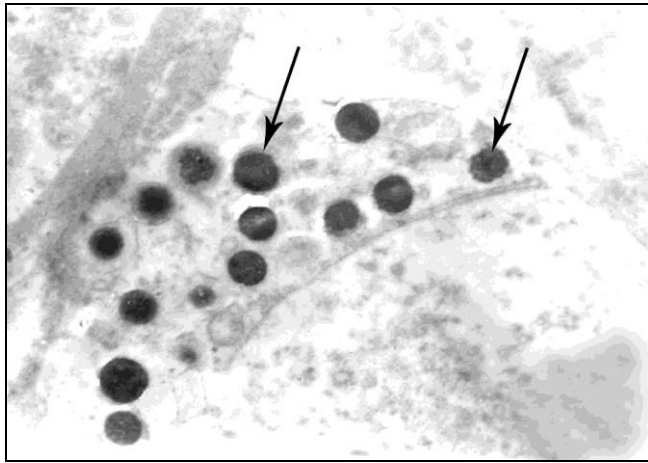


Fig 5: TEM image of a 152 PND glomerulus (Group III) showing electron-dense JG granules in the mesangium (arrow). TEM 15440x

Discussion

The GFB's trilaminar architecture—comprising glomerular endothelium, GBM, and podocytes—supports the glomerular tuft's function. In Group I, a dual GBM with distinct epithelial and endothelial lamina densa was observed, consistent with early developmental stages reported in rats (Sariola *et al.*, 1984; Abrahamson, 1987) [12, 2]. Podocytes exhibited flat cytoplasmic sheets and limited endothelial fenestrations, aligning with findings in rats (Abrahamson, 1985; Watanabe *et al.*, 1996; Şimşek *et al.*, 2009; El-gammal *et al.*, 2010) [1, 16, 13, 5]. In Groups II and III, the dual GBM fused into a single layer shared by endothelial cells and podocytes, with filtration slits bridged by slit diaphragms. This mature structure resembles observations in dogs (Bulger *et al.*, 1979) [4] and humans (Kanwar and Venkatachalam, 1992; Haraldsson *et al.*, 2008) [8, 7]. Endothelial fenestrations, opening into the mesangium, facilitate plasma filtration, consistent with mammalian studies (Kriz and Kaissling, 1992) [9]. The GBM's electron-dense, amorphous nature and increased fenestrations in older groups suggest progressive maturation, as noted in rats (El-gammal *et al.*, 2010) [5].

Conflict of Interest

The authors declare no conflicts of interest.

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