**Title: Nox5 Deletion Confers Renal Protection in a Rabbit Model of Diabetic Kidney Disease**

**Background**

Oxidative stress, resulting from elevated reactive oxygen species (ROS), is a key driver of diabetic kidney disease (DKD). Among ROS-generating enzymes, NADPH oxidase 5 (NOX5) has emerged as a major contributor to DKD pathogenesis. However, the absence of NOX5 in rodents has limited prior studies to in vitro systems and transgenic mice expressing human NOX5. In contrast, rabbits naturally express NOX5, providing a physiologically relevant model to investigate its role in DKD.

**Aims**

Our aim was to generate a novel Nox5 knockout rabbit model and conduct a comprehensive investigation into the role of NOX5 in DKD.

**Methods**

A novel Nox5 knockout (KO) rabbit model was generated using CRISPR/Cas9 gene-editing technology. Wild-type (WT) and Nox5 KO rabbits aged 8–10 weeks were randomized to receive a single intravenous injection of alloxan (150 mg/kg) to induce diabetes or saline as a control. After 16 weeks of sustained hyperglycaemia, all animals were humanely euthanized. Kidney tissues, along with plasma and urine samples, were collected for comprehensive analysis of renal parameters, including albuminuria, renal injury, fibrosis, oxidative stress, endoplasmic reticulum (ER) stress, and apoptosis.

**Results**

Diabetic WT rabbits developed significant renal injury, demonstrated by a 13-fold increase in albuminuria, a 2-fold increase in mesangial expansion, and a 6-fold increase in renal fibrosis (fibronectin). These changes were associated with elevated renal oxidative stress (nitrotyrosine, 5-fold), ER stress (CHOP, 6-fold), and apoptosis (Bax/Bcl-2 ratio, 6-fold) compared to non-diabetic controls. In contrast, diabetic Nox5 KO rabbits exhibited marked attenuation of these responses, including a 70% reduction in albuminuria, a 40% reduction in fibrosis, and reductions in oxidative stress (75%), ER stress (55%), and apoptosis (75%) relative to diabetic WT rabbits.

**Conclusion**

These findings provide the first direct evidence implicating NOX5 in the pathogenesis of DKD, highlighting it as a potential therapeutic target for human DKD.