

Placental DEPTOR inhibition restores maternal vasodilation in FGR mice.

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BACKGROUND

- Fetal growth restriction (FGR) is a common pregnancy complication characterized by a reduction in expected fetal growth.¹
- The mechanistic target of rapamycin (mTOR) kinase is the catalytic subunit of two complexes, called mTOR complex 1 and 2 (mTORC1 and mTORC2). Studies have shown mTORC1 and mTORC2 are decreased in human placentas from FGR pregnancies as well as in animal models of nutrient-restriction FGR, suggesting placental mTOR signaling links maternal nutrient availability to fetal growth.^{2,3,4,5,6}
- DEP-domain-containing mTOR-interacting protein (DEPTOR) is an endogenous inhibitor of mTOR signaling that downregulates mTORC1 and mTORC2 activity.⁷ It has been shown that silencing DEPTOR in the placenta increases trophoblast amino acid transport and mitochondrial respiration.^{8,9}
- Vasodilation of the uterine arteries (UtA) is a key component of this adaptation as pregnancy progresses. Consistently, reduced UtA vasodilation and blood flow has been observed in human FGR pregnancy and FGR animal models compared to normal fetal growth pregnancies.^{10,11}

METHODS

Animals. Pregnant females were divided into two dietary groups: a control group was fed a diet comprised of 20% protein, while a low protein group was fed a diet comprised of only 6% protein. At GD14.5, pmGENIE-shRNAmir constructs targeting DEPTOR (FGR+DEPTOR) or scrambled sequences (FGR+Scr) were delivered to each placenta via sonoporation in the FGR group. Control diet dams received pmGENIE-shRNAmir construct with scrambled sequence (Control+Scr). At GD18.5, dams were euthanized; right and left UtA were isolated and either used for myography or histological sectioning.

Wire Myography. Isolated vessels (Figure 1) were mounted in a small-vessel wire myograph (Figure 2). UtA were constricted with 120 mM KCl to establish viability. The full contractile response to phenylephrine was determined across 1 nM - 100 μ M range. Vasodilation was tested by pre-constricting the vessels with 10 μ M PE for 15 min and then applying increasing concentrations of vasodilators: acetylcholine (ACh, 0.1 nM - 100 μ M); bradykinin (BK, 0.1 nM - 1 μ M); or the nitric oxide (NO) donor sodium nitroprusside (SNP, 0.1 nM - 100 μ M).



Figure 1

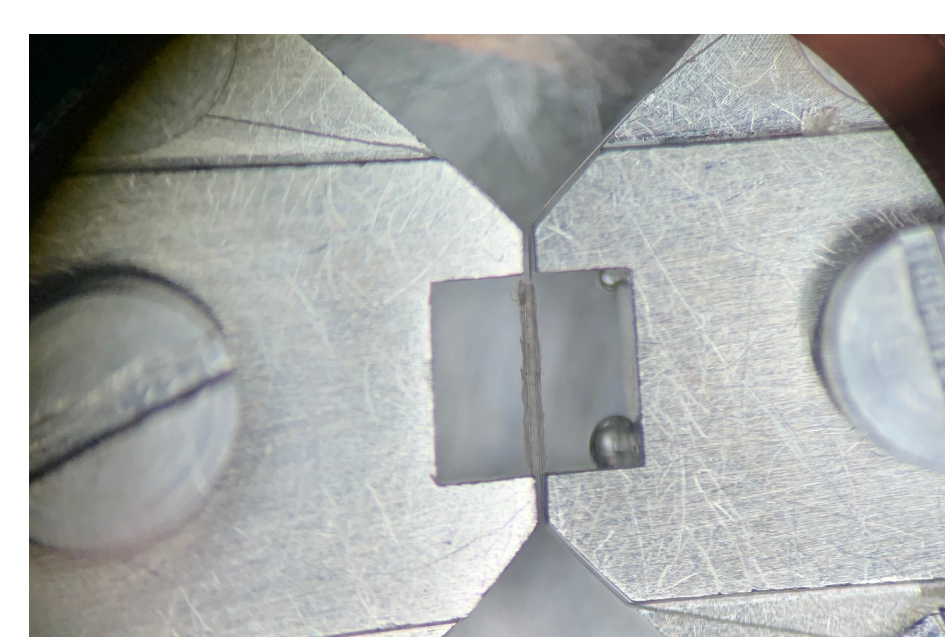


Figure 2

Histology. Samples were attached to slides and underwent a hematoxylin and eosin staining process. Stained vessels were imaged via bright field microscopy. Lumen perimeter and cross-sectional area (CSA) were measured by determining the area encompassing the tunica intima and tunica media, excluding the luminal area.

RESULTS

Maternal UtA Vascular Responses

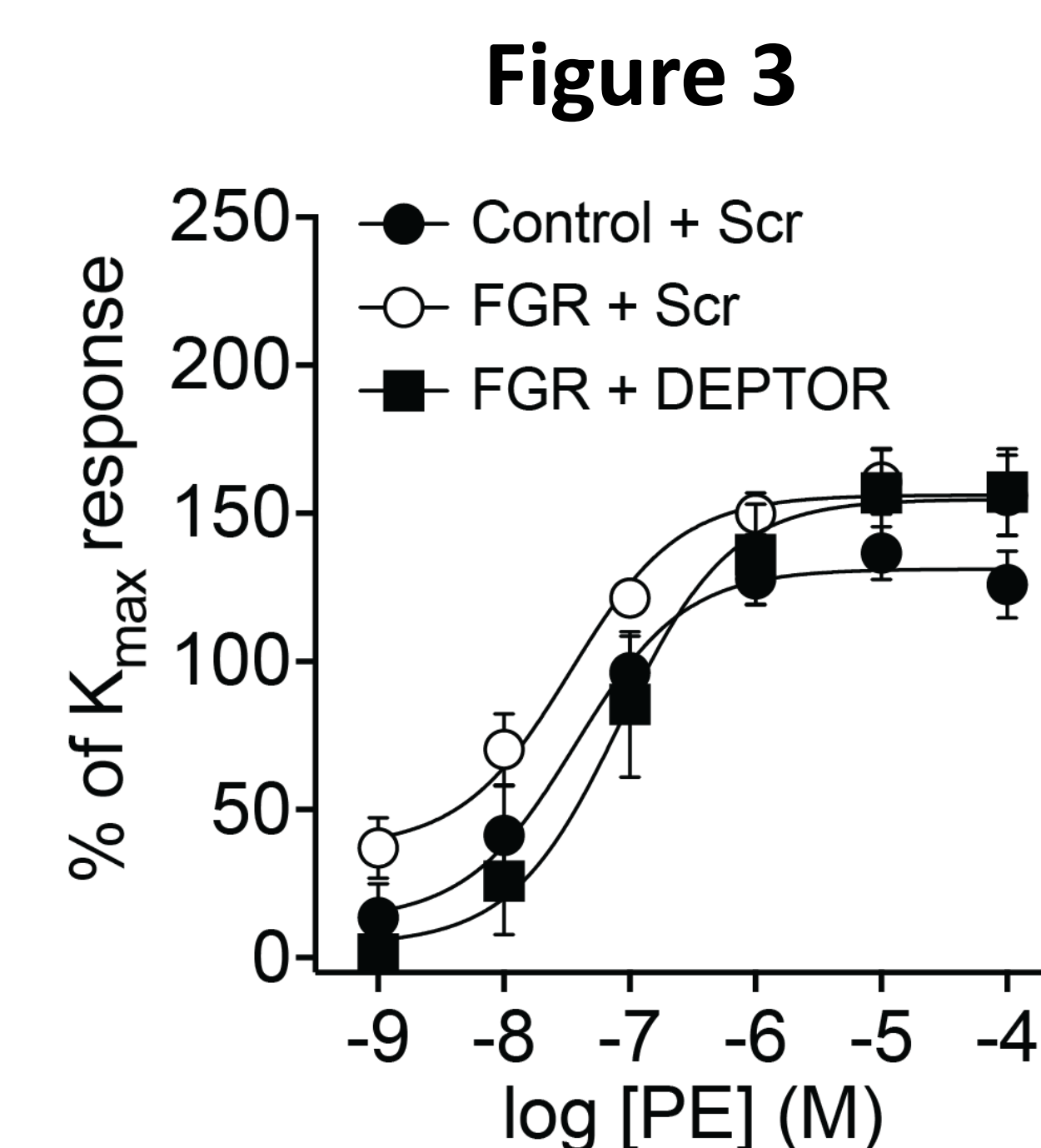


Figure 3. Contractile response curves to phenylephrine (PE) in Control+Scr, FGR+Scr, and FGR+DEPTOR pregnant mice. Vasoconstriction of uterine arteries was similar between all groups with no significant differences.

Figure 4

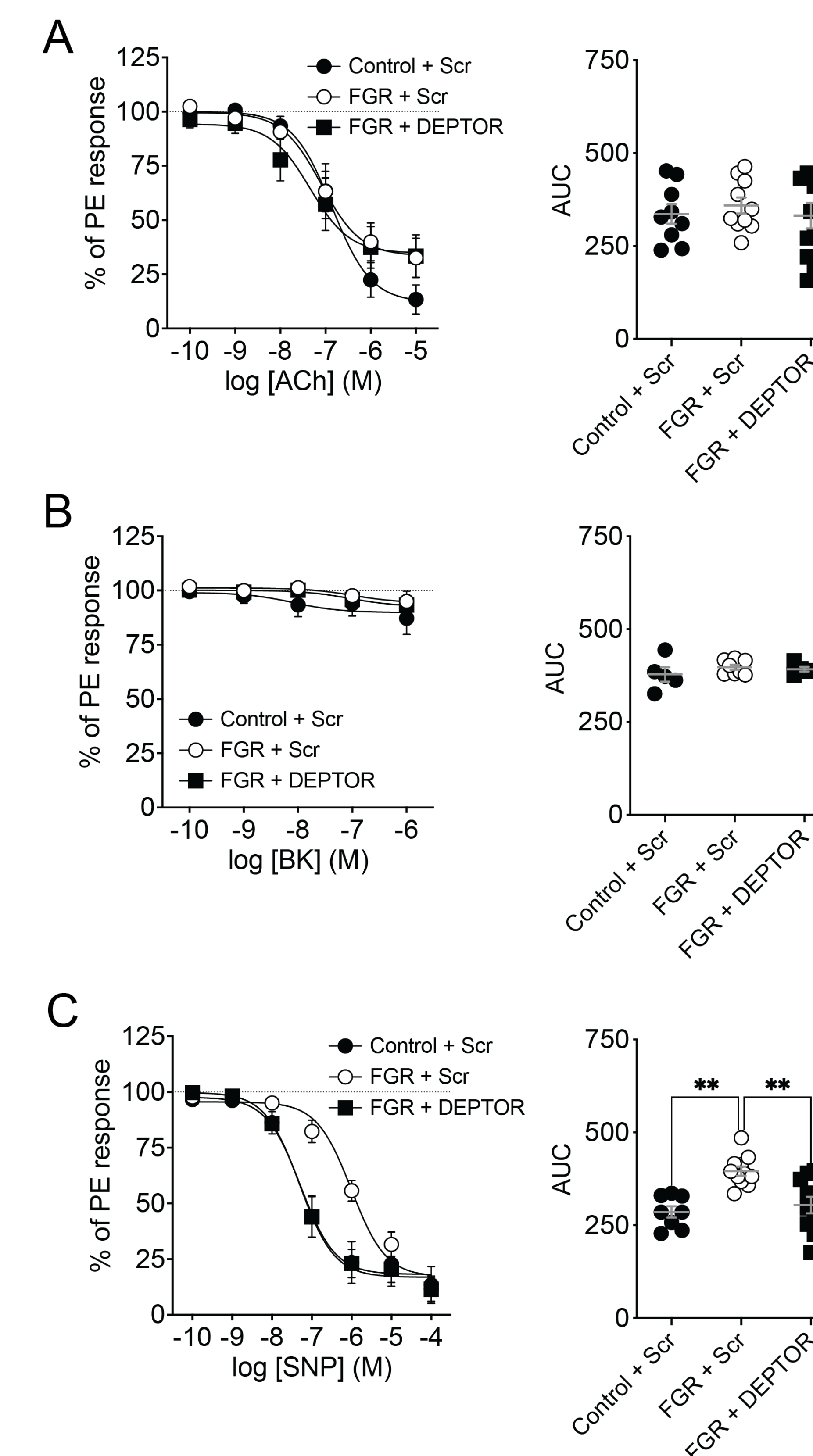


Figure 4. Vasodilation in response to ACh was similar in FGR+Scr vs. Control+Scr mice as well as FGR+Scr vs. FGR+DEPTOR mice (Figure 4A). BK induced minimal vasodilation in UtA that was not different between groups (4B). UtA from FGR+Scr mice showed reduced vasodilation in response to the NO donor SNP compared to Control+Scr (4C, $p = 0.0007$). UtA from FGR+DEPTOR presented increased SNP responses compared to UtA from FGR+Scr mice (4C, $p = 0.002$).

Maternal UtA Vascular Structural Analysis

Figure 5

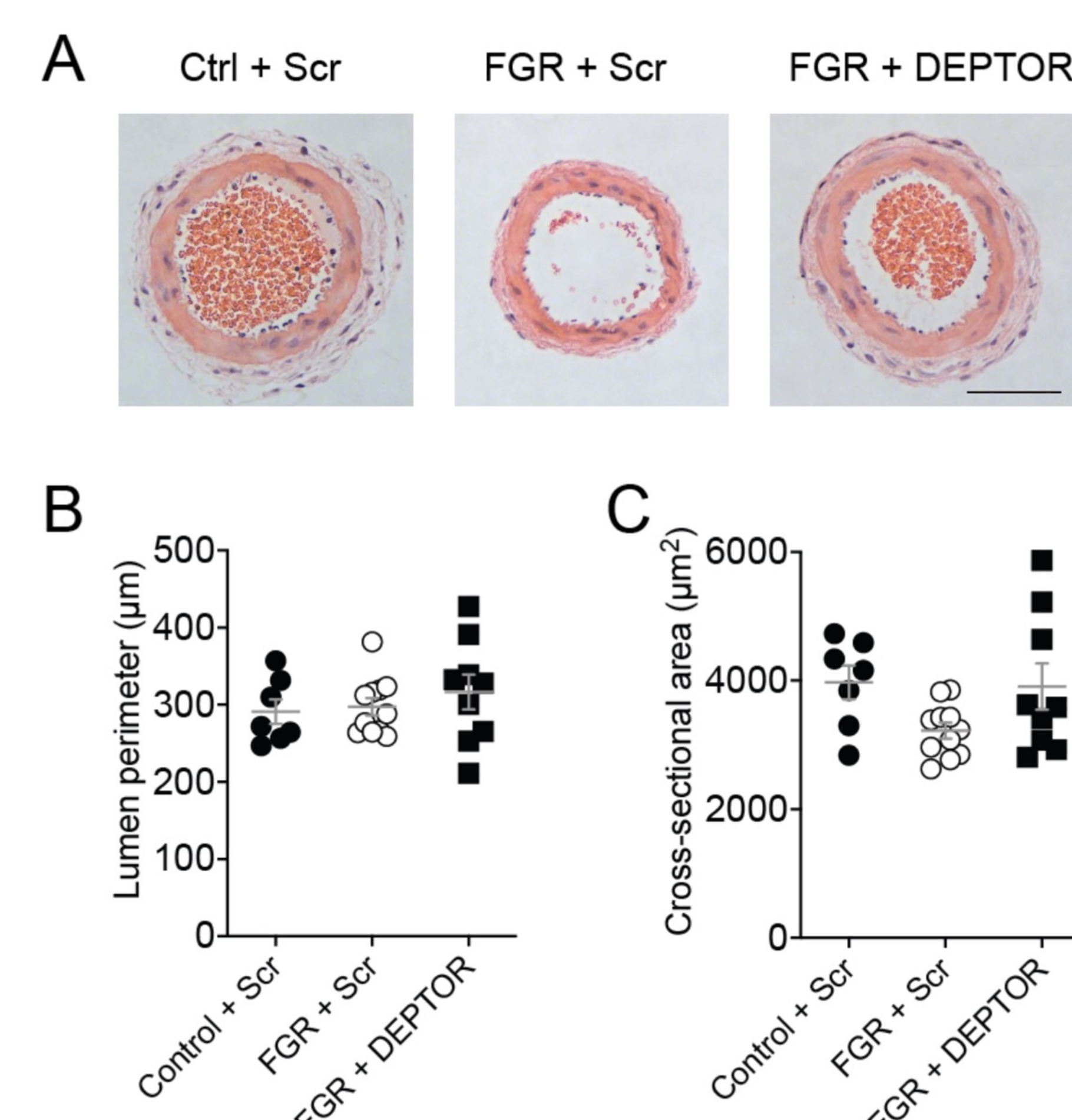


Figure 5. Histologic cross sections of Ctrl+Scr, FGR+Scr, and FGR+DEPTOR uterine arteries with respective cross-sectional area analysis; scale represented as 50 μ M on FGR + DEPTOR photo (Figure 5A). Lumen perimeter was not significantly different between groups (5B). Cross-sectional area of FGR+Scr appeared to decrease compared to Control+Scr (5C).

CONCLUSIONS

The results from this study suggest **placental mTOR signaling contributes to regulate uterine vasodilation** during murine pregnancy. By inhibiting placental DEPTOR, the NO-dependent vasodilatory responses in UtA were restored, suggesting an important role for mTOR signaling in FGR. Understanding the various complexes involved in the pathophysiology of FGR can lead to the development of targeted therapeutics and treatments in the human population.

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