known as angiogenesis [1-3]. In advanced stages of the disease, the patients may experience loss of vision, usually secondary but not limited, to tractional retinal detachment [1,4]. e formation and contraction of the membranes and glial tissue that is accompanying the new vessels is the major risk factor [4]. Other causes of visual loss in patients with proliferative retinopathies are: vitreous hemorrhages, subhyaloid hemorrhages involving the macula, subretinal hemorrhage, macular ischemia and cystoid macular edema among others [4-6].

Angiogenesis is one of the most studied processes in the human eye. It is a complex mechanism that involves stimulation, proliferation and migration of vascular endothelial cells, as well as proteolytic breakdown and degradation of the capillary basement membrane and extracellular matrix [7]. Growth factors have a regulatory activity over angiogenesis. Depending on the type, they can stimulate or inhibit the phenomenon and can be found in all the retinal layers, vitreous and choroid of patients with proliferative diseases [2,8,9]. Vascular endothelial growth factor (VEGF) is a dimeric glycoprotein overexpressed under hypoxic conditions.[10] It is one of the most potent angiogenic growth factors as well as one of the most studied. It is a mitogen glycoprotein of 46 kDa with various subtypes and has been identi ed as a key factor in the retina angiogenesis process [10-12].

Age-related macular degeneration (AMD) is the leading cause of vision loss among individuals of 50 years or older with an incidence that reaches 40 to 50% at 60 years of age [13,14]. Choroidal neovascularization (CNV) accounts for roughly 10% of the cases, but is responsible for 90% or so of the cases with severe visual loss [15]. Increased levels of VEGF have been found in patients with active CNV and treatments directed to blocking its biological activity have reported promising results [16-18].

Bevacizumab (Avastin®, Genetech Inc, San Francisco CA.) is a

Rapamycin is a potent broad-spectrum antibiotic which has demonstrated antineoplastic and antiangiogenic activity [21,22]. It is presumed that the antiangiogenic activity is due to its property to downregulate VEGF [21,22] erefore the aim of this study is to evaluate the safety and e cacy of intravitreal rapamycin and the combination of rapamycin and bevacizumab versus bevacizumab alone as well as the variation on the VEGF expression levels in each group on an animal model of CNV.

Material & Methods

e study was reviewed and approved by the hospital internal review board and the Institutional Animal Care and Use Committee. All the procedures were performed according to the statement for the use of animals in ophthalmic and visual research from the Association of Research in Vision and Ophthalmology (ARVO).

We included twelve eyes from twelve Landrace pigs. We divided the population into four di erent groups of three animals each (Group A, B, C and D). Pharmacological mydriasis was induced on all right eyes with 3 drops of tropicamide (0.8%) and phenylephrine (5%) every 10 minutes, three times. Choroidal neovascularization was induced in all animals' right eyes, with the use of a double frequency Nd: YAG laser (Ophthalas 532 Eye Lite, Alcon Labs, Dallas Forth Worth, Tx) using 30 burns of 1.5 W of power, a spot diameter of 500 µm and a burn duration of 300 ms. e burns were placed above and temporal to the *area centralis*, outside the main vascular arcade. CNV was successfully induced in all eyes, and was evident 5 days a er laser administration in fundus examination and uorescein angiography (data not shown).

A er checking for the successful induction of CNV, all animals were sedated with ketamine hydrochloride (0.1%) at a dose of 1 µg/Kg and locally anesthetized with topical drops of lidocaine hydrochloride (4%). A er surgical preparation and drape (only the right eye), instillation of drops of povidone iodine (10%) in conjunctival fornices and the placement of an eyelid speculum, animals on group A received an intravitreal injection of 0.1ml balanced salt solution and served as a control group; group B received an intravitreal injection of rapamycin (1mg/ml) 0.1ml; group C received 0.1ml intravitreal injection of bevacizumab (2.25mg/ml) and group D received 0.2ml intravitreal injection of rapamycin and bevacizumab (same doses). Intraocular pressure was assessed a er injection and 5 minutes later. If there was a high intraocular pressure, an anterior chamber paracentesis was done for pressure control.

Twenty one days a er intravitreal injections, animals were euthanized and enucleated. Immediately, a piece of the retina (0.5mm x 0.5ml) was harvested and placed on a sterile eppendorf tube (Eppendorf AG, Hamburg, Germany) for mRNA extraction, using a commercially available extraction kit (total RNA microprep kit, Stratagene, La Jolla, CA) according to the manufacturer's instructions. e rest of the eye was xed on a solution of 4% paraformaldehyde and preserved in para n for histological analysis.

Reverse transcription was carried out using TaqMan Reverse Transcription Reagents (Applied Biosystems, Carlsbad, CA). Real-time Polymerase Chain Reaction (RT-PCR) was performed with a set of primers and probes, specially designed to detect VEGF mRNA of all isoforms. e expression levels of VEGF were determined by using a linear regression model (SPSS so ware, version 16.0 Sigma Stat; Systat So ware, Inc., San Jose, CA, USA).

e tissue preserved in para n was cut on sections of 10µm and placed on glass slides. e slides were placed in a solution of Triton (0.05%) and PBS La l9 0 0 9 42.5197alysiing sol5197alyspan ⁄AMCID 249 ⁄BDC