Online Resource 5

Cyclic GMP Protects Endothelial Progenitors from Oxidative Stress

Angiogenesis

Anna Maria Curatola, Jie Xu, and Karen D. Hendricks-Munoz
Department of Pediatrics
New York University School of Medicine
550 First Ave.
New York, NY 10016
annamaria.curatola@med.nyu.edu
Staining with cGMP antibodies identifies vascular structures in the developing EBs. EBs were exposed to 60% O2 in the absence or presence of VEGF-A (40ng/ml) or MMQ (0.6µm) and then allowed to recover in normoxia for one week. At the end of the incubation, EBs were fixed with 4% paraformaldehyde in PBS (20 min, 4°C), incubated in 0.1% Triton X-100 (10 min, 4°C) and double-stained with antibodies against PECAM-1 (a, c and e) and cGMP (Chemicon International, rabbit polyclonal antibodies, 1:3000) (b, d and f), followed by appropriate secondary antibodies. cGMP staining co-localized with PECAM staining in the different culture conditions (arrows), but the strength of staining differed. A weak cGMP staining identified both abnormal and VEGF-stimulated vascular structures. cGMP staining was stronger in MMQ-treated cultures and localized in vascular structures. Scale bar = 100µm.