

DNA demethylating agent 5-azacytidine (5-azaC) impairs proliferation and survival of rat trophoblast cells

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INTRODUCTION

Our previous results have shown that pretreatment with the antioxidant N-tert-Butyl- α -phenylnitron (PBN) improved some developmental parameters (survival, overall growth, and limb morphogenesis) in fetal rats whose mothers were treated by the epigenetic drug 5-azacytidine during the gestation. The question remained whether the PBN could improve the growth of the placenta in the same model. .

MATERIAL AND METHODS

On the 12th and 13th days of gestation Fisher rats were pretreated by an i.v. injection of PBN (40 mg/kg) and one hour later by an i.p. injection of 5-azacytidine (5mg/kg). On the 15th and 20th days of gestation placentae were isolated. Their weight, immunohistochemically detected and stereologically quantified Proliferating Cell Nuclear Antigen (PCNA) expression and apoptotic index were compared to sham controls, those treated with PBN and/or 5-azacytidine (5-azaC) and statistically evaluated.

	Control	PBN	PBN+5azaC	5azaC
No of placentas	65	113	103	71



5azaC PBN+5azaC PBN control

RESULTS

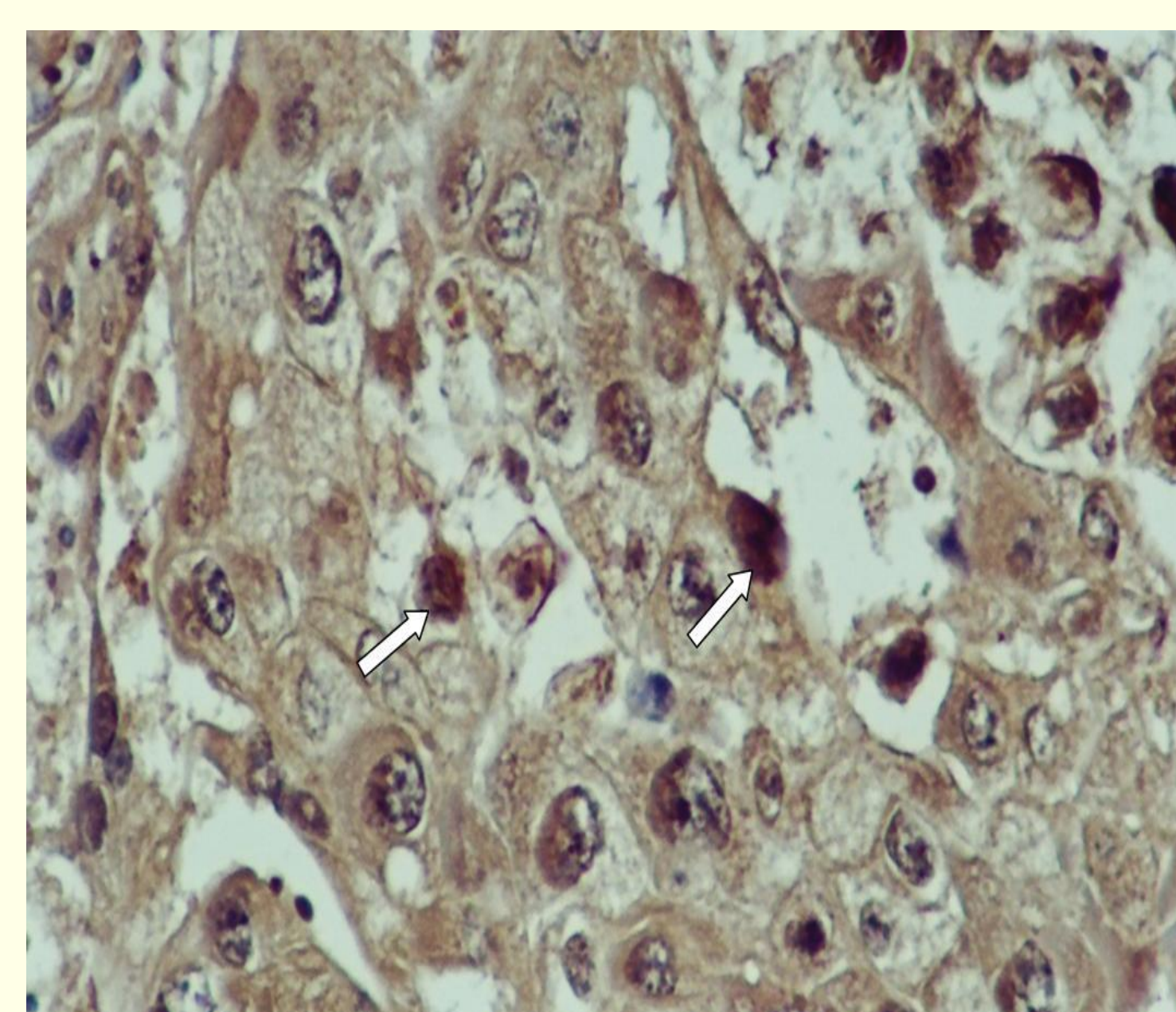
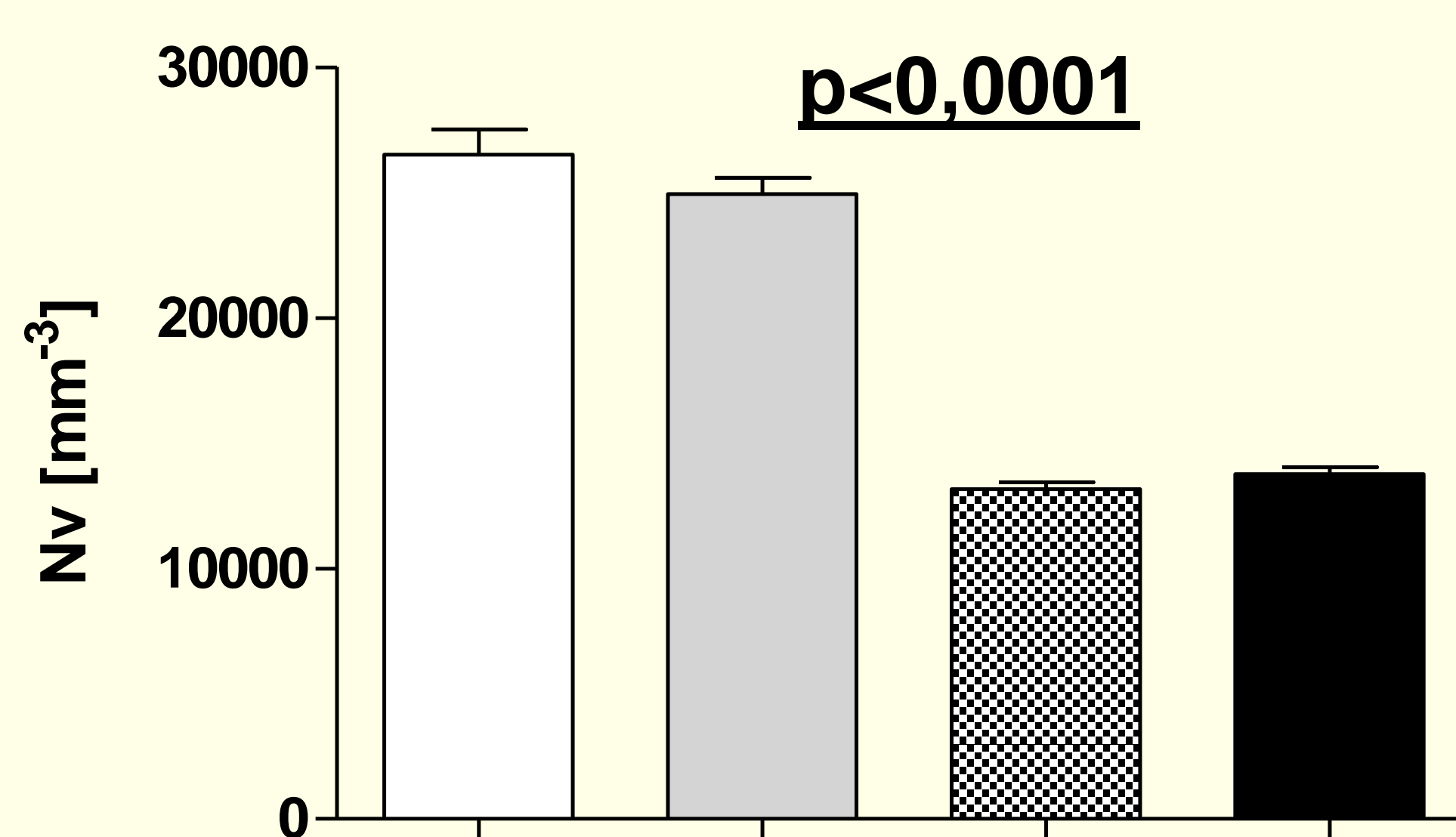
PBN significantly improved overall growth of the 15-days-old-placenta when applied before 5azaC. However, apoptotic index was the same in all groups of samples.

In 20-days-old samples treated with 5-azaC overall growth was the same, while numerical density of the PCNA signal was significantly lower than in PBN-treated or controls. Apoptotic index in 20-days-old samples treated with 5-azaC was significantly higher than in PBN-treated controls or sham controls, PBN exerted a small beneficial impact which was not statistically significant.

Proliferation and apoptosis in the 20-days-old placenta.

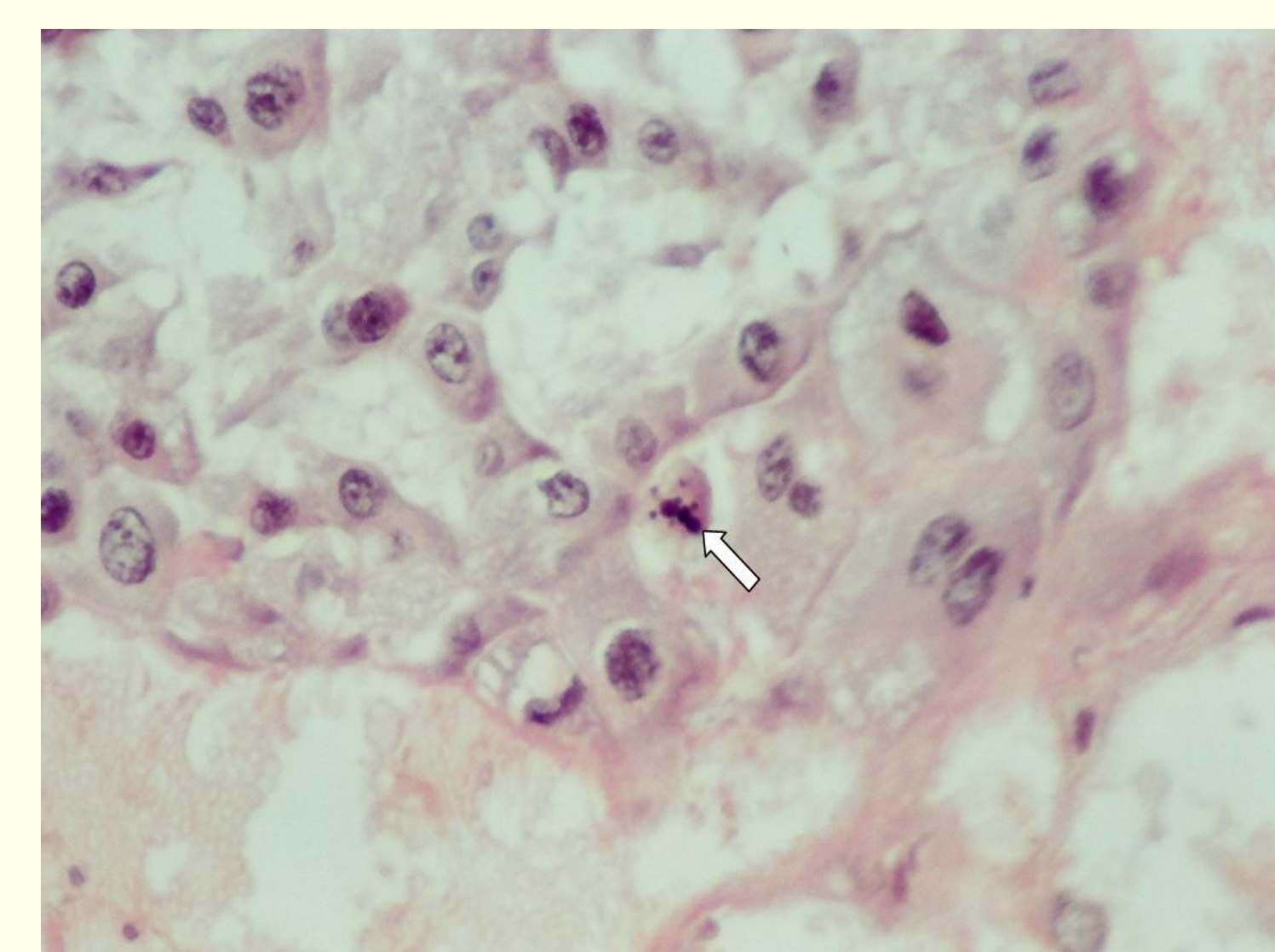
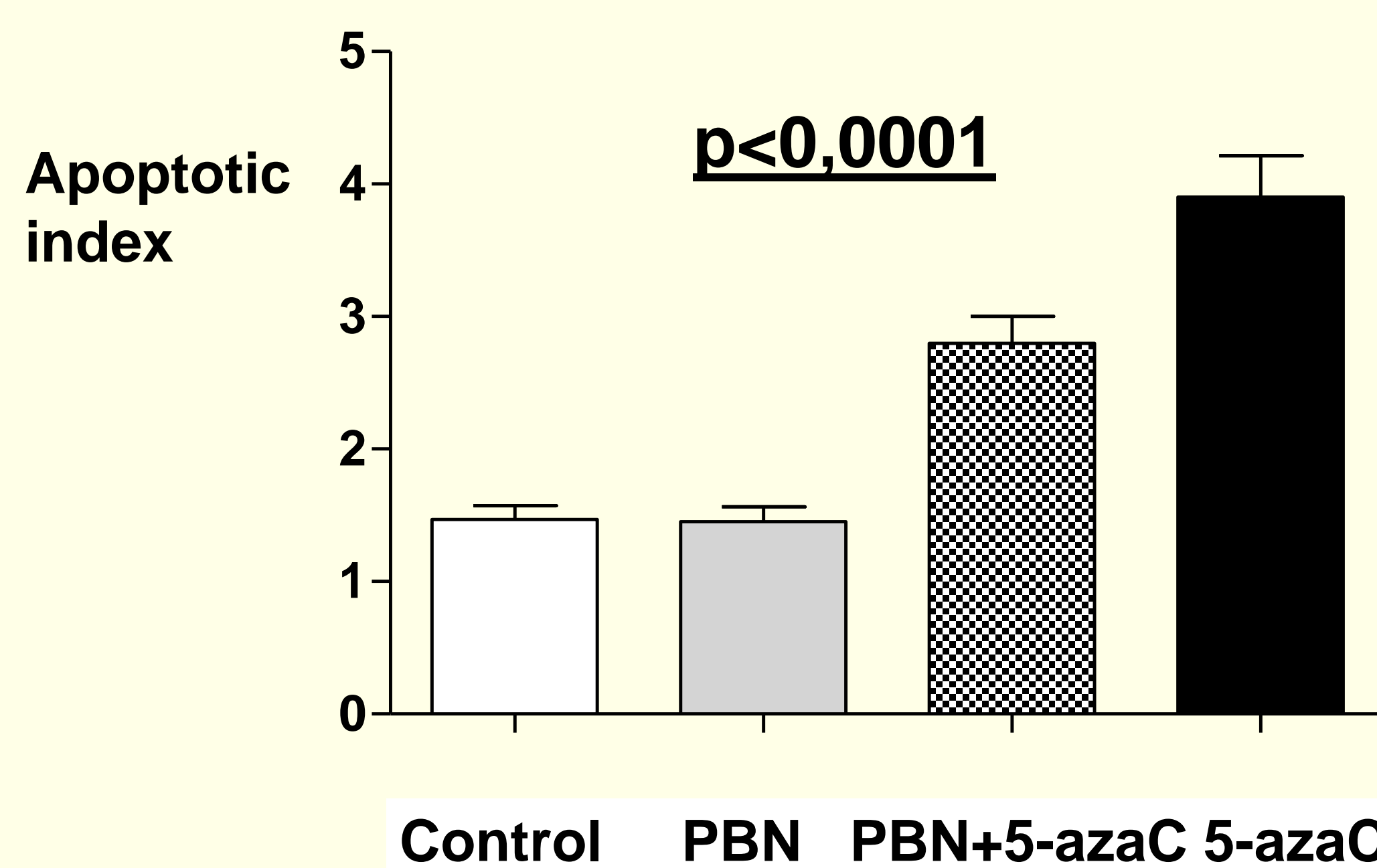
Dunn post-hoc test. $p < 0,0001$

Numerical density



PCNA in trophoblast. DAB, hematoxylin, x200.

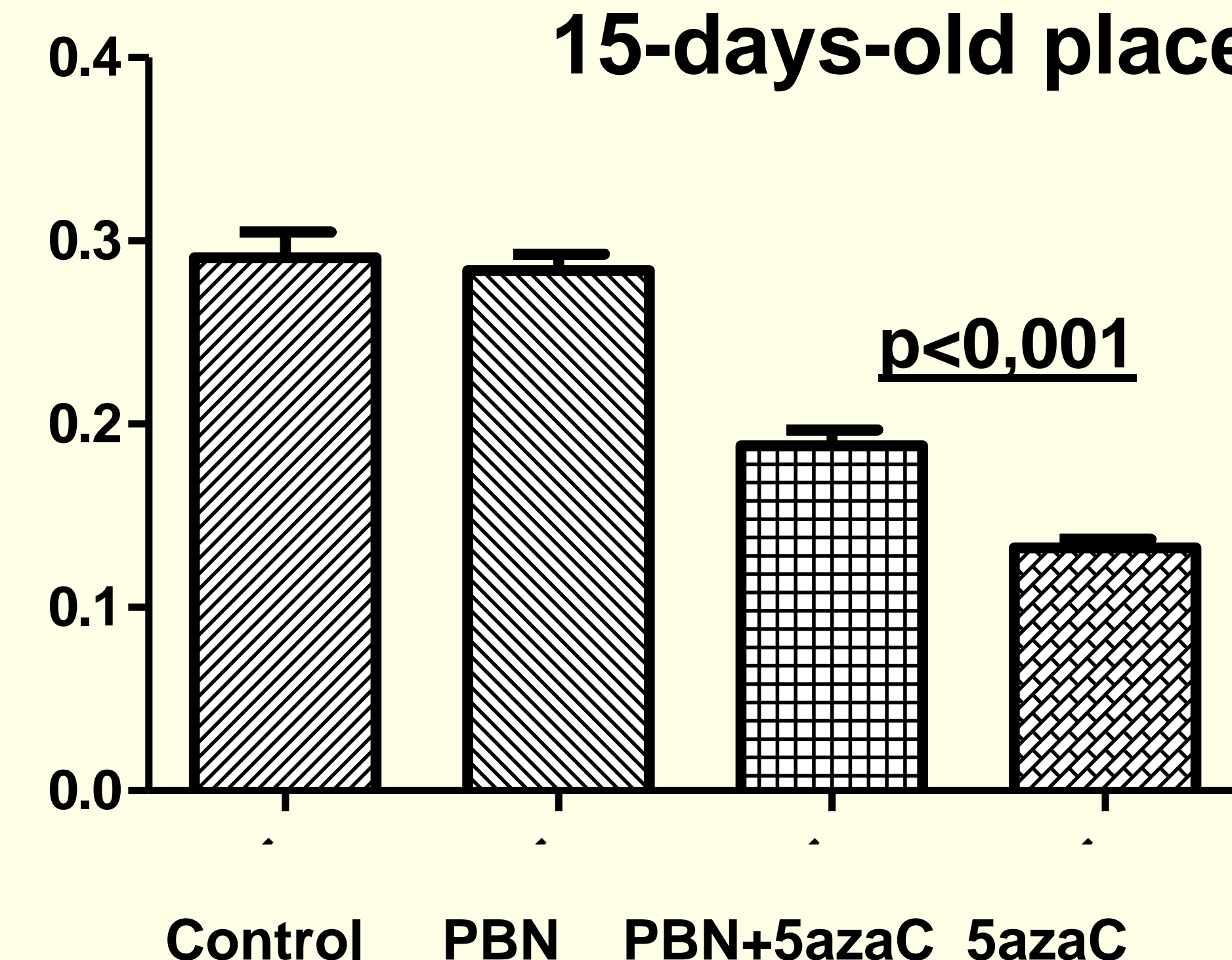
Apoptotic index



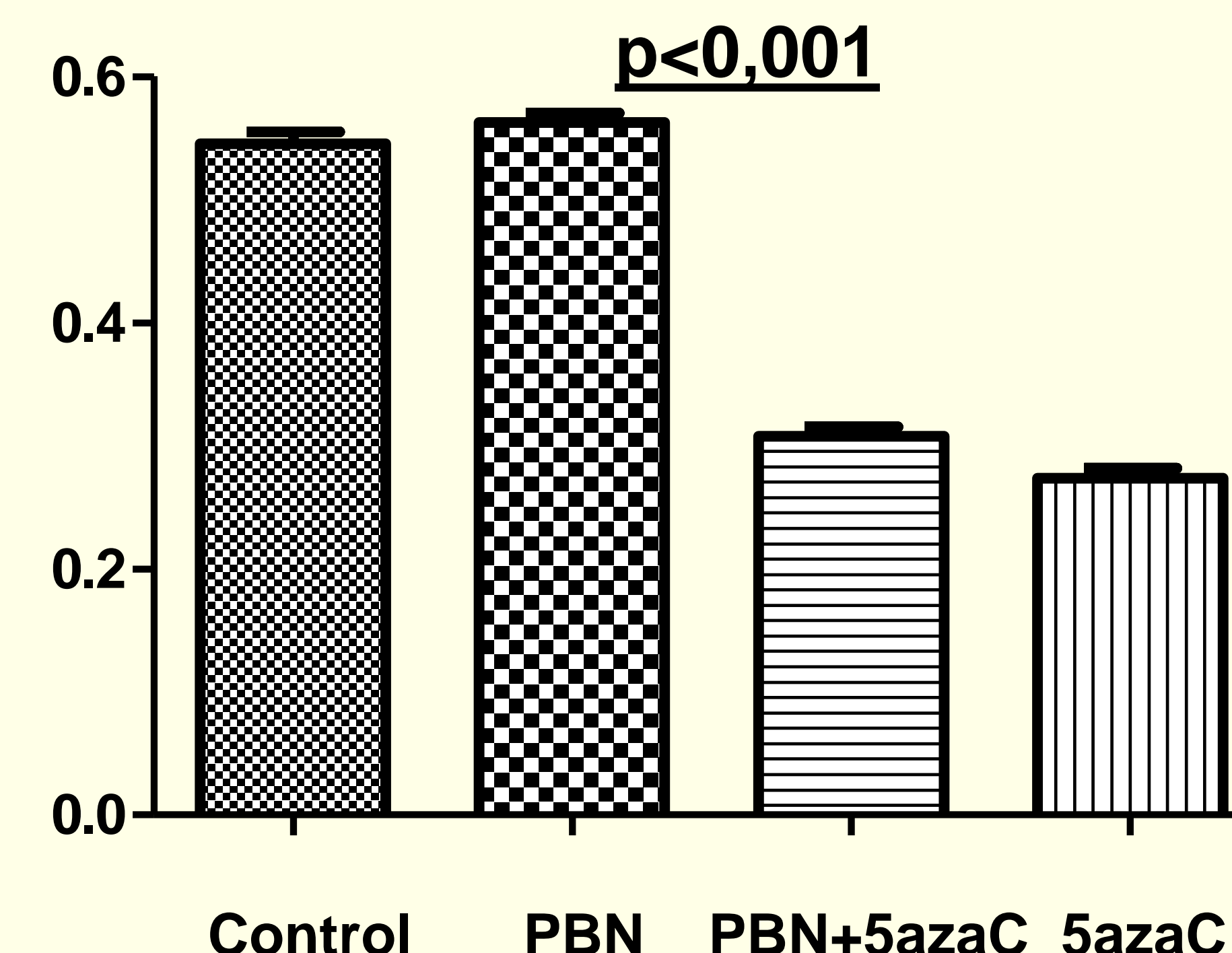
Apoptotic cell (arrow) in trophoblast. HE, x400

Weight (g)

15-days-old placenta



20-days-old placenta



CONCLUSION

PBN expressed similar positive effect on the overall placental growth as on the growth of fetuses when used as a pretreatment to 5-azaC. This can lead to conclusion that impact of 5-azaC could at least partially depend on activation of oxidative stress pathways within the placenta itself.