

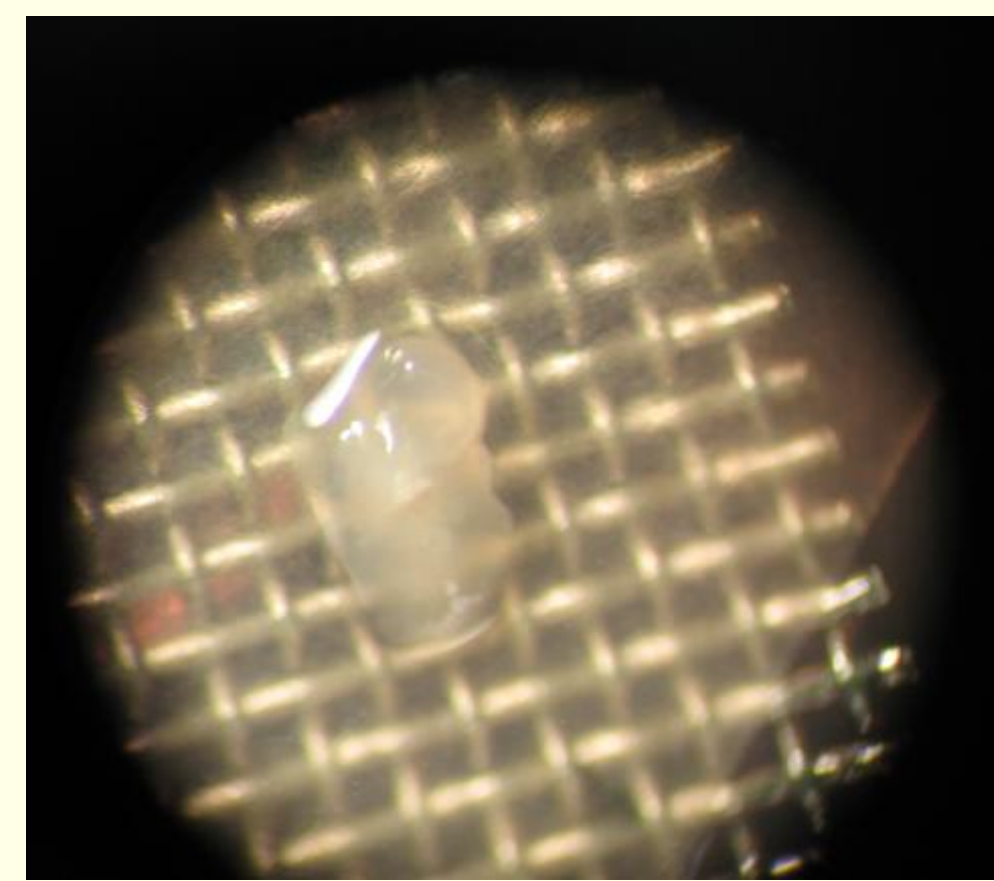
Rat limb bud in an *ex vivo* system for embryotoxicity testing

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INTRODUCTION

Ex vivo models are alternative methods that are used to explore developmental potential of various tissues or organs isolated from the organisms in order to avoid confounding and complex *in vivo* environment especially for embryotoxic assays.

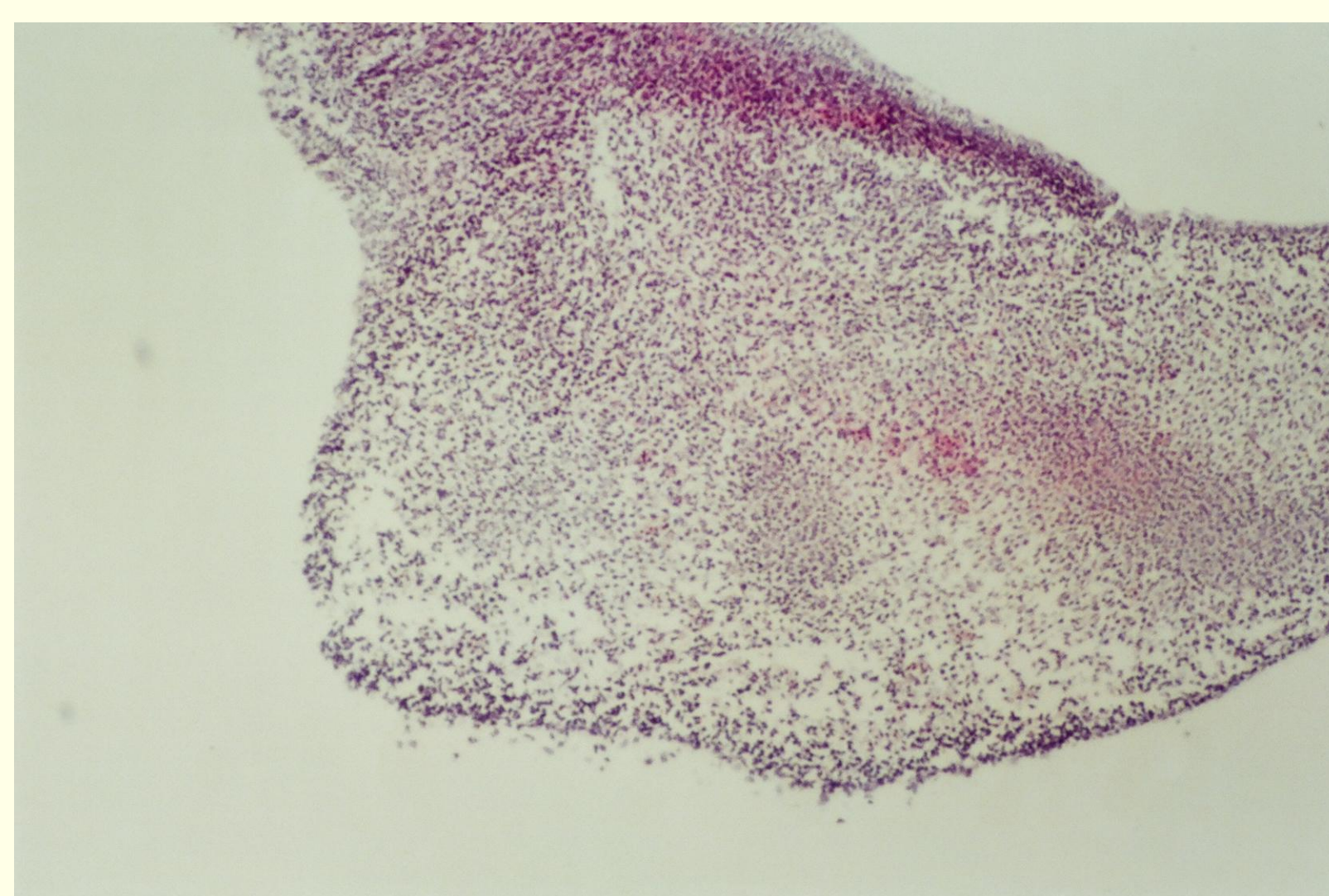


MATERIAL AND METHODS

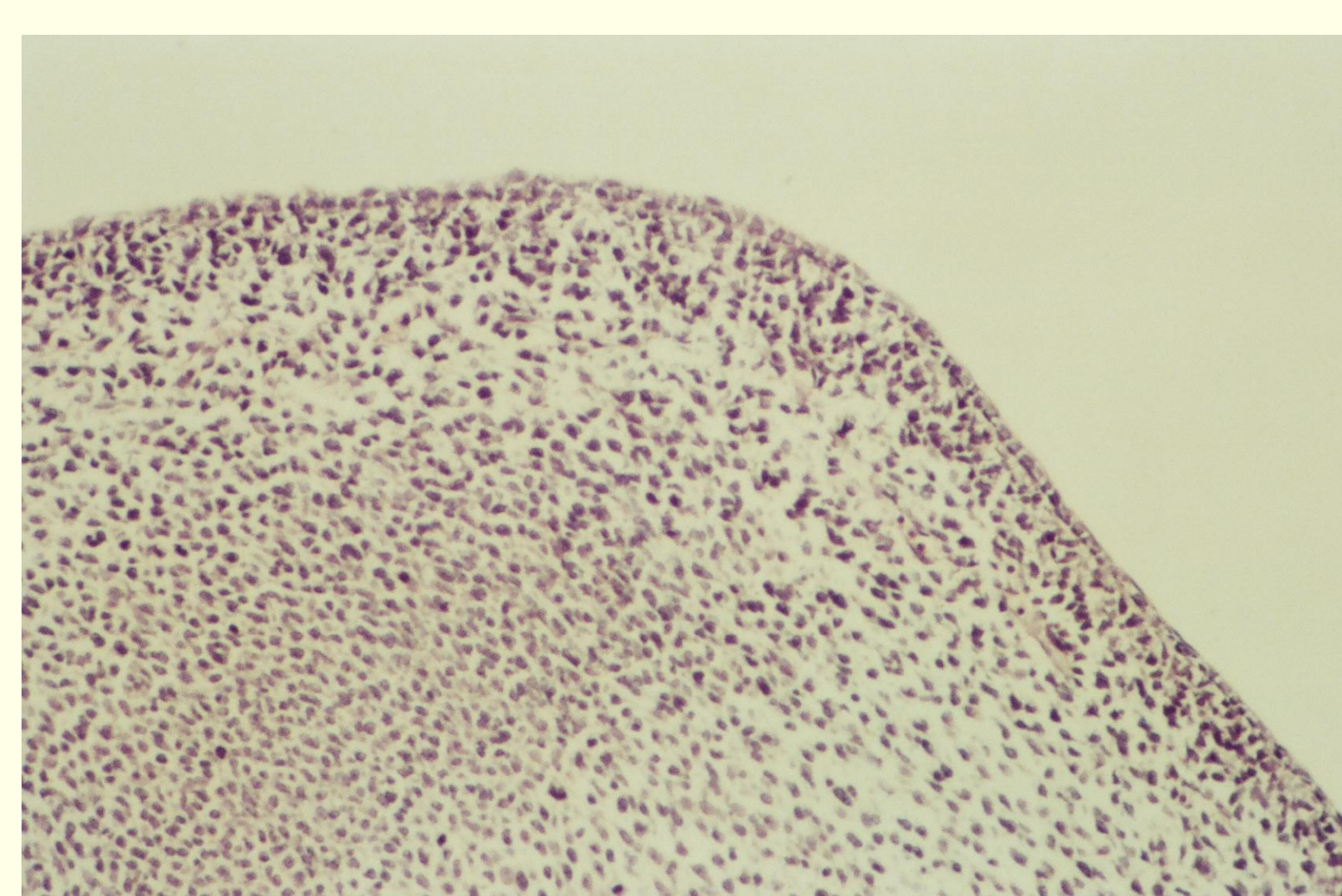
Fisher rat fore- and hind-limb buds were microsurgically removed under the dissecting microscope from 13- and 14-days-old embryos and placed on a lens paper supported by a stainless steel grid where they spent three days or two weeks at the air-liquid interface. Eagle's Minimum Essential Medium was supplemented with 50% rat serum and changed every other day. Samples were processed by routine histology, embedded in paraffin and uninterrupted serial sections were stained by HE, Masson trichrome or Azan stain.

RESULTS

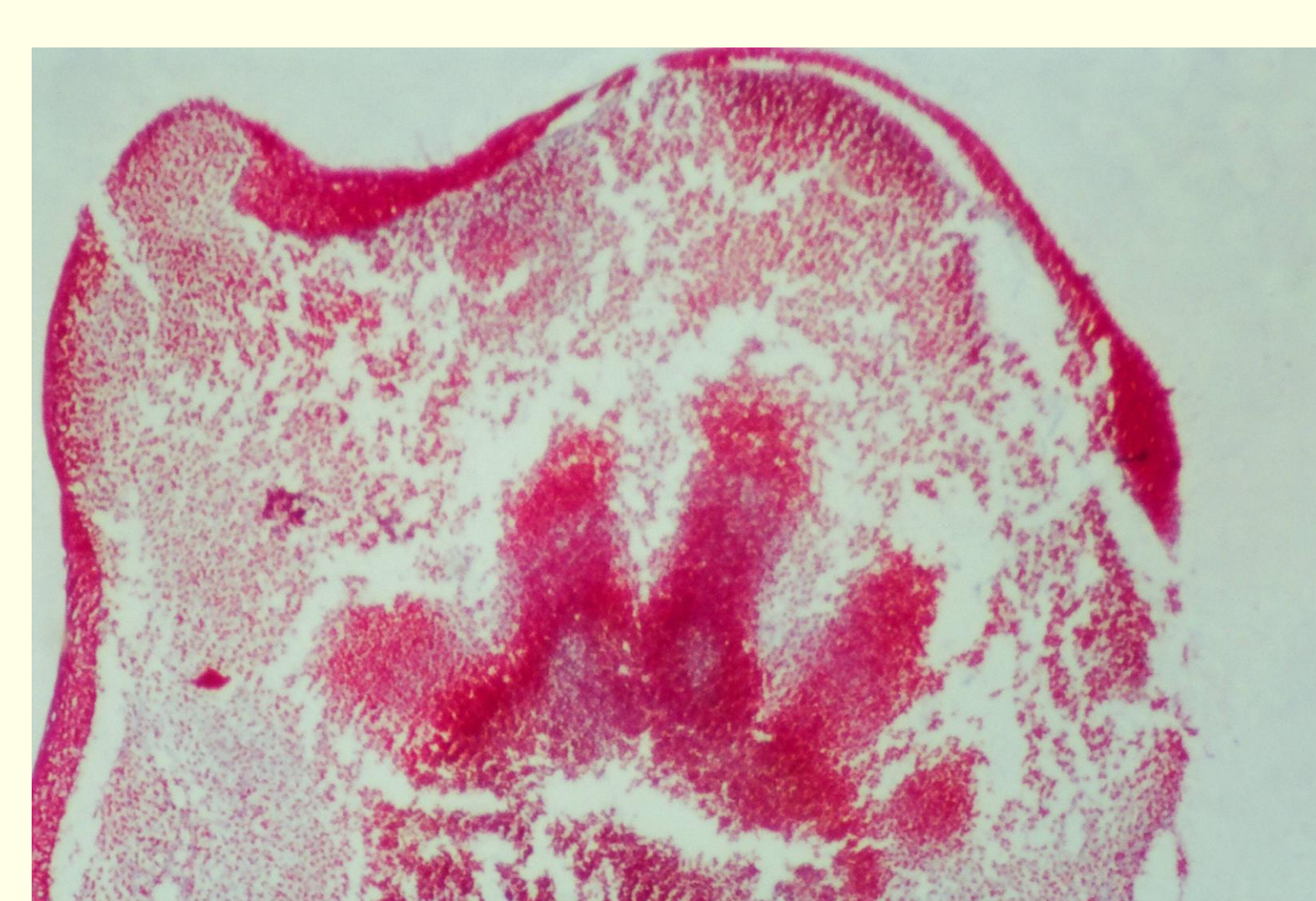
In isolated limb bud immature epithelium covering its surface was present. During the 3-day culture period, stratified epithelium developed. In limb buds that spent two-weeks in culture keratinization of the stratified epithelium and fully developed stratum granulosum could be discerned in some explants.



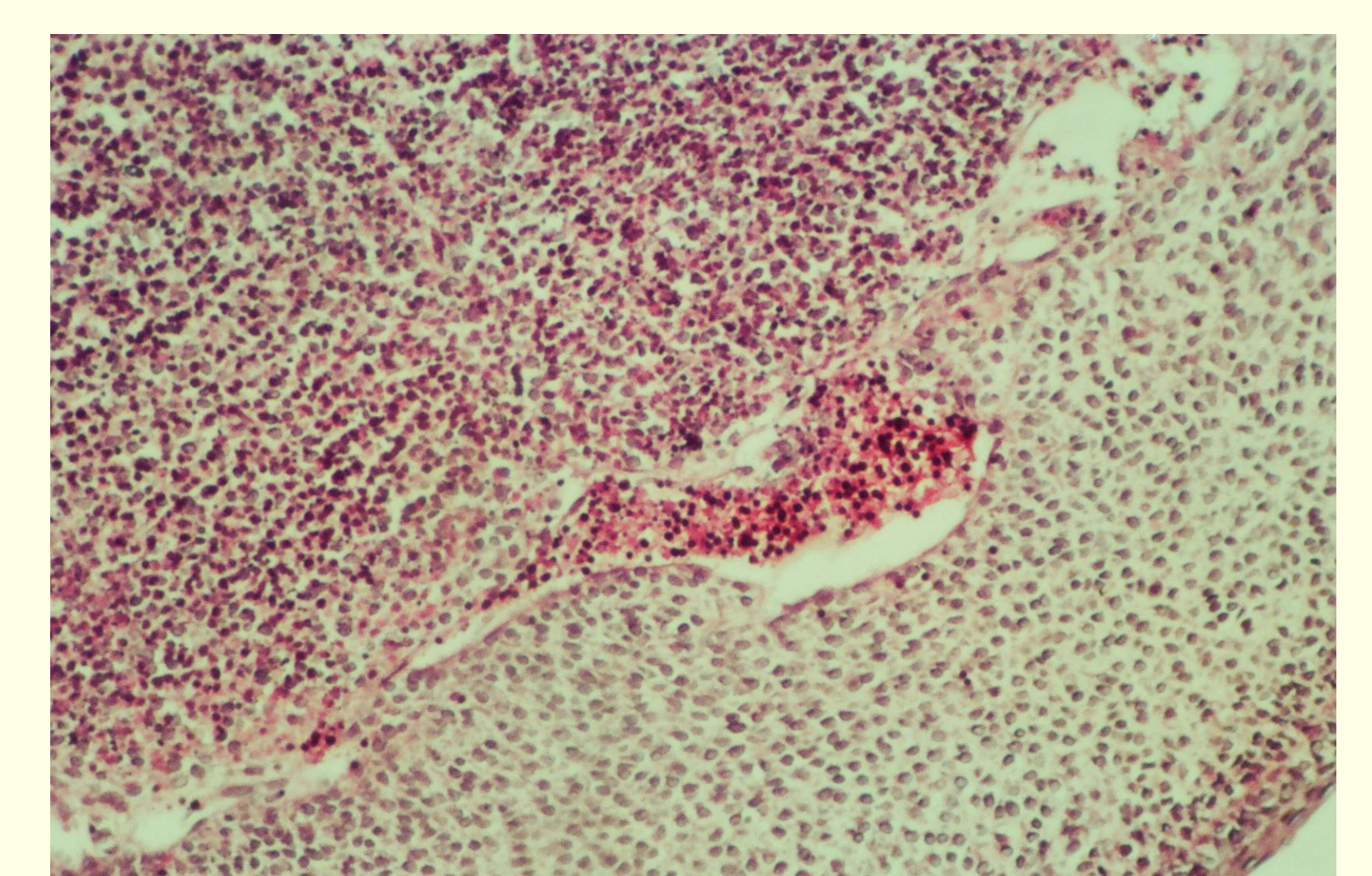
13-days-old embryo, hind-limb bud, HE X40



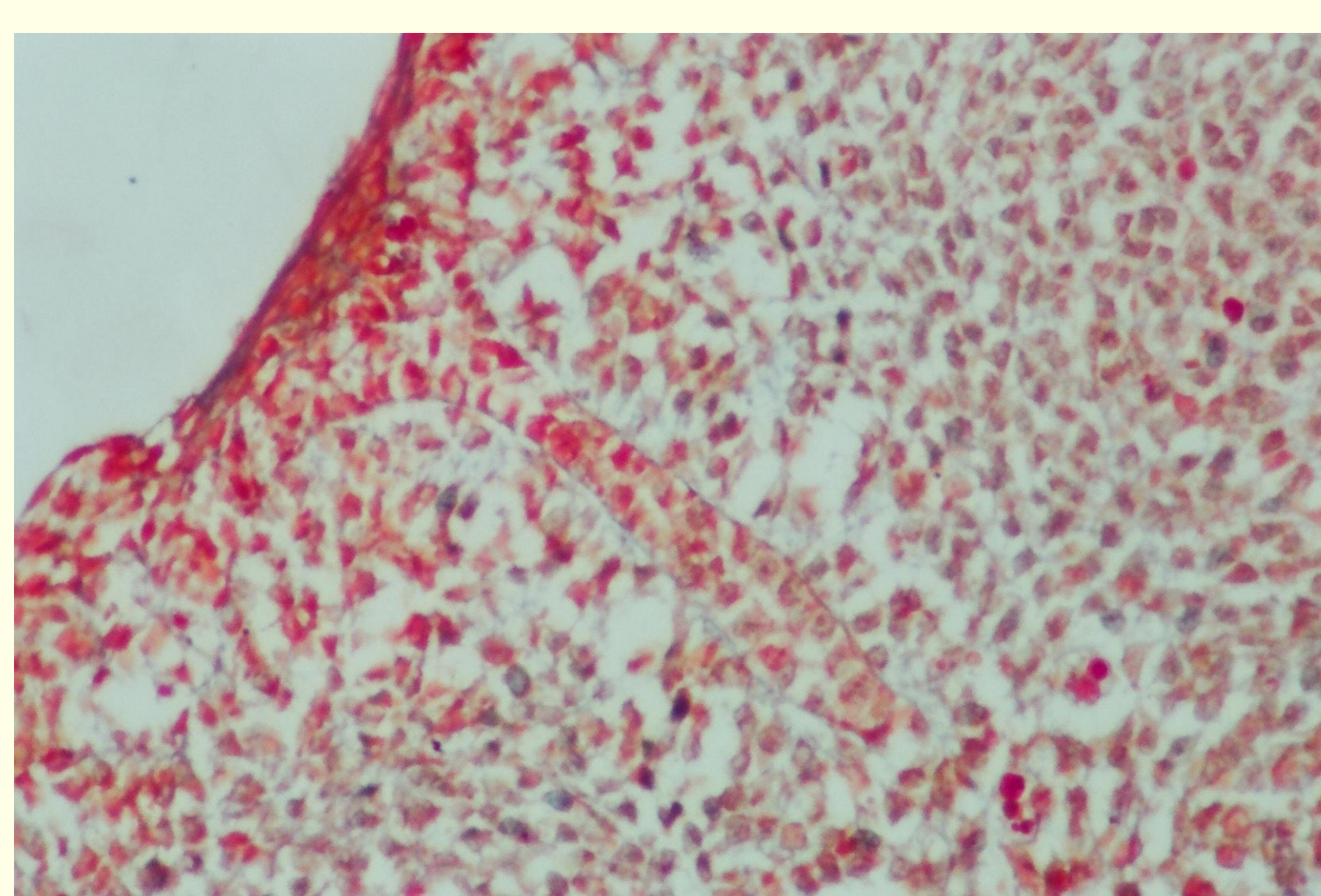
13-days-old embryo, fore-limb bud, HE X100



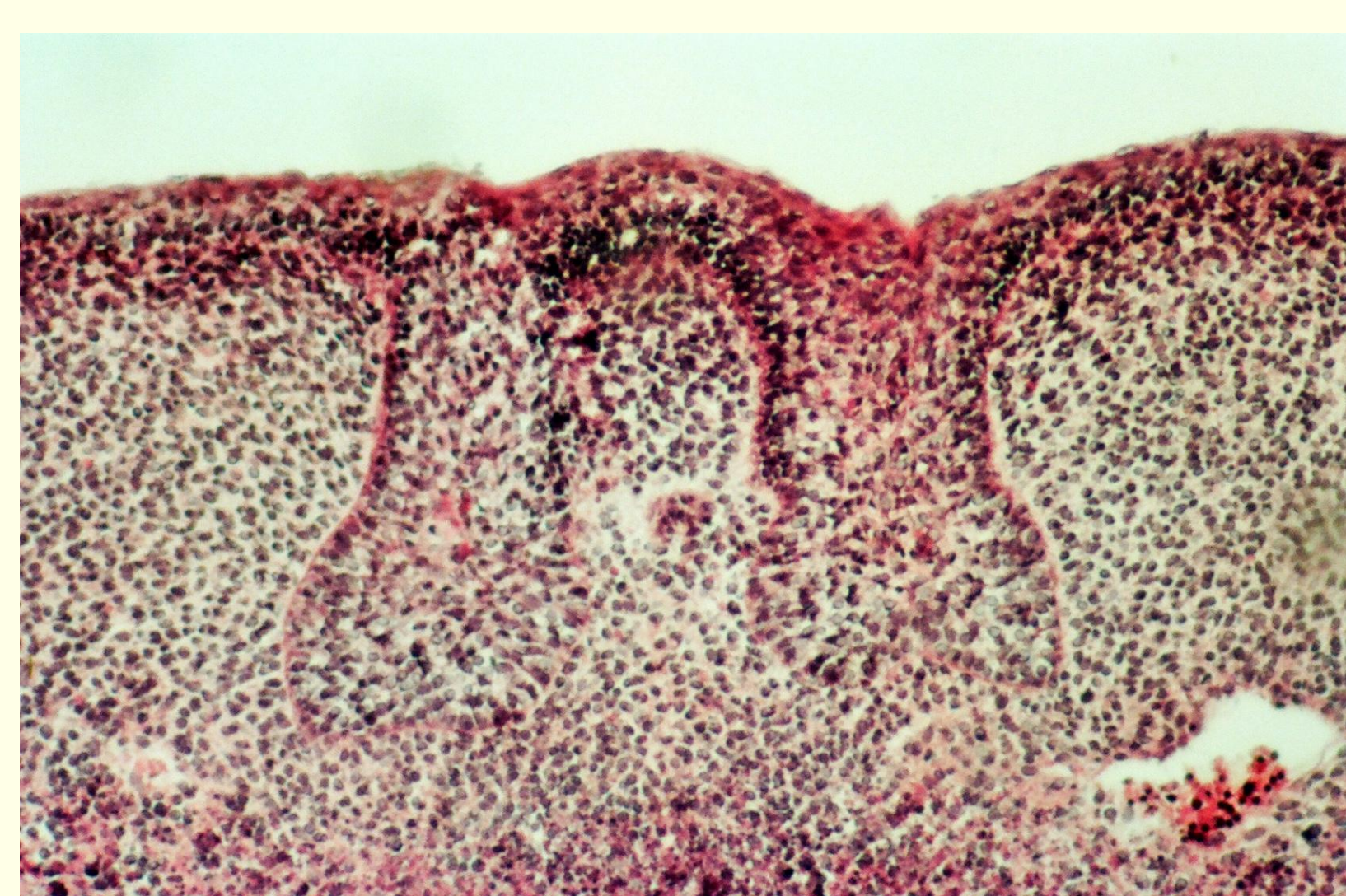
Ex vivo culture 13-days-old embryo, fore-limb bud, control. Azan X40



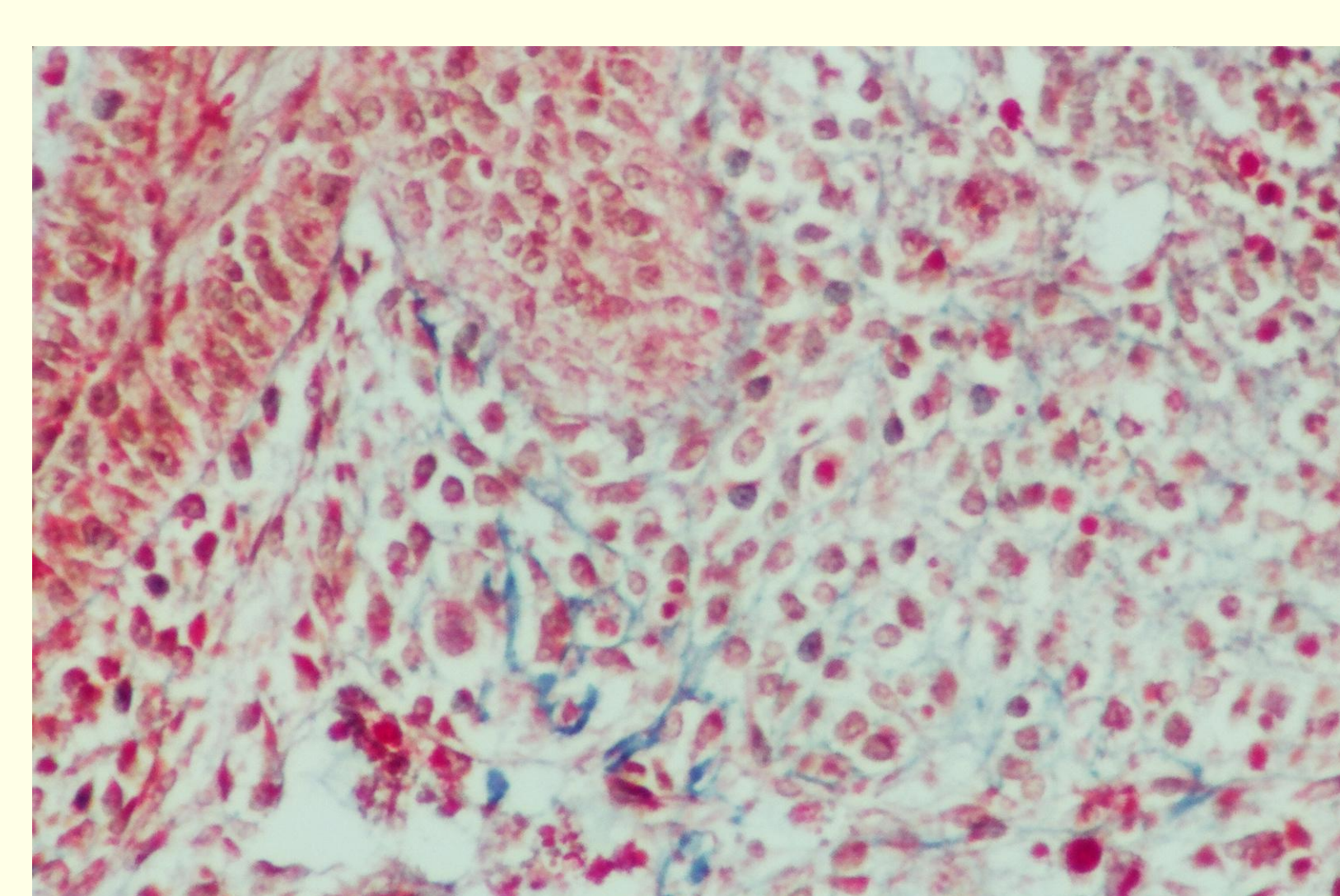
Ex vivo culture 13-days-old embryo, fore-limb bud, 5-azaC. HE X100



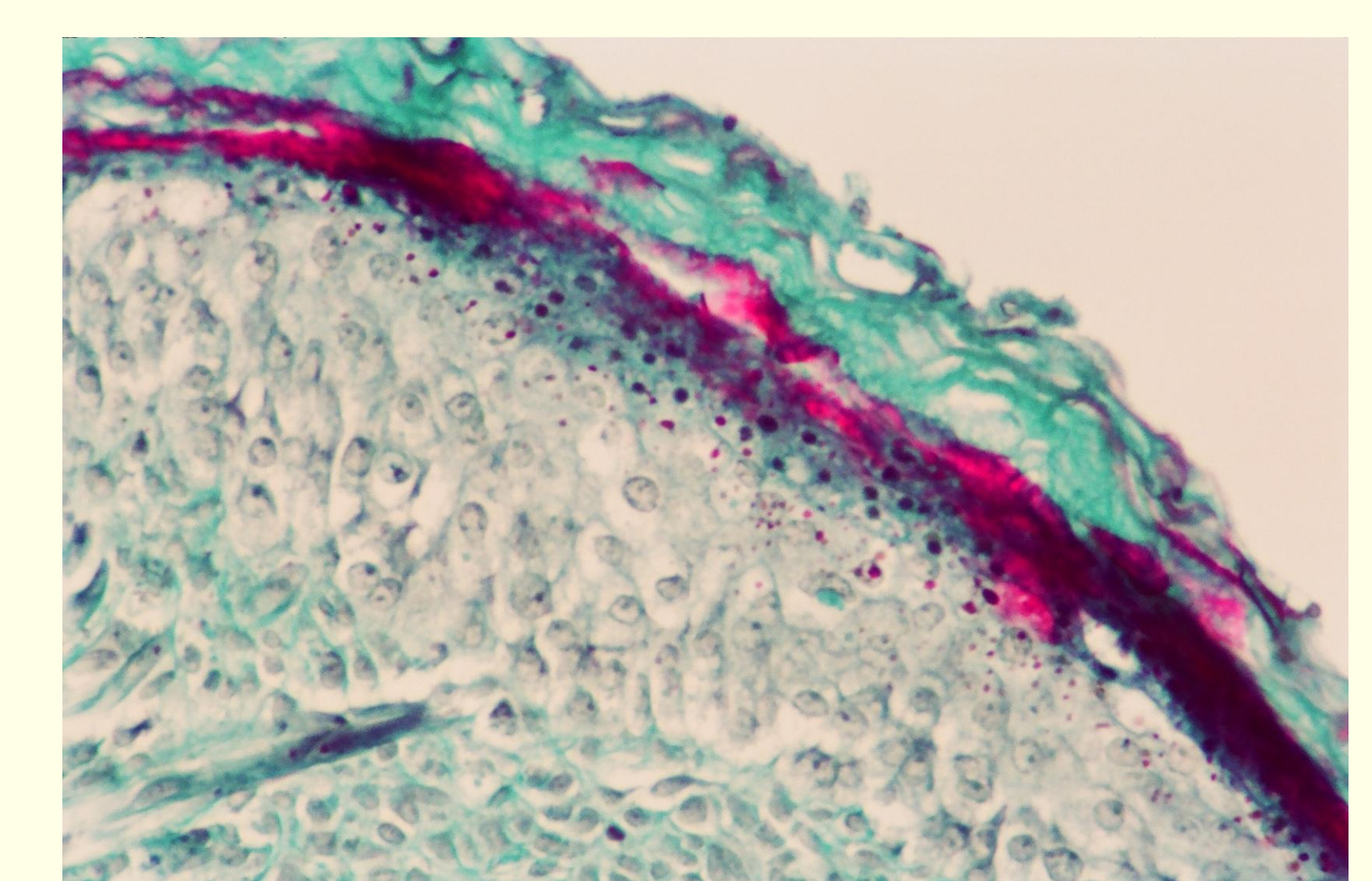
Ex vivo culture 13-days-old, hind-limb bud, control. Azan X200



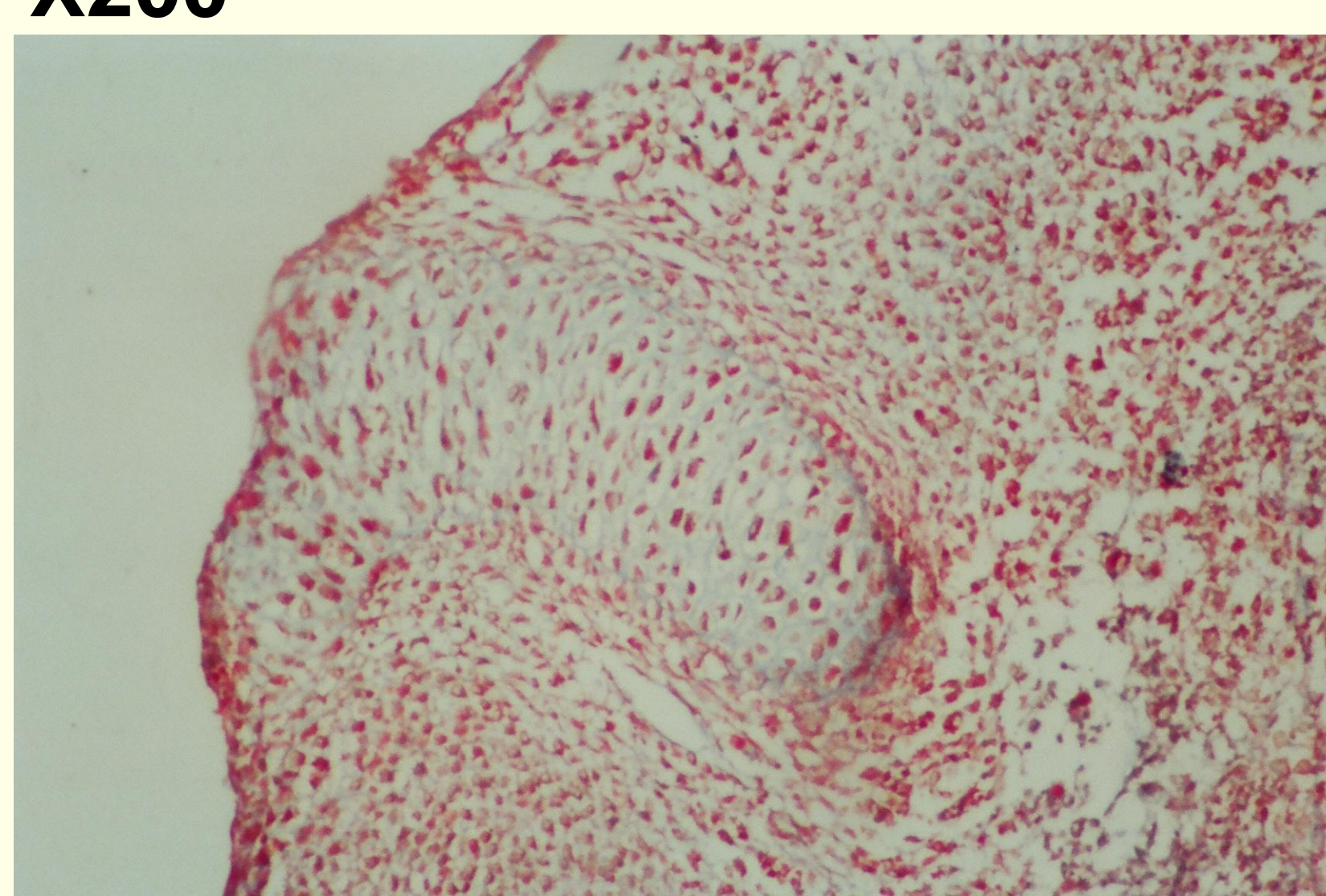
Ex vivo culture 13-days-old, hind-limb bud, 5-azaC. HE X100



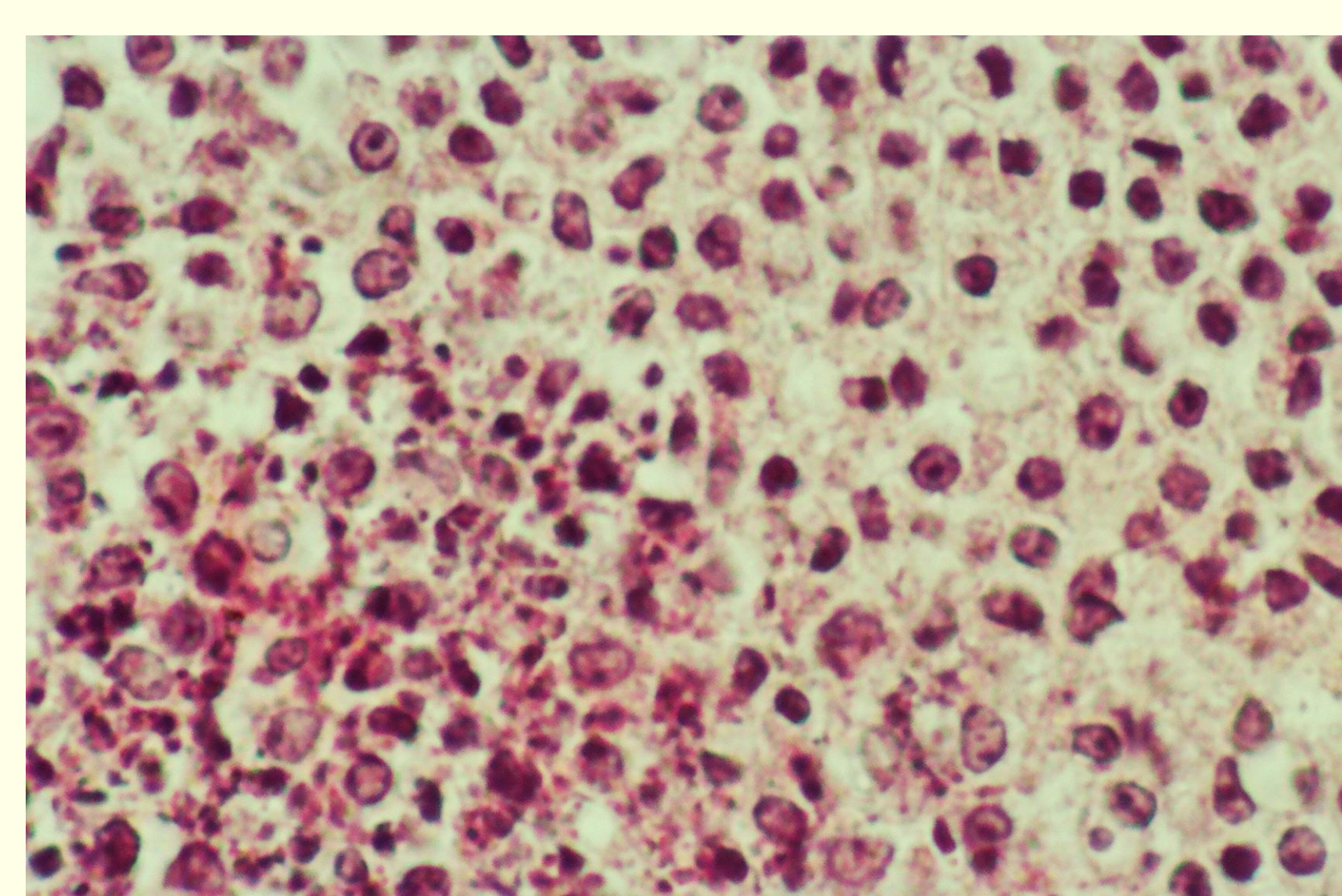
Ex vivo culture 13-days-old, hind-limb bud, 5-azaC. Azan X200



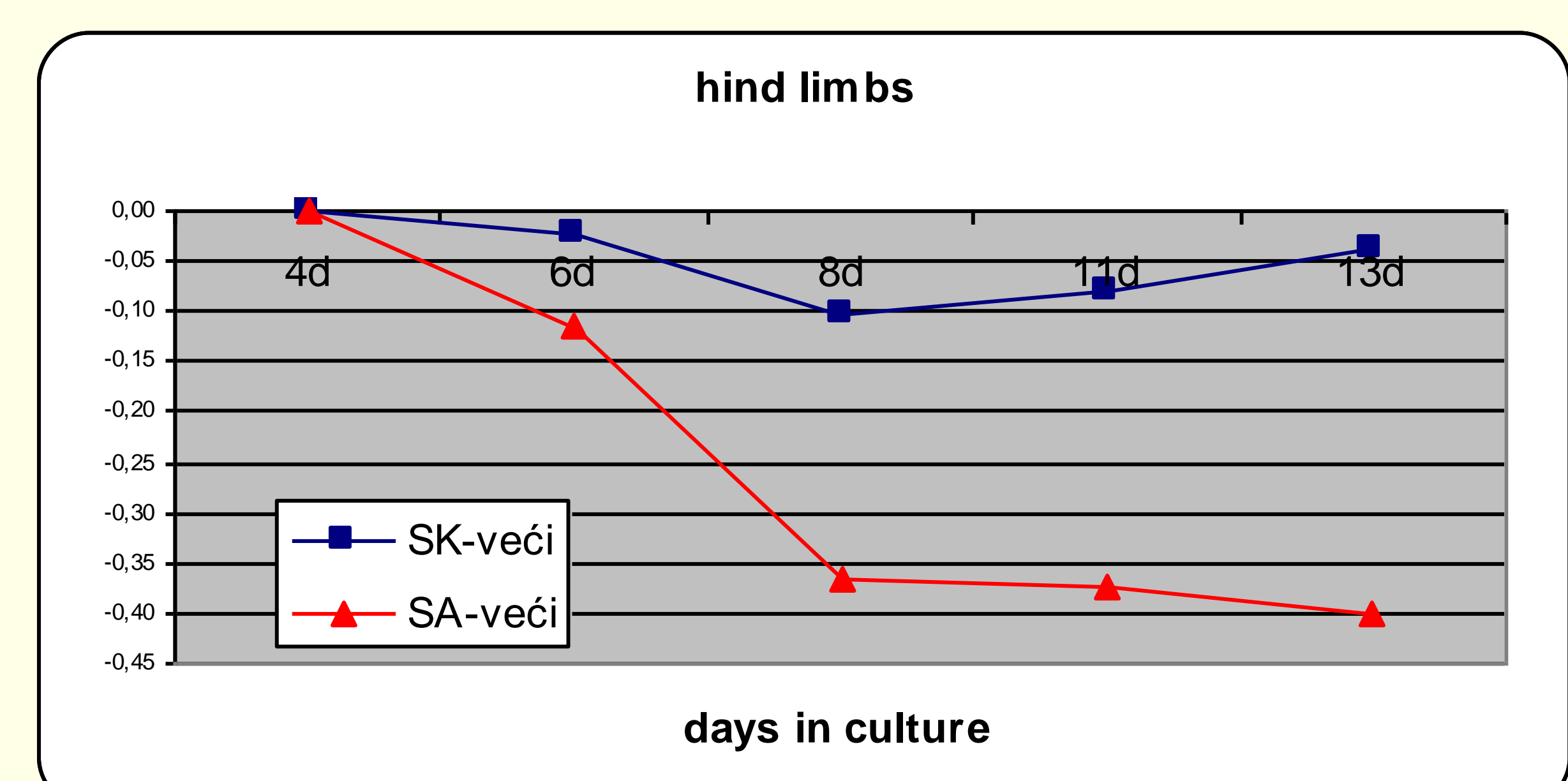
Ex vivo 13-days-old embryo, hind-limb bud, control. Masson X200



Ex vivo culture 13-days-old embryo, fore-limb bud, control. Azan X100



Ex vivo culture 13-days-old embryo, fore-limb bud, control. HE X100



CONCLUSION

It can be concluded that developmental parameters such as overall growth and differentiation of specific tissues in this mammalian model system make it adequate to screen for embryotoxic substances such as the epigenetic drug 5-azacytidine used in this investigation.