牛胚胎滋养层巨细胞的体外分离培养

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摘要 建立牛胚胎滋养层细胞的体外分离、培养方法。取怀孕牛45~60天的完整子宫, 无菌条件下收集胚胎子叶, 采用胶原酶消化方法分离细胞。将细胞随机分为两组, 一组用差异贴壁法纯化滋养层细胞, 另一组未纯化则用含15%胎牛血清和滋养层细胞生长添加剂 (trophoblast growth supplement, TGS)的DMEM培养基培养细胞。采用台盼蓝和Hoechst 33342细胞染色液对细胞活力及形态学进行分析, 采用免疫细胞化学法进行细胞纯度鉴定。结果显示, 本实验分离的细胞经台盼蓝染色后记录细胞存活率大于90%, 核染可见典型双核特征。纯化组细胞经差异贴壁法纯化后双核滋养层巨细胞(binucleate trophoblast giant cells, TGC)纯度可达95%, 未纯化组细胞中TGC只占45%~50%左右。抗细胞角蛋白抗体检测呈阳性, 抗波形蛋白抗体检测呈阴性。细胞经过4次传代后, 可以存活60天。本试验成功建立了一种快速、简便、能够培养高纯度牛胚胎滋养层细胞的方法, 为进一步研究滋养层细胞与相关病原侵入机制打下了基础。

关键词 细胞培养; 牛; 滋养层巨细胞

双核滋养层巨细胞(binucleate trophoblast giant cells, TGC)是由单核滋养层细胞通过核分裂形成的, 在母体怀孕期间从绒毛膜上皮移行到子宫上皮, 在子宫上皮处TGC与单一的上皮细胞融合, 成为三核母-子杂合细胞[1]。TGC在胚胎发育第17天形成, 直到生产后一直存在, 数量随着妊娠期增加而减少15%~20%。TGC不仅能够产生和释放各种内分泌、旁分泌和自分泌活性激素, 例如胎盘催乳素(placental lactogen, PL)、泌乳刺激素相关蛋白(prolactin-related proteins, PRL)、妊娠相关糖蛋白(pregnancy associated glycoproteins, PAG)等, 同时也能产生、释放前列腺素和类固醇[2]。由于与子宫上皮密切接触, TGC在母体-胚胎的相互关系中发挥重要作用, 与多种妊娠相关疾病的发生、发展与其增殖、功能调节的失常有密切关系。由滋养层细胞、毛细血管内皮细胞以及二者之间基膜所构成的胎盘屏障, 是各种营养物质和某些药物、病毒、激素等从母体进入胎儿的必经之路[3]。体外培养的滋养层细胞为研究各种物质通过胎盘屏障的机制提供了细胞模型, 是研究垂直传播疾病发病机制的细胞学基础。本文通过对滋养层细胞进行体外分离和培养, 以期获得能满足试验要求的高纯度的滋养层细胞。

1 材料与方法

1.1 材料

1.1.1 实验动物 取怀孕45~60天的牛子宫(确保子宫完整, 以免在外界环境中打开造成污染), 保存在10 ℃的无菌容器内。

1.1.2 主要试剂 DMEM培养粉、胶原酶Ⅰ(collagen I)购自美国Gibco公司; 标准胎牛血清购自美国Hyclone公司; 滋养层细胞生长添加剂(trophoblast growth supplement, TGS)购自上海麦莎生物科技有限公司; 青霉素、链霉素购自华北制药厂; Monoclonal anti-pan cytokeratin FITC conjugate clone C-11鼠抗人、猪等角蛋白抗体和Monoclonal anti-Vimentin clone V9鼠抗人波形蛋白抗体购自美国Sigma公司; 荧光素酶标记羊抗鼠IgG (Rb anti-Mo IgG/FITC)购自北京博奥森生物技术有限公司。

1.2 原代细胞培养及纯化

用70%酒精清洗整个子宫, 在无菌操作台上将子宫剪开, 用手将6~7个与母体肉阜相连的胚胎子叶分开并剪下, 置于PBS (含青霉素、链霉素)中反复清洗6~8遍, 尽量去除内膜组织。充分剪碎子叶, 获得细胞组织匀浆, 加入适量消化液 (含0.1 g胶原酶Ⅰ的100 ml PBS液用0.22 μm的过滤器除菌)消化约

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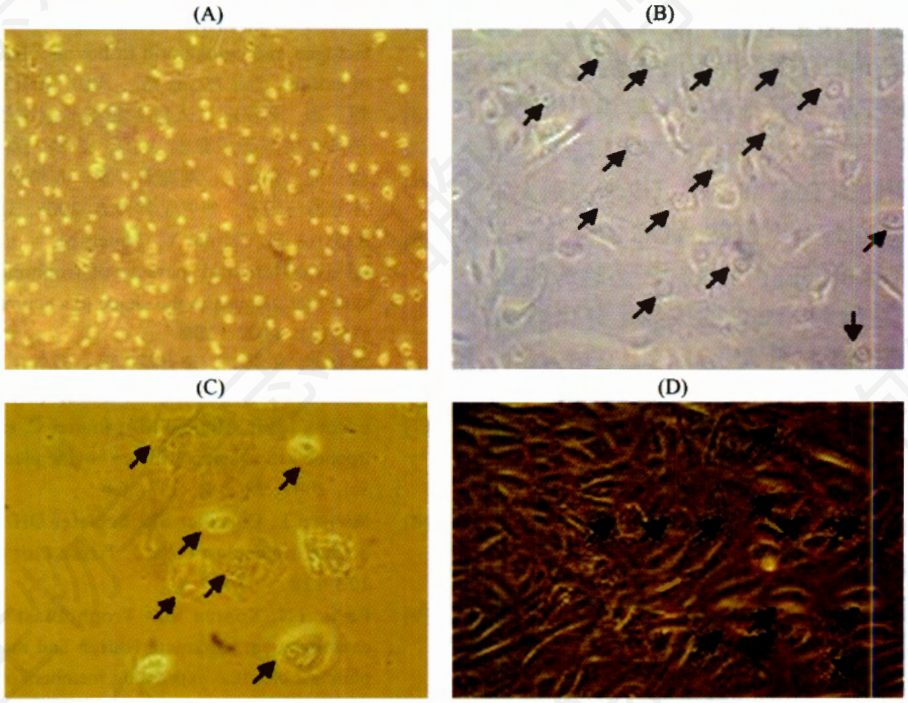

Fig.1　Culture and purify of TGC

A: isolated binucleate cells from bovine placenta (3rd day, 10×); B: binucleate cells become attached to the dish, whereupon the edges of the cells become extended on the substratum (7th day, 10×); C: cells cultured after purification (10th day, 40×); D: cells cultured without purification (10th day, 40×). Arrows represent TGC.

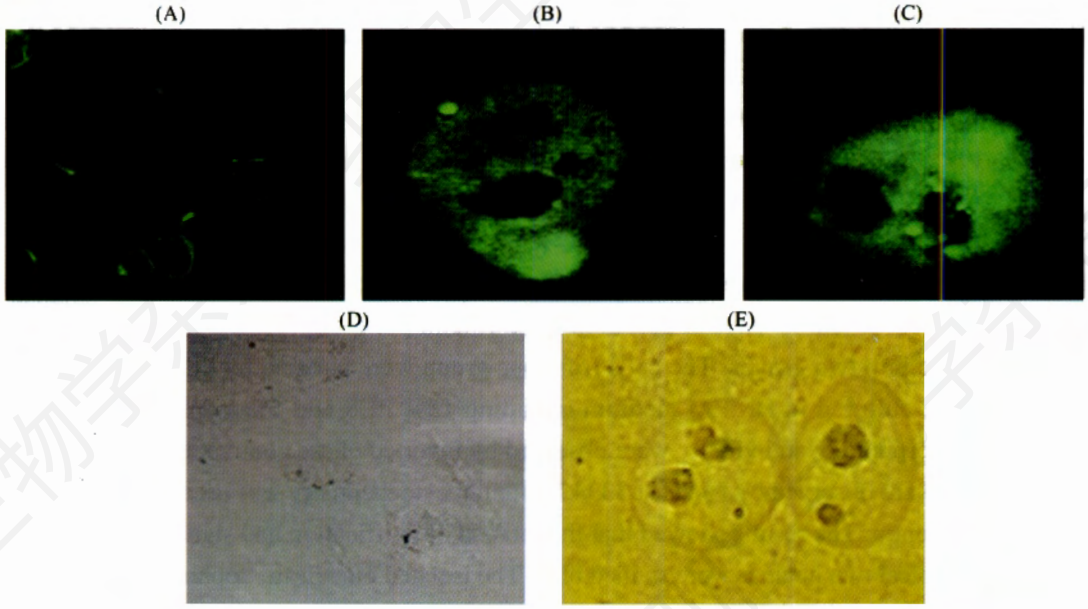

Fig.2　Results of immunocytochemistry

A, B and C: the cells are cytokeratin positive; D: the cells are vimentin negative; E: nuclear staining revealed that the cells regularly displayed two nuclei. Magnification: A is 40× and others are 400×.

中采用了 DMEM (含 15% FCS)添加 TGS 的培养基, 增加了必需和非必需氨基酸、维生素、激素、生长因子和多种矿物质等胚胎滋养层细胞营养成分, 以达到模拟与体内最相近的生长环境, 促进细胞的生长。

对于滋养层细胞的鉴定, 目前国际上尚无统一的标准, 但较认同的分子标志有细胞角蛋白和波形蛋白[1]。角蛋白是构成上皮细胞骨架蛋白, 波型蛋白是内皮细胞及间质细胞的标志蛋白。滋养层细胞是上皮样细

胞，即抗角蛋白抗体染色阳性、波形蛋白染色阴性。试验分离、培养的TGC角蛋白和波形蛋白表达均符合上皮细胞特征，只是细胞纯度与其它结果略有不同[1,7,8]。有研究发现，PL阳性的TGC缺乏角蛋白的表达，而PL阴性的TGC和多核上皮细胞则大量表达角蛋白[2]。Shimada等[10]证实，TGC的分化与呈下降表达趋势的细胞角蛋白和呈上升表达趋势的PL相关。有关PL表达检测，TGC的成熟、分化与PL之间的关系还需进一步试验研究。

滋养细胞是母-胎界面唯一与母体免疫系统直接接触的胎儿细胞，在胚胎植入、母-胎免疫耐受过程中发挥重要作用。体外分离培养的滋养层细胞与体内的真实情况最为接近，为研究各种物质通过胎盘屏障进入胎儿的具体机制打下了细胞学基础，也为滋养细胞及相关疾病的研究提供重要的实验基础，促进了滋养细胞及其相关疾病的研究进展。

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In Vitro Culture of Trophoblast Giant Cells from Bovine Placentomes

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Abstract　To establish a convenient method of purification and culture bovine trophoblast cells. Healthy placental tissues between 45 and 60 days of gestation were obtained and digested by collagen I, and then the cells were randomly divided into two groups. The cells from one group were purified by different speeding adherence. Finally, the cells were cultured in DMEM medium including 15% FCS and 5% trophoblast grouth supplement (TGS). The cellular morphology and vitality were observed by inverted phase contrast microscope and trypan blue staining. The nucleus were observed by Hoechst 33342. Immunocytochemistry was used to examine the expression of cytokeratin and vimentin. The results showed that the method of purification had significant effect. The livability was above 90% and typical two nucleus were stained blue. The isolated binucleate trophoblast giant cell (TGC) were positive for cytokeratin and negative for vimentin throughout the duration of the experiment. The purified cells contained 95% TGC and unpurified cells contained only 45%–50% TGC. Cells could passage four generations and survived for sixty days. We had developed an efficient culture system for differentiated TGC enabling future research in this field.

Key words　cell culture; bovine; trophoblast giant cell

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