

猪原始生殖嵴(PGCs)细胞分离、建系培养

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从五指山近交系(WZSP)培育群中,先后选用自然或同期发情11头5-8月龄青年母猪,分别于授精后25~29d采集胎儿75个,进行原始生殖嵴(PGCs)细胞分离、培养等建系技术研究。培养液为DMEM+F12(1:1),并含15%胎牛血清、0.1mmol/L巯基乙醇、40ng/ml hSCF、20ng/ml hLIF、2.0mmol/L谷胱甘肽,另添加或不添加20ng/ml bFGF作为对比试验。用STO细胞作饲养层,在37.5℃、5.0%CO₂和湿润的气相中进行培养。分别冷冻保存胚胎生殖嵴细胞(EG)细胞系11个,其中2个EG细胞系分别传至2~4代。并进行了染色鉴定、冻存、解冻实验等。研究发现:(1)WZSP27~29d胎儿的生殖嵴易观察和采集,更有利于建立猪EG细胞系。(2)以WZSP为材料,进行猪EG细胞培养建系时,较国外Shim(1997)、Piedrahita(1998)、Mueller(1999)报道的提前2天出现细胞克隆点(3天对5天)。(3)以DMEM为WZSP EG细胞建系基础的培养液,添加bFGF与否均可建系,但添加bFGF和其它因子可更早得到更多的克隆细胞。(4)在本研究条件下,冻存EG细胞解冻后尚能复苏。为今后系统鉴定其特性、嵌合体制作、筛选最佳EG细胞系以及探索种系传递提供了材料和可能。

ESTABLISHMENT OF PORCINE PRIMORDIAL GERM CELL(PGCs) *IN VITRO* CULTURE

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Abstract: Inbred of Wu Zhishan miniature pig (WZSP) at 5 to 8 Months of age, which were used as embryo donors. The total 75 fetuses was collected from 14 donors at day 25 to 29 of pregnancy (estrus = day 0), which was used for *in vitro* culture, clone, isolation, passage of porcine embryonic germ (PEG). The results were shown that: (1) The PGCs of fetuses between days 27-29 of pregnancy (estrus = 0) was easily to collect and more suitable to set up PEGs. (2) The PEGs clones with WZSP was coming out 2 days early than Shim (97), Piedrahita (98) and Mueller (99) reported previously. (3) PEG's will be survival *in vitro* after thawing.

Key words: Primordial Germ Cell (PGCs); Wu Zhishan miniature Pig (WZSP); *In Vitro* Culture

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