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研究报告

不同供体细胞及其处理对猪核移植重构胚体外发育的影响

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摘要

系统探讨了体细胞的组织来源及培养代数对猪核移植重构胚发育的影响。体外成熟培养40~44 h的猪卵母细胞去核后, 将经血清饥饿(0.5%FBS)培养2~9天、0.1 mg/L Aphidicolin(APD)培养+0.5% FBS培养2~9天或一般培养法(10% FBS)培养的卵丘细胞、颗粒细胞、输卵管上皮细胞和耳皮成纤维细胞, 直接注射到去核的卵母细胞质中, 或注射到卵周隙中, 再经电融合(100 V/mm, 30 [mu]s, 电脉冲1次)构建重构胚。重构胚以钙离子载体A23817 或电脉冲结合6-DMAP 激活处理, 体外培养6天。耳皮成纤维细胞和颗粒细胞经0.1 mg/L APD + 0.5% FBS培养处理后的重组胚卵裂率, 均高于血清饥饿和一般培养处理的同种供体细胞($P<0.01$)。卵丘细胞、颗粒细胞经0.1 mg/L APD + 0.5% FBS处理后进行核移植的分裂率和发育率均高于输卵管上皮细胞和耳皮成纤维细胞($P<0.05$)。以猪颗粒细胞为核供体时, 电融合法的重构胚分裂率显著高于胞质内注入法($P<0.05$), 但囊胚发育率无显著差异($P>0.05$)。培养3代和6代的猪颗粒细胞以及培养6代和10代的耳皮成纤维细胞, 其具有正常二倍染色体的细胞比例均无显著差异($P>0.05$); 以这2种细胞不同培养代数做供体进行核移植时, 各代之间核移胚的体外分裂率、囊胚发育率无显著差异($P>0.05$)。这些结果表明: (1) 猪耳皮成纤维细胞和颗粒细胞经培养传代所建立起来的细胞系相对比较稳定; (2) 0.1 mg/L APD预培养处理供体细胞能提高猪体细胞核移植的效果, 血清饥饿培养则无明显效果; (3) 猪颗粒细胞和耳皮成纤维细胞等均可做供核细胞, 核移植后都能得到体细胞克隆的囊胚, 但前者的效果略优于后者, 且其核移植效果不受供核细胞培养代数的影响; (4) 电融合核移植胚胎的发育率高于胞质内直接注入法, 但两者的总体效率相近。

关键词 猪 体细胞核移植 细胞周期

分类号

Effects of different donor cells on the development of nuclear- trans-ferred porcine embryos

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Abstract

<P>This study was undertaken to systematically examine the effects of different donor cells and numbers of passages on the development of nuclear-transferred porcine embryos, so as to establish a preliminary procedure for porcine cloning. Porcine oocytes obtained at slaughter were matured in vitro for 40—44 h and then enucleated in manipulation medium containing 5 mg/mL cytochalasin B. Fibroblast cells (FC), oviduct epithelial cells (OEC), granulosa cells (GC) and cumulus cells (CC) after 3—9 passages in 10% FBS-supplemented culture medium were either treated by serum starvation (0.5% FBS for 2—9 days), 0.1 mg/mL aphidiconlin (APD) for 1

day and 0.5% FBS for 2—9 days or left untreated in complete medium for 2—9 days. They were transferred into enucleated oocytes by microinjection or electric fusion (100 V/mm, 30 ms and 1 pulse). Reconstituted embryos were activated with a combination of calcium ionophore A23187 or electric pulse and 6-DMAP, and cultured for 6 days, to evaluate their cleavage and embryonic development. The cleavage rate of embryos reconstructed with FC and GC pretreated with 0.1 mg/mL APD + 0.5% FBS were significantly higher than that of serum starvation group and control group (<I><SPAN lang=EN-US style="FONT-SIZE: 10pt; LAYOUT-GRID-MODE: line; FONT-FAMILY: 'Times New Roman'; LETTER-SPACING: 0.2pt; mso-bidi-font-size: 12.0pt; mso-fareast-font-family: 方正书宋简体; mso-font-kerning: 1.0pt; mso-ansi-language: EN-US; mso-fareast-language: ZH-CN; mso-bidi-language: AR-SA">P</SPAN></I><SPAN lang=EN-US style="FONT-SIZE: 10pt; LAYOUT-GRID-MODE: line; FONT-FAMILY: 'Times New Roman'; LETTER-SPACING: 0.2pt; mso-bidi-font-size: 12.0pt; mso-fareast-font-family: 方正书宋简体; mso-font-kerning: 1.0pt; mso-ansi-language: EN-US; mso-fareast-language: ZH-CN; mso-bidi-language: AR-SA"><0.01</SPAN>). There was a significant difference in the cleavage rate and embryonic development among embryos derived from GC, CC and FC, OEC pretreated with 0.1 <SPAN lang=EN-US style="FONT-SIZE: 10pt; LAYOUT-GRID-MODE: line; FONT-FAMILY: Symbol; LETTER-SPACING: 0.2pt; mso-bidi-font-size: 12.0pt; mso-fareast-font-family: 方正书宋简体; mso-font-kerning: 1.0pt; mso-ansi-language: EN-US; mso-fareast-language: ZH-CN; mso-bidi-language: AR-SA; mso-bidi-font-family: 'Times New Roman'">m</SPAN><SPAN lang=EN-US style="FONT-SIZE: 10pt; LAYOUT-GRID-MODE: line; FONT-FAMILY: 'Times New Roman'; LETTER-SPACING: 0.2pt; mso-bidi-font-size: 12.0pt; mso-fareast-font-family: 方正书宋简体; mso-font-kerning: 1.0pt; mso-ansi-language: EN-US; mso-fareast-language: ZH-CN; mso-bidi-language: AR-SA">g/mL</SPAN> APD + 0.5% FBS. The cleavage rate of embryos reconstructed with GC by electrofusion was significantly higher than that by microinjection (<I><SPAN lang=EN-US style="FONT-SIZE: 10pt; LAYOUT-GRID-MODE: line; FONT-FAMILY: 'Times New Roman'; LETTER-SPACING: 0.2pt; mso-bidi-font-size: 12.0pt; mso-fareast-font-family: 方正书宋简体; mso-font-kerning: 1.0pt; mso-ansi-language: EN-US; mso-fareast-language: ZH-CN; mso-bidi-language: AR-SA">P</SPAN></I><SPAN lang=EN-US style="FONT-SIZE: 10pt; LAYOUT-GRID-MODE: line; FONT-FAMILY: 'Times New Roman'; LETTER-SPACING: 0.2pt; mso-bidi-font-size: 12.0pt; mso-fareast-font-family: 方正书宋简体; mso-font-kerning: 1.0pt; mso-ansi-language: EN-US; mso-fareast-language: ZH-CN; mso-bidi-language: AR-SA"><0.05</SPAN>), but no difference was found in the proportion of embryos that developed to blastocysts. About 75% to 85% of GC at 3 and 6 passages, and FC at 6 and 10 passages had a normal karyotype, and resulted in similar cleavage rate and blastocyst development. These results indicate that: (1) FC and GC can be cultured up to 9 passages and maintain a relatively stable karyotype; (2) Treatment of donor cells with 0.1 mg/mL APD prior to nuclear transfer can improve the efficiency of somatic cell nuclear transfer in buffalo but serum starvation is inefficient in our system; (3) Both FC and GC cells can be used as the donor karyoplasts for nuclear transfer, and their efficiency is not influenced by the culture passages. (4) The development of reconstructed embryos by electrofusion is higher than that by microinjection, but there is no difference in the overall efficiency between the two methods.</P>

Key words pigs somatic cells nuclear transfer cell cycle

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