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Title	Effects of culture media and cycloheximide treatment following electrical stimulation on parthenogenetic development of in vitro-matured porcine oocytes
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thologic changes in other breeds or animals affected with GM₁ gangliosidosis. This finding was considered as the characteristic lesion of severe GM₁ gangliosidosis in Shiba dogs, probably indicating that most of the storage materials accumulate within central nervous sys-

tem throughout the life. In addition, it is suggested that the affected dogs also have retardation of myelinogenesis (hypomyelinogenesis) associated with disturbance of oligodendroglial maturity.

Effects of culture media and cycloheximide treatment following electrical stimulation on parthenogenetic development of *in vitro*-matured porcine oocytes

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Recently, the birth of cloned piglets by somatic cell nuclear transfer has been reported, but the success rates are very low. This may be due to the inadequacy in the *in vitro* culture conditions for maturation and development, and activation treatment. To address this problem, the effects of media for maturation and subsequent development, and activation treatment on the parthenogenetic development of *in vitro*-matured porcine oocytes were examined.

First, *in vitro*-matured porcine oocytes were activated by electrical stimulation, and then cultured for 7 days either in NCSU-23 medium, which is widely used for porcine embryo culture system, or mSOF medium supplemented with 20 amino acids, which is successfully used for ovine and bovine embryo culture. Eighteen percent of parthenotes developed to blastocysts when they were cultured in NCSU-23, while parthenotes ceased to develop at the 2-4 cell stage when they were cultured in mSOF. Secondly, *in vitro*-matured porcine oocytes were activated by electrical stimulation combined with or with-

out exposure to 10 µg/ml cycloheximide (CHX) for 6 hr, and then cultured in NCSU-23 for 7 days. Combined treatment of electrical stimulation with CHX caused higher frequency of cleavage (63%) and blastocyst formation (54%) than electrical stimulation alone (32 and 19%, respectively). Next experiment was conducted to examine effects of supplementation with epidermal growth factor (10 ng/ml) to maturation medium on the development of parthenotes activated by electrical stimulation and CHX, but no beneficial effect was observed. To examine the effect of supplementation with fetal calf serum (FCS) to culture medium on parthenogenetic development, parthenotes were cultured in NCSU-23, and then transferred to NCSU-23 supplemented with 10% FCS at various time after electrical stimulation (0, 96 and 120 hr, respectively). When parthenotes were cultured in NCSU-23 supplemented with FCS from the beginning of culture or in FCS-free NCSU-23 until 96 hr after electrical stimulation then transferred to NCSU-23 supplemented with FCS, development to blastocysts

was suppressed and cell number of blastocysts was reduced. Parthenotes cultured in FCS-free NCSU-23 until 120 hr after electrical stimulation, and then transferred to NCSU-23 supplemented with FCS, developed to blastocysts at a similar rate with those cultured in FCS-free NCSU-23 throughout.

These results suggest that the 20 amino acids and FCS in the culture media inhibit the early development of porcine embryos, and that combined treatment of electrical stimulation and CHX may be effective for the induction of activation in nuclear transferred porcine embryos.

The role of epidermal growth factor in prostaglandin production
by the uterine endometrium in cow : studies in isolated-cultured cells and tissues

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The present study examined the role of epidermal growth factor (EGF) in the regulation of prostaglandin (PG) production to understand the regulatory mechanisms of PG production in the uterine endometrium.

The effect of EGF on PGE₂ and PGF_{2α} production by endometrial cell culture in the presence of oxytocin (OT) was compared between two stages, days 5 to 10 and days 11 to 17, of the estrous cycle. EGF increased PGE₂ production and PGE₂/PGF_{2α} production ratio regardless of the stage of the estrous cycle. Oxytocin receptor (OT-R) mRNA levels were compared before and after culture using endometrial cells on days 3, 7, 14 and 17 of estrus. Prior to culture, levels of OT-R expression were higher on days 7 and 14 compared to the other days; however, the difference in the levels of OT-R expression on all days became

comparable after culture. Levels of EGF-R mRNA expression was higher in the endometrial tissues at EGF treatment on day 17 than the other days. Finally, the effect of EGF on PG production in the presence of OT was compared among endometrial tissues obtained on days 3, 7-8, 14 and 17 of estrus. EGF increased PGE₂ production regardless of the days after estrus. Production of PGF_{2α} was lower in the tissues on days 7-8 than the other days.

The present results demonstrated that isolated uterine endometrial cells were not suitable materials to compare uterine endometrial functions between different stages, and that EGF increased PGE₂/PGF_{2α} production ratio in all estrous stages.