A Combination of Three Growth Factors Can Enhance Porcine Embryo Development During In Vitro Culture

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Introduction

With the increasing interest in using pigs as an animal model for studies of human diseases, there is a need to increase the efficiency of creating pig embryos in vitro. The objective of this study has been to investigate the effects of three growth factors, human fibroblast growth factor 2 (FGF2), human leukemia inhibitory factor (LIF), and human insulin-like growth factor 1 (IGF-1), on embryo developmental competence during porcine in vitro culture. These cytokines were chosen on the basis of their ability to improve the self-renewal capabilities of porcine pluripotent stem cells.

Methods

Ovaries from prepubertal gilts were obtained from Smithfield slaughterhouse in Milan MO, and follicles less than 15mm in size were aspirated and matured for 42 h in our standard lab oocyte maturation system (M199 supplemented with EGF, LH, and FSH). After oocyte maturation cumulus cells were stripped, and only oocytes with a 1st polar body at MII stage with homogenous cytoplasm were selected for fertilization. Oocytes were co-incubated with sperm (final concentration of 0.25X 10^6) in modified Tris buffered medium for 4 h. Next the oocytes were washed in culture medium MU1 (PZM3 + 1.69mM arginine), placed in Nunc 4-well plates in 5% carbon dioxide at 38.5°C overnight, and then moved to an incubator with 5% oxygen 5% CO2 and 90% nitrogen. Then, on day 4, growth factors were added to the medium, and the embryos cultured to day 6 when development to blastocyst was assessed. Percentage data were arcsin transformed and analyzed by two-way ANOVA with Fisher LSD multiple comparison test (significance, P < 0.05)

Summary

There were no differences in the percent of oocytes exposed to sperm that developed to the blastocyst stage between when only FGF2 or only LIF was added to the culture compared to controls lacking FGF2 and LIF. However, in the second experiment when a combination of 40 ng/ml FGF2 and 20 ng/ml LIF was added to the cultures on d4, significantly more blastocysts formed as compared to the control group. Finally the effects of adding the third growth factor, IGF-1 (20 ng/ml), alone or in combination with the other two factors on d4 was examined. Embryos were cultured under four different conditions: 1, Control; 2, FGF2 + LIF; 3, IGF-1; 4, FGF2 + LIF + IGF-1. IGF-1 alone or in combination with FGF2 and LIF yielded significantly better production of blastocysts than the controls, while the combination of all three growth factors yielded the highest numbers of blastocysts among all groups.