FGF2, LIF, and IGF-1 Added Together During Maturation and Culture Improve Somatic Cell Nuclear Transfer Embryo Development and Overall Efficiency

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Introduction
Culture systems for in vitro maturation of immature oocytes are generally suboptimal compared to the in vivo environment. Pigs are becoming increasingly useful as models for human disease; therefore, it is imperative that maturation and culture media are improved for production of high quality embryos. Somatic cell nuclear transfer (SCNT) is a technique often used to produce genetically engineered pigs that relies upon appropriately matured oocytes. The goal of this study has been to examine the efficiency of porcine blastocyst production from zygotes generated via SCNT following oocyte maturation in a medium that had been supplemented with three growth factors, human fibroblast growth factor 2 (FGF2), human leukemia inhibitory factor (LIF) and human insulin-like growth factor 1 (IGF-1). In combination, these three cytokines have a major positive effect on the nuclear maturation of immature porcine oocytes, with a consequent improvement in the progression of the in vitro fertilized oocytes to blastocyst (abstract numbers 531 and 343).

Materials and Methods
Prepubertal gilt ovaries were obtained from Smithfield slaughterhouse in Milan, MO. Follicles were aspirated and cumulus oocyte complexes were either matured for 42 hours in M199 supplemented with EGF, LH and FSH, our control medium; or in M199 supplemented with EGF, LH, FSH and 40 ng/ml FGF2, 20 ng/ml LIF, and 20 ng/ml IGF-1, referred to as FLI medium. Cumulus cells were removed at 42 hours and metaphase II oocytes were selected based on the protrusion of a polar body. SCNT was completed on control and FLI matured oocytes with porcine fetal fibroblasts as donor cells. Reconstructed zygotes were cultured in MU2 (PZM3+1.69 mM arginine+5μM PS48) in 5% CO2, 5% O2, 90% N2 at 38.5°C until D6 when blastocyst formation was assessed in four replicate experiments. Data were analyzed by ANOVA. In experiment 2, COCs were matured for 42 hours in FLI medium and then on day 2 of culture, the same three cytokines were added to the medium and then blastocyst percentage was assessed.

Results

Figure 1. Effects of FGF2, LIF and IGF-1 during maturation on blastocyst development first looking at blastocyst percentage based on the number of MII oocytes and then the overall efficiency based on total COCs matured. Five replicates, n=200, 198, respectively.

Figure 2. Effects of supplementation of FGF2, LIF and IGF-1 on day 2 of embryo culture on subsequent blastocyst development. Three replicates, n=113, 114, respectively. p<0.04.

Embryo Transfer
Transfer of SCNT embryos that originated from maturation with FLI to two surrogates resulted in successful pregnancies, with an average of 6 live piglets born.

Summary
The addition of FLI into maturation improves the development of reconstructed SCNT embryos to the blastocyst stage. In addition, the overall efficiency of the number of starting COCs that made it to blastocyst was also greatly improved. In the second experiment, using FLI matured oocytes and then adding FLI on day 2 of culture further improved development to blastocyst stage. Embryo transfer of FLI matured SCNT embryos did result in successful pregnancies and live piglets. Together, this data illustrate that FLI supplementation appears to provide better cytoplasmic as well as nuclear maturation of oocytes than unsupplemented medium and the further addition of these factors to embryo culture medium leads to improved development to blastocyst of SCNT embryos.