Effect of cytokine supplemented maturation medium on bovine somatic cell nuclear transfer embryo development

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Introduction

In vitro maturation (IVM) is an important process in the *in vitro* production of embryos. It has been recently shown that three cytokines (FGF2, LIF, IGF1) have increased the efficiency of *in vitro* maturation, blastocyst production and *in vivo* development in pig (Yuan et al., PNAS 2017. 114(29): E5796-E5804). IVM in medium supplemented with cytokines doubled the blastocyst rate and quadrupled the litter size when transferred. We have investigated whether this medium will have similar effects in the bovine model. Cytokine supplemented medium will be referred to as "FLI medium".

Objectives

- To assess the effect of FLI medium on IVM in bovine SCNT and IVF in vitro development
- To assess the effect of FLI medium on oocyte and blastocyst quality
- To assess the effect of FLI medium on bovine SCNT pregnancy and birth rate

Methods

Oocyte Maturation Cumulus oocyte complexes were matured for 21 hours in either our standard maturation medium (TCM-199 [Gibco], containing 10% FBS, 0.5 µg/mL FSH, 5 µg/mL LH, and 100 U/mL penicillin/streptomycin) or maturation medium supplemented with 20 ng/mL human LIF, 20 ng/mL human IGF1 and 40 ng/mL human FGF2.

GSH/ROS Staining MII oocytes were incubated in 20 µM CellTracker Blue CMF2HC or 10 µM H2DCFDA to measure GSH and ROS levels, respectively. Fluorescence was observed under ultraviolet light and measured using ImageJ software.

Somatic Cell Nuclear Transfer After IVM, the first polar body and metaphase plate were removed from MII oocytes. Donor cells were placed in the perivitelline space and fused with ooplasts in 0.28 M sorbitol fusion medium (0.1 mM calcium, 0.5 mM magnesium, 0.5 mM hepes, 1 g/mL BSA) by a single pulse of 1.75 kV/cm for 22 microseconds. Fused embryos were activated by exposure to 5 µM ionomycin for 5 minutes, followed by a 4 hour incubation in 2 mM DMAP and 10 µg/mL cycloheximide.

In Vitro Fertilization after IVM cumulus oocytes complexes were placed in fertilization medium with frozen thawed sperm as described by (Bavister et al. 1977), with modifications.

In Vitro Culture Embryos were cultured in groups of 45 in 50 µL droplets of SOF medium under mineral oil at 38.5 °C with 5% CO2. Cleavage and blastocyst rates were assessed at day 2 and day 8, respectively.

Blastocyst Cell Count day 8 blastocysts were fixed, all cells stained with Hoescht and trophectoderm cells with CDX-2 and Alexa Fluor 488, a goat anti-mouse IgG secondary antibody. Blastocysts were imaged using a Zeiss fluorescent microscope. Cells were counted using ImageJ software (NIH, Bethesda, MD, USA).

Embryo Transfer Day 7 blastocyts were transferred to synchronized recipients. Initial pregnancy was determined by ultrasound on Day 40.

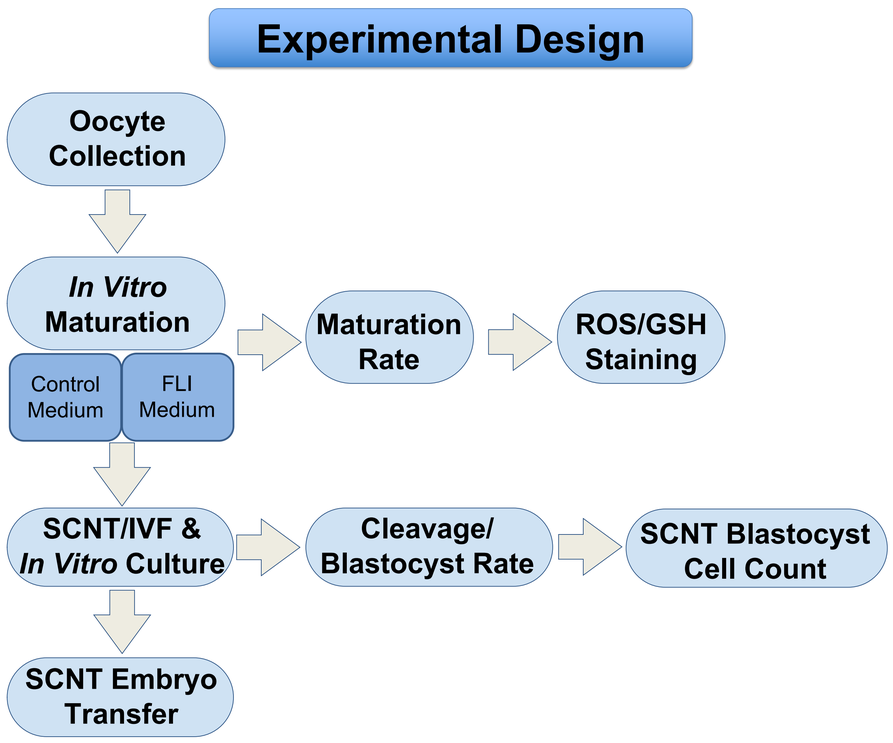

Figure 1. Chart outlining experimental design

Results

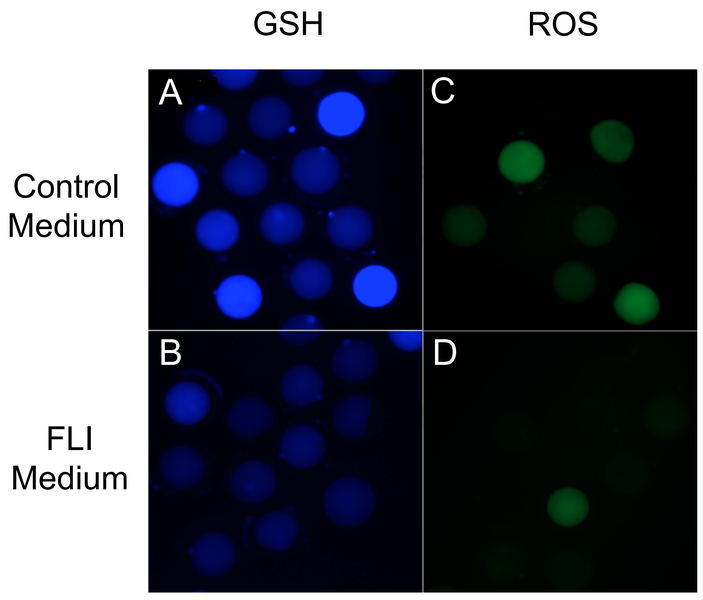

Figure 2. Fluorescent images of *in vitro* matured bovine oocytes. (A-B) Oocytes were stained with CellTracker Blue CMF2HC to detect intracellular GSH levels. Relative GSH levels were significantly higher in oocytes matured in control medium *(P* <0.05). (C-D) Oocytes were stained with 20,70-dichlorodihydro-fluorescein diacetate to detect intracellular ROS levels. Relative ROS levels were also significantly higher in oocytes matured in control medium compared to those matured in FLI medium *(P* <0.05).

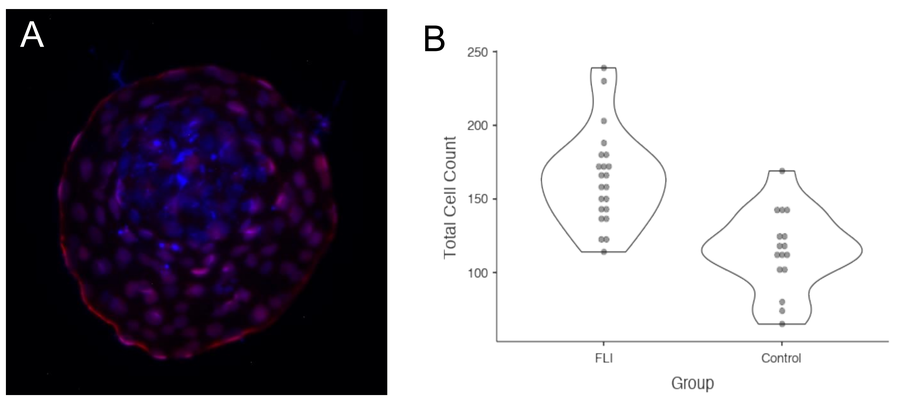

Figure 3 Fluorescent images of fixed stained SCNT blastocysts. (A) Blastocyst cell DNA was stained with Hoescht and trophectoderm cells were stained with CDX-2 antibodies. (B) Violin plot of total cell count data. Total cell count was significantly higher in FLI embryos*(P* <0.05). There was no significant difference in trophectoderm and inner cell mass cell ratio.

Table 1 Effect of FLI medium on oocyte maturation

	Oocytes (n)	Mature Oocytes (n)	Maturation Rate (%)
FLI Medium	885	709	80.2±2.3%[a]
Control Medium	822	549	66.8±1.8%[b]

Table 2 Effect of FLI medium on IVF embryo development

	Oocytes (n)	Cleaved Embryos (n)	Cleavage Rate (%)	Blastocysts / Cleaved Embryo (n)	Blastocyst Rate (%)
FLI Medium	518	435	84.0±1.3%	155	35.6±2.1%[a]
Control Medium	551	428	77.7±1.9%	117	27.3±1.9%[b]

Table 3 Effect of FLI medium on SCNT embryo development

	Recon-structed Embryos (n)	Cleaved Embryos (n)	Cleavage Rate (%)	Blasto-cysts (n)	Blastocyst Rate (%)
FLI Medium	446	418	93.94±1.1%	181/446	40.6±5.1%[a]
Control Medium	300	272	90.9±1.2%	73/300	24.3±2.9%[b]

Table 4 Effect of FLI medium on SCNT pregnancy development

	Transfers (n)	Pregnancies (n)	Pregnancies (%)	Births (n)	Births (%)
FLI Medium	48	25	50.3±20.0%[a]	*in progress*	*in progress*
Control Medium	31	9	29.0±20.1%[b]	*in progress*	*in progress*

- Percentages are means ± SEM. 4, 3, 8, and 4 replicates were performed in tables 1-4, respectively. Data were analyzed by One-way ANOVA in Tables 1-3. Data in Table 4 were analyzed using chi-square (the Jamovi Project 2020)
- a, b values within a column with a different superscript are significantly different.

Summary

- FLI medium improved bovine IVM rate and oocyte quality as evidenced by the decreased ROS level.
- Oocyte matured in FLI medium exhibited greater blastocyst formation rate following IVF and SCNT. Additionally, total cell number in FLI SCNT blastocysts was greater than in the control group.
- Furthermore, pregnancy rate (Day 40) was also improved in the FLI group. We continue to monitor these pregnancies.

Future work

- Measurement of mRNA levels will be performed to identify differentially expressed genes in FLI treated group.
- RNA sequencing will be performed on cumulus cell samples.

References

Yuan, Ye, et al. "Quadrupling efficiency in production of genetically modified pigs through improved oocyte maturation." *Proceedings of the National Academy of Sciences* 114.29 (2017): E5796-E5804.