SI Appendix

Quadrupling efficiency in production of genetically modified pigs through improved oocyte maturation

Short title: Cytokine-induced enhancement of oocyte maturation

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SI Materials and Methods

Chemicals. All chemicals were purchased from Sigma–Aldrich (St. Louis, MO) unless indicated otherwise.

Quantitative PCR (qPCR). RNA was extracted from a pool of 100 COCs for each sample by using the RNeasy micro kit (Qiagen, Germantown, MD). Total RNA was suspended in 12 µL H_2O and was converted to cDNA by using the SuperScript VILO kit (Life Technologies, Carlsbad, CA). Q-PCR was conducted on each of the samples for genes of interest by means of iTaq™ Universal SYBR Green Supermix (Bio-Rad Laboratories). Primers were designed from the most representative public ID sequence through use of Oligo5.0 software (Molecular Biology Insights, Inc.) except for primers for *EREG*, which have been previously reported (*1*). Primers are listed in Table S3. All qPCR reactions were run in triplicate for every biological replicate on the CFX Connect™ Real-Time P System (Bio-Rad Laboratories). We tested several housekeeping genes; *CALR* was selected as a stable reference gene for this experiment.

Western blot analysis of cumulus cell samples. Cumulus cells were removed from oocytes by repeated pipetting of COCs in a centrifuge tube in the presence of 0.1 % w/v hyaluronidase. Cell suspensions containing only cumulus cells were transferred to centrifuge tubes and centrifuged at 300 × g for 5 min. Cell pellets were frozen in radioimmunoprecipitation assay buffer containing a cocktail of protease inhibitors at -80 ^{o}C before western blotting (*2*). Denatured protein samples (3 µg) were analyzed by separation on a 10% SDS/PAGE gel (Biorad), transferred to PVDF membranes (Biorad) and incubated in phospho-p44/22 MAPK primary antibody (Cell Signaling, Catalog no. 4370, 1:2000) overnight. Anti-rabbit HRP-coupled secondary antibody was used to visualize bands by using SuperSignal West Dura chemiluminescent substrate (Thermo Scientific). Images were acquired with the Fuji LAS 3000 imaging system (Fujifilm Medical Systems). The same blot was then stripped with Restore-Plus Western blot stripping buffer (Thermo Scientific), incubated with 5% (w/v) nonfat dry milk, and re-probed with MAPK antibody (Cell Signaling, Catalog no. 4695, 1:1000) to detect the amount of total MAPK. Band

intensity was measured as the integrated intensity with ImageJ software and normalized to the background intensity.

Supplemental Figures

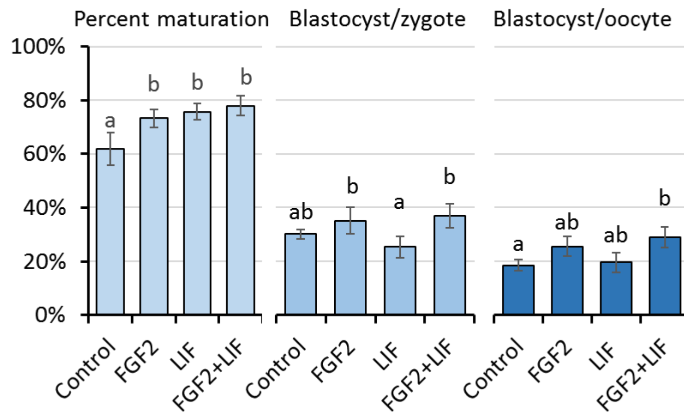

Fig. S1. Effects of FGF2 and LIF present in porcine IVM medium on nuclear maturation and subsequent blastocyst development following IVF. Data are reported as means ± SEM. Different superscripts ^{ab} denote a significant difference from the control, $P < 0.05$. The experiments were replicated five times with a total of 1455 oocytes.

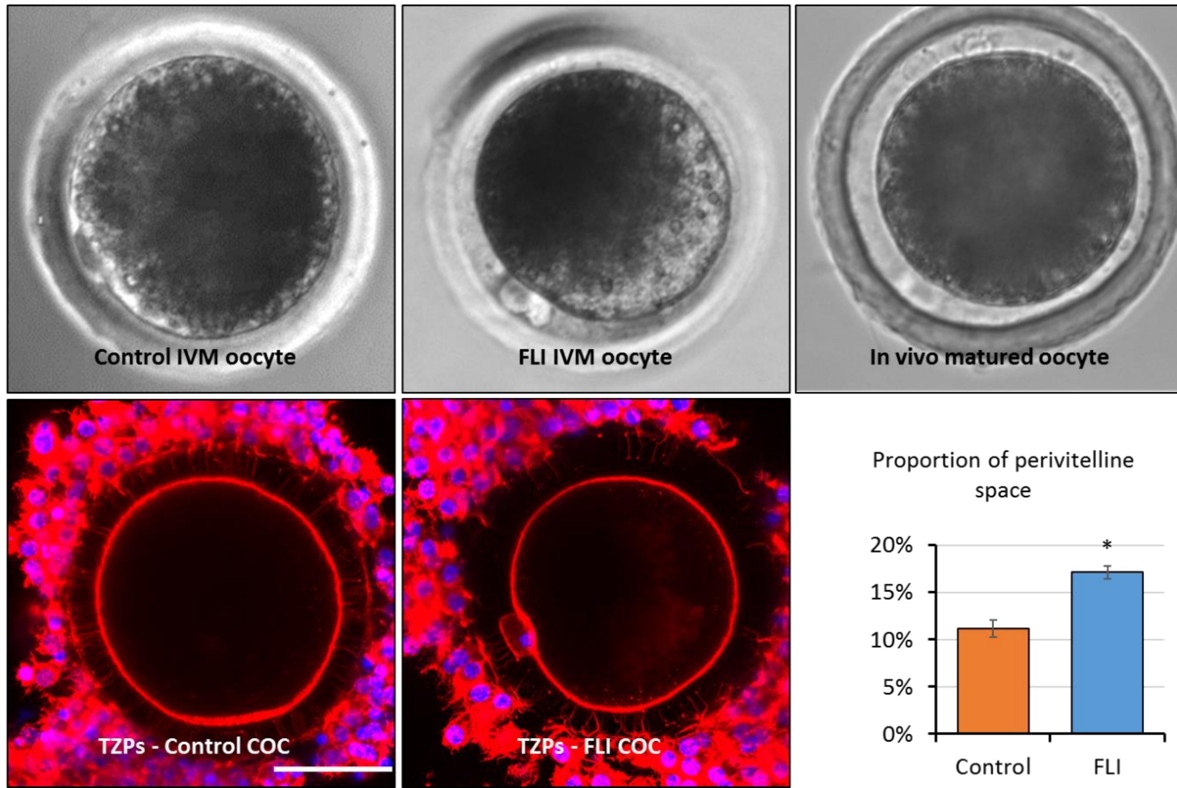

Fig. S2. Representative images of porcine oocytes matured in control and FLI medium in comparison to an in vivo matured oocyte (top). Representative Z-stack confocal Images of TZPs in COC (bottom left and middle, Scale bar=50 µm) were acquired after 42 h IVM after fixation and staining with rhodamine phalloidin. The proportion of perivitelline space (bottom right) was calculated by (area under zona - area of ooplasm) / Area under zona. Images of ten MII oocytes from each group were collected, and areas measured with image J software.

Supplemental Tables

Table S1. The relative decline of cumulus cell expansion index between Control and FLI treatment groups at 6 to 7 h of culture. P values were obtained by performing a Student's t test to compare values at any time point for Control and FLI groups. Asterisks denote a significant difference between control and treatment, $P < 0.05$.

Time (h)	Control		FLI		P-value
	Mean	SEM	Mean	SEM	
5.75	89.4%	1.2%	85.0%	2.6%	0.08
5.875	89.6%	1.4%	84.5%	2.5%	0.05
6.00	89.0%	1.5%	84.5%	2.5%	0.07
6.125	88.5%	1.5%	84.2%	2.6%	0.09
6.25	89.8%	0.7%	84.5%	2.6%	0.04*
6.375	91.1%	1.7%	84.6%	2.3%	0.02*
6.50	90.5%	1.5%	84.4%	2.5%	0.03*
6.625	90.9%	1.7%	84.5%	2.7%	0.04*
6.75	90.9%	1.6%	84.5%	2.8%	0.04*
6.875	90.9%	1.9%	85.0%	2.6%	0.05
7.00	91.5%	2.4%	84.5%	2.8%	0.04*
7.125	91.5%	2.3%	85.0%	2.7%	0.05
7.25	91.2%	2.4%	85.6%	2.8%	0.08

Table S2. Composition of oocyte maturation media.

Component	Source	Control medium	FLI medium
TCM 199	Gibco, Catalog no. 11150-059	-	-
Glucose	Sigma, Catalog no. g7021	3.05 mM	3.05 mM
Sodium pyruvate	Sigma, Catalog no.p4562	0.91 mM	0.91 mM
Cysteine	Sigma, Catalog no.c7352	0.57 mM	0.57 mM
EGF	Sigma, Catalog no.s4127	10 ng/ml	10 ng/ml
LH	Sigma, Catalog no. L5269	0.5 μg/ml	0.5 μg/ml
FSH	Sigma, Catalog no. F2293	10 ng/ml	10 ng/ml
polyvinyl alcohol	Sigma, Catalog no. p8136	0.1 % w/v	0.1 % w/v
human LIF	Millipore, Catalog no. LIF1050	0	20 ng/ml
human IGF1	Peprotech, Catalog no. CYT-022	0	20 ng/ml
human FGF2	Made in-house*	0	40 ng/ml

* Human recombinant FGF2 was purified from a yeast strain obtained as a gift from Dr. Bradley Olwin's laboratory at University of Colorado, Boulder, CO (3, 4).

Table S3. Primer information

Gene		Primer Sequence	Amplicon length	Tm	Accession number or reference
AREG	Forward Reverse	GATCCTCTGCTCAGCCCATTATG CCTCACTTCCCGAGGACATC	140	54	NM_214376
EREG	Forward Reverse	AAGACAATCCAGGTGTGGCTCAAG CGATTTTTGTACCATCTGCAGAAA	276	58	(1)
BTC	Forward Reverse	GCTGTCATCCTCTTCGGAAA AGGCATTTTGTAGCTCGCAC	161	54	FR694924
HAS2	Forward Reverse	GAAGTCATGGGCAGGGACAATTC TGGCAGGCCCTTTCTATGTTA	407	54	NM_214053
PTGS2	Forward Reverse	TCGACCAGAGCAGAGAGATGAGAT ACCATAGAGCGCTTCTAACTCTGC	260	55	NM_214321
TNFAIP6	Forward Reverse	GAGAAGCTCTGCTACGTCG CCAGACAGCACCGTGTTGG	255	58	U07786
CYP11A1	Forward Reverse	TTCCAGAAGTATGGTCCCATTTA TGAGCATGGGGACACTAGTGTGG	501	58	NM_214427
BAD	Forward Reverse	TTGCCAGCCGAGATTAACCCTAAC CACGCGGGCTTTATTAGCACGTTT	100	60	XM_003122573
TP53	Forward Reverse	GGAACAGCTTTGAGGTGCGTGTTT ATACTCGCCATCCAGTGGCTTCTT	182	60	NM_213824
CALR	Forward Reverse	CCGCCGCTGAACCCACTATT GGGCTCAAATCTGGCCGACA	206	60	XM_013990652.1

Supplemental Video Legends

Video S1. A 42 h time-lapse video to monitor cumulus cell expansion in control medium (10x magnification).

Video S2. A 42 h time-lapse video to monitor cumulus cell expansion in FLI medium (10x magnification).

References

1. Y. Yamashita *et al.*(2007) Hormone-induced expression of tumor necrosis factor alpha-converting enzyme/A disintegrin and metalloprotease-17 impacts porcine cumulus cell oocyte complex expansion and meiotic maturation via ligand activation of the epidermal growth factor receptor. *Endocrinology* 148(12):6164-6175.
2. M. Amita *et al.* (2013) Complete and unidirectional conversion of human embryonic stem cells to trophoblast by BMP4. *Proc Natl Acad Sci U S A* 110(13):E1212-21.
3. Rapraeger AC, Guimond S, Krufka A, & Olwin BB (1994) Regulation by heparan sulfate in fibroblast growth factor signaling. *Methods Enzymol* 245:219–240.
4. Fedorov YV, Jones NC, & Olwin BB (1998) Regulation of myogenesis by fibroblast growth factors requires beta-gamma subunits of pertussis toxin-sensitive G proteins. *Mol Cell Biol* 18(10):5780-5787.