

DMBA acts on cumulus cells to desynchronize nuclear and cytoplasmic maturation of pig oocytes

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Supplementary information

Supplementary Table S1: The information of DMBA concentration used in vitro in previous reports.

Level of material	Species and material type	DMBA concentration	Culture/treatment time	Reference	Note
	Human granulosa cell	5 μ M	12h	Bengtsson et al., 1988	+HCG
	Human T cell	1,3, 10, 30 μ M	100sec	Archuleta et al., 1993	+10% FBS
Somatic cell	Mice thymus cell	3,10,30 μ M	4h	Burchiel et al., 1992	+10% FBS
	Mice langerhans cell	32 μ M, 64 μ M	24h	Modi et al., 2012	+10% FBS
	Pig ovarian granulosa cell	5 μ M	48h	Becedas et al., 1993	+10% FCS
	Mice ovary	50nM	4days	Sobinoff et al., 2011	+5% FCS
Ovary	Mice ovary	1 μ M	72h,6days	Matikainen et al., 2001	+3mg/ml BSA
	Neonatal rat ovary	12.5nM,75nM	1,2,4,8days	Madden et al., 2014	+1mg/ml BSA +1mg/ml Albumax
	Mytilus sperm	0,5,10,15,20 μ M	10min	Togo and Morisawa, 2004	Seawater only
Germ cell	Ascidian oocyte	10, 20 μ M	1h	Lambert., 2005, 2011; Robert et al., 1999	Seawater only
	Pig COC	0.01,0.1,1,10 μ M	24h	Su et al., 1999	+hypoxanthine - hormones

Abbreviation in Supplementary Table S1

COC:cumulus -oocyte complexe; HCG:human chorionic gonadotropin; FCS:fetal calf serum; FBS:fetal bovine serum; BSA: bovine serum albumin.

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Supplementary Table S2: Comparison of composition of culture medium used in our present study and referred report.

Composition of culture medium	Su et al. 1999 ¹	In the present study
Basic medium 199	With	With
Pyruvate	0.23mM	-
Glutamin	2mM	-
Bovine serum albumin	3mg/ml	-
Penicillin	100IU/ml	-
Streptomycin	100IU/ml	-
Hypoxanthine	4mM	-
Sodium pyruvate	-	0.91mM
Polyvinyl Alcohol	-	1mg/ml
D-glucose	-	3.05 mM
Gentamincin	-	1µg/ml
L-cysteine	-	0.57mM
Follicle stimulating hormone	-	0.5µg/ml
Luteinizing hormone	-	0.5µg/ml
Epidermal growth factor	-	10µg/ml

1. Su, Y. Q., Xia, G. L., Byskov, A. G., Fu, G. D. & Yang, C. R. Protein kinase C and intracellular calcium are involved in follicle-stimulating hormone-mediated meiotic resumption of cumulus cell-enclosed porcine oocytes in hypoxanthine-supplemented medium. *Mol Reprod Dev.* **53**, 51-58 (1999).

Supplementary Table S3: Primers used for real-time PCR.

Genes	Gene Bank accession No.	Primer sequence (5'-3')	Tm (°C)	Product size (bp)
Kdm6a	XM_003360275.3	F: GCAGGCTCAGTTGTGTAACC R: GGTTTACATGCCTGCTGTGC	60	150
Setd2	XM_005669478.1	F: TAGCTCGCAAGCTGACTCAT R: ACAGCCCCAAACTTCTGCAT	60	139
Ezh2	NM_001244309.1	F: TGCAACACCCAATACTTACAAGC R: ACTCTTTTGCTCCCTCCAAGT	60	101
G9a	NM_001101823.1	F: GGAGGAGCTGGGGTTTGAC R: CAGAGGTGGCTGCTGAGTTG	60	254
Eed	XM_013979231.1	F: TGTGACTATTCTTGGGCGATTT R: TGGCTTTATGAGGATCTTCTACTT	60	153
Suz12	XM_013981159.1	F: TCAGGATATTCATCGCCAACC R: CTTCGGATTCAAGAAATTCAGACA	60	134
Ywhag	XM_003124396.4	F: GGCCATGAAGAACGTGACAG R: CATCCTGACATACGGCCTCC	60	224
Caspase3	XM_005671704	F: TTTGCGTGCTTCTAAGCCAT R: GGCAGGCCTGAATTATGAAA	60	147
Bcl2	AF216205	F: CGTCCCAGCTCCACATCACC R: AGTGCCCCACCGAAGGAGAA	60	130
Bax	XM_003127290.4	F: GCCGAAATGTTTGCTGACG R: GCAGCCGATCTCGAAGGA	60	157

Supplementary Figures

Supplementary Figure S1: Effects of DMBA exposure on cumulus expansion of

COCs. COCs were photographed using digital camera and the ratio of cumulus cell expansion was calculated according to the previously described method (Li et al., 2016). **(a)** Representative images of COCs taken at 0, 24 and 44h. Scale bar, 500 μ m.

(b) The extent of the relative cumulus expansion of COCs presented as a ratio of the total two-dimensional area of each COCs at different time points to the original size of COCs at 0h. *** indicate significant differences at $P < 0.001$ level between groups.

Supplementary Figure S2: The effect of CBX on PB1 rate of porcine CDOs.

PB1 rate of CDOs was not affected by 50 μ M CBX incubation for 44h.

Supplementary Figure S3: Full-length blot to detect p-ERK1/2, γ H2A.X and

β -tubulin proteins. Lysate from three replicates of control and 20 μ M treated pig oocytes (see Methods for details) was run on the same gel and protein was transferred onto membrane. The membrane was cut in two parts containing the proteins above and below the 25 kDa marker. The upper part of the membrane was used to detect p-ERK1/2 (phospho T202/Y204) and the lower part was used to detect γ H2A.X (phospho S139). After p-ERK1/2 detection, the upper part of the membrane was re-probed with anti- β -tubulin antibody.

Supplementary Figure S4: Distribution of nuclear stages of porcine oocytes from

COCs treated by DMBA during IVM. **(a)** Representative images of porcine oocytes at different nuclear stages during IVM. Scale bar, 100 μ m. **(b-h)** Nuclear stages of porcine oocytes from COCs collected at 12h, 18h, 24h, 30h, 36h, 44, 72h, respectively.

* indicates the significant difference at 0.05 level. * indicates significant differences at

P < 0.05 level between groups.

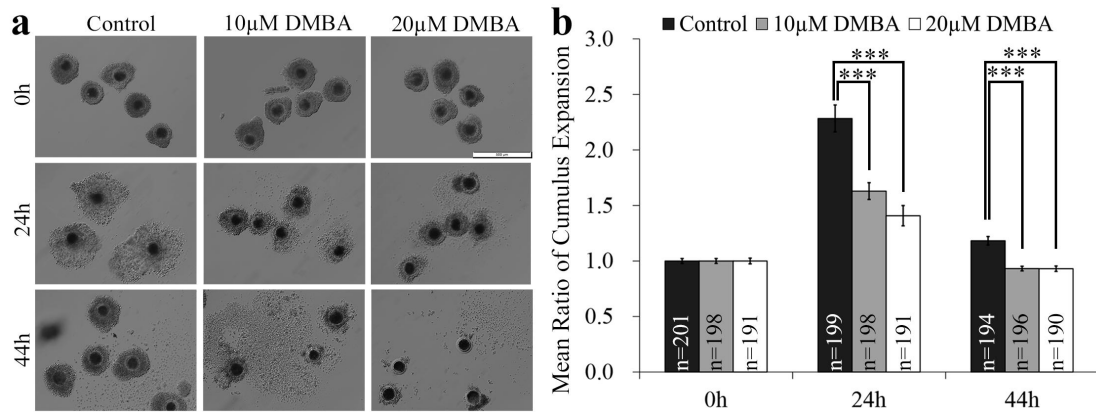
Supplementary Figure S5: Analysis of 1-cell arrested parthenotes from different

groups. (a) Developmentally arrested 1-cell embryos were stained with Hoechst33342 to show different numbers of pro-nuclei, from 1 to 6. Scale bar, 100 μ m.

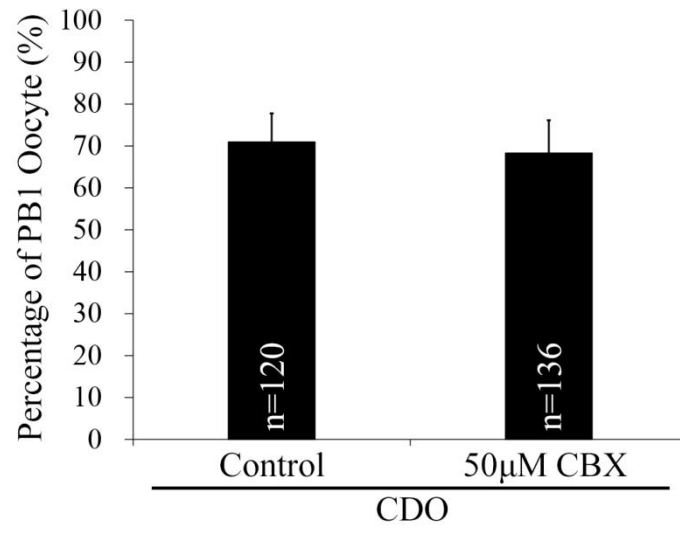
(b) Graph to show the rate of 1-cell arrested parthenotes in control and 20 μ M DMBA treated COC and CDO groups. (c) Graph to show the rate of 1-cell arrested parthenotes with different number of pronuclei. *** indicate significant differences at

P < 0.001 level between groups.

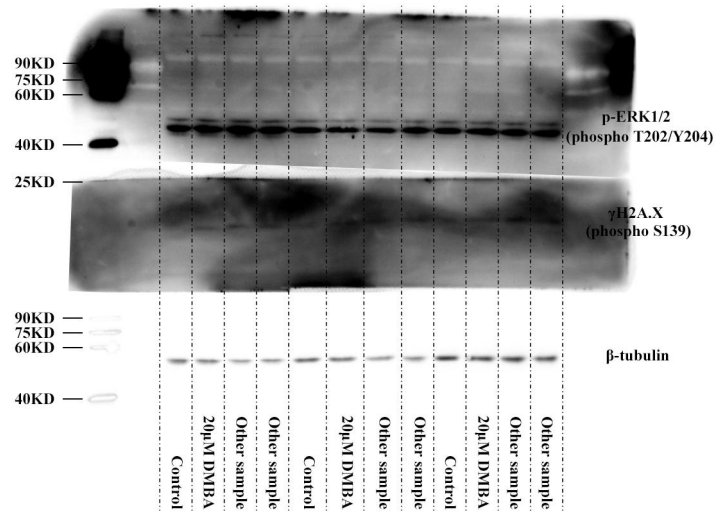
Supplementary Figure S1



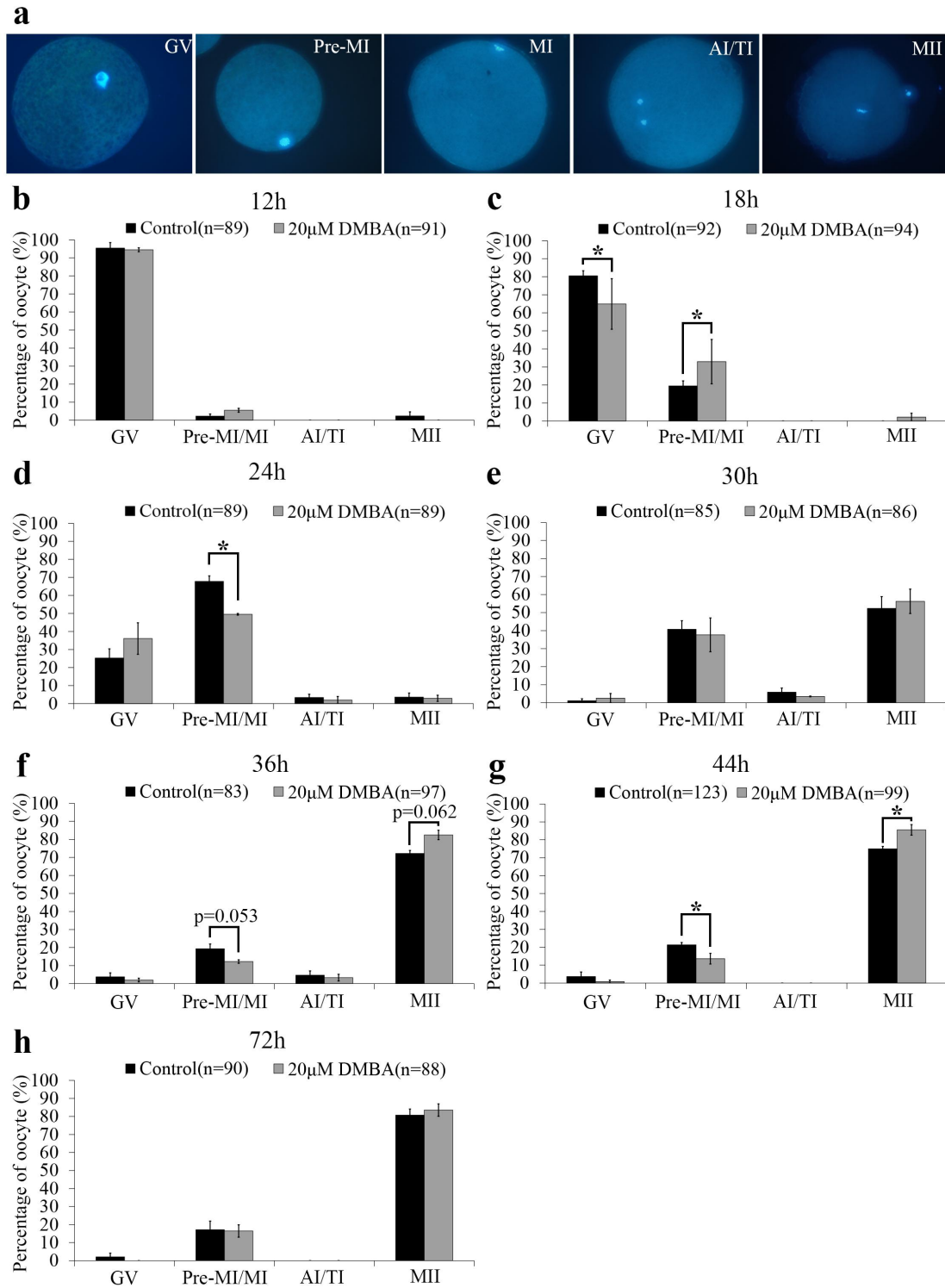
Supplementary Figure S2



Supplementary Figure S3



Supplementary Figure S4



Supplementary Figure S5

