

Supplementary Material

Follicular environment as a predictive tool for embryo development and kinetics in cattle

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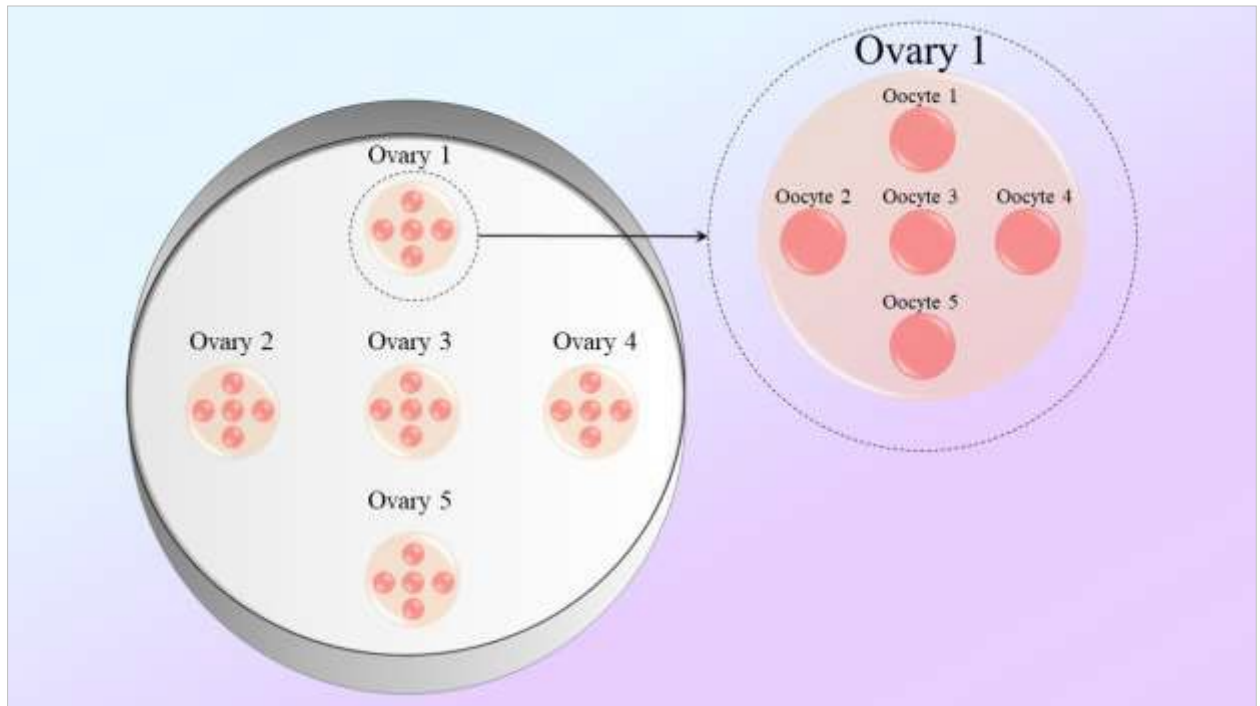


Fig. S1. Schematics representing the oocytes position in the dish for *in vitro* maturation. Each dish was prepared containing 5 droplets of 50 μ L of medium for maturation (under mineral oil), and there were 5 wells containing one oocyte from each follicle in each droplet. The follicles respective follicular fluids were collected and maintained at -80 $^{\circ}$ C until the analysis.

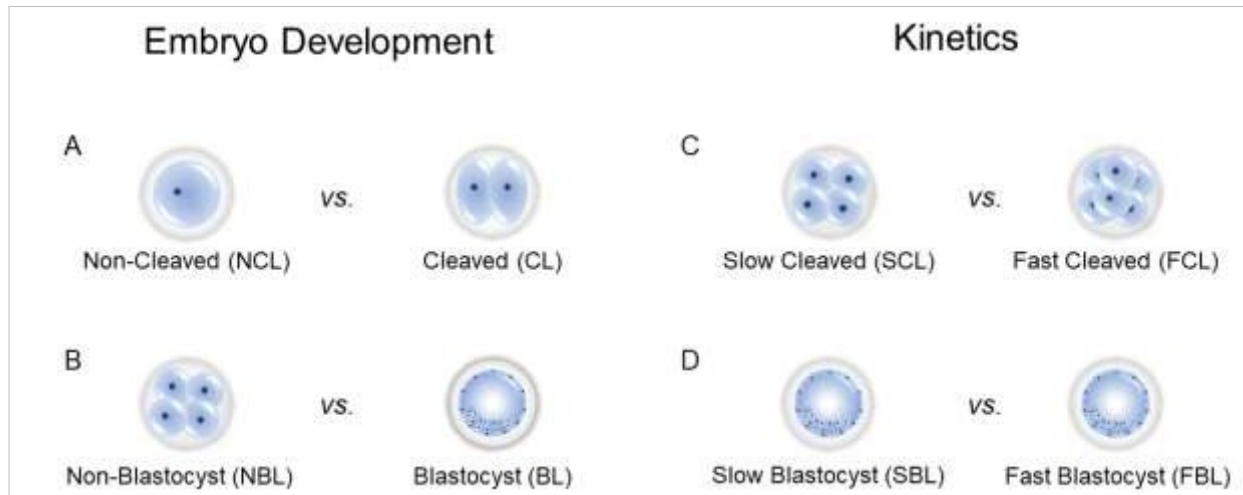


Fig. S2. Schematics representing definition of the groups and comparisons included in the present study, according with embryo development stage and kinetics of cleavage. The embryo development was assessed considering presence of cleavage (A) and development to the blastocyst stage (B). Kinetics was analyzed considering cleavage speed (C) and development speed in order to generate a blastocyst (D).

Table S1. Individual functions of genes included in the gene expression experimentThe functions were determined based on the biological processes from Uniprot (*uniprot.org*)

Gene	Name	TaqMan Code	Uniprot Entry	Biological Function	General Biological Process
ADCY3	Adenylate cyclase 3	Bt04289077_m1	F1MPC9	acrosome reaction, cGMP biosynthetic process	Cell signaling
ADCY6*	Adenylate cyclase 6	Bt03816767_m1	E1B9K3	cGMP biosynthetic process, signal transduction	Cell signaling
ADCY9	Adenylate cyclase 9	Bt04287024_m1	E1BM79	cGMP biosynthetic process, signal transduction	Cell signaling
BDNF	Brain-derived neurotrophic factor		Q95106	Cell-cell signaling	Cell signaling
BMP15	Bone Morphogenetic Protein 15	Bt03286494_u1	Q6PX77	granulosa cell development, ovarian follicle development	Cell signaling
DNMT1	DNA (Cytosine-5-)-Methyltransferase 1	Bt03224737_m1	Q24K09	maintenance of DNA methylation	Cell signaling
EGFR	Epidermal growth factor - receptor	AJT96D7	F1N7F3	cell morphogenesis, cell proliferation	Cell signaling
FOXO3	Forkhead box O3	Bt03649334_s1	F1N4K5	cell differentiation	Cell signaling
GREM1*	Gremlin	Bt03255355_m1	A2VE87	cell-cell signaling	Cell signaling
GUCY1B3*	Guanylate cyclase 1, soluble, beta 3	Bt03215602_m1	P16068	Cellular response to nitric oxide, cGMP biosynthetic process	Cell signaling
H1FOO	H1 histone family, member O, oocyte-specific	Bt03228652_g1	Q3HNG7	negative regulation of stem cell differentiation	Cell signaling
H2AFZ*	H2A histone family, member Z	Bt03216346_g1	P0C0S4	DNA binding	Cell signaling
H3F3A	H3 histone, family 3A	Bt03278804_g1	Q5E9F8	nucleosomal DNA binding	Cell signaling
HAS2	Hyaluronan synthase 2	Bt03212695_g1	O97711	cell adhesion	Cell signaling
HDAC2	Histone deacetylase 2	Bt03244871_m1	F1MFZ7	positive regulation of cell proliferation	Cell signaling
HMBS*	Hydroxymethylbilane synthase	Bt03234763_m1	Q2KIN5	Heme biosynthetic process	Cell signaling

HPRT1*	Hypoxanthine-guanine phosphoribosyltransferase	Bt03225311_g1	Q3SZ18	GMP catabolic process	Cell signaling
IGFBP2*	Insulin-Like Growth Factor Binding Protein 2	Bt01040719_m1	P13384	regulation of insulin-like growth factor receptor signaling pathway	Cell signaling
IGFBP4	Insulin-Like Growth Factor Binding Protein 4	Bt03259500_m1	Q05716	regulation of insulin-like growth factor receptor signaling pathway	Cell signaling
IMPDH1*	IMP (inosine 5'-monophosphate) dehydrogenase 1	Bt00995384_m1	A0JNA3	GTP biosynthetic process	Cell signaling
IMPDH2	IMP (inosine 5'-monophosphate) dehydrogenase 2	Bt03226238_g1	Q3SWY3	GTP biosynthetic process	Cell signaling
NOS2*	Nitric oxide synthase 2, inducible	Bt03249597_m1	Q27995	inflammatory response	Cell signaling
NOS3	Nitric oxide synthase 3	Bt03217679_m1	P29473	nitric oxide mediated signal transduction	Cell signaling
NPPA	Natriuretic peptide A (ANP)	Bt03223175_g1	P07501	cGMP biosynthetic process, female pregnancy	Cell signaling
NPPB	Natriuretic peptide B (BNP)	Bt04301375_g1	P13204	cGMP biosynthetic process	Cell signaling
NPPC	Natriuretic peptide C (CNP)	Bt03212844_m1	P55206	cGMP biosynthetic process	Cell signaling
NPR1*	/Natriuretic peptide receptor 1	Bt04297034_g1	E1BN71	cGMP biosynthetic process	Cell signaling
NPR2	Natriuretic peptide receptor 2	Bt04316732_m1	P46197	cGMP biosynthetic process	Cell signaling
NPR3*	Natriuretic peptide receptor 3	Bt03212867_m1	P10730	osteoclast proliferation	Cell signaling
OOSP1	Oocyte-secreted protein 1	Bt03233533_g1	Q2Q0J1	may be involved in cell differentiation	Cell signaling
PDE5A	Phosphodiesterase 5A, cGMP-specific	Bt03214261_m1	Q28156	cGMP catabolic process	Cell signaling

PTGS2	Prostaglandin-endoperoxide synthase 2	Bt03214489_m1	O62698	inflammatory response, ovarian cumulus expansion, positive regulation of embryonic development	Cell signaling
TFAM	Transcription Factor A, Mitochondrial	Bt03260078_m1	Q0II87	positive regulation of transcription, DNA-templated	Cell signaling
ATP5L	ATP synthase, H ⁺ transporting, mitochondrial Fo complex subunit E	Bt03210836_g1	Q28852	ATP synthesis coupled proton transport	Energy metabolism
CPT1B	Carnitine O-palmitoyltransferase 1, muscle isoform	Bt03244645_m1	Q58DK1	fatty acid beta-oxidation, transport	Energy metabolism

CPT2	Carnitine O-palmitoyltransferase 2, mitochondrial	Bt03233823_m1	Q2KJB7	fatty acid metabolic process, transport	Energy metabolism
PFKP	Phosphofructokinase	Bt04316551_m1	E1BCW3	fructose 6-phosphate metabolic process	Energy metabolism
SDHA	Succinate dehydrogenase complex flavoprotein subunit A	Bt04307509_m1	P31039	anaerobic respiration, oxidation-reduction process	Energy metabolism
SLC2A1*	Solute carrier family 2 (facilitated glucose transporter), member 1	Bt03215314_m1	P27674	glucose transport	Energy metabolism
SLC2A4	Solute carrier family 2 (facilitated glucose transporter), member 4	Bt03215316_m1	Q27994	cellular response to insulin stimulus	Energy metabolism
ACACA*	Acetyl CoA carboxylase	Bt03213389_m1	Q9TTS3	fatty acid biosynthetic process	Lipid metabolism
AGPAT1	1-acylglycerol-3-phosphate	Bt03224587_g1	Q95JH2	glycerophospholipid biosynthetic process	Lipid metabolism
	O-acyltransferase 1				Lipid metabolism
AGPAT9*	1-acylglycerol-3-phosphate	Bt04292093_m1	Not Found	-	Lipid metabolism
	O-acyltransferase 9				Lipid metabolism
DGAT1*	Diacylglycerol O-acyltransferase 1	Bt03251719_g1	Q8MK44	triglyceride biosynthetic process	Lipid metabolism
FASN*	Fatty Acid synthase	Bt03210485_m1	Q71SP7	fatty acid biosynthetic process	Lipid metabolism
FDX1*	Adrenodoxin, mitochondrial	Bt03217449_m1	P00257	lipid metabolism	Lipid metabolism
LIPE	Lipase, hormone-sensitive (HSL)	Bt03253691_m1	P16386	lipid catabolic process	Lipid metabolism
LPL*	Lipoprotein lipase	Bt03240493_m1	P11151	lipid metabolism	Lipid metabolism

PLIN2*	Perilipin 2	Bt03212182_m1	Q9TUM6	may be involved in development and maintenance of adipose tissue	Lipid metabolism
PLIN3*	Perilipin 3	Bt03230537_m1	Q3SX32	Not Described	Lipid metabolism
PNLIPRP2	Pancreatic lipase-related protein 2	Bt03267914_m1	A5PK46	phospholipid catabolic process	Lipid metabolism
PNPLA2	Patatin-like phospholipase domain containing 2	Bt03234129_g1	Q2KI18	lipid homeostasis	Lipid metabolism
ATF4*	Activating transcription factor 4	Bt03221057_m1	Q3ZCH6	positive regulation of apoptotic process, response to ER stress, response to oxidative stress	Cellular stress
BAX*	BCL2-Associated X Protein	Bt03211777_g1	O02703	Intrinsic apoptotic signaling pathway in response to DNA damage	Cellular stress
BCL2	B-cell CLL/lymphoma 2	Bt04298952_m1	F6R2C4	Actin filament organization	Cellular stress
BID	BH3 interacting domain death agonist	Bt03241255_m1	Q17QH5	Activation of cysteine-type endopeptidase activity involved in apoptotic process	Cellular stress

CASP3	Caspase 3, apoptosis-related cysteine peptidase	Bt03250954_g1	Q08DY9	execution phase of apoptosis	Cellular stress
CASP9	Caspase 9, apoptosis-related cysteine peptidase	Bt04282453_m1	E1BNU2	execution phase of apoptosis	Cellular stress
CAT*	Catalase	Bt03228713_m1	P00432	hydrogen peroxide catabolic process	Cellular stress
DDIT3*	DNA-damage-inducible transcript 3	Bt03251320_g1	P35638	apoptotic signaling pathway in response to ER stress	Cellular stress
DICER1	Dicer 1, Ribonuclease III	Bt03217754_m1	Q6TUI4	apoptotic DNA fragmentation	Cellular stress
GDF9	Growth differentiation factor 9	Bt03223996_m1	Q9GK68	regulation of apoptotic process	Cellular stress
GLRX2	Glutaredoxin 2	Bt03229700_m1	Q32L67	response to hydrogen peroxide	Cellular stress
GPX1*	Glutathione Peroxidase 1	Bt03259217_g1	P00435	lipid metabolism	Cellular stress
GPX4	Glutathione peroxidase 4	Bt03259611_m1	Q9N2J2	response to oxidative stress	Cellular stress
HSPA1A	Heat shock 70kDa protein 1A	Bt03292670_g1	Q27975	negative regulation of transcription from RNA polymerase II promoter in response to stress	Cellular stress
HSPA5*	78 kDa glucose-regulated protein		Q0VCX2	positive regulation of cell migration	Cellular stress
IGF1R	Insulin-Like Growth Factor 1 Receptor	Bt03649217_m1	Q05688	negative regulation of apoptotic process	Cellular stress
NDUFA1*	NADH:ubiquinone oxidoreductase subunit A1	Bt03216720_g1	Q02377	oxidation-reduction process	Cellular stress
PGK1*	Phosphoglycerate Kinase 1	Bt03225854_mH	Q3T0P6	cellular response to hypoxia	Cellular stress
PRDX1	Peroxiredoxin-1	Bt03223684_m1	Q5E947	cell redox homeostasis, cell proliferation	Cellular stress
PRDX3*	Peroxiredoxin 3	Bt03214402_m1	P35705	cell redox homeostasis	Cellular stress
SOD1	Superoxide dismutase 1, soluble	Bt03215423_g1	P00442	apoptotic DNA fragmentation	Cellular stress
TXNRD1	Thioredoxin reductase 1	Bt03215471_m1	O62768	response to oxygen redical	Cellular stress
XBP1*	X-Box Binding Protein 1	Bt03227621_g1	Q3SZZ2	endoplasmatic reticulum stress	Cellular stress

AKR1B1	Aldo-Keto Reductase Family 1, Member B1	Bt03218049_g1	P16116	Catalyzes NADPH-dependent reduction	Other cellular functions
AREG	Amphiregulin	Bt03271014_m1	A5PJE7	Not Described	Other cellular functions
CD36*	CD36 molecule (thrombospondin receptor)	Bt03212335_mH	P26201	regulation of energy homeostasis	Other cellular functions
CLIC3*	Chloride intracellular channel protein 3	Bt03263038_m1	A6QL90	chloride transmembrane transport	Other cellular functions
GATM*	Glycine amidinotransferase	Bt03237896_m1	Q2HJ74	creatine biosynthetic process	Other cellular functions
GFPT2	Glutamine-fructose-6-phosphate transaminase 2	Bt03250351_m1	Q08DQ2	UDP-N-acetylglucosamine metabolic process	Other cellular functions
KRT8*	Keratin 8	Bt03225178_g1	P05786	link the contractile apparatus to dystrophin	Other cellular functions
LUM	Lumican	Bt03211920_m1	Q05443	collagen fibril organization	Other cellular functions
MTIF3	mitochondrial translational initiation factor 3	Bt03231844_m1	Q32KZ1	ribosome disassembly	Other cellular functions

NLRP5	NLR Family, Pyrin Domain Containing 5	Bt03218031_m1	Q647I9	ATP binding associated with first embryonic cell divisions	Other cellular functions
PTX3	Pentraxin 3, long	Bt03249011_m1	Q0VCG9	regulation of innate resistance to pathogens	Other cellular functions
RPL15	60S ribosomal protein L15	Bt03288449_g1	Q5EAD6	cytoplasmic translation	Other cellular functions
RPLP0	Ribosomal protein, large, P0	Bt03218086_m1	Q95140	ribosome disassembly	Other cellular functions
RPS25	40S ribosomal protein S25	Bt03220440_g1	Q56JX5	Not Described	Other cellular functions
STAT3*	Signal Transducer And Activator Of Transcription 3	Bt03259871_g1	P61635	cellular response to leptin stimulus	Other cellular functions
TNFAIP6	Tumor necrosis factor, alpha-induced protein 6	Bt03210223_m1	Q5W1C4	cell adhesion	Other cellular functions
VCAN	Versican	Bt03217633_m1	P81282	cell adhesion	Other cellular functions
VNN1	Vanin 1	Bt03220248_m1	Q58CQ9	hydrolysis one of the carbamide linkages in D-pantheine	Other cellular functions
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	Bt03210912_g1	P10096	glucose metabolic process	Endogenous control
ACTB	Actin, beta	PA5-16914	P60712	cell motility	Endogenous control
PPIA	Peptidylprolyl Isomerase A	Bt03224617_g1	P62935	protein folding	Endogenous control

*Indicates genes that showed significant expression in at least one comparison.