

Supplementary Material

Effect of heifer age on the granulosa cell transcriptome after ovarian stimulation

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Table S1. All ten animals from the present study with follicle size, total number of aspirated follicles, number of oocytes in maturation and number of viable embryos (blastocysts)

The percentage of embryos refers to the ratio between the number of oocytes in maturation and the number of viable embryos

OPU Date	Name	Age at OPU (months)	Total Aspirated Follicles	Follicles 1-4 mm	Follicles 5-6 mm	Follicles 7-10 mm	Follicles 11-15 mm	Follicles > 15 mm	Oocytes in Maturation	Number of Viable Embryos	Embryos %
	Group 8 months										
2014-10-13	10989	7.75	15	0	4	10	1	0	12	7	58 %
2014-10-13	10990	7.72	16	2	2	9	3	0	7	3	43 %
2014-10-13	10991	7.98	10	0	2	6	2	0	9	6	67 %
2014-10-13	10992	7.98	9	0	0	9	0	0	9	4	44 %
2014-10-13	10993	8.71	15	0	2	11	2	0	11	6	55 %
2014-10-13	10994	8.02	27	0	2	20	5	0	20	9	45 %
2014-10-13	10995	7.65	23	0	2	16	5	0	23	5	22 %
2014-10-13	10996	7.79	32	0	7	21	4	0	23	4	17 %
2014-10-13	10997	8.28	15	0	5	5	5	0	10	2	20 %
2014-10-13	10998	8.54	5	0	3	2	0	0	4	0	0 %
	Average	8.04	16.7	0.2	2.9	10.9	2.7	0	12.8	4.6	37.09%
	Group 11 months										
2015-01-07	10989	10.58	13	0	4	9	0	0	10	7	70 %
2015-01-07	10990	10.55	17	0	0	11	6	0	11	4	36 %
2015-01-07	10991	10.81	7	0	3	4	0	0	8	7	88 %
2015-01-07	10992	10.81	5	0	1	4	0	0	2	2	100 %
2015-01-07	10993	11.53	6	0	1	5	0	0	2	1	50 %
2015-01-07	10994	10.84	16	0	0	11	5	0	10	5	50 %
2015-01-07	10995	10.48	31	0	3	25	3	0	10	7	70 %
2015-01-07	10996	10.61	18	0	2	13	3	0	15	2	13 %
2015-01-07	10997	11.10	11	0	2	7	2	0	8	4	50 %
2015-01-07	10998	11.37	21	0	7	14	0	0	17	4	24 %
	Average	10.87	14.5	0	2.3	10.3	1.9	0	9.3	4.3	50.07%
	Group 14 months										
2015-04-01	10989	13.34	12	0	4	6	2	0	11	5	45 %
2015-05-11	10990	13.31	7	0	0	7	0	0	7	4	57 %

2015-04-01	10991	13.57	3	0	2	0	1	0	2	1	50 %
2015-04-01	10991	14.88	10	0	1	7	2	0	10	4	40 %
2015-04-01	10992	13.57	5	1	1	2	1	0	3	2	67 %
2015-04-01	10993	14.29	9	0	2	5	2	0	8	8	100 %
2015-04-01	10994	13.60	23	0	0	18	5	0	19	8	42 %
2015-04-01	10995	13.24	13	0	2	10	1	0	12	8	67 %
2015-04-01	10996	13.37	11	0	3	8	0	0	10	4	40 %
2015-04-01	10997	13.86	11	0	2	7	2	0	8	6	75%
2015-04-01	10998	14.13	22	0	5	16	1	0	21	8	38 %
	Average	13.74	11.5	0.1	2	7.8	1.5	0	10.1	5.6	57.11%

Table S2. Primers used in real-time PCR experiments

Gene symbol	Primer sequence	Product size (pb)	Annealing temperature	GenBank accession number
GAPDH	Fwd: CCAACGTGTCTGTTGTGGATCTGA	218	57	NM_001034034.2
	Rev: GAGCTTGACAAAGTGGTCGTTGAG			
ACTB	Fwd: ATCGTCCACCGCAAATGCTTCT	102	57	NM_173979.3
	Rev: GCCATGCCAATCTCATCTCGTT			
BCL2L1	Fwd: GCA GGT ATT GGT GAG TCG GAT	116	57	NM_001077486.2
	Rev: TGT TCC CGT AGA GTT CCA CAA			
CKB	Fwd: CCA ATA GCC ACA ACA CGC TG	322	57	NM_001015613.1
	Rev: TTG AGG TCG GTC TTG TGC TC			
FOXA2	Fwd: GAACAAAGCGGGCCTGGATAACC	447	57	XM_002692160.4
	Rev: GACTTCCCTGCAACGACAGCAA			
TNFAIP6	Fwd: CAAGGGCAGAGTTGGATAACC	230	57	NM_001007813.2
	Rev: TGTGCCAGTAGCAGATTTGG			
IGF2	Fwd: CACGCGCAGAACACCAAGTCAT	140	57	NM_174087.3
	Rev: TGGGATTGCGAGCGATAAAGGT			

Table S3. (A) Upstream regulators identified by IPA in granulosa cells from 8-month-old heifers after FSH stimulation and coasting, compared to themselves at 14 months of age

Upstream regulator	Exp fold change	Molecule type	Predicted activation state	Activation z-score	<i>P</i> -value of overlap
beta-estradiol		chemical - endogenous mammalian	Activated	3.067	9.13E-23
tretinoin		chemical - endogenous mammalian	Activated	2.889	2.30E-10
TCF7L2	1.549	transcription regulator	Activated	2.446	2.38E-05
Growth hormone		group	Activated	2.439	1.02E-04
SP1	-1.804	transcription regulator	Activated	2.438	1.32E-08
KLF4		transcription regulator	Activated	2.438	6.79E-08
Ins1		other	Activated	2.274	6.37E-06
TP53		transcription regulator	Activated	2.256	4.26E-22
LDLR	-1.768	transporter	Activated	2.21	3.04E-04
PRDM1		transcription regulator	Activated	2.19	1.71E-03
SOD1	-2.013	enzyme	Activated	2.132	2.68E-06
CD3E	-2.118	transmembrane receptor	Activated	2.116	1.01E-03
hexachlorobenzene		chemical toxicant	Activated	2.111	6.22E-05
IFNA2		cytokine	Activated	2.039	4.55E-06
F2	5.346	peptidase	Activated	2.037	1.36E-06
PRL		cytokine	Activated	2.029	2.01E-10
NR5A2	-2.697	ligand-dependent nuclear receptor	Activated	2.01	2.16E-03
PDGF BB		complex	Activated	2.005	1.22E-06
IL19		cytokine	Inhibited	-2	7.20E-03
BTRC		enzyme	Inhibited	-2	1.56E-02
LRP5		transmembrane receptor	Inhibited	-2	1.56E-02
IL12A		cytokine	Inhibited	-2.022	1.62E-04
SPI1		transcription regulator	Inhibited	-2.023	4.39E-05
hyaluronic acid		chemical - endogenous mammalian	Inhibited	-2.06	1.59E-02
CLEC7A		transmembrane receptor	Inhibited	-2.161	4.68E-03
PI3K (complex)		complex	Inhibited	-2.169	2.86E-05
SENPI		peptidase	Inhibited	-2.2	2.00E-03
PRKCQ	-2.025	kinase	Inhibited	-2.236	1.24E-02
CD2		transmembrane receptor	Inhibited	-2.284	1.34E-02
P38 MAPK		group	Inhibited	-2.363	3.47E-04
NR0B2		ligand-dependent nuclear receptor	Inhibited	-2.387	2.25E-02

SOX7		transcription regulator	Inhibited	-2.449	1.20E-02
GRB2		kinase	Inhibited	-2.449	2.25E-02
NOTCH1	-2.431	transcription regulator	Inhibited	-2.517	5.29E-04
KRAS	4.066	enzyme	Inhibited	-2.545	5.47E-13
PAX1		transcription regulator	Inhibited	-2.646	5.50E-03
STAT5B		transcription regulator	Inhibited	-2.75	4.95E-02
STAT5A		transcription regulator	Inhibited	-2.795	6.28E-05
GATA4		transcription regulator	Inhibited	-3.553	1.75E-03

Table S3. (B) Upstream regulators identified by IPA in granulosa cells from 11-month-old heifers after FSH stimulation and coasting, compared to themselves at 14 months of age

The predicted upstream regulator, the expression differential (based on microarray probe), molecule type, predicted activation state, z score and statistical significance of the overlap (p value) are shown

Upstream regulator	Exp fold change	Molecule type	Predicted activation state	Activation z-score	P-value of overlap
EGF		growth factor	Activated	2.728	1.52E-06
PRL		cytokine	Activated	2.705	8.20E-05
PTH		other	Activated	2.608	1.16E-06
TP53		transcription regulator	Activated	2.459	8.35E-05
IL10RA		transmembrane receptor	Activated	2.449	5.07E-03
TGFB1		growth factor	Activated	2.4	2.20E-06
TO-901317		chemical reagent	Activated	2.365	1.65E-04
EGR2		transcription regulator	Activated	2.353	1.23E-04
retinoin		chemical - endogenous mammalian	Activated	2.229	2.95E-06
Ins1		other	Activated	2.207	5.56E-03
NCOA2		transcription regulator	Activated	2.186	2.07E-05
CTGF		growth factor	Activated	2.18	1.79E-04
NR5A2		ligand-dependent nuclear receptor	Activated	2.178	7.57E-05
PGR		ligand-dependent nuclear receptor	Activated	2.174	1.34E-02
Vegf		group	Activated	2.138	9.94E-04
hydrogen peroxide		chemical - endogenous mammalian	Activated	2.077	4.74E-04
forskolin		chemical toxicant	Activated	2.026	2.43E-07
8-bromo-cAMP		chemical reagent	Activated	2.008	3.31E-05
Growth hormone		group	Activated	2	4.13E-03
miR-16-5p		mature microRNA	Inhibited	-2.621	7.70E-05

Table S4. (A) Upstream regulators identified by IPA from the differential gene expression peculiar to 8-month-old heifers after FSH stimulation and coasting, relative to 14-month-olds

Upstream regulator	Exp fold change	Molecule type	Predicted activation state	Activation z-score	<i>P</i> -value of overlap
beta-estradiol		chemical – endogenous mammalian	Activated	3.184	8.80E-18
beta-carotene		chemical – endogenous mammalian	Activated	2.515	3.07E-06
CREM		transcription regulator	Activated	2.45	1.36E-02
ADRB		group	Activated	2.429	1.15E-02
NPPB		other	Activated	2.425	4.45E-02
IL10RA		transmembrane receptor	Activated	2.401	1.05E-01
ABCA1		transporter	Activated	2.383	3.18E-04
LIF		cytokine	Activated	2.259	1.51E-01
INSIG2		other	Activated	2.236	7.58E-03
SYVN1		transporter	Activated	2.232	3.02E-04
IRF7		transcription regulator	Activated	2.225	4.75E-01
PCGEM1		other	Activated	2.207	2.77E-01
INSIG1	-1.575	other	Activated	2.203	6.97E-06
ADRB2		g-protein coupled receptor	Activated	2.19	1.14E-01
SKIL		transcription regulator	Activated	2.155	1.34E-02
WNT1		cytokine	Activated	2.136	1.51E-03
POR	1.827	enzyme	Activated	2.132	1.05E-04
GFI1		transcription regulator	Activated	2.073	6.85E-05
CD38	1.547	enzyme	Activated	2.02	6.79E-08
Igm		complex	Activated	2.005	8.54E-04
ACTL6A		other	Inhibited	-2	2.12E-01
MBTD1	1.718	other	Inhibited	-2	4.36E-01
FSH		complex	Inhibited	-2.032	9.11E-07
KMT2D		transcription regulator	Inhibited	-2.047	6.55E-04
P38 MAPK		group	Inhibited	-2.05	2.06E-03
KAT5		transcription regulator	Inhibited	-2.121	3.23E-01
caspase		group	Inhibited	-2.138	2.91E-03
Ige		complex	Inhibited	-2.143	4.56E-01
GSK3B		kinase	Inhibited	-2.144	4.58E-02
SREBF1		transcription regulator	Inhibited	-2.149	4.04E-06
ICOS		transmembrane receptor	Inhibited	-2.158	2.18E-01
MSTN		growth factor	Inhibited	-2.167	4.74E-01

SLC16A3		transporter	Inhibited	-2.173	4.99E-04
TLR5		transmembrane receptor	Inhibited	-2.176	2.25E-01
ATF2		transcription regulator	Inhibited	-2.186	2.38E-01
RICTOR		other	Inhibited	-2.208	1.07E-06
Lh		complex	Inhibited	-2.213	2.75E-04
CSF1R		kinase	Inhibited	-2.213	7.62E-03
USP7	1.675	peptidase	Inhibited	-2.216	2.78E-02
hyaluronic acid		chemical - endogenous mammalian	Inhibited	-2.217	1.11E-01
BMP7		growth factor	Inhibited	-2.224	4.29E-02
RELA		transcription regulator	Inhibited	-2.231	6.13E-06
T3-TR-RXR		complex	Inhibited	-2.236	2.94E-01
ARHGAP21		other	Inhibited	-2.236	3.30E-01
IL12A		cytokine	Inhibited	-2.257	1.91E-03
uric acid		chemical - endogenous mammalian	Inhibited	-2.297	1.79E-01
KRAS	2.039	enzyme	Inhibited	-2.318	3.44E-09
ADAM17	-1.562	peptidase	Inhibited	-2.338	7.33E-02
PRKAA2		kinase	Inhibited	-2.359	3.12E-03
AXIN1		other	Inhibited	-2.377	6.19E-03
ZEB1		transcription regulator	Inhibited	-2.383	7.33E-02
CEBPD	2.08	transcription regulator	Inhibited	-2.414	1.08E-02
PLAGL1		transcription regulator	Inhibited	-2.423	1.17E-03
MTORC1		complex	Inhibited	-2.433	3.27E-02
RASSF5		other	Inhibited	-2.556	8.43E-05
PTGER2		g-protein coupled receptor	Inhibited	-2.577	2.01E-03
Gsk3		group	Inhibited	-2.578	3.21E-02
NR5A1		ligand-dependent nuclear receptor	Inhibited	-2.664	6.35E-02
D-fructose		chemical - endogenous mammalian	Inhibited	-2.815	2.87E-02
palmitic acid		chemical - endogenous mammalian	Inhibited	-2.92	8.49E-06
MTOR		kinase	Inhibited	-3.003	2.92E-08

Table S4. (B) Upstream regulators identified by IPA from differential gene expression peculiar to 11-month-old heifers after FSH stimulation and coasting, relative to 14-month-olds

Predicted upstream regulator, expression differential (based on microarray probe), molecule type, predicted activation state, z score and statistical significance of the overlap (*P*-value) are shown

Upstream regulator	Exp fold change	Molecule type	Predicted activation state	Activation z-score	<i>P</i> -value of overlap
PRL		cytokine	Activated	2.752	4.35E-05
cyclic AMP		chemical – endogenous mammalian	Activated	2.589	2.18E-04
STAT3		transcription regulator	Activated	2.571	7.33E-04
MKNK1		kinase	Activated	2.236	1.12E-04
CREB1		transcription regulator	Activated	2.232	4.03E-03
butyric acid		chemical – endogenous mammalian	Activated	2.183	1.71E-02
ESR1		ligand-dependent nuclear receptor	Activated	2.144	3.14E-04
KLF3		transcription regulator	Inhibited	-2	7.21E-02
IRF3		transcription regulator	Inhibited	-2	8.18E-03
IRF7		transcription regulator	Inhibited	-2.219	1.02E-03

Table S4. (C) Pathway analysis (IPA) of differential gene expression peculiar to 8-month-old heifers after FSH stimulation and coasting, relative to 14-month-olds

The affected canonical pathway, *P*-value, ratio (based on DEGs) and the z score are shown

Ingenuity canonical pathways	<i>P</i> -value	Ratio	z-score
MIF-mediated glucocorticoid regulation	4.37E-02	2.00E-01	2.646
Fc epsilon RI signaling	2.75E-02	1.52E-01	2.357
VEGF signaling	1.45E-02	1.65E-01	2.324
EIF2 signaling	3.47E-02	1.37E-01	2.324
Fc γ 3 receptor-mediated phagocytosis in macrophages and monocytes	4.79E-02	1.52E-01	2.324
Phospholipase C signaling	5.89E-03	1.47E-01	2.309
Signaling by Rho family GTPases	2.04E-02	1.37E-01	2.268
Rac signaling	8.71E-04	1.92E-01	2.132
Thrombin signaling	7.08E-04	1.67E-01	2.121
Insulin receptor signaling	2.24E-02	1.50E-01	2.065

Cyclins and cell cycle regulation	3.24E-02	1.67E-01	-2.111
RhoGDI signaling	1.95E-02	1.45E-01	-2.132
PTEN signaling	1.58E-04	2.07E-01	-3