

A new microfluidic concept for successful *in vitro* culture of mouse embryos

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Supplementary material

Figure S1

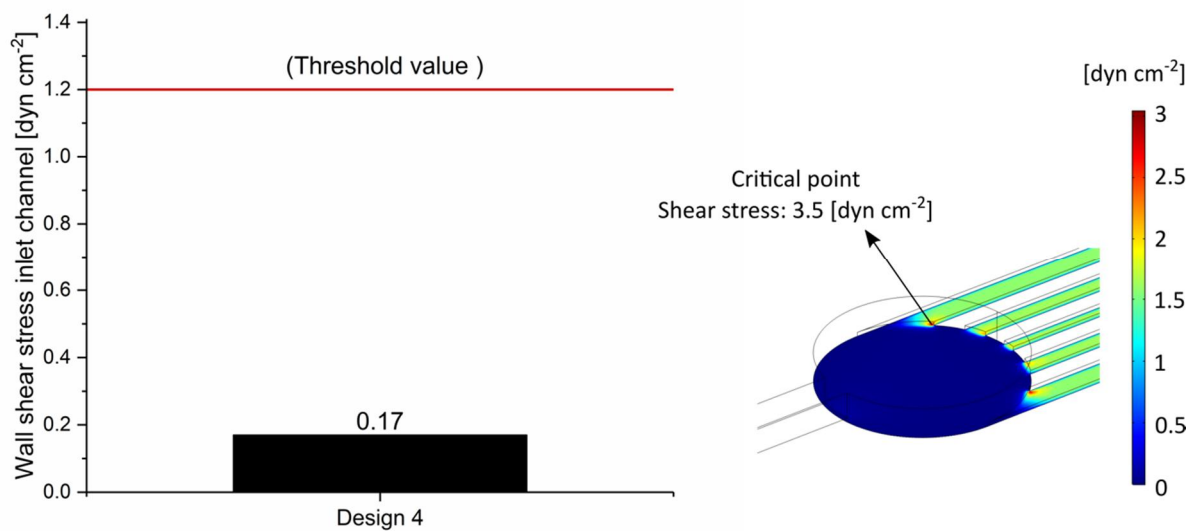


Figure S1. a) Computationally estimated inlet channels wall shear stress for the microfluidic device in its final version (Design 4), and b) fluid flow computational model of shear stress field surface plot during manual loading. The colour spectrum bar shows the shear stress field generated in the fluid systems.

Figure S2

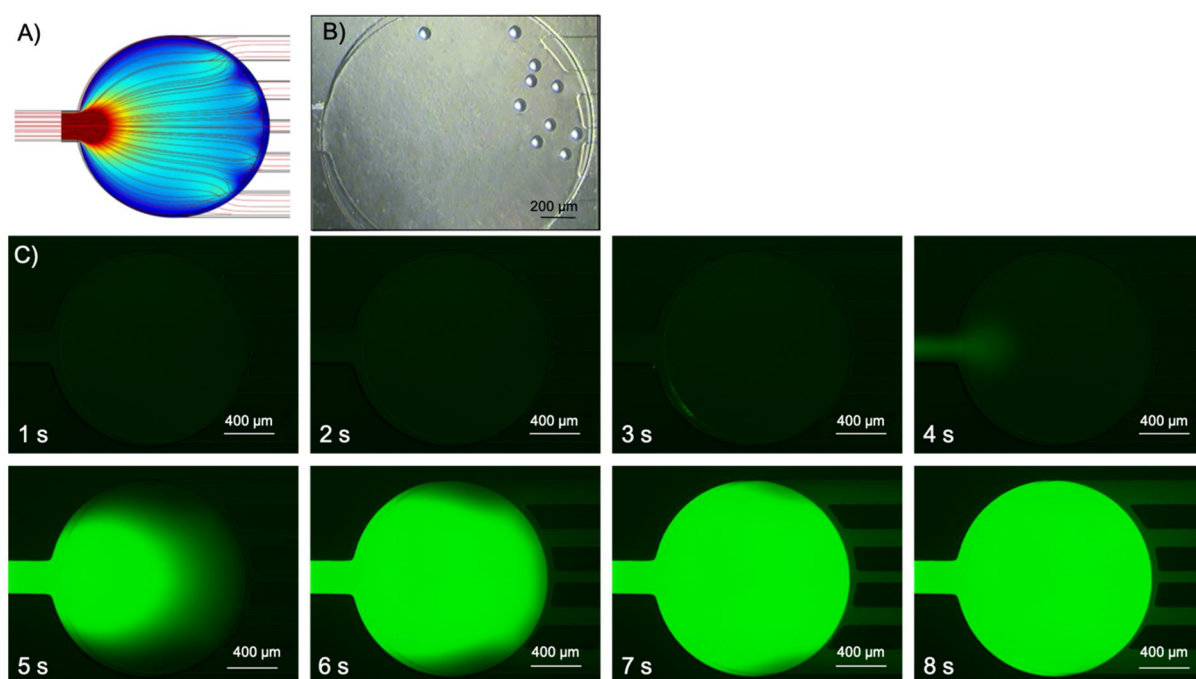


Figure S2. Real flow characterization within the culture chamber. A) Velocity magnitude surface and stream lines plot. B) Polystyrene beads spread within the culture chamber. C) Microfluidic device filled with a 0.05 mg ml^{-1} fluorescein solution.

Figure S3

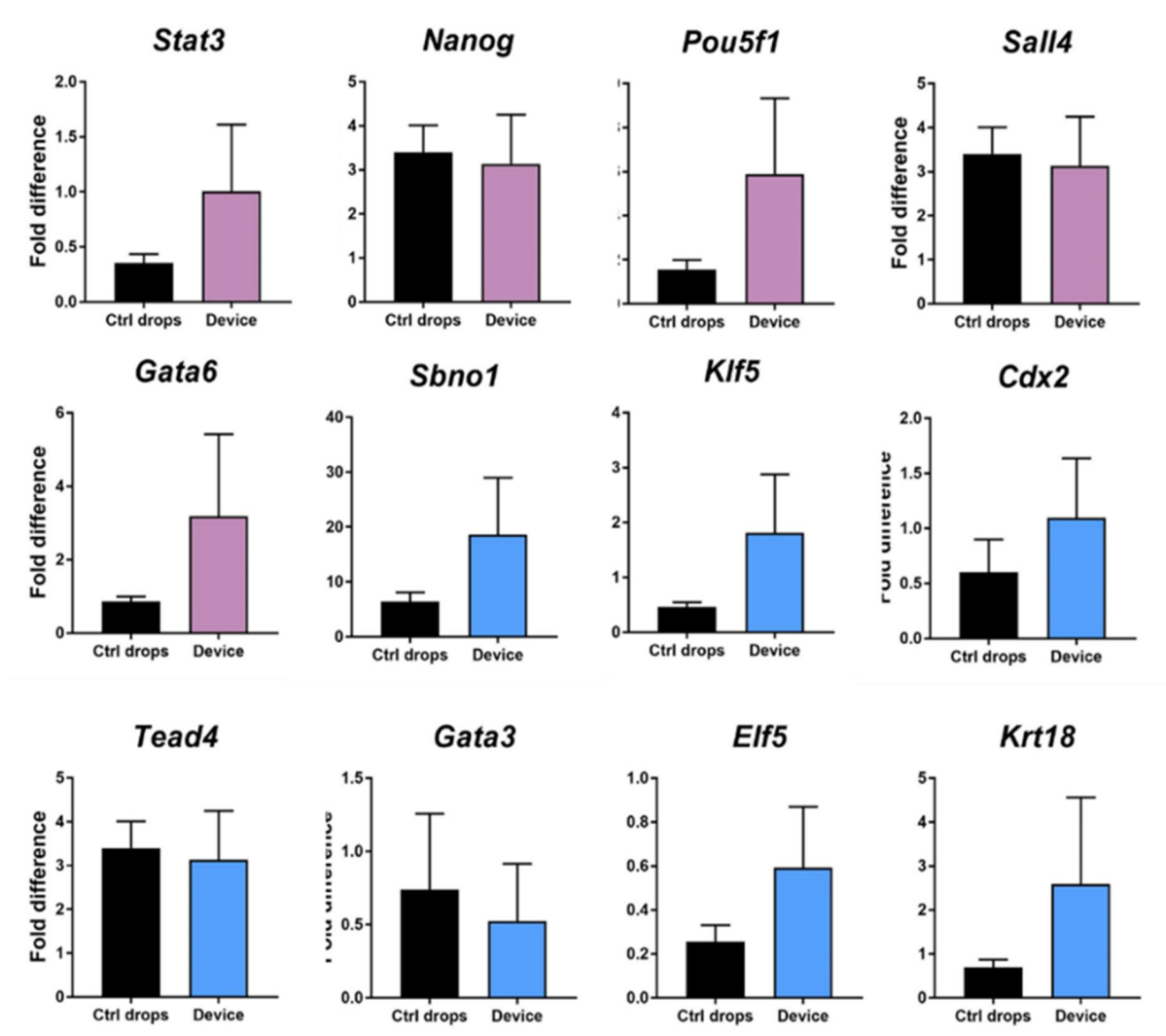


Figure S3. Relative mRNA expression of selected genes (purple: markers of ICM/epiblast differentiation; blue: markers of trophectoderm differentiation) in blastocysts (n=10) cultured in the device, compared with control (n=10). Data are presented as mean $\pm$ SEM for the numbers of embryos shown.

Figure S4

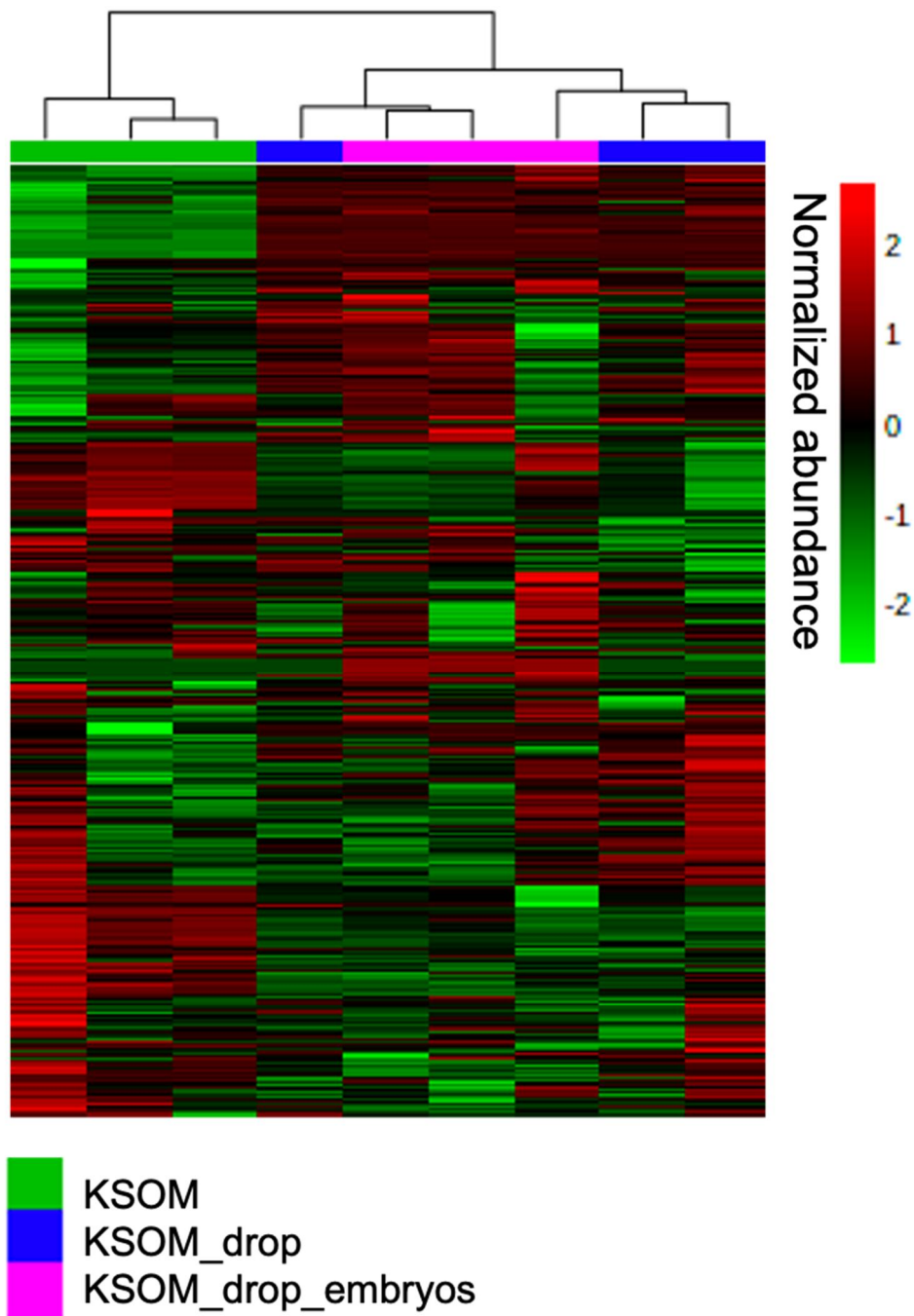


Figure S4. Heatmap analysis of media samples collected from control microdrop with and without embryos. Sample replicates are visualized in columns column based on hierarchical clustering, with metabolites presented on individual rows. Species are colored based on normalized abundance from red (high) to green (low).

Table S1. Summary of gene symbol, accession number, product length and primer sequences of the genes library used in this work.

Symbol	Name	Accession	Size (bp)	Forward	Reverse
Nnat	neuronatin	NM_010923	174	TTGAATCCCACCTTTACCAA	CCTGTCTCCAGGAGCTTACA
Mkrn	Makorin, ring finger protein, 1	NM_018810	218	TTGTGGGTATCTGCCTCATT	AGAGCAACAAAATGCAAAGG
Kcnq1ot1	KCNQ1 overlapping transcript 1	NR_001461	234	CCCAAACCTTAGTCCTCCAT	TAACAAAGGGCACACGGTAT
AIRN	antisense Igf2r RNA	NR_002853	111	AGCTTGATCCACTTGCAAAC	CTGGATGTCCCTGTTAGGTG
Impact	impact, RWD domain protein	NM_008378	227	TACAGCACCGCCTTCTTATC	CTTCTTCGGACTCAACCTCA
Snrpn	small nuclear ribonucleoprotein N	NM_013670	202	AAAACAGCCAGAACGTGAAG	AGCCTGGGGAATAGGTACAC
Xist	In multiple Geneids	X59289	161	TGTGGTCTGCTTTGTCTTCA	ACAAAGAACAAGTGGGGTGA
Phlda2	pleckstrin homology-like domain, family A, member 2	NM_009434	69	CGTGGAGCACACCTCTAAGT	CGGAAGTCGATCTCCTTGTA
Cdkn1c	cyclin-dependent kinase inhibitor 1C (P57)	NM_001161624	185	TCACCAGCTTCAGATTACCC	GTTGGTATGGGCAGTACAGG
Igf2r	insulin-like growth factor 2 receptor	NM_010515	174	CTGCATTGGCTTGACTCCT	TATAAGGCAGAGGGTTGCAG
Meg3	maternally expressed 3	NR_003633	152	TTAGCAGCAGTGGACATGAA	GGCTGTGAAGGAAAGACAGA
Dnmt3b	DNA methyltransferase 3B	NM_001003961	155	ACTTGGTGATTGGTGGAAGC	CCAGAAGAATGGACGGTTGT
Dnmt3c	Aka Sycp1	XM_021192798	241	TGATGATCAATGCCATCAAG	ATGTCGTCTTTGCCATTTCAT
Dnmt3l	DNA (cytosine-5-)-methyltransferase 3-like	NM_019448	179	CATCCCTACCTACGGGTTCT	AGAGGACTGTCAGCATCAGG
Tet1	tet methylcytosine dioxygenase 1	NM_001253857	142	AAGAAGAGGAAATGCGAGGT	GGCCATTTACTGGTTTGTG
Tet2	tet methylcytosine dioxygenase 2	NM_001040400	200	ATGCAGAGAGATGCCTTCAC	GCCGTGTAGCTGTAGATCGT
Mbd1	methyl-CpG binding domain protein 1	NM_013594	84	AAAGAGGACTGTGGGGTGTG	CCGCTCACACTTGACAGTAAA
Mbd2	methyl-CpG binding domain protein 2	NM_010773	205	AGACCCTTCTGTCTGCTGTG	AGTGCCTCCTCCAGTTTCTT
Mbd3	methyl-CpG binding domain protein 3	NM_013595	246	GTGGATTGAGTGCCTTTGAC	TGATGTCGTATCTGTCACC
Ehmt2	euchromatic histone lysine N-methyltransferase 2	NM_147151	222	CCCGTTACTATGGCAACATC	ACTTCTCAGAGCCACACTGG
Hat1	Histone acetyltransferase 1	NM_026115	150	GTGCCGTGGAGAAGAACTA	CAGTTTCATCATCCCCAAG
Zfp57	zinc finger protein 57	NM_001013745	85	TTAACCCACCTCAAGATCCA	TGAGTGTGTTGGATGAGTGG
Trim28	tripartite motif-containing 28	NM_011588	166	AGTAAGTGGGCCAGGTATCC	GCAGAGTGAGCAACTCCATT
Uhrf1	ubiquitin-like, containing PHD and RING finger domains, 1	NM_010931	150	ATGGCCAACTATAACGTGGA	CATGATCCGACAGTTGTTGA
Cdx2	caudal type homeobox 2	NM_007673	150	TCAGGGGAAGACATGGTTTA	AGGCTGATAGCTTCATGTGG
Elf5	E74-like factor 5	NM_010125	112	GTGCACCCTGAATACTGGAC	CGCTGATGTTGAAGTGACAG
Gata3	GATA binding protein 3	NM_008091	189	CCCTTTATTCCTCCGTGTCT	GAGAGGGGTCGTTTAATGGT
Krt18	keratin 18	NM_010664	173	ACCTGAGGGCTCAGATCTTT	GGTGTCTATCTACCACCTTGC
Sbno1	sno, strawberry notch homolog 1 (Drosophila)	NM_001081203	193	GCAGCTGAGCTTTACTGGAG	CTGGTGAGCAGACCAGAACT
Tead4	TEA domain family member 4	NM_011567	62	TCACCTGCTCTACGAAGGTC	CTCCGTCTCAACTTTCTCCA
Klf5	Kruppel-like factor 5	NM_009769	248	CACCATGCCAAGTCAGTTTC	TCTCCAGATCCGGGTTACTC
Nanog	Nanog homeobox	NM_028016	207	GGACAGGTTTCAGAAGCAGA	CAATGGATGCTGGGATACTC
Klf2	Kruppel-like factor 2 (lung)	NM_008452	187	GGCTAGATGCCTTGTGAGAA	CACCACTACCGTGATTCCTC
Klf4	Kruppel-like factor 4 (gut)	NM_010637	232	TCAAGTTCCAGCAAGTCAG	AAACTTCCAGTCACCCCTTG
Pou5f1	POU domain, class 5, transcription factor 1	NM_013633	160	AAGCAACTCAGAGGGAACCT	GGTGATCCTCTTCTGCTTCA
Gata4	GATA binding protein 4	NM_008092	246	TCTCACTATGGGCACAGCAG	CGAGCAGGAATTTGAAGAGG
Gata6	GATA binding protein 6	NM_010258	168	TAGAAATGCTGAGGGTGAGC	ACAGAGCCACTGCTGTTACC

Serpinh1	serine (or cysteine) peptidase inhibitor, clade H, member 1	NM_009825	239	GTGTCACTGGGTGGTAAAGC	TTGGAGTGTTCGCAGTTGTA
Sal4	sal-like 4 (Drosophila)	NM_175303	125	TTAAGCAGCCATGTGTCTCA	GGTAGCTTGGCTTGTTCAT
Stat3	signal transducer and activator of transcription 3	NM_213659	187	ATCTGTAACCACAGGGCAAA	GTAAGCTGAGTGAGCGAAGC
Slc2a1	solute carrier family 2 (facilitated glucose transporter), member 1	NM_011400	163	TGCCAAGCTAATCTGTAGGG	GAATGGGCGAATCCTAAAT
Slc2a3	solute carrier family 2 (facilitated glucose transporter), member 3	NM_011401	204	TTTTCCACAGGTCACTGGAT	GTTAGAGGAGTCGCCTTTCC
Slc2a8	solute carrier family 2, (facilitated glucose transporter), member 8	NM_019488	197	CGCCTGTACCTCACTTGACT	GGAAAGAGCCAGAAAGAACC
Folr1	folate receptor 1	NM_001252552	163	AAGAGGACTGTCAGCAGTGG	TCACACAGAGCAGCAGATGT
Slc19a1	solute carrier family 19 (folate transporter), member 1	NM_031196	85	GGAGCAGGTGACTAACGAGA	GTAGTCGGTGAGCAGGAAGA
Slc46a1	solute carrier family 46, member 1	NM_026740	137	GTTCCAGCAGTTTCTCTAGA	CAAGGGTGGATAGACTGTGG
Mtr	5-methyltetrahydrofolate-homocysteine methyltransferase	NM_001081128	144	GAGGAAGGACAAGCAACAGA	TTCCTCCATTCCAGTACCAA
Shmt1	serine hydroxymethyltransferase 1	NM_009171	125	TCTTTGGTGGGTGTTTCTGT	AATAGCTTTGCCTCACATGC
Shmt2	serine hydroxymethyltransferase 2	NM_028230	102	GCTCAAAGACCCAGAGACAA	CCTCAACGTTTCATCAAATCC
Mthfd1	Methylenetetrahydrofolate dehydrogenase (NADP+ dependent), methenyltetrahydrofolate cyclohydrolase, formyltetrahydrofolate synthase	NM_138745	213	ATTGCACTCAAGCTGGTAGG	TGTGTAAGCCTTGGAAGAG
Bhmt	Betaine-homocysteine methyltransferase	NM_016668	157	TGTGGCAAACAGGGATTAT	TGATGTGGTAGGGCTCAAAT
Ahcy	S-adenosylhomocysteine hydrolase	NM_016661	193	AGCTGAATGTGAAGCTGACC	AGGCTACTGCAAAGTTGGTG
Slc16a1	solute carrier family 16 (monocarboxylic acid transporters), member 1	NM_009196	156	AGAGGTTCTCCAGTGCTGTG	GATAGATACCCGCGATGATG

Table S2. List of compounds that were significantly ($p < 0.05$, $FC > 2$) up-regulated uniquely in day 5 embryo culture PDMS-media vs day 0 KSOM.

(-)-11-hydroxy-9,10-dihydrojasmonic acid 11-beta-D-glucoside	gamma-Glutamylvaline
(-)-AS 115 22453	Gemcitabine
(-)-trans-Carveol glucoside	Gingerenone B
(+)-Mahanimbicine	Gln Cys Tyr 4996
(+)-Neoisomenthol	Gln Ile Lys 7055
(1S,2R,4R,8S)-p-Menthane-2,8,9-triol 2-glucoside	Gln-Cys-Lys
(2E)-hexenedioylcarnitine	Gln-Ser-Arg
(2R,3R)-heptane-1,2,3-triol	Glu Ile Asn 9712
(3R,7R)-1,3,7-Octanetriol	Glu Met Tyr 4441
(3S,4S)-3-hydroxytetradecane-1,3,4-tricarboxylic acid	Glutamylglutamine
(4-ethyl-2-methoxyphenyl)oxidanefulfonic acid	Glutamylmethionine
(4-Methylphenyl)acetaldehyde	Glutamylvaline
(4E)-6-hydroxy-1-(4-hydroxy-3-methoxyphenyl)tetradec-4-en-3-one	Glutarylarginine
(5-Chloro-1H-indol-2-yl)(4-methyl-1-piperazinyl)methanone	Glutarylglutamine
(S)-2-Acetolactate	Gly Leu 19822
(S)-3-Mercaptohexyl pentanoate	Gly Phe Val 10506
(S)-a-Amino-2,5-dihydro-5-oxo-4-isoxazolepropanoic acid N2-glucoside	Glycerol
(S)-Homostachydrine	Glycerol 1-propanoate
(S)-Reticuline	Glycerol tributanoate
(S1)-Methoxy-3-heptanethiol	Glycerol tripropanoate
[(1E)-5-oxo-1,7-diphenylhept-1-en-3-yl]oxy)sulfonic acid	Glycyltyrosine
[2-hydroxy-5-[3-(4-hydroxyphenyl)propanoyl]phenyl]oxidanefulfonic acid	Hexaethylene glycol
1-(sn-Glycero-3-phospho)-1D-myo-inositol	Hexanethioic acid S-propyl ester
1-[2-Methyl-3-(methylthio) allyl]cyclohex-2-enol	His Gln Ala 4582
1-Ipomeanol	His Ile Phe 3204
1-Isothiocyanato-2-phenylethane	His Ile Val 9187
1,2-Dehydrosalsolinol	His Val Val 3965
1,3-Dimethyluric acid	Histidinyl-Lysine
1,7-Dimethylguanosine	Homocysteinesulfonic acid
10-Hydroxy-8-nor-2-fenchanone glucoside	HoPhe-Nap-OH 1154
17-phenyl trinor Prostaglandin E2 serinol amide 20818	Hordatine B

9-Methylxanthine	N-Methyl-1-deoxynojirimycin
9,9-dimethoxy-nonanoic acid	N-Methylethanolaminium phosphate
Abiraterone sulfate	N-Nonanoylglycine
Abu-Lys-OH 773	N-Phenylacetylglutamic acid
Acetaldehyde	N,N-Bis(2-hydroxyethyl)-2-aminoethanesulfonic acid
Acetazolamide	N,N-Dimethylformamide
Acetylglycine	N1,N8-Diacetylspermidine
Acuminoside	N2-Maltosylarginine
Ajmaline	N5-Carboxyaminoimidazole ribonucleotide
Ala Asn Met 11107	N8-Acetylspermidine
Ala Ile Ser 11844	Nap-Thr-OH 626
Ala Lys Phe 11848	Necatorine
Alfuzosin	Neosaxitoxin
Alizapride	Nepafenac
Allyl thiohexanoate	Netilmicin
Amiprilose 31522	Nigakinone
Arginyl-Arginine	Norfenfluramine
Arginyl-Hydroxyproline	O-Acetyls erine
Arginyl-Isoleucine	O-sebacoylcarnitine
Arginyl-Phenylalanine	Orciprenaline
Arginyl-Valine	Pantothenamide
Armillaripin	Pantothenic acid
Asn-Arg-Arg	Pantothenol
Asn-Ser-Arg	Penciclovir
Asp Asn Val 1875	Penicillin G
Asp His Met 12861	Penicillin V
Asp Trp Met 13629	Pentaethylene glycol
Asparaginyl-Valine	Pentapropylene glycol
Asymmetric dimethylarginine	Phaseolic acid
Benzylamine	Phe Gly Val 10456
Beta-Guanidinopropionic acid	Phe Tyr Val 20210
Bis (2-hydroxypropyl) amine 26447	Phenylalanyl-Arginine
Brassicinal B	Picrocrocin
Bumetanide	Pirbuterol
Butyl ethyl malonate	Piromidic Acid 17891
Butyl lactate	Portuloside A
Butyramide	Prenyl glucoside
Caffeoyl tyrosine	Pro Lys Asn 10821
Calystegine B5	Pro-Pro-Lys
Capryloylglycine	Propafenone
Carnitine-d3	Propionylcarnitine
Casomorphin	Pseudoecgonine
Chitotriose	Pterolactam
CHOLINE-41	Pyridine N-oxide glucuronide
Chrysophanol 1-triglucoside	Pyridoxamine
Cibulins	PyroGlu-Lys-Lys
Ciclopirox 21996	Quinaprilat
cis-4-Hydroxyproline	Quinone
Clausarinol	R-2-Hydroxy-3-methylbutanoic acid 3-Methylbutanoyl
Cys Glu Phe 13468	S-(3-Methyl-2-butenyl) 2-methylpropanethioate
Cys Phe Ser 4336	S-Adenosylmethionine
Cysteinyl-Gamma-glutamate	S-Propyl 1-propanesulfinothioate
D-Glucosaminic acid	SAICAR
D-Glucosaminide	Ser Asn Asn 16756
D-glycero-L-galacto-Octulose	Seryllysine
Dehydromatricaric acid	Spermidine
Dendryphiellic acid B	Sphingosine 1-phosphate (d19:1-P)
Deoxyribose	Succinic acid
Deoxythymidine diphosphate-l-rhamnose	Succinylacetone
Dexfenfluramine	Sufentanil
Diacetone-D-galacturonic acid	Sulfadiazine
Dibromodichloromethane	Sulfallate
Dibutyl malate	Swainsonine
Didesmethyl doxepin	Tetraethylene glycol
Diethyl oxalpropionate	Tetrapropylene glycol
Dihydro-2,4,6-tris(2-methylpropyl)-4h-1,3,5-dithiazine	Theobromine
Dihydroergocornine	Thiiranebutanenitrile
Dihydrolipoamide	Thorium
Dimethicone	Thymine
Dimethyl sulfoxide	Toxin T2 tetrol
Divinyl sulfide	trans-S-(1-Propenyl)-L-cysteine
dUDP	Triethyl citrate
Echitamine	Triethylene glycol
Echothiophate	Trifluridine
Erinapyrone A	Tripropylene glycol

Etamiphylline	Tyrosine methylester
Ethanol, 2-[2-(2-butoxyethoxy)ethoxy]-	Tyrosyl-Hydroxyproline
Ethiofencarb	Tyrosyl-Tyrosine
Fluconazole	Val Pro His 10192
Fluoxymesterone	Val Pro Leu 2286
Flupirtine	Val-Gly-Val
Furcelleran	Valyl-Valine
Gamma-Butyrolactone	Xanthine
Gamma-glutamyl-L-putrescine	xi-2,5-Dihydro-2,4-dimethylthiazole
gamma-Glutamyl-S-methylcysteine sulfoxide	xi-4-Hydroxy-4-methyl-2-cyclohexen-1-one
gamma-Glutamylisoleucine	Zanamivir
gamma-Glutamylleucine	Zapotidine

Table S3. List of compounds that were significantly ($p < 0.05$, $FC > 2$) up-regulated uniquely in day 4 embryo culture PS-media vs day 0 KSOM.

(9xi,10xi,12xi)-9,10-Dihydroxy-12-octadecenoic acid	Carboxytolbutamide
(Z)-1-(Methylthio)-5-phenyl-1-penten-3-yne	Chrycorin
{[3,4,5-trihydroxy-5-(hydroxymethyl)oxolan-2-yl]methoxy}sulfonic acid	Cilastatin
1-(4-Fluorobenzyl) piperazine 28321	cis-2-Methylnaconitate
1-(4-hydroxyphenyl)-7-phenylheptane-3,5-dione	Dimethylallylpyrophosphate
1,2,3-Tris(1-ethoxyethoxy)propane	Eribofuran
1,2,3,4-Tetrahydro-b-carboline-1,3-dicarboxylic acid	Flavoxate
1,9-Nonanedithiol	Gancaonin A
15(R)-hydroperoxy-EPE	Histidiny-Hydroxyproline
18-Carboxy-dinor-LTE4	Homolanthionine
2-Hydroxyglutarate	Hydroxyprolyl-Threonine
2-Methoxy-3-methyl-9H-carbazole	Hydroxytyrosol 1-O-glucoside
2,4-Dimethylpyridine	Indanone
3-[3,4-dihydroxy-2-(5-hydroxy-3,7-dimethylocta-2,6-dien-1-yl)phenyl]propanoic acid	Isogemichalcone B
3-Carbamoyl-2-phenylpropionic acid	Isoginkgetin
3-Deaza-2'-deoxyadenosine 28523	KH7 23352
3,4,5-trihydroxy-6-[(2-methylpropanoyl)oxy]oxane-2-carboxylic acid	L-2-Amino-3-(oxalylamino)propanoic acid
3,4,5-trihydroxy-6-[[3,6,7-trihydroxy-2-(4-hydroxyphenyl)-4-oxo-3,4-dihydro-2H-1-benzopyran-5-yl]oxy]oxane-2-carboxylic acid	L-Proline
3b,8b-Dihydroxy-6b-(3-chloro-2-hydroxy-2-methylbutanoyloxy)-7(11)-eremophilene-12,8-olide	Lanthionine ketimine
4-Ethyl-2-methylthiazole	Leucyl-Leucine
4-Methylbenzoic acid	Maltol
4,8 Dimethylnonanoyl carnitine	Meropenem
5-Hydroxyomeprazole	Met Thr Tyr 14215
5-Oxoproline	Mevalonic acid
Abu-Phe-OH 156	N-(4-aminobutyl)-3-(4-hydroxy-3-methoxyphenyl)oxirane-2-carboximidic acid
Acetylpterosin C	N-Caffeoyltryptophan
Ala Lys Gln 2386	Nb-Feruloyltryptamine
Alanyl-Arginine	O-SUCCINYL-L-HOMOSERINE-34
Alpha-Lactose	Ornithine
Arginyl-Asparagine	Parvisoflavone A
Arginyl-Glycine	Pinocembrin 7-apiosyl-(1->5)-apiosyl-(1->2)-glucoside
Arginyl-Lysine	S-Allylcysteine
Arginyl-Tyrosine	Saccharopine
Aspartylphenylalanine	Talaromycin A
Avenanthramide C	Theaflavin
Benzeneacetamide-4-O-sulphate	Tryptophyl-Gamma-glutamate
	Tyramine glucuronide
	Valyl-Isoleucine