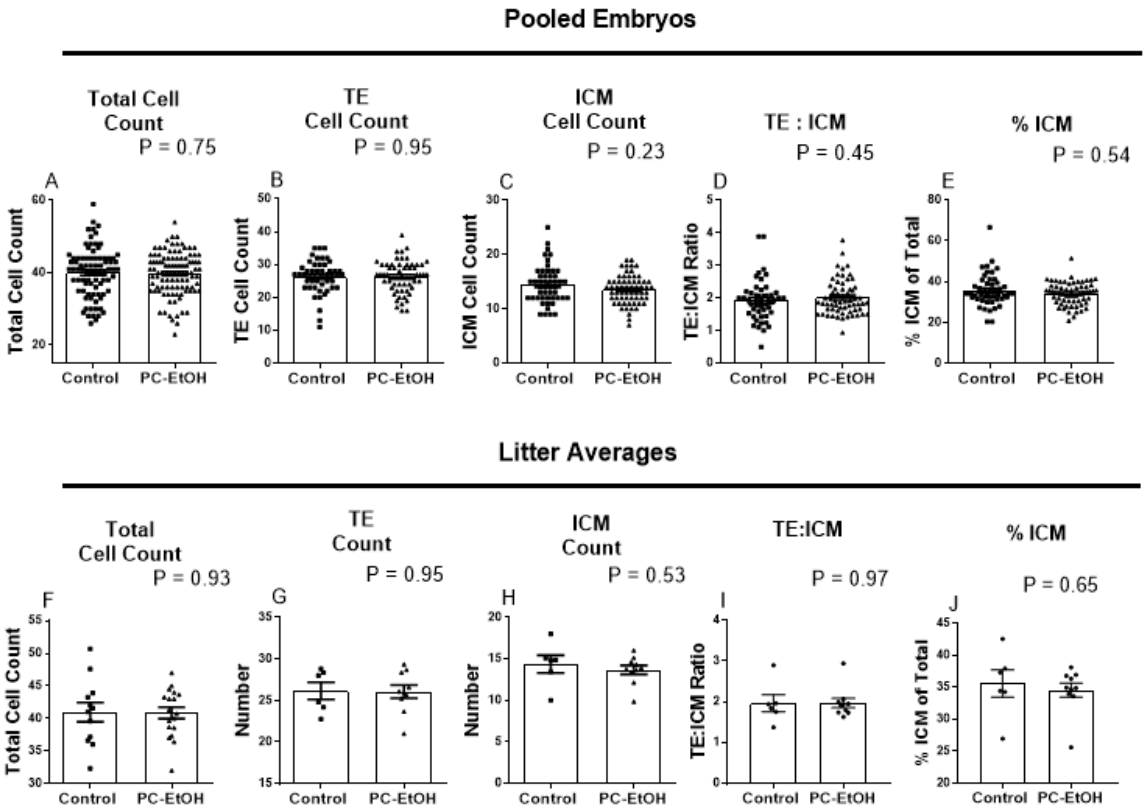

Figure S1. *In vivo* PC-EtOH exposure does not alter cell counts of E5 blastocyst. (A) Total cell count. (B) Trophectoderm (TE) cell count. (C) Inner cell mass (ICM) count. (D) Ratio of trophectoderm to inner cell mass (TE:ICM). (E) Percentage inner cell mass count of total cell count (% ICM). Litter averages were assessed for (F) Total cell count, (G) TE count, (H) ICM count, (I) TE:ICM and (J) % ICM. All data are presented as mean ± SEM and analysed with student's t tests. Non-parametric Mann-Whitney tests were used on ICM and %ICM pooled groups due to unequal variances. Total cell counts in panel (A) and (F) contain 12-18 dams per treatment, with 82-106 embryos. Panels (B-E) and (G-J) have 6-10 dams per treatment, with 49-62 embryos.

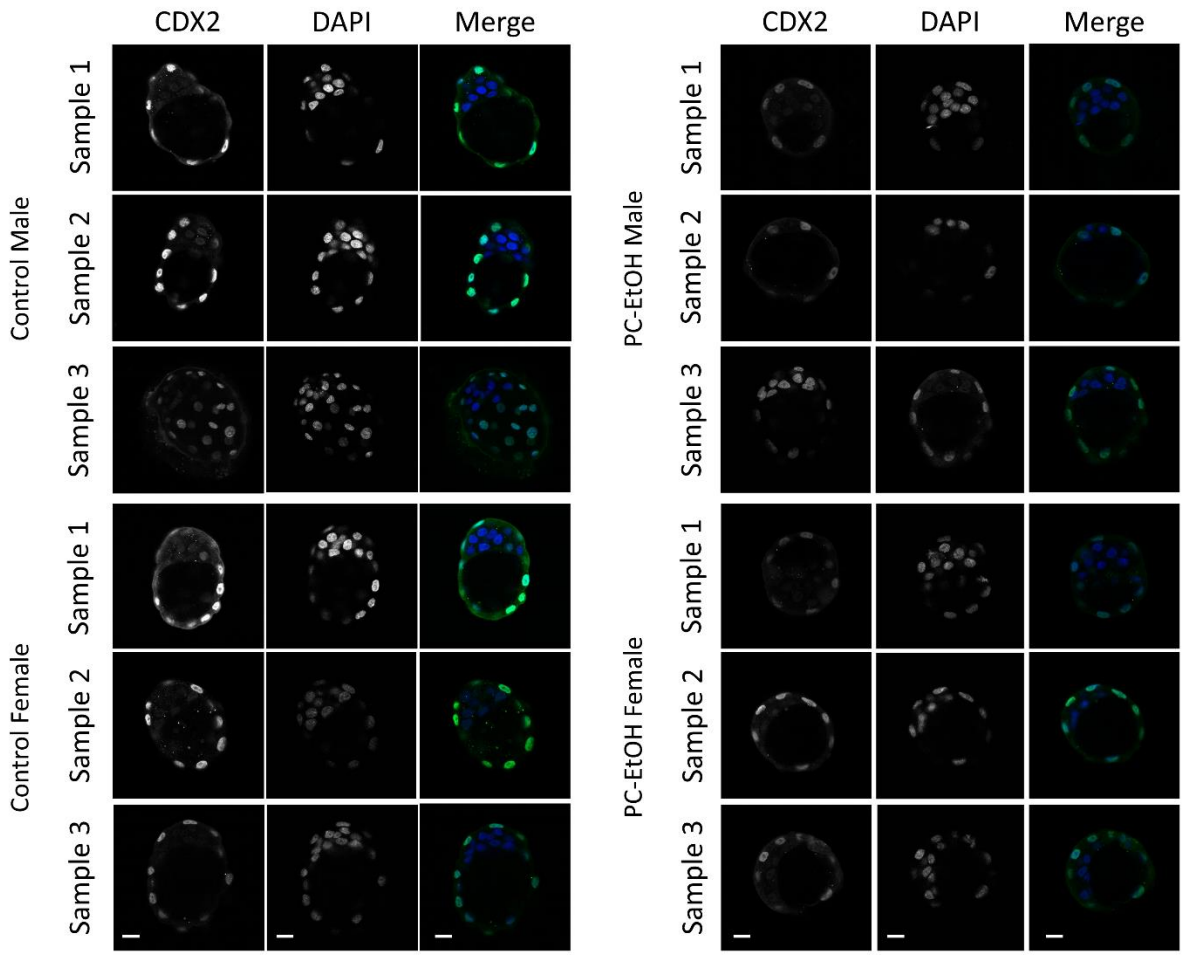

Figure S2. Representative images of CDX2 immunofluorescence from *in vivo*-derived PC-EtOH exposed embryos at E5. Scale bars: 20um.

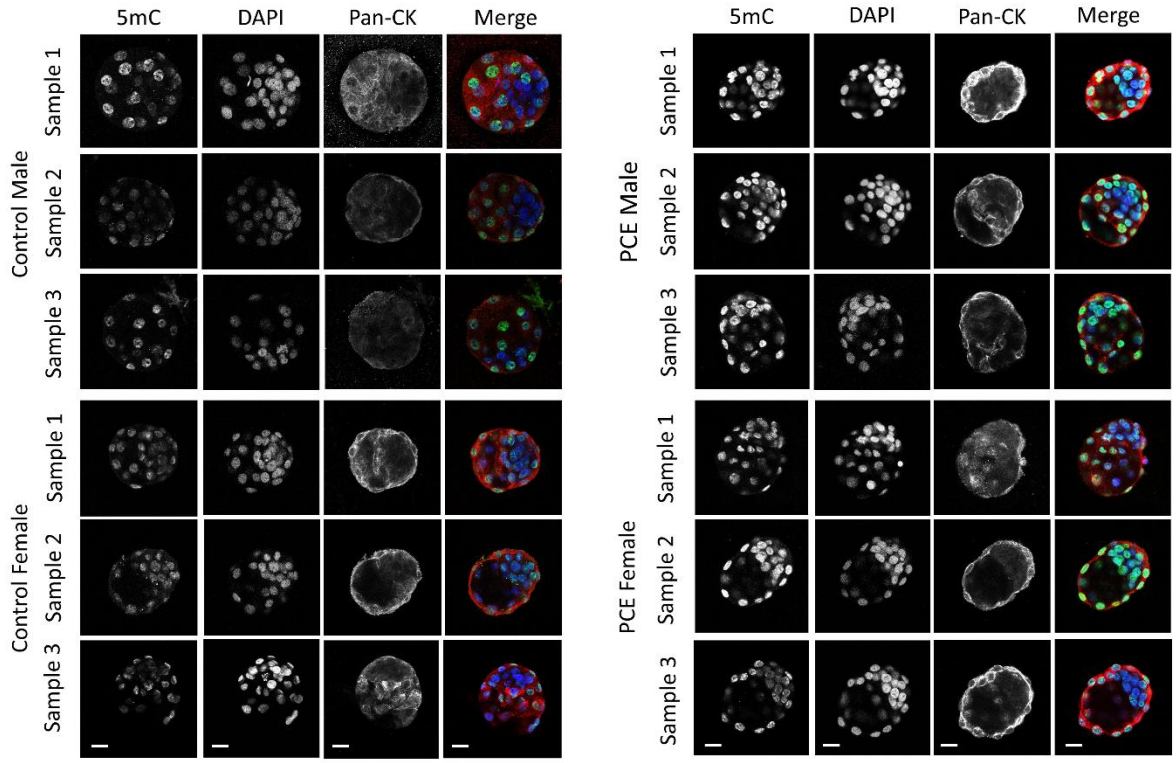

Figure S3. Representative images of 5mC immunofluorescence from *in vivo*-derived PC-EtOH exposed embryos at E5. Pan-cytokeratin (Pan-CK) marks trophectodermal cells. Scale bars: 20um.

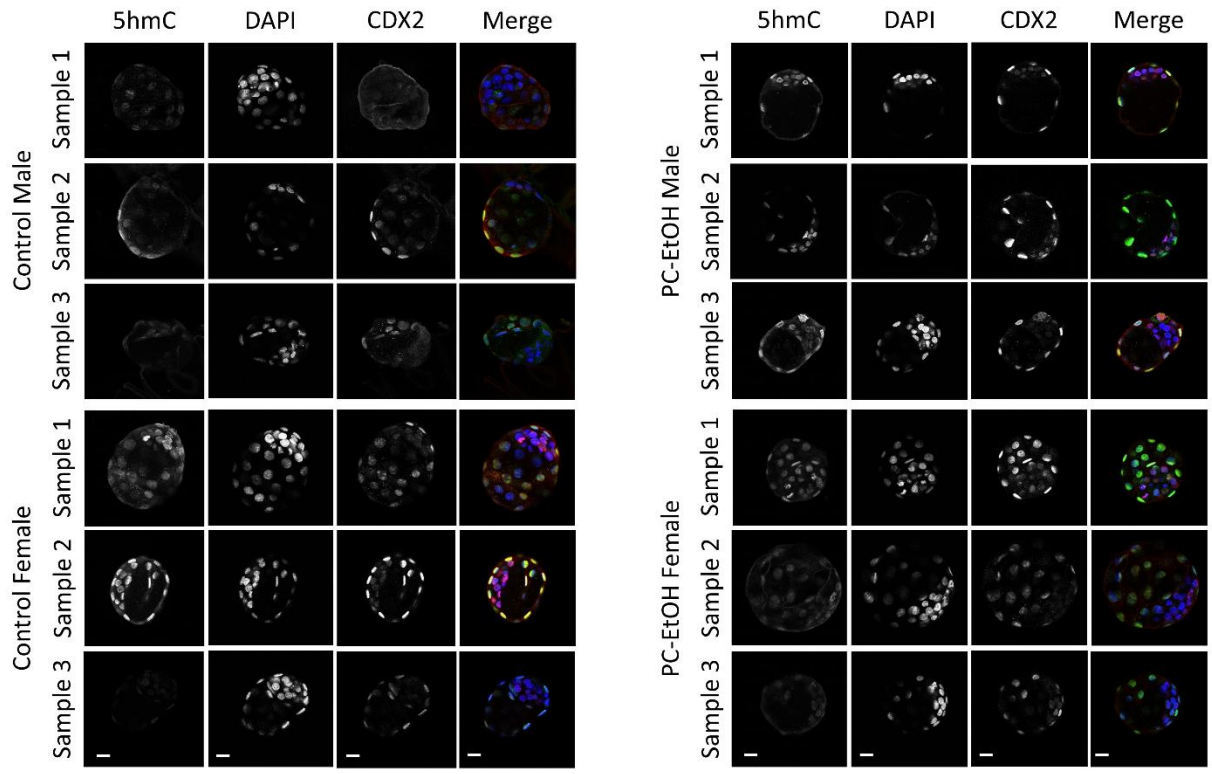

Figure S4. Representative images of 5hmC immunofluorescence from *in vivo*-derived PC-EtOH exposed embryos at E5. CDX2 marks trophectodermal cells. Scale bars: 20um.

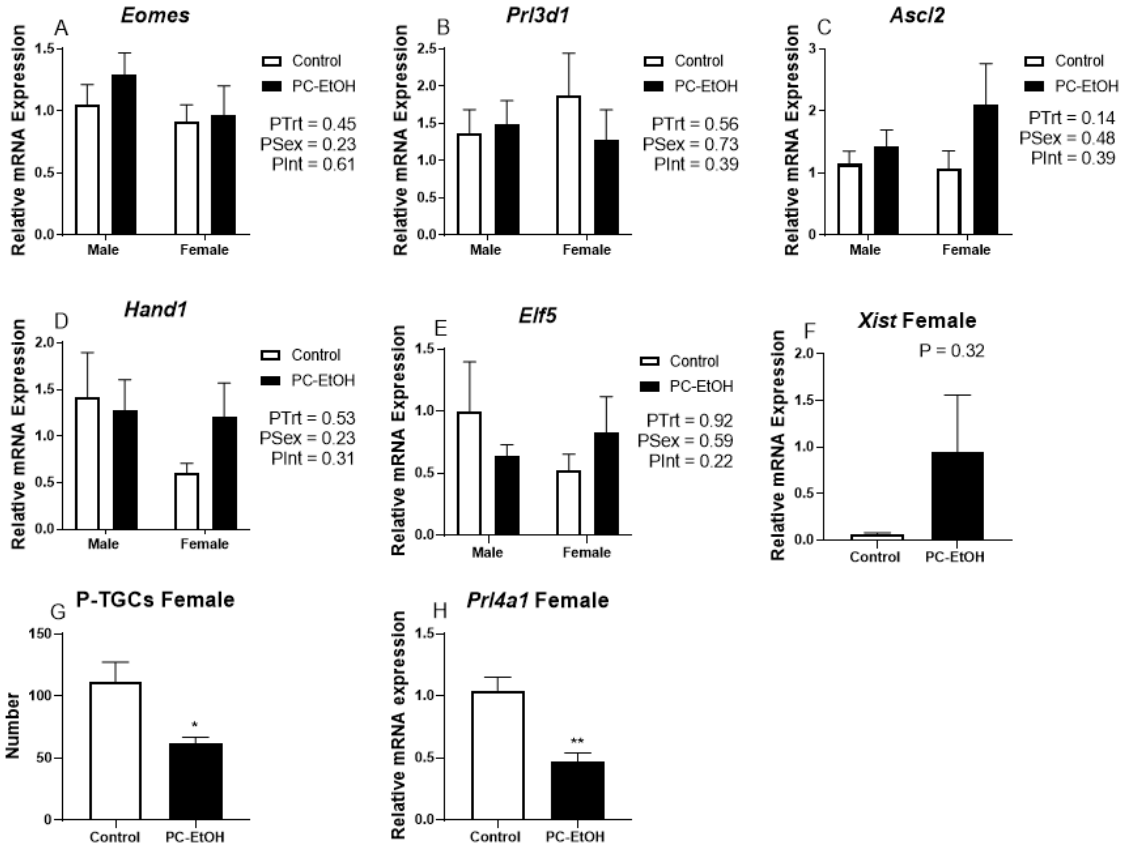

Figure S5. Gene expression assays and female-specific parameters of trophoblast outgrowths from *in vivo* derived control and PC-EtOH derived embryos. All data are presented as mean ± SEM. Panels (A-E) were analysed by two-way ANOVA. Panel (F) was analysed by a non-parametric Mann Whitney test, while (G and H) were analysed by Students t tests. (A-F and H) *n* = 7-10/sex from 3-4 litters/ treatment. Panel (G), *n* = 6-11 per sex from 4 litters/treatment. Gene expression is relative to control male group (A-E) or control female group (F), and standardised to a geometric mean of 2 housekeepers (*18S* and *Rpl13a*). Gene expression assays were carried out in duplicate. Control (white bars), PC-EtOH (black bars). *P<0.05, **P<0.01.

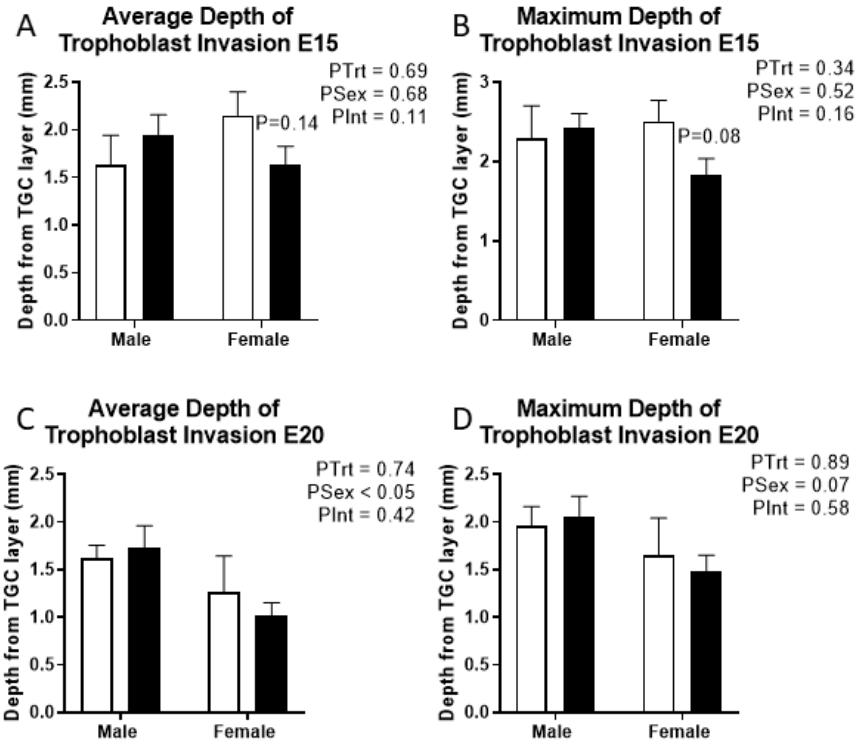

Figure S6. PC-EtOH does not alter trophoblast invasion depth. Data shows mean ± SEM, analysed by two-way ANOVA. *P<0.05. Control (white bars), PC-EtOH (black bars). E15; *n* = 5-8/ sex from 5-7 litters per treatment, E20; *n* = 3-6/ sex from 3-4 litters per treatment.

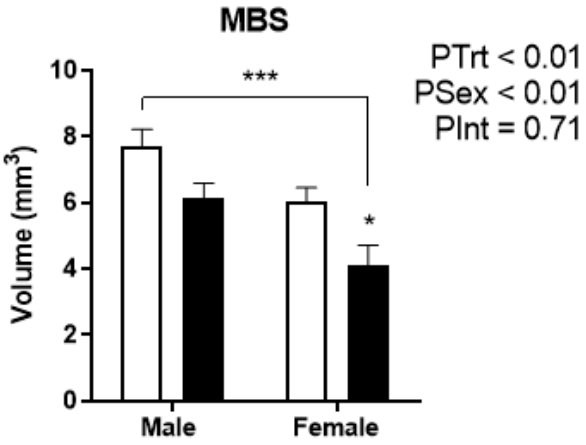

Figure S7. **PC-EtOH causes reduced MBS volume in both sexes.** Data shows mean ± SEM, analysed by two-way ANOVA. *P<0.05. Control (white bars), PC-EtOH (black bars). E15; *n* = 7-9/sex, 5-7 litters/treatment.

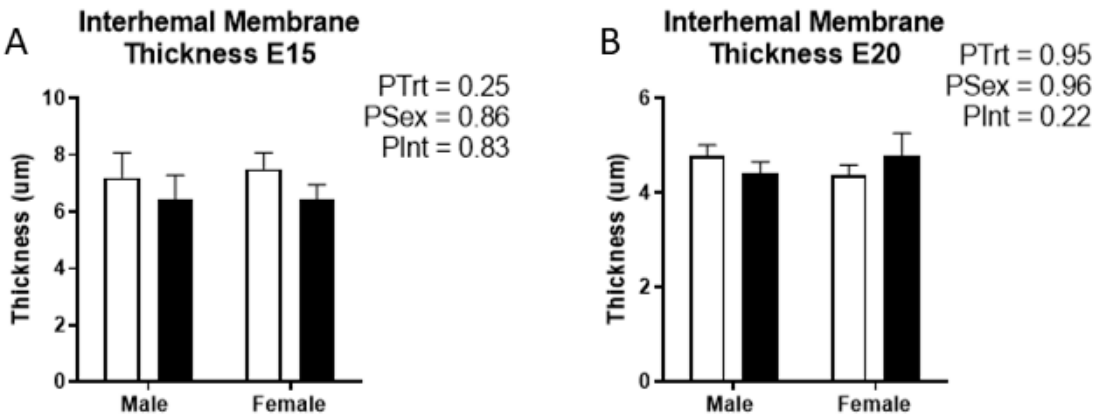

Figure S8. PC-EtOH does not alter interhaemal membrane thickness. Thickness of the interhemal membrane was assessed in ImageJ at E15 (A) and E20 (B). Data shows mean ± SEM, analysed by two-way ANOVA. *P<0.05. Control (white bars), PC-EtOH (black bars). E15; *n* = 4-6/sex from 3-4 litters/treatment, E20; *n* = 3-5/sex from 3-4 litters/treatment.

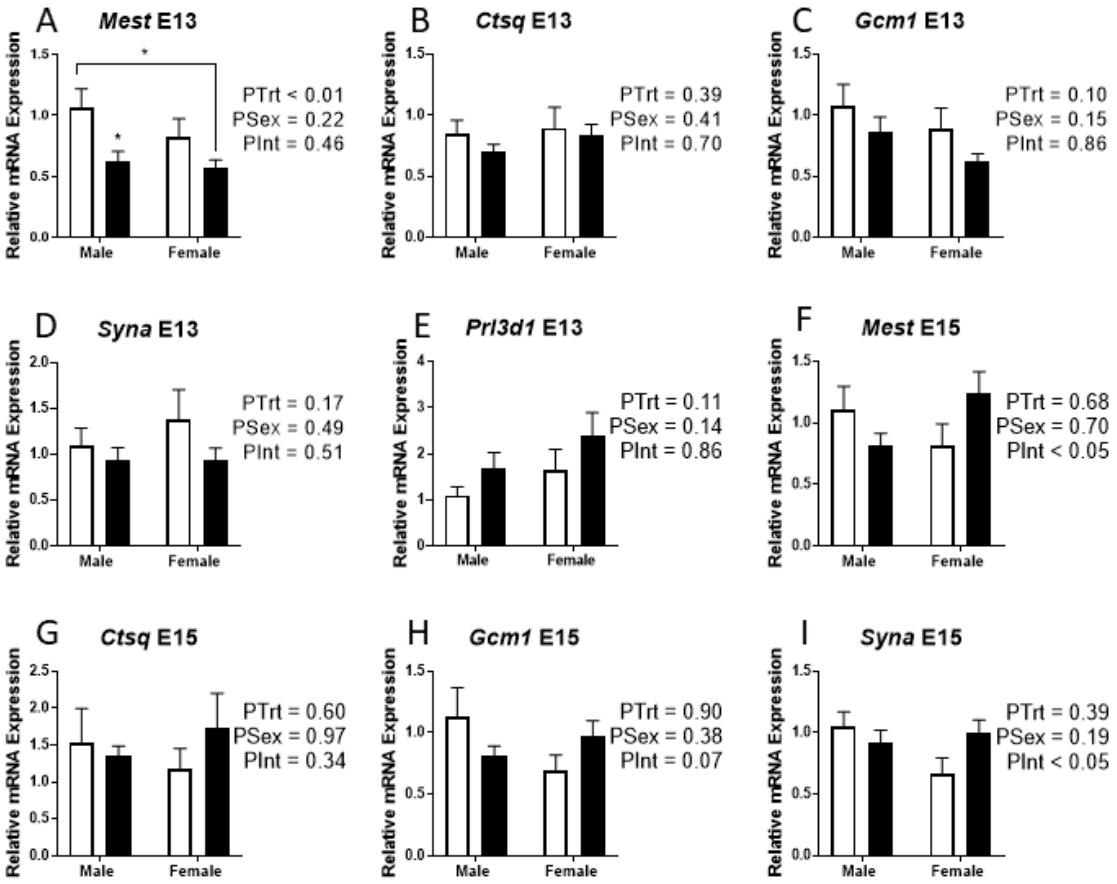

Figure S9. **Expression profiles of gene markers of placental differentiation in E13 whole placenta and the E15 labyrinth**. *Mest* (fetal endothelial cells) at E13 (A) and E15 (F). *Ctsq* (sinusoidal trophoblast giant cells) at E13 (B) and E15 (G). *Gcm1* (syncytiotrophoblast layer II) at E13 (C) and E15 (H), *Syna* (syncytiotrophoblast layer I) at E13 (D) and E15 (I). *Prl3d1* (parietal trophoblast giant cells) at E13 (E). Data shows mean ± SEM, analysed by two-way ANOVA. *P<0.05. Control (white bars), PC-EtOH (black bars). E13; *n* = 7-8/sex from 4-5 litters/treatment, *n* = 7-8/sex from 7-8 litters/treatment, expression data is relative to the geometric mean of *18S* and *Rpl13a*, and standardised to average expression of the control male group for each age.

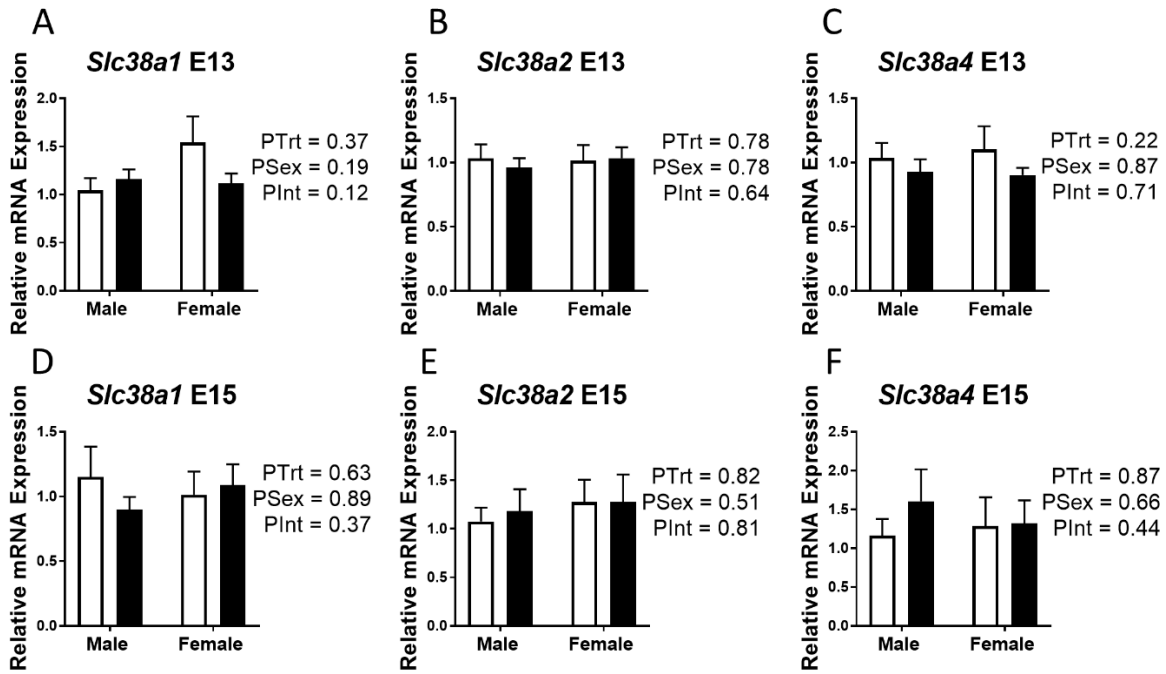

Figure S10. PC-EtOH does not alter placental system A amino acid transporter expression. Expression of nutrient transporters were assessed in the E13 whole placenta and E15 labyrinth. System A amino acid transporters - *Slc38a1*, *Slc83a2*, and *Slc38a4* were assessed at E13 (A-C) and E15 (D-F). Data shows mean ± SEM, analysed by two-way ANOVA. Control (white bars), PC-EtOH (black bars). Expression assays (A-J) E13; *n* = 7-8/sex from 4-5 litters/treatment, *n* = 7-8/sex from 7-8 litters/treatment, expression data is relative to the geometric mean of *18S* and *Rpl13a*, and standardised to average expression of the control male group for each age.

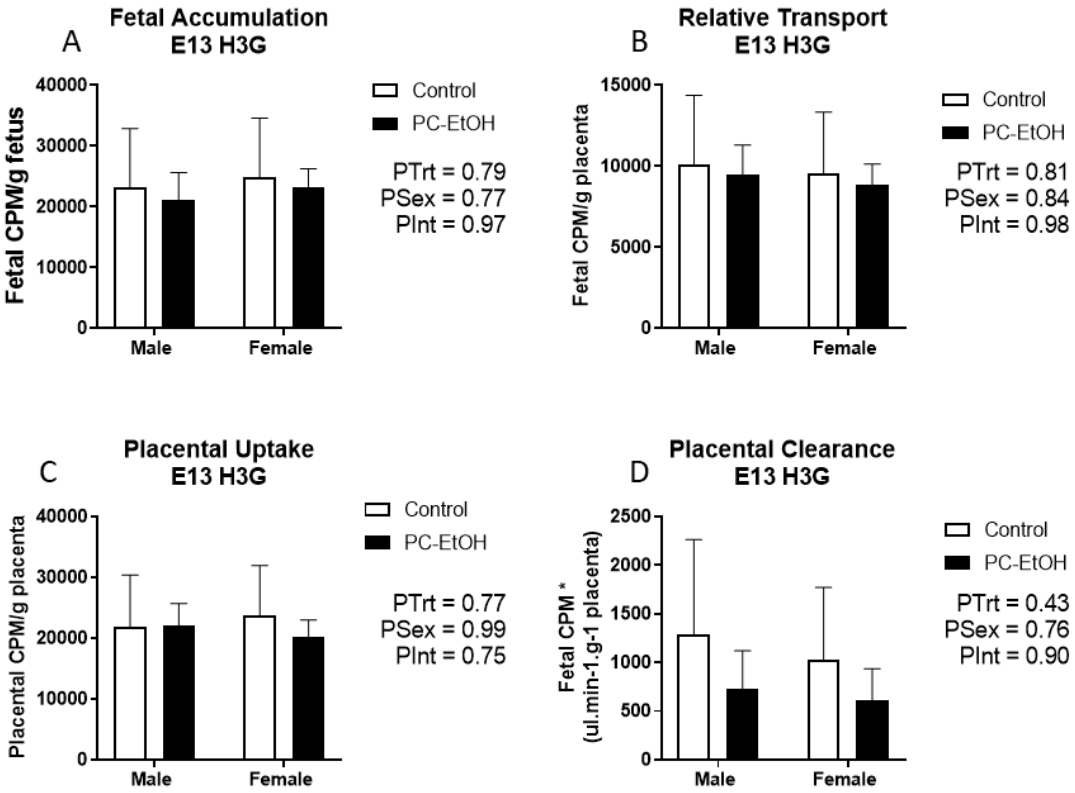

Figure S11. Quantification of placental glucose transport at E13 after PC-EtOH. Total glucose was assessed using glucose analogue H3-O-methyl-D-glucose (H3G) at E13. Assays assessed fetal accumulation (fetal CPM/g fetus, A), relative placental transport (fetal CPM/g placenta, B), placental uptake (placental CPM/g placenta, C), and placental clearance (fetal CPM *ul.min-1.g-1 placenta, D). Data shows mean ± SEM, analysed by two-way ANOVA. Control (white bars), PC-EtOH (black bars). *n* = 18-29 from 3-4 litters per treatment.

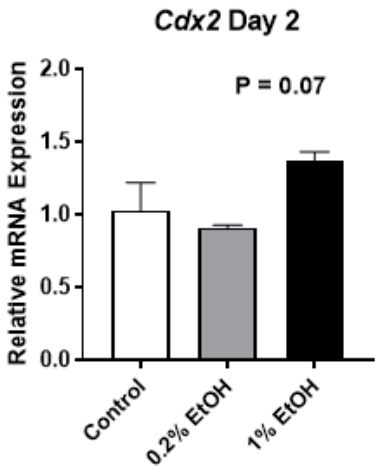

Figure S12. *Cdx2* expression assays from cultures of mouse trophoblast stem cells at 2 days of differentiation. Control (white bars), 0.2% EtOH (grey bars), 1% EtOH (black bars). All data analysed by one-way ANOVA with Tukey's post-hoc tests. All data represented by mean ± SEM. *n* = *3* per treatment.

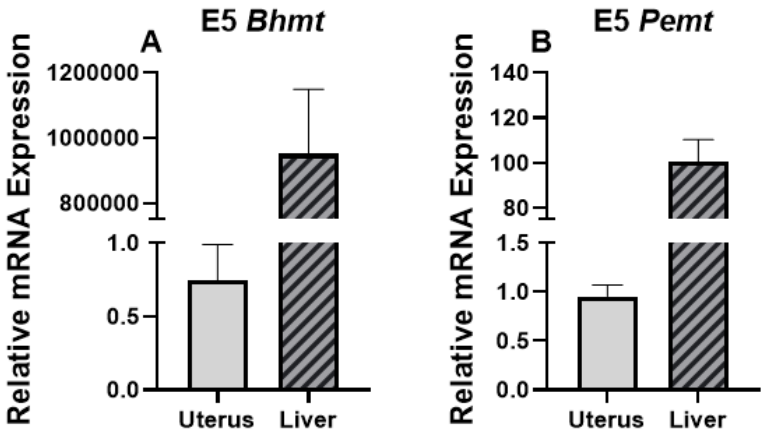

Figure S13. Comparison of choline metabolism genes in the uterus and liver at E5. Expression was assessed for *Bhmt* (A) and *Pemt* (B) in uterus (grey bars) and liver samples (striped bars). All data are presented as mean ± SEM. For gene expression assays, *n* = 6/treatment (E5 uterus) and *n* = 8/treatment (E5 livers) from 6-8 litters per treatment. Expression is relative to control group at E5, and standardised to geometric mean of *Rpl19* and *B-actin*.

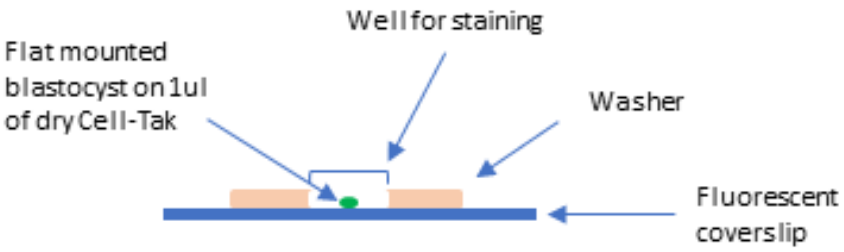

Figure S14. Staining chambers for immunofluorescent analysis of blastocysts. Fluorescent coverslips were attached to washers with paraffin wax and cooled to room temperature. 1ul of Cell-Tak was applied to the centre of the chamber. When dry, the blastocyst was placed onto this area with minimal volume of PBS. The blastocyst can be seen flattening at this stage. The well is subsequently flooded with PBS and staining procedures can continue.

Treatment	Aberrant	Oocytes	Morulae	Blastocysts	Average Blastocysts / Litter	Average Embryos/ Litter
Control	15/274 (5.6%)	13/274 (4.8%)	1/274 (0.4%)	240/274 (89.2%)	10.91 ± 0.74	12.46 ± 0.91
PC-EtOH	8/285 (2.8%)	4/285 (1.4%)	2/285 (0.7%)	271/285 (95.1%)	10.54 ± 0.68	10.96 ± 0.67
P Value	*P = 0.38*	P<0.05	*P = 0.64*	*P = 0.70*		*P = 0.18*

Table S1. Pre-implantation embryos derived from uterine flushings of E5 mothers. Aberrant counts do not include 2- and 4-cell embryos. Data was analysed using student's t tests. Non-parametric Mann-Whitney tests were used on oocytes and aberrant groups due to unequal variances. *n* =22-33 litters/treatment. Data presented as percentages or mean ± SEM.

	E5			E7			Two-way ANOVA		
	Control	**PC-EtOH**	***Statistics***	**Control**	**PC-EtOH**	***Statistics***	**PTrt**	**PAge**	**PInt**
Estrogen response genes									
Esr2	1.13 ± 0.19	1.78 ± 0.33	*P = 0.12*	7.52 ± 1.04	5.29 ± 0.82	*P = 0.11*	*PTrt = 0.38*	*PAge < 0.0001*	*PInt = 0.11*
Muc1	1.04 ± 0.14	0.69 ± 0.21	*P = 0.18*	0.33 ± 0.07	0.50 ± 0.10	*P = 0.22*	*PTrt = 0.45*	*PAge < 0.001*	*PInt < 0.05*
Hbegf	1.13 ± 0.26	1.15 ± 0.38	*P = 0.78*	0.15 ± 0.02	0.23 ± 0.03	*P = 0.07*	*PTrt = 0.43*	*PAge < 0.0001*	*PInt = 0.99*
Vegfa	1.04 ± 0.14	0.86 ± 0.20	*P = 0.47*	0.96 ± 0.08	1.04 ± 0.10	*P = 0.51*	*PTrt = 0.69*	*PAge = 0.70*	*PInt = 0.29*
Fgf2	1.05 ± 0.14	0.84 ± 0.05	*P = 0.31*	0.73 ± 0.09	0.63 ± 0.05	*P = 0.37*	*PTrt = 0.09*	*PAge < 0.01*	*PInt = 0.53*
Fgf9	1.05 ± 0.13	1.45 ± 0.46	*P = 0.93*	0.37 ± 0.03	0.34 ± 0.07	*P = 0.32*	*PTrt = 0.26*	*PAge < 0.001*	*PInt = 0.20*
Lif	1.20 ± 0.33	1.34 ± 0.42	*P = 0.79*	-	-	-	-	-	-
Usag1	1.01 ± 0.29	0.55 ± 0.14	*P = 0.19*	0.18 ± 0.02	0.22 ± 0.03	*P = 0.34*	*PTrt = 0.07*	*PAge < 0.0001*	*PInt < 0.05*
Ncoa6	1.05 ± 0.15	0.91 ± 0.25	*P = 0.63*	0.81 ± 0.10	0.75 ± 0.11	*P = 0.67*	*PTrt = 0.48*	*PAge = 0.18*	*PInt = 0.78*
Progesterone response genes									
Ihh	1.24 ± 0.36	1.29 ± 0.51	*P = 0.17*	0.09 ± 0.01	0.22 ± 0.06	*P = 0.32*	*PTrt = 0.69*	*PAge < 0.0001*	*PInt = 0.87*
Areg	1.73 ± 1.01	2.16 ± 0.89	*P = 0.42*	-	-	-	-	-	-
Hand2	1.03 ± 0.11	0.96 ± 0.11	*P = 0.66*	6.92 ± 0.49	6.71 ± 0.62	*P = 0.79*	*PTrt = 0.78*	*PAge < 0.0001*	*PInt = 0.89*
Igfbp1	1.35 ± 0.31	1.22 ± 0.57	*P = 0.84*	3.89 ± 0.70	2.83 ± 0.38	*P = 0.20*	*PTrt = 0.32*	*PAge < 0.01*	*PInt = 0.44*
dNK cell function genes									
Prf1	-	-	-	1.18 ± 0.19	1.04 ± 0.27	*P = 0.66*	-	-	-
Others									
Cnr1	-	-	-	1.14 ± 0.21	0.74 ± 0.09	*P = 0.09*	-	-	-
Klf5	-	-	-	0.96 ± 0.15	1.94 ± 0.36	*P < 0.05*	-	-	-
Cyp26a1	1.17 ± 0.25	1.34 ± 0.44	*P = 0.74*	6.38 ± 1.11	8.02 ± 0.77	*P = 0.24*	*PTrt = 0.34*	*PAge < 0.0001*	*PInt = 0.44*

Table S2. Expression of uterine genes during receptivity (E5) and post-implantation (E7) decidualisation. All data are presented as mean ± SEM and analysed by two-way ANOVA. Due to significant differences in age, treatments were analysed at each gestational age with Student's t tests. *n* = 6/treatment (E5) and *n* = 10/treatment (E7) from 5-7 litters per treatment. Gene expression is relative to control group at E5, and standardised to geometric mean of *Rpl19* and *B-actin*.

	Control		PC-EtOH		
Variables	**Male**	**Female**	**Male**	**Female**	**Statistics**
Fetus (mg)	38.385 ± 2.592	34.957 ± 2.283	40.011 ± 3.158	35.263 ± 1.427	*PTrt = 0.70* *PSex = 0.12* *PInt = 0.79*
Placenta (mg)	92.274 ± 4.070	86.704 ± 2.817	88.015 ± 5.817	86.439 ± 6.205	*PTrt = 0.65* *PSex = 0.48* *PInt = 0.69*
PW:BW ratio (mg/mgbw)	2.417 ± 0.068	2.499 ± 0.107	2.218 ± 0.132	2.487 ± 0.297	*PTrt = 0.55* *PSex = 0.33* *PInt = 0.60*
	Control		**PC-EtOH**		**Statistics**
Litter size (number)	15.73 ± 0.384		14.20 ± 0.389		*P < 0.05*
Resorptions (number)	0.636 ± 0.244		0.800 ± 0.200		*P = 0.61*
Sex Ratio (M:F)	1.038 ± 0.177		1.057 ± 0.194		*P = 0.95*
Male Fetuses (%)	48.62 ± 3.121		48.59 ± 4.194		*P = 0.99*

Table S3. Fetal and Placental Biometry at E13. All data are presented as mean ± SEM and *n* = 5-11/ sex from 4 litters/treatment for fetal and placental weights, and PW (placental weight):BW (body weight) ratio. *n* = 4-11/sex from 8-9 litters/treatment for remaining parameters. Note: litter size includes resorptions. M; male, F; female.

	E13					E20				
	Control		PC-EtOH			Control		PC-EtOH		
Variables	**Male**	**Female**	**Male**	**Female**	**Statistics**	**Male**	**Female**	**Male**	**Female**	**Statistics**
WP Vol E13 (mm^3)	26.78 ± 1.42	27.57 ± 1.48	26.08 ± 1.82	25.49 ± 1.82	*PTrt = 0.40 PSex = 0.95 PInt = 0.68*	167.56 ± 12.06	164.78 ± 12.49	177.71 ± 9.06	174.51 ± 7.97	*PTrt = 0.60 PSex = 0.78 PInt = 0.98*
Lab Vol (mm^3)	3.24 ± 0.32	3.80 ± 0.39	3.64 ± 0.17	3.88 ± 0.32	*PTrt = 0.48 PSex = 0.23 PInt = 0.63*	119.58 ± 9.85	121.12 ± 9.97	128.41 ± 6.69	119.27 ± 7.79	*PTrt = 0.70 PSex = 0.67 PInt = 0.55*
JZ Vol (mm^3)	1.88 ± 0.20	2.21 ± 0.26	2.36 ± 0.19	2.22 ± 0.24	*PTrt = 0.29 PSex = 0.68 PInt = 0.31*	47.98 ± 3.88	43.66 ± 3.33	49.29 ± 4.05	52.80 ± 3.72	*PTrt = 0.17 PSex = 0.92 PInt = 0.31*
Dec Vol (mm^3)	21.66 ± 1.18	21.83 ± 1.52	20.08 ± 1.82	19.40 ± 1.64	*PTrt = 0.20 PSex = 0.87 PInt = 0.78*	-	-	-	-	-
FBS Vol (mm^3)	0.46 ± 0.06	0.54 ± 0.05	0.54 ± 0.06	0.65 ± 0.08	*PTrt = 0.19 PSex = 0.15 PInt = 0.83*	20.02 ± 1.66	19.32 ± 1.40	22.23 ± 1.97	18.73 ± 1.92	*PTrt = 0.64 PSex = 0.23 PInt = 0.42*
MBS Vol (mm^3)	0.81 ± 0.13	1.09 ± 0.24	0.92 ± 0.10	1.14 ± 0.09	*PTrt = 0.63 PSex = 0.13 PInt = 0.82*	46.48 ± 4.44	39.67 ± 3.37	41.56 ± 3.22	39.34 ± 3.95	*PTrt = 0.50 PSex = 0.23 PInt = 0.55*
Lab Troph Vol (mm^3)	1.97 ± 0.16	2.16 ± 0.13	2.18 ± 0.08	2.10 ± 0.17	*PTrt = 0.64 PSex = 0.72 PInt = 0.36*	53.07 ± 5.46	62.13 ± 5.72	64.62 ± 5.46	61.19 ± 3.89	*PTrt = 0.32 PSex = 0.59 PInt = 0.25*
Total SA (FBS + MBS, cm^2)	5.20 ± 0.57	6.14 ± 0.58	6.18 ± 0.36	6.55 ± 0.48	*PTrt = 0.20 PSex = 0.23 PInt = 0.60*	202.64 ± 16.05	208.31 ± 12.71	225.92 ± 11.91	207.54 ± 15.06	*PTrt = 0.44 PSex = 0.66 PInt = 0.40*
FBS SA (cm^2)	2.43 ± 0.31	2.99 ± 0.21	3.11 ± 0.200	3.40 ± 0.34	*PTrt = 0.07 PSex = 0.15 PInt = 0.63*	78.42 ± 6.38	84.96 ± 4.90	96.71 ± 5.27	84.92 ± 7.14	*PTrt = 0.14 PSex = 0.67 PInt = 0.14*
MBS SA (cm^2)	2.77 ± 0.30	3.14 ± 0.38	3.07 ± 0.18	3.15 ± 0.22	*PTrt = 0.61 PSex = 0.45 PInt = 0.62*	124.23 ± 10.13	123.35 ± 9.41	129.20 ± 7.67	122.62 ± 9.02	*PTrt = 0.82 PSex = 0.69 PInt = 0.76*
Lab Weight: Lab Vol (g/mm^3) x100	-	-	-	-	-	0.31 ± 0.03	0.29 ± 0.01	0.28 ± 0.04	0.30 ± 0.02	*PTrt = 0.93 PSex = 0.79 PInt = 0.54*
JZ Weight: JZ Vol (g/mm^3) x100	-	-	-	-	-	0.032 ± 0.02	0.33 ±0.05	0.34 ±0.05	0.32 ±0.03	*PTrt = 0.88 PSex = 0.98 PInt = 0.67*

Table S4. Stereological investigation of the placenta at E13 and E20. All data are presented as mean ± SEM, analysed by two-way ANOVA. E13; *n* =5-7/sex from 4 litters/treatment, E20; *n* = 6-12/sex from 4-6 litters/treatment. Dec; decidua. FBS; fetal blood space. Lab; labyrinth. Lab Troph; labyrinth trophoblast. JZ; junctional zone. MBS; maternal blood space. SA; surface area. Vol; volume. WP; whole placenta.

Gene	Direction	Sequence (5'-3')	Product (bp)
Mest	Forward (T3)	CTGCTCTGCACTCATGGAAG	478
	Reverse (T7)	CCGTCTTTGAGGAGCTTTTG	
Prf1	Forward (T3)	AGCCAGTGTCTCAAGCGAAT	414
	Reverse (T7)	CAGTCCTGGTTGGTGACCTT	
Prl8a2 (dPRP)	Forward (T3)	TGCATCAGTTCCTGCTTGTC	871
	Reverse (T7)	TTTCCCTGTTATGCGACACA	

Table S5. *In situ* hybridisation primers and product length. T3; AATTAACCCTCACTAAAGGG and T7; TAATACGACTCACTATAGGG sequences were added to the beginning of forward and reverse primers respectively. Note; *Prl8a2* is also known as decidual prolactin-related protein (dPRP).

Gene	Forward Primer	Reverse Primer	Primer Efficiency
Bhmt	AAG AGG GGA ATC TTA GAA CG	TTC CAG TGC AAA GAC AAA TC	2.151
Rpl13a	GCA CAA GAC CAA AAG AAG	CGC TTT TTC TTG TCA TAG GG	2.033
RplO	GAG TGA CAT CGT CTT TAA ACC	AAG CAT TTT GGG TAG TCA TC	2.023
Prl3d1	AGA CCT TAT ACA ACA GGA CTC	ATG GCA AAA GAT GAG TGT C	1.992
Hand2	CTC CAA AAT CAA GAC TCT GC	CAT TCA GCT CTT TCT TCC TC	1.963
Ncoa6	AAA AGA TCT TCT CGA CCT G	GTA TCA AGT CAT CTT CCT GC	1.989
Usag1 (Sostdc1)	ACT GGA TCG AAA TAG TCG AG	TCC AGT ACT TTG TTC CGT AG	1.945
Elf5	ATT TGT CAG AGA CTT GCT TC	TTA TAG TAG TAT TCT AGG GCT C	1.989
Esr1	ATA TGA TCA ACT GGG CAA AG	CAT TTA CCT TGA TTC CTG TCC	2.021
Pemt	CTA GGC TGG GCA CTT ATG	CTT CAA ACA GGA GAG CAA C	1.931
Pgr	TCT AAT CCT GAA TGA GCA GAG	GAC TTT CAT ACA GAG GAA CTC	2.018
Fgf2	AAA CTC GGA TCC AAA ACG	TGT CTA AAG AGA GTC AGC TC	1.998
Fgf9	ACT ATA AAT GCT TCA TGC GG	CAA TAA ATC AAG CAA GTG GC	1.960
Igfbp-1	AAA CTG AAA GTT GTT TCC TCC	ATA CAA ACC CAC TTG TAC ATC	1.969
Cyp26a1	CAA GCA GCG AAA GAA GGT GA	CTT CAG CAA TCA CTG GCA CG	1.940
Cnr1 (CB1)	CAC CCA TGG CTG AGG GTT CC	CCC ACG TAG AGG AGG TCT GT	1.985
Klf5	AAT CCA AAT TTA CCT GCC AC	TGC AAC CAT CAT AAT CAC AG	1.909
Ihh	AAA GAC GAG GAG AAC ACC	AAGATTCCTCTGAGTGATGG	1.924
Muc1	CGG AAG TCA ATG TGA ATG AG	CAA AAT ACA GAC CAG TAC CAG	1.955
Xist	CCT ACC TGT AAT ACA CAA TGC C	GGT CGT ATG TAA AGC TGG C	2.055
Hprt	ACT GGT AAA ACA ATG CAG AC	CCT GAA GTG CTC ATT ATA GTC	2.066

Table S6. SYBR primer sequences and efficiencies. All probes are designed for the rat unless otherwise stated.

Gene of Interest	ID #
18S	4333760F
Ascl2	Rn00580387_m1
Areg	Rn00567471_m1
Bmp2	Rn00567818_m1
Cited1	Rn00821880_g1
Ctl1 (SLC44a1)	Rn00585181 m1
Ctl2 (SLC44a2)	Rn01486877 m1
Ctsq	Rn01448613_m1
Eomes	Rn01746545_m1
G6pd	Rn00566576_m1
Gcm1	Rn00820824_g1
Glut1 (Slc2a1)	Rn01417099_m1
Glut3 (Slc2a3)	Rn00567331
Hand1	Rn00572139_m1
Hbegf	Rn01405658_m1

Gene of Interest	ID #
Igf2	Rn01454518_m1
Igf2r	Rn01636937_m1
Lif	Rn00573491_g1
Mest	Rn01500324_m1
Ogt	Rn00820779_m1
Prf1	Rn00569095_m1
Prl4a1	Rn00566830_m1
Nr2f2 (CoupTF-II)	Rn00756179_m1
Rlim	Rn01473927_m1
Rpl19	Rn00821265_g1
Sry	Rn04224592_u1
Syna	Rn01500024_m1
Slc38a1 (Snat1)	Rn00593696_m1
Slc38a2 (Snat2)	Mn00628416_m1
Slc38a4 (Snat4)	Rn00593742_m1

Table S7. Taqman Primer information.