Supplementary Material

Timing of exogenous progesterone administration is critical for embryo development and uterine gene expression in an ovine model of maternal constraint

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Table S1. Candidate and reference gene ID, accession number, forward and reverse primer sequences, amplicon sizes (base pairs, bp) and primer efficiency tested by reverse-transcription PCR (RT-qPCR)

	Gene identifier[1]	NCBI accession	Forward primer sequence	Reverse primer sequence	Efficiency (%)	Amplicon size (bp)	References[2]
Day 6	DGAT2	XM_012096077.1	CCCTCCAGGACCTGTTTTCC	TGAGCCAGCAATCGGTACAG	97	159	
	FGF7	XM_012180603	ATCAGGACAGTGGCTGTTGG	CCGCTGTGTGTCCATTTAGC	97	182	
	FGF10	NM_001009230.1	TTGAGAACGGGAAGGTCAGC	AGCAACAACTCCGATTTCCAC	104	89	
	HGF	XM_012176562.1	TGGTGTTTCACAAGCAATCCA	AGGTCATGCATTCAACTTCTGAAC	97	79	Satterfield *et al.* (2006)
	IGF1	NM_001009774.3	TCAGCAGTCTTCCAACCCAA	CAAGCACAGGGCCAGATAGA	105	110	
	PGR	XM_012095331.1	TGGTATTTGGACTAGGATGGAGA	AAACTCCTGTGGGATCTGCC	108	149	
	PTGS2	NM_001009432.1	CAGAGCTCTTCCTCCTGTGC	CAGATTTGTGCCCTGGGGAT	99	142	
	MSTN	NM_001009428.1	GATCTTGCTGTAACCTTCCC	GTGGAGTGCTCATCACAATC	99	125	Jeanplong *et al.* (2013)
Day19	CTSL	XM_004004094.2	ACAGCCAAGGGAAACATAGCT	TCTCCTGCCTTTGAAAGCCA	102	102	
	IGF1	NM_001009774.3	TCAGCAGTCTTCCAACCCAA	CAAGCACAGGGCCAGATAGA	105	110	
	IGF1R	XM_012098367.1	GGAATGGGTCATGGACGGAG	GGGCAGAGCAATCATCAGGT	99	89	
	LGALS15	NM_001009238.1	CGGGAGTTGGCAGAAGGAAG	CAAACTGGCAGATGGGCTTG	100	131	
	MUC1	XM_012107297.1	ACAGAGGGATTTTCTGGGCC	AGCTGACTGAACTGTGCCTT	100	123	
	PGR	XM_012095331.1	TGGTATTTGGACTAGGATGGAGA	AAACTCCTGTGGGATCTGCC	108	149	
	PTGS2	NM_001009432.1	CAGAGCTCTTCCTCCTGTGC	CAGATTTGTGCCCTGGGGAT	99	142	
	RSAD2	XM_004005669.2	GCAGGCTGGTGAAGTTCTGT	TGCTCGTCAAAGCTGTCACA	103	148	
	SERPIN	NM_001009304.1	GCTTCCCAAGATTGACCCCA	TCCGTGGTTAAGGCATGCTC	97	138	

	SPP1	NM_001009 224.1	TGCAGTGATTTGCTTCT GCC	GCTTCTGAGATGGGTCA GGC	107	146	
Reference genes	*RPL19*	XM_004012 836.2	ATGCCCGAGAAGGTAA CGTG	GTCTGCCTTCAGCTTGT GGA	101	180	
	SF1	XM_004019 657.1	GAGAGTTGGCTCGCTT GAAT	CCCCTCCACACTTGGTA CAC	101	120	Paten *et al.* (2014)
	TBP	XM_015097 549.1	AGAATAAGAGAGCCCC GCAC	GCTCCCCACCATGTTCT GAA	101	177	

[1]Gene names: *CTSL* = Cathepsin L, *DGAT2* = Diacylglycerol-O-acyltransferase 2, *FGF7* = Fibroblast growth factor 7, *FGF10* = Fibroblast growth factor 10, *HGF* = Hepatocyte growth factor, *IGF1* = Insulin like growth factor 1, *IGF1R* = Insulin like growth factor 1 receptor, *LGALS15* = Endometrial galectin 15, *MUC1* = Mucin 1, *MSTN* = Myostatin, *PGR* = Progesterone receptor, *PTGS2* = Prostaglandin-endoperoxide synthase 2, *RSAD2* = Radical S-adenosyl methionine domain containing 2, *SERPIN* = Ovine uterine serine proteinase inhibitor, SPP1 = Secreted phosphoprotein 1, *RPL19* = Ribosomal protein L 19, *SF1* = Splicing factor 1, *TBP* = TATA box binding protein.

[2]Forward and reverse primer sequences were used from these references where indicated, but checked against Geneious and NCBI primer BLAST as outlined in the text.

Table S2. Pregnancy day 19 uterine horn mRNA expression levels in in pregnant Cheviot (C) ewes that were and were not administered exogenous progesterone (P4) for various time periods from day 0-6 (CP4$^{0-3}$, CP4$^{0-6}$ CP4$^{2-4}$, CP4$^{3-6}$ and CnP4) for combined uterine horns

Fold change is expressed relative to levels in control Suffolk (S) ewes that were not administered exogenous progesterone (SnP4, n=12). Data is normalised with *RPL19*, *SF1* and *TBP*. Data is shown as fold change with 99% confidence intervals (given in parenthesis). If confidence intervals do not include 1, then mRNA expression levels are significantly different from SnP4 control (bold). Different superscripts indicate that mRNA expression levels differ between CP4$^{0-3}$, CP4$^{0-6}$ CP4$^{2-4}$, CP4$^{3-6}$ and CnP4 treatment groups ($P<0.01$). Gene names: *CTSL* = Cathepsin L, *IGF1* = Insulin like growth factor 1, *IGF1R* = Insulin like growth factor 1 receptor, *LGALS15* = Endometrial galectin 15, *MUC1* = Mucin 1, *PGR* = Progesterone receptor, *PTGS2* = Prostaglandin-endoperoxide synthase 2, *RSAD2* = Radical S-adenosyl methionine domain containing 2, *SERPIN* = Ovine uterine serine proteinase inhibitor, SPP1 = Secreted phosphoprotein 1, *RPL19* = Ribosomal protein L 19, *SF1* = Splicing factor 1, *TBP* = TATA box binding protein

	Combined horns: ipsilateral and contralateral to CL				
	CnP4	CP4$^{0-3}$	CP4$^{0-6}$	CP4$^{2-4}$	CP4$^{3-6}$
n	12	8	4	20	10
CTSL	**1.15 (1.11 – 1.19)^b**	1.04 (0.63 - 1.71)^{bc}	**0.43 (0.32 - 0.57)^a**	**1.28 (1.27 - 1.30)^c**	**1.21 (1.09 - 1.35)^{bc}**
IGF1	**0.90 (0.83 - 0.98)^a**	**1.34 (1.33 - 1.35)^c**	1.21 (0.51 – 2.89)^{abcd}	**1.51 (1.32 - 1.72)^d**	1.14 (0.98 - 1.31)^b
IGF1R	0.84 (0.70 – 1.01)^a	**1.20 (1.05 - 1.36)^b**	0.94 (0.49 - 1.82)^{abc}	**1.43 (1.14 - 1.78)^c**	0.92 (0.73 - 1.17)^a
LGALS15	1.20 (1.00 - 1.45)^c	**0.60 (0.37 - 0.96)^b**	**0.34 (0.29 - 0.39)^a**	0.93 (0.76 - 1.14)^{bc}	**0.73 (0.50 - 1.08)^{bc}**
MUC1	**1.04 (1.03 - 1.05)^a**	**1.81 (1.34 – 2.46)^b**	1.04 (0.85 - 1.26)^a	**1.84 (1.75 - 1.93)^b**	**1.54 (1.36 - 1.76)^b**
PGR	**1.35 (1.18 - 1.54)^c**	**0.74 (0.66 – 0.84)^a**	1.12 (0.54 - 2.32)^{abc}	0.93 (0.76 - 1.14)^b	0.96 (0.71 - 1.28)^{ab}
PTGS2	0.98 (0.79 - 1.21)^a	1.02 (0.63 - 1.64)^{ab}	1.49 (0.72 – 3.09)^{ab}	**1.42 (1.20 - 1.68)^b**	**1.38 (1.04 - 1.83)^b**
RSAD2	1.02 (0.66 - 1.56)	0.83 (0.54 - 1.25)	-[1]	1.12 (0.83 - 1.50)	0.80 (0.46 - 1.40)
SERPIN	0.63 (0.29 - 1.37)	1.52 (0.41 - 5.69)	0.60 (0.10 – 3.43)	1.11 (0.58 - 2.12)	1.29 (0.58 – 2.91)
SPP1	**2.19 (1.15 - 4.18)^b**	**2.25 (1.10 - 4.63)^b**	0.52 (0.18 – 1.51)^a	**1.71 (1.02 - 2.89)^b**	**2.69 (1.44 – 5.02)^b**

[1] Value omitted due to lack of biological replicates to reliably examine expression once outliers were removed.