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Supplementary Material

Epigenetics and developmental programming of welfare and production traits in farm animals

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Supplementary Materials S1. Major epigenetic mechanisms governing cell identity

DNA methylation

DNA methylation in mammalian cells is predominantly targeted to cytosines of the palindromic CpG dinucleotide sequence. ("p" refers to the phosphodiester bond that connects the bases "C" and "G"). DNA is duplicated prior to cell division by semi-conservative DNA replication to ensure that the daughter cells receive a full copy of the genome. Following DNA replication, DNA methyltransferases (Dnmts) copy the methylation pattern of the parent DNA strand onto the newly synthesized daughter strand; the methylation status of a ‘parent CpG’ serves as template for the ‘daughter CpG’. Such ‘maintenance methylation’ is therefore a mechanism that transmits epigenetic information ‘on the back’ of DNA to descendants of a given cell.

Mammalian DNA has millions of CpG dinucleotides. These potential methylation sites are unevenly distributed throughout the genome. Regions of high CpG-density speckle a genome that is otherwise characterised by a relative depletion of this dinucleotide. Approximately 70% of gene promoters are CpG-rich (Saxonov *et al.* 2006). The majority of CpG-rich promoter regions remain completely unmethylated throughout development and adult life. Biologically important exceptions are CpG-rich sequences of imprinted genes and gene promoters present on the inactive X chromosome in somatic cells of females. Dense promoter methylation is generally associated with gene inactivity.

We still do not fully understand how cell type specific DNA methylation patterns emerge. The genotype exerts a strong influence and provides a blueprint for DNA methylation patterns found in adult tissues (Silva and White 1988; Gertz *et al.* 2011). However, evidence suggests that these DNA methylation patterns are

subtly altered during the life-course of an animal by environmental, physiological and stochastic events (Jaenisch and Bird 2003; Whitelaw and Whitelaw 2006). Plasticity and modulation of DNA methylation patterns in response to environmental signals, likely processes involved in fetal programming, are thought to have particular impact during critical periods of development when cell fates are specified.

Thus, measuring differences in DNA methylation has become an important approach to explain phenotypic differences observed, for example in monozygotic twins and inbred animals. Measurements, however, are complicated by the presences of additional cytosine-modifications. The TET family of enzymes oxidise methylated cytosines to 5-hydroxymethylcytosine (5-hmC), 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC) (Ito *et al.* 2011). At present, routine epigenetic screens fail to efficiently distinguish between the different types of cytosine modifications in DNA. TET-oxidised cytosines may represent intermediate steps of a demethylation process that removes the epigenetic mark from DNA. These cytosine modifications may also play other, as yet unidentified roles in gene expression and DNA metabolism. Whatever their purpose, current findings indicate that there is a cross-talk between DNA methylation/modifications and the second epigenetic memory system, which is based on chromatin structure and histone modifications.

Histone modifications

Chromosomal DNA of eukaryotic cells is always in contact with certain nuclear proteins; this type DNA-protein complex is called chromatin. The core unit of chromatin is the nucleosome (see **Fig. S1**). It is a structure that consists of a 146 base-pair DNA sequence that is wound around an octamer-complex composed of two histone proteins each (H2A, H2B, H3 and H4). Chromatin is a dynamic structure and its configuration ranges from open, transcriptionally active 'euchromatin', to condensed transcriptionally silent 'heterochromatin'. Large portions of the genome are packed and organised into heterochromatin in differentiated cells. Genes poised for expression in a given cell-lineage are thought to emanate from 'euchromatic' chromosomal loops that provide access to transcription factors. Multi-subunit protein complexes are capable of remodelling the chromatin structure by repositioning of nucleosomes, leading to changes in established, lineage-specific gene expression patterns. Chromatin and gene expression are further influenced by posttranslational modifications of histones.

N-terminal tails of histones protrude from the nucleosome-core and extend beyond the associated DNA. This structural property permits communication with surrounding nuclear factors. A host of enzymes has been identified that can either add - or remove - an ever-expanding list of modifications to histone tails (reviewed by Bannister and Kouzarides 2011; Arnaudo and Garcia 2013). Many of these post-translational histone modifications promote or inhibit gene transcription and influence the general chromatin structure. The numerous possible combinations of histone modifications add to the complexity of epigenetic gene regulation. For most of these combined modification patterns the biological function(s) remain to be decoded. Generally, genomic regions with modification-rich histone tails are associated with gene regulation and expression. For example, histone H3 usually has three methyl-groups added to the fourth lysine (H3K4me3) in promoters of transcriptionally active genes. An overview of presently known histone modifications is provided in **Fig. S1**. Unlike DNA methylation, epigenetic inheritance of region-specific histone modifications from mother to daughter cells is only rudimentarily understood. Likewise, we are only beginning to unravel signalling pathways, environmental cues and cellular factors that determine how histone modifications are laid down.

Non-coding RNAs

Small RNAs and long-noncoding RNAs (lncRNAs) are the two broad classes of biological ribonucleic acids known to participate in epigenetic processes such as transcriptional silencing, chromatin remodelling and DNA methylation. For instance, methylation and inactivation of transposable genetic elements can be mediated by piRNAs, a class of small (26-31 nucleotides), non-coding RNAs. These piRNAs bind

specialised protein-complexes and are thought to recruit Dnmts to repetitive elements present within the genome (Carmell *et al.* 2007).

lncRNAs were first identified to play prominent roles in epigenetic phenomena such as X-inactivation and genomic imprinting. With the advent of new sequencing technologies that allow profiling of a cell's entire transcriptome it became apparent that thousands of genomic loci express lncRNAs (Ulitsky and Bartel 2013). Thus, we are only starting to understand the specific roles of these non-coding RNAs in epigenetic regulation. The lncRNA *HOTAIR*, for example, associates with the Polycomb repressive complex 2 (PRC2) and is necessary to promote methylation of histone H3 at lysine 27 in certain chromosomal domains (Rinn *et al.* 2007). Intriguingly, a recent study demonstrated that oestradiol induces transcription of the lncRNA *HOTAIR* (Bhan *et al.* 2013). It is therefore reasonable to speculate that non-coding RNAs are mechanistically linked with environmental programming of the reproductive system.

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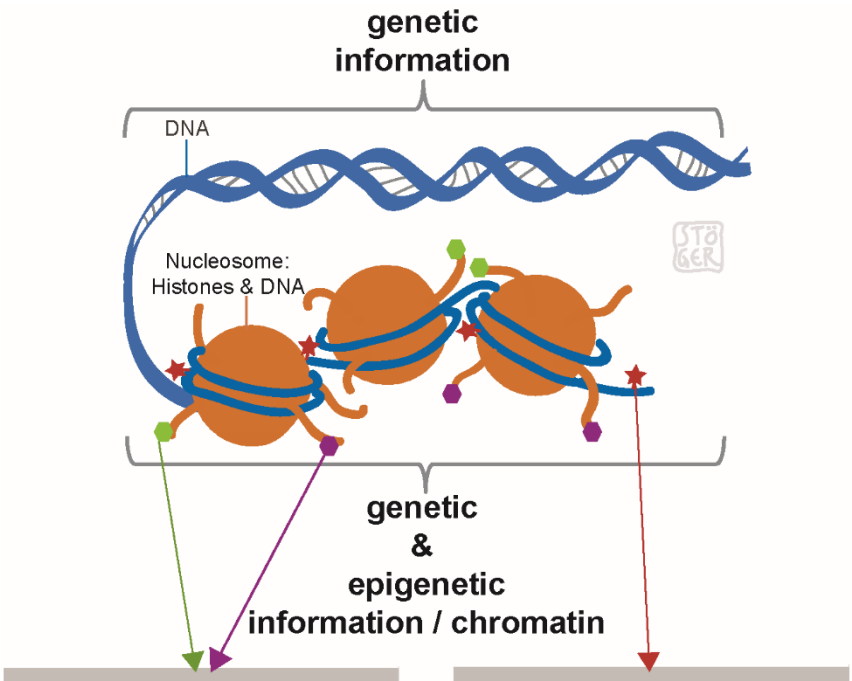

histone modifications	
modification type	**modified amino acid**
Acetylation	Lysine
ADP-ribosylation	Lysine
Biotinylation	Lysine
Butyrylation	Lysine
Crotonylation	Lysine
Formylation	Lysine
Glutathionylation	Cysteine
Hydroxylation	Tyrosine
Malonylation	Lysine
Methylation	Lysine, Arginine
O-glcNAcylation	Serine
Phosphorylation	Serine, Threonine
Propionylation	Lysine
Succinylation	Lysine
Sumoylation	Lysine
Ubiquitylation	Lysine

cytosine modifications	
modification type	**modified base**
Methylation	Cytosine (5-mC)
Hydroxylation	Cytosine (5-hmC)
Formylation	Cytosine (5-fC)
Carboxylation	Cytosine (5caC)

Fig. S1. Epigenetic mechanisms program and regulate gene expression patterns and thereby influence the phenotype without changing the DNA sequence (genetic information) of a cell. Well-defined epigenetic mechanisms include DNA modifications of the cytosine base and post-translational modifications of histone proteins which, together with around 146 base pairs of DNA, form the nucleosome, a core unit of chromatin.

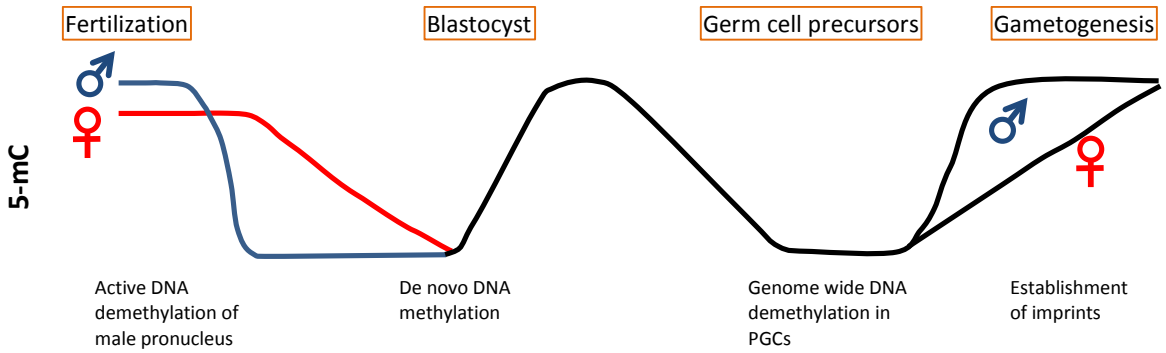

Figure 2.2. DNA methylation in pre-implantation embryos and germ cells. Removal of DNA methylation marks during embryo development prevents the transmission of epimutations between generations. Two major waves of methylation reprogramming take place during development: 1- rapid demethylation of the paternal genome takes place after fertilization. The maternal DNA is demethylated gradually during cleavage divisions. De novo methylation is established in a tissue specific manner during germ layer differentiation; 2- germ cell precursors undergo genome wide demethylation and erasure of imprinted loci during fetal development. During gametogenesis the germ cells acquire new imprinted methylation marks in a parent-of-origin specific manner.

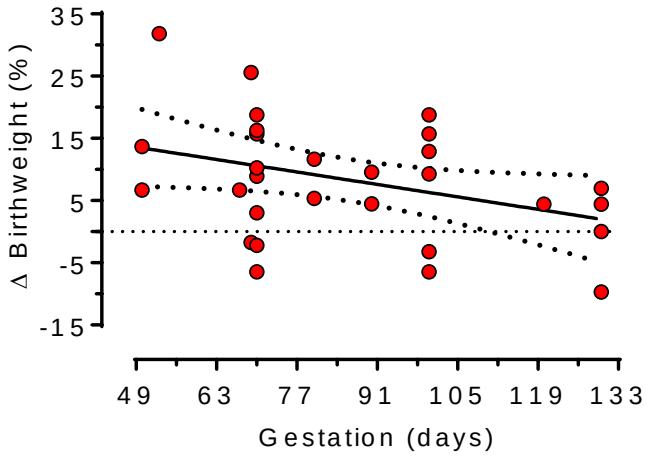

Fig. S3. Birth-weight responses to shearing (increase (%) relative to unshorn ewes) at different stages of gestation, showing 95% confidence intervals. $R^2 = 0.13$ ($P = 0.042$). Mean responses are combined for male and female twin and single lambs from the following studies: (Morris *et al.* 1999; Morris *et al.* 2000; Revell *et al.* 2000; Cam and Kuran 2004; Corner *et al.* 2006; Kenyon *et al.* 2006; Corner *et al.* 2007b, 2007a; Jenkinson *et al.* 2009; Banchero *et al.* 2010; Mousa-Balabel and Salama 2010; Sphor *et al.* 2011).

Table S1. Effect of heat stress in cattle on gestation length and birth weight (data from Tao and Dahl 2013)

Gestation (days)		Fetus / birth weight (kg)		Reference
Heat stress	**Control**	**Heat stress**	**Control**	
281	281	36.6*	39.7	(Collier *et al.* 1982)
		40.6	43.2	(Wolfenson *et al.* 1988)
		33.7*	37.9	(Avendaño-Reyes *et al.* 2006)
274	278	40.8*	43.6	(Adin *et al.* 2009)
		31.0*	44.0	(do Amaral *et al.* 2009)
		39.5*	44.5	(do Amaral *et al.* 2011)
274	277	41.6*	46.5	(Tao *et al.* 2011)
272	276	36.5*	42.5	(Tao *et al.* 2012)

* Statistically significant reduction relative to control calves.

Table S2. Studies investigating progeny stress responses as a consequence of maternal stress or under-nutrition in sheep

Study	Gestation day	Effect on progeny
A. Under-nutrition		
(Bloomfield *et al.* 2003)	105 to 115	Increased ACTH response to CRH/AVP challenge, and increased baseline concentrations of cortisol and ACTH
	105 to 125	No effect on ACTH response to CRH/AVP challenge, or baseline concentrations of cortisol and ACTH.
(Gardner *et al.* 2006)	0 to 30	CRH/AVP challenge produced a lower ACTH and cortisol response in female, but not male, UN lambs.
(Chadio *et al.* 2007)	30 to 100	No effect on ACTH and cortisol response to CRH at 2 months old.
	1 to 30	Increased ACTH and cortisol response to CRH at 2 months old.
(Hernandez *et al.* 2010)	2 to 30	No effect at 4 months of age on cortisol response to isolation.
		Reduced cortisol response to isolation at 18 months of age.
(Long *et al.* 2010b)	28 to 105	No change in response to CRH/AVP or ACTH. Reduced ACTH and cortisol response to environmental stressors.
B. Maternal stress		
(Roussel *et al.* 2004)	110 to term	No effect on cortisol response to isolation.
(Roussel-Huchette *et al.* 2008)	110 to term	No effect on cortisol response to isolation
(Fisher *et al.* 2010)	135 to 138	Reduced febrile and cortisol responses to endotoxin challenge

Table S3. Sheep studies investigating progeny behaviour as a consequence of maternal stress or under-nutrition

Study	Maternal treatment (Stage of gestation)	Effect on progeny
(Erhard and Rhind 2004)	UN 0 to 95	Higher activity during restraint in male, but not female, lambs. Longer approach latency to novel object. Male UN lambs more active in response to startle than controls. In a maze test male lambs from UN ewes showed reduced learning speed. Behavioural laterality of lambs was also altered
(Hernandez *et al.* 2010)	UN 0 to 30	Lambs born to UN ewes made fewer escape attempts during a five minute isolation test at 4 months of age.
(Simitzis *et al.* 2009)	UN 31 to 100	No effect on response to isolation with a novel object at 2, 3, 4 and 5 months of age.
(Hernandez *et al.* 2009)	UN 0 to 30	Behavioural laterality of lambs was altered
(Roussel *et al.* 2004)	Stress 110 to 150	At 8 months of age: increased jumping during isolation; increased activity after exposure to novel object; increased exploration of novel object; higher frequency of changes between light and dark compartment.
(Roussel-Huchette *et al.* 2008)	Stress 110 to 150	At 3 months of age: males spent increased time close to novel object; males reduced jumping in novel arena test. No effect on female lambs.
(Coulon *et al.* 2011)	Stress 110 to term	Reduced activity in human approach test.
(Coulon *et al.* 2015)	Stress 100 to term	Impaired cognition in maze test; negative cognitive bias
(Averós *et al.* 2015)	Stress 62 to term	Increased fearfulness in novel arena; altered sociality

Table S4. Effects of altered fetal nutrition on muscle fibre formation in sheep (from Brameld and Daniel 2008)

ME metabolisable energy; **LD** *longissimus dorsi*; **ST** *semitendinosus*; **VL** *vastus lateralis*; 1º Primary muscle fibres; 2º Secondary muscle fibres; I Slow oxidative (SO) fibres; IIa Fast oxidative glycolytic (FOG) fibres; IIx/IIb Fast glycolytic (FG) fibres.

Time of challenge	Nutritional challenge (% ME requirements)	Fetal/postnatal offspring age (d)	Muscle(s) studied	Fibre effects	Reference
A. In utero					
-18 to 6d	50% vs 150%	75	ST	Decreased total no. 2º fibres No change total no. 1º fibres Decreased 2º:1º fibre ratio No change in diameters	(Quigley *et al.* 2005)
28-78d	50% vs 100%	78	LD	Decreased 2º:1º fibre ratio	(Zhu *et al.* 2004)
B. Post-natal					
30-70d	50% vs 100%	14	LD, VL, ST[a]	Fast fibres: decreased density, increased diameters Slow fibres: increased density Decreased fast: slow ratio	(Fahey *et al.* 2005)
55-95d	50% vs 100%	14	VL	Fast: increased diameter Slow: No effects	(Fahey *et al.* 2005)
55-95d	50% vs 100%	14	LD, ST	Fast: No effects Slow: No effects	(Fahey *et al.* 2005)
85-115d	50% vs 100%	14	LD, VL, ST	Fast: No effects Slow: No effects	(Fahey *et al.* 2005)
30-85d	50% vs 100%	119	ST (No effects in LD, VL)	IIb/IIx: Increased density (no./μm^2) I, IIa: No effects	(Daniel *et al.* 2007)
30-70d	50% vs 100%	168	LD (No effects in ST, VL)	Fast: increased density (no/μm^2), decreased diameter Slow: No effects	(Daniel *et al.* 2007)
-30 to 100d	70% vs 100%	203 vs 185	ST	No change in total no. fibres	(Nordby *et al.* 1987)
28-78d	50% vs 100%	240	LD	Increased total no. fibres ($P<0.1$) Increased % IIb, decreased %IIa No effects on % I and IIx	(Zhu *et al.* 2006)

Table S5. Effects of altered fetal nutrition on muscle fibre formation in pigs (from Brameld and Daniel 2008)

ST *semitendinosus*; **RF** *rectus femoris*; **LD** *longissimus dorsi*; **CSA** Cross-sectional Area ; 1º Primary muscle fibres; 2º Secondary muscle fibres; HE High Energy (42.8 MJ DE/d); SE Standard Energy (30 MJ DE/d); LE Low Energy (18.5 MJ DE/d); %SO percentage of slow oxidative (type I) fibres; %FOG percentage of fast oxidative glycolytic (type IIa/IIx) fibres; %FG percentage of fast glycolytic (type IIb) fibres; [a] Lightest birthweight pigs only (vs Heaviest birthweight).

Time of challenge	Nutritional challenge (% ME requirements)	Fetal/postnatal offspring age (d)	Muscle(s) studied	Fibre effects	Reference
A. In utero					
Gestation and lactation	50 vs 0 ppm L-carnitine	1dpn	ST	↑ muscle CSA, ↑ total no. fibres	Musser *et al.* 2001 (Abstract)
Gestation and lactation	50 vs 0 ppm L-carnitine	21dpn	ST	↑ muscle CSA [a], ↑ total no. fibres	Musser *et al.* 2001 (Abstract)
d25-55	5 vs 2.5 kg diet/d	35 dpn	ST	→ total no. fibres , → total 2º fibre no., ↑ 2º:1º fibre ratio	Dwyer *et al.* 1995
d50-80	5 vs 2.5 kg diet/d	35 dpn	ST	→ total no. fibres , → total 2º fibre no., ↑ 2º:1º fibre ratio	Dwyer *et al.* 1995
d25-80	5 vs 2.5 kg diet/d	35 dpn	ST	→ total no. fibres , → total 2º fibre no., ↑ 2º:1º fibre ratio	Dwyer *et al.* 1995
B. Post-natal					
d25-50	2.2 vs 3.0 kg feed/d	61 dpn	ST	→ 1º fibre no./area, ↑ 2º fibre no./area, ↑ 2º:1º fibre ratio	Gatford *et al.* 2003
d25-70	Ad lib vs restricted control	153 vs 159 dpn (104kg)	ST	→ Total fibre no. , → total no. 2º fibres, → total no. 1º fibres, → 2º:1º fibre ratio, → 1º or 2º fibre diam	Nissen *et al.* 2003
d0-50	HE vs SE vs LE	(104kg)	ST (red)	%SO: LE>SE>HE, %FOG: No effects, %FG: HE>SE>LE	Bee 2004
d0-50	HE vs SE vs LE	(104kg)	ST (white)	%SO: No effects , %FOG: LE=SE>HE [a], %FG: HE>LE [a]	Bee 2004
d0-50	HE vs SE vs LE	(104kg)	RF, LD	%SO: No effects , %FOG: No effects, %FG: No effects	Bee 2004

Table S6. Effects of fetal nutrition on adiposity in sheep

Time of challenge	Nutritional challenge	Effect on fetal growth	Fetal/postnatal offspring age	Effect on adiposity	Reference
A. In utero					
0-130d	Maternal food restriction70%	Fetal weight decreased	130 d	Decreased perirenal fat and total carcass fat	(Luther *et al.* 2007)
Throughout	Overnourished adolescent dam – placental insufficiency IUGR	Fetal weight decreased	130 d	Increased relative perirenal fat weight	(Matsuzaki *et al.* 2006, Redmer *et al.* 2012)
28 -80d	Maternal food restriction 60%	Fetal weight decreased	140 d	Increased perirenal fat mass	(Bispham *et al.* 2003)
B. Post natal					
115 – 124d	Maternal overnutrition 160%	No effect	30 d	Increased subcutaneous fat	(Muhlhausler *et al.* 2006)
Throughout	Placental restriction by carunclectomy IUGR	Low birth weight	45 d	Increased visceral fat	(De Blasio *et al.* 2007)
Throughout	Overnourished adolescent dam - placental insufficiency IUGR vs control-fed dams	Low birth weight	77 d	Increased total body fat (DXA)	(Wallace *et al.* 2011b)
Throughout	Overnourished adolescent dam - placental insufficiency IUGR	Low vs normal birth weight	77 d	No effect, but female>male	(Wallace *et al.* 2013)
Embryo donor	Overnutrition 170-190% for 5 months	No effect	120 d	Females fatter, males no effect	(Rattanatray *et al.* 2010)
Embryo donor	Overnutrition for 4 months then food restriction 70% for 1 month	No effect	120 d	No effect	(Rattanatray *et al.* 2010)
28 – 78d	Maternal food restriction 50%	No effect	120 d	Increased backfat (males)	(Ford *et al.* 2007)
30 – 70d	Maternal food restriction: 50%	No effect	120 d	No effect subcutaneous backfat depth, omental fat mass and perirenal fat mass; but females>males	(Daniel *et al.* 2007)
-	-	Range	150 d	Positive correlation between total body fat (DXA) vs birth weight	(Muhlhausler *et al.* 2008)

Table S6. (continued)

Time of challenge (gestation)	Nutritional challenge	Effect on fetal growth	Fetal/postnatal offspring age	Effect on adiposity	Reference
30 – 70d	Maternal food restriction: 50%	No effect	180 d	Increased intramuscular fat (LD and ST muscles), particularly in Males. No effect subcutaneous backfat depth, omental fat mass and perirenal fat mass; but females>males.	(Daniel *et al.* 2007)
Throughout	Overnourished adolescent dam - placental insufficiency IUGR vs control-fed dams	Low birth weight	240 d	No effect, but female>male (DXA)	(Wallace *et al.* 2011b)
28 – 78d	Maternal food restriction 50%	No effect	270 d	Increased kidney-pelvic fat (males)	(Ford *et al.* 2007)
105d - term	Maternal food restriction 50%	Low birth weight	6 months	Increased abdominal:subcutaneous fat ratio	(Khanal *et al.* 2015)
105d - term	Maternal overnutrition 150%	No effect	6 months	Increased abdominal:subcutaneous fat ratio	(Khanal *et al.* 2015)
0 – 30d	Maternal food restriction 50%	No effect	12 months	No effect	(Gardner *et al.* 2005)
110d - term	Maternal food restriction 50%	No effect	12 months	Increased perirenal and omental fat mass	(Gardner *et al.* 2005)
Throughout	Maternal overnutrition 150% - obese dam	No effect	19 months	No effect (DXA)	(Long *et al*. 2010a)
Throughout	Maternal overnutrition 150% - obese dam	No effect	22 months	Increased total body fat (DXA)	(Long *et al.* 2010a)
-	Twins vs singles	Low birth weight	2 years	Increased total body fat (DXA)	(Hancock *et al.* 2012)
Throughout	Twinning and placental embolization	Low birth weight	2.3 years	Increased abdominal fat mass	(Louey *et al.* 2005)
Periconception, -61– 0d, -61 – 30d or 2 – 30d	Maternal food restriction: ~50%	No effect	3 – 4 years	Increased total body fat (DXA) and increased perirenal fat mass – in males; no effect in females.	(Jaquiery *et al.* 2012)
28-78d	Maternal food restriction: 50%	No effect	6 years	No effect (DXA and perirenal and omental fat mass), but plasma leptin increased	(George *et al.* 2012)

Table S7. Effects of altered fetal nutrition on appetite regulation in sheep

CART, cocaine- and amphetamine-regulated transcript; **POMC,** pro-opiomelanocortin; **NPY,** neuropeptide Y; **AGRP,** agouti-related peptid

Time of challenge	Nutritional challenge	Fetal/offspring age	Hypothalamic neuropeptide changes	Appetite/Food intake	Reference
A. In utero					
Throughout	Maternal overnutrition: 150%	75 d	No effect CART, POMC, NPY, AGRP	-	(Breton *et al.* 2011)
Throughout	Overnourished adolescent dam - placental insufficiency IUGR	130 d	Decreased CART No effect POMC, NPY, AGRP	-	(Adam *et al.* 2011a)
0 – 130d	Maternal food restriction: 70%	130d	Increased NPY, AGRP No effect POMC, CART		(Adam *et al.* 2015)
130 – 140d	Fetal glucose infusion	140 d	Increased POMC No effect CART, NPY, AGRP	-	(Muhlhausler *et al.* 2005)
115-145d	Maternal food restriction: 50%	145 d	Increased NPY	-	(Warnes *et al.* 1998)
B. Post natal					
30 – 80d	Maternal food restriction: 50%	7 d	Decreased NPY No effect POMC, AGRP	-	(Sebert *et al.* 2009)
Throughout	Low birth weight, increase fetal no.	7 d	-	No effect	(Villette and Theriez 1983)
Throughout	Placental restriction by carunclectomy IUGR	15 d	-	Increased	(De Blasio *et al.* 2007)
Throughout	Overnourished adolescent dam - placental insufficiency IUGR	21 d	-	No effect	(Adam *et al.* 2013)
115 – 124d	Maternal overnutrition 160%	30 d	Increased POMC	Increased 1-3wks No effect at 4wks	(Muhlhausler *et al.* 2006)
Throughout	Low birth weight, increase fetal No.	35 d	-	No effect	(Vilette and Theriez 1981)
Throughout	Low birth weight - Overnourished adolescent dam - placental insufficiency IUGR	77 d	No effect CART, POMC, NPY, AGRP	-	(Adam *et al.* 2013)
30 – 70d	Maternal food restriction: 50%	17 wks	-	No effect	(Daniel *et al.* 2007)
30 – 70d	Maternal food restriction: 50%	24 wks	-	No effect	(Daniel *et al.* 2007)
30 – 80d	Maternal food restriction: 50%	12 months	No effect POMC, NPY, AGRP	No effect	(Sebert *et al.* 2009)
-60d - term	Maternal obesity: 150%	19 months	-	Increased	(Long *et al.* 2010)

105d - term	Maternal food restriction: 75%	2 years	-	No effect	(Sibbald and Davidson 1998)
28-78d	Maternal food restriction: 50%	6 years	-	Increased	(George *et al.* 2012)

Table S8. Impact of early life nutrition on fetal gonadal development, hypothalamic-pituitary-gonadal function and adult fertility in sheep

Nutritional exposure	Period of exposure	Litter size	Effect on fetal / birth wt.	Life-stage 1^0 endpoints measured	Main effect(s) reported[c]	Study size and gender	Reference
Maternal UN[a]	0 to 62d GA[b]	Singletons, twins	None	Fetal d62	Delayed ovarian follicular development	11 females	(Borwick *et al.* 1997)
Maternal UN[a]	0 to 30, 31 to 50, 31 to 65, 65 to 110d GA[b]	Singletons, twins	None	Fetal d50, 65 or 110	Delayed ovarian follicular development & stage specific effects on markers of apoptosis	130 females	(Rae *et al.* 2001, Lea *et al.* 2006)
Maternal UN[a]	0 to 30, 31 to 50, 31 to 65, 65 to 110d GA[b]	Singletons, twins	None d50 or 65, 15%↓at d110	Fetal d50, 65 or 110	No effect on testes mass, transient (d50) effect on steroidogenic capacity. No effect on Sertoli cell number or markers of apoptosis (d110)	113 males	(Rae *et al.* 2002b, Andrade *et al.* 2013)
Maternal UN[a]	28 to 78d GA[b]	Singletons, twins	None	Fetal d78	Increased oxidative DNA damage in oogonia	12 females	(Murdoch *et al.* 2003)
Maternal ON[d] /reduced fetal nutrient supply	4 to 103d GA[b]	Singletons[e]	None	Fetal d103	Reduced primordial & total follicle number; No effect on seminiferous cord or Sertoli cell no.	11 females 17 males	(Da Silva *et al.* 2002)
Maternal ON[d] /reduced fetal nutrient supply	4 to 131d GA[b]	Singletons[e]	31% ↓	Fetal d131	Reduced primordial & total follicle number. Higher pituitary LHβ mRNA	19 females	(Da Silva *et al.* 2003)
Maternal UN[a]± high selenium	50 to 135d GA[b]	Singletons	None	Fetal d135	Variable effects of UN and selenium on proliferation in ovarian follicles and blood vessels	32 females	(Grazul-Bilska *et al.* 2009)
Maternal UN[a]	70d GA[b] to term	Singletons	12% ↓	Neonatal d2	Reduced Sertoli cell number	25 males	(Bielli *et al.* 2002)

Maternal UN/ON[f]	-82 to 70, 71 to 100, 100 to 126d GA[b]	Singletons, twins	None	Pre-pubertal (2 months)	Blastocyst production in vitro highest in females exposed to ON mid-late pregnancy	36 females	(Kelly *et al.* 2005)
Maternal ON[d] /reduced fetal nutrient supply	4d GA[b] to term	Singletons[e]	31% ↓ female 47% ↓ male	Pre-adult (10 months)	No effect on age at puberty, normality or number of ovarian cycles; Delayed onset of puberty, lower testosterone, reduced testes volume	28 females 14 males	(Da Silva *et al.* 2001)
Maternal UN[a]	0 to 30 (UN1), 31 to 100d GA[b] (UN2)	Twins (artificially reared)	None	Pre-adult (10 months)	No effect on onset of puberty. Higher FSH post GnRH challenge & lower Sertoli cell number- UN2	19 males	(Kotsampasi *et al.* 2009a)
Maternal UN[a]	0 to 30 (UN1), 31 to 100d GA[b] (UN2)	Twins (artificially reared)	None	Pre-adult (10 months)	No effect on onset of puberty or LH surge parameters. Higher FSH post GnRH challenge- UN1	17 females	(Kotsampasi *et al.* 2009b)
Maternal UN[a]	0 to 95d GA[b]	Singletons, multiples	None	Adult (20 months)	Reduction in ovulation rate No effect on testes size or semen quality	49 females 32 males	(Rae *et al.* 2002a)
Maternal UN or ON	1 to 39d, 40-90d GA[b]	Singletons, multiples	Not reported	Adult (mated at 8 months)	No effect on conception rate, litter size or number of lambs weaned	60 females	(Munoz *et al.* 2009)
Maternal UN[a]	100d GA[b] to 14 weeks postnatal age	Twins	18% ↓	Pre-pubertal (7months), Adult (18months)	No effect on hypothalamic -pituitary function at either stage	2 cohorts of 28 females each	(Borwick *et al.* 2003)
Maternal UN[a]	28 to 78d GA[b]	Singletons	Not reported	Adult (12 & 24 months)	Lower progesterone in one cycle - both years. Reduced pregnancy rate in year 2	14 females	(Long *et al.* 2010b)
Maternal UN[a]	0 to 35d GA[b]	Singletons	None	Adult (18 & 30 months)	No effect on natural ovulation rate (7 measures) or after PMSG[g]	~170 females	(Parr *et al.* 1986)

Maternal supplementation	50d GA[b] to term, term to 100 days postnatal	Singletons, multiples	Pregnancy supplemented ↑14%	Adult (3 pregnancies)	No effect on ovulation rate. Higher lifetime incidence of multiple births in supplemented groups (lactation > pregnancy)	450 females	(Gunn *et al.* 1995)
Variable fetal nutrient supply[h]	Pregnancy	Singletons	Not applicable – as per study design	Adult (median of 3 pregnancies)	Reduced average number of lambs per litter in females born at both birth weight extremes	2427 females	(Gardner *et al.* 2009)
High stocking density/ low available nutrition[i]	0d GA[b] to 3months, 3 to 15months, >15months postnatal age	Singletons, twins	None	Adult (up to 9 years & 8 pregnancies)	Fewer lambs born if stocking density high from conception to weaning but only if also high in adult life	283 females	(Langlands *et al.* 1984)
Undernutrition	2 to 15 weeks postnatal age	Singletons	Not reported	Adult (up to 7 years, 4-6 pregnancies)	Lower lifetime incidence of multiple births	499 females	(Rhind *et al*. 1998)

[a]UN, undernutrition (typically 0.5-0.7 x maintenance in adult ewes); [b]GA, gestational age; [c]where effects reported, minimum $P<0.05$ relative to optimally nourished reference control group; [d]ON, overnutrition (typically 2 x maintenance in adolescent ewes); [e]singleton pregnancies derived by embryo transfer using a single sire; [f]UN/ON, undernutrition 0.7x maintenance and overnutrition 1.5 x maintenance in adult ewes, 2x2x2 factorial design; [g]PMSG, pregnant mares serum gonadotrophin; [h]birth weight as a proxy for variable fetal nutrient supply, lambs categorised as relatively small or large at birth if 2 standard deviations below or above the mean birth weight, respectively; [i]High versus low stocking density during three periods, 2x2x2 factorial design.

Table S9. Summary of impact of early life nutrition on reproductive function in cattle

UN, undernutrition; GA, gestational age; d, day; AFC, antral follicle count; AMH, anti-mullerian hormone; FSH, follicle stimulating hormone; [a]birth weight as a proxy for variable fetal nutrient supply, neonatal follicle parameters determined after calves died as a consequence of dystocia at <31d of age, adult follicle data obtained by ultrasound prior to breeding. [b]birth weight as a proxy for variable fetal nutrient supply, 3 equal sized groups based on lowest, average and highest birth weight.

Nutritional exposure	Period of exposure	Type	Effect on fetal / birth wt.	Life-stage 1[o] endpoints measured	Main effect(s) reported[c]	Study size and gender	Reference
Variable fetal nutrient supply[a]	Pregnancy	Beef cattle	Not applicable – as per study design	Neonatal Adult (12-14 months)	Birth weight positively associated with AFC (neonatal and adult life). Decreased pregnancy rate when AFC is low.	181 females 406 females	(Cushman *et al.* 2009)
Maternal UN (0.6 x maintenance)	-11 to 110d GA	Beef cattle	None	Pre-pubertal (7, 18, 35 weeks) and adult (56, 86 weeks)	No effect on age at puberty. Diminished ovarian reserve; lower AFC at 7, 18, 56, 86 weeks, lower AMH and higher FSH	23 females	(Mossa *et al.* 2013)
Maternal low/ high protein (2 x 2 factorial)	0 to 93, 93-180d GA	Beef cattle	8%↓ by 2nd trimester for low protein	Pre-pubertal and adult (5, 23 months)	Reduced primordial, primary and AFC after low-high protein in first two thirds of gestation	36 females	(Sullivan *et al.* 2009)
Maternal protein supplementation / improved pasture (2 x 2 factorial)	Late gestation, early lactation	Beef cattle	None	Adult (up to start of second breeding season)	No effect on age at puberty. Earlier first calving and higher pregnancy rates following supplementation (protein) in late gestation	170 females	(Martin *et al.* 2007)
Slow or rapid growth by varying maternal nutrition	30-90dGA to term, birth to weaning	Beef cattle	24% ↓	Adult (30 months)	Reduced ovarian weight and large follicle diameter after prenatal growth	162 females	(Wilkins *et al.* 2006)

					restriction. No effect of postnatal growth		
High or low weight gain	Weaning to 15months	Beef cattle	Measured but not reported	Adult (15months)	Weight gain category did not impact AFC or overall pregnancy rate	212-300 females	(Eborn *et al.* 2013)
Variable fetal nutrient supply[b]	Pregnancy	Dairy cattle	24% ↓ low<high	Adult (spanning two service periods)	Low birth weight did not impact fertility in first service period, protective against abnormal ovarian cycles in second.	65 females	(Swali and Wathes 2006)
Variable postnatal growth (on- farm data)	30 to 180, 181-450d postnatal age	Dairy cattle	Not reported	Adult (first calving)	Suboptimal growth increased age to first breeding and age at calving	392 females	(Brickell *et al.* 2009)
Maternal low/ high protein (2 x 2 factorial)	0 to 93, 93-180d GA	Beef cattle	8%↓ by 2nd trimester for low protein	Pre-pubertal (5 months)	Baseline (but not GnRH stimulated) FSH higher after low dietary protein in first two thirds gestation.	33 males	(Sullivan *et al.* 2010)

Table S10. Summary of impact of specific endocrine disrupting chemicals on different aspects of reproductive axis function in sheep

Chemical exposure	Period of exposure	Stage 1° endpoints measured	Main effect(s) reported	No. of exposed & control animals (gender)	Reference
Bisphenol A	30 to 90d GA	21 months postnatal	Hypothalamic GnRH & ESR2 mRNA ↓, ESR1 ↑	12 (females)	(Mahoney and Padmanabhan 2010)
Bisphenol A	4 to 11 weeks postnatal age	11 weeks postnatal	Pulsatile LH secretion ↓	12 (females)	(Evans *et al.* 2004)
Bisphenol A	2 to 4 months postnatal age	4 months postnatal	Basal LH & LH pulse frequency ↓	18 (females)	(Collet *et al.*, 2010)
Bisphenol A	30 to 90d GA	6 to 40 weeks postnatal	Duration of first breeding season ↑, LH surge ↓at induced cycle	26 (females)	(Savabieasfahani *et al.* 2006)
Bisphenol A	30 to 90d GA	Fetal,65 and 90d GA	Ovarian steroidogenic genes x 2 ↑ (d65), microRNA ↓(45 at d65, 11 at d90)	19 (females)	(Veiga-Lopez *et al.* 2013)
Bisphenol A	4 to 11 weeks postnatal age	11 weeks postnatal	Uterine weight ↑, altered uterine ESR1 & 2 distribution	12 (females)	(Morrison *et al.* 2003)
Bisphenol A	1 to 4d postnatal	30 days postnatal	Ovarian weight ↑, primordial follicles ↓, antral follicles ↑ number of atretic antral follicles ↑	22 (females)	(Rivera *et al.* 2011, 2015)
Sewage Sludge[a]	0 to 110d GA	Fetal, 110d GA	Hypothalamic GnRH, GnRHR & GALRs mRNA ↓, pituitary GALRs ↓	18 (10 female, 8 male)	(Bellingham *et al.* 2010)
Sewage Sludge[a]	0 to 110d GA	Fetal, 110d GA	Hypothalamic & pituitary kisspeptin mRNA ↓, pituitary kisspeptin, LHβ & ERα↓	39 (not reported)	(Bellingham *et al.* 2009)
Sewage Sludge[a]	0 to 110d GA	Fetal, 110d GA	Perturbed ovarian development	23 (females)	(Fowler *et al.* 2008)
Sewage Sludge[a]	0 to 110d GA	Fetal, 110d GA	Testis weight, gonocytes, Sertoli & Leydig cell no. ↓, plasma inhibin & testosterone ↓	19 (males)	(Paul *et al.* 2005)
Sewage Sludge[a]	Conception to 7 months postnatal	19 months postnatal	Spermatogenic abnormalities in 42% of exposed males	24 (males)	(Bellingham *et al.* 2010)
Methoxychlor	30 to 90d GA	21 months postnatal	Hypothalamic GnRH & ESR2 mRNA ↓	11 (females)	(Mahoney and Padmanabhan 2010)

Methoxychlor	30 to 90d GA	40 weeks postnatal	LH surge delayed at induced cycle	26 (females)	(Savabieasfahani *et al.* 2006)
Octylphenol	70d GA to birth	At birth	Pituitary FSH mRNA & protein, testis weight, Sertoli cell no. ↓	Not reported	(Sweeney *et al.* 2000)
Octylphenol	70d GA to birth or weaning, birth to weaning	1 year postnatal	Morphologically abnormal sperm ↑ (birth to weaning group only)	22 (males)	(Sweeney *et al.* 2007)
Octylphenol	70d GA to birth or weaning, birth to weaning	Up to 10 months (end of first season)	All OP groups, onset of puberty ↑, duration of first breeding season ↑	19 (females)	(Wright *et al.* 2002)
Polychorinated biphenyl (2 types)	Conception to birth	60 days postnatal	GnRH induced LH ↑, advanced follicle dynamics	26 (females)	(Kraugerud *et al.* 2012)

[a]Dams maintained on plots fertilised with sewage sludge (SS) throughout their breeding lives (typically at least 3 years) prior to mating. SS, by product of waste water treatment from domestic, industrial and agricultural sources (Stevens *et al*. 2003). Bisphenol A, used in the production of polycarbonated plastic and epoxy resins (vom Saal and Hughes 2005); Methoxychlor, a pesticide (ATSDR 2002); Octylphenol, non-ionic surfactant used in the production of detergents (White *et al.* 1994); Polychlorinated biphenyls, industrial pollutants now banned but abundant in environment (Lindenau and Fischer 1996)