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1 **Title: Prenatal adversity modulates the quality of maternal care via the exposed offspring.**

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11 Keywords: fetal programming; maternal behaviour; placental signalling; prenatal adversity;
12 ultrasonic vocalisation

13

14 Abbreviations: HFD, high fat diet; LPD, low protein diet; USV, ultrasonic vocalisation

15

16 **Abstract**

17 Adversities in pregnancy, including poor diet and stress, are associated with increased risk of
18 developing both metabolic and mental health disorders later in life, a phenomenon described as
19 fetal programming or developmental origins of disease. Predominant hypotheses proposed to
20 explain this relationship suggest that the adversity imposes direct changes to the developing fetus
21 which are maintained after birth resulting in an increased susceptibility to ill health. However,
22 during pregnancy the mother, the developing fetus and the placenta are all exposed to the adversity.
23 The same adversities linked to altered offspring outcome can also result in suboptimal maternal
24 care which is considered an independent adverse exposure for the offspring. Recent key
25 experiments in mice reveal the potential of prenatal adversity to drive alterations in maternal care

26 through abnormal maternal-pup interactions and via alterations in placental signalling. Together,
27 these data highlight the critical importance of viewing fetal programming holistically paying
28 attention to the intimate, bidirectional and reiterative relationship between mothers and their
29 offspring.

30

31 **1 Introduction**

32 One of the most common adversities to blight pregnancy is overnutrition, which is estimated to
33 impact one third of all pregnancies in developed countries. Further, there is an increasing burden
34 to developing countries^[1]. Obesity in pregnancy is specifically associated with higher risk of
35 pregnancy complications and poorer outcomes for children. These include the increased risk of
36 neurologic disorders including attention deficit/hyperactivity disorder, autism and schizophrenia as
37 well as metabolic syndrome – findings that have, at least in principle, been reproduced in a number
38 of animal models ^[2]. The reported association between obesity and other prenatal adversities with
39 later life illnesses has led to suggestions that the exposure induces direct changes to the fetus which
40 persist into adulthood increasing susceptibility to disease – a relationship which is often referred to
41 as fetal programming ^[3] or developmental origins of disease ^[4]. However, in humans exposures
42 rarely occur in isolation nor are they limited to pregnancy, and there are different patterns and long-
43 term consequences of fetal adversities depending on their timing, nature and magnitude.
44 Considerable progress in our understanding of the mechanisms underpinning the fetal
45 programming phenomenon has been made using animal models but until recently little attention
46 has been paid to the impact of prenatal adversities on the mother's health and behaviour, and how
47 the combination of prenatal adversity and suboptimal maternal care could contribute to offspring
48 outcomes ^[5]. This is important because variations in maternal care in rodents, independent of
49 prenatal exposures, have been linked to altered offspring behaviour and persistent changes in the
50 offspring brain ^[6]. High fat diet (HFD) ^[7-9], low protein diet (LPD) ^[10], chronic, psychological stress ^[11],

51 physical restraint ^[12], chronic corticosterone administration ^[13] and vitamin D deficiency ^[14] in
52 pregnancy are just some of the stressors that have been reported to induce changes in maternal
53 behaviour in animal models. There is very little data on the consequences specifically of high fat diet
54 in a human pregnancy on maternal care but maternal obesity is a well known risk factor for maternal
55 depression and anxiety ^[15] and there are studies that link maternal obesity to lower quality maternal
56 attachment ^[16] and maternal parenting stress ^[17]. Consequently, adversities in the prenatal period
57 may contribute to altered outcomes either directly by impacting the fetus or indirectly by altering
58 maternal care giving, or potentially by both routes. This imposes considerable complexities in the
59 interpretation of studies characterising the causes and consequences of early life adversity. Recent
60 studies have begun to address gaps in our knowledge and, through careful experimental design,
61 demonstrate that both prenatal and postnatal communication between offspring and mother has
62 the potential to influence postpartum maternal care potentially contributing to longer term
63 outcomes.

64

65 **2 High fat diet influences maternal behaviour through changes to offspring**

66 In a recent study published in *Proceeding of the Royal Society B*, Baptissart and colleagues employed
67 a high fat diet (HFD) intervention with cross fostering to dissect apart the contribution of the
68 stressor of obesity and HFD in pregnancy to alterations in maternal behaviour ^[18]. HFD has
69 previously been reported to result in alterations in maternal care but in all but one of these studies,
70 dams continued on the dietary alteration while their behaviour was being assessed (**Table 1**). In this
71 study, female C57BL/6 mice were fed either a control diet (10% calories from fat) or a HFD (45%
72 calories from fat) from 3 weeks of age to 9 weeks of age. Prior to mating, dams fed on the HFD
73 gained more weight and were less glucose tolerant than dams fed the control diet. After mating to
74 males maintained on a control diet, pregnant dams remained on their respective diets throughout
75 pregnancy and while mothering their pups. At birth, four experimental groups were generated: 1)

76 dams fed a control diet caring for control diet-exposed offspring (CT:ct); 2) dams fed a HFD caring
77 for HFD-exposed offspring (HF:hf); 3) dams fed the control diet caring for HFD-exposed offspring
78 (CT:hf); and 4) dams fed a HFD caring for control diet-exposed offspring (HF:ct) (**Figure 1a**). In all
79 cases, pups were either fostered within groups or across groups to control for the disruption of this
80 event, and pup sex was balanced. HF:hf dams spent less time interacting with their pups and nesting,
81 and more time on non-interactive behaviours (exploration, wall-rearing) than CT:cf dams,
82 essentially as previously reported [7, 8]. However, dams nursing mis-matched pups (CT:hf and HF:ct)
83 did not clearly align with either matched pairing. This demonstrated that the HFD is not purely acting
84 as a stressor on the dam altering her behaviour. Instead, both the prenatal and postnatal
85 environment contribute to the altered maternal behaviour. Further analysis in a generalised linear
86 model identified *in utero* exposure of the fetus as the strongest predictor of the postnatal maternal
87 behaviour i.e. pups exposed *in utero* to the HFD appeared to be influencing the behaviour of dams
88 not exposed to the diet. This remarkable study demonstrates that an adversity experienced by the
89 fetus *in utero* has the potential to alter the mother's behaviour postpartum.

90

91 **3 Offspring communication regulated by imprinting influence maternal behaviour**

92 The newborn is known to elicit maternal care through many different interactions, any one of which
93 could be impacted during fetal development. Newborns influence maternal care-giving behaviour
94 through suckling [19], through calls in the form of ultrasonic vocalisations (USVs) [20] and, potentially,
95 through body temperature changes, as recently reviewed [21]. Although maternal HFD has not been
96 reported to impact suckling behaviour [22], HFD-exposed offspring can exhibit alterations in USVs [23].
97 Pup USVs normally increase in intensity and frequency during separations from the mothers, hence
98 the term “whistles of loneliness” [24]. These communications from the pups are known to stimulate
99 a number of maternal behaviours including nest building, pup retrieval and nursing [20]. Seven day
100 old pups exposed gestationally to a HFD (60% calories from fat) reportedly vocalise less than non-

101 exposed controls (13.5% calories from fat) when isolated from their mothers ^[23]. Therefore, HFD in
102 pregnancy could alter maternal behaviour by impacting the offspring's ability to communicate
103 postnatally. While Baptissart and colleagues did not measure USVs in their study and findings from
104 different HFD studies vary (**Table 1**), nonetheless the observation that prenatally exposed pups can
105 influence a foster mother's behaviour postnatally means that studies in animal models linking
106 prenatal adversity to later life health must be carefully interpreted. Adversities in pregnancy may
107 disrupt maternal care indirectly by changing the way in which the offspring communicate with their
108 mothers after they are born (**Figure 2**).

109
110 We recently reported reduced USVs in pups with loss-of-function of *Paternally expressed gene 3*
111 (*Peg3*) ^[25]. *Peg3* null pups born to wild dams make significantly less USVs when separated from their
112 mothers than wild type pups (**Figure 1b**). Consistent with the importance of USVs in pup retrieval
113 ^[26], wild type dams who carried and cared for these low vocalising pups were significantly slower to
114 sniff and then to retrieve their pups. We observed no changes in a nest building behaviour nor in
115 the dams' direct interactions with their pup during the nest building task. There was, however, a
116 marked difference in maternal anxiety between the dams carrying and caring for wild type pups and
117 those that carried and cared for *Peg3* null pups with dams exposed to the *Peg3* null pups displaying
118 higher levels of anxiety in the elevated zero maze test. Loss of *Peg3* expression has a significant
119 negative impact on placental development and fetal growth ^[27, 28]. Importantly, *Peg3* mutant mice
120 display both metabolic ^[29] and behavioural disorders as adults ^{[27] [30]}. The reason this study is
121 relevant to research into fetal programming is because *Peg3* belongs to the remarkable family of
122 imprinted genes that are expressed exclusively or predominantly from one parental allele as a
123 consequence of epigenetic events initiated in the parental germline and consolidated after
124 fertilisation ^[31]. Changes in epigenetic gene regulation induced by the prenatal adversity have been
125 suggested as a mechanism underpinning the fetal programming phenomenon, recently reviewed

126 [32]. Epigenetic marks, which are by definition inherited through the cell cycle, play a key role in
127 maintaining a cellular memory of gene transcription patterns. Therefore, environmental exposures
128 that alter epigenetic marks can, in theory, be “remembered” by the organism even after the
129 exposure stops.

130

131 **4 Prenatal adversities alter the expression of imprinted genes**

132 A number of interventions in pregnancy have been linked to the altered expression of imprinted
133 genes in the offspring (**Table 2**). As an example, we recently showed that a low protein diet
134 restricted to pregnancy results in loss of paternal silencing of the imprinted gene *Cdkn1c* in the
135 offspring maintained into adulthood [33]. This formally demonstrates that adversity in pregnancy can
136 influence the epigenetic processes that maintain allelic gene expression in the developing fetus.
137 High fat diet, in combination with prenatal obesity or just during pregnancy, has not been shown to
138 impact expression of *Peg3*. Further work is therefore required to demonstrate *Peg3* responds
139 epigenetically to prenatal adversity. Moreover, loss of expression is a considerable insult to
140 development and it will need to be shown that more modest changes in gene expression have a
141 phenotypic consequence that could impact another individual’s behaviour.

142

143 **5 Placental imprinting modulates maternal behaviour**

144 Interpreting studies on the interaction between prenatal adversities and later life outcomes is
145 further complicated by the potential of placental endocrine dysfunction to alter outcomes for
146 mother and offspring. The placenta is a fetally-derived organ predominantly recognised for its role
147 as a sophisticated transportation system bringing nutrients to the fetus and removing waste. Less
148 well recognised is the function of the placenta as the signalling coordinator of pregnancy. The
149 placenta manufactures vast quantities of hormones that act on the mother to establish and
150 maintain the adaptations necessary for pregnancy [34] and promote fetal brain development [35].

151 Hormones produced by the placenta include placental lactogen-like hormones (Prls) some of which
152 are known to bind and activate the prolactin receptor ^[36]. This receptor is required for the
153 appropriate induction of maternal care in mice ^[37] with a key site of action being the medial preoptic
154 area of the hypothalamus ^[38]. Infusion of placental lactogen directly into this area of the brain
155 induces maternal care in the non-pregnant rodent ^[39]. These indirect infusion experiments highlight
156 the potential function of the placenta in the programming of maternal care. We recently tested this
157 theory in a novel mouse model in which we were able to manipulate the size of the placental
158 endocrine compartment by genetically altering the expression of the imprinted gene *Phlda2*. *Phlda2*
159 negatively regulates the major endocrine lineage of the mouse placenta ^[40]. We exposed wild type
160 female mice to fetuses with different doses of *Phlda2*, and thus to different doses of placental
161 hormones. As the dose of placental hormones increased, we observed increased maternal nurturing
162 and pups grooming ^[41]. This experiment formally demonstrates that imprinted genes expressed in
163 the placenta, and regulated by epigenetic marks, can influence the behaviour of mothers. This opens
164 the possibility that prenatal adversities in pregnancy could influence maternal behaviour via
165 alterations in the placenta mediated by imprinted genes (**Figure 2**).

166

167 **6 Potential for prenatal adversity to alter placenta signalling**

168 A number of studies report changes in placental hormones and/or placental endocrine lineages
169 after exposures of pregnant females to a variety of stressors (**Table 3**). One study examining
170 overnutrition in pregnancy specifically assayed the expression of placental hormones and reported
171 a significant decrease in the expression of two hormones ^[42]. In another study, changes in fat
172 content of the maternal diet altered the expression of a number of hormones in the placenta in a
173 sexually dimorphic manner ^[43]. Evidence that maternal stressors impact the expression of imprinted
174 genes that regulate development of placental endocrine lineages is less well established. A focused
175 study on the consequences of an obesogenic diet on the placental expression of imprinted genes

176 reported increased expression of several imprinted genes including *Igf2* and a non-significant
177 increase in expression of *Phlda2* ^[44]. In rats, LPD resulted in decreased expression of placental *Ascl2*
178 ^[45]. As well as diet, the infection status of the dams appears to be important for placental imprinted
179 gene expression. Challenging pregnant dams with *Campylobacter rectus*, a periodontal pathogen
180 associated with adverse pregnancy outcomes, resulted in decreased placental expression of several
181 imprinted genes including *Ascl2* and *Igf2* ^[46]. Together, these data support an interaction between
182 maternal stressors and alterations in the expression of imprinted genes. However, few studies have
183 examined allelic expression changes in the placenta and it is not clear whether these changes in
184 expression occur as a result of changes in imprinting, changes in the expression of the normally
185 active allele or changes in cellular composition, which must be addressed.

186

187 **7 Conclusions and Outlook**

188 In conclusion, there is considerable experimental evidence that the environment mothers
189 experience in pregnancy can alter her behaviour towards her offspring. There is emerging evidence
190 that adverse exposures may act not directly on the mother but indirectly via her developing fetus
191 and associated placenta. Together, these data highlight the critical importance of viewing fetal
192 programming holistically paying attention to the intimate, bidirectional and reiterative relationship
193 between mothers and offspring (**Figure 2**).

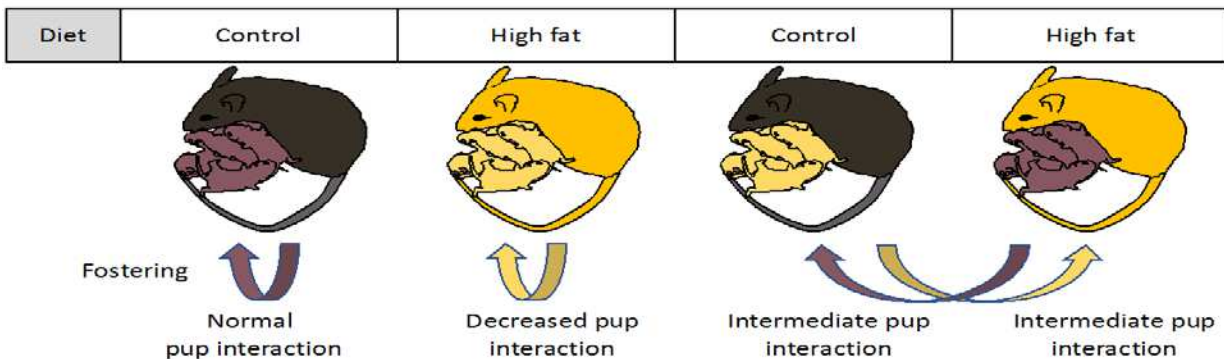
194 **Figure legends**

195 **Figure 1. Neonatal and placental influences on maternal behaviour**

196 Dietary influence on maternal behaviour via the exposed neonate. Obese wild type dams exposed
 197 to high fat diet (HFD) in pregnancy give birth to pups that can influence a normal weight, non-HFD
 198 exposed dam's behaviour. Arrows indicate fostering of pups to generate matched and mis-matched
 199 groups.

200 Programming of maternal care by placental imprinting. Wild type dams exposed to fetuses with
 201 different gene doses of the maternally expressed *Phlda2* gene (doses given in top row of table) and
 202 consequently different doses of placental hormones (doses given in bottom row of table) show
 203 alterations in pup focused behaviours consistent with the role of placental hormones in inducing
 204 maternal care. Enhanced behaviour is maintained even when "programmed" dams are given pups
 205 from another dam.

a)



b)

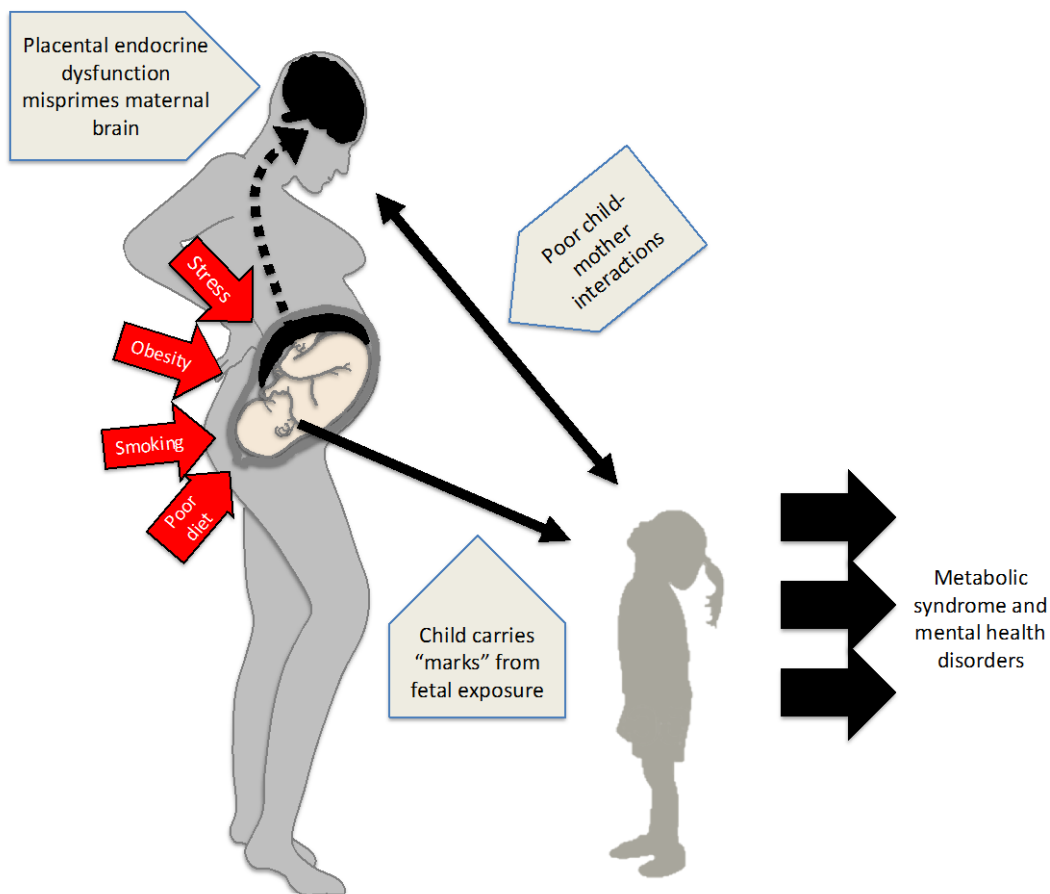
2X	1X	0x	Fetal <i>Phlda2</i>	1x	0x
50%	100%	200%	Placental hormones	100%	200%



206

207 **Figure 2. Prenatal adversity and the intimate, bidirectional and reiterative relationship between**
208 **mother and offspring.**

209 Prenatal adversities expose the mother, the developing fetus and the placenta. Alterations to the
210 fetus have the potential to change the way the child interacts with their mother after birth (solid
211 arrow), resulting in suboptimal maternal care. Alterations to the placenta have the potential to
212 misprogram maternal behaviour (dotted arrow) also resulting in suboptimal maternal care. These
213 misaligned reiterative interactions between mother and child (solid double headed arrow) further
214 contribute to poor outcomes for children later in life.



215

216 **Tables**

217

218 **Table 1. High fat diet protocols associated with alterations in maternal behaviour**

219 Only rodent studies focused on high fat diet protocols and maternal behaviour are reported

Species	Diet	Duration of HFD	Response to HFD	Reference
Sprague-Dawley rats	45% v. 25% v. 5% fat by weight	One week pre mating for duration	Decreased and delayed non-postural nursing Increased postural nursing Decreased total nursing Increased pup grooming Increased self grooming More time with litter	[47]
Sprague-Dawley rats	60% v. 17% calories from fat	From day 2 of gestation for duration	Dark phase/week one: Increased arch back nursing Increased total nursing Decreased resting	[9]
Wistar rats	45% v. 18% calories from fat	From day 1 of gestation for duration	P3 to P8 Decreased licking and grooming of pups	[7]
C57BL/6 mice	58% v. 10.5% calories from fat	10 weeks pre mating to E15.5	Increased frequency of cannibalistic episodes	[48]

C57BL/6 mice	45% v. 10% calories from fat	6 weeks pre mating for duration	Decreased pup interactions/increased exploration	[18]
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220

221 **Table 2. Prenatal adversities resulting the altered expression of imprinted genes.**

222 Only studies explicitly reporting altered expression of imprinted genes are reported. For mouse
 223 studies the first day of visible plug is referred to as embryonic day (E) 0.5. For rat studies, first day
 224 of observable sperm can be referred to as gestational day (GD) 1. LPD = low protein diet; HFD = high
 225 fat diet; QPCR = quantitative real time polymerase chain reaction.

226

Species	Stressor	Duration	Findings	Reference
ICR mice	50% food restriction	E12.5 to E16.5	QPCR: decreased brain <i>Cdkn1c</i> and <i>Snrpn</i> ; increased liver <i>H19</i> , <i>Grb10</i> , <i>Peg3</i> (male), <i>Igf2r</i> (female) and <i>Zac1</i> (female) at E16.5	[49]
C57BL/6 mice	LPD (8% calories from protein) v. Control (20%)	E0.5 to term	QPCR at P21: decreased liver <i>Gnas</i>	[50]
Wistar rats	Intraperitoneal dexamethasone at GD15	GD15-GD20	QPCR at GD20: increased liver <i>Igf2</i> , <i>Cdkn1c</i> , <i>Grb10</i> and <i>H19</i> ; decreased placental <i>Igf2</i>	[51]

Cdkn1c- FLucLacZ 129S2/SvHsd	LPD (8.1% calories from protein) v control (18.3%)	E0.5 to E18.5	QPCR and Imaging: reactivation of paternal <i>Cdkn1c</i> allele	[33]
ICR mice	50% food restriction	E12.5 to E16.5	QPCR at E16.5: Increased placental <i>Peg3</i>	[49]
C57BL/6 mice	HFHS (30% calories from fat, 36% sugar) v control diet (11% fat, 7% sugar)	E0.5 to E15.5	QPCR at E15.5: increased placental <i>Igf2</i> (non-significant increase in <i>Phlda2</i> and <i>Cdkn1c</i>)	[44]
C57BL/6 mice	Cafeteria (58% calories from fat) v. control (10.5%)	12 weeks premating to E14.5	QPCR: increased placental <i>Igf2</i> (male only).	[52]
Sprague- Dawley rats	HFD (60% calories from fat) v. control (13.5%)	GD2 to GD21	QPCR: increased placental <i>Igf2</i> (female only).	[53]
Sprague- Dawley rats	LPD (4.6% calories from protein) v. Control (19%)	GD1 to GD14 or GD18	QPCR: decreased <i>Ascl2</i> day 18.	[45]

CD1 mice	LPD (6% calories from protein) v. control (22%)	E4.5 to E17.5 with or without oral gavage of <i>Heligmosomoides bakeri</i> worms	Microarray: LPD only - increased placental <i>Igf2</i> .	[54]
BALB/c mice	Intra-chamber injection of live <i>Campylobacter rectus</i> strain 314 at E7.5	E7.5 to E16.5	Microarray: decreased placental <i>Ascl2, Igf2, Cdkn1c, Peg3</i>	[46]

227

228

229 **Table 3. Prenatal adversities associated with alterations consistent with placental endocrine**
 230 **dysfunction.**

231 Only changes in members of the placental lactogen-like gene family (*PrIs*) or placental endocrine
 232 lineages are reported. Where publications state “placental prolactin” in late gestation, they likely
 233 refer to placental lactogens. In mice, day of visible plug is embryonic (E) day 0.5 and length of
 234 gestation is 19-20 days depending on strain. In rats, day of sperm cell detection in female is day 1
 235 and length of gestation is 21-24 days depending on strain

236

Species	Stressor	Duration	Findings	Reference
Dietary Stressors				
C57BL/6 mice	HFHS (30% calories from fat,	From E0.5 to E15.5	QPCR: decreased placental <i>Prl2b1</i> and <i>Prl7b1</i>	[42]

	36% sugar) v control diet (11% calories from fat, 7% sugar)			
NIH Swiss mice	LFD (10% calories from fat) versus “control” (26% calories from fat) v. HFD (54% calories from fat)	From 30-35 weeks pre mating to E12.5	Microarray: changes in male/female ratio of <i>Prl2c3</i> , <i>Prl3b1</i> , <i>Prl3d2</i> , <i>Prl5a1</i> and <i>Prl7c1</i> .	[43]
Dorset Horn×Mule sheep	Moderate v. high levels of nutrition	After transfer of day 4 embryos (Border Leicester/Scottish Blackface x Dorset Horn) until day 100 of gestation	Radioimmunoassay: low maternal serum placental lactogen	[55]
Swiss Webster (ND4) mice	50% food restriction	From E1.5 to E11.5	Histology: reduced junctional zone Microarray: decreased placental <i>Prl8a8</i> .	[56]
Fischer 344 rat	LPD (5% calories from protein) v. 20% alcohol in	From day 6 to day 20	Radio-receptor assay:	[57]

	water v. control (18% calories from protein)		decreased maternal serum and placental levels of “prolactin” (both LPD and alcohol)	
C57BL/6 mice	LPD (6% calories from protein) v. Control (20%)	Two weeks premating to E10.5, E17.5 or E18.5	Histology: reduced junctional zone QPCR: decreased <i>Prl3a1</i> at E18.5 (non-significant decrease in <i>Prl5a1</i> and <i>Prl8a8</i>)	
Fischer 344 rat	LPD (5% calories from protein) v. control (20% calories from protein)	From day 6 to day 20	Radio-immuno assay: decreased maternal serum levels of rat “prolactin”	[58]
Sprague- Dawley rats	LPD (6% calories from protein) pair matched with control (20% calories from protein)	From day 6 to day 19	Northern: Decreased placental <i>Prl6a1</i> ; Western: Reduced <i>Prl6a1</i> secretion from explant cultures.	[59]
Sprague- Dawley rats	LPD (4.6% calories from protein) v. control (19% calories from protein)	From conception to day 14 or day 18	QPCR: decreased placental <i>Prl5a1</i> and <i>Prl2c1</i> day 14 and 18	[45]

CD1 mice	LPD (6% calories from protein) v. control (22% calories from protein) with or without oral gavage of <i>Heligmosomoides bakeri</i> worms	From E4.5 to E17.5	Microarray: LPD only - decreased placental "prolactin",	[54]
Other maternal stressors				
Holtzman rats	Continuous infusion of dexamethasone	From day 13 to day 20	Mini-array analyses and Northern: decreased <i>Prl8a8</i> , <i>Prl3b1</i> , <i>Prl6a1</i> and <i>Prl3d4</i> ; <i>In situ</i> hybridisation: mislocalisation of spongiotrophoblast into labyrinth	[60]
Suffolk sheep	Heat stress (40°C for 9 hours per day then 30°C for 15 hours/day; 40% humidity) v. thermoneutral	From day 64 to day 136-141	Radioimmunoassay: reduced maternal serum placental lactogen (by >60%).	[61]

	(18-20°C; 30% humidity).			
Fisher rats	Chromium (IV) in tap water	From day 7 to Day 19	Northern blot: decreased placental <i>Prl3d1</i> and <i>Prl3b1</i> ; <i>Prl4a1</i> , <i>Prl8a2</i> . Radioimmunoassay: decreased maternal serum <i>Prl3d1</i> and <i>Prl3b1</i> ; Histology: reduced “spongiotrophoblast”	[62]
CD1 mice	Perfluorooctanoic acid by gavage	From E10.5 to E15.5	Histology: Decrease in parietal trophoblast giant cells, glycogen cells and sinusoidal trophoblast giant cells; Northern: decreased placental <i>Prl3b1</i> , <i>Prl7a1</i> and <i>Prl7a2</i> .	[63]
Sprague-Dawley rats	Triclosan by gavage	From day 6 to day 20	Radio-immunoassay: decreased maternal serum “prolactin”	[64]
CD1 mice	Reduced utero-placental perfusion pressure	From E12.5 to E16.5-E18.5	<i>In situ</i> hybridisation: reduced area of junctional zone.	[65]

237

238

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241 **Conflict of Interest**

242 The author declares no conflict of interest.

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