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**Glucocorticoid programming of intrauterine development**

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36 **ABSTRACT**

37

38 Glucocorticoids are important environmental and maturational signals during intrauterine  
39 development. Towards term, the maturational rise in fetal glucocorticoid concentrations decreases  
40 fetal growth and induces differentiation of key tissues essential for neonatal survival. When cortisol  
41 levels rise earlier in gestation as a result of suboptimal conditions for fetal growth, the switch from  
42 tissue accretion to differentiation is initiated prematurely, which alters the phenotype that develops  
43 from the genotype inherited at conception. While this improves the chances of survival should  
44 delivery occur, it also has functional consequences for the offspring long after birth. Glucocorticoids  
45 are, therefore, also programming signals that permanently alter tissue structure and function during  
46 intrauterine development to optimise offspring fitness. However, if the postnatal environmental  
47 conditions differ from those signalled *in utero*, the phenotypical outcome of early life glucocorticoid  
48 overexposure may become maladaptive and lead to physiological dysfunction in the adult. This  
49 review focusses on the role of glucocorticoids in developmental programming, primarily in farm  
50 species. It examines the factors influencing glucocorticoid bioavailability *in utero* and the effects that  
51 glucocorticoids have on the development of fetal tissues and organ systems, both at term and earlier  
52 in gestation. It also discusses the windows of susceptibility to glucocorticoid overexposure in early  
53 life together with the molecular mechanisms and long term consequences of glucocorticoid  
54 programming with particular emphasis on the cardiovascular, metabolic and endocrine phenotype of  
55 the offspring.

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57

58 **1. Introduction**

59

60

61 Glucocorticoids are important stress hormones in adult animals but have a wider range of functions  
62 in the fetus. In late gestation, they act as maturational signals that ensure the fetus is mature  
63 enough to survive the transition to extra-uterine life at delivery [1]. Earlier in gestation,  
64 glucocorticoids can act as signals of suboptimal environmental conditions and modify fetal  
65 development in relation to the available resource for intrauterine growth. While improving the  
66 likelihood of survival both *in utero* and at birth, this early overexposure to glucocorticoids adapts the  
67 phenotype that develops from the genotype inherited at conception with life-long functional  
68 consequences [2-11]. Glucocorticoids are, therefore, also programming signals that permanently  
69 alter tissue structure and function during intrauterine development to optimise offspring fitness [12,  
70 13]. Previous reviews of glucocorticoid programming have tended to concentrate on the human  
71 implications and/or the experimental studies of short lived, laboratory species like mice, rats and

72 guinea pigs [2-9]. In contrast, this review examines the role of glucocorticoids in developmental  
73 programming with particular emphasis on the longer-lived farm species like sheep, pigs, cattle and  
74 horses.

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76

## 77 **2. Fetal glucocorticoid exposure**

78

79 There are a number of different mechanisms by which glucocorticoid concentrations can rise in the  
80 fetal circulation (Figure 1). For most of gestation, the primary source of cortisol in fetal ovine plasma  
81 is the mother [19]. Glucocorticoids cross the placenta readily by diffusion down a materno-fetal  
82 concentration gradient which exists in normal conditions in all species studied to date including pigs,  
83 cattle, sheep, pigs and horses [1,3]. Consequently, increases in maternal glucocorticoid  
84 concentrations induced by stressful conditions, such as isolation, transport, undernutrition and  
85 housing conditions, can lead to raised concentrations in the fetus [20]. However, the degree of fetal  
86 overexposure to the higher maternal glucocorticoid concentrations is minimised by the presence in  
87 the placenta of the enzyme, 11 $\beta$ -hydroxysteroid dehydrogenase type 2 (11 $\beta$ HSD2, Figure 1). This  
88 isoform of the enzyme converts active glucocorticoids into their inactive keto-metabolites and,  
89 hence, acts as a barrier to transplacental glucocorticoid transfer [14]. Amongst species, placental  
90 11 $\beta$ HSD2 activity appears to be positively related to the magnitude of the materno-fetal  
91 glucocorticoid gradient and is influenced by gestational age and a range of environmental factors  
92 including glucocorticoid concentrations on both sides of the placenta (Figure 1). In addition, in the  
93 hemochorial type of placenta, there are multidrug resistance transporters, which transfer  
94 xenobiotics like synthetic glucocorticoids from the trophoblast cells back into the maternal  
95 circulation (Figure 1). Abundance of these transporters are also regulated developmentally and by  
96 glucocorticoids but whether they are present in the epitheliochorial placenta of ruminants, pigs and  
97 horses remains unknown [9]. Fetal glucocorticoid concentrations can, therefore, be altered  
98 independently of maternal levels by manipulating the effectiveness of the placental barrier to  
99 materno-fetal glucocorticoid transfer. Glucocorticoid programming in early to mid-gestation,  
100 therefore, depends on the level of stress experienced by the mother during pregnancy, her HPA  
101 responses and ensuing cortisol concentration, and on the glucocorticoid permeability of the  
102 placenta.

103

104 Later in gestation when the fetal hypothalamic-pituitary-adrenal (HPA) axis has developed  
105 functionally, fetal glucocorticoid concentrations can also rise independently of maternal levels by

106 direct cortisol secretion from the fetal adrenal glands (Figure 1). This occurs via activation of the  
107 HPA axis in response to adverse intrauterine conditions like hypoxia and hypoglycaemia caused, for  
108 example, by cord occlusion, placental insufficiency, poor uterine perfusion or maternal alterations in  
109 dietary composition or calorie intake [20]. Development of fetal HPA responsiveness to adverse  
110 stimuli varies in timing between species and with both early glucocorticoid overexposure and  
111 repeated insults during late gestation [13,21]. Closer still to term, fetal cortisol concentrations rise  
112 naturally in the absence of adverse stimuli as part of the normal sequence of prepartum  
113 maturational events that ensure viability at birth [1; Figure 2]. The magnitude and timing of this  
114 normal prepartum cortisol surge also varies widely between species (Figure 2) and can be activated  
115 earlier than normal by poor nutritional conditions either around the time of conception or during  
116 late gestation [1,18,22,24]. Its timing is also influenced by the number of fetuses in sheep [22]. In  
117 some species like the horse, the main perinatal rise in cortisol concentrations occurs immediately  
118 after not before birth [21, 25]. The window of susceptibility to glucocorticoid programming in late  
119 gestation will, therefore, vary with species in relation to environmental conditions *in utero* and the  
120 development and responsiveness of the fetal HPA axis.

121

122 Fetal glucocorticoid overexposure can also occur as a result of clinical use of synthetic  
123 glucocorticoids like dexamethasone and betamethasone during pregnancy. These drugs are 20 times  
124 more potent than the natural hormones and are cleared more slowly from the circulation [26]. They  
125 are often used to treat conditions with an inflammatory component such as joint and respiratory  
126 problems, allergic reactions and endotoxic shock in several species [26-28]. They are also given  
127 routinely to healthy pregnant women threatened with pre-term delivery to improve neonatal  
128 viability of their infants [26]. Since the onset of labour is co-ordinated with maturation through the  
129 prepartum cortisol rise in many ruminants, synthetic glucocorticoids are also used to induce delivery  
130 of viable offspring at or near term in cattle and sheep [29-31]. Experimentally, these drugs have  
131 been used extensively in the longer-lived farm species to investigate the likely long-term  
132 physiological consequences for the human infant of antenatal glucocorticoid treatment [10,11].

133

134 The developmental effects of the glucocorticoids are determined ultimately by their bioavailability  
135 within the tissues. In turn, this depends on expression of the glucocorticoid (GR) and  
136 mineralocorticoid receptors (MR) to which the glucocorticoids bind [14]. These receptors vary in  
137 abundance between fetal tissues and with gestational age [32-34]. Their expression can also be  
138 influenced by glucocorticoid concentrations *per se* [35]. In addition, fetal tissues express 11 $\beta$ HSD,  
139 both the type 2 isoform found in the placenta and the type 1 isoform which reactivates the

140 biologically inactive metabolites [14]. Activity of the two isoforms varies between tissues and both  
141 isoforms are regulated developmentally and by fetal oxygen, nutrient and glucocorticoid availability  
142 in a tissue specific manner [32-35]. Consequently, overexposure to glucocorticoids is determined not  
143 only systemically by the circulating concentrations but also locally within the tissues themselves.  
144 Since synthetic glucocorticoids are poorly inactivated by 11 $\beta$ HSD2 and bind only to the GR [14], their  
145 bioavailability and actions can differ from those of the natural glucocorticoids.

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147

### 148 **3. Glucocorticoid programming**

149

#### 150 *3.1 Glucocorticoids and fetal development*

151 Maturationally, glucocorticoids affect development of a wide range of fetal tissues, particularly  
152 those essential for immediate neonatal survival like the lungs, liver, gut and kidneys [1,13]. During  
153 late gestation, experimental manipulation of fetal cortisol concentration in fetal sheep by fetal  
154 adrenalectomy and exogenous cortisol infusion have shown that cortisol induces changes in tissue  
155 expression of cytostructural proteins, receptors, enzymes, ion channels and growth factors [1,3].  
156 These changes lead to alterations in tissue morphology, biochemical composition, metabolism and  
157 hormone sensitivity with functional consequences for multiple organ systems in the fetus. For  
158 example, in addition to their well established role in pulmonary maturation [1,26], glucocorticoids  
159 increase fetal blood pressure during late gestation via effects on the fetal heart and blood vessels  
160 [36]. Similarly, the fetal liver develops the capacity of gluconeogenesis close to term as a result of  
161 cortisol-induced increases in glycogen deposition and gluconeogenic enzyme activities [37].  
162 Glucocorticoids, therefore, activate many of the physiological processes that have little or no  
163 function *in utero* but which are vital at birth like pulmonary gas exchange, hepatic gluconeogenesis  
164 and thermogenesis [1]. They also affect development of many other tissues like the brain and  
165 skeletal muscle which are important for offspring viability and fitness in the longer term [38, 39].  
166 Consequently, delivery before adequate intrauterine exposure to rising cortisol concentrations leads  
167 to functional immaturity at birth, poor neonatal viability and/or a failure to thrive postnatally [40].  
168 This scenario is likely to be particularly important in twin-bearing and polytocous species like sheep  
169 and pigs in which the timing of fetal HPA activation can differ between littermates.

170

171 The maturational effects of the glucocorticoids are mediated, in part, by changes in the circulating  
172 concentrations and tissue bioavailability of a range of other hormones [41]. In fetal sheep, the  
173 functioning of several endocrine systems including the HPA axis itself is affected by the prepartum

174 cortisol surge via changes in endocrine cell populations, enzyme activities and hormone receptor  
175 abundance (Figure 3). This leads to parturition increases in fetal plasma concentrations of several  
176 hormones in addition to cortisol, including tri-iodothyronine ( $T_3$ ), leptin and adrenaline [20]. In turn,  
177 these hormones have independent effects on development of a range of fetal tissues [41]. For  
178 instance, terminal differentiation of mononucleated cardiac myocytes to their binucleated form is  
179 initiated by the parturition cortisol surge but depends on activation of specific tissue deiodinases  
180 and the concomitant increase in fetal  $T_3$  bioavailability [42,45]. The changes in the set point and  
181 sensitivity of the endocrine axes induced by the parturition cortisol surge also prepare the fetus for  
182 the new homeostatic challenges of extrauterine life. For example, the cortisol induced increases in  
183 the adrenal activity of phenylethanolamin-N-methyl transferase (PNMT) and the hepatic abundance  
184 of  $\beta$ -adrenoreceptors mean that neonates can secrete adrenaline in response to stressful conditions  
185 like hypoglycaemia and respond to the circulating adrenaline and produce glucose endogenously  
186 [37,46,47].

187

188 Early increases in the fetal glucocorticoid concentration also trigger tissue differentiation in the fetus  
189 [1,13]. However, in sheep, the effects of preterm cortisol administration do not entirely recapitulate  
190 the maturational changes in tissue differentiation induced by the increase in cortisol concentrations  
191 towards term. For example, adrenal PNMT abundance is increased by the parturition cortisol surge  
192 but is decreased by cortisol administration at 100 days of gestation [46,48]. Similarly, cortisol  
193 depresses hepatic IGF-II expression at term but not earlier in gestation [49]. The transcriptome  
194 observed in the fetal lung and heart after early cortisol infusion also differs from that seen at term  
195 [50,51]. This probably reflects, in part, the ontogenic changes in tissue abundance of GR and/or  
196 other hormone receptors.

197

198 By simulating tissue differentiation, cortisol reduces tissue accretion *in utero* [1,13]. As a result, the  
199 overall rate of fetal growth declines as cortisol concentrations rise in fetal sheep towards term and in  
200 response to adverse intrauterine conditions [13]. The parturition decline in growth rate can be  
201 prevented by fetal adrenalectomy and can to be stimulated prematurely by infusing cortisol into  
202 either the fetus or mother earlier in gestation [13,52]. In several species including laboratory and  
203 farm animals, maternal administration of synthetic glucocorticoids in late gestation has also been  
204 shown to reduce offspring size, both shortly after administration and at delivery longer after the  
205 period of treatment [26,53,54; Table 1]. Similar reductions in fetal growth have been seen with  
206 administration of synthetic glucocorticoids directly to fetal sheep although the effects appear to be  
207 less pronounced than with maternal administration [53]. This suggests that the growth inhibitory

208 effects of synthetic glucocorticoids may be mediated, in part, by maternal metabolic changes or  
209 actions on the placenta [10, 103].

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### 212 *3.2 Glucocorticoids and placental development*

213 Reductions in placental weight are seen in response to administration of both natural and synthetic  
214 glucocorticoids during mid to late gestation in sheep and other species [104]. These changes are  
215 associated with reduced expression of anti-apoptotic markers and increased expression of pro-  
216 apoptotic factors in the ovine placenta [105]. The growth inhibitory effects are more pronounced  
217 with maternal than fetal administration and tend to persist after cessation of treatment [105]. They  
218 may also be sex-linked [10]. In sheep, both maternal and fetal cortisol administration alter the gross  
219 placental morphology with proportionately fewer of the more everted placentomes [106,107].  
220 Although the functional significance of this shift in placentome distribution remains unclear  
221 [106,108,109] placentas with fewer everted C and D type placentomes transport more glucose on a  
222 weight specific basis when fetal cortisol concentrations are high [106]. In general, glucocorticoids  
223 reduce placental glucose transport via effects on the transplacental glucose concentration gradient,  
224 placental glucose consumption and/or placental expression of the glucose transporters, dependent  
225 on the species [104]. They also reduce the active transport of amino acids across the placenta and  
226 alter placental amino acid metabolism in some species [104]. Glucocorticoid-induced changes in  
227 placental transport phenotype also vary with time both during the period of treatment and after it  
228 has ended [104]. In addition, there are alterations in the endocrine function of the placenta in  
229 response to raising glucocorticoid concentrations, which involve a wide range of hormones and  
230 changes in both their synthesis and metabolism (Figure 2). Again, these effects can be sex-linked  
231 and are often dependent on gestational age at the time of glucocorticoid exposure. For example,  
232 placental 11 $\beta$ HSD2 gene expression is increased by dexamethasone administration to ewes at 30%  
233 of gestation in males alone but decreased by treatment later in pregnancy in both sexes  
234 [10,105,109].

235

236 The changes in placental endocrine and transport function induced by the prepartum cortisol surge  
237 are part of the normal sequence of events leading to labour and delivery of viable neonates [1,40].  
238 However, their induction by glucocorticoid overexposure earlier in gestation may alter fetal growth  
239 and development independently of any direct effects of the glucocorticoids on the fetal tissues *per*  
240 *se* [1,3,13]. For example, the reduction in placental lactogen production and its maternal  
241 concentration in response to early glucocorticoid overexposure may alter maternal metabolism and,  
242 hence, nutrient allocation to the gravid uterus [109]. Similarly, glucocorticoid induced changes in

243 the production of progesterone and other progestagens may influence maternal insulin resistance  
244 and appetite with indirect consequences for intrauterine growth [110]. In addition, changes in  
245 placental phenotype induced by early glucocorticoids overexposure may persist or appear only after  
246 restoration of normal concentrations to affect fetal development long after original insult [104].

247

248

### 249 *3.3 Postnatal outcomes of early overexposure to glucocorticoids*

250 Glucocorticoid exposure *in utero* has been shown to affect organ growth and the functioning of  
251 physiological systems in the offspring after birth in sheep, pigs and cattle (Table 1). In particular,  
252 there are abnormalities in postnatal cardiovascular and metabolic function that are associated with  
253 overt hypertension and glucose intolerance by adulthood. These changes involve a wide range of  
254 tissues including the brain, blood vessels, kidneys, heart and skeletal muscle as well as several  
255 endocrine systems (Table 1). Similar findings have been made in adult rodents and humans  
256 overexposed to glucocorticoids prenatally [2-9]. Because of the central role of glucocorticoids in  
257 regulating adult cardiovascular and metabolic function, many of these studies have concentrated on  
258 programming of the HPA axis *per se* (Table 1). In sheep, pigs and cattle, early prenatal overexposure  
259 to either natural or synthetic glucocorticoids can alter both basal and stimulated cortisol  
260 concentrations postnatally and, hence, the physiological responses to homeostatic challenges (Table  
261 1). Indeed, amongst species, maternal glucocorticoid administration during late pregnancy has been  
262 shown to programme postnatal HPA function at every level of the axis from the hippocampus to  
263 glucocorticoid bioavailability in the peripheral tissues [11]. Taken together, these studies have  
264 shown that glucocorticoids overexposure *in utero* affects the same range of tissues and cellular  
265 processes in the adult as seen in the fetus [1,3,13]. However, the specific postnatal outcomes of  
266 intrauterine glucocorticoid overexposure depend on gestational age at its onset, its severity and  
267 duration and on whether exposure was to natural or synthetic glucocorticoids (Table 1).

268

269 Natural and synthetic glucocorticoids have different programming effects. Maternal administration  
270 of cortisol but not dexamethasone at 20% of ovine pregnancy causes fasting hyperglycaemia while  
271 dexamethasone but not cortisol increases their initial insulin response to glucose administration in  
272 the adult male offspring [53]. In contrast, both cortisol and dexamethasone administration at this  
273 stage of pregnancy give rise to hypertension in the adult offspring [61]. However the mechanism by  
274 which hypertension is induced differs with cortisol increasing peripheral resistance but not cardiac  
275 output while dexamethasone enhances cardiac output but not peripheral resistance in the adult



276 sheep [4]. Different synthetic glucocorticoids also appear to have different programming effects  
277 (Table 1), although few, if any, studies have specifically compared the postnatal consequences of  
278 dexamethasone and betamethasone treatment *in utero*.

279

280 In sheep, glucocorticoids have been shown to have programming effects on postnatal phenotype  
281 with administration from as early as 27 days of pregnancy right up until term (Table 1). However, the  
282 specific outcomes depend on gestational age at onset of treatment (Table 1). For instance, maternal  
283 administration of a single course of dexamethasone leads to hypertension in the adult offspring  
284 when given at 27 and 80 days but not at 64 days of pregnancy [63,64]. In contrast, dexamethasone  
285 has little effect on glucose tolerance or insulin sensitivity of adult female offspring with  
286 administration at either 27 days or 64 days of gestation [64]. Similarly, maternal dexamethasone  
287 treatment early in pregnancy appears to have little effect on HPA function but, later in gestation, it  
288 decreases HPA responsiveness of the adult offspring [69,111]. Furthermore, multiple doses of  
289 betamethasone over a 14-d period in late pregnancy have subtly different effects on HPA function  
290 and glucose-insulin dynamics of the adult offspring than single doses given at the same gestational  
291 age as that at the start of the more prolonged treatment [86,87]. Longer periods of dexamethasone  
292 treatment at lower doses also have little effect relative to a single treatment at the higher, clinically  
293 relevant doses during the same period of gestation [4,112]. Consequently, the dose of synthetic  
294 glucocorticoid administered as well as its duration and timing in pregnancy is important in  
295 determining the phenotypical outcome.

296

297 Another factor influencing the apparent extent of programming is the postnatal age at which the  
298 outcomes are assessed (Table 1). Some glucocorticoid-programmed changes in postnatal growth  
299 and physiological function are apparent immediately after birth while others only become evident  
300 later in life as the animal ages, reaches key life course events like weaning, puberty or pregnancy or  
301 experiences adverse conditions after birth [113; Table 1]. For example, hypertension is not seen in  
302 the neonatal lamb after intrauterine dexamethasone overexposure, although there are changes in  
303 the baroflexes indicative of resetting of the neural mechanisms of blood pressure control even at  
304 this early stage of postnatal life [56]. Hypertension is evident at 4 months of age at about the time  
305 weaning is complete and becomes progressively more pronounced with increasing age thereafter  
306 [57]. Overall, the experimental studies suggest that glucocorticoid-programmed metabolic  
307 dysfunction appears later in ovine life than the cardiovascular abnormalities and is often not  
308 detected until adulthood (Table 1). Metabolic changes may also only be detected in one sex (Table

309 1). For instance, altered glucose-insulin dynamics are seen in 4-5 year-old male but not female  
310 offspring overexposed to dexamethasone at 27 days of gestation [55,64]. In addition, there is  
311 emerging evidence in rodents and other species that maternal diet and pre-existing conditions such  
312 as intrauterine growth restriction can influence the fetoplacental responses to glucocorticoid  
313 administration, which, in turn, are likely to affect programming of postnatal phenotype [114,115].

314

#### 315 *3.4 Developmental windows of glucocorticoid programming*

316 Collectively, the experimental studies summarised in Table 1 suggest that there are specific stages in  
317 development when glucocorticoid overexposure is most likely to result in an altered postnatal  
318 phenotype. The first window of susceptibility is probably during pre-implantation development  
319 when lineage specification occurs and cells are segregated into trophectoderm and inner cell mass.  
320 Certainly, undernutrition during this period of pregnancy has effects on development of the fetal  
321 HPA axis and other organ systems much later in gestation [18,24]. The second vulnerable period for  
322 glucocorticoid programming is during organogenesis which occurs between days 7 and 30 of  
323 gestation in sheep embryos. This also covers the period of implantation and formation of the ovine  
324 placenta [10]. Indeed, compromised development of the metanephric kidney is likely to be a  
325 significant contributory factor in the hypertension seen in adult offspring of ewes treated with  
326 dexamethasone at 27 days of gestation [4]. After completing organogenesis, there is a relatively long  
327 period of gestation when the fetus is gaining mass and developing the neural and endocrine  
328 mechanisms regulating homeostasis. During this period, excess glucocorticoids appear to act by  
329 changing the kinetics of the cell cycle to slow growth and set the responsiveness of the regulatory  
330 mechanisms. When tissues have developed sufficient GR or at critical concentrations or duration of  
331 exposure, glucocorticoids can switch the cell cycle from proliferation to differentiation prematurely,  
332 with permanent effects on total cell number and/or the balance of different cell types within an  
333 organ [13]. For example, cortisol induced differentiation of cardiomyocytes from the  
334 mononucleated form, which can divide, to the binucleated type, which cannot, means that cell  
335 number is fixed and that cardiac growth depends primarily on cell hypertrophy rather than  
336 hyperplasia thereafter [42]. Finally, in some species, there appears to be a window of susceptibility  
337 to glucocorticoid programming in the period immediately after birth, which may be particularly  
338 important in species like the horse in which terminal differentiation of tissues is not complete at  
339 birth [21]. Neonatally, cortisol overexposure may occur either directly due to sickness or  
340 maladaptation *ex utero* or indirectly via changes in milk composition and its glucocorticoid content  
341 as a result of maternal stress or abnormal mammary development [116,117]. Certainly,

342 experimental overexposure of healthy newborn foals to cortisol for 5 days after birth alters both  
343 HPA and pancreatic  $\beta$  cell function later in life (Table 1).

344

### 345 *3.5 Molecular mechanisms of glucocorticoid programming*

346 At the molecular level, there appears to be two broad mechanisms by which glucocorticoids act to  
347 programme development. First, bound to their receptors, they may act as enhancer binding proteins  
348 that activate or repress expression of genes via interaction with glucocorticoid response elements in  
349 the promotor or other regulatory regions of the genome [118]. With genes that trigger key  
350 developmental stages, their altered expression at inappropriate times in the normal sequence of  
351 events may have permanent effects on the subsequent pattern of development. This type of  
352 glucocorticoid-induced change in expression of specific genes may occur either early in  
353 development, for example during cell lineage specification and mitochondrial biogenesis, or later in  
354 gestation during differentiation of sub-populations of cells within tissues such as the liver or  
355 endocrine pancreas. The outcomes of these discrete gene expression events may, therefore, be  
356 global in terms of cell metabolism and oxidative stress or specific to certain tissues or cell types. In  
357 rodents, increased oxidative stress is a common feature of glucocorticoid programming along with  
358 changes to the relative numbers of the different endocrine cell types within the Islets of Langerhans  
359 [2-9].

360

361 Secondly, glucocorticoids may alter the epigenome with more long term consequences for gene  
362 expression throughout life [119]. Changes in DNA methylation and histone modifications have been  
363 observed both globally and in specific tissues in postnatal offspring glucocorticoid overexposed *in*  
364 *utero* [35,119]. In particular, there are tissue specific changes in the methylation status of the  
365 regulatory regions of the GR gene which influence expression of these receptors and, hence,  
366 postnatal glucocorticoid responsiveness [33, 118]. Maternal dexamethasone administration during  
367 pregnancy has been shown to reduce placental transport of folate required for one carbon  
368 metabolism and DNA methylation while, conversely, dietary supplementation with folate  
369 ameliorates, in part, the fetoplacental growth restriction induced by this treatment [115,121]. In  
370 addition, there is emerging evidence for postnatal changes in expression of various non-coding and  
371 microRNAs after early life glucocorticoid overexposure [39]. Glucocorticoids, therefore, affects the  
372 developing epigenome through a number of different routes with dynamic consequences for  
373 epigenetic marks throughout the lifespan of the animal. However, to date, most of the information

374 about the molecular mechanisms of glucocorticoid programming has been derived from studies in  
375 rodents and guinea pigs so the extent to which they apply to farm species remains unclear.

376

#### 377 **4. Conclusions**

378

379 Glucocorticoids have a number of roles during intrauterine and early neonatal development. Not  
380 only are they essential for normal maturation close to term, they also act as important signals of  
381 environmental compromise earlier in gestation. The glucocorticoid triggered switch from tissue  
382 accretion to differentiation improves offspring fitness by maximising the chances of the fetus  
383 surviving into adulthood. Prenatally, early activation of this switch ensures that fetal growth is  
384 commensurate with the nutrient supply *in utero* and that fetal tissues are sufficiently mature to  
385 function *ex utero* should delivery occur. Postnatally, the glucocorticoid-induced adaptations in  
386 phenotype and, particularly the resetting of the homeostatic control mechanisms, will help the  
387 offspring to thrive in a postnatal environment matching that signalled to it *in utero*. However, when  
388 pre- and post-natal environments are mismatched in laboratory species, the glucocorticoid-induced  
389 changes in offspring phenotype can become maladaptive and lead to early onset of cardiometabolic  
390 dysfunction characteristic of old age [2-9]. Recent findings have also shown that the effects of early  
391 life glucocorticoid overexposure can persist inter-generationally with changes in F2 placental  
392 phenotype and HPA function after dexamethasone treatment of their pregnant grandmothers [122-  
393 125]. This raises the possibility that glucocorticoids may also have an important evolutionary role in  
394 the transgenerational inheritance of phenotypical traits. However, the extent to glucocorticoid  
395 overexposure during early development influences lifespan and transgenerational inheritance in  
396 longer lived farm species remains largely unknown.

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## 761 **Acknowledgements**

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

763 We are grateful to all the members of the Department of Physiology, Development and  
764 Neuroscience who have helped with studies described here. We would also like to thank the BBSRC,  
765 the Horserace Betting Levy Board and the Centre for Trophoblast for their financial support.

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767 **Figure legends**

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769 **Figure 1:** Schematic diagram showing the sources of cortisol in the fetal circulation and the role of 11  
770 beta-hydroxysteroid dehydrogenase as a placental barrier to materno-fetal cortisol transfer in  
771 sheep. Open arrows = major cortisol movements. Dashed arrow = minor cortisol movement.

772  Stimulatory effect.  Inhibitory effect.

773 Data from references 14-18.

774

775 **Figure 2:** Fetal cortisol concentrations with respect to proximity to delivery in different species.  
776 Length of pregnancy: Pig 115 days (filled circles), Sheep 145 days (open circles), Human 280 days  
777 (filled triangle), Cow 280 days (filled squares), Horses (Pony) 335 days (open triangles). Data from  
778 references 1,22,23.

779

780 **Figure 3:** The endocrine systems affected by natural and synthetic glucocorticoids in fetal sheep  
781 together with the cellular and molecular processes within these endocrine systems influenced by  
782 prenatal glucocorticoid exposure. Data from references 1-11,42-44.

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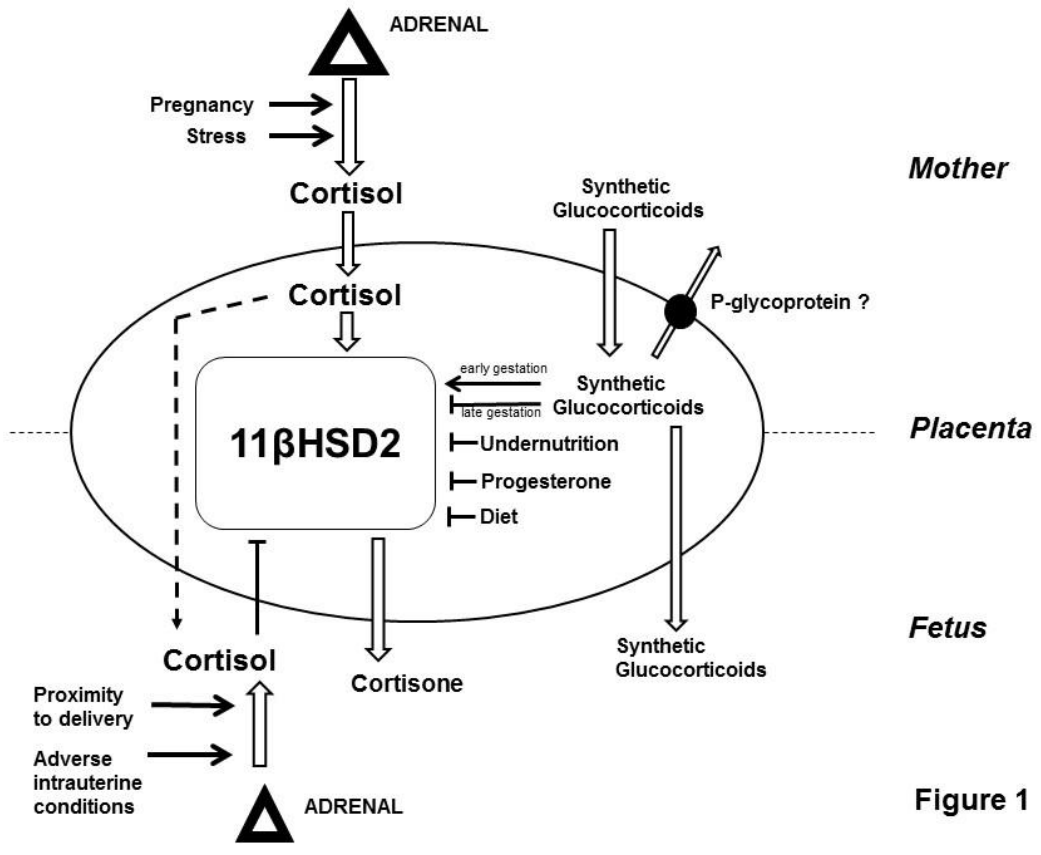
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*Mother*

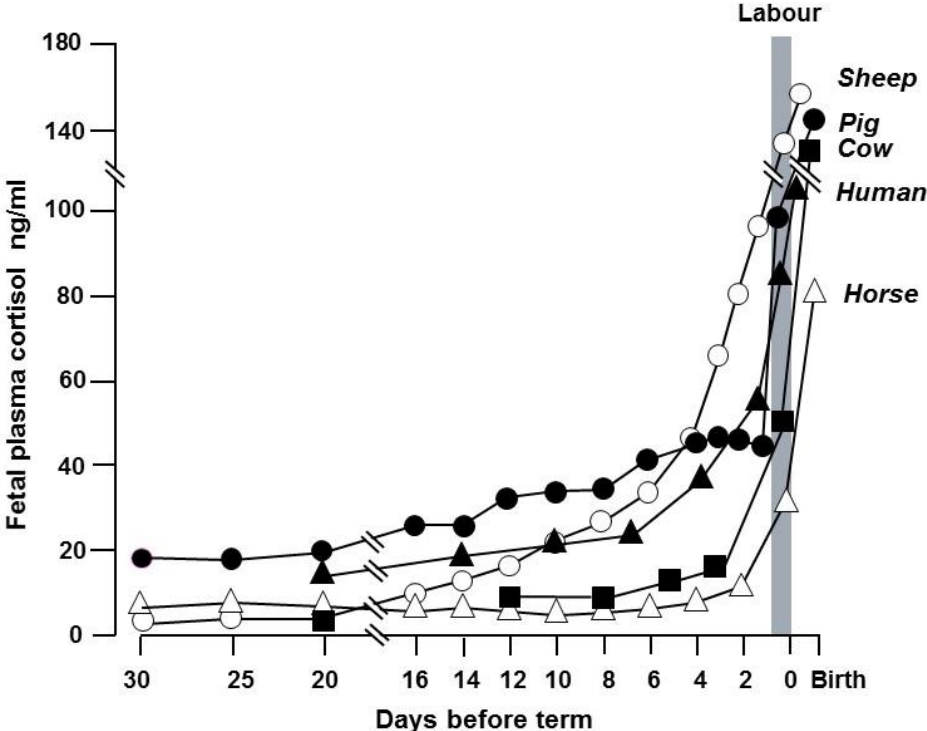
*Placenta*

*Fetus*

**Figure 1**

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Figure 2



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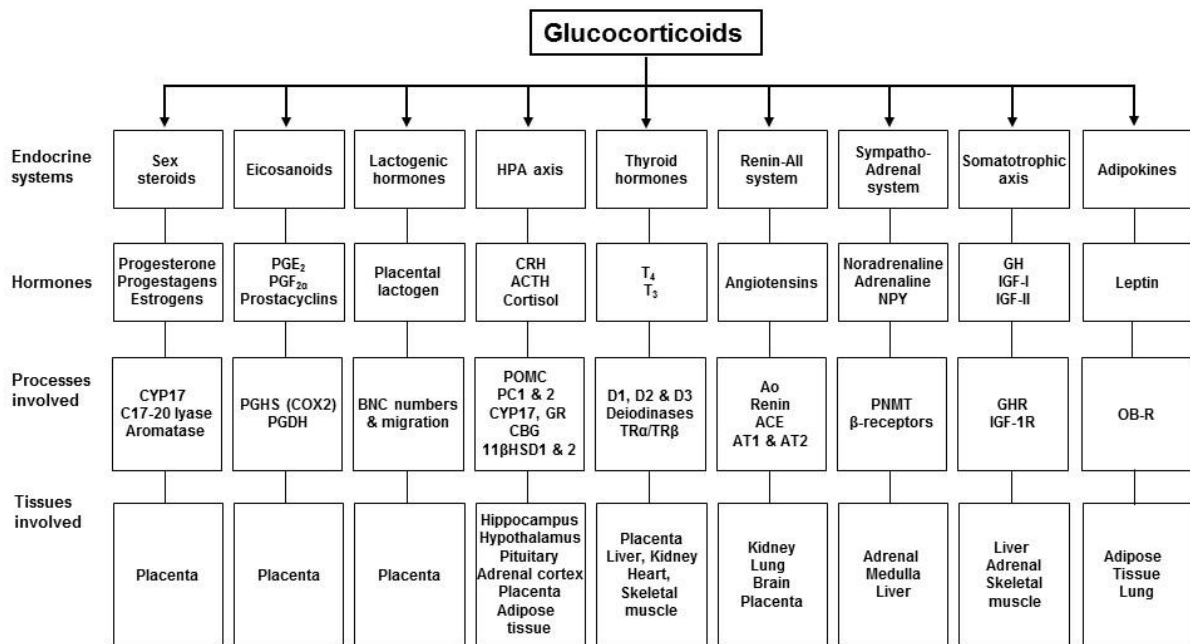
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**Table 1:** The postnatal outcome of early life overexposure to glucocorticoids in farm animals.

	Species	Agent	Stage of pregnancy at overexposure	Postnatal outcomes	Age at outcome N = Neonate J = Juvenile A = Adult	Sex of Offspring M = Male F = Female	Reference
Maternal Synthetic Glucocorticoid Overexposure	Sheep	Dex	20%	↓Birth weight and abdominal circumference	N	M	55
				Altered baroreflexes, ↑sympathetic activity	N	M & F	56
				Altered vasodilatory responses	N	M & F	56
				Hypertension	J (4 mo)	M & F	57
					A (1.5-2 yr)	M & F	58
					A (5-6 yr)	F	59
					A (7 yr)	M & F	60
				Left ventricular hypertrophy ↑Cardiac output	A (7 yr)	F	58
					A (7 yr)	F	58
				Altered endothelial superoxide production	J (4 mo)	M & F	61
				Altered cardiac mitochondrial function	J (6 mo)	M	62
				Altered brain RAS function ↑brainstem AT1 receptor abundance	A (4-5 yr)	M	63
					A (7 yr)	F	60
			↓Nephron number	A (5-6 yr)	F	59	
				A (7 yr)	M & F	58	
			↑Mean glomerular volume ↑single nephron glomerular filtration rate	A (7 yr)	M & F	60	
				A (5-6 yr)	F	59	
			↑Glucose stimulated insulin secretion ↑Glucose tolerance	A (4 yr)	M	54	
				A (4 yr)	M	54	
↑sensitivity to inhibition of lipolysis by insulin	A (5 yr)	F	64				
30%	↑pituitary-adrenal responsive to stress	N (30 d)	F not M	65			
	↓liver, adrenal, pituitary & kidney weight	J (7 mo)	F	66			
	↓pituitary-adrenal responsiveness	J (7 mo)	F not M	67			
	↑Hippocampal GR mRNA	J (7 mo)	M not F	67			
	↑Hypothalamic AVP & GR mRNA	J (7 mo)	M not F	67			
	↓Pituitary POMC mRNA	J (7 mo)	F not M	67			

			↑Adrenal ACTH receptor, StAR and 3βHSD mRNA	J (7 mo)	M not F	67	
		70%	↓body weight and CRL	N	F	68	
			↓basal and stimulated HPA function	A (2.5-3.5 yr)	F	69	
			↓Glucose tolerance	A (2.5-3.5 yr)	F	69	
			↓Insulin secretion	A (2.5-3.5 yr)	F	69	
		70-82%	↓ brain weight and size	N	M & F	54	
		98%	↑UCP1 content in brown adipose tissue	N	M & F	70	
			↑Prolactin receptor abundance	N	M & F	70	
			↑Relative fat mass	A (16 mo)	F not M	71	
	Beta	55%	↑sympathetic and HPA responses	J (40 d)	F	72	
				↑basal and ACTH stimulated cortisol secretion	A (1.5 yr)	F not M	73
				Hypertension	J (6 mo) A (1-2yr)	M & F M	74 75
			Altered systemic and renal RAS function	J (6 mo)	M	76	
			↓plasma renin and All concentrations	J (6 mo)	M & F	77	
			↑All stimulated ROS production	J (6 mo)	M & F	76	
			Altered renal All responsiveness	J (6 mo) A (1-1.5 yr)	M & F M & F	78 79	
			↓nephron number	J (6 mo)	M & F	80	
			↓glomerular filtration rate	J (6 mo)	M	80	
			Altered cerebral vascular tone and reactivity	A (1.5 yr)	F	81	
			↑plasma leptin	A (1.5 yr)	M & F	73	
			↑ leptin inhibition of adrenal function	A (1.5 yr)	M not F	73	
			72-84%	↓Brain weight	J (6 wk) A (3.5 yr)	M & F M & F	66 82
				↓Lung weight	J (12 wk)	M & F	66
				↓testicular development	J (6 & 12 wk)	M	83
		↓Body weight		J (12 wk)	M & F	84	
		Hypotension		J (12 wk)	M & F	82	
		↓plasma T <sub>3</sub> levels		J (6 & 12 wk)	M & F	66	
		↓plasma IGF-I & IGFBP levels	J (12 wk)	M & F	85		

				↓hypothalamic AVP & CRH mRNA	J (6 & 12 wk)	M & F	65	
				↓pituitary POMC, PC1 & PC2 mRNA	J (6 & 12 wk)	M & F	65	
				↓ pituitary CRH/AVP responsiveness	J (7 mo)	F not M	67	
				↓Pituitary GR	J (7 mo)	M & F	67	
				↑basal and ACTH stimulated cortisol levels	A (1 yr)	M & F	86	
				↑pituitary CRH/AVP responsiveness	A (2 yr)	M & F	86	
				↓adreno-cortical ACTH responsiveness	A (3 yr)	M & F	87	
				↓basal ACTH and cortisol levels	A (3 yr)	M & F	87	
				↓plasma glucose levels	J (12 wk)	M & F	65	
				Insulin resistance	J (6 mo)	M & F	84	
				Glucose intolerant	A (1.5 yr)	M & F	84	
				↑Glucose stimulated insulin secretion	A (1.5 yr)	M & F	84	
				↑Fasting insulin:glucose ratio	A (2 & 3 yr)	M & F	88	
				↑Hepatic glucose-6-phosphatase activity	A (3.5 yr)	M & F	88	
	Horse	Dex	95%	↓Body weight	N	M & F	89	
				↓ CRL and adreno-cortical ACTH responsiveness	N	M & F	90	
Maternal Cortisol Overexposure	Sheep	Cortisol	20%	↑renal Na <sup>+</sup> -K <sup>+</sup> ATPase α-subunit	J (2 mo)	M & F	59	
				↓glomerular number	A (4-5 yr)	F	59	
				↑single nephron GFR	A (4-5 yr)	F	59	
				Hypertension	A (1.5 yr)	M & F	63	
					A (4-5 yr)	F	59	
				Fasting hyperinsulinaemia	A (4 yr)	M	54	
		↑Glucose stimulated insulin secretion	A (4 yr)	M	54			
			50-72%	↓Body weight	N	M & F	90	
		Periodic isolation	72%-term	↑Birth weight	N	M & F	92	
				↑Basal Cortisol concentration	N	M & F	92	
		Pig	Cortisol	33-50%	↑body weight	J (5 mo)	M	93
			ACTH	40%	↑basal LH	N	F	94
	40-65%			↑plasma CBG & ↑adrenomedullary cells	N	M & F	95	
	40-73%			↑adrenal cortex:medulla ratio	N & J (60 d)	M & F	96	
			↑Hypothalamic CRH and adrenal ACTH receptor	N	M & F	96		
			↓Hypothalamic endorphin	J (30 d)	M & F	96		

				↑Pituitary POMC mRNA ↑HPA stress responsiveness	J (60 d) J (11 wk)	M & F F	96 96
			75-93%	↓Body weight ↑plasma CBG & 5HT ↓Relative adrenal weight ↑adrenal cortex area Altered brain neurotransmitter system	N & J N & J J (4 wk) J (4 wk)	M & F M & F M & F M & F	95 95 95 95
		Social mixing	35-56%	↑hypothalamic CRH expression to social stress ↑Cortisol response to social stress	J (9 wk) J (9 wk)	F F	97 97
			67-97%	↑Cortisol response to social stress	J (9 wk)	F	97
	Cow	ACTH	20-50%	↑Body weight ↑Cortisol secretion to restraint	N (at birth) J (5 mo)	M & F M & F	98 98
		Transport	20-50%	↑Cortisol secretion to restraint ↓Cortisol clearance ↑Heart rate increment to restraint	J (5 mo) J (5 mo) J (5 mo)	M & F M & F M & F	98 98 98
Fetal Synthetic Glucocorticoid Overexposure	Sheep	Beta	72-84%	↑Glucose-stimulated insulin secretion Glucose intolerant ↓Basal insulin concentration ↑Basal insulin concentration ↑Hepatic glucose-6-phosphatase activity	J (6 mo) A (1 yr) A (2 yr) A (3 yr) A (3.5 yr)	M & F M & F M & F M & F M & F	83 83 88 88 88
				↓ Pituitary CRH/AVP responsiveness ↑ Adreno-cortical ACTH responsiveness ↓Basal and stimulated ACTH concentration ↑ Adreno-cortical ACTH responsiveness	A (1 yr) A (1 yr) A (2 yr) A (2 yr)	M & F M & F M & F M & F	86 86 87 87
				↓Brain weight	A (3.5 yr)	M & F	82
Neonatal Glucocorticoid Overexposure	Sheep	Dex	3-4 days	Altered NMDA receptor kinetics	5 d	M & F	99
	Horse	ACTH	1-5 days	↓Glucose stimulated insulin secretion ↑Basal cortisol concentrations Altered pituitary sensitivity to hypoglycaemia	J (2 & 12 wk) J (12 wk) A (1 & 2 yr)	M & F M & F M & F	100 101 102

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831 Dex=dexamethasone, Beta=betamethasone, ACTH=Adrenocorticotrophic hormone, All=Angiotensin II, AT1=Angiotensin receptor type 1, AVP=Arginine  
832 vasopressin, CBG=Corticosteroid binding globulin, CRH=Corticotropin releasing hormone, CRL=Crown rump length, GR= Glucocorticoid receptor,  
833 GRF=glomerular filtration rate, HPA=hypothalamic-pituitary-adrenal, 3 $\beta$ HSD=hydroxysteroid dehydrogenase, 5HT=5-hydroxytryptamine, IGF-I=Insulin-like  
834 growth factor I, IGFBP=Insulin-like growth factor binding protein, LH=Luteinising hormone, NMDA=N-methyl-D-aspartate, POMC=Pro-opiomelanocortin,  
835 RAS=Renin-angiotensin system, ROS=Reactive oxygen species, StAR=Steroidogenic acute regulatory protein, T<sub>3</sub>=Tri-iodothyronine, UCP1=Uncoupling  
836 protein 1.

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