

1 **EFFECTS OF MATERNAL DEXAMETHASONE TREATMENT ON**
2 **PANCREATIC β CELL FUNCTION IN THE PREGNANT MARE AND**
3 **POSTNATAL FOAL.**

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39 **ABSTRACT**

40 **Reasons for performing the study:** Synthetic glucocorticoids are used to treat inflammatory
41 conditions in horses. In other pregnant animals, glucocorticoids are given to stimulate fetal
42 maturation with long-term metabolic consequences for the offspring if given pre-term.
43 However, their metabolic effects during equine pregnancy remain unknown.

44 **Objective:** Thus, this study investigated the metabolic effects of dexamethasone
45 administration on pregnant pony mares and their foals after birth.

46 **Study Design:** Pancreatic β cell function was measured in pregnant pony mares and their
47 foals following maternal administration of dexamethasone or saline in late gestation.

48 **Methods:** Three doses of dexamethasone (200 μ g/kg im) were given to 6 pony mares at 48h
49 intervals beginning at \approx 270 days of pregnancy. Control saline injections were given to 5
50 mares using the same protocol. After fasting overnight, pancreatic β cell responses to
51 exogenous glucose were measured in the mares before, during and after treatment. After
52 birth, pancreatic β cell responses to exogenous glucose and arginine were measured in the
53 foals at 2 and 12 weeks.

54 **Results:** In mares during treatment, dexamethasone but not saline increased basal insulin
55 concentrations and prolonged the insulin response to exogenous glucose. Basal insulin and
56 glucose concentrations still differed significantly between the two groups 72h post-treatment.
57 Dexamethasone treatment significantly reduced placental area but had little effect on foal
58 biometry at birth or subsequently. Foal β cell function at 2 weeks was unaffected by maternal
59 treatment. However, by 12 weeks, pancreatic β cell sensitivity to arginine, but not glucose,
60 was less in foals delivered by dexamethasone than saline treated mares.

61 **Conclusions:** Dexamethasone administration induced changes in maternal insulin-glucose
62 dynamics, indicative of insulin resistance, and had subtle longer term effects on postnatal β
63 cell function of the foals. The programming effects of dexamethasone in horses may be
64 mediated partially by altered maternal metabolism and placental growth.

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73 **INTRODUCTION**

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75 In several species, synthetic glucocorticoids like dexamethasone are administered clinically
76 for a range of conditions, often with an inflammatory component. In horses, the anti-
77 inflammatory properties of these drugs are used to treat joint and respiratory problems as well
78 as endometritis, allergic reactions and endotoxic shock [1-4]. They have also been given to
79 regulate ovarian function in non-pregnant mares [3-5]. In humans, synthetic glucocorticoids
80 are used to treat a similar range of inflammatory diseases, but they are also given routinely to
81 healthy pregnant women threatened with preterm delivery to improve neonatal viability of
82 their infants [6]. During pregnancy, these drugs mimic the normal rise in endogenous
83 glucocorticoids seen in fetuses near term, which promotes maturation of fetal tissues and, in
84 some species, also triggers labour [7]. Synthetic glucocorticoids are, therefore, used to induce
85 delivery in cattle and sheep at or near term [8]. In mares, synthetic glucocorticoids appear to
86 be less effective at inducing delivery in late gestation and can be detrimental to pregnancy
87 outcome if given too close to full term [9, 10]. However, early delivery of viable foals has
88 been observed in response to maternal dexamethasone administration between 315 and 322
89 days of gestation [11-13].

90

91 In healthy non-pregnant animals, administration of synthetic glucocorticoids at the anti-
92 inflammatory doses has a number of side effects including metabolic actions that leads to
93 hyperglycaemia, insulin resistance and to type 2 diabetes if given in excess [14,15]. In non-
94 pregnant horses, dexamethasone causes rapid changes in glucose-insulin dynamics and
95 insulin resistance, which can persist for several days after cessation of treatment [16-20].
96 Synthetic glucocorticoids are also thought to increase the risk of laminitis, particularly in
97 horses prone to the disease [21]. In comparison, relatively little is known about the maternal
98 metabolic outcomes of dexamethasone treatment in pregnant animals, although pregnancy is
99 associated with a natural state of insulin resistance in many species including the horse [22-
100 24]. Indeed, there are many more studies of the metabolic consequences of this treatment for
101 the postnatal offspring than for the pregnant mother *per se* [25-27]. Studies in pregnant rats,
102 guinea pigs, sheep and non-human primates have shown that maternal administration of
103 synthetic glucocorticoids during late pregnancy alters fetal development and induces
104 postnatal abnormalities in cardiovascular, metabolic and endocrine function in the offspring
105 [7, 24-26]. In particular, there are changes in glucose metabolism in the adult offspring,
106 which are due, in part, to altered secretion and action of insulin [25-27]. However, nothing is

107 known about the metabolic consequences of dexamethasone treatment of pregnant horses
108 either for the mare or her foal after birth, although there are changes in maternal progestagen
109 concentrations and adreno-cortical function of the newborn foal after dexamethasone
110 administration to pregnant mares near term [13]. This study, therefore, examined pancreatic β
111 cell function in pregnant mares and their foals after birth following dexamethasone
112 administration in late gestation. Postnatally, pancreatic β cell function was tested using both
113 glucose and arginine, as they act through different mechanisms to secrete insulin and are
114 known to be effective in both fetal and newborn foals [28-30].

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117 **METHODS**

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119 **Animals**

120 A total of 11 pregnant pony mares of known gestational age were used. They were housed in
121 individual stables and fed hay *ad libitum* and concentrates twice a day (Dodson and Horrell^a
122 Mare and young stock mix, 1 kg/100kg 12.5 MJ/kg, 14% Crude protein, 4.5% Oils, 8%
123 Crude fibre). All mares delivered spontaneously without assistance. On the day of birth the
124 foals were treated with equine tetanus antitoxin (1000IU, Intervet Ltd. UK) and remained
125 with their mothers throughout the experimental period. One foal from a control mare had
126 limb deformities and was euthanised at 48h on veterinary advice. At the end of the entire
127 study, the animals were either rehomed (n=9 mares, n=5 foals) or euthanized (n=2 mares, n=5
128 foals; 200 mg/kg of sodium pentobarbitone^b) to provide tissue for other research studies. All
129 studies were carried out under the UK Animal (Scientific Procedures) Act 1986 after
130 permission from the Animal Welfare and Ethical Review Board of the University of
131 Cambridge.

132

133 **Experimental procedures**

134 **Mares:** The mares were assigned randomly to be treated intramuscularly with either
135 dexamethasone (200 μ g/kg i.m, Dexamethasone 21-phosphate^c in 0.9% w/v saline, n=6) or
136 the equivalent volume of saline as a control (0.9% w/v, n=5) on three occasions at 48 h
137 intervals beginning at a mean gestational age of 267.0 ± 12.0 days that was similar in the two
138 treatment groups (Saline, 271.0 ± 19.0 days; n=5; Dexamethasone, 263.0 ± 16.0 days; n=6;
139 $P > 0.05$, Term ~ 335 days). The two groups were of similar bodyweight at the onset of

140 treatment (Saline, 273.0 ± 19.0 kg; n=5; Dexamethasone, 251.0 ± 6.5 kg; n=6; $P>0.05$). Four
141 days before injections began, a long stay catheter (16 gauge^d) with an extension tube was
142 inserted into a jugular vein under local anaesthesia (Intra-Epicane^e). Between 08.00-08.30 h
143 daily, the jugular catheter was flushed with heparinised saline to maintain patency. An
144 intravenous glucose tolerance test was carried out 48 h before treatment began (pre-
145 treatment), then, again 24 h after the second injection (during treatment) and, finally, 72 h
146 after the third injection of saline or dexamethasone (post-treatment). In each test, blood
147 samples (10 ml) were taken at 30, 15 and 0 min before and at 5, 15, 30, 45, 60, 90 and 120
148 min after intravenous administration of glucose (0.5 g/kg, Dextrose, 40% w/v^f) for the
149 measurement of plasma glucose and insulin at all times and plasma L-lactate at 0 min. After
150 glucose administration, the catheter was flushed with 20 ml of saline (0.9% w/v). Mares were
151 without food overnight before the glucose tolerance test and did not receive their morning
152 ration of concentrates until after the glucose tolerance test was complete. Water was freely
153 available at all times. At the end of the experimental period, the catheter was removed and the
154 mares were monitored twice daily for signs of impending delivery.

155

156 **Foals:** At birth, the foal and placenta were weighed and measured. Placental area was
157 measured by laying the placenta on a plastic sheet, cutting around the area and weighing the
158 resulting template. Using the weight of a known area of the plastic sheeting, the total
159 placental area was calculated. After birth, the foals were weighed and measured weekly until
160 12 weeks. Blood samples (5 ml) were taken by venipuncture from the jugular vein of all foals
161 on the day of birth (Day 1) for measurement of plasma cortisol concentrations. At 10-12 days
162 of postnatal age, the jugular vein was catheterized as described for the mares. Beginning at 2
163 weeks, glucose (0.5 g/kg, Dextrose, 40% w/v^f,) followed by arginine (100 mg/kg^g) were
164 given intravenously over 5 min at a 48-72 h interval at doses known to be effective at
165 stimulating insulin secretion in newborn foals [29]. On each occasion, the catheter was
166 flushed with with saline (10 ml, 0.9% w/v). Blood samples (5 ml) were taken from the
167 jugular catheter at 30, 15 and 0 min before and at 5, 15, 30, 45, 60, 90 and 120 min after
168 substance administration. At 2 weeks, foals remained with their mothers throughout the
169 experiments but were muzzled to prevent suckling from 1 h before sampling began until the
170 end of the sampling period. At the end of this series of experiments, the jugular catheter was
171 removed and the foal was re-catheterised at ≈ 12 weeks. The pancreatic β cell challenges
172 were then repeated in the same order using the same protocol as at 2 weeks. To minimize

173 stress, the older foals were not muzzled but were separated from their mothers by a barrier
174 which allowed sight and interaction but no suckling for 3 h before sampling began until the
175 end of the sampling period.

176

177 **Biochemical analyses**

178

179 Blood samples were added to tubes containing either heparin or EDTA and centrifuged
180 immediately. The plasma was stored at -20 °C until analysis of plasma metabolite and
181 hormone concentrations. Plasma glucose and lactate concentrations were measured using a
182 glucose-lactate analyser^h. Plasma α -amino nitrogen concentrations were determined on
183 deproteinised plasma by the colourimetric method of Evan *et al.* [31] using glycine as a
184 standard as an index of the arginine concentrations. Plasma insulin concentration was
185 measured by an ELISA assayⁱ validated for use with equine plasma [32]. The intra- and inter-
186 assay coefficients of variation for the insulin assay were 3.4% and 13% respectively. Plasma
187 cortisol was assessed using an ELISA^j validated for equine plasma as described previously
188 [33]. The intra- and inter-assay coefficients of variation for this assay were 4.3% and 8.9%
189 respectively.

190

191 **Statistical analyses**

192 All values are expressed as means (\pm SEM). Statistical comparisons between groups were
193 made using Student's t-test, or one-way or two-way ANOVA with repeated measures (time)
194 followed by Turkey *post hoc* test, as appropriate. When time or treatment was identified as
195 significant factors by two-ANOVA, the two treatment groups were analysed separately by
196 one way ANOVA. The responses to glucose and arginine administration were measured as
197 delta concentrations from baseline values at 0 min. Insulin data was normalized by log
198 transformation, where required. For each challenge, the area under the curve (AUC) for the
199 glucose (AUCG), α -amino nitrogen (AUCAN) and insulin (AUCI) responses was calculated
200 as the integrated plasma concentration after administration of glucose or arginine from 0-120
201 min above the baseline concentration at 0 min for all positive values. The area above the
202 curve (AAC) for the hypoglycaemic response to insulin was calculated in the same way. All
203 statistical analyses were performed using Sigma-Stat^k and considered significant when
204 $P < 0.05$.

205

206 **RESULTS**

207

208 **Effects of dexamethasone treatment on the pregnant mares.**

209

210 *Basal insulin and metabolite concentrations*

211 Plasma concentrations of glucose, lactate and insulin after an overnight fast did not differ
212 between the two groups of mares before treatment began (Table 1). However, 3 days after
213 beginning treatment, fasted concentrations of plasma glucose, lactate and insulin were
214 significantly higher in dexamethasone than saline treated animals (Table 1). Three days after
215 finishing dexamethasone treatment, fasting concentrations of lactate and insulin were not
216 significantly different from the pre-treatment values (Table 1). However, post-treatment
217 fasting levels of insulin were higher in dexamethasone- than saline-treated mares (Table 1).
218 In contrast, fasting concentrations of plasma glucose in dexamethasone-treated mares post-
219 treatment were lower than both their own pre-treatment values and the post-treatment
220 concentrations in saline-treated mares (Table 1). There were no changes in the fasting
221 concentrations of glucose, lactate or insulin in mares receiving saline with time over the
222 treatment period (Table 1).

223

224 *Pancreatic β response to glucose*

225 The increment and maximal concentration of plasma glucose after glucose administration did
226 not differ significantly with time over the treatment period in either group of mares or
227 between the two groups of mares at any time over the treatment period (Figure 1A, Table 1).
228 The AUCG were also unaffected by treatment (Figure 2A). In common with previous
229 findings [23], the insulin response to glucose administration varied widely between pregnant
230 mares, even pre-treatment (Figure 2B). Pre-treatment, there were no significant differences
231 in the insulin increment, maximal insulin concentration or time course of the insulin response
232 to glucose between saline- and dexamethasone-treated mares (Table 1, Figure 1B). However,
233 during dexamethasone treatment, the insulin response to glucose was more prolonged than
234 seen in mares receiving saline (Figure 1B). The maximum increment and the maximal
235 concentrations of plasma insulin were also greater in the dexamethasone than saline group of
236 mares during treatment (Table 1). In the dexamethasone- but not the saline-treated mares, the
237 area under the insulin curve (AUCI) during treatment was greater than their respective pre-
238 treatment values (Figure 2B). Relative insulin secretion, measured as the ratio of the AUCI
239 to AUCG, showed the same profile with treatment as the AUCI (Figure 2C).

240

241 ***Delivery***

242 Mares delivered uneventfully at a mean gestational age of 335.0 ± 2.9 days (Saline, $338.0 \pm$
243 0.9 days; $n=5$, 2 male & 3 female foals: Dexamethasone, 333.0 ± 5.3 days; $n=6$, 3 males 3
244 females, $P>0.05$). At delivery, placental weight was similar while gross placental area was
245 significantly less in dexamethasone- than saline-treated mares (Table 2).

246

247

248 **Effects of dexamethasone treatment on the foals**

249

250 ***Biometry***

251 All foals stood and suckled within 1h of delivery, and were classified as mature by clinical
252 criteria [34]. On the day of delivery, plasma cortisol concentrations were similar in foals
253 delivered by dexamethasone (22.3 ± 2.7 ng/ml, $n = 6$) and saline treated mares (21.8 ± 4.0
254 ng/ml, $n = 5$, $P>0.05$). At birth, foals of dexamethasone-treated mares tended to be smaller
255 than those of saline treated mares but there were no statistically significant differences in
256 body weight, crown rump length or height at the withers between the two groups of newborn
257 foals ($P=0.07$, all cases, Table 2). Only femur length of the newborn foals was significantly
258 shorter after maternal dexamethasone treatment (Table 2). Birth weight per cm^2 of placenta
259 was unaffected by maternal treatment (Saline, 3.26 ± 0.18 g/cm^2 , $n = 5$; Dexamethasone, 3.13
260 ± 0.20 g/cm^2 , $n = 6$, $P>0.05$). None of the morphometric measurements of the foals differed
261 significantly between the two treatment groups at either 2 or 12 weeks (Table 2). There were
262 also no differences in the growth rate or fractional growth rate of any of the body
263 measurements of the foals between the two treatment groups over the first 12 weeks after
264 birth ($P>0.05$, data not shown). In addition, there were no differences in the plasma cortisol
265 concentrations between the two treatment groups at 2 or 12 weeks (2 weeks; Saline, $40.5 \pm$
266 4.8 ng/ml, $n = 4$; Dexamethasone, 40.3 ± 6.2 ng/ml, $n = 6$: 12 weeks; Saline 27.5 ± 2.6 ng/ml,
267 $n = 4$; Dexamethasone, 35.0 ± 3.4 ng/ml, $n = 6$, $P>0.05$ both ages)

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271 ***Pancreatic β cell responses***

272 *Glucose:* At 2 and 12 weeks, there were no significant differences in the basal concentrations
273 of plasma glucose, α -amino nitrogen or insulin before administration of glucose or arginine
274 between the two treatment groups (Table 3). At both ages, there were also no significant
275 differences in the incremental or maximal concentrations of glucose or insulin in response to
276 glucose administration between the two treatment groups (Figure 3A & B, Table 3).
277 However, the maximal increment and the maximum concentration of plasma glucose and the
278 AUCG were greater at 12 weeks than 2 weeks, irrespective of maternal treatment (Figure 3A,
279 Table 3). The insulin response to glucose administration was also more prolonged at 12 than
280 2 weeks in both treatment groups (Fig 3B). However, there were no significant differences in
281 AUCI or relative insulin secretion in the foals with either maternal treatment or increasing
282 age (Table 3).

283

284 *Arginine:* The incremental and maximal concentration of plasma α -amino nitrogen in
285 response to arginine administration in the foals was unaffected by maternal treatment at 2
286 weeks (Figure 3C, Table 3). The incremental and maximal concentrations of insulin and the
287 AUCI in response to arginine were unaffected by maternal treatment at this age (Figure 3D,
288 Table 3). However, at 12 weeks, the increment in plasma α -amino nitrogen concentration
289 was more prolonged and significantly greater in foals of dexamethasone treated mares
290 (Figure 3C). Consequently, relative to controls receiving saline, the AUCAN was
291 significantly greater in 12 week old foals of dexamethasone- than saline-treated mares
292 (Figure 3D, Table 3). The increment in insulin concentration was less in the dexamethasone
293 than saline group of 12 week old foals at 5 min after arginine administration although not at
294 the later sampling times (Figure 3D). As a result, relative insulin secretion, the ratio of AUCI
295 to AUCAN, was significantly less in the dexamethasone than saline group of foals at 12
296 weeks but not at 2 weeks of postnatal age (Table 3). At 12 weeks, the insulin response to
297 arginine administration was more prolonged than in the younger foals, irrespective of
298 maternal treatment (Figure 3D).

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302 **DISCUSSION**

303 The study shows for the first time that treatment of pregnant pony mares at \approx 270 days of
304 gestation with the synthetic glucocorticoid, dexamethasone, induces transient maternal
305 hyperinsulinaemia, indicative of increased insulin resistance. During treatment, basal fasting
306 concentrations of plasma insulin and the increment in plasma insulin in response to
307 exogenous glucose were significantly greater in dexamethasone-treated mares than in control
308 mares receiving saline. The maternal AUCI was also significantly greater during treatment
309 with dexamethasone but not saline. Maternal dexamethasone treatment had no effect on the
310 length of gestation but reduced gross placental area at delivery. Despite these changes,
311 dexamethasone treatment of pregnant mares had relatively little effect on their offspring with
312 only subtle changes in pancreatic β cell function in their 12 week old foals.

313

314 **Maternal effects**

315 Several previous studies have shown that dexamethasone at doses similar to those used here
316 induces hyperglycaemia, hyperinsulinaemia and insulin resistance in non-pregnant horses
317 [16-20]. These changes are seen in response to single and multiple doses of dexamethasone
318 and begin within 2 h of administration with recovery taking up to 2 week after multiple
319 dosing [1, 16, 18, 19]. Similar increases in peripheral insulin resistance are seen in non-
320 pregnant horses with hyperadrenocorticism induced by pars intermedia dysfunction [35]. In
321 the current study, dexamethasone treatment of pregnant mares caused fasting
322 hyperinsulinaemia, lactacidaemia and an enhanced pancreatic β cell response to exogenous
323 glucose without any changes in glucose dynamics or fasting hyperglycaemia. At this stage of
324 pregnancy, mares are already insulin resistant and have a significant feto-placental glucose
325 requirement [22, 24, 36]. Dexamethasone treatment, therefore, appears to further increase
326 maternal insulin resistance without the concomitant changes in glycaemia observed in non-
327 pregnant horses. This suggests that, during dexamethasone treatment, any reduction in
328 glucose uptake by insulin resistant maternal tissues is balanced by an increase in glucose
329 transfer to the rapidly growing fetus that is insulin independent [36]. Maternal
330 hyperinsulinaemia and insulin resistance together with elevated whole body glucose disposal
331 have been seen in pregnant rats treated with dexamethasone in late gestation when glucose
332 demands of the gravid uterus are high [37]. Increased lactate production has also been
333 observed previously in response dexamethasone treatment in human and other species [38,

334 39]. In the current study, the insulin-glucose dynamics of the dexamethasone-treated mares
335 were still abnormal 72 h after ceasing treatment with significant differences in basal fasting
336 concentrations of both insulin and glucose between the two treatment groups at this time.
337 Indeed, in dexamethasone-treated mares, fasting glucose concentrations were lower post-
338 treatment than pre-treatment, although post-treatment insulin concentrations were not
339 significantly different from the pre-treatment values. Since dexamethasone would have
340 cleared from the maternal circulation by 72 h post-treatment [18], these findings indicate that
341 insulin sensitivity of the dexamethasone-treated mares may have been greater post- than pre-
342 treatment, consistent with previous findings in non-pregnant horses receiving dexamethasone
343 [17, 20].

344

345 **Foal effects**

346 In the current study, dexamethasone treatment had little apparent effect on gestational length
347 or prepartum maturation as all foals were mature at birth, and stood and sucked within the
348 normal time, irrespective of maternal treatment [34]. At delivery, placental area was smaller
349 after maternal dexamethasone treatment, consistent with the known growth inhibitory effects
350 of synthetic glucocorticoids on the placenta in other species [26, 40]. This led to a tendency
351 for smaller foals after maternal dexamethasone treatment but only femur length was reduced
352 significantly at birth. In previous studies of dexamethasone administration, foal birth weight
353 was also unaffected, although crown rump length was reduced at dexamethasone doses
354 similar to those used here [11-13]. In other species, maternal administration of synthetic
355 glucocorticoids at a similar dose and stage of gestation reduces fetal weight and results in
356 lower birth weight [7, 25-27]. Collectively, these observations suggest that dexamethasone
357 can restrict fetal bone growth but may be less effective at inhibiting growth of fetal somatic
358 tissues in horses than other species.

359

360 Maternal dexamethasone treatment had little effect on pancreatic β cell sensitivity to glucose
361 of the foals 2 and 12 weeks after birth. The insulin responses of the foals to glucose were
362 similar in the two treatment groups at both ages and resembled those published previously for
363 age-matched foals of mares receiving no treatment [41]. In other species, maternal
364 dexamethasone treatment during late pregnancy alters glucose-stimulated insulin secretion in

365 the offspring, although at older postnatal ages than studied here [25-27]. However, there were
366 developmental changes in equine β cell responses to glucose over the first 12 week of
367 postnatal life, irrespective of maternal treatment. Insulin secretion in response to exogenous
368 glucose switched from a monophasic response at 2 weeks to a more biphasic pattern of
369 response at 12 weeks, without any significant age-related change in AUCI. This resembled
370 the developmental profile of glucose-stimulated insulin secretion seen previously in foals of
371 untreated mares [41]. Since the AUCG was significantly greater at 12 weeks than 2 weeks of
372 age, the current findings suggest that insulin sensitivity decreases with age over the first 12
373 weeks of postnatal life, irrespective of maternal treatment, in keeping with previous findings
374 in older untreated foals [42].

375

376 In contrast to β cell glucose sensitivity, the insulin response to arginine was affected by
377 maternal dexamethasone treatment by the time the foals were 12 weeks old. The increments
378 in α -amino-nitrogen and AUCAN were greater and the initial insulin increment and relative
379 insulin secretion were less in the dexamethasone- than saline-treated group of 12 week old
380 foals. Collectively, these findings suggests that arginine may be less effective at stimulating
381 insulin release and that insulin may be less effective at stimulating tissue amino acid uptake
382 in 12 week old foals after maternal dexamethasone treatment. Arginine depolarises β cells
383 directly through ATP-dependent K^+ channels whereas glucose acts indirectly on these
384 channels via generation of ATP [28]. In fetal horses, the pancreatic β cell response to glucose
385 but not arginine increases near term indicating that there is prepartum maturation of the
386 insulin secretory pathways upstream of β cell depolarization [30]. The current findings
387 suggests that maturation of the insulin secretory pathway continues after birth and is
388 influenced by maternal dexamethasone treatment, particularly at and/or downstream of the
389 depolarising K^+ channels. However, further studies are required to establish the extent to
390 which these changes in β cell sensitivity to arginine are due directly to dexamethasone
391 exposure *in utero* or indirectly to the maternal metabolic and other physiological alterations
392 that affect perinatal development of the foal. Certainly, maternal glucocorticoid overexposure
393 in late pregnancy is known to influence mammary development and milk quality in the mare
394 [13].

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399 **CONCLUSIONS**

400 Maternal dexamethasone treatment has metabolic actions in the mare during late pregnancy
401 but relatively little effect on the growth or pancreatic endocrine function her foal after birth.
402 However, there were treatment differences in placental area and femur length of the foal at
403 birth. There are also differences in the pancreatic β cell response of the foals to arginine after
404 maternal dexamethasone treatment, which became evident between 2 and 12 weeks. This
405 indicates that dexamethasone treatment during pregnancy can have longer term metabolic
406 consequences for the offspring in horses as occurs in other species [25-27]. Certainly,
407 glucocorticoid overexposure of foals immediately after birth is known to have metabolic and
408 endocrine effects long after weaning [41, 43]. Taken together, the current findings suggest
409 that dexamethasone may have programming effects during equine pregnancy, possible due to
410 maternal metabolic changes and placental growth restriction.

411

412 **Author declaration**

413 The authors declare that they have no competing interests.

414

415 **Ethical Animal Research**

416 All studies were carried out under the UK Animal (Scientific Procedures) Act 1986 (ASPA)
417 after permission from the Animal Welfare and Ethical Review Board of the University of
418 Cambridge. Animals to be re-homed were discharged from the Act with veterinary approval.

419

420 **Manufacturer's addresses**

421 ^aDodson and Horrell Ltd, Islip, Northamptonshire, UK.

422 ^bPentoject, Animal Care Ltd., Dunnington, York, UK.

423 ^cDexamethasone 21-phosphate, Sigma-Aldrich Ltd, Dorset, UK.

424 ^dArrow International Inc, Reading, PA, USA.

425 ^eIntra-Epicane, Arnolds Veterinary Products, Shrewsbury, Shropshire, UK.

426 ^fArnolds Veterinary Products, Shrewsbury, Shropshire, UK.

427 ^gSigma-Aldrich Co. St. Louis, MO, USA.

428 ^hYellow Springs 2300 Stat Plus, YSI Ltd., Farnborough, UK.

429 ⁱMercordia, Uppsala, Sweden.

430 ^jSiemens Medical Solutions Diagnostics, Los Angeles, CA, USA.

431 ^kStatistical Software version 2.0, Point Richmond, CA, USA.

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433

434 **Authorship**

435 ALF devised the study. VLA and NBH carried out the experiments. OAV and JKJ undertook
436 the biochemical analyses. ALF and OAV drafted the manuscript. All authors commented and
437 contributed to the draft.

438

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441

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454 **FIGURE LEGENDS**

455 **Figure 1:** Mean \pm SEM increments in the plasma concentrations of (A) glucose and (B)
456 insulin from basal 0 min values in response to glucose administration (at 0 min) pre-, during
457 and post-treatment of pregnant mares with dexamethasone (filled symbols, n=6) or saline
458 (open symbols, n=4-5). Details of the dosing regimen are given in the text. *Significant
459 increment from basal value either for a specific sampling time and treatment group when
460 given singly or for both groups when spanning a range of sampling times (t-test, $P<0.05$).

461

462 **Figure 2:** Mean \pm SEM values of the area under the response curve (AUC) for (A) glucose,
463 AUCG, and (B) insulin, AUCI and (C) relative insulin secretion (AUCI:AUCG) in pregnant
464 mares pre-, during and post-treatment with dexamethasone (filled columns, n=6) or saline
465 (open columns, n=4-5). Details of the dosing regimen are given in the text. Within each
466 treatment group, columns with different superscripts are significantly different from each
467 other (one-way ANOVA, $P<0.05$).

468

469 **Figure 3:** Mean \pm SEM increments from basal 0min values in the plasma concentrations of
470 (A) glucose and (B) insulin in response to glucose administration at 0 min and of (C) α -amino
471 nitrogen and (D) insulin in response to arginine administration at 0 min in foals at 2 and 12
472 weeks delivered by mares treated with dexamethasone (filled symbols, n=6) or saline (open
473 symbols, n=4). *Significant increment from basal value either for a specific sampling time
474 and treatment group when given singly or for both groups when spanning a range of sampling
475 times (t test, $P<0.05$). † significantly different from value in the saline treatment group (two-
476 way ANOVA, $P<0.05$). # significantly different from values at 2 weeks in both treatment
477 groups (two-way ANOVA, $P<0.05$).

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612 **Table 1:** Mean \pm SEM basal concentrations of plasma glucose, L-lactate and insulin after an
 613 overnight fast and the maximum concentrations of glucose and insulin in response to glucose
 614 administration (0.25 g/kg) pre-treatment (-48 h from 1st dose), during treatment (+24 h after
 615 2nd dose) and post-treatment (+72 h after 3rd dose) of pregnant mares with 3 doses of
 616 dexamethasone (Dex, im, 200 μ g/kg) or saline (im, 0.9%w/v) at 48 h intervals during late
 617 pregnancy.

			Pre- treatment	During treatment	Post- treatment
Basal values	Glucose mmol/l	Saline	6.39 \pm 0.91	5.53 \pm 0.44	5.65 \pm 0.41
		Dex	6.98 \pm 0.76 ^a	7.24 \pm 0.33 ^{a*}	4.80 \pm 0.04 ^{b*}
	L-Lactate mmol/l	Saline	1.42 \pm 0.30	1.50 \pm 0.29	1.06 \pm 0.08
		Dex	1.41 \pm 0.10 ^a	2.58 \pm 0.27 ^{b*}	1.05 \pm 0.13 ^a
	Insulin ng/ml	Saline	340 \pm 260	60 \pm 20	56 \pm 10
		Dex	440 \pm 160 ^{ab}	1130 \pm 250 ^{b*}	201 \pm 80 ^{a*}
Maximal values	Glucose mmol/l	Saline	19.18 \pm 1.67	16.93 \pm 0.89	20.55 \pm 2.21
		Dex	19.13 \pm 1.18	20.63 \pm 1.93	17.57 \pm 0.57
	Insulin ng/ml	Saline	805 \pm 315	860 \pm 412	983 \pm 441
		Dex	1173 \pm 278 ^a	2542 \pm 271 ^{b*}	1178 \pm 234 ^a

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 619 Values within rows with different superscripts are significantly different from each other
 620 (P<0.05, one-way ANOVA) * Significantly different from value in the saline treated group at
 621 the same time (P<0.05, t-test).

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Table 2 : Mean \pm SEM biometry measurements of the placenta at birth and of the foals at birth, 2 weeks and 12 weeks of postnatal age delivered by mares treated with 3 doses of saline (n=4-5) or dexamethasone (n=6, 200 μ g/kg) at 48 h intervals during late pregnancy.

	Measurement	Age	Treatment	
			Saline	Dexamethasone
Placenta	Weight (kg)	Birth	2.27 \pm 0.20	1.88 \pm 0.20
	Area (cm ²)	Birth	8290 \pm 510	6870 \pm 340*
Foal	Weight (kg)	Birth	26.9 \pm 2.0	21.6 \pm 1.8
		2 weeks	42.1 \pm 5.0	34.2 \pm 2.7
		12 weeks	100.1 \pm 7.4	85.4 \pm 3.8
	Crown rump length (cm)	Birth	74.3 \pm 2.6	68.3 \pm 3.4
		2 weeks	82.2 \pm 3.7	74.7 \pm 2.9
		12 weeks	116.5 \pm 2.2	109.8 \pm 2.3
	Height at wither (cm)	Birth	80.7 \pm 2.5	73.9 \pm 2.3
		2 weeks	86.3 \pm 3.0	81.3 \pm 2.2
		12 weeks	103.4 \pm 2.9	98.8 \pm 1.5
	Femur length (cm)	Birth	25.7 \pm 0.3	23.0 \pm 0.7*
		2 weeks	29.0 \pm 1.8	27.0 \pm 0.8
		12 weeks	37.3 \pm 1.3	35.0 \pm 0.7

*Significantly less than value in the saline treated group (P<0.02, t-test)

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Table 3: Mean \pm SEM vales of basal (fasted) and maximal concentrations of glucose, α -amino nitrogen and insulin, the area under of the curve (AUC) of the glucose, α -amino nitrogen and insulin responses and the relative insulin secretion in response to administration of glucose (0.5 g/kg) or arginine (100 mg/kg) in foals at 2 weeks and 12 weeks of postnatal age delivered by mares treated with saline (n=4) or dexamethasone (Dex, n=6) during late pregnancy.

		2 weeks	12 weeks
Glucose administration			
Basal glucose mmol/l	Saline	7.92 \pm 0.29	6.78 \pm 0.30
	Dex	7.70 \pm 0.36	6.88 \pm 0.52
Maximum glucose mmol/l	Saline	21.08 \pm 1.12	26.80 \pm 1.67#
	Dex	20.87 \pm 1.11	24.67 \pm 1.18#
AUC glucose (AUCG) mmol/l/min	Saline	268 \pm 23	702 \pm 104#
	Dex	277 \pm 21	599 \pm 29#
Basal insulin ng/l	Saline	194 \pm 115	50 \pm 10#
	Dex	60 \pm 20	27 \pm 5#
Maximum insulin ng/l	Saline	730 \pm 310	386 \pm 52
	Dex	470 \pm 150	272 \pm 62
AUC insulin (AUCI) ng/l/min	Saline	14309 \pm 4150	24680 \pm 5630
	Dex	14425 \pm 5855	17869 \pm 4100
Relative insulin secretion AUCI: AUCG	Saline	58 \pm 22	38 \pm 10
	Dex	53 \pm 21	30 \pm 8
Arginine administration			
Basal α -amino nitrogen mmol/l	Saline	2.67 \pm 0.13	2.16 \pm 0.10#
	Dex	2.40 \pm 0.19	2.01 \pm 0.11#
Maximum α -amino nitrogen mmol/l	Saline	3.18 \pm 0.04	3.08 \pm 0.24
	Dex	3.33 \pm 0.34	3.00 \pm 0.21
AUC α -amino nitrogen (AUC α AN) mmol/l/min	Saline	21 \pm 7	11 \pm 2
	Dex	24 \pm 7	24 \pm 2†
Basal insulin ng/l	Saline	63 \pm 27	55 \pm 11
	Dex	96 \pm 55	40 \pm 14
Maximum insulin ng/l	Saline	247 \pm 45	227 \pm 17
	Dex	265 \pm 61	155 \pm 31
AUC insulin (AUCI) ng/l/min	Saline	5195 \pm 1608	7620 \pm 1438
	Dex	6622 \pm 2456	5170 \pm 1625
Relative insulin secretion AUCI:AUC α AN	Saline	538 \pm 378	764 \pm 161
	Dex	873 \pm 598	212 \pm 54†

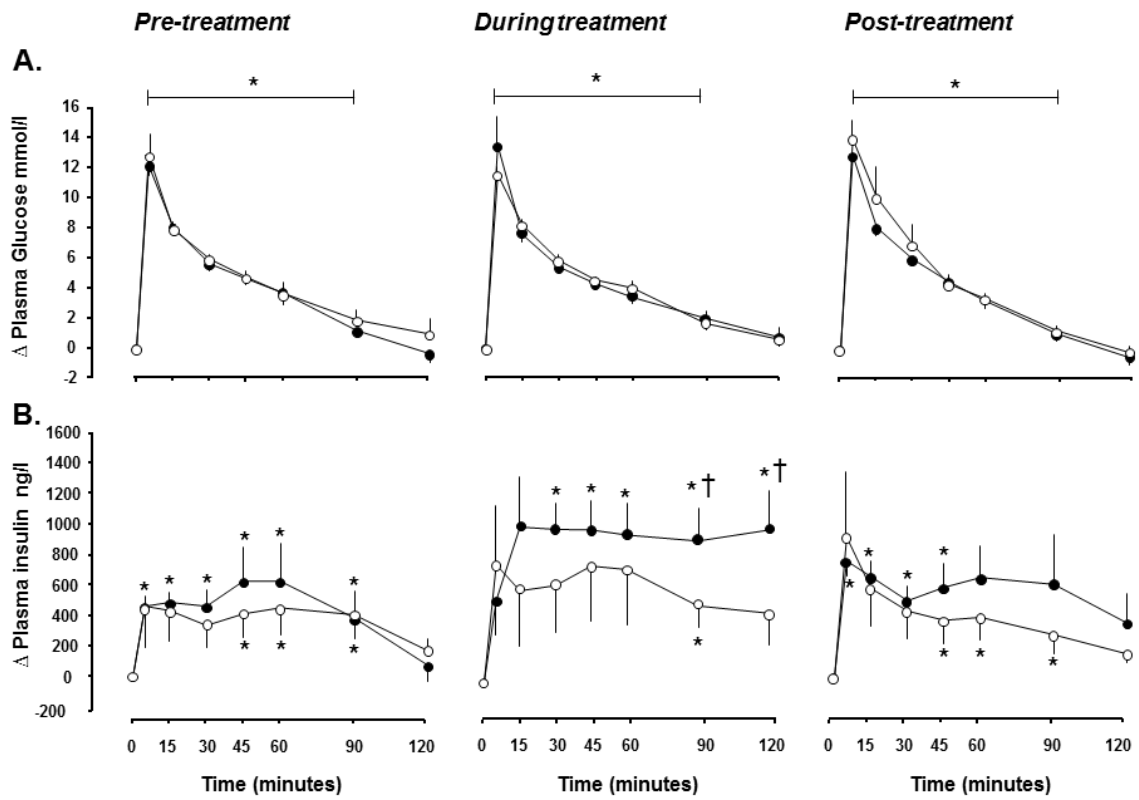
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634 # Significantly different from value in the same group at 2 weeks (P<0.01, t-test).

635 †Significantly different from value in the saline treated group at the same age (P<0.05, t-test).

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637 **Figure 1**



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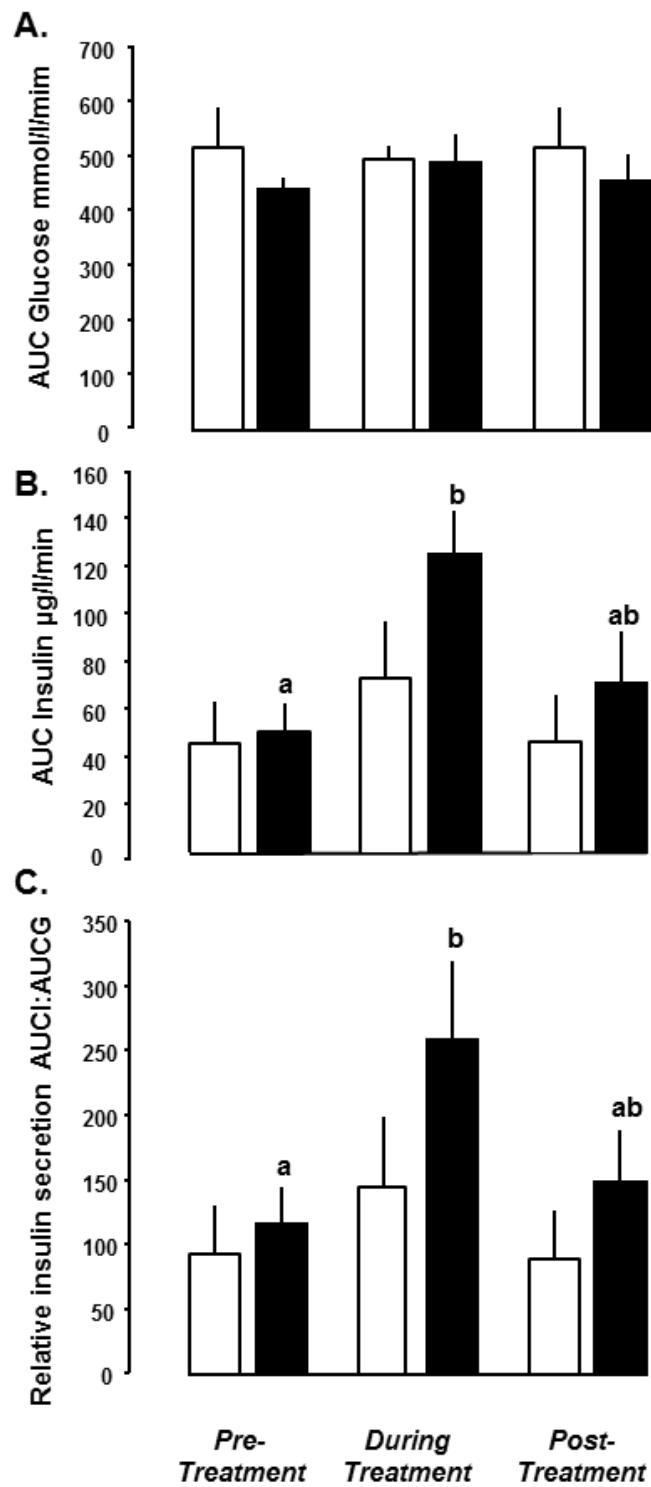
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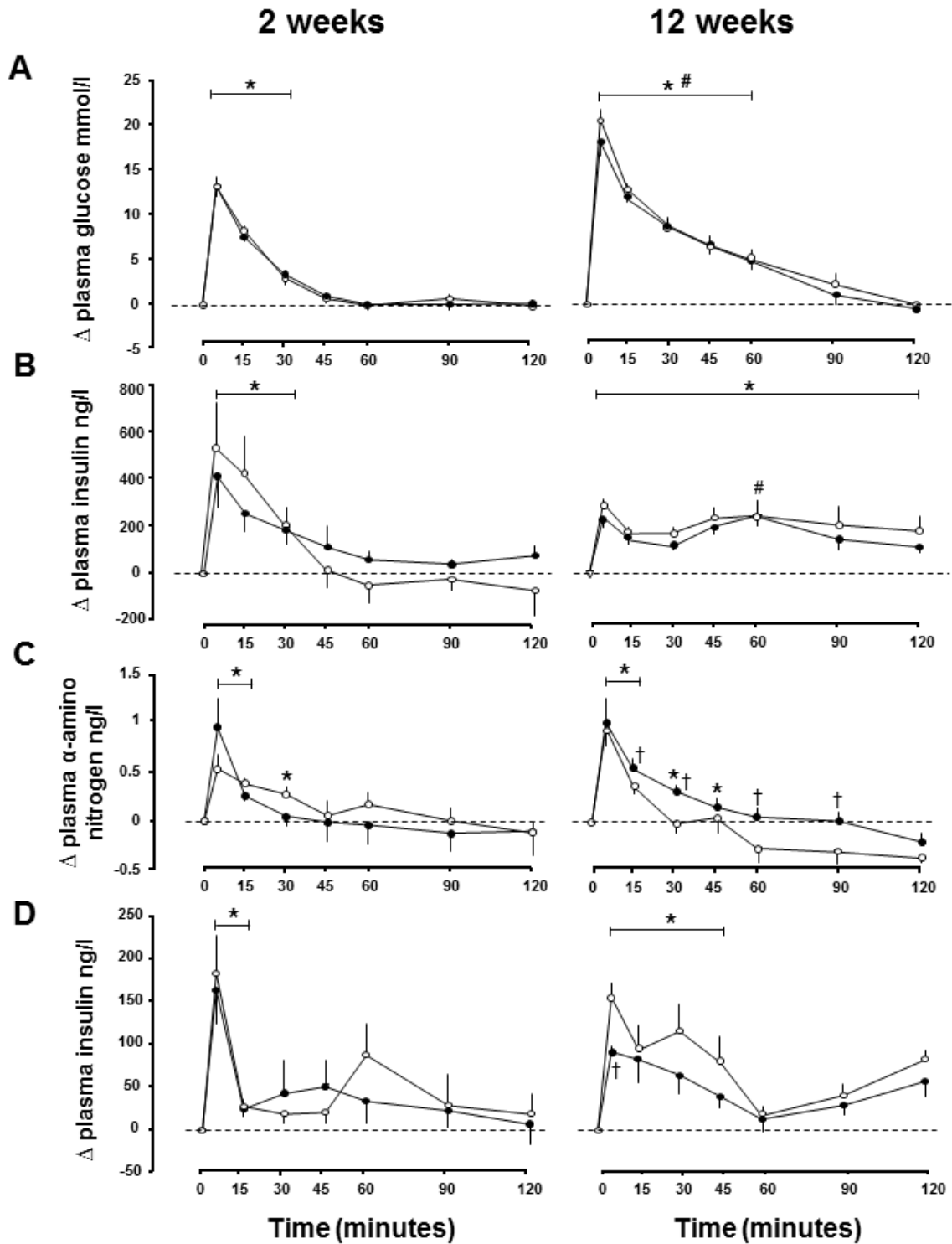
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