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

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ABSTRACT

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

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

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

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

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

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

INTRODUCTION

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

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

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

METHODS

Animals

A total of 11 pregnant pony mares of known gestational age were used. They were housed in individual stables and fed hay *ad libitum* and concentrates twice a day (Dodson and Horrell [a] Mare and young stock mix, 1 kg/100kg 12.5 MJ/kg, 14% Crude protein, 4,5% Oils, 8% Crude fibre). All mares delivered spontaneously without assistance. On the day of birth the foals were treated with equine tetanus antitoxin (1000IU, Intervet Ltd. UK) and remained with their mothers throughout the experimental period. One foal from a control mare had limb deformities and was euthanised at 48h on veterinary advice. At the end of the entire study, the animals were either rehomed (n=9 mares, n=5 foals) or euthanized (n=2 mares, n=5 foals; 200 mg/kg of sodium pentobarbitone[b]) to provide tissue for other research studies. All studies were carried out under the UK Animal (Scientific Procedures) Act 1986 after permission from the Animal Welfare and Ethical Review Board of the University of Cambridge.

Experimental procedures

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

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

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

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

Biochemical analyses

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

Statistical analyses

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

RESULTS

Effects of dexamethasone treatment on the pregnant mares.

Basal insulin and metabolite concentrations

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

Pancreatic β response to glucose

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

Delivery

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

Effects of dexamethasone treatment on the foals

Biometry

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

Pancreatic β cell responses

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

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

DISCUSSION

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

Maternal effects

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

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

Foal effects

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

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

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

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

CONCLUSIONS

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

Author declaration

The authors declare that they have no competing interests.

Ethical Animal Research

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

Manufacturer's addresses

[a]Dodson and Horrell Ltd, Islip, Northamptonshire, UK.

[b]Pentoject, Animal Care Ltd., Dunnington, York, UK.

[c]Dexamethasone 21-phosphate, Sigma-Aldrich Ltd, Dorset, UK.

[d]Arrow International Inc, Reading, PA, USA.

[e]Intra-Epicane, Arnolds Veterinary Products, Shrewsbury, Shropshire, UK.

[f]Arnolds Veterinary Products, Shrewsbury, Shropshire, UK.

[g] Sigma-Aldrich Co. St. Louis, MO, USA.

[h]Yellow Springs 2300 Stat Plus, YSI Ltd., Farnborough, UK.

[i]Mercordia, Uppsala, Sweden.

[j]Siemans Medical Solutions Diagnostics, Los Angeles, CA, USA.

[k]Statistical Software version 2.0, Point Richmond, CA, USA.

Authorship

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

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FIGURE LEGENDS

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

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

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

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

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

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

Table 2 : Mean ± 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 µg/kg) at 48 h intervals during late pregnancy.

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

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

Table 3: Mean ± 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 ± 0.29	6.78 ± 0.30
	Dex	7.70 ± 0.36	6.88 ± 0.52
Maximum glucose mmol/l	Saline	21.08 ± 1.12	26.80 ± 1.67#
	Dex	20.87 ± 1.11	24.67 ± 1.18#
AUC glucose (AUCG) mmol/l/min	Saline	268 ± 23	702 ± 104#
	Dex	277 ± 21	599 ± 29#
Basal insulin ng/l	Saline	194 ± 115	50 ± 10#
	Dex	60 ± 20	27 ± 5#
Maximum insulin ng/l	Saline	730 ± 310	386 ± 52
	Dex	470 ± 150	272 ± 62
AUC insulin (AUCI) ng/l/min	Saline	14309 ± 4150	24680 ± 5630
	Dex	14425 ± 5855	17869 ± 4100
Relative insulin secretion AUCI: AUCG	Saline	58 ± 22	38 ± 10
	Dex	53 ± 21	30 ± 8
Arginine administration			
Basal α-amino nitrogen mmol/l	Saline	2.67 ± 0.13	2.16 ± 0.10#
	Dex	2.40 ± 0.19	2.01 ± 0.11#
Maximum α-amino nitrogen mmol/l	Saline	3.18 ± 0.04	3.08 ± 0.24
	Dex	3.33 ± 0.34	3.00 ± 0.21
AUC α-amino nitrogen (AUCαAN) mmol/l/min	Saline	21 ± 7	11 ± 2
	Dex	24 ± 7	24 ± 2†
Basal insulin ng/l	Saline	63 ± 27	55 ± 11
	Dex	96 ± 55	40 ± 14
Maximum insulin ng/l	Saline	247 ± 45	227 ± 17
	Dex	265 ± 61	155 ± 31
AUC insulin (AUCI) ng/l/min	Saline	5195 ± 1608	7620 ± 1438
	Dex	6622 ± 2456	5170 ± 1625
Relative insulin secretion AUCI:AUCαAN	Saline	538 ± 378	764 ± 161
	Dex	873 ± 598	212 ± 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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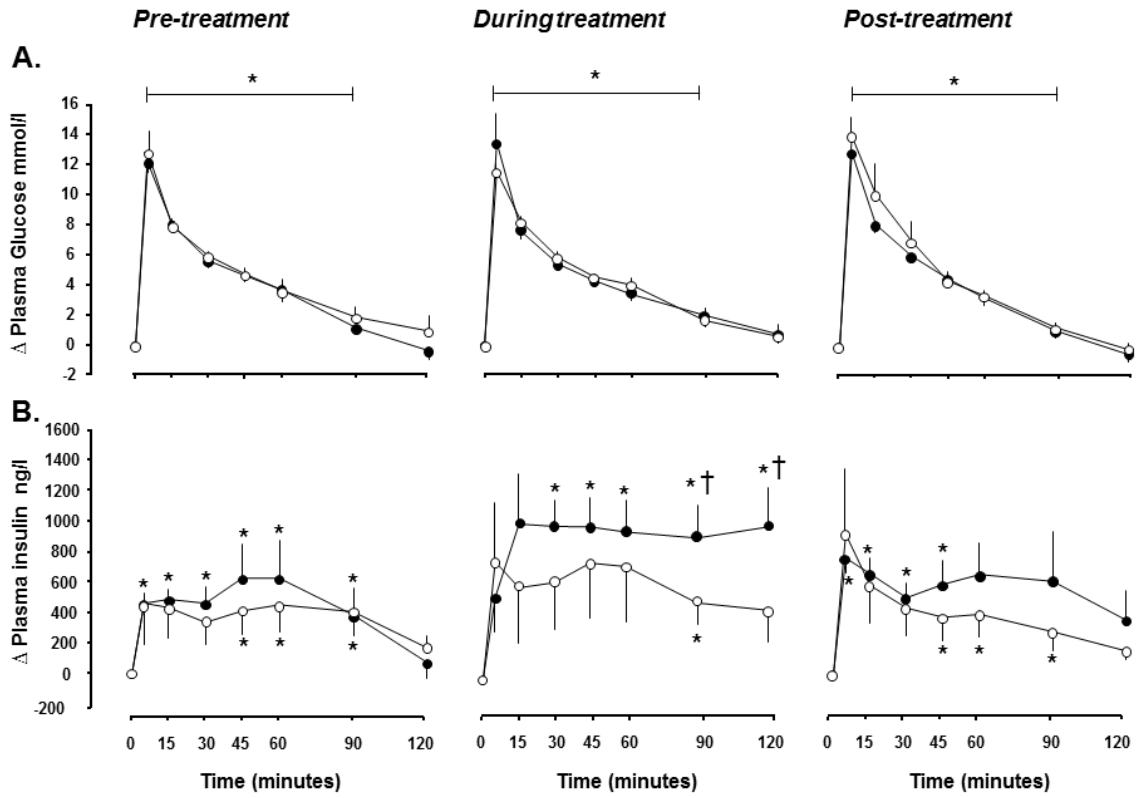
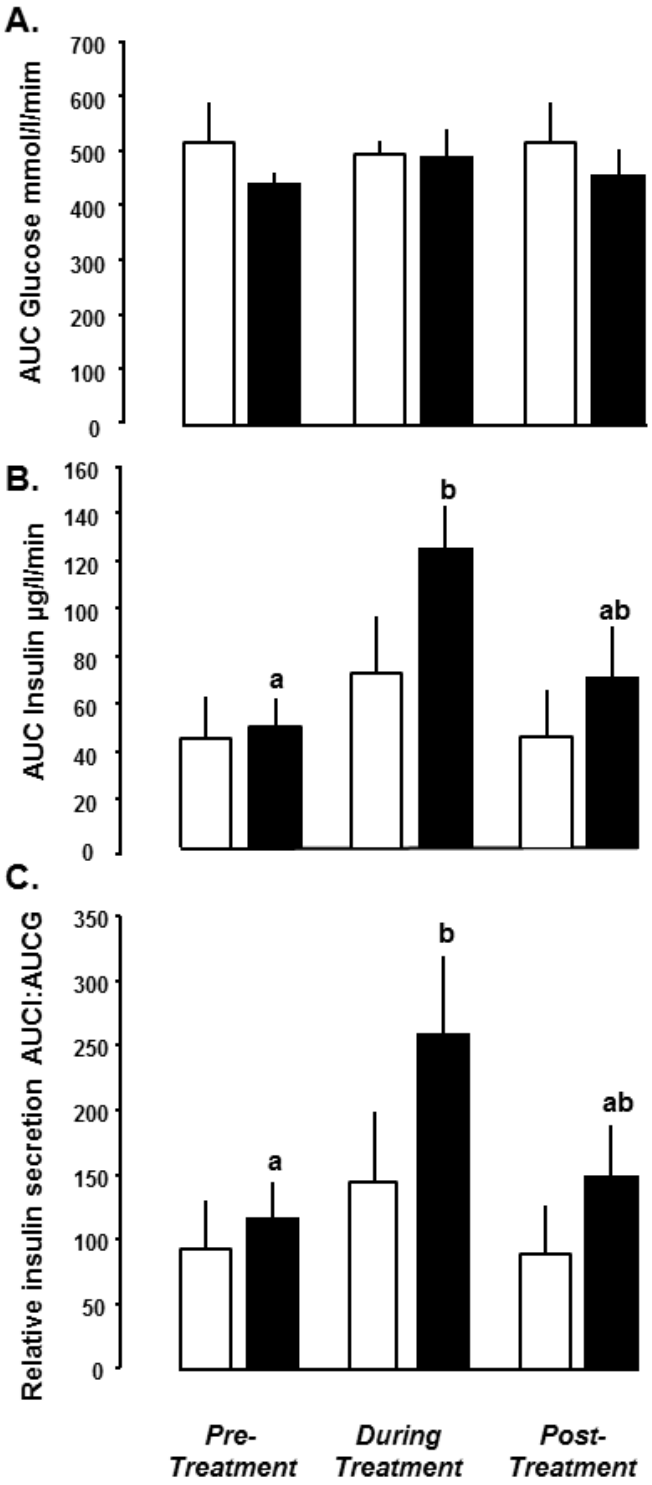
Figure 1


Pre-treatment
During treatment
Post-treatment
A.
Δ Plasma Glucose mmol/l
B.
Δ Plasma insulin ng/l
Time (minutes)
Time (minutes)
Time (minutes)

649 **Figure 2**



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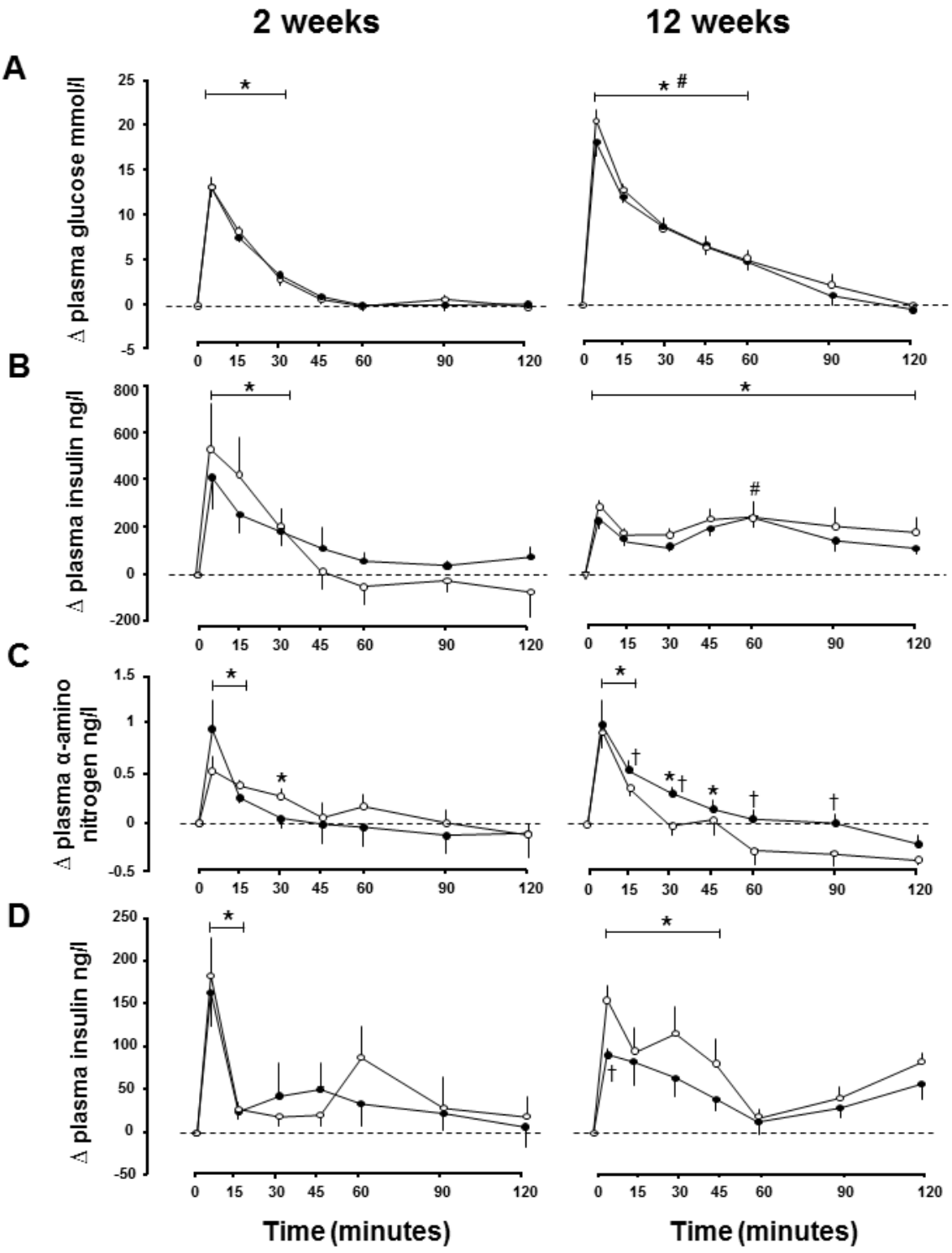
653 **Figure 3**



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