PHYSIOLOGICAL DEVELOPMENT OF THE EQUINE FETUS DURING LATE GESTATION

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41 **SUMMARY**

42 In many species, the pattern of growth and physiological development *in utero* has an important role in
43 determining not only neonatal viability but also adult phenotype and disease susceptibility. Changes in
44 fetal development induced by a range of environmental factors including maternal nutrition, disease,
45 placental insufficiency and social stresses have all been shown to induce adult cardiovascular and
46 metabolic dysfunction that often lead to ill health in later life. Compared to other precocious animals,
47 much less is known about the physiological development of the fetal horse or the longer term impacts
48 on its phenotype of altered development in early life because of its inaccessibility *in utero,* large size
49 and long lifespan. This review summaries the available data on the normal metabolic, cardiovascular
50 and endocrine development of the fetal horse during the second half of gestation. It also examines the
51 responsiveness of these physiological systems to stresses such as hypoglycaemia and hypotension
52 during late gestation. Particular emphasis is placed on the role of the equine placenta and fetal
53 endocrine glands in mediating the changes in fetal development seen towards term and in response to
54 nutritional and other environmental cues. The final part of the review presents the evidence that the
55 early life environment of the horse can alter its subsequent metabolic, cardiovascular and endocrine
56 phenotype as well as its postnatal growth and bone development. It also highlights the immediate
57 neonatal environment as a key window of susceptibility for programming of equine phenotype. Although
58 further studies are needed to identify the cellular and molecular mechanisms involved, developmental
59 programming of physiological phenotype is likely to have important implications for the health and
60 potential athletic performance of horses, particularly if born with abnormal body weight, premature or
61 dysmature characteristics or produced by assisted reproductive technologies, indicative of an altered
62 early life environment.

63

64 **INTRODUCTION**

65

66 Compared to other species, much less is known about the physiology of the fetal horse. Its long and
67 variable gestational period, large size and relative inaccessibility within a diffuse placenta make studying
68 development *in utero* in the conscious state particularly challenging in equids [1]. As a consequence,
69 the physiological data available on fetal horses are largely from ponies between mid and late gestation

and relate to specific organ and tissue systems that can be studied without complex interventions [1-3]. For instance, there are very few equine studies investigating the effects of manipulating fetal concentrations of hormones known to be important in controlling physiological development *in utero* in other species. This review concentrates on (i) the three aspects of physiological development that have been studied most extensively in the equine fetus, namely metabolic, cardiovascular and endocrine development, and (ii) the developmental programming of equine physiological phenotype by environmental cues during intrauterine and early neonatal life.

GROWTH AND METABOLIC DEVELOPMENT

The equine fetus grows linearly during the second half of gestation and gains 75% of its final birth weight between mid and late gestation [4]. The fetal nutrient requirements for tissue accretion and oxidative metabolism, therefore, increase rapidly over this period as fetal mass rises. This places a significant drain on maternal nutritional resources and is accompanied by increasing maternal insulin resistance as pregnancy advances [5-7]. In turn, this increases the availability of maternal glucose for feto-placental use and is associated with ontogenic changes in the rates of fetal and utero-placental metabolism towards term [8, 9]. Maternal body condition score (BCS) as a proxy index of maternal nutrient availability does not appear to be related to foal birthweight over the normal range consistent with the concept that the fetus has priority over the mother for available nutrients [10, 11]. However, at the extremes of the BCS range or when there are acute reductions in BCS during pregnancy there is evidence for a positive correlation between maternal BCS and foal birthweight [12, 13].

Absolute rates of umbilical glucose uptake increase between 180 and 290 days of gestation but then remain stable until term (≈ 335 days), despite a trend for an increasing transplacental gradient in the glucose concentration [9]. Consequently, the weight specific rates of fetal glucose uptake decline progressively towards term (Figure 1A). Similar ontogenic decreases in glucose utilisation per kg body weight are observed between 180 days and term [9]. In contrast, the umbilical uptake of oxygen continues to rise progressively between mid and late gestation in line with fetal weight [9], so that there is no change in the weight specific rate of fetal oxygen consumption throughout the second half of gestation (Figure 1B). As a result of these changes, glucose tends to make a smaller contribution to

fetal oxidative metabolism with increasing gestational age towards term, despite the increasing requirement for energy of the growing fetus [8]. The gestational fall in glucose metabolism is ameliorated, in part, by increasing the distribution of uterine glucose uptake away from the utero-placental tissues towards the fetus and by the onset of utero-placental production of lactate [9]. Placental delivery of lactate into the umbilical circulation becomes significant in the last 20% of gestation [8], although the rate of delivery declines towards term when expressed per kg fetal body weight (Figure 1C). In sheep, fructose is also synthesised from glucose in the placenta and released into the umbilical circulation for oxidative use by the fetus [14]. Like sheep, fetal fructose concentrations are high in the horse but the source and metabolic fate of this fructose in fetal horses remains unknown [1]. In addition, the equine placenta is lipid permeable and can synthesise lipid in late gestation [15]. Fat may, therefore, be a more important oxidative fuel in fetal horses near term than in other species [16].

The equine placenta undergoes a number of other functional and structural changes during the later part of gestation, which facilitate nutrient transfer to the fetus [17]. Unlike the cotyledonary ovine placenta, the diffuse equine placenta increases in weight and macroscopic area right up until term [4]. The fetal villi continue to elongate and branch throughout the second half of equine pregnancy, which increases the total villous area for exchange 5 fold between mid and late gestation [18]. The distance between the maternal and fetal blood vessels also decreases with increasing gestational age towards term [19], which will enhance transplacental transfer of substances transported by simple diffusion, such as oxygen [17]. In contrast to the ovine placenta, there is little evidence for gestational changes in the abundance of glucose transporters (GLUTs) in the equine placenta towards term [20, 21]. Spatial localisation of the different GLUTs on the equine placental membranes in mid-late gestation shows that GLUT1 and GLUT3 are the predominant isoforms and are used sequentially to transfer glucose from the maternal to the fetal circulation in line with findings in other species with an epitheliochorial type of placenta [21, 22]. Given the kinetics of the different isoforms, localisation of GLUT3 at the maternal-fetal interface may aid glucose transport to the equine fetus, particularly at lower glucose concentrations [17, 21]. Gene expression of other GLUT isoforms, including those sensitive to insulin, has been detected in pre-implantation equine embryos [23], but whether these isoforms are involved in placental transport of glucose and other hexoses like fructose in late gestation remains unclear. In addition, a range of environmental factors have been shown to alter the morphological and transport characteristics

of the equine placenta at term, including season of the year, nutrition, maternal size and the genetically determined demands of the fetus for growth (Table 1). Collectively, these studies have shown that the equine placenta can adapt to help support fetal growth when nutrient availability is restricted *in utero* (Table 1).

The normal reduction in mass of utero-placental tissue between the maternal and fetal circulations towards term may contribute to the gestational decrease in utero-placental glucose consumption and account for the increased proportion of uterine glucose uptake delivered to the equine fetus between mid and late gestation [8]. In contrast, distribution of uterine oxygen uptake between the fetal and uteroplacental tissues does not change with gestational age, which suggests that, like the fetus, uteroplacental tissues may change their preferred oxidative substrate in late gestation. Nevertheless, weight specific rates of glucose consumption by equine uteroplacental tissues are high compared to other species near term, which adds to the metabolic burden on the mare in late pregnancy [17]. When utero-placental glucose availability is restricted in late gestation by short term fasting of the mare, maternal and fetal concentrations of lipids and free fatty acids rise in association with enhanced utero-placental production of prostaglandins and the early onset of labour [30, 31]. This suggests that utero-placental metabolism is responsive to substrate availability and, by switching to fat metabolism when glucose availability is limited, the utero-placental tissues may increase production of arachidonic acid, the precursor of prostaglandin synthesis [8, 15, 30].

Equine fetuses appear to have a limited capacity for endogenous glucose production in late gestation compared to other precocial species [16]. They have lower activities of key gluconeogenic enzymes and store less glycogen in their livers than sheep fetuses at a similar stage of late gestation [17, 32]. They do not activate glucogenesis close to term or in response to short term maternal fasting, unlike ovine fetuses [9, 33]. Fetal glucose utilisation, therefore, falls by a greater extent in fetal horses than sheep during maternal undernutrition [9, 33]. Moreover, during short term maternal fasting, fetal horses use proportionately more of their available glucose for oxidative metabolism, which suggests that they have limited ability to switch to alternative fuels when glucose availability is reduced acutely [9]. There is, therefore, a tight metabolic balance between the mare and her gravid uterus in late gestation, which may have adverse consequences for pregnancy outcome if maternal nutrient availability is lower or fetal

nutrient demands are higher than normal for the stage of gestation [31, 34]. This may explain, in part, the variable gestational length in mares and their inability to carry twins to full term [34].

CARDIOVASCULAR DEVELOPMENT

In common with other species, fetal blood pressure increases with gestational age towards term in the horse. It rises from a mean value of 30-35 mmHg at 150 days to about 80-90 mmHg at term in association with a decline in heart rate from 120 to 80 beats per minute (Figure 2) [35, 36]. These changes occur gradually between 150 and 300 days and then accelerate towards term (Figure 2) with a further decrease in heart rate in the last 30 minutes before birth [35-37]. They are accompanied by increases in the fetal concentrations of several vasoactive hormones including adrenaline, noradrenaline and vasopressin and by elevated fetal plasma and pulmonary concentrations of the angiotensin converting enzyme responsible for producing angiotensin II, another potent circulating vasoconstrictor [35, 36, 39]. Vasoconstriction of peripheral vessels and pressor responses to fetal administration of phenylephrine, angiotensin II and vasopressin are also all greater at 300 than 200 days of gestation with further maturational changes during the immediate neonatal period [36, 40, 41]. This may reflect changes in receptor density, efficiency of intracellular receptor coupling or an increased mass, or contractility, of cardiac muscle and vascular smooth muscle.

In contrast, fetal cardiac baroreceptor sensitivity decreases over the last third of gestation [41], which suggests that there may be central resetting of baroreflex function to accommodate the ontogenic rise in basal blood pressure. In turn, this rise in blood pressure would help maintain placental perfusion. Indeed, blood flow to the placenta and hind limbs of the fetal horse increases in line with the rise in blood pressure towards term [1, 36]. In the hind limbs, there is also an ontogenic fall in basal vascular resistance which leads to an increase in the weight specific flow towards term [36]. In contrast, the gestational rise in umbilical flow does not keep pace with the increase in fetal weight, despite the rise in fetal blood pressure, so umbilical flow per kg fetus decreases by 50% between mid and late gestation [9].Consequently, weight specific umbilical flow is low in the horse compared to other species in late gestation, probably due, in part, to the lack of a ductus venosus in the fetal horse [1]. Less is known about the vascular shunts in the fetal circulation of equids than other species, although there are

morphological changes in the foramen ovale of the fetal equine heart towards term which may aid the functional closure of this shunt at birth [42]. Taken together, the maturational changes in the cardiovascular systems towards term prepare the foal for the loss of the low resistant placental pathway and for the greater flexibility in blood flow required to support new postnatal activities such as exercising muscles, gastrointestinal nutrient absorption and regulated heat loss through the skin.

ENDOCRINE DEVELOPMENT

In all species studied to date including the horse, there are significant changes in the functioning of fetal endocrine glands during late gestation [43]. These lead to gestational changes in the circulating concentrations and tissue bioavailability of a range of hormones in both normal and adverse conditions. In the horse, many of the ontogenic changes in endocrine function occur in the last 1-2% of gestation, much closer to term than in other precocious species [2, 44].

Pancreas: Basal plasma concentrations of insulin change little between mid and late gestation or during the perinatal period in fetal horses (Figure 3A), despite increasing sensitivity of the fetal pancreatic β cells to glucose over this period of gestation [45]. Exogenous administration of glucose does not evoke insulin secretion before about 200 days of gestation. Thereafter, there is a prompt β cell response to exogenous glucose, which increases in magnitude between 260 and 290 days and then again close to term when the fetus is maturing in preparation for delivery [46]. In contrast, the response of fetal pancreatic β cells to the amino acid, arginine, changes little with gestational age, even in the immediate prepartum period [46]. These observations suggest that, in late gestation, there are maturational changes in the glucose signalling pathway of fetal β cells upstream of the mechanism of insulin vesicle release used by both glucose and arginine. Much less is known about pancreatic α cell function in the fetal horse. Glucagon concentrations increase in fetal horses during late gestation to peak at birth (Figure 3B). Equine pancreatic α cells also respond to arginine from late gestation onwards but appear to be relatively insensitive to changes in fetal glycaemia [47]. Thus, glucagon does not appear to be a glucoregulatory hormone *in utero* consistent with the limited glucogenic capacity of the fetal horse

whereas insulin probably regulates fetal glucose utilisation and growth in relation to glucose availability in this species as occurs in other animals [16, 51].

Sympatho-adrenal system: Basal circulating concentrations of the catecholamines, adrenaline and noradrenaline, increase in fetal horses in the period immediately before birth and peak at birth or shortly thereafter (Figure 3C). For most of this period, noradrenaline levels are 2-5 fold higher than those of adrenaline (Figure 3C). Sympatho-adrenal responses to asphyxia and hypoglycaemia also increase during late gestation and again immediately after birth [49]. These gestational changes are probably due to increased innervation of the adrenal medulla and/or increased effectiveness of the splanchnic nerves at releasing catecholamines. Before birth, there is little adrenaline secretion in response to either asphyxia or hypoglycaemia but, by 7-14 days after birth, these responses are rapid and significantly greater than those to noradrenaline [44]. These observations suggest that activation of phenyl-N-methyl-tranferase (PNMT), the enzyme responsible for adrenaline synthesis, occurs very close to term in the adrenal medulla of fetal equids [52]. The poor adrenergic response of the fetal horse to stimuli may explain in part the lack of glucagon secretion in response to hypoglycaemia because adrenaline is known to be a fetal α cell secretogue in other species [16, 47].

Hypothalamic-pituitary-adrenal axis: Activation of the fetal hypothalamic-pituitary-adrenal (HPA) axis in the period before birth is important for many of the maturational processes essential for neonatal survival (Figure 3D & E) [53]. This activation results in a prepartum rise in cortisol concentrations in the fetal circulation (Figure 3E), which induces functional and structural changes in a wide range of fetal tissues that have to assume new roles at birth. In fetal horses, the prepartum cortisol surge occurs very late in gestation compared with other species and is driven by maturational changes at all levels of the HPA axis (Figure 3D & E) [54, 55]. The adrenal content of the enzymes responsible for cortisol production increase in late gestation and adrenal weight doubles over the last 5% of gestation, primarily due to growth of the zona fasiculata [50, 56]. Adrenocortical sensitivity to exogenous and endogenous adrenocorticotrophic hormone (ACTH) increases between 290 days and term in parallel with the changes in adrenal size and steroidogenic capacity, and with the increased pituitary release of ACTH in response to stressful stimuli, such as hypoglycaemia [50, 57]. The gestational changes in the pulsatile pattern of ACTH levels in normal unstressed conditions also suggest that there are changes in the

249 release of the hypothalamic releasing factors, corticotropic releasing hormone and arginine
250 vasopressin, and/or in the abundance of their receptors on the pituitary corticotrophs towards term in
251 the fetal horse [57].
252
253 In fetal horses, the plasma cortisol concentration in late gestation is positively correlated with blood
254 pressure, pressor and peripheral vasoconstrictor responses to vasoactive hormones, the slope of the
255 cardiac baroreflex, plasma and pulmonary ACE concentrations, adrenal PNMT activity, hepatic
256 glycogen content and the plasma concentrations of tri-iodothyronine, adrenaline and vasopressin [32,
257 35, 36, 40, 41, 44, 49, 58]. These relationships suggest that, in common with other species, cortisol has
258 an important role in the prepartum maturation of these and other tissue and organ systems in the horse
259 [2, 53]. However, the window for equine maturation is narrow and close to term, which may explain the
260 range of neonatal maladjustment conditions seen clinically in foals compared to other precocious
261 species [59]. These conditions include overt prematurity, dysmaturity and neonatal maladaptation
262 syndrome [60].
263
264 The late activation of the HPA axis in fetal horses may also have consequences for the timing and onset
265 of labour in the mare [61]. During late pregnancy, the fetal adrenal glands appear to be the primary
266 source of pregnenolone (P5) that is required for utero-placental production of the progestagens
267 essential for maintaining quiescence of the equine myometrium [62]. Since P5 is also the precursor of
268 cortisol, the prepartum onset of adrenal cortisol secretion probably accounts for the reduction in fetal
269 P5 concentrations observed in the days preceding birth (Figure 3F). In turn, this reduces the utero-
270 placental P5 supply, utero-placental progestagen synthesis and the maternal progestagen
271 concentrations with consequences for uterine contractile activity [44, 63]. The onset of labour may,
272 therefore, be linked to fetal maturation via the prepartum changes in adrenal steroid synthesis in fetal
273 equids as occurs in other species, although the range of neonatal immaturity syndromes suggests that
274 this link is not as tightly coupled in horses as seen in sheep and other ruminants [44, 60]. In part, this
275 may relate to the ability of utero-placental tissues to increase prostaglandin production, independently
276 of fetal HPA axis activation and changes in the progestagenic environment in certain circumstances
277 [31, 63].
278

Gonads: The gonads of the horse fetus expand and then regress in size during the second half of gestation due to altered growth of the interstitial cells synthesising steroids including the oestrogen precursor, dehydroepiandrosterone [64, 65]. Maternal oestrogen concentrations therefore closely parallel the weight profile of the fetal gonads during the second half of pregnancy and are reduced rapidly by fetal gonadectomy at 250 days of gestation [66]. In contrast, gonadectomy of the fetal horse had little effect on maternal progestogen concentrations in late pregnancy but did reduce maternal prostaglandin concentrations during labour at term in association with weak uterine contractions [65]. In addition, compared to their sham-operated controls, gonadectomised foals were growth restricted at birth at term, indicative of a reduced transplacental supply of nutrients and/or oxygen associated perhaps with the lack of oestrogen dependent changes in utero-placental blood flow [63, 65, 66]. Furthermore, gonadectomised foals were dysmature at birth with poor neonatal viability, which suggests a more complex set of interactions than previously thought may exist between the fetal gonads, HPA axis and the placenta in co-ordinating fetal maturation with the onset of equine labour at term.

Collectively, the prepatum endocrine changes in the fetus activate many of the physiological systems and homeostatic mechanisms that have little or no function *in utero* but are essential for survival *ex utero* including glucoregulation, thermoregulation and the maintenance of blood pressure, pO_2 and perfusion of key tissues such as the brain. Onset of these regulatory processes support the novel postnatal functions like locomotion and intermittent feeding as well as the responses to new environmental stressors such as diurnal temperature variations and the presence of predators. Certainly, when the normal prepartum maturational changes in endocrine function are circumvented by acute maternal illness, placentitis or clinical induction of labour, neonatal viability is poor, even during the period of gestation between 320 and 360 days considered full term in the horse [59, 60]. In many species the prepartum endocrine changes, particularly in the HPA axis, tightly synchronise fetal maturation at the cell, tissue and systems levels with the mechanisms controlling the onset of labour to maximise the chances of offspring survival at birth [53]. However, the relationship between gestational age, fetal maturation and parturition appears to be more complex and less well co-ordinated in the horse [59, 60]. Consequently, foals are not always ready for extrauterine life when born at full term and, conversely, they can be delivered physiologically mature well before 320 days and survive when they would not be expected to do so on the basis of gestational age alone [59]. In part, this reflects the long

gestational period, the natural variation in gestational length, the high demands for glucose by the gravid uterus and the very short time frame for final prepartum maturation in the horse relative to other species producing precocious offspring.

DEVELOPMENTAL PROGRAMMING OF PHYSIOLOGICAL PHENOTYPE

Changes in fetal growth and development induced by alterations in placental development and maternal dietary intake may have consequences for the foal long after birth [67, 68]. In other species, both epidemiological and experimental studies have shown that environmental conditions during early life have an important role in determining the adult physiological phenotype that develops from the genotype inherited at conception, even when there is little if any change in birth weight [2, 69]. However, compared to other species, relatively little is known about the developmental programming of the adult equine phenotype, partly because of its long lifespan [3].

Changes in the metabolic, cardiovascular and endocrine phenotype have been observed in neonatal and juvenile foals in response to maternal undernutrition, altered dietary composition and glucocorticoid administration during late pregnancy (Table 2). Similarly, variations in birth weight caused by maternal parity and embryo transfer between different equine breeds are associated with differences in the endocrine and metabolic profiles of neonatal and older pre-weaning foals (Table 2) and with changes in the postnatal growth rate [83, 84]. To date, the physiological studies have concentrated on insulin-glucose dynamics and on the functioning of the HPA axis and cardiovascular system with a greater emphasis on newborn and pre-weaning foals than older animals [82, 67]. In particular, there are postnatal changes in glucose tolerance, insulin secretion and sensitivity, and in the HPA and catecholaminergic responses to hypoglycaemic and hypotensive stimuli (Table 2). In addition, these metabolic and endocrine changes are often accompanied by alterations in postnatal growth and bone development [7, 29, 71]. To date, the pre- and post-natal studies suggest that developmental programming of equine physiological phenotype by environmental conditions *in utero* tracks from intra- to extra-uterine life and is related to alterations in resource allocation to the fetus, mediated, in part, by the accompanying changes in placental development (Table 1). This potential role of the placenta in

339 developmental programming also has implications for the phenotype of foals produced by assisted
340 reproductive technologies because use of *in vitro* culture and a recipient uterus unprimed by a natural
341 embryo produces an abnormal environment for development that can alter embryonic cell fate decisions
342 and trophoblast differentiation epigenetically [68, 85, 86].
343
344 In addition to the prenatal manipulations, experimental overexposure to cortisol in the days immediately
345 after birth leads to altered glucose-insulin dynamics and HPA function in newborn, pre-weaning and
346 young adult horses (Table 2). There are alterations in pituitary secretion of ACTH and in the apparent
347 sensitivity of the adrenal cortex to the circulating ACTH which suggest that the ACTH secreted is less
348 bioactive in the adult animals exposed to excess cortisol neonatally [82, 83]. Similarly, insulin secretion
349 by the β cells in response to both glucose and amino acids is altered by neonatal glucocorticoid
350 overexposure in an age-related manner with muscle specific changes in insulin receptor abundance by
351 adulthood [79, 80]. In general, the postnatal physiological changes induced by neonatal cortisol
352 overexposure become more pronounced with increasing age and appear to be sex-linked in some
353 instances (Table 2) [2, 80, 87]. These findings have implications for the phenotype of premature and
354 dysmature foals that have naturally elevated cortisol concentrations in the immediate neonatal period
355 [48, 88].
356
357 Overall, the findings suggest that, in common with other species, environmental cues during early life
358 have an important role in determining the physiological phenotype of the horse (Figure 4), with
359 implications for its growth, health and athletic performance in later life [2, 89]. Indeed, adult
360 susceptibility to conditions such as hyperlipidaemia, metabolic syndrome, laminitis and exercise-
361 induced pulmonary dysfunction may all be related to environmental conditions experienced during early
362 life. In addition, the studies undertaken to date highlight the late gestation and immediate neonatal
363 period as key windows of developmental programming in the horse (Figure 4), consistent with the
364 structural and functional maturation of equine tissues so close to term. However, further studies are
365 needed to determine the extent and molecular mechanisms of this programming, particularly in older
366 horses.
367
368

FIGURE LEGENDS

Figure 1: Mean (±SD) rates of umbilical uptake of (A) glucose, (B) oxygen and (C) lactate by the fetal ponies at different gestational ages (term ≈ 335 days). N = 4-6 individual fetuses at each gestational age. Columns with different letters are significantly different in value from each other (one way ANOVA with Holm-Sidak *post doc* test, $P<0.05$). † Not significantly different from zero (t-test for significant of a single mean, $P>0.05$). Data from [8, 9].

Figure 2: Mean (±SD) values of (A) arterial blood pressure and (B) heart rate of fetal ponies at different gestational ages (term ≈ 335 days). N = 4-9 individual fetuses at each gestational age. Columns with different letters are significantly different in value from each other (one way ANOVA with Holm-Sidak *post hoc* test, $P<0.05$). Data from [35, 36].

Figure 3: Mean (±SEM) plasma concentrations of A) insulin (n=9 fetuses), B) glucagon (n=4-8 fetuses), C) catecholamines (adrenaline, filled circles; noradrenaline, open circles, n=8-9 fetuses), D) ACTH (n=6 fetuses), E) cortisol (n=6 fetuses) and (F) prenenolone (P5, n=4 fetuses) in fetal ponies with respect to days from birth. Data from [36, 44, 46-50].

Figure 4: Schematic diagram showing the environmental cues that have developmental consequences for the fetal and newborn foal with subsequent outcomes for their physiological phenotype in later life.

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Figure 1

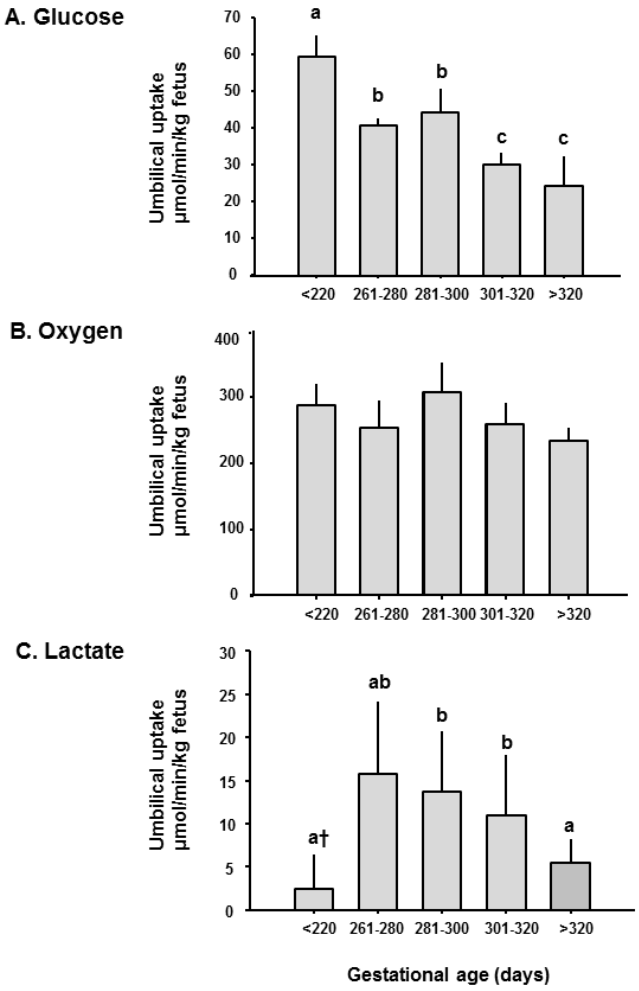

A. Glucose
Umbilical uptake µmol/min/kg fetus
70
60
50
40
30
20
10
0
a
b
b
c
c
<220
261-280
281-300
301-320
>320
B. Oxygen
Umbilical uptake µmol/min/kg fetus
400
300
200
100
0
<220
261-280
281-300
301-320
>320
C. Lactate
Umbilical uptake µmol/min/kg fetus
30
25
20
15
10
5
0
a†
ab
b
b
a
<220
261-280
281-300
301-320
>320
Gestational age (days)

FIGURE 2

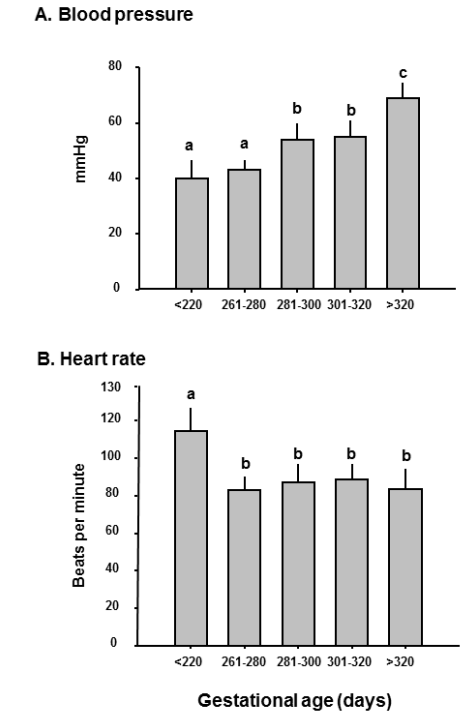

A. Blood pressure
mmHg
80
60
40
20
0
a
a
b
b
c
<220
261-280
281-300
301-320
>320
B. Heart rate
Beats per minute
130
120
100
80
60
40
20
0
a
b
b
b
b
<220
261-280
281-300
301-320
>320
Gestational age (days)

FIGURE 3

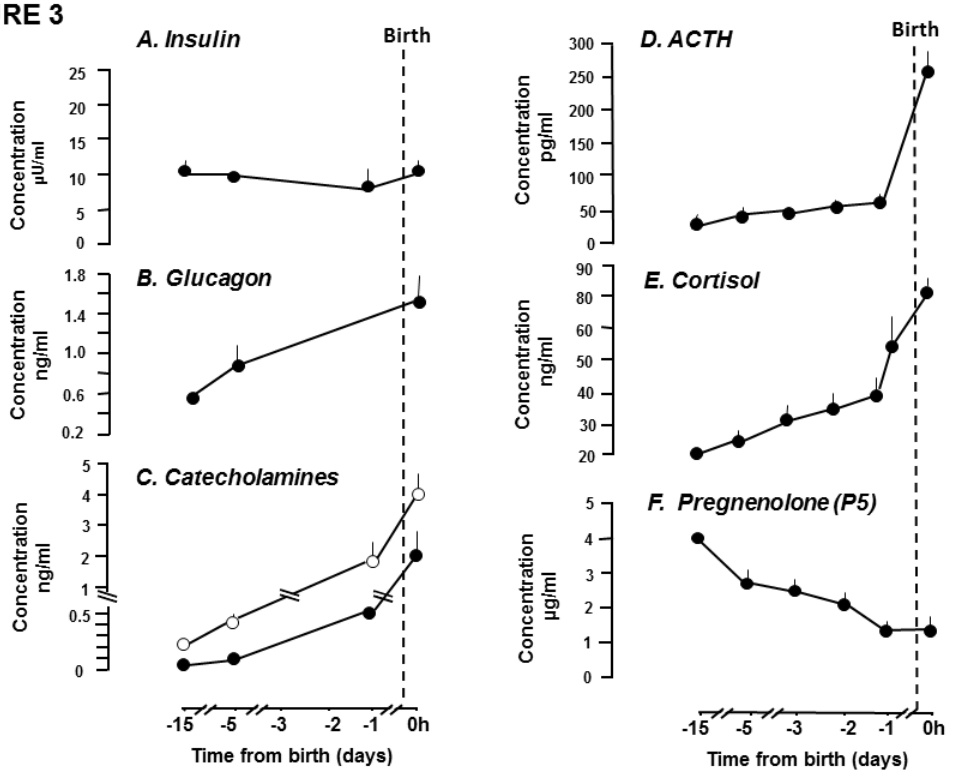
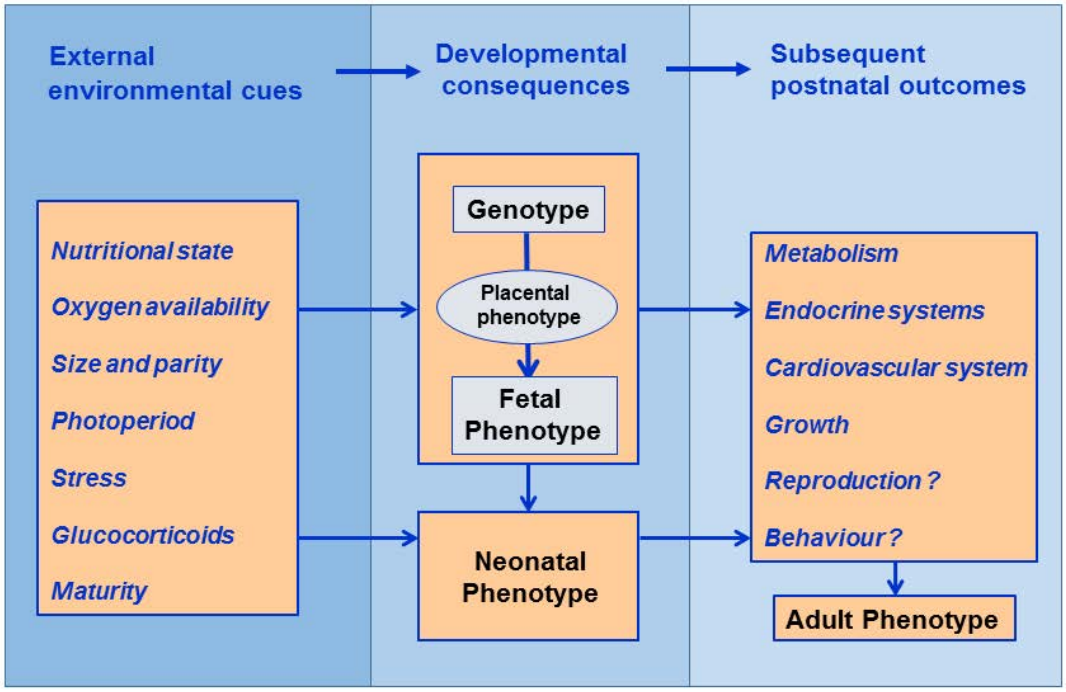

A. Insulin
Birth
Concentration µU/ml
25
20
15
10
5
0
B. Glucagon
Concentration ng/ml
1.8
1.4
1.0
0.6
0.2
C. Catecholamines
Concentration ng/ml
5
4
3
2
1
0.5
0
-15
-5
-3
-2
-1
0h
Time from birth (days)
D. ACTH
Birth
Concentration pg/ml
300
250
200
150
100
50
0
E. Cortisol
Concentration ng/ml
90
80
60
50
40
30
20
F. Pregnenolone (P5)
Concentration µg/ml
5
4
3
2
1
0
-15
-5
-3
-2
-1
0h
Time from birth (days)

Figure 4



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