

Tutorial Article

Fetal programming for athletic performance in the horse: potential effects of IUGR

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Introduction

Fetal growth and development depends upon the intrauterine environment responsible for supplying nutritional, metabolic and endocrine requirements, the majority of which are derived directly or indirectly from the maternal source. Fetal growth retardation has been defined as a condition of pregnancy in which the developing fetus undergoes a pathological process modifying its growth potential by reducing its growth rate (Cambell 1989). Recently, the term has also been used to include reduced cellular structure and function.

The pathway between supply and demand extends from the outer milieu of the maternal being, through the interfaces of her alimentary and respiratory surfaces, to the maternal/placental arteriovenous alignment in the microcotyledons and, ultimately, via the umbilical vein into the fetal circulation, with its unique absence, in the case of the fetal horse, of a *ductus venosus*, thereby entailing that all fetal blood passes through the liver. The system is completed via the umbilical arteries, enabling exchanges between fetal and maternal pathways to occur in the reverse direction (**Fig 1**).

Any disturbances in these pathways, to or from the fetus, may modify fetal development and growth (referred to as intrauterine growth retardation: IUGR), ranging from severe and immediate effects, evidenced by abortion or neonatal maladjustment, to more subtle long-term deficits, which may not become apparent clinically until later in the individual's life. These latter effects were first proposed by Professor David Barker and his colleagues working in Southampton to the effect that a baby's nourishment, before birth and during infancy, programmes the development of risk factors, such as raised blood pressure, serum fibrinogen and factor VIII concentrations and glucose intolerance in the individual; and from epidemiological studies, these factors were postulated as being key determinants of coronary heart disease in later life (Barker 1992, 1994).

Here, we summarise the hypothesis that an adverse

fetal environment may impose substantial limits on future health and athletic performance of the horse, within the context of knowledge regarding other species, including man.

Comparative studies

Following early epidemiological investigations (Barker and Ormond 1986), many more observations have been made among the human population and on experiments in animals, particularly sheep (e.g. McMillen *et al.* 2001). These authors described a range of pathophysiological factors which result in perturbation or restriction of fetal growth; and the cardiovascular, neuroendocrine and metabolic adaptations of the fetus to these stimuli, depending upon their nature, timing and intensity. McMillen *et al.* (2001) emphasised the critical importance of these adaptations both for immediate survival and long-term health outcomes in affected individuals.

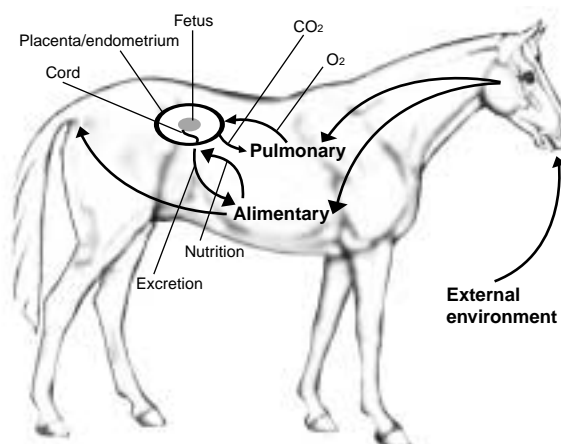


Fig 1: Representation of the pathway extending through the 1) external environment to the fetus, 2) interfaces at the maternal lungs and alimentary tract, 3) somatic homeostasis of the mare, 4) uterine placental 'barrier' and 5) the umbilical cord and fetal circulation.

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Programming

Lucas (1991) defined programming as a process in which a stimulus or insult, at a critical period of development, has lasting lifelong effects. Tissue growth spurts in the fetus have been identified in many species (Davies 1981); and organ, tissue and cell growth curves are asynchronous (Reed *et al.* 1989), therefore presenting 'windows' of susceptibility to deleterious developmental effects. A neurological growth spurt occurs in the human fetus at 15–20 weeks gestation, i.e. 39–52% of term, whereas in the horse a growth spurt in CNS myelin appears to occur around 270 days, 79% term (Sweasey *et al.* 1982).

The term 'programming of the fetus' was summarised by Desai and Hales (1997) in an elegant presentation entitled '*Role of fetal and infant growth in programming metabolism in later life*'. The concept of fetal programming describes the sequential and interrelated aspects of functional and structural development which prepares the fetus for birth and immediate adjustment postnatally. These changes underwrite the processes of maturity that sustain both life and performance in later life of the individual. It is upon the normality of these

developmental processes, occurring *in utero*, that the individual is able to sustain a wide range of performance, from a sedentary and relatively unchallenging existence to that of high performance and, importantly, to meet the challenges of infection or extreme metabolic or environmental demands.

Nutritional disturbances

The relationship between impaired nutritional intake (restricted intake on the maternal side or through diffusion pathways between maternal and fetal circulations) has featured largely in both clinical observations and experimental studies in human and animal species, respectively. In 1946, Barcroft concluded a chapter on '*Relative claims of the fetus and mother to available nutritive material*' by stressing the importance of further research on the effects of maternal malnutrition on fetal development. Further, Hammond (1961) observed that anatomical and physiological development of an animal at birth may be modified by circumstances occurring prenatally.

Malnutrition impairing pancreatic beta cell growth and function was described in studies on malnourished children from

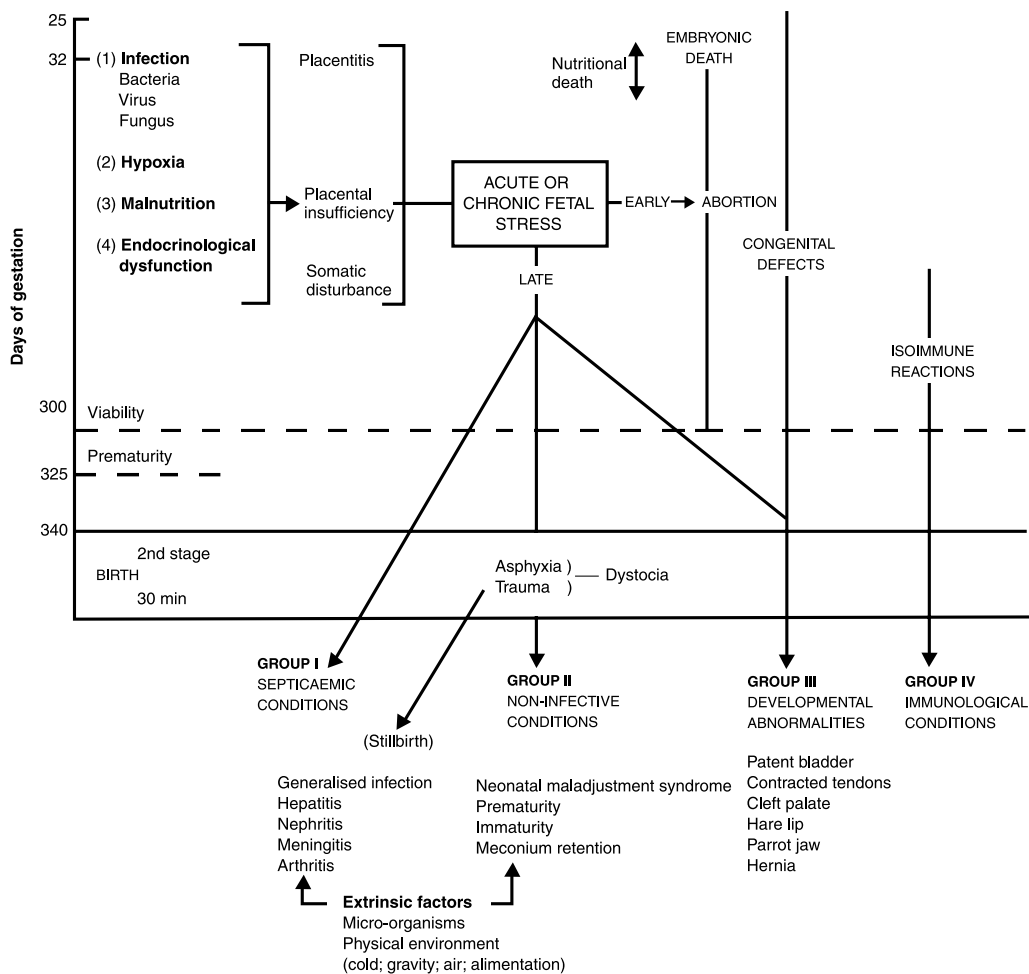


Fig 2: Intrinsic and extrinsic factors which may affect fetal development (Rossdale 1972a). This illustrates postnatal problems that may be encountered clinically as a result of these disturbances and which are well recognised. However, as discussed in the text, the **Barker hypothesis** has opened the avenue to a better understanding of the relationship between intrauterine growth-retarding effects and conditions in later life.

the developing countries; and glucose intolerance found even after recovery in those suffering nutritional deficits (Milner 1971). Hales and Barker (1992) proposed a hypothesis concerning the prevalence of noninsulin-dependent diabetes (*type 2*) in human populations, where poor fetal and early postnatal nutrition imposes mechanisms of nutritional thrift upon the growing individual, resulting in impaired development of the endocrine pancreas and increased susceptibility to diabetes. Studies in experimental animals showed that these changes can be reproduced by subjecting either fetal or early postnatal animals to general protein/calorie malnutrition (Weinkove *et al.* 1974). For a review of intrauterine programming of the endocrine pancreas, see Fowden and Hill (2001).

Maternal protein-undernourished rat fetuses show long-term morphological changes in β -cells, insulin secretion, blood pressure and hepatic metabolism (see Desai and Hales 1997). The measurement of insulin precursors, 32-33 proinsulin, has thrown light on the understanding of noninsulin-dependent diabetes in man (Rhodes and Alarcon 1994); and is currently the subject of study in horses (N. Holdstock and A.L. Fowden, personal communication) as a potential means of diagnosing the IUGR-affected foal *in vivo*.

Micronutrient-induced disturbances (vitamins, minerals) during early fetal life have been recently studied (for review, see Ashworth and Antipatis 2001). These authors state that deficiency of specific micronutrients, such as zinc, may result in a greater incidence of fetal malformation and embryo resorption than general undernutrition; moreover, the range of micronutrients that affect development, number of critical developmental stages, diverse biochemical systems and types of tissue, combine to increase the risks from inappropriate micronutrient status. The authors proposed a unifying hypothesis that micronutrient-induced disturbances affect the balance between the generation of free oxygen radicals and production of antioxidants. These studies are based on the rat, pig, mouse, rhesus monkey and man; similar studies are lacking for the horse.

Endocrine disturbance

Hormones play a central role in the control of fetal growth and development (Fowden 1995; Fowden and Forhead 2001), acting on tissue accretion and differentiation. Insulin stimulates fetal growth via its effect on mitosis and nutrient availability for tissue accretion, whereas cortisol acts on tissue differentiation and maturation.

Nutritional availability *in utero* is a major influence on circulating levels of hormones (e.g. insulin, thyroxine, cortisol and prostaglandins) and growth factors which, in turn, regulate uptake and metabolism of nutrients by the fetus. Nutrient availability also regulates the duration of pregnancy in any given individual (Fowden 1989; Fowden and Forhead 2001). When nutrient availability is limited, fetal concentrations of insulin and thyroxine fall, while those of prostaglandins and cortisol rise (Fowden 1985). These endocrine changes occur whether the substrate is restricted by maternal undernutrition, placental insufficiency or by reduction in uterine or umbilical blood flows (Fowden 1985). These endocrine changes are interdependent,

e.g. an increase in cortisol, due to undernutrition of the fetus, curtails growth and the demand for nutrients; thyroxine regulates oxygen consumption in the fetus and decreases during episodes of hypoxia, enabling the rate of oxygen utilisation to be matched to supply (Fowden *et al.* 1998). Changes in endocrine status of a fetus, therefore, may have profound significance on body growth and microstructural elements of specific organ systems in individuals affected by disturbances at the placental/endometrial interface.

Fetal corticosteroids contribute to the maturation of many organ systems and regulate the transition from intrauterine to extrauterine life. This is achieved via activation of the fetal hypothalamic pituitary adrenal (HPA) axis and has been studied most intensively in the sheep (for review, see Mathews *et al.* 1995). In this species, the fetal adrenal cortex exhibits a triphasic pattern of secretory activity, highly active at Days 55–60 and at term, Day 145, but relatively unresponsive to ACTH between Days 80–125 (Challis and Brooks 1989). In contrast, in the fetal foal, there is a poor response to ACTH administration for the majority of gestation and instead maximal responses are obtained in the immediate *postpartum* period (Silver *et al.* 1984; Silver and Fowden 1994). The placental enzyme 11 β -HSD converts active cortisol to inactive cortisone in the mare; therefore, the placenta forms a barrier to cortisol transfer between mare and fetus (Chavatte *et al.* 1995) which is a mechanism that protects the fetus against maternal levels of the hormone. The transplacental cortisol gradient is higher in the horse than in the sheep, where 2 isoforms of the enzyme have been identified, i.e. *types 1* and *2* (Yang 1997). Fetal cortisol and 11 β -HSD2 activity in fetal sheep also plays a role in parturition in this species (Clarke *et al.* 2002) by regulating local actions within the placental tissues, but this has not yet been confirmed in the horse.

It is of interest that the fetal foal lives in a higher oxygen milieu than the fetal lamb (Comline and Silver 1970); this may relate to the level at which hypoxaemia becomes injurious to the foal compared to the lamb in terms of survival and development *in utero* and postnatally.

Another potential influence on normal growth and development is the finding that the fetal foal deaminates little amino acid and has limited glucogenic capacity (Fowden 1997); it is, therefore, more dependent on transplacental supply of glucose than other species studied. Further, the rates of umbilical uptake and uteroplacental consumption of glucose are relatively high, the gravid uterus accounting for 70% of the maternal rate of glucose utilisation in late gestation (Evans 1971). These high rates of glucose consumption *in utero* may, in part, explain the vulnerability of the equine fetus to nutritional challenge during gestation (Fowden *et al.* 1994).

Clinical consequences of IUGR

The clinical consequences of IUGR may be illustrated by the individual that:

1) is reduced in size and/or appears undernourished at birth;

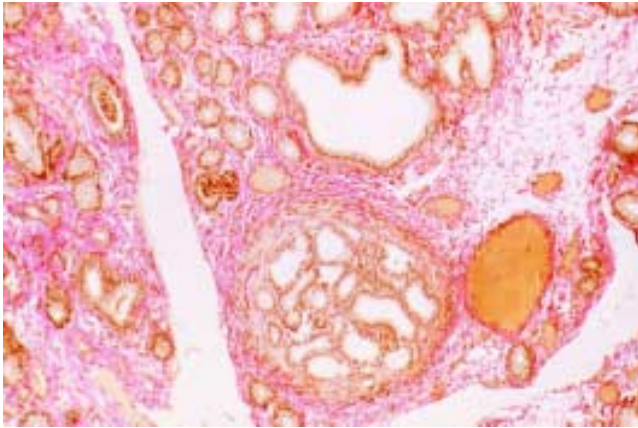


Fig 3: A view of chronic endometritis and gland nest formation associated with disturbed placental attachment and function by Bracher et al. (1996).

- 2) exhibits signs of maladjustment, as judged by the criteria of normality for the species. In precocious species, such as the foal or lamb, signs are more obvious at birth than in altricial species, such as man;
- 3) has suffered subtle growth retardating effects *in utero* and may, in consequence, have functional deficits that are never exposed or identified because of the individual's way of life. This is in contrast to another individual exposed to more severe environmental challenges in which clinical signs appear.

The following examples are given to illustrate these clinical aspects of IUGR.

Sudden infant death syndrome (SIDS)

It has been postulated that IUGR is associated with an increased risk of sudden infant death syndrome (SIDS) in which affected infants have reductions in both weight and length, suggesting that responsible mechanisms start early in pregnancy (Buck *et al.* 1989). Employing stereological techniques (Sterio 1983), it has been reported that SIDS infants have markedly fewer renal glomeruli (Hinchcliffe *et al.* 1992), pulmonary terminal bronchiolar ducts, phrenic nerve

myelinated axons and neocortical neurons, compared to non-SIDS infants (Sibbons *et al.* 1998). These authors also hypothesised that SIDS victims have limitations in reserve capacity in some organs critical to survival in the face of physiological challenges during extrauterine existence.

Pulmonary effects

Placental insufficiency resulting in fetal substrate deprivation, hypoxia, and cigarette smoking in human subjects are known to cause fetal pulmonary hypoplasia (Harding 1995) and other disorders that continue through the postnatal period into later life (Shaheen and Barker 1994), including an increased risk of chronic obstructive pulmonary disease (Barker *et al.* 1991).

Muscle function

Numerous contractile and metabolic functions depend on muscle, including breathing, locomotion, posture and thermogenesis; defects in muscle development could, therefore, impair any of these functions. IUGR may result in disproportionate reductions in muscle mass and selective atrophy of *type 2* (fast twitch) fibres in adults (Dauncey 1998).

Neuronal damage and hypotension

Frequent, brief episodes of asphyxia are more injurious to neuronal integrity than are a single, longer insult (Mallard *et al.* 1995). One of the most common causes of fetal asphyxic episodes is cord occlusion. In the equine, a long cord is necessary for successful delivery, a feature which may make this species more susceptible to cord twisting (occlusions) and resultant asphyxic episodes. During asphyxia, blood circulation is redistributed giving priority to perfusion of the brain and heart, through activation of peripheral chemoreceptors and endocrine responses that include secretion of arginine vasopressin (AVP) and catecholamines (Iwamoto 1993). The postnatal consequences of asphyxia may range from the severely disadvantaged newborn that may fail to survive to subtle neurological deficits that are diagnosed only when the individual faces specific challenge of an intellectual (in human individuals) or physical nature.



Figs 4a,b: The allantoic and chorionic surfaces of grossly normal placentae and those associated with full-term normal foals. Note the diffuse nature of the placenta, the relatively short cord and clear unstained amnion.

Osteochondrosis (OC)

Osteochondrosis is a general term for a disturbance in the physiological process of bone and cartilage formation in the articular epiphyseal cartilage complex of the growing individual (Hurtig and Pool 1996). The primary lesion affects the differentiation and maturation of the cartilage cells and surrounding matrix destined to be replaced by bone. There is a strong breed predisposition; ponies are not commonly affected, but Thoroughbreds, Standardbreds and some other performance breeds have a relatively high incidence (for references, see Jeffcott 1991; van Weeren and Barneveld 1999). This may be due to variables of genetics, size environment/management factors or biomechanical stress. However, the disease is also very common in pigs, and congenital predisposing influences resulting from IUGR would, therefore, seem worthy of investigation. Preliminary reports of microstructural development, during *in utero* development, suggest there are differences between ponies and Thoroughbreds and that the latter may suffer a degree of IUGR compared with the former (Beech *et al.* 2001).

The exact aetiology of OC is still contentious, but there is growing evidence that insulin-induced effects on growth hormone associated with feeding of carbohydrate-enriched diets postnatally may play a role (Jeffcott and Henson 1998). Pool (1993) refers to inherent metabolic defects in the cartilage of some individuals. Cases of OC occur in foals age less than one month and the search for congenitally derived susceptibility might prove rewarding.

The process of mineralisation of bone tissue during prenatal life is related closely to regulation of calcium and phosphorus metabolism at the time. This is influenced by 1) mineral metabolism of the mother, 2) placental activity and metabolism and 3) the endocrine regulation of the fetus itself (Royer 1981). There appear to be few reports of prenatal microdevelopment in the horse, but the milieu of fetal development is important to these aspects and it seems reasonable to hypothesise that IUGR has not only the demonstrable effects at birth, but also long-term consequences programmed into the individual as a result.

Equine IUGR

Clinical background

The concept of prenatal origins of newborn foal conditions (**Fig 2**) was established in the 1970s (Rossdale 1972a,b; Rossdale and Leadon 1975). The term dysmaturity was coined from the human literature (Gruenwald 1963) to describe foals showing signs of immediate postnatal maladjustment and weakness, i.e. premature-like but delivered in the full-term period of >320 days gestation (Rossdale 1976). The terms IUGR and placental deprivation (Gruenwald 1963) denote clinical signs of malnourishment and physiological deficits inherent in those individuals born following a gestation involving interference with normal placental transfer of metabolites and nutritional requirements *in utero*.

In the 1960s, increasing emphasis was being placed upon the prenatal origin of conditions and diseases of the newborn in both human and veterinary medicine. A summary of known intrinsic and extrinsic factors that may affect fetal development adversely and thereby interfere with adaptive processes was presented by Rossdale (1972a), as illustrated in **Figure 2**. At that time, emphasis was almost entirely upon neonatal conditions arising from prenatal and intranatal events (Rossdale 1972b; Rossdale and Leadon 1975). Rossdale (1972a) classified neonatal conditions into 4 groups, of which *Group II* encompassed those individuals with neonatal maladjustment syndrome (NMS), previously known as Barker's (Reynolds 1930), convulsive and allied syndromes (Mahaffey and Rossdale 1957) or respiratory distress syndrome (Mahaffey and Rossdale 1959); *Group II* also included individuals with dysmaturity, immaturity or prematurity. Twenty years later, NMS was recognised as a syndrome divided into 2 categories according to aetiology and pathogenesis starting prenatally or intranatally (for a review see Hess-Dudan and Rossdale 1996a,b). The conditions of prematurity and dysmaturity are essentially ones of common origin, as their clinical signs are the result of prenatal disturbance at some period of gestation (see **Fig 2**); and the distinction is based on an arbitrary definition related to gestational age, i.e. 320 days.

IUGR and the older individual

The epidemiological observations of the relationship between IUGR and conditions in later life, as proposed by Barker and Ormond (1986), would seem to have particular relevance to horses whose purpose is primarily athletic performance of an exacting nature, such as racing, 3-day eventing and similar pursuits. van Velzen *et al.* (1995) drew attention to similarities in pathogenesis of postnatal conditions in human and equine 'infants' due to IUGR. In 1998, it was hypothesised (Rossdale and Ousey 1998a) that the 'second day syndrome' (Rossdale and Leadon 1975), i.e. foals whose condition deteriorates markedly on the second day *postpartum*, is the result of intrauterine growth retarding effects that disturb pulmonary and metabolic function. The signs of this become apparent in foals during the first few days *postpartum*, as the demands for extrauterine adaptation and athletic performance are imposed on this precocious species within a few hours of birth.

Clinical range of potential IUGR effects

The range of possible effects of IUGR on the neonate and older horse have been proposed (Rossdale and Ousey 1998b) as follows:

- 1) pulmonary failure in the newborn resulting in atelectasis and respiratory distress, particularly on the second and third day *postpartum*;
- 2) failure of the individual to establish an immediate postnatal respiratory rhythm;
- 3) disturbances in microstructure resulting in imbalanced relationships between the terminal bronchiolar/alveolar/

arteriolar proportions, thereby predisposing to exercise-induced pulmonary haemorrhage (EIPH);

- 4) neuropathies involving idiopathic laryngeal hemiplegia and denervation of laryngeal muscles (Duncan *et al.* 1991) which might originate from effects of IUGR on myelination or selective neuronal apoptosis;
- 5) other conditions, including hyperlipaemia (Love 1990), chronic obstructive pulmonary disease (COPD), OC (see above) and myopathies.

Desai and Hales (1997) emphasised that fetal growth and development is controlled by genetic factors determined by the fetal genome and environmental factors, such as maternal nutrition, that alter the expression of the fetal genome. This concept may be applied to horses, particularly with respect to the narrow genetic base of Thoroughbreds whose linearity stems from 30 founders only, 27 of these being male; 10 founder females account for 72% of maternal and 1 stallion for 95% of paternal lineages (Cunningham *et al.* 2001). With a narrow genetic base in the population, it might be expected that performance would have a correspondingly narrow presentation. This is not the case, however, which suggests that the environment *in utero* may play a significant role in influencing the athletic expression of the genome.

Possible routes of fetal disturbance during pregnancy

In the pathway between the external environment and fetal wellbeing (**Fig 1**), possible routes of interference may occur as follows:

- 1) the maternal interface with the external environment;
- 2) the alimentary mucosa;
- 3) the maternal pulmonary air sacs/blood circulation;
- 4) disturbances in maternal metabolic and homeostatic mechanisms;
- 5) the endometrium;
- 6) disturbances due to a) placental pathology and b) associated endocrine changes;
- 7) disturbances within the a) umbilical and fetal blood circulation and b) maternal uterine circulation.

These routes of potential interference are discussed briefly below.

1) The external environment

The mare may be subjected to environmental stress resulting from extreme exertion or disturbances, such as hunting or low-flying aircraft in their vicinity, the need for extreme exertion or any factor causing flight. The subject of maternal stress is usually addressed in terms of the risk of abortion, rather than potential effects on fetal growth and development.

Maternal inappetence or deficiencies in nutritional quality or quantity of intake may affect the fetus, both by deficiency and excess, imbalance in composition and in temporal terms of the

stage of pregnancy (Fowden *et al.* 1994). However, there are few reports correlating nutritional intake of the mare with specific long-term consequences in the foal as it matures in later life.

Important external environmental influences associated with abortion include infectious agents such as herpesvirus, arteritis and leptospirosis (Acland 1993), whereas bacterial and fungal infections may, in association with placental pathology, result in IUGR. The ingestion of noxious substances, such as tall fescue endophyte (Boosinger *et al.* 1995; Brendemuehl *et al.* 1995) presents definitive risks, as does the administration of drugs; but most reports, with respect to pregnancy, have dealt with resulting fetal death and abortion, rather than that of fetal stress and postnatal consequences. In horses, most studies have concentrated on long-term developmental effects with respect to postnatal dietary regimes. The subject of cause and effect relationships of all the above mentioned elements on the fetal horse require epidemiological and experimental studies that await finance and motivation to be performed.

2) The alimentary interface and digestive processes

Nutrition is important from the very early stages of gestation, but the relationship between pathology or functional disturbance of the mare's gut and fetal consequences deserves further study. Enteropathies occur which may be long-term or temporary and may, correspondingly, limit the absorption of essential elements of nutrition at critical stages of fetal development. When these occur at the interface of the maternal alimentary tract, the mare's metabolism may be able to compensate and maintain fetal nutrition at adequate and normal levels. However, possible long-term effects on postnatal development have not been investigated in such cases.

An interesting case illustrates this point. A 12-year-old Thoroughbred mare, lactating and 90 days pregnant, was referred due to severe weight loss (N. J. Wingfield Digby, personal communication). Investigation indicated a malabsorption syndrome, confirmed by negative glucose absorption test. Lymphocytic-enteritis, possibly of autoimmune origin, was diagnosed from multiple intestinal biopsies. Three months later, following therapy with corticosteroids and antimicrobial drugs, the mare's condition had improved and a positive glucose absorption test was recorded. An apparently healthy colt, of normal birthweight, was delivered at 342 days gestation. At age 7 months a cardiac arrhythmia was found, consisting of extrasystoles.

This case shows that maternal alimentary malabsorption does not always cause fetal growth retardation, but the finding of a cardiac anomaly may indicate a relationship with fetal deprivation during the early stage of pregnancy. It has been shown in several species that normal muscle development is dependent on thyroid, growth, insulin and steroid hormones (Dauncey 1998) and, during undernutrition, the resulting increase in cardiac thyroid hormone receptors ($\text{Tr}\alpha_2$) affects cardiac contractile ability and reduced α -myosin transcription (White and Dauncey 1998). Although conclusions cannot be based on one equine case, this illustrates the potential relationship of prenatal disturbance and subtle postnatal effects.



Fig 5a: Placenta of a foal delivered prematurely with 50% of its placental surface avillous. The foal survived, however, but was delivered 'small for dates' and suffered IUGR in consequence of the loss of placental attachment.



Fig 5c: A grossly enlarged and thickened oedematous chorion associated with a foal suffering from IUGR at full term. The oedema was presumed to be the result of circulatory disturbance in the fetus. The foal showed severe signs of dysmaturity and did not survive.

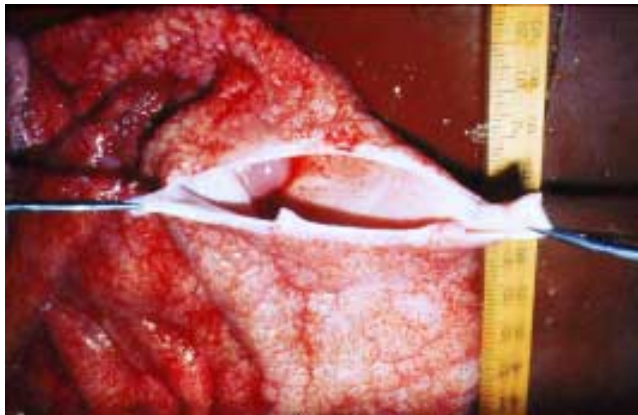


Fig 5b: Placenta of foal delivered at full term suffering necrosis at cervical pole. The artery to this area had inflammatory changes which may have been the cause of ischaemia or the result of the inflammatory process ascending into the artery.

3) Pulmonary deficits

Chronic obstructive pulmonary disease and hypersensitivity of airways (Robinson 2001) are common features of broodmares, but there is no evidence to suggest that this affects the delivery of oxygen to the fetus or the accumulation of carbon dioxide in the maternal circulation to such a degree as to reduce the normal exchange of gases at the placental uterine interface.

4) Maternal conditions affecting homeostasis

Any condition which affects the thermoregulatory, endocrine or metabolic state of the mare may influence the uterine circulation and, thereby, affect the milieu of the fetus. The risk of this occurring depends on the nature of the condition, its severity and, probably, the period for which it lasts, in addition to the stage of gestation at which it occurs. Conditions which produce endotoxins or increased levels of

prostaglandin, or cause endocrine imbalances, may result in abortion or have deleterious effects on fetal development through the passage of substances across the placenta or by causing disturbances in blood flow on either side of the uterine/placental interface. Anaesthesia is a high risk procedure in the pregnant mare and may cause periods of fetal hypoxia (Taylor 1997).

The consequences of colic and abdominal surgery have not received extensive epidemiological studies of cause and effect, although anecdotal accounts exist and there are reports of follow-up to cases (Santschi *et al.* 1991). From these, it would seem that, as far as abortion is concerned, the results are greatly influenced by the individual and there is no generality as to outcome. With respect to intrauterine growth retarding effects, the evidence is even less and most anecdotal accounts fail to convince because of the large number of variables involved in postnatal growth, development and soundness of each individual. Once again, there must be value for future studies in this regard if we are to understand the subject of the effect of maternal disturbances relative to the programming of the equine fetus for future life.

5) The maternal endometrium

Studies have shown that chronic changes (**Fig 3**) in the endometrium adversely affect the relationship between the chorion and the endometrium (Bracher *et al.* 1996; Flood 1996). Bracher *et al.* (1996) found failure of attachment of the placenta in areas where cysts were present; and chorionic macro- and microvilli appeared shorter in 2 subfertile mares with lower weight of the fetus compared with healthy controls. It was hypothesised that lengthening of the pathway between the maternal and fetal circulations or the restriction of blood supply and uterine gland production disturbs the transfer of material both to and from the fetal circulation or to the placenta itself. This latter element is important because nourishment of the placenta is essential to both the health and length of the pathway between maternal and fetal circulations.

6a) The placental endometrial interface and placental pathology

The structure of this interface has been well described by Stevens and Samuel (1975); the morphology of the placenta by Whitwell and Jeffcott (1975) and its pathology by Whitwell (1988), Cottrill (1991) and Cottrill *et al.* (1991). In the context of IUGR, pathology may impede diffusion or restrict diffusion pathways between maternal and fetal circulations.

The functional relationship of the normal maternal/fetal interchange across the placental 'barrier' has been investigated, from the early pioneering work of the late Robert Comline and Marian Silver (Comline *et al.* 1975) to more recent studies by Fowden and Silver (1995) and Allen *et al.* (2002). Studies in several species (Schneider 1996) demonstrate that major features of remodelling of the placenta occur during early gestation in villous surface area and in diffusion distance between maternal and fetal circulations. There is oversupply of nutrient available to the fetus in early gestation but, in late pregnancy, the balance reduces, thereby putting the fetus at risk of growth retardation (Schneider 1996).

Clinical appraisal of these relationships in the horse is largely empirical and based on the condition of the placenta when it is expelled relative to the health status of the individual foal (Figs 4a,b). It is clear that placental pathology, at the time of delivery, is evidence of potential injurious processes to the survival prospects of the foal; and the extent of the pathology of the placenta has been noted in relation to the chances of survival (Figs 5a–c). For example, villous atrophy/hypoplasia, placentitis, thickening or oedema of the allantochorion or amnion have been associated with fetal death or delivery of low birthweight live foals (Whitwell 1988; Rossdale *et al.* 1991). Diminution of the placental area of function, increase in length of diffusion pathways (Fig 6) and disturbances in endocrine balance all appear to form part of the pathogenesis of the effects on the fetus, but the nature and extent of these disturbances in the fetus have yet to be elucidated.

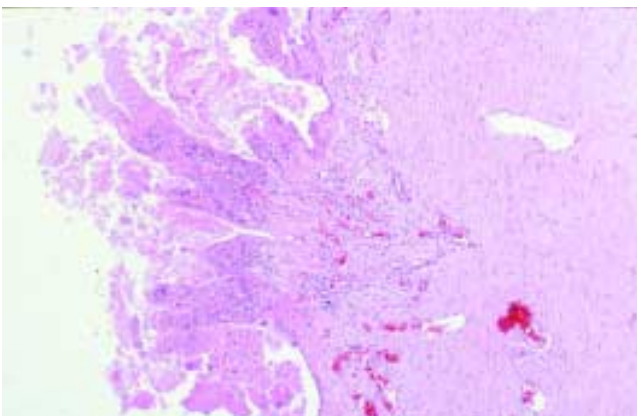


Fig 6: Histopathology of a section of placenta that has been grossly thickened by an inflammatory process associated with mycotic infection. This illustrates the destruction/lengthening of diffusion pathways at the endometrial/placental interface.

6b) Associated endocrinology of placental pathology

The relationship between placental disturbance and changes in endocrine production and/or metabolism has been identified from studies on maternal plasma progestagen concentrations in cases of twins and where placental pathology exists in cases of singletons (Rossdale *et al.* 1991). The progestagens involved are, predominantly, 5 α -pregnane-3,20-dione (5 α -DHP), 5 α -pregnane-3 β ,20 α -diol (b α -diol) and 20 α -hydroxy-5 α -pregnan-3-one (20 α -5P), all of which rise at term (Holtan *et al.* 1975, 1991; Rossdale *et al.* 1991). The finding of precocious increments (Rossdale *et al.* 1991) in cases where placentitis develops during the period 200–300 days gestation is evidence of altered endocrine status of the individual; raising questions both as to associated changes in other hormone pathways in the feto/placental/ endometrial unit, and consequent changes in fetal growth and development at a microstructural and functional level.

Adrenocortical function has been shown to play a central role in the final stages of fetal maturation for postnatal survival (Nathanielsz *et al.* 1975; Fowden and Silver 1995). In the horse, compared with the sheep and pig, plasma cortisol concentrations increase relatively late, i.e. a few days prior to parturition (Fig 7). This supports the conclusion that readiness for birth (i.e. final stages of preparation; Rossdale and Silver 1982) presents a relatively brief window of opportunity for delivery of the individual with normal adrenocortical function, even in the full-term period of 320–340 days gestation. Conversely, when the fetus is stressed, as in cases of placental pathology, either naturally-occurring or induced experimentally, precocious adrenocortical function may be present as early as 280 days

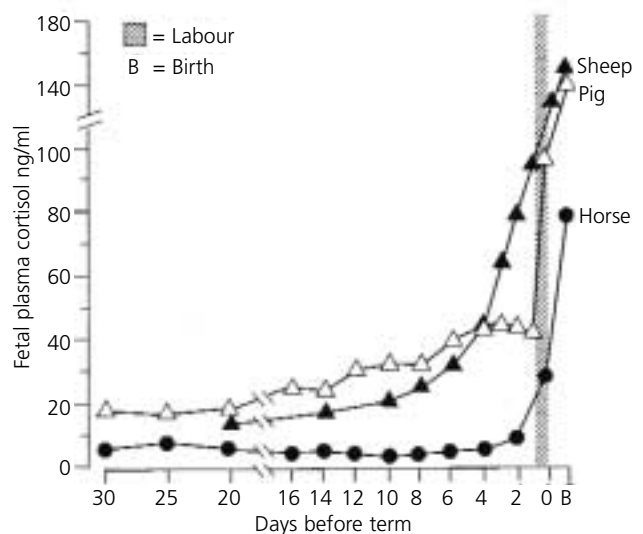


Fig 7: Showing the late rise that occurs in fetal cortisol concentrations before birth of the foal compared with the rise that occurs in the sheep and the pig (from Silver and Fowden 1988). Cortisol plays a central role in maturation, particularly in the final 'switching on' of the fetal foal for postnatal adaptation and in the initiation of parturition.

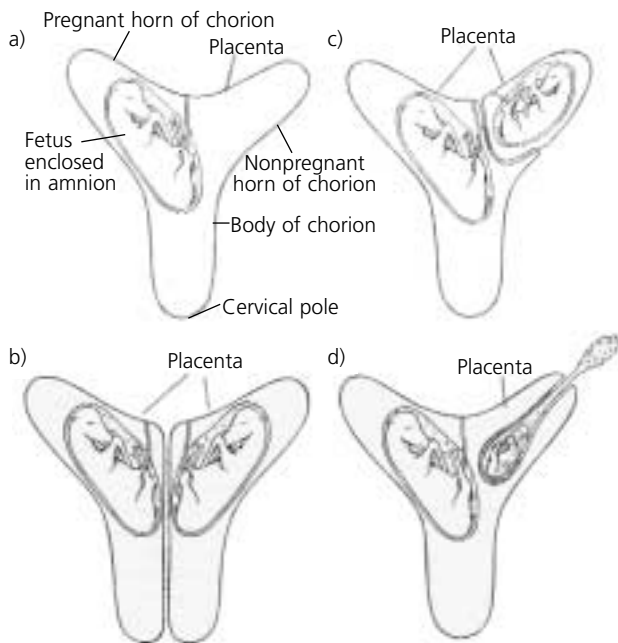


Fig 8: Illustrating the competition for the placentae for attachment in the case of twins. a) Singleton; b) twins of about equal size; c) twins of dissimilar size; d) one twin undergoing mummification. Twins are a natural model of intrauterine growth retarding effects (from Jeffcott and Whitwell 1973, with permission).

gestation (Rossdale *et al.* 1991; Ousey and McGladdery 2000; LeBlanc 2001). In contrast, if a mare is induced to foal by exogenous administration of drugs or through derangement of intrinsic physiological processes of parturition (usually with no placental pathology or mammary development), hypoadrenocortical function is present in the newborn foal (Rossdale and Silver 1982; Silver *et al.* 1984).

7a) Fetal circulation

The fetal circulation deserves special consideration, because of the diffuse nature of the placenta and its extended length, of some 365 cm between cardiac outlet and inlet. The circulation courses through the fetal aorta, umbilical cord of, on average, 55 cm length (Whitwell and Jeffcott 1975), and allantoic arteries, chorionic capillaries, into and out of the microcotyledonous vessels, thence returning through the venous system and the liver to the cardiac inlet to complete the circle.

There is little information available in the horse regarding pressure gradients in this cardiovascular circuit. In lambs, mean arterial pressure increases during pregnancy from mean 30 to 60 mmHg at term (Dawes 1968), with a corresponding increment in cardiac output (Silver *et al.* 1982). The control of flow in the fetal foal must be finely balanced, especially in supplying the peripheral areas of placenta at the cervical pole, and in view of the absence of a *ductus venosus*. It has been postulated that some cases of the commonly experienced placental pathology in this region are the result of ischaemia associated with an overlong cord

(Smith *et al.* 1999), in addition to those caused by ascending infection (LeBlanc *et al.* 2001).

The circulation may also be impeded by pressure on the umbilical cord causing restriction to veins, arteries or both. This may come about by entanglement with the fetal limbs or by compression between the fetal body and uterine wall. Such compression may be accentuated by the presence of cysts or pedunculated structures attached to the amniotic or allantoic portions of the cord. Compression may be sufficiently severe to result in a fatal period of fetal hypoxaemia or of a lesser chronic nature, leading to sufficient interference to arrest fetal development but not to fetal death. In these circumstances, the risks of neuronal damage are high, as discussed above. Long cords (>80 cm) have been suggested as a cause of fetal death and abortion (Whitwell and Wood 1992); and their length may add to the chance of both entanglement with the fetal limbs and compression against the uterine wall, and may also add significantly to cardiovascular considerations of blood flow. Silver *et al.* (1982) calculated that <2% of total umbilical arterial blood flow perfused the fetal membranes, compared with 5% in the sheep. However, the diffuse nature of the placenta may require a higher proportion of flow to satisfy the nutritional requirements of the nonmicrocotyledon tissue and, therefore, make this tissue at more risk of ischaemia and subsequent pathology.

7b) Maternal uterine circulation

Age-related changes of uterine arteries (Nambo *et al.* 1995), involving hyperplasia of elastic fibres, may also play a part in the development of chronic endometritis and in diminishing perfusion in the maternal microcotyledons.

Models of IUGR in the horse

Twins are a natural model of IUGR in the horse because of competition between the 2 placentae for attachment to the endometrium (Fig 8). The outcome of such pregnancies is either fetal death or undersize as a result of an undernourished state, with poor prospects for survival of one or both members (Ginther and Douglas 1982).

There are several experimental models of IUGR which have been developed in the horse:

- placental separation or ligation of vessels in mid to late gestation, designed for the purposes of studying progestagen metabolism (Rossdale *et al.* 1991);
- ascending placental infection (Leblanc *et al.* 2001);
- implantation of Thoroughbred embryos into pony mares, and *vice versa*, in order to compare placental microstructural development, fetal and postnatal growth and endocrine changes (Allen *et al.* 1998, 2002). These authors found that the extent of fetal development was related to maternal size, with the gross placental area, size and density of microcotyledons being the primary operative controlling mechanisms.

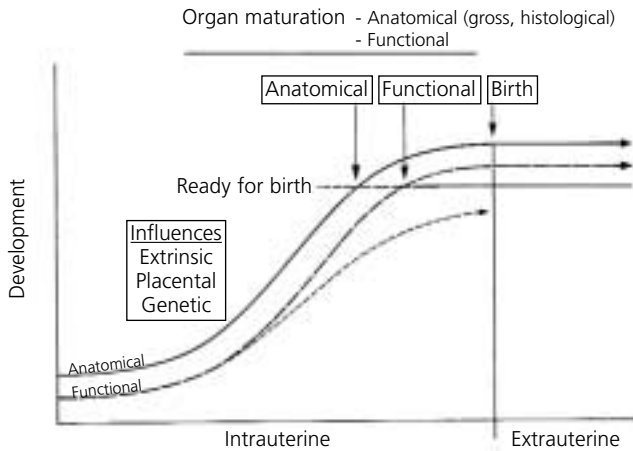


Fig 9a: During intrauterine growth and development, anatomical structures develop in advance of functional elements until both are in such a state as to support extrauterine existence (e.g. pulmonary air ducts and alveoli develop in advance of the surfactant system). Intrauterine growth retardation may reduce functional capacity to a level which will not support extrauterine existence or, more subtly, be responsible for conditions that become apparent only in later life (e.g. heart disease) as discussed in the text.

In these models, the effect on foals resulting from the disturbed pregnancies have been noted as abortion, premature delivery, dysmaturity or apparent normality at full-term birth. However, in some of the latter, skeletal problems became apparent in later development. The resulting outcomes for foals under these experimental conditions, therefore, mirrored those seen under natural conditions.

Consequences of deprivation on the equine fetus

The term stress has been used to describe conditions of challenge (Figs 9a,b) occurring to the fetus *in utero*, for example placentitis, or other deleterious circumstances (Rossdale 1976a), such as hypoxaemia, malnutrition or acidaemia. Acute or chronic states are recognised. The end point of stressful conditions *in utero* depends upon their nature, severity, stage of gestation and duration, and includes fetal death, neonatal maladjusted states or more subtle effects in the individuals, apparent in later life (Fig 9b). The most obvious clinical conditions are abortion, dysmaturity, maladjustment and prematurity. However, potential effects in later life include abnormalities of skeletal development, i.e. angulations, OC and developmental joint disease. Therefore, stress of any origin or pathogenesis causing IUGR may condemn the affected individual to a range of conditions presenting in the adaptive period in the first 4 days *postpartum*, in the period of further development to maturity or beyond.

Parameters of normality for several organ systems, adaptive behaviour and physiology of the newborn foal have been well documented (Barnes *et al.* 1975; Nathanielsz *et al.* 1975; Jeffcott *et al.* 1982; Rossdale *et al.* 1984a,b; Rossdale and McGladdery 1991). Although some foals may appear to be normal with respect to physical and behavioural criteria, they may not display functional maturity of various organ systems. Similarly, even if their physiology appears normal at rest, organ systems may not be able to maintain adequate

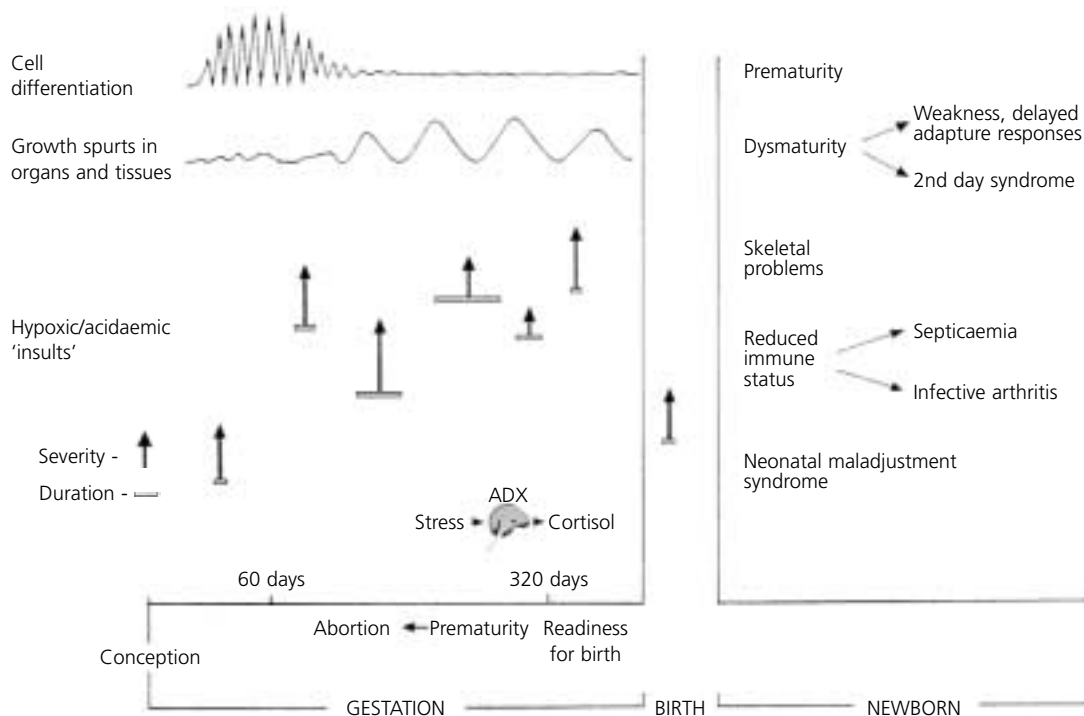


Fig 9b: Illustration of prenatal periods of fetal stress (insult) varying in duration and intensity (arrows) in relation to cell differentiation and growth spurts (Rossdale 1997). Some of the resulting conditions are listed on the right.

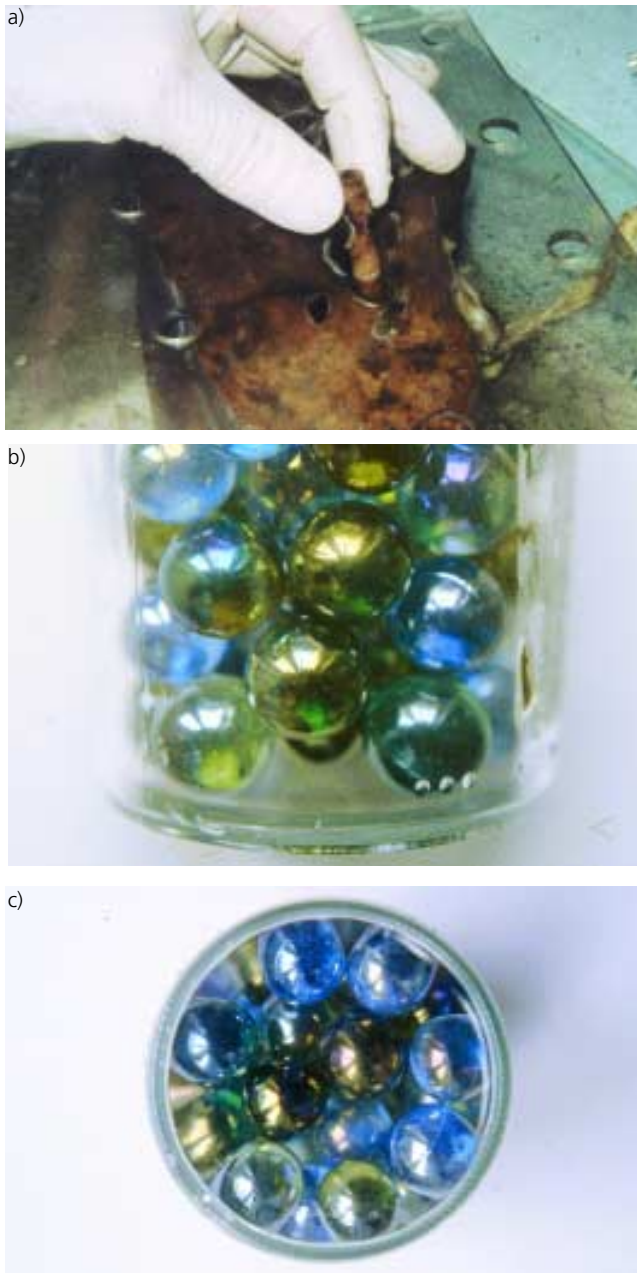


Fig 10: Stereology refers to a method of determining organ or tissue microstructure by a precise and orderly approach (Sterio 1984). a) illustrates the taking of samples from the lung. From these, the microstructures (e.g. number of terminal bronchiolar ducts) can be calculated employing a 3-dimensional microscope. The system allows, for example, the number of glomeruli in the kidney to be calculated in a repeatable and unbiased manner. The difference between counting, in a 3-dimensional (b) compared to a 2-dimensional (c) manner, is illustrated by the glass beads in a jar; in (b) all the beads can be counted.

homeostatic balance under stressful conditions, thereby resulting in clinical conditions in later life. It has been hypothesised that the pathogenesis of conditions such as exercise-induced pulmonary haemorrhage (EIPH) or neuropathy may include microstructural deficiencies acquired *in utero* (Rossdale and Ousey 1998a,b).

Microstructural studies of equine IUGR

Relatively little is known regarding organ development (organogenesis) in the equine fetus, foal or older individual. Development and growth have been studied, employing changes in mass or dimensions of the whole body or specific organs or tissues (Douglas and Ginther 1975; Platt 1984). *Postmortem* studies or those employing ultrasonography *in vivo* have compared gross mass organ development in relation to gestational age (Adams-Brendemuehl and Pipers 1987; Ginther 1992). Detailed histological studies of specific organs, e.g. adrenals, gonads, lungs and pituitary, have correlated development with gestational age. The number of functional units required for organ sufficiency, including compensatory potential, is currently under study (Beech *et al.* 2001).

The approach to a dedicated system of 3-dimensional appraisal of microstructure developed by Sterio (1984) opened a novel way to determine the effects of IUGR on organ microstructural development in individuals (Figs 10a–c). This approach has rendered some interesting avenues of investigation, albeit in morbid and not living specimens. Similar studies are beginning to emerge in horses examining the stereological development of various organ systems, including the placenta (Allen *et al.* 2002). One study, reported by Beech *et al.* (2001), has shown that the lungs of ponies are more developed at birth compared with Thoroughbreds; and that lung development in Thoroughbreds continues after birth, a unique micromorphogenic postnatal development in this species compared to others studied. In another study, Holdstock *et al.* (2001) have shown that the microstructural development of kidneys is equivalent in ponies and Thoroughbreds at birth. Further studies are being conducted to examine whether the development of these organs is affected by intrauterine disturbances and illnesses encountered during pregnancy and in later life, respectively.

The relationship between organ function and its microstructural development is the objective of further studies currently being performed in Newmarket on the microanatomy of laryngeal nerves (M. O'Donnell, personal communication).

Material is currently being collected for a study aiming to classify the stereology of the equine recurrent laryngeal nerve in horses observed to have normal and abnormal grades of arytenoid asymmetry. Sections of nerve collected *postmortem* in a series of horses subjected to euthanasia for other reasons are currently being analysed. Where possible, clinical data collected *antemortem* include examination at exercise and endoscopic evaluation of the larynx at rest. It is hoped that any comparisons drawn will highlight differences between left and right nerves and also differences between various grades of asymmetry. In Cambridge, a study on insulin sensitivity and pancreatic microstructure is in progress. These studies aim to develop clinical tests which can be used *in vivo* to identify the presence of IUGR-related microstructural deficits of specific organ systems in a particular individual.

Conclusions

There is abundant evidence from many species, including man, that fetal programming for postnatal organ functions may be disturbed by untoward events occurring at any point in the pathway between the external environment of the 'mother' and the *in utero* environment of her fetus. The resulting disturbance in physiological equilibrium may present as clinical conditions in later life. Little is known of this relationship in the horse. It is improbable that this species is different, although breeds such as the Thoroughbred, selected for athletic performance, may be more at risk than native breeds selected less intensively.

Further studies of the relationship between fetal perturbations and postnatal conditions in the horse should include attempts to correlate placental structure and pathological evidence in the fetus/newborn/mature individual employing epidemiological and microstructural techniques. Although experimental models (see above) are useful, their use may be limited due to ethical and financial restraints. Studies in the field of the naturally-occurring pathogenesis would seem, therefore, to be both practical and necessary. Nor should it be overlooked that the information thereby obtained may contribute important comparative information on a subject of great human medical importance. The suggestion from preliminary microstructural studies that the horse is unique in postnatal development of pulmonary tissue opens the way for further avenues of understanding and potential for therapy. The development of diagnostic tests *in vivo* is also of paramount importance both for the horse and for man.

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References

- Acland, H.M. (1993) Abortion in mares. In: *Equine Reproduction*, Eds: A.O. McKinnon and J.L. Voss, Lea & Febiger, Philadelphia. pp 554-562.
- Adams-Brendemuehl, C. and Pipers, F.S. (1987) *Antepartum* evaluations of the equine fetus. *J. Reprod. Fert., Suppl.* **35**, 565-573.
- Allen, W.R., Stewart, F., Ball, M., Fowden, A., Ousey, J.C. and Rossdale, P.D. (1998) The influence of maternal size on fetal and postnatal development in the horse. *Equine vet. J.* **30**, 457.
- Allen, W.R., Wilsher, S., Turnbull, C., Stewart, F., Ousey, J.C., Fowden, A. and Rossdale, P.D. (2002) The influence of maternal size on, placental, fetal and postnatal growth in the horse: 1. Development *in utero*. *Reproduction* (In Press).
- Ashworth, C.J. and Antipatis, C. (2001) Micro-nutrient programming of development throughout gestation. *Reprod.* **122**, 527-535.
- Barcroft, J. (Ed) (1946) The relative claims of the foetus and mother to available nutritive material. In: *Researches on Pre-natal Life*, Blackwell Scientific Publications, Oxford. pp 52-66.
- Barker, D.J. (1992) *The Fetal and Infant Origins of Adult Disease*, British Medical Journal Books, London.
- Barker, D.J. (1994) *Mothers, Babies and Diseases in Later Life*, British Medical Journal Publishing Group, London.
- Barker, D.J. and Ormond, C. (1986) Infant mortality, child nutrition and ischaemic heart disease in England and Wales. *Lancet* **1**, 1077-1081.
- Barker, D.J.P., Godfrey, K.M., Fall, C., Osmond, C., Winter, P.D. and Shaheen, S.O. (1991) Relation of birth weight and childhood respiratory infection to adult lung function and death from chronic obstructive airway disease. *Br. med. J.* **306**, 817.
- Barnes, R.J., Nathanielsz, P.W., Rossdale, P.D., Comline, R.S. and Silver, M. (1975) Plasma progestagens and oestrogens in foetus and mother in late pregnancy. *J. Reprod. Fert., Suppl.* **23**, 617-623.
- Beech, D.J., Sibbons, P.D., Rossdale, P.D., Ousey, J.C., Holdstock, N.B., Chavatte, P. and Ansari, T. (2001) Organogenesis of lung and kidney in Thoroughbreds and ponies. *Equine vet. J.* **33**, 438-445.
- Boosinger, T.R., Brendemuel, J.P., Schumacher, J., Bransby, D.I., Kee, D. and Shelby, R.A. (1995) Effects of short-term exposure to and removal from the fescue endophyte *Acremonium coenophialum* at Day 300 of gestation on pregnant mares and foal viability. In: *Biology of Reproduction Monograph*, Series 1, Eds: D.S. Sharp and F.W. Bazer, Society for the Study of Reproduction, Madison, Wisconsin. pp 61-69.
- Bracher, V., Mathias, S. and Allen, W.R. (1996) Influence of chronic endometritis (endometriosis) on placental development in the mare. *Equine vet. J.* **28**, 180-188.
- Brendemuel, J.P., Williams, M.A., Boosinger, T.R. and Ruffin, D.C. (1995) Plasma progesterone, triiodothyronine and cortisol concentrations in postdate gestation foals exposed *in utero* to the tall fescue endophyte *Acremonium coenophialum*. In: *Biology of Reproduction Monograph*, Series 1, Eds: D.S. Sharp and F.W. Bazer, Society for the Study of Reproduction, Madison, Wisconsin. pp 49-53.
- Buck, G.M., Cookfair, D.L., Michalek, A.M., Nasca, P.C., Standfast, S.J., Sever, L.E. and Kramer, A.A. (1989) Intrauterine growth retardation and risk of sudden infant death syndrome (SIDS). *Am. J. Epidemiol.* **129**, 874-884.
- Campbell, S. (1989) The detection of intrauterine growth retardation. In: *Foetal Growth*, Eds: F. Sharp, R.B. Fraser and R.D.G. Milner, Royal College of Obstetricians and Gynaecologists, London. pp 251-261.
- Challis, J.R.G. and Brooks, A.N. (1989) Maturation and activation of hypothalamic-pituitary-adrenal function in fetal sheep. *Endocr. Rev.* **10**, 182-204.
- Chavatte, P., Rossdale, P.D. and Tait, A.D. (1995) 11 β -Hydroxysteroid dehydrogenase (11BHD) in equine placenta. *Proc. Am. Ass. equine Practns.* **41**, 264-265.
- Clarke, K.A., Ward, J.W., Forhead, A.J., Giussani, D.A. and Fowden, A.L. (2002) Regulation of 11 β -hydroxysteroid dehydrogenase type 2 activity in ovine placenta by fetal cortisol. *J. Endocrin.* **172**, 1-8.
- Comline, R.S. and Silver, M. (1970) PO₂, PCO₂, and Ph levels in the umbilical and uterine blood of the mare and ewe. *J. Physiol.* **209**, 587-608.
- Comline, R.S., Hall, L.W., Lavelle, R. and Silver, M. (1975) The use of intravascular catheters for long-term studies on the mare and fetus. *J. Reprod. Fert., Suppl.* **23**, 583-588.
- Cottrill, C.M. (1991) Placental evaluation in the field. *Equine vet. Educ.* **3**, 204-207.
- Cottrill, C.M., Jeffers-Lo, J., Ousey, J.C., McGladdery, A.J., Ricketts, S.W., Silver, M. and Rossdale P.D. (1991) The placenta as a determinant of fetal well-being in normal and abnormal equine pregnancies. *J. Reprod. Fert., Suppl.* **44**, 591-601.
- Cunningham, E.P., Dooley, J.J., Splan, R.K. and Bradley, D.G. (2001) Micro satellite diversity, pedigree relatedness and the contributions of founder lineages to thoroughbred horses. *Anim. Genet.* **32**, 360-364.
- Dauncey, M.J. (1998) Potential impact of intrauterine growth retardation on muscle function: a cellular and molecular analysis. *Equine vet. J.* **30**, 460.
- Davies, D.P. (1981) Physical growth from fetus to early childhood. In: *Scientific Foundations of Paediatrics*, 2nd edn., Eds: J.A. Davies and J. Dobbing, William Heinemann Medical Books Ltd., London.

- pp 304-329.
- Dawes, G.S. (1968) *Foetal and Neonatal Physiology*, Year Book Medical, Chicago.
- Desai, M. and Hales, C.N. (1997) Role of fetal and infant growth in programming metabolism in later life. *Biol. Rev.* **72**, 329-348.
- Dobbing, J. and Sands, J. (1970) Timing of neuroblast multiplication in developing human brain. *Nature (Lond.)* **226**, 639-640.
- Douglas, R.H. and Ginther, O.J. (1975) Development of the equine fetus and placenta. *J. Reprod. Fert., Suppl.* **23**, 503-505.
- Duncan, I.D., Reifenrath, P., Jackson, K.F. and Clayton, M. (1991) Preferential denervation of the adductor muscles of the equine larynx II: nerve pathology. *Equine vet. J.* **23**, 99-103.
- Evans, J.W. (1971) Effects of fasting, gestation, lactation and exercise on glucose turnover in horses. *J. anim. Sci.* **33**, 1001-1004.
- Flood P.F. (1996) Ever since Daisy: today's endometrium and tomorrow's placenta. *Equine vet. J.* **28**, 170-172.
- Fowden, A.L. (1985) Pancreatic endocrine function and carbohydrate metabolism in the fetus. In: *Perinatal Endocrinology*, Eds: E.B. Albrecht and G. Pepe, Perinatal Press, Ithaca, New York. pp 71-90.
- Fowden, A.L. (1989) The endocrine regulation of fetal metabolism and growth. In: *Advances in Fetal Physiology: Reviews in Honour of G.C. Liggins*, Eds: P.D. Gluckman, B.M. Johnston and P.W. Nathanielsz, Perinatal Press, Ithaca, New York. pp 223-243.
- Fowden, A.L. (1995) Endocrine regulation of fetal growth. *Reprod. Fert. Dev.* **7**, 351-363.
- Fowden, A.L. (1997) Comparative aspects of fetal carbohydrate metabolism. *Equine vet. J., Suppl.* **24**, 19-25.
- Fowden, A.L. and Silver, M. (1995) Comparative development of the pituitary-adrenal axis in the fetal foal and lamb. *Reprod. domest. Anim.* **30**, 170-177.
- Fowden, A.L. and Forhead, A.J. (2001) The fetal origins of cardiovascular and lung disease In: *Lung Biology in Health and Disease*, Vol. 151, Ed: D.J.P. Barker, Marcel Dekker, New York. pp 199-228.
- Fowden, A.L. and Hill, D.J. (2001) Intra-uterine programming of the endocrine pancreas. *Br. med. Bull.* **60**, 123-142.
- Fowden, A.L., Ralph, M.M. and Silver, M. (1994) Nutritional regulation of utero placental prostaglandin production in pregnant ewes and mares during late gestation. *Expt. clin. Endocrin.* **102**, 212-221.
- Fowden, A.L., Li, J., Forhead, A.J. and Silver, M. (1998) Hormones as nutritional signals during intrauterine development. *Equine vet. J.* **30**, 468.
- Ginther, O.J. (Ed.) (1992) Embryology and placentation. In: *Reproductive Biology of the Mare*, 2nd edn., Equiservices, Wisconsin. pp 345-418.
- Ginther, O.J. and Douglas, R.H. (1982) The outcome of twin pregnancies in mares. *Theriogenol.* **18**, 237-242.
- Gruenewald, P. (1963) Chronic fetal distress and placental insufficiency. *Biol. Neonat. (Basel)* **5**, 215-265.
- Hales, C.N. and Barker, D.J.P. (1992) Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. *Diabetologia* **35**, 595-601.
- Hammond, J. (1961) Effect of nutrition on the stage of development of the young at birth in farm animals. In: *Somatic Stability in the Newly Born*, Ed: G.E.W. Wolstenholme and M. O'Connor, J & A Churchill Ltd, London. pp 5-9.
- Harding, R. (1995) Sustained alterations in postnatal respiratory function following sub-optimal intrauterine conditions. In: *Progress in Perinatal Physiology*, Eds: R. Harding, G. Jenkin and A. Grant, CSIRO, Australia. pp 431-441.
- Hess-Dudan, F. and Rossdale, P.D. (1996a) Neonatal maladjustment syndrome and other neurological signs in the newborn foal: Part 1. *Equine vet. Educ.* **8**, 24-32.
- Hess-Dudan, F. and Rossdale, P.D. (1996b) Neonatal maladjustment syndrome and other neurological signs in the newborn foal: Part 2. *Equine vet. Educ.* **8**, 79-83.
- Hinchcliffe, S.A., Lynch, M.R.J., Sargent, P.H., Howard, C.V. and van Velzen, D. (1992) The effect of IUGR on the development of renal nephrons. *Br. J. Obstetr. Gynec.* **99**, 296-301.
- Holdstock, N.B., Rossdale, P.D., Beech, D.J., Ansari, T. and Sibbons, P.D. (2001) Microanatomical development of the equine kidney and defects associated with intra-uterine growth retardation. *Pferdeheilkunde* **17**, 659-661.
- Holtan, D.W., Ginther, O.J. and Estergreen, V.L. (1991) 5 α -pregnanes in pregnant mares. *J. anim. Sci.* **41**, 359. (Abstr.)
- Holtan, D.W., Houghton, E., Silver, M., Fowden, A.L., Ousey, J. and Rossdale, P.D. (1991) Plasma progestagens in the mare, fetus and newborn foal. *J. Reprod. Fert., Suppl.* **44**, 517-528.
- Hurtig, M.B. and Pool, R.R. (1996) Pathogenesis of equine osteochondrosis. In: *Joint Disease in the Horse*, Eds: C.W. McIlwraith and G.W. Trotter, W.B. Saunders Co., Philadelphia. pp 362-383.
- Iwamoto, H.S. (1993) Hormonal regulation of the cardiovascular system, fluids and electrolytes. In: *Perinatal and Pediatric Pathophysiology - A Clinical Perspective*, Eds: P.D. Gluckman and M.A. Heymann, Edward Arnold, London. pp 334-340.
- Jeffcott, L.B. (1991) Osteochondrosis in the horse: searching for the key to pathogenesis. *Equine vet. J.* **23**, 331-338.
- Jeffcott, L.B. and Whitwell, K.E. (1973) Twinning as a cause of fetal and neonatal loss in the Thoroughbred mare. *J. comp. Path.* **83**, 91-106.
- Jeffcott, L.B. and Henson, F.M.D. (1998) Studies on growth cartilage in the horse and their application to the aetiopathogenesis of dyschondroplasia (osteochondrosis). *Vet. J.* **156**, 177-192.
- Jeffcott, L.B., Rossdale, P.D. and Leadon, D.P. (1982) Haematological changes in the neonatal period of normal and induced premature foals. *J. Reprod. Fert., Suppl.* **32**, 537-544.
- LeBlanc, M.M., Calderwood Mays, M.B., Sheerin, B.R., Hendry, J.M. and O'Donnell, L.J. (2001) Route of fetal infection in a model of ascending placentitis in the mare. *Pferdeheilkunde* **17**, 689.
- Love, S. (1990) Hyperlipaemia comes of age (Editorial). *Equine vet. Educ.* **2**, 171-172.
- Lucas, A. (1991) Programming by early nutrition in man. In: *The Childhood Environment and Adult Disease* (Ciba Foundation Symposium 156), Eds: G.R. Brock and J. Whelan, John Wiley, Chichester. pp 38-55.
- Mahaffey, L.W. and Rossdale, P.D. (1957) Convulsive and allied syndromes in newborn foals. *Vet. Rec.* **69**, 1277-1289.
- Mahaffey, L.W. and Rossdale, P.D. (1959) A convulsive syndrome in newborn foals resembling pulmonary syndrome in the newborn infant. *Lancet* **1**, 1223-1225.
- Mallard, E.C., Williams, C.E., Johnston, B.M. and Gluckman, P.D. (1995) Neuronal damage in the developing brain following intrauterine asphyxia. In: *Progress in Perinatal Physiology*, Eds: R. Harding, G. Jenkin and A. Grant, CSIRO, Australia. pp 647-653.
- Mathews, S.G., Lu, F., Yang, K. and Challis, J.R.G. (1995) Hypothalamic pituitary adrenal function in the sheep fetus. *Reprod. Fert. Dev.* **7**, 509-516.
- McMillen, I.C., Adams, M.B., Ross, J.T., Coulter, C.L., Simonetta, G., Owens, J.A., Robinson, J.S. and Edwards, L.J. (2001) Fetal growth restriction: adaptations and consequences. *Reproduction* **122**, 195-204.
- Milner, R.D.G. (1971) Metabolic and hormonal responses to glucose and glucagon in patients with infantile malnutrition. *Pediatr. Res.* **5**, 33-39.
- Nambo, Y., Oikawa, M., Yoshihara, T., Kuwano A. and Katayama, Y. (1995) Age-related morphometrical changes of arteries of uterine wall in mares. *J. vet. med. Ass.* **42**, 383-387.

- Nathanielsz, P.W., Rossdale, P.D., Silver, M. and Comline, R.S. (1975) Studies on foetal, neonatal and maternal cortisol. *J. Reprod. Fert., Suppl.* **23**, 625-630.
- Ousey, J.C. and McGladdery, A.J. (2000) Clinical diagnosis and treatment of problems in the late pregnant mare. *In Pract.* **22**, 200-207.
- Platt, H. (1984) Growth of the equine fetus. *Equine vet. J.* **16**, 247-252.
- Pool, R.R. (1993) Difficulties in definition of equine osteochondrosis: differentiation of developmental and acquired lesions. *Equine vet. J., Suppl.* **16**, 5-112.
- Reed, G.B., Bain, M.D. and Volland, J. (1989) Overview of antenatal and neonatal period. In: *Diseases of the Fetus and Newborn. Pathology, Radiology and Genetics*, Eds: G.B. Reed, A.C. Claireaux and A.D. Bain, Chapman and Hall Medical, London. pp 3-39.
- Reynolds, E.B. (1930) Clinical notes on some conditions met with in the mare following parturition and in the newly born foal. *Vet. Rec.* **10**, 227.
- Rhodes, C.J. and Alarcon, C. (1994) What β -cell defect could lead to hyperproinsulinemia in NIDDM? Some clues from recent advances made in understanding the proinsulin-processing mechanisms. *Diabetes* **43**, 511-517.
- Robinson, N.E. (2001) International workshop on Equine Chronic Airway Disease. *Equine vet. J.* **33**, 5-19.
- Rossdale, P.D. (1972a) Modern concepts of neonatal disease in foals. *Equine vet. J.* **4**, 1-12.
- Rossdale, P.D. (1972b) Differential diagnosis and treatment of equine neonatal disease. *Vet. Rec.* **91**, 581-588.
- Rossdale, P.D. (1976) A clinician's view of prematurity and dysmaturity in Thoroughbred foals. *Proc. Roy. Soc. Med.* **69**, 27-28.
- Rossdale, P.D. (1997) Advances in equine perinatology (1956-1996): a tribute. *Equine vet. Educ.* **9**, 273-277.
- Rossdale, P.D. and Leadon, D. (1975) Equine neonatal disease: a review. *J. Reprod. Fert., Suppl.* **23**, 685-690.
- Rossdale, P.D. and Silver, M. (1982) The concept of readiness for birth. *J. Reprod. Fert., Suppl.* **32**, pp 507-510.
- Rossdale, P.D. and McGladdery, A.J. (1991) Perinatology: a clinical concept. *Equine vet. Educ.* **3**, 208-214.
- Rossdale, P.D. and Ousey, J.C. (1998a) Aims of the workshop and terminology of equine IUGR. In: *The Dorothy Russell Havemeyer Foundation, Third International Workshop on Equine Perinatology: Comparative Aspects*, Eds: P.D. Rossdale and J.C. Ousey, Equine Veterinary Journal, Newmarket. pp 1-2.
- Rossdale, P.D. and Ousey, J.C. (1998b) Abnormal intrauterine development: potential consequences for survival and athletic performance in the horse. In: *The Dorothy Russell Havemeyer Foundation, Third International Workshop on Equine Perinatology: Comparative Aspects*, Eds: P.D. Rossdale and J.C. Ousey, Equine Veterinary Journal, Newmarket. pp 5-6.
- Rossdale, P.D., Ousey, J.C., Dudan, F.E., Leadon, D.P., Cash, R.S.G., Reddy, R., Silver, M., Fowden, A.L., Broughton-Pipkin, F. and Jeffcott, L.B. (1984a) Studies on equine prematurity. 1: Methodology. *Equine vet. J.* **16**, 275-278.
- Rossdale, P.D., Ousey, J.C., Silver, M. and Fowden, A.L. (1984b) Studies on equine prematurity. 6: Guidelines for assessment of foal maturity. *Equine vet. J.* **16**, 300-302.
- Rossdale, P.D., Ousey, J.C., Cottrill, C.M., Chavatte, P., Allen, W.R. and McGladdery, A.J. (1991) Effects of placental pathology on maternal plasma progesterone and mammary secretion calcium concentrations and on neonatal adrenocortical function in the horse. *J. Reprod. Fert., Suppl.* **44**, 579-590.
- Royer, P. (1981) Growth and development of bony tissues. In: *Scientific Foundations of Paediatrics*, Eds: J.A. Dobbing and J. Dobbing, Heinemann Medical Books, London. pp 571.
- Santschi, E.M., LeBlanc, M.M. and Western, P.G. (1991) Progesterone, oestrone sulphate and cortisol concentrations in pregnant mares during medical and surgical disease. *J. Reprod. Fert., Suppl.* **44**, 627-634.
- Schneider, H. (1996) Ontogenic changes in the nutritive function of the placenta. *Placenta* **17**, 15-26. (Erratum in *Placenta* **17**, 268)
- Shaheen, S.O. and Barker, D.J.P. (1994) Early lung growth and chronic airflow obstruction. *Thorax* **49**, 533-536.
- Sibbons, P.D., Ansari, T., Beech, D.J., Pahal, N., Howard, C.V. and van Velzen, D. (1998) Micro-anatomical defects in kidneys, lungs, brain, phrenic nerve and diaphragm, in SIDS infants: a stereological study. *Equine vet. J.* **30**, 458.
- Silver, M. and Fowden, A.L. (1988) Induction of labour in domestic animals: endocrine changes and neonatal viability. In: *The Endocrine Control of the Fetus*, Eds: W. Künzel and A. Jensen, Springer-Verlag, Berlin. pp 417-425.
- Silver, M. and Fowden, A.L. (1994) *Prepartum* adrenocortical maturation in the fetal foal: responses to ACTH1-24. *J. Endocr.* **142**, 417-425.
- Silver, M., Barnes, R.J., Comline, R.S. and Burton, G.J. (1982) Placental blood flow: some fetal and maternal cardiovascular adjustments during gestation. *J. Reprod. Fert., Suppl.* **31**, 139-160.
- Silver, M., Ousey, J.C., Dudan, F.E., Fowden, A.L., Knox, J., Cash, R.S.G. and Rossdale, P.D. (1984) Studies on equine prematurity 2: Postnatal adrenocortical activity in relation to plasma adrenocorticotrophic hormone and catecholamine levels in term and premature foals. *Equine vet. J.* **16**, 278-286.
- Smith, K.C., Blunden, A.S., Whitwell, K.E., Dunn, K.A. and Wales, A.D. (1999) A ten year survey of causes of abortion and neonatal death in British horses. *J. equine vet. Sci.* **19**, 584.
- Sterio, D.C. (1983) The unbiased estimation of number and sizes of arbitrary particles using the disector. *J. Microsc.* **134**, 127-136.
- Steven, D.H. and Samuel, C.A. (1975) Anatomy of the placental barrier in the mare. *J. Reprod. Fert., Suppl.* **23**, 579-582.
- Sweasey, D., Patterson, D.S.P. and Leadon, D.P. (1982) Chemical composition of the spinal cord in the normal developing fetus and in the premature foal. *J. Reprod. Fert., Suppl.* **32**, 563-567.
- Taylor, P.M. (1997) Anaesthesia for pregnant animals. *Equine vet. J., Suppl.* **24**, 1-6.
- van Velzen, D., Howard, C.V. and Sibbons, P.D. (1995) Pathology in human and equine infants: comparative aspects. In: *Workshop on Equine Perinatology: Dorothy Russell Havemeyer Foundation*, Ed: P.D. Rossdale, R & W Publications, Newmarket. pp 23.
- van Weeren, P.R. and Barneveld, A. (1999) Study design to evaluate the influence of exercise on the development of the musculoskeletal system of foals up to age 11 months. *Equine vet. J., Suppl.* **31**, 4-8.
- Weinkove, C., Weinkove, E.A. and Pimstone, B.L. (1974) Insulin release and pancreatic islet volume in malnourished rats. *S. Afr. med. J.* **48**, 1888.
- White, P. and Dauncey, M.J. (1998) Postnatal under nutrition markedly up regulates cardiac $\alpha 1$ and $\alpha 2$ thyroid hormone receptor gene expression. *Proc. Nutr. Soc.* **57**, 79A.
- Whitwell, K.E. (1988) Infective placentitis in the mare. In: *Equine Infectious Diseases*, Vol. 5, Ed: D.G. Powell, University Press, Kentucky. pp 172-180.
- Whitwell, K.E. and Jeffcott, L.B. (1975) Morphological studies on the fetal membranes of the normal singleton foal at term. *Res. vet. Sci.* **19**, 44-55.
- Whitwell, K.E. and Wood, J. (1992) The length of the umbilical cord: association with abortion in Thoroughbreds and investigation into factors influencing length. *Proc. British Equine Veterinary Association Congress* **31**, 53-56.
- Yang, K. (1997) Placental 11β -hydroxysteroid dehydrogenase: barrier to maternal glucocorticoids. *Rev. Reprod.* **2**, 129-132.