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The impact of *in utero* nutritional programming on small ruminant performances

C.J. Ashworth*, C.M. Dwyer, T.G. McEvoy*, J.A. Rooke* and J.J. Robinson*****

Scottish Agricultural College, Sustainable Livestock Systems Group
*Roslin BioCentre, Roslin, Midlothian, EH25 9PS, Scotland (United Kingdom)
**Bush Estate, Penicuik, Midlothian, EH26 0PH, Scotland (United Kingdom)
***Craibstone Estate, Bucksburn, Aberdeen AB21 9YA, Scotland (United Kingdom)

Abstract. The current resurgence of interest in foetal programming of post-natal production traits in small ruminants, notably sheep, is embracing reproductive performance, behaviour and the development of the immune system. It is also posing questions regarding effects on appetite and longevity. The earlier known effects on wool production and carcass composition are being revisited. Much emphasis is being placed on understanding the cellular and molecular mechanisms that are involved, in particular the alterations in the expression of genes involved in prenatal growth and development. Unlike the early studies in which the emphasis was on altered nutrition during the foetal stages of development, it is the pre-implantation embryo that is now attracting attention. The findings are demonstrating the exquisite sensitivity of the early cleavage stage embryo to its environment with permanent carryover effects on the new-born and adult. Nonetheless more quantitative data are required to estimate the economic impact of *in utero* programming in commercial sheep and goat production systems. It is suggested that in farming practice, some of the most important effects of *in utero* nutritional programming may be those relating to behaviour and immune competence with the associated production and product quality benefits coming from improvements in health and welfare.

Keywords. Ruminants – Foetal programming – Nutrition – Carcass composition – Wool production – Reproduction – Health – Behaviour – Appetite.

Impact de la programmation nutritionnelle* in utero *sur les performances des petits ruminants

Résumé. *La reprise actuelle de la recherche sur le rôle de la programmation fœtale des caractères de production post-nataux chez les petits ruminants, entre autres le mouton, prend maintenant en compte les performances reproductives, le comportement et le développement du système immunitaire. Le rôle de cette programmation sur l'appétit et la longévité est présenté et les effets sur la production de laine et la composition de la carcasse, effets déjà connus, sont rediscutés. La compréhension des mécanismes cellulaires et moléculaires impliqués, en particulier les altérations de l'expression des gènes impliqués dans la croissance et le développement prénatal, est développée. Contrairement aux études précédentes dans lesquelles l'intérêt était porté sur les conséquences des modifications nutritionnelles pendant les phases fœtales du développement, l'attention est maintenant portée sur la phase préimplantatoire de l'embryon. Les résultats montrent que les premières phases de la division de l'embryon sont très sensibles aux modifications de l'environnement pouvant entraîner certains effets permanents sur le nouveau-né et l'adulte. Cependant, plus de données quantitatives seraient nécessaires afin d'estimer, pour les productions commerciales d'ovins et de caprins, l'impact économique de la programmation utérine. Il est suggéré que, parmi les pratiques d'élevage, la gestion du comportement (bien-être) et celle du système immunitaire (santé) sont parmi celles qui influencent le plus la programmation nutritionnelle in utero, et impliquent une amélioration des performances zootechniques.*

Mots-clés. *Ruminant – Programmation fœtale – Nutrition – Composition de la carcasse – Production de laine – Reproduction – Santé – Comportement – Appétit.*

I – Introduction

There are numerous examples of the impact of nutrition during foetal life on programming of subsequent production among small ruminants. Although most of the experimental data are for sheep, it is likely that if investigated, similar effects would be found for goats. In addition to the size and viability of the newborn, other production and health traits can be altered. These include carcass composition and wool production, reproductive performance, behaviour and the susceptibility to disease and stress. There is also the question as to whether there may be effects on appetite (McMillen *et al.*, 2005). Longevity is an important production trait in breeding animals but apart from the effects on viability at birth we are unaware of any direct observations of *in utero* nutritional effects on longevity in small ruminants. However, in rats, prenatal exposure to a maternal low protein diet shortened life span (Sayer *et al.*, 2001) and accelerated reproductive ageing in female progeny (Guzmán *et al.*, 2006). The known adult health problems associated with *in utero* growth retardation in a range of species are indicative of an adverse effect on longevity (Wu *et al.*, 2006).

With regard to adult health problems the major organs that are affected by nutritional programming are the heart, kidneys and liver (Ashworth *et al.*, 2005). For each organ there is evidence that the nutritional effects can be programmed during early embryo development, their origin being alterations in gene expression, in particular of the imprinted genes (Fleming *et al.*, 2004; Sinclair and Singh, 2007). The rapidly-expanding knowledge of the sensitivity of mammalian embryos to their environment, as seen in alterations in their gene expression, has been the subject of recent reviews (Fleming *et al.*, 2004; Wrenzycki *et al.*, 2004, 2005; Young *et al.*, 2004; Sinclair *et al.*, 2007) and this aspect will not be considered in detail here. Rather the focus will be on the subsequent impact on production and health.

II – Carcass composition

The legacy of *in utero* growth retardation in terms of the subsequent growth and body composition of lambs that are given milk to appetite is as follows: (i) lower absolute and higher relative growth rates in the first two weeks of life with no difference in absolute growth rates thereafter; (ii) lower rates of gain and absolute amounts of muscle protein and DNA; (iii) higher amounts of body fat and lower amounts of minerals at fixed weights; and (iv) smaller mature sizes (Greenwood and Bell, 2003). These effects are seen when there is a reduction in birthweight. However, transitory periods of undernutrition imposed before day 85 of pregnancy, i.e. when the majority of muscle differentiation and fibre formation occurs (Fahey *et al.*, 2005a), can adversely affect foetal muscle development without decreasing birthweight (Zhu *et al.*, 2004, 2006; Fahey *et al.*, 2005b). The important question, in terms of animal production, is whether these effects on foetal muscle can influence quantitatively, subsequent lean meat production. Early studies, such as those by Nordby *et al.* (1987) and Krausgrill *et al.* (1999) involving undernutrition from mating until day 100 and day 70 of gestation respectively looked solely for effects in lambs slaughtered at either 58 or 35 kg respectively and found no evidence for an influence of early pregnancy undernutrition, apart from its association with more tender meat in the study of Krausgrill *et al.* (1999; see Table 1). Furthermore, when one looks at the results of other studies in Table 1 there is general agreement between those of Zhu *et al.* (2004) and Fahey *et al.* (2005b) with regard to the effects on foetal muscle. There is also broad agreement between the observations of Zhu *et al.* (2006) and Daniel *et al.* (2007) with regard to the increase in the expression of type IIB myofibres when the animals were slaughtered at 8 months and 44/55 kg respectively. However, as shown in the study of Daniel *et al.* (2007), these effects had no material influence on the gross chemical composition of the lambs at their 44 kg commercial slaughter weight. Interestingly, there was a small depression in carcass protein and an increase in the fat: lean ratio at the higher slaughter weight of 55 kg (see Table 1), which again is in agreement with the increase observed by Zhu *et al.* (2006) in intramuscular fat at 8 months of age in the lambs from ewes undernourished from days 28 to 78 of gestation (Table 1).

An important point emerging from Table 1 is that the effects of undernutrition on foetal muscle occur at a very early stage of *in utero* development. In the Fahey *et al.* (2005b) study involving 3 groups of ewes that received 0.5 × maintenance rations during different stages of pregnancy, only ewes that were underfed during the earliest period (30-70 days of pregnancy) gave birth to lambs with altered muscle characteristics on day 14 of life. There was no effect of undernutrition from days 55 to 95 or from days 85-115 of pregnancy on the number of muscle fibres in new-born lambs. The decrease in muscle weight following undernutrition from days 85 to 115 reflected the adverse effect on foetal growth and birthweight of undernutrition at this time. It can be argued therefore that the early critical window occurs before day 55, a view supported by the recent observation of Costello *et al.* (2007) that undernutrition (0.5 × requirements) in the first month of gestation reduced skeletal muscle capillary density in day 127 singleton Welsh Mountain foetuses. Indeed the data of Quigley *et al.* (2005) indicate that there can be pronounced adverse effects of undernutrition on foetal muscle development even earlier; in their case during the period from 18 days before mating until 6 days after mating (Table 1). This observation together with those showing stimulatory effects on foetal muscle growth of altering the *in vivo* environment of ovine embryos (Maxfield *et al.*, 1998) begins to reveal the exquisite sensitivity of the early cleavage-stage embryo to its environment and how it may impact on subsequent foetal and post-natal development.

Table 1. Overview of the effects of feed restrictions during specific periods of gestation on muscle development of offspring (M = maintenance; MHC = myosin heavy chain)

Period of restriction (days of gestation)	Level of restriction	Effect	Reference
0 to 100	0.7 M	- None when slaughtered at 58 kg liveweight	Nordby *et al.* (1987)
0 to 70	Body weight loss, kg (%) Expt 1 – 9.2 (19) Expt 2 – 13.7 (32) Expt 3 – 11.4 (28)	- Foetuses lighter but birthweights and age at 35 kg not affected - No effect on muscle weight or DNA content at 35 kg but meat more tender	Krausgrill *et al.* (1999)
28 to 78	0.5 M	- Reduction in secondary myofibres in foetuses at day 78	Zhu *et al.* (2004)
28 to 78	0.5 M	- Decrease in myofibres and increase in ratio of myosin IIB to other isoforms at 8 months of age - Increase in intramuscular fat	Zhu *et al.* (2006)
30 to 70	0.5 M	At 2 weeks of age: - Fewer fast and more slow fibres; more MHC-slow and fewer MHC-fast expression	Fahey *et al.* (2005b)
55 to 95	0.5 M	- No effect	
85 to 115	0.5 M	- Decrease in muscle weight	
30 to 70 30 to 85	0.5 M	- No effects on carcass composition at 17 weeks (~44 kg) but at 24 weeks (~55 kg) a small decrease in protein and an increase in fat:lean ratio - Increase in fat fibres in 30-70 d restricted group and myosin IIA and IIB in females and IIB in males in 30-85 d restricted group	Daniel *et al.* (2007)
–18 to 6	0.5 M	- 20% reduction in muscle fibre number in day 75 foetuses arising from a reduced secondary to primary fibre ratio - Tendency towards a reduction in protein:DNA ratio in muscle	Quigley *et al.* (2005)

The mechanisms involved in embryonic programming are still the subject of speculation, but seem likely to involve epigenetic modifications in DNA methylation which, in the case of undernutrition, may come about through inadequate amounts of those nutrients (methionine, choline, folate and vitamin B_{12}) on which DNA methylation depends (Sinclair *et al.*, 2007).

In addition to the effects of early *in utero* undernutrition on the development of foetal muscle, subsequent carcass composition and meat tenderness, there is another aspect that merits consideration. This relates to the observations of Zhu *et al.* (2006) that the increase, at 8 months of age, in the intramuscular fat of lambs from ewes undernourished in early pregnancy, was accompanied by a 25% reduction in carnitine palmitoyltransferase-1, a key enzyme in the control of fatty acid oxidation. As pointed out by Zhu *et al.* (2006), such a scenario would make skeletal muscle less sensitive to insulin, thereby raising intriguing questions regarding the maternal metabolism of these animals if used for breeding, in that a reduced sensitivity of maternal skeletal muscle to insulin is a feature of pregnancy and is accentuated by undernutrition (Bell and Ehrdardt, 2000).

III – Wool production

It is over 50 years since the first observations were made of an adverse effect of *in utero* undernutrition on adult wool production with the effect shown to arise from a decrease in the production of secondary wool follicles in the foetus. Now researchers are applying contrasting *in utero* nutritional regimens to genetically-identical Merino foetuses from bisected embryos, in order to bring more precision to quantitative estimates of the *in utero* nutritional effects. The experiments are being carried out in Western Australia and the degree of undernutrition is commensurate with that occurring in Autumn-lambing Merino ewes in that region. A summary of the effects on clean fleece weights is presented in Table 2.

Table 2. Effects of in utero undernutrition on clean fleece wool production as assessed in experiments using identical twin foetuses

Period of differential feeding	Nutritional regimens	Effects of undernutrition on:		Reference
		Birth weight	Wool weight	
50 to 140 days of gestation	Maintenance of ewe weight *vs* ~12 kg (18%) loss of weight	0.5 kg (9%) lighter	0.1 kg (8%) less at 0.4 years of age and 0.14 kg (3.3%) less between 0.4 and 1.4 years	Kelly *et al.* (1996)
Experiment 1: 50 to 140 days of gestation	9 *vs* 18 kg (14 *vs* 28%) loss of weight	0.5 kg (9%) lighter	0.1 kg (8%) less at 4 months of age and 0.17 kg (3.4%) less/year to 6.4 years of age	Kelly *et al.* (2006)
Experiment 2: 50 days of gestation to weaning at 12 weeks of age	3 kg (5%) loss of weight in gestation and 4 kg gain in lactation *vs* 10 kg (15%) loss in gestation and 10 kg loss in lactation	0.5 kg (11%) lighter	0.4 kg (44%) less at 4 months of age and 0.24 kg (5.3%) less/year to 4.4 years of age	

While the percentage reductions (8% at 0.4 years of age falling to 3 to 4% per year in adults) may appear small, these persist throughout adult life (Table 2). It is therefore on this lifetime effect that decisions have to be made regarding whether or not to provide one-off supplementary feeding to their dams during pregnancy. Because the effect arises from a reduction in the number of secondary wool follicles, there is an accompanying increase of up to 0.3 µm in wool fibre diameter and thus an associated decrease in fine wool quality, particularly from the mid-side and leg regions where the adverse effect of undernutrition on secondary fibre numbers is most pronounced. Interestingly, as shown by the data in Table 2 (Kelly *et al.*, 2006; Experiment 2), extending undernutrition into lactation magnifies the differences in wool yield in 4-month old animals but this post-natal nutritional effect largely disappears in adults. The current Australian Lifetime Wool Production Project is verifying the permanent nature of the effects of ewe nutrition on progeny wool production (http://www.lifetimewool.com.au). Furthermore, embracing the impact of *in utero* nutrition on lifetime wool production changes the economically optimal ewe management strategy to one in which the ewe is better fed; extensive field testing is demonstrating that this is achieved by supplementary feed at key times rather than by reducing stocking rate (A. Thompson, pers. comm., 2007).

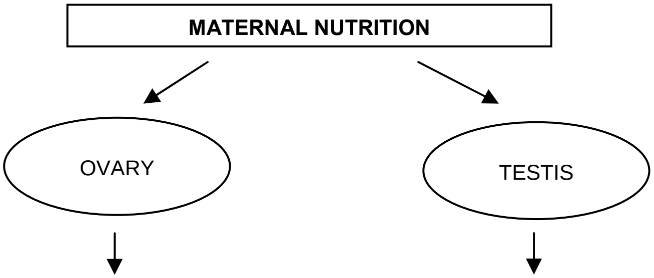
IV – Reproduction

1. The female

Despite numerous studies in ewes showing that undernutrition during a number of different stages of gestation can reduce folliculogenesis in foetal ovaries (see Rhind *et al.*, 2001; Rhind, 2004; Ashworth *et al.*, 2005 for reviews), there is very little quantitative information on down-stream effects on reproductive performance. Rae *et al.* (2002) observed a 20% reduction in ovulation rate at 20 months of age in the progeny of Scottish Blackface ewes that received a 0.5 × maintenance as opposed to a maintenance diet during the first 95 days of gestation. Although the effect of this specific window of 0.5 × maintenance feeding on the development of the foetal ovary appears to be similar to that observed when the same nutritional regimen is applied either during the first 30 days of gestation, the subsequent 20 days or indeed the day 50 to day 65 period that spans germ cell meiosis, there are no data for subsequent effects on ovulation rates. Neither is there information to confirm that the observed 20% decrease in ovulation rate for the days 0 to 95 period of undernutrition is sustained throughout life, or that it results in decreased lamb production, although data from the extensive hill ewe management study (Gunn *et al.*, 1995) that stimulated the experiments on foetal ovarian development imply that it probably is a programmed lifetime suppression of ovulation rate and litter size. Nonetheless, in the apparent absence of observations for *in utero* undernutrition on progeny embryo survival, it is pertinent to note that Kelly *et al.* (2005) found that oocytes collected from 9-week old ewe lambs born to ewes given a sub-maintenance (0.7 × maintenance), diet from 71 to 110 and/or 101 to 126 days of gestation had a lower efficiency of *in vitro* blastocyst production than those from lambs of ewes that received 1.5 × maintenance during these periods. This finding implies an *in utero* nutritional effect on oocyte quality and therefore provides some justification for investigating whether there is, in addition to the ovulation rate effect, an embryo survival component in the *in utero* nutritional programming of litter size. In this regard, it may be pertinent to note that Lea *et al.* (2006) observed that restricted nutrition (0.5 × requirement) during the first 110 days of gestation, increased the primordial follicle expression of the apoptosis-regulatory gene, Mcl1, which has a role in peri-implantation embryo survival. Furthermore if, as seems likely, cell proliferation and apoptosis are important in the programming of *in utero* ovarian effects, it is interesting to note that Arndt *et al.* (2006) have shown reduced cell proliferation in foetal ovarian primordial follicles following high levels of dietary selenium. This effect on cell proliferation was, similar to that which they observed with a 60% maintenance feeding regimen.

2. The male

Impairment of foetal growth by overfeeding adolescent ewe lambs during pregnancy delayed, by 5 weeks, the sustained increase in testosterone concentrations that occurs at puberty (da Silva *et al.*, 2001), yet this nutritional regimen did not appear to have any adverse effect on Sertoli cell numbers in the foetal testes (da Silva *et al.*, 2003). In contrast, Bielli *et al.* (2002) found significantly lower (by 20%) Sertoli cell numbers in the testes of newborn lambs from ewes that just maintained their weight during the second half of gestation compared with those that gained 17%. Lower Sertoli cell numbers (34% reduction) were also observed in the testes of foetuses of ewes grazed continuously on sewage sludge treated pastures (Paul *et al.*, 2005). Here the reduction in Sertoli cells was associated with a similar percentage reduction in testes weight and a 12-15% reduction in foetal weight. It was also accompanied by reductions of 36 and 38% respectively in foetal blood testosterone and inhibin A concentrations. In none of these studies were the subsequent effects on spermatozoa production and fertilizing ability reported. A summary of the *in utero* effects on the development of the foetal ovary and testis as well as associated gaps in our knowledge is given in Fig. 1.



- Alteration in the timing of the transition from primordial to primary follicles
- Shift in primordial and total follicle numbers
- Possible effects on oocyte quality
- Alterations in ovulation and litter size
- Embryo survival (?)

- Alteration in Sertoli cell number
- Alteration of foetal testicular steroidogenesis
- Shift in pubertal testosterone rise
- Spermatozoa number (?)
- Fertilizing ability (?)

Fig. 1. Overview of *in utero* nutritional effects on the development of the foetal gonads and the subsequent impact on reproduction (data sources: Rhind *et al.*, 2001, Rae *et al.*, 2002, Rhind, 2004 and Lea *et al.*, 2006 for the ovary; and da Silva *et al.*, 2001, Bielli *et al.*, 2002 and Paul *et al.*, 2005 for the testis).

V – Health

In numerous recent studies the pregnant ewe has been used as a model for testing *in utero* nutritional effects on the structural integrity of the cardiovascular and renal systems with a view to understanding the role of foetal nutrition in the development of adult hypertension (reviewed by Ashworth *et al.*, 2005 and Symonds *et al.*, 2007). While these studies may be very important in the context of human health, their direct relevance to the long term health of small ruminants in commercial production systems is not immediately apparent, although there may be associated behavioural effects (see later) that are important. Of more immediate relevance to small ruminant farmers is the possible role that *in utero* nutrition plays in the development of the neonate's immune system.

1. Passive immunity

In utero nutritional effects that decrease placental efficiency are associated with reduced neonatal vigour (Dwyer *et al.*, 2005, sheep; Konyali *et al.*, 2007, goat) and an increased risk of sub-optimal acquisition of passive immunity. This raises interesting questions regarding the origins of placental efficiency, particularly as Kakar *et al.* (2005) found that undernutrition during the peri-conception period increased the number of trophectoderm (placenta forming) cells in day 6 sheep embryos, and Dwyer *et al.* (2005) suggested that the higher placental efficiency of Blackface hill ewes compared with the Suffolk breed may be the result of an adaptation to their poorer nutritional environment.

Fisher and MacPherson (1991) found that cobalt deficiency during the first half of pregnancy reduced neonatal lamb vigour and the acquisition of passive immunity. Although Mitchell *et al.* (2007) found no adverse effect of a less severe degree of cobalt deficiency during the peri-conception period on the time taken by lambs to stand and find the udder, their early life (first 3 days) activity and investigatory behaviour was reduced. There is therefore sufficient evidence linking peri-conception and/or early foetal nutrition to neonatal well-being to justify further study.

There are also important recent findings showing an adverse effect of high levels of iodine in ewe mineral supplements on the newborn lamb's ability to absorb colostral IgG (Boland *et al.*, 2005) and vitamin E (Boland *et al.*, 2006), and therefore in its early-life ability to combat infection. Although the daily intake of iodine in these studies was grossly in excess of requirement it mimicked commercial production systems involving free access to mineral blocks. The critical upper limit for iodine was around 10 mg/kg of concentrate dry matter (Rose *et al.*, 2007) with higher concentrations causing a rapid decline in IgG absorption. Although the mechanism for the impaired absorption is unknown, associated alterations in the newborn lamb's thyroid hormone status (Boland *et al.*, 2007; Rose *et al.*, 2007) point to precocious gut maturation and premature termination of pinocytosis; there is also the possibility of a compromised level of absorption. In this regard, the sensitivity of the development of the ovine foetal gastrointestinal tract to *in utero* nutrition is well documented both in feeding-level experiments (Trahair *et al.*, 1997) and in those involving foetal oesophageal infusions (Trahair and Sangild, 2000).

2. Active immunity

In contrast to the well documented evidence in man and laboratory animals that *in utero* nutrition can influence the development of those components of the active immune system (thymic and splenic lymphocyte proliferation and splenic natural killer cell activity) that are important in combating adult disease, there have been few studies with ruminants (Cronjé, 2003). Many of the laboratory animal studies predate the current phase of interest in foetal programming that started in the early 1990s. For example, Beach *et al.* (1983) demonstrated that the offspring of mice deprived of adequate zinc during pregnancy had impaired immunological competence and this persisted, albeit in an attenuated form, in the F2 and F3 generations.

In the case of small ruminants, the development of the main organs (thymus, spleen and lymph nodes) of the immune system takes place in early pregnancy (see Table 3) implying, from the results of laboratory animal studies, that their function could be compromised by early *in utero* nutrition. The post-natal expression of pro-inflammatory cytokines, produced by the Th-2 subset of helper T cells that are thought to mediate resistance to gastrointestinal parasitism (Colditz, 2003) and are part of the acute phase response that follows stimulation of the immune system, are sensitive to *in utero* nutrition (Godfrey *et al.*, 1994). This observation prompted Paganoni (2005) to test for *in utero* nutritional effects on susceptibility to gastro-intestinal parasitism. Ewes either maintained body condition between mating and day 90 of pregnancy or lost one unit of body condition (3 down to 2 on a five point scale). No significant effect of the early *in utero* undernutrition on the faecal worm egg counts of the progeny between 7 and 27 months of age was found but the author suggested that this might have been due to the relatively low larval challenge. In view of the estimated costs of gastro-intestinal parasitism to the sheep industry (£84 million annually for Great Britain; Nieuwhof and Bishop, 2005), further *in utero* studies that embrace greater diversity of nutrient supply and larval challenge, are warranted. There may even be a case for investigating whether there are *in utero* nutritional effects on post natal susceptibility to other endemic diseases such as footrot, mastitis, coccidiosis and blow-fly strike. Such studies, however, should embrace the possibility that enhancing the immune response may result in a transitory impairment of nutrient economy (Sykes and Greer, 2003). Equally, it should be borne in mind that *in utero* nutritional effects on the immune system might occur indirectly through alteration in the metabolism of glucose, a major immune system substrate. In this regard, a reduction in both the circulating concentration of glucose and its response to insulin was noted by Cronjé and Adams (2002) for the progeny of ewes subjected to the same degree of early pregnancy undernutrition as was used in the study of Paganoni (2005) referred to earlier.

Table 3. Ontogeny of components of the ovine foetal immune system (from literature reviewed by Cronjé, 2003 and Paganoni, 2005)†

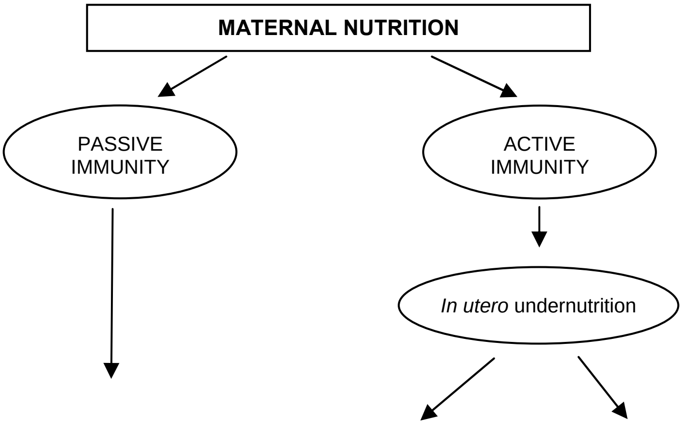
Foetal age (days)	Components of immune system
35	Start of T cell colonisation of thymus
50 to 60	T cell colonisation of spleen and lymph nodes
80 to 90	Cellular composition and immunohistology of thymus same as that at birth
70 to 75	Recirculation of lymphocytes
100 to birth at ~150 days	Exponential growth of spleen and lymph nodes

†The early timing of T cell colonisation, particularly for the thymus implies that the critical window for the nutritional programming of the immune system may be very early pregnancy.

Currently, it is unclear, even from the results of studies on laboratory species, to what extent the effects of prenatal undernutrition on the development of the immune system are direct as opposed to indirect through an alteration in the sensitivity of the foetal hypothalamic-pituitary-adrenal axis. In sheep, this axis seems to be particularly sensitive to relatively modest restrictions in feed intake with a 15% restriction for the first 70 days of gestation causing foetal down-regulation and post natal up-regulation (Hawkins *et al.*, 2000) and a 30% reduction from 2 months before mating to one week post-mating causing foetal up-regulation (Edwards and McMillen, 2002). In terms of the *in utero* nutritional programming of both the passive and active immune systems, there is now sufficient information (see Fig. 2 for overview) to indicate that this is an important aspect of small ruminant research that is worthy of further investigation.

VI – Behaviour

There is now evidence of early *in utero* nutritional effects on offspring behaviour. For example, in ewes, undernutrition during the first 95 days of pregnancy (0.5 maintenance *vs* maintenance) resulted in offspring which, when tested at 18 months of age, exhibited greater activity in a novel environment (Erhard *et al.*, 2004). They also took longer than their adequately-nourished contemporaries to approach a novel object introduced to their environment. These hyper-active responses may have important health implications; they may also influence mothering ability and as a result the neonatal survival of their offspring. In addition, this increased stress reactivity can adversely affect ease of handling and responses to normal on-farm procedures, many of which are intrinsically stressful. Research with laboratory species indicates that behavioural effects can be programmed during the early cleavage stages of embryo development. Our recent finding that sub-clinical cobalt deficiency in embryo donor ewes resulted in newborn lambs that spent less time interacting with their dams than lambs from embryos donated by cobalt-adequate ewes (Mitchell *et al.*, 2007) confirms the importance of the nutritional status of the early cleavage-stage embryo on post-natal behaviour.



- Low peri-conception Co/VitB12 reduced IgG in 2 and 4 week-old lambs
- High I intakes in late pregnancy reduced IgG and Vit E absorption by newborn lamb

- Attenuation of the acute phase response that follows stimulation of the immune system

- Up-regulation of the +HPA-axis with possible adverse effects on the immune system
- Reductions in glucose concentrations and insulin sensitivity with possible adverse effects on the immune response

+Hypothalamic-pituitary-adrenal

Fig. 2. Overview of *in utero* nutritional effects on the programming of the immune system (data sources: Fisher and MacPherson, 1991 and Boland *et al.*, 2006 for passive immunity; and Edwards and McMillen, 2002, Cronjé and Adams, 2002 and Cronjé, 2003 for active immunity).

VII – Appetite

In view of the importance of appetite to the efficiency of animal production and the evidence from laboratory animal studies of prenatal and early life effects on food intake and food preferences (Cripps *et al.*, 2005) there have been remarkably few attempts, in farm animals, to test whether appetite is altered by *in utero* nutrition. Sibbald and Davidson (1998) found no effect of moderate nutritional restriction of ewes during the last 6 weeks of pregnancy and lactation on the voluntary food intake of their lambs between 17 weeks and 2 years of age. They did, however, allude to the possibility of later appetite effects associated with a reduction in liveweight and body condition, particularly in males. Recently, Ford *et al.* (2007) have reported that feed restriction (0.5 × maintenance) of ewes between days 28 and 78 of gestation resulted in male progeny that grew faster and deposited more fat in their carcasses than their *in utero* adequately nourished contemporaries possibly as a result of a programmed increase in appetite although voluntary food intake was not measured. In this study the effect on growth rate and adiposity occurred in the absence of an effect on birthweight but was associated with an elevated insulin response to infused glucose preweaning (63 days of age) followed by a lowered insulin response, indicative of pancreatic β-cell dysfunction, in later life (250 days of age).

In a review that embraces programming of the appetite regulatory system in sheep, McMillen *et al.* (2005) concluded that the higher relative voluntary intake of low as opposed to high birthweight lambs in early life (Greenwood *et al.*, 1998) could be the result of early programming of the appetite regulatory neuropeptide network. This network develops during foetal life and elements of it are responsive, before birth, to nutritionally-sensitive signals such as glucose, insulin and leptin (McMillen *et al.*, 2006). Still missing from this aspect of foetal programming however, are quantitative estimates for the effects on short- and long-term appetite of the range of nutrient intakes that small ruminants experience during pregnancy in their natural environments.

VIII – Conclusions

Although there are numerous examples of prenatal programming of post-natal production and health, much of the recent research has been directed at obtaining greater understanding of the cellular and molecular mechanisms that are involved. Great emphasis is being placed on defining the associated alterations in gene expression, yet for many of the effects there is a paucity of quantitative production data relevant to farming systems. It may well be that some of the most important *in utero* effects, from the point of view of practical sheep and goat production, will come from a better understanding of the role of *in utero* nutrition in programming postnatal and adult behaviour and in the acquisition of immune function with the benefits to production coming from improved health and welfare.

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