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Focus feeding to improve reproductive performance in male and female sheep and goats – How it works and strategies for using it

D. Blache and G.B. Martin

UWA Institute of Agriculture M082, Faculty of Natural & Agricultural Sciences, University of Western Australia, 35 Stirling Highway, Crawley, WA 6009 (Australia)

Abstract. Sheep and goat production in Mediterranean regions is increasingly reliant on "focus feeding" strategies for improving reproductive performance while maximising pasture utilisation and returns on investment in supplements. The strategies have been developed through the integration of four research areas. First, is the nutritional needs for each stage of the reproductive process, from conception to puberty to the start of productive life. Second is the links between reproduction and energy homeostasis, an area of research that led us to the concept of "metabolic status" and a better understanding of: (i) the main physiological (hormonal, neuronal) processes; (ii) genetic influences on the sensitivity of reproduction to changes in metabolic status; and (iii) temporal relationships among these interactions. The main conclusion from this work is that animals use multiple signals (insulin, leptin, ghrelin, kisspeptin) to measure the quantity, quality and timing of variations in metabolic status. The third research area is practical strategies for early adoption by farmers of some aspects of "focus feeding": improving sperm production, ovulation rate and colostrum production. Fourth, we are investigating factors preventing adoption of the riskier aspects of "focus feeding", with an emphasis on two areas: (i) the effects of nutrition on embryo mortality, fetal programming of adult performance, and postnatal survival; and (ii) low-cost techniques for controlling the timing of pregnancy in flocks and herds so that nutritional management can be focused and cost-effective for each stage of the reproductive process. These techniques include ultrasound and a hormone-free option, the male effect.

Keywords. Nutrition – Reproduction – Embryo – Fetus – Metabolic status – Small ruminants.

La supplémentation ciblée pendant une courte période pour améliorer les performances de reproduction chez le mâle et la femelle des ovins et caprins – Comment agit-elle et les stratégies de son utilisation

Résumé. La production ovine et caprine dans les zones méditerranéennes dépend de plus en plus des stratégies de supplémentation ciblée pendant une courte période pouvant à la fois améliorer la reproduction, optimiser l'utilisation des parcours et réduire le recours aux suppléments. Ces stratégies reposent sur quatre axes de recherche. Les deux premiers axes concernent : (i) les besoins nutritionnels pendant la reproduction, de la conception à la puberté jusqu'au début de la carrière de production ; et (ii) les relations entre la reproduction et l'homéostasie énergétique. Ce travail a conduit au concept d'état métabolique pour mieux comprendre : (i) les mécanismes physiologiques (hormonaux et nerveux) ; (ii) les effets génétiques sur la sensibilité de la fonction de reproduction face à l'état métabolique ; et (iii) les relations temporelles entre ces interactions. La principale conclusion est que les signaux utilisés pour mesurer le niveau, la qualité et la rythmicité de l'état métabolique sont multiples (insuline, leptine, ghréline, kisspeptine). Le troisième axe cible les stratégies pratiques pour l'adoption par les éleveurs d'effets connus de la supplémentation ciblée pendant une courte période comme l'amélioration de la production de sperme, du taux d'ovulation, et de la production de colostrum. Enfin, les facteurs qui empêchent l'adoption des aspects risqués de la supplémentation ciblée pendant une courte période ont été étudiés, principalement : (i) les effets de la nutrition sur la mortalité embryonnaire, la programmation fœtale des performances de l'adulte et la survie postnatale ; et (ii) les techniques peu coûteuses, comme l'ultrasonographie et l'effet bélier, qui aident à contrôler et synchroniser la gestation afin que la conduite alimentaire soit plus précise et plus rentable.

Mots-clés. Nutrition – Reproduction – Embryon – Fœtus – État métabolique – Petits ruminants.

I – Introduction

An energy cost is attached to the physiological and behavioural components of the reproductive process as illustrated by the poor reproductive success observed in sheep and goats raised under conditions where nutritional inputs are limited (Lindsay *et al.*, 1993). It is possible to improve the reproductive performance of sheep and goats by using short, targeted feeding regimes, "focussed feeding" (Martin *et al.*, 2004). Successful focussed feeding targets the underlying mechanisms that have evolved to allow sheep and goats to reproduce in changeable environments. However, reproductive performance can be optimised further, the biological limits of the animal being theoretically the only limitation to the development of new strategies. In this paper, we consider strategies that are already in use, such as nutritional stimulation of ovulation rate or sperm production, and the biology behind them, and we propose research to further improve reproductive capacity.

II – Energy requirements for reproduction

Reproduction is considered energetically more demanding for females than males because of the requirements for gestational development and milk production, but nutritional limitations affect the reproductive process in both genders. The genders differ in the timing of maximum energy demand –in males, high energy intake boosts spermatogenesis before fertilization and provides energy reserves to support sexual and aggressive behaviour during mating (review: Martin and Walkden-Brown, 1995). In females, most energy is invested after fertilization, often with a considerable delay (Horton and Rowsemitt, 1992). For example, in grazing ewes, the process of ovulation has no impact on energy balance and the first 3 months of gestation costs only 3% of daily energy expenditure, but the cost builds rapidly to 20% over the last 2 months before parturition (Fierro and Bryant, 1990).

Therefore, for maximum efficiency, the nutrition of the animal must be precisely matched to each stage of the reproductive process. The availability of energy within the animal is usually quantified as "energy balance" –the difference between the pool of disposable energy and the amount of energy expended, including the demands for maintenance. However, this concept gives the impression that the situation is passive. We thus prefer to use the term "metabolic status" –it includes an integrative dimension that is illustrated by the number and variety of physiological control processes that influence reproductive function (Fig. 1).

III – Physiological basis of the links between metabolic status and reproduction – The blueprints for focussed feeding strategies

To be successful, reproduction must at least exceed the lower limit at which a constant number of animals is maintained and this is only feasible if the reproductive regulatory systems resonate with the metabolic regulatory systems (Blache *et al.*, 2007a). This requires a level of fine-tuning that is made possible by interactions among a multiplicity of hormonal and neural signals, the genetic make-up of the animal and a temporal component that is an important aspect of nutrition-reproduction cross-talk.

1. Hormonal, metabolite and neuronal pathways

In the control of the reproductive process, the secretion of hypothalamic GnRH, and consequently pituitary LH and FSH, is central to both sexes. When the production of GnRH pulses increases above a threshold frequency, in response to external or internal factors, males will produce sperm and females will ovulate. The system that controls GnRH secretion is also the final common pathway via which gonadal activity is influenced by most factors that have a high-level impact on reproductive strategy, including socio-sexual cues, photoperiod, and energy balance (review: Martin *et al.*, 1994, 2004). However, some of the tissues involved in the process of reproduction, including

the pituitary gland, gonads and mammary glands, can also respond to metabolic inputs independently of changes in the GnRH signals, although not to the extent of switching the reproductive process on or off. The GnRH signal is always dominant, so the systems that regulate reproduction via changes in GnRH pulse frequency need to respond accurately, and in an integrative fashion, to changes in metabolic status. To perform their integrative roles, metabolic regulatory systems originating from a variety of organs use a variety of signals such as nutrients, hormones, and neural connections.

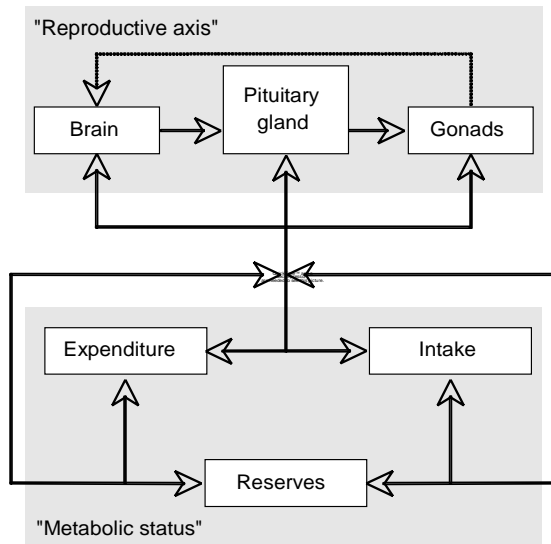


Fig. 1. Schematic representation of the relationship between metabolic status and reproductive axis. Arrows represent signalling systems utilising hormones, metabolites or neuronal inputs. Metabolic status comprises three compartments connected to each other by similar signalling systems. All signals coming from metabolic status converge on reproductive axis. This convergence is: 1) physical because the hormones and metabolites circulate in blood; 2) structural because the same organs (eg brain) can be affected by multiple signals; 3) temporal because the signals can act in cascade over time, one signal affecting the production of another. The interactions between signals can operate on a very short time scale but also can be time-delayed by the "metabolic memory".

A. Direct inputs by nutrients

Critical nutrients, such as amino acids, fatty acids and carbohydrates can signal the amount of energy that is readily available, from digestion and from stores. A diverse variety of somewhat uncoordinated experimental observations in this field makes it difficult to provide a coherent overall picture. For example, in goats, deprivation of lipid availability at cellular level does not reduce the activity of GnRH neurons (Ohkura *et al.*, 2004) whereas, in rams, supplements of fatty acids, a major currency in energy transactions in ruminants, increase GnRH secretion (Blache *et al.*, 2000). In female sheep, pulsatile LH secretion is not affected by intravenous infusion of neurotransmitter precursors or large-neutral amino acids (Downing *et al.*, 1995, 1996, 1997). The role of glucose is also not clear –in male sheep, intra-abomasal, intracerebral and intravenous infusions of glucose failed to stimulate LH secretion (Miller *et al.*, 1995; Boukhliq *et al.*, 1997). In contrast, in goats and

young sheep, glucose deprivation at cellular level (using 2-deoxyglucose) or peripheral level (using insulin injections), decreases GnRH neuronal activity (Kittok, 1999; Ohkura *et al.*, 2004). Overall, nutrients seem to have permissive effects on GnRH secretion because highly deficient situations are strongly inhibitory, but these experimental models are often extreme and might not reflect normal processes in grazing animals. On the other hand, some nutrient-based signals could trigger the endocrine systems described below and stimulate the reproductive axis.

B. Endocrine systems linking metabolic status and GnRH secretion

Reproduction can be influenced by many metabolic endocrine systems because each component of metabolic status produces specific hormonal signals, many of which have been shown to affect the reproductive endocrine axis. In the next section, the hormonal systems have been grouped according to their relationship with the three compartments of metabolic status for energy (Fig. 1) although, as discussed below, none of the hormonal systems are affected by changes in only one compartment.

a] Endocrine factors linked to the amount of energy readily available

Insulin secretion is affected by the amount of energy available and it is involved in the control of reproduction in male sheep: (i) a high plane of nutrition leads to high concentrations of insulin in both plasma and cerebrospinal fluid (Miller *et al.*, 1998; Zhang *et al.*, 2004, 2005); (ii) with a restricted diet or with diabetes, infusion of a low dose of insulin into the third cerebral ventricle increases LH pulse frequency to values similar to those seen in well-fed animals (Miller *et al.*, 1995, 2002; Tanaka *et al.*, 2000); (iii) insulin receptors are present in the hypothalamus (Blache *et al.*, 2002); and (iv) following an acute dietary supplement, the increase in insulin secretion coincides with the increase in LH pulse frequency (Zhang *et al.*, 2004). Thus, the accumulated evidence strongly suggests a major role for insulin in the GnRH response to variations in metabolic status. Insulin may also act at ovarian level (Downing *et al.*, 1995).

The suggestion that GH could provide nutritional inputs to brain centres that control gonadotrophic outputs (Monget and Martin, 1997) is supported by the presence of mRNA for GH receptors in the bovine hypothalamus and pituitary gland (Lucy *et al.*, 1998). Energy balance certainly affects plasma concentrations of GH under a wide variety of conditions (Bossis *et al.*, 1999) but, in male sheep at least, it is not likely to be involved in the stimulation of GnRH secretion because an increase in nutrition induces a decrease in plasma GH concentrations (Miller *et al.*, 1998).

IGF-I and its receptor have been found in the rat hypothalamus (Schechter *et al.*, 1994) so IGF-I might play a direct role in the control of GnRH neurons. Concentrations of IGF-I are affected by diet in male sheep and peripheral administration of physiological doses of IGF-I inhibits LH secretion, but this is probably through an action at pituitary level rather than by a change in GnRH pulse frequency (Adam *et al.*, 1998). Indeed, IGF-I concentrations in blood and CSF are not affected by diet (Miller *et al.*, 1998) and we have been unable to demonstrate that IGF-I infusion into the third ventricle affects LH pulse frequency (Blache *et al.*, 2000). It therefore seems unlikely that, in male sheep at least, IGF-I is involved in the effects of changes in nutritional status on the brain's reproductive centres.

A most interesting candidate is ghrelin, a gut hormone that is the endogenous ligand of the GH secretagogue (GHS) receptor. In rodents, the distribution of GHS receptors overlaps that of GnRH in the arcuate nucleus (St-Pierre *et al.*, 2003) and ghrelin decreases the secretion of LH (Fernández-Fernández *et al.*, 2004). Interestingly, ghrelin and GHS receptors are also expressed in the testis of humans and rats (Tena-Sempere, 2005) and, in sheep, ghrelin secretion is inhibited by an increase in feed intake and stimulated by fasting (Sugino *et al.*, 2004). In cows, ghrelin concentrations decrease during progress from early to mid- to late lactation, suggesting greater ghrelin production during the postpartum period of negative energy balance (Itoh *et al.*, 2005). Clearly, we need research on the role of ghrelin and reproduction in small ruminants, especially because ghrelin seems to be responsive to all three components of energy balance (intake, storage and expenditure).

b] Endocrine signals linked to the amount of energy reserves

The role played by adipose tissue in metabolic homeostasis has evolved from that of a passive energy reserve to that of a very active endocrine regulator of a many functions, including thermoregulation, food intake, metabolism, immunity, and cardiovascular function, as well as reproduction (Ahima, 2005). Of the 20 or more endocrine products of adipose tissue, leptin seems to be the most important regulator of reproductive activity in ruminants (review: Chilliard *et al.*, 2005). On the other hand, leptin is also the most studied of the adipocyte hormones and future research might reveal key roles for the others. The expression and release of leptin, and the sensitivity of gonadal and brain tissues to leptin, are all altered by short- and long-term changes in metabolic status. Moreover, numerous experiments in female and male sheep have shown that leptin can affect the neuroendocrine systems that control the activity of the reproductive axis (reviews: Adam *et al.*, 2003; Chilliard *et al.*, 2005). A recent study in dairy cattle has illustrated the close link between leptin concentrations and LH pulse frequency during the post-partum period (Kadokawa *et al.*, 2006), although a triggering role for leptin in the termination of anoestrus is not always supported (Chagas *et al.*, 2006). Leptin is also found in the blood and milk of lactating goats, and milk leptin decreases dramatically after parturition, but its role is yet to be elucidated (Whitley *et al.*, 2005). The consensus emerging from the large body of literature is that the role of leptin is permissive rather than triggering (Blache *et al.*, 2007a). A most important observation is that, in ovariectomised ewes fed a restricted diet for almost a year, LH pulse frequency responds to re-feeding before any increase in leptin concentrations is detected (Szymanski *et al.*, 2007) suggesting that leptin is not involved in rapid GnRH responses to sudden increases in energy availability.

Adiponectin, another hormone exclusively produced by adipose tissue, stimulates energy expenditure without any effect on feed intake when it is infused into the cerebral ventricle of the rat, in contrast to leptin (Ahima, 2005). In male rat pituitary cells in culture, adiponectin reduces the expression of GnRH receptor and decreases the secretion of LH (Malagón *et al.*, 2006). An effect of adiponectin on the activity of GnRH neurons is yet to be demonstrated.

c] Endocrine signals linked to energy expenditure

Thyroid hormones play a role in the seasonality of reproduction in sheep and goats, probably through inputs into endogenous cerebral rhythms (Karsch *et al.*, 1995; Walkden-Brown *et al.*, 1996). In goats, circulating thyroid hormones are a good indicator of metabolic status (Todini *et al.*, 2007). However, in mature male sheep, the concentrations of thyroid hormones in plasma and CSF are not affected by an acute nutritional supplement that increases LH pulse frequency, suggesting that they play no role in the rapid response of the GnRH neurons (Miller *et al.*, 1998; Zhang *et al.*, 2004, 2005). Overall, there is little evidence for thyroid hormones being important in the link between energy balance and reproduction in adults.

d] Interactions among the signalling systems

Several levels of integration are needed because metabolic status at any given time depends on the status of all three key compartments –intake, storage and expenditure. Essential interactions are managed by several hormonal systems, perhaps best exemplified by leptin secretion because it is affected by intake and expenditure as well as storage (mass of adipose tissue). Leptin can stimulate the activity of three other endocrine systems involved in controlling the reproductive axis: (i) pancreatic insulin in fasted cattle (Zieba *et al.*, 2003); (ii) pituitary GH in female sheep (Henry *et al.*, 2001) but not normal-fed cattle (Zieba *et al.*, 2005); and (iii) thyroid hormones (Flier *et al.*, 2000). In addition, leptin secretion is affected by hormonal systems such as insulin, thyroid, growth factors and stress hormones (Zieba *et al.*, 2005). Leptin expression and secretion are also controlled by the products of digestion and absorption, and by autonomic neural activity (Penicaud *et al.*, 2000).

C. Brain pathways

It is important to note that the ultimate level of integration is within the central nervous system (Fig. 1). The brain mechanisms involved in the sensing of metabolic status, and in the connection of metabolic status to GnRH neuronal activity, are poorly understood. Some sort of "metabolic sensor" is thought to be localised in the arcuate nucleus and median eminence because receptors for leptin and insulin are found in these areas (Blache *et al.*, 2002; Chilliard *et al.*, 2005). Possible links, such as neuropeptide-Y neurons, have been identified between GnRH cells and the neurons containing insulin and leptin receptors. Other neuropeptides, such as cocaine-and amphetamine-regulated transcript, pro-opiomelanocortin, and agouti-related peptide, all involved in the control of feed intake, could be acting as "metabolic sensors", integrating peripheral signals and regulating the GnRH pulse generator (reviews: Blache *et al.*, 2002, 2006). A most promising candidate is the recently discovered neuropeptide, kisspeptin –the kisspeptin receptor is found in over 75% of GnRH neurons in male rats (Irwig *et al.*, 2004) and kisspeptin can be seen as major player in the processing of inputs into the systems that regulate GnRH and LH secretion (Messenger *et al.*, 2005).

IV – Genetic influences on the sensitivity of reproductive function to changes in metabolic status

In perhaps all small ruminants, the timing of reproduction is affected by photoperiod, but some genotypes respond more strongly than others, often reflecting the geography of their origin. For example, the breeding season of the Merino is primarily controlled by integration of an endogenous annual rhythm with responses to changes photoperiod, just as it is in the Suffolk, but Merinos are not as strongly affected by photoperiod as Suffolks, reflecting the latitude of their historical origins (Martin *et al.*, 2002). The effect of genotype on responsiveness to photoperiod also affects responsiveness to nutritional stimuli (Blache *et al.*, 2003). In Merino rams, the time of year has no major influence on the response of the GnRH neurons to stimulation by nutrition. In contrast, in Suffolk rams, LH pulse frequency responds to a feed supplement during the breeding season, but not during the non-breeding season when the reproductive axis is dominated by the inhibitory effects of photoperiod (Martin *et al.*, 2002). Thus, nutrition can only influence the GnRH pulse generator when there is no inhibitory effect of photoperiod, either because the photoperiod is stimulatory for photosensitive genotypes, or because it is virtually irrelevant (genotypes that are not very photosensitive). It is our view that photoperiod exerts a "filtering" effect on both nutritional and social inputs, and that this filtering effect is determined by genotype (Blache *et al.*, 2003).

1. Temporal components inherent in interactions with metabolism

Dynamic aspects of the responses to nutritional inputs, the effects of time *per se*, need to be considered if we are to understand the long-term effects of nutrition on reproduction (e.g., fetal programming). In rams, the GnRH response to an abrupt change in nutrition is initially rapid and robust, but then fades over the next few weeks (Martin *et al.*, 1994; Zhang *et al.*, 2004). The rapidity of the response is consistent with the dynamics of the autonomic nervous system and could involve input from the digestive viscera to the brain via the vagus nerve, as is the case for satiety responses following a meal (Woods *et al.*, 2004). However, hormonal systems can also react very quickly to the absorption of some nutrients (e.g., volatile fatty acids, glucose). In rams, plasma insulin concentrations respond about 3 h before GnRH following an abrupt increase in intake (Zhang *et al.*, 2004). In contrast, the long-term effect of nutrition on the testis, measured on a scale of weeks, seems to be independent of changes in the primary, GnRH-based, control system (Hötzel *et al.*, 1995). The mechanism involved has not been studied further but, again, leptin might be implicated because, in the rat at least, the testis contains leptin receptors and leptin can inhibit testicular steroidogenesis (Tena-Sempere *et al.*, 2001). On an annual timescale, the role of nutritional inputs, as well as the types of response to those inputs, can vary substantially, especially in genotypes that undergo seasonal changes in appetite (Rhind *et al.*, 2002). Control systems that might mediate these effects could include ghrelin, leptin and the brain neuropeptides listed above

because their expression is modulated by photoperiod (Adam *et al.*, 2000; Alila-Johansson *et al.*, 2004).

In sheep, previous metabolic status influences the reproductive response of the animals to an increase in energy availability. With respect to adipose stores in mature rams, animals in low body condition, but not high body condition, show a robust and repeatable increase in LH pulse frequency in response to an increase in intake. In low body condition, the leptin response is blunted but the response to insulin is not (Zhang *et al.*, 2005). These observations suggest that: (i) neither insulin nor leptin are necessary for inducing an increase in GnRH pulse frequency in response to an increase in food intake; and (ii) leptin secretion does not always respond to an influx of nutrients. This is consistent with the notion of a "metabolic memory" that modulates the stimulatory effect of nutrient intake according to the level of energy reserves or energy expenditure (Blache *et al.*, 2007b). Recently, the processes that might underpin "metabolic memory" have been investigated in sheep and cattle and the data suggest that leptin and insulin play central roles (Chilliard *et al.*, 2005). In addition to leptin and insulin, "metabolic memory" could involve growth factors and nutrients (e.g., short-chain fatty acids) that affect leptin secretion in whole-animal studies and in isolated adipocytes (Chilliard *et al.*, 2005).

V – Strategies for focus feeding that are ready for adoption

Decades of research on nutrition-reproduction interactions have led to the development of "focus feeding", the success of which depends on the timing and quality of the dietary stimulus as well as the metabolic history of the animals (particularly "metabolic memory"). Focus feeding is already being used to boost sperm production, increase ovulation rate and improve offspring survival (Fig. 2).

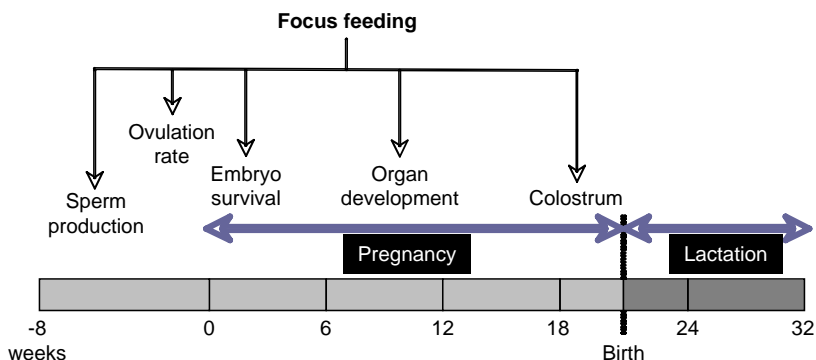


Fig. 2. Targets for focus feeding that are known to affect reproductive output in small ruminants.

Timing, quantity and quality of supplements are not indicated because they vary between sheep and goats, and among genotypes and environments. Redrawn after Martin *et al.* (2004).

1. Sperm production and sexual behaviour

Feeding supplements to rams and bucks for 8 weeks before mating ensures that their testicular size and sperm production are maximal (review: Martin and Walkden-Brown, 1995). These responses are reliable in genotypes that are not dominated by photoperiod, but also can be used in more photoperiod-sensitive breeds, such as the Corriedale (Pérez-Clariget *et al.*, 1998; Martin *et al.*, 2002). Feeding before mating needs to be well controlled for both sexes because the sexual behaviour of males and females is reduced by severe decreases in intake. The effect of severe under-nutrition in the ram has been attributed to general weakness (Parker and Thwaites, 1972).

On the other hand, over-nutrition decreases mating behaviour simply because the increase in weight leads to clumsiness (Okolski, 1975). Among adult ewes, severe undernutrition may alter sexual behaviour because poor body condition leads to irregular oestrous cycles or acyclicity (Allen and Lamming, 1961).

2. Maximize potential litter size

Nutrition can increase potential litter size by increasing ovulation rate in both goats and sheep. There are responses to long-term increases in body weight, the "static effect", and also responses to very short and well-timed supplements (e.g., 4 days in the final stages of the oestrous cycle), the "acute effect" (review: Scaramuzzi *et al.*, 2006). Both the "static" and "acute" effects of nutrition alter ovarian folliculogenesis (Vinoles *et al.*, 2002, 2005), apparently via direct actions on the ovary by leptin, IGF-I, insulin, and glucose (review: Scaramuzzi *et al.*, 2006).

In addition to increasing the number of offspring produced during each breeding season, the total number of offspring produced over a lifetime can be increased in both goats and sheep by using a high plane of nutrition to advance the time of puberty (Fasanya *et al.*, 1992; Robinson *et al.*, 2006). In Savanna Brown goats, at least, this seems to be independent of body weight (Fasanya *et al.*, 1992).

3. Colostrum production and lamb survival

Increases in litter size are economically and ethically justified only if all the offspring survive until sale or reproductive maturity. The survival of lambs is mainly compromised during the first few hours after birth because of the massive heat loss, especially during the coolest months of the year. To produce heat, the lamb will utilise brown fat but that represents only 2.0-4.5% of its body weight so is quickly depleted (review: Nowak and Poindron, 2006). It is therefore essential for the lamb to have access to a high quantity of quality colostrum during the first hours after birth. The colostrum provides energy and immunoglobulins and, in addition, it will facilitate the establishment of the ewe-lamb bond (Dwyer *et al.*, 2003; Nowak and Poindron, 2006). The problems caused by inadequate fat reserves and colostrum are exacerbated in twin-bearing ewes (Nowak and Poindron, 2006), so feeding strategies have been designed to optimise birth weight and colostrum production. This "focus feeding" needs to be fine-tuned for single- and twin-bearing females, and for different genotypes (Nowak and Poindron, 2006). The necessary degree of precision is only possible if the number of fetuses is known (ultrasonography) and if there is a good synchronisation of conceptions in the flock or herd. In ewes, colostrum production during the first 12 hours after parturition can be doubled by feeding 30% above maintenance requirements for the final 10 days before parturition (review: Banchemo *et al.*, 2006). However, the nature of the supplement is crucial –starchy feedstuffs such as maize have been shown to stimulate colostrum production, but protein-rich lupin grain is less reliable (review: Banchemo *et al.*, 2006). The need for precision management is clear because, at the end of pregnancy, over-nutrition can increase birth mass and cause distocia.

VI – Development of the next phase of focus feeding

Nutrition during pregnancy also affects embryo survival and fetal programming of adult performance. Paradoxically, both under- and over-feeding are listed among the factors suggested to cause embryo loss in the first few weeks after fertilisation (Abecia *et al.*, 2006). Overfeeding seems to change the liver blood flow and consequently increase the clearance of progesterone (Parr *et al.*, 1993). Underfeeding is thought to act on progesterone delivery to the uterus which is important for the development of the placenta and fetus (Abecia *et al.*, 2006). Remarkably, there is an additive effect of under-nutrition before and after mating. Underfeeding from 60 days before until 30 days after conception, which induces only a moderate 15% loss of body mass in ewes, increases the number of premature births, with some lambs born up to 20 days before term (Bloomfield *et al.*, 2003). Whilst demonstrating the importance of nutrition, this phenomenon raises questions about mechanisms that lead to delayed responses to subtle changes in body weight. The

"Bloomfield effect" might be one of the most dramatic illustrations of the concept of "metabolic memory".

Effects of nutrition over longer time scales are clearly evident in "fetal programming". It has long been known that low birth weight is associated with poor life-time performance in production animals (review: Bell, 1984) mainly because inadequate nutrition of the mother during the development of the fetal organs (days 60 and 120 of pregnancy) will alter the function of those organs in later life. In humans, under-nutrition during pregnancy alters many physiological functions at adult age, including those that control metabolism and the hypothalamo-pituitary-adrenal axis (McMillen and Robinson, 2005). In sheep, the number of Sertoli cells is reduced by around 20% in lambs born from undernourished mothers (Bielli *et al.*, 2002) suggesting that the male reproductive axis could be programmed during fetal life. Undernutrition can also have serious effects on the metabolism of the offspring –lambs from undernourished ewes have a greater requirement for amino acids early in life and are insulin-resistant (Greenwood *et al.*, 2002). Surprisingly, over-nourished adolescent ewes also give birth to low liveweight lambs that show metabolic dysfunction and impaired regulation of food intake later in life (Wallace, 2000; Bouret and Simerly, 2006). Changes in insulin sensitivity and in the hormonal balance that controls the metabolic status of animals should have dramatic consequences on animals born with low live weight, particularly their ability to accurately regulate their reproductive function in response to variations in nutrition.

1. Low-cost techniques for controlling and synchronising the reproductive process

The main limitation of focus feeding is the need for accurate timing to ensure the success and economic viability of feed supplements. One option for controlling the time of ovulation is the "male effect" –the induction of ovulation by the sudden introduction of novel males to ewes or does that are reproductively quiescent because they are out of season or lactating (review: Walkden-Brown *et al.*, 1999). In addition to giving us control over the timing of births, the male effect offers synchronised mating (adequate for AI) and therefore synchronised parturition in the flock or herd. Although attractive from the perspective of "clean, green and ethical" management, the male effect has several limitations that require serious research input (review: Martin *et al.*, 2004). Most important are variation in the proportion of females that respond and, during the first cycle after the introduction of the male, the high frequency of non-functional corpora lutea accompanied by an unpredictable display of fertility and oestrus (review: Chemineau *et al.*, 2006).

Advances are being made, however, we now know that the mode of presentation of the male to the females can affect the efficiency of the male effect as shown by work done during the transition period between non-breeding and breeding seasons (Hawken *et al.*, 2007). Ewes can be synchronised by a sequence of three short (24 h) exposures to vasectomised rams every 17 days, leading to over 90% of the ewes lambing during a 10-day period. This does not match the outcomes with pharmacological synchronisation (3-4 days), but it is better than a randomly cycling flock and within the constraints necessary for implementing focus feeding.

We also know now that previous experience with mature rams can improve the responses of young ewes to the male stimulus (Gelez and Fabre-Nys, 2006b). The emerging knowledge of the brain pathways involved in the male effect (Gelez and Fabre-Nys, 2006a) offers new avenues to improve its efficiency and therefore its value for farmers.

A major problem is that the "male effect" is particularly effective in some genotypes, particularly those of Mediterranean origin (e.g., Merino), and either marginally useful or completely ineffective in others. In very seasonal genotypes, the male effect might be ineffective because of the poor quality of the signals (behavioural, olfactory, visual) from the rams, as has been suggested for the "buck effect" (Delgadillo *et al.*, 2006). Importantly, the responses depend on the nutrition of the animals and, in seasonal genotypes, the response is blocked when the animals are under long days (Blache *et al.*, 2003; Véliz *et al.*, 2006). The nature of the interactions among the male effect, nutrition and photoperiod needs to be investigated further.

VII – Conclusions

Breeds that have evolved in environments where nutritional supplies vary over time, such as the Mediterranean region, are the best breeds for "clean, green and ethical" animal production. Because they are well adapted to the climate, they are very responsive environmental manipulation as a method for controlling and improving their reproductive output. Based on solid knowledge of the physiology responsible for the natural tuning of reproductive function to metabolic status, research will lead us towards simple, flexible and low-cost nutritional tools with which producers can improve the reproduction of their flock or herd. The timing of the dietary treatments and the nature of the diet will need to be defined according to the requirements of the animal, a basic rule in nutrition, but also according to the past history of the animals because of the carry-over effects of nutrition that are illustrated by metabolic memory and fetal programming. Finally, it is important to remember that these tools can only increase reproductive output within biological limits and that they should be developed with specific respect to the genotype of animal and its environment.

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