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Overview

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Introduction

Ruminants have played essential roles in the development of many civilizations. Their diversity of form and function between, and indeed within, species, their adaptations to cope with environmental extremes and their ability to utilize cellulosic plants and plant by-products not suitable for direct consumption by man are invaluable attributes. Interestingly, approximately one-third of the world's domesticated ruminants are in the Indian subcontinent and South East Asia. Here, their primary role is to provide draught power with milk and meat being subsidiary, but also important. Currently, ruminants provide draught power for approximately half of the world's crop production (Council of Agricultural Science and Technology, 1999).

To farmers, ruminant reproduction is critical to the viability of their ruminant livestock enterprises. To researchers, it represents a fascinating network of neuroendocrine and biochemical transactions for which a clear mechanistic understanding requires complementary investigations at whole animal, organ, tissue, cellular and molecular levels. Within the present symposium the benefit of a complementary research approach is further reinforced by the inclusion of species and breed diversity. Therefore, it seems appropriate that this overview should also be complementary and not just a reiteration of material presented during the symposium. For this reason it represents personal thoughts, questions and views prompted by the breadth and depth of the excellent scientific contributions. It is also interspersed with references to published data that hopefully complement the symposium proceedings and are both relevant and interesting.

Energy balance and ovulation

From a quantitative viewpoint the energy required to fuel ovulation as an event must be trivial. It is thus not surprising that ovulation can occur, as in the case of the high-yielding dairy cow, when the body is in negative energy balance. Yet, if the negative energy balance is excessive it is delayed. Ovulation is also delayed in working ruminants by the body energy deficit created by exercise (Zerbini *et al.*, 1993). Interestingly, in this case, there is a partial effect of work *per se* that is independent of energy balance.

Although energy balance is important to reproductive success, its link to ovulation is complex. Its main weakness as a practical index of nutritional status is the lack of information on its composition. In addition, attempting to interpret the link between energy balance and ovulation in ruminants in terms of deviations from what are regarded as homeostatic 'norms' for the blood concentrations of metabolites is probably too simplistic. In contrast to the GnRH pulse generator of the nutritionally responsive Merino ram, which responds almost immediately to improved nutrition, in a manner similar to small female rodents in the

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wild (Bronson, 1998), the link between energy balance and ovulation in female domestic ruminants probably involves longer-term homeorhetic (Bauman, 2000) mechanisms. These bring into play energy-balance driven adaptations in key tissues (adipose, skeletal muscle) and organs (liver, brain, ovary) mediated by alterations in the expression and function of hormone receptors and nutrient transporters, leading to modifications in gene expression. However, against this backdrop, short-term improvements in nutritional status during the few days preceding oestrus can increase the number of ova shed in polyovular species such as sheep.

Stress and the reproductive axis

Compared with life in the wild, domesticated ruminants could be perceived to enjoy relatively stress-free environments with an assured food supply that avoids the daily, and reduces the circannual, fluctuations of intake seen in the wild. Although this is partly true, their higher levels of production invariably lead to physiological conflict that forces domesticated ruminants into quite severe negative energy balance. However, even when food is available *ad libitum*, the high-yielding dairy cow in negative energy balance and regarded by many nutritionists as an animal under considerable metabolic stress, opts for major day-to-day fluctuations in her food intake (Forbes, 2001). Why she does so is not known; neither is the effect, if any, of these oscillations in intake on her GnRH pulse generator. Clearly, other forms of stress do result in acute suppression of GnRH release; an interesting question is whether there is genetic variation in domestic ruminants for this effect. In feral species, there are genotype differences in the effects of transport-induced stress on the reproductive axis which are linked to the *Agouti* locus (Hayssen, 1998). Variation in this locus occurs in domestic ruminants and is best understood in sheep in which, in the context of reproduction, a dominant mutation (A^{wh}) suppresses out-of-season breeding activity (Dýrmundsson and Adalsteinsson, 1980). Interestingly, in cattle, polymorphisms in another neuroendocrine mediator of reproduction, the leptin gene, appear to result in higher milk yields without associated adverse effects on fertility (Liefers *et al.*, 2002).

Fecundity genes

The story of the identification of the Inverdale gene has been described as 'a tale of remarkable coincidences' (Davis, 1994). The same is true for the identification of the Booroola gene. Early events in the discovery of both genes illustrate the important role that farmers can play in observing, conserving and bringing unusual production traits in their livestock to the attention of geneticists. The reports of the actual identification of the genes, the oocyte-derived bone morphogenetic proteins (BMP), are examples of the enormous benefits to knowledge that can accrue from the integration of the full range of research approaches (whole animal to molecular) that are now available to investigators in the biological sciences. The value of these findings is likely to impinge on human reproduction and health and, thus, extend beyond sheep production for which the desirable goal is twin lambs of uniform birth weight.

In view of the recent finding that the Booroola genotype has a mutation of the BMPR-IB gene which probably involves loss of BMP-6 growth factor activity, it seems pertinent to recall that Landau *et al.* (1995) observed that carriers of the gene exhibited quite a marked response in ovulation rate to improved nutrition (mean $\pm$ SE 3.29 ± 0.27 versus 2.46 ± 0.31), whereas non-carriers did not respond (1.44 ± 0.25 versus 1.36 ± 0.34). The nutritional treatment, which comprised 100 g supplementary rumen undegraded starch per ewe per day during the

3 weeks before mating, had no clear effect on plasma insulin but increased whole animal glucose entry rate by more than 70%.

Prenatal programming

The inheritance from *in utero* life of a legacy of environmentally driven changes that affect the size, vigour, health and growth of the neonate, and its subsequent adult production characteristics can no longer be regarded as a solely fetal phenomenon; rather it extends to the early cleavage stage embryo. There is even evidence for oocyte effects that are acquired before fertilization. Surprisingly some of the nutritionally mediated effects, particularly those involving the oocyte and early embryo, occur at relatively modest levels of undernutrition. Are they therefore challenging the validity of the long-established view that the maternal body, through the catabolism of its tissues, is very successful at protecting the conceptus from the adverse effects of undernutrition? Perhaps the enforced level of maternal tissue catabolism is too trivial or not sufficiently general to ensure tissue release of an essential nutrient that the diet is failing to supply. Or is it that cell differentiation, organogenesis and tissue hyperplasia, processes that are very sensitive to alterations in gene expression, are much more responsive to nutritionally generated signals than is organ and tissue hypertrophy? In specific circumstances these effects on gene expression probably also embrace alterations in genomic imprinting.

Often the reported detrimental effect of inadequate *in utero* nutrition on adult production traits of domestic animals is seen only in breeds with an above average quantitative expression of the trait. An example is the reduction in adult wool production in Merino ewes as a result of late prenatal nutrient restriction (for a review, see Black, 1983). It is also interesting that the adverse effects of undernutrition during early pregnancy on the development of the fetal ovary and subsequent adult ovulation rate (Rae *et al.*, 2002) have been observed in Scottish Blackface sheep, a breed well recognized for the exquisite sensitivity of its ovulation rate to nutrition. These examples imply that the genotype of the fetus may be important in the expression of some forms of prenatal programming.

Although many production, behavioural and health traits are currently being extensively investigated for prenatal programming effects, appetite seems to be a notable exception, yet the magnitude of its expression is central to animal production and human health. 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. However, they did allude to the possibility of later effects, particularly in the males, operating through differences in body fatness. Other prenatal stages would appear to merit investigation.

In practical animal production some of the most important consequences of prenatal effects on the growth and development of the fetus are seen at birth. These involve the vigour and thermoregulatory competence of the newborn animal. Because of the wide range of causes of intrauterine-growth retardation including heat stress, under- and over-nutrition, increases in litter size and sibling embryo mortality during the implantation phase, it is important to characterize their effects in terms of placental function, fetal metabolism and neonatal physiology. A better understanding of the similarities and differences between the various forms of placental and fetal growth restriction offers new opportunities for reducing neonatal mortality and improving animal welfare. However, interpretation of optima for neonatal physiology may not be easy. For example, periconceptional undernutrition in ewes is associated with increased stimulation of the fetal–pituitary–adrenal axis (Edwards and McMillen, 2002), an observation which, in human epidemiology studies, would be considered detrimental to adult health. However, the recent observations of Leenhouwers *et al.* (2002) indicate that, in pigs, selection for improved survival at birth leads to increased piglet cortisol concentrations.

Fertility of dairy cows

The thoroughness of current research into improving fertility of dairy cows leaves little opportunity to contribute new thoughts that are worthy of consideration. The uncoupling of the growth hormone–insulin-like growth factor axis is just one of the many aberrant features of low fertility high-yielding dairy cows, but is it solely an energy-deficit-driven phenomenon, as is generally assumed? Bell *et al.* (2000) alluded to the possibility that an insufficiency of protein or indeed the specific amino acid, methionine, might also be involved.

Low oestradiol is another aberrant feature, yet oestradiol research seems to have slipped from centre stage despite the hormone having been given a central rôle in the Soboleva *et al.* (2000) model of follicular development and ovulation in sheep and cattle. That said, the inadequacy of the dominant follicle, as a contributing factor to poor fertility, is widely accepted. The importance of the nature of the dominant follicle is further reinforced by the recent observation of Townson *et al.* (2002) that dairy cows that ovulate after two follicular waves are less fertile than those that do so after three waves.

One component of infertility of dairy cows that is a cause of concern to farmers is an apparent high rate of pregnancy loss during the second and third month after insemination. However, when investigated scientifically, the levels are usually found to be low (approximately 7%) and similar to those for maiden heifers (for example, see Selke *et al.*, 2002). However, a value of approximately 24% presented at this symposium for high-yielding Holsteins in the USA indicates that the picture may be changing. It certainly would appear to warrant close monitoring.

In terms of the successful establishment of pregnancy, the emphasis in this symposium has been on factors, in particular nutrition, influencing oocyte and embryo quality rather than uterine endometrial competence. Although many would argue that in the absence of infection, uterine effects on the establishment of pregnancy are minor, McMillan and Donnison (1999) have presented convincing evidence in embryo transfer studies in cattle for a specific recipient effect, genetic in origin, and operating at the endometrium. In view of the general low immune status of the high yielding dairy cow and absence of immediate postpartum sucking-induced physiotherapy of her uterine endometrium, uterine effects may also be contributing to her declining fertility.

The reproductive technologies

At a time of unprecedented advances in gamete and somatic cell manipulations for the production of embryos, it seems churlish to draw attention to the lack of a satisfactory routine laboratory-based test for assessing the fertilizing ability of cryopreserved semen. However, such a test is much needed. So too, it would appear, is better application of pregnancy diagnostic tests to avoid the culling of in-calf dairy cows on the erroneous assumption that they failed to conceive. Indeed, diagnostics are central to most, if not all, of the assisted reproduction technologies. It is proving just as difficult to provide tests for embryo quality as it is for semen quality. Deviations from the 'considered norms' for morphology, metabolism and gene expression in the embryo are difficult to interpret. Although some deviations may result in embryo or fetal loss or aberrant conceptus development others will inevitably reflect adaptations to ensure developmental normality; the key will be the ability to separate the 'compensatory and benign' from the 'aberrant and damaging'.

Lessons for research

The symposium provides a superb example of the benefits to knowledge that can accrue from the integration of the extended range of science disciplines and techniques that are now available to study ruminant reproduction. It also provides numerous examples of both the usefulness and limitations of animal models for studying problems of human reproduction, likewise for *in vitro* as opposed to whole animal approaches. In some cases, the nature of the problem under investigation may be species, breed or indeed strain specific and, therefore, not suitable for the adoption of a model approach. It is important, for ethical as well as scientific reasons, to establish whether this is so at an early stage in the research programme. At the same time, the usefulness and power of simplified experimental models to study complex phenomena should not be dismissed. Here a recent pertinent example is the use of the developing chick embryo as a model for studying the effects of prenatal stress in mammalian species (Lay and Wilson, 2002).

The future

As Sir Kenneth Blaxter so eloquently said '. . .we are in a monopoly situation as far as our children's children are concerned and it must be our purpose to provide the fabric of their future from the threads of understanding that rest in our hands'. Clearly, as far as ruminant reproduction is concerned a much stronger 'fabric' now exists than in 1986 when Sir Kenneth made this statement. However, much remains to be done in improving the efficiency of reproduction in domestic ruminants, but at the same time safeguarding their health and welfare. As well as providing new understanding and solutions to the chronic wastage and inefficiency that still occur in some of our domestic ruminant species and breeds, there is a need to make the new reproductive technologies and, indeed, some of the older ones much more reliable and robust than is currently the case. There is also the challenge of convincing the public of the value of the new technologies, not just for the conservation of the biodiversity of our ruminant livestock when under threat, as during the recent UK foot and mouth disease outbreak, but also for the widespread improvement of reproductive efficiency and rapid introduction of desirable production traits. At the same time it will be important to consider the implications for fertility of dairy cows of industry developments such as robotic milking and the desire, perhaps, of some milk producers to opt for extended lactations. The spread in organic production systems and greater reliance on forage species with a natural resistance to pest attack may well introduce new problems; see Gutzwiller (1993) for an example of a clover variety, the natural high content of cyanogenetic glycosides of which interferes with selenium metabolism and fetal thyroid function leading to reduced neonatal viability in lambs. No longer driven solely by financial concerns but increasingly by public opinion, it is inevitable that ruminant production and, therefore, reproduction will face additional problems. Ideally the knowledge base and research skills should be such as to allow us to act in a pre-emptive manner.

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