The consequences of epigenetics and fetal programming for English beef and sheep producers

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Executive summary

1. **Aims and objectives:** A comprehensive review of the literature is provided which offers a critical analysis of data pertaining to how fetal programming and epigenetics influence contemporary beef and sheep production traits. In places the review draws heavily on knowledge and experience gained from a wide variety of species but, in particular, rodents and humans where the majority of data resides.

   In this article we provide:
   a. An assessment of possible risk factors and likely exposures affecting beef and sheep production
   b. Evidence of the extent to which commercially important traits are affected
   c. An analysis of effects of specific environmental factors (e.g. parental diet, maternal stress)
   d. A comprehensive overview of epigenetic mechanisms and their role in fetal programming of subsequent development and health
   e. Key industry 'take-home' messages
   f. A list of future research needs

2. **Overview of document:** The article is divided into 7 sections which provide detailed overviews of (i) the general field of developmental programing, (ii) epigenetics and its role in long-term development, (iii) programming of health and wellbeing, (iv) programming of body composition, (v) programming of fertility, (vi) impact of advanced reproductive technologies and (vii) industry relevance and recommendations.

3. **Context:** Although it has been known for some 70 or so years that fetal development can have a lifelong impact on offspring growth, it is only since the 1980s that the extent and impact of prenatal exposure to malnutrition or stress on adult health and disease became fully appreciated. This was first identified through human cohort studies (such as those investigated by Barker or the Dutch 'Hunger Winter' studies), and was subsequently explored in detail in rodent models. The relatively recent discovery that some of these effects are present not only in offspring of the mother that experienced the dietary or stressful event but also in her grand-offspring, generated a great deal of interest concerning the mechanisms of inheritance.

4. **General issues:** Research in this area currently follows three lines of enquiry: (i) investigations (mostly in rodents) into the mechanisms (including epigenetic) by which *in utero* environmental impacts arise, and whether these effects can be reversed; (ii) studies of the longer term consequences of various forms of prenatal insult (generally in humans and rodents) directed towards non-communicable diseases and offspring behaviour, and (iii) theoretical considerations relating to the evolutionary nature of these mechanisms. This review focuses on research concerning the first two of these issues. It is noteworthy that the vast majority of studies which have investigated this phenomenon have tended to focus on the long-term consequences of negative environmental factors such as malnutrition and maternal stress.

5. **Epigenetics:** Every cell within the body retains a copy of the entire genetic code (i.e. the whole genome) of the organism, although not all this information is utilised by every cell. Different cell types are 'programmed' to use the genetic code selectively to achieve their functions. This cell-type specific 'programming' is established during normal development and involves epigenetic mechanisms. Epigenetic processes allow gene expression patterns to differ between cells

without alterations or mutations to the underlying DNA. Epigenetics can thus be described as: "*the study of mitotically and/or meiotically heritable changes in gene function that are not explained by changes in DNA sequence"*. Patterns of gene expression can be inherited across many cell cycles, with adult tissues carrying the 'memories' of 'modified genes' from embryonic development; or even from previous generations. Two classic memory systems exist in mammals that are based on epigenetic programming of the genome: (i) DNA methylation and (ii) associated higher-order chromatin & histone modifications. A detailed overview of our contemporary understanding of these mechanisms and others is provided in Section 2.

6. **Stage of pregnancy:** In recent times cohort studies in humans have tended to focus on early gestation, including the peri-conceptional period, which is a time when the mammalian genome is most sensitive to epigenetic modifications. In studies with ruminants, moderately severe undernutrition up to 30 days after mating does not affect birth weight or growth rate but produces offspring that show symptoms of 'metabolic syndrome' (e.g. hypertension, insulin resistance). In some studies with sheep offspring also have behavioural disturbances and reduced survivability. In contrast, mild undernutrition during this period is associated with increased placental development and enhanced embryo survival. Poor nutrition in late gestation is reliably associated with reduced birth weights which, through impacts on offspring behaviour, thermoregulation and body reserves are associated with increased mortality. Shearing the housed pregnant ewe increases lamb birth weight by increasing dietary intakes, although improved lamb survival and post-natal growth rates are not always evident. The impact of gestational nutrition on calf weight has been less convincingly demonstrated. However, calf weight is reduced when cows experience either heat or cold stress which, in the case of heat stress, may be associated with reduced feed intakes. In addition, birth weight is reduced in cases where mothers experienced ill health (of various forms) during pregnancy.

7. **Stress during pregnancy:** Pregnant farm animals may be exposed to many factors that can elicit physiological stress responses (e.g. transport, human contact, predators, housing). In pregnant rodents and humans these exposures are known to cause permanent and long lasting impacts on the developing offspring, particularly influencing behaviour, and stress reactivity. Very few studies have considered the impact of stress during pregnancy on offspring responses in cattle and sheep. In general, studies suggest that offspring behaviour is altered by exposure of the mother to stressful events, particularly if this occurs during early to mid-pregnancy; and that male offspring are more affected than females. Somewhat paradoxically, exposure to stress during late pregnancy may have positive impacts such as increasing offspring birth weight.

8. **Immunity:** As the immune system develops largely *in utero* in farm livestock, immune function is likely to be susceptible to the effects of the maternal environment. In lambs the absorption of immunoglobulins from colostrum is affected by maternal intake of micronutrients (e.g. cobalt, vitamin E) as well as macronutrients (e.g. protein). A recent and largely unexplored concept in farm animals relates to the hologenome (i.e. the genome of the host plus all microorganisms associated with the host). In mammals microbial symbionts are vertically transmitted to offspring initially via the birth canal and subsequently from milk and the surrounding environment. This has been shown to affect the development of the immune system in humans (associated with allergies, cancer and inflammatory bowel disease). In ruminants this is thought to affect the population of microbes that inhabit the rumen.

9. **Muscle development and carcass composition:** Muscle mass, is an important potential target for epigenetic mechanisms as, in cattle and sheep, the proliferation of muscle fibres occurs *in utero*. Lambs and calves are, therefore, born with a fixed number of muscle fibres, with subsequent growth occurring by hypertrophy (increase in fibre size). When nutritional insults on the pregnant ewe occur during early gestation (a critical period for muscle development) then effects on muscle fibre number and type can be detected in young offspring, but these effects tend to be lost (or are too difficult to detect) in older sheep. Such studies, however, are few and offspring were often subsequently placed on high-planes of nutrition, which probably induced an element of compensation; although the nature (i.e. mechanisms, including epigenetics) of how such compensation may come about has not been explored.

10. **Body fat, appetite and feed efficiency:** In humans and rodents, poor prenatal dietary intakes of energy, protein and micronutrients are associated with increased risk of adult obesity in offspring. In cattle and sheep there also appears to be some evidence of long-term programming of adiposity although, perhaps surprisingly, the development of adipose tissue in ruminants is less well understood than that of muscle. In sheep, nutritional restriction in early gestation, or low birth weight, is associated with increased adiposity, particularly in older (i.e. over 6 months) male offspring. Unlike muscle fibres, there is no evidence to suggest that the number of adipocytes (or precursor cells) is set at a specific stage of life. There is certainly considerable scope to explore this area further, and also how muscle and lipid metabolism can influence residual feed intake and overall feed efficiency. In this context it is noteworthy that although nutritional challenges *in utero* can alter the developing hypothalamic appetite-regulatory circuits in fetal cattle and sheep, there is no evidence that these changes result in alterations in subsequent food intake in current animal production systems. However, emerging data that epigenetic changes in anorexigenic genes could be of lasting significance for appetite drive deserves further study in livestock species.

11. **Reproduction and fertility:** In female cattle and sheep, lifetime supply of potentially fertilizable oocytes (eggs) is established before birth and cannot be replenished thereafter. In males new spermatozoa are produced continually after puberty, but the number of Sertoli cells which are the primary determinant of sperm production and testes size in adult life is determined by proliferation during the fetal, neonatal and peripubertal periods. There certainly appears to be effects of malnutrition *in utero* on development of both male and female gonads. However, there is little evidence for an effect of prenatal nutrition on the onset of puberty in sheep or cattle, and the main impact appears to be on the number of ovarian follicles. There is some evidence of a reduction in ovulation rate and litter size in ewes malnourished during pregnancy, but larger scale studies are required to confirm these observations and their significance in commercial practice. Likewise in cattle, there is some evidence of effects of early pregnancy malnutrition on ovarian follicle reserve in offspring leading to poor subsequent fertility; but here the evidence is even more limited. There is also limited evidence for a negative impact of prenatal undernutrition on fertility of males, although very few long-term follow-up studies have been conducted in this area.

    Environmental chemicals, including so called ‘endocrine disrupting compounds’ (EDCs) have the potential programme various components of the reproductive axis (i.e. brain- pituitary-gonad-uterus) to malfunction in later life, and so affect fertility. There is certainly evidence in rodents to support such effects. Cattle and sheep grazing sewage-sludge treated pastures are exposed to higher than normal levels of such compounds, and so are potentially most at risk. To date the most worrying implication of EDC research relates to the high incidence of spermatogenic abnormalities in male offspring. Effects on female fertility are less evident, but there is a distinct lack of long-term follow-up studies for both sexes in both cattle and sheep. Given the well-known phenomenon of 'bioaccumulation' is there an increased risk for humans consuming milk and meat products from ruminants grazing sludge-treated pastures?

12. **Advanced reproductive technologies (ART):** The potential of these technologies to enhance reproductive rate of beef cattle and sheep, either within genetic improvement programmes or in commercial herds and flocks, has not been fully realised in the UK. CAP associated structural problems within the beef and sheep sectors are partly to blame for the lack of technical innovation and industry uptake in the past, but there have been issues with regard to success rates and fetal development leading to 'Large Offspring Syndrome'. The main issues pertained to early pregnancy losses and large calves/lambs at birth with associated obstetrical complications and morbidity. The available evidence indicates that there are no obvious long-term effects on animal production and health, although there have been few studies in this area. Subsequent refinements to methods of *in vitro* embryo production seem to have mitigated these adverse effects (although the situation requires monitoring). Developments in the use of sexed semen (e.g. for single-sexed once-bred heifer systems) and genomically evaluated sexed embryos offer huge potential advantages for livestock improvement programmes, recognised and practiced in various countries across the world; none more so than Brazil where it seems that these technologies work better in *indicus* than *taurine* breeds of cattle. The successful uptake of these technologies within the UK beef and sheep sectors requires improvements in the general level of reproductive management and on-farm facilities for handling livestock.

13. **Industry relevance and recommendations:** There are a number of commercially relevant traits that have not been considered in beef cattle and sheep, and aspects of normal agricultural practice that haven't been investigated. These omissions primarily reflect the nature and level of research funding in the past, which has primarily been research council and charity based, and where there has been a clear biomedical slant. The limited data that does exist pertains mostly to sheep.

    In this article we have considered the following traits that have been investigated to a greater or lesser extent: (a) neonatal survival, (b) growth rate and feed conversion, (c) whole-body and carcass composition, (d) animal behaviour, and (e) reproductive potential and fertility. Prenatal risk factors that can influence these traits include: (i) parental nutrition, (ii) gestational stress, (iii) environmental chemicals, and (iv) breeding technologies.

    Consequently, key take-home messages and recommendations include:

    A. *Nutrition during pregnancy*: Adherence to existing standard dietary recommendations for macro- and micro-nutrients should avoid suboptimal *in utero* development that could have negative long-term effects on offspring growth and health. However, there is a lack of information for beef cattle and sheep to predict effects on carcass composition. EBLEX funded studies, therefore, could establish KPIs on commercial herds and flocks to validate/refine these recommendations, and to quantify the extent to which early life development may impact on long-term performance (both physical and financial performance). Data collection should include ewe/cow body condition at key stages of the annual production cycle and birth weight, ultimately with corresponding data on carcass yields. Another key trait to monitor is fertility across successive parities.

B. *Gestational stress*: This is an area that has been under investigated in both beef cattle and sheep. Evidence from rodent and human studies indicates that these effects are real. Factors such as housing, stocking density and handling during pregnancy are all worthy of further investigation.

C. *Environmental chemicals*: As around 73% of sewage sludge is dispensed on agricultural land, so there is a need to assess the effects that this may have on grazing livestock. The available evidence indicates effects on the development of male reproductive organs in sheep, but long-term consequences for ram fertility have not been properly ascertained; and effects in beef cattle have not been established. There is also the issue of bioaccumulation and, consequently, effects in humans consuming meat from animal grazing sludge-treated pastures.

D. *Advanced breeding technologies*: A watching brief on 'Large Offspring Syndrome' is recommended should activity in this area pick up again. These technologies have much to offer for livestock improvement, but the UK lags behind other countries, particularly those in North and South America. Improved standards of reproductive management (i.e. for sperm/egg/embryo donors and recipients) in both beef herds and sheep flocks are required. Improved handling facilities are needed as well as an improved awareness of factors that affect fertility. There is scope also to develop our understanding of why these technologies are so much more successful in *Bos indicus* and opposed to *Bos taurine* cattle. This extends to establishing a better understanding of their underlying fertility, which also differs between these two sub species.