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MANAGEMENT SOLUTIONS

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Sheep Reproduction in Australia

Current Status and Potential for Improvement through Flock Management and Gene Discovery

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PREFACE

This review has been written on behalf of Meat and Livestock Australia in accordance with the Terms of Reference as provided on page 3. It aims to assess the current reproductive performance of the Australian sheep flock and to identify areas of flock management and research where potential exists for improvement.

The review is written in three Sections:

Section 1 – Reproduction in the ewe – from germ cells to lambing (author SK Walker)
Section 2 – Neonatal mortality and early growth in sheep (author DO Kleemann)
Section 3 – Molecular events in establishment and maintenance of pregnancy (author CS Bawden)

Each section consists of a number of Chapters and, at the end of each, a summary of important points relevant to that Chapter is provided. The final chapter of each Section contains an overview of the information reviewed together with a list of recommendations for flock management and/or future research. Each Section is complete with its own bibliography.

It is the firm belief of the authors that this review provides a solid basis for future improvements in the reproductive performance of the national sheep flock. It is conservatively estimated that implementation, on a national basis, of the management and research strategies outlined, would improve lamb marking percentages by 10-20%. This increase equates to an increase in income from the national flock of between $125,000,000 to $250,000,000 per annum.

SUMMARY

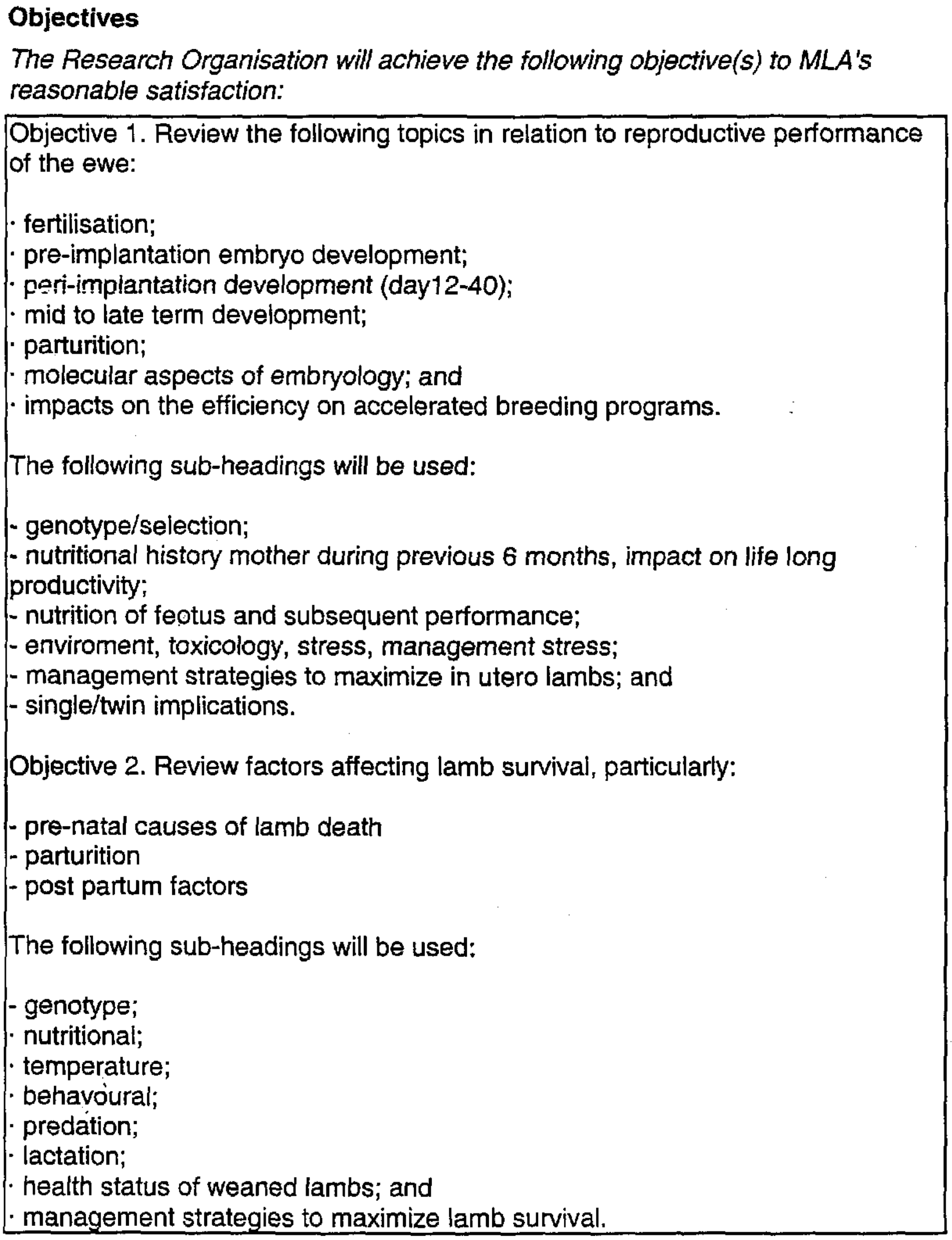
Reproductive efficiency of the national flock is below acceptable levels given the advances made in pasture improvement and animal husbandry. Two factors are primarily responsible – high levels of embryo mortality and lamb mortality. Conservative estimates of each are 25% and 20 – 30% respectively. It is calculated that halving the magnitude of each would result in an additional 28 lambs weaned per 100 ewes mated. Hence, there is adequate potential in the national flock to improve flock performance and the realisation of this potential would improve flock income very significantly.

Many factors influence the components of reproduction but nutrition is clearly the most important. There are at least six phases of the reproductive life-cycle (from development of the germ cell until the production of the newborn) that are responsive to nutritional conditions. These phases have unique nutritional requirements and it is proposed that the application of a differential nutrition strategy to meet these requirements would markedly improve reproductive efficiency. Emphasis in the application of this strategy is on improving oocyte and embryo quality as compared with yesteryear when emphasis was on improving the number of growing follicles and, ultimately, ovulation rate. These improvements should lead to enhanced rates of embryo survival and, possibly, improved placental development by way of altered trophoblast function. Furthermore, the data reviewed on lamb mortality indicate that management and selection strategies to improve lamb survival are numerous and there is potential to further develop an array of options. Again, nutrition during pregnancy is an important factor and when manipulated in conjunction with strategies to improve oocyte quality, significant increases in lamb survival should be obtained.

Improvements in flock performance through the application of new management strategies should be augmented through advances made in the identification of genes that regulate important reproductive steps. Genes of interest are those involved in granulosa cell function in pre-antral and antral follicles as well as those involved in differentiation of the trophectoderm and subsequent function of the placenta. Such studies should be richly complemented with the determination of mRNA and protein species present in the oocyte cytoplasm.

TERMS OF REFERENCE

(precisely as provided by Dr P.A. Speck, Meat and Livestock Australia, June, 2002)



Objectives

The Research Organisation will achieve the following objective(s) to MLA's reasonable satisfaction:

Objective 1. Review the following topics in relation to reproductive performance of the ewe:

- fertilisation;
- pre-implantation embryo development;
- peri-implantation development (day12-40);
- mid to late term development;
- parturition;
- molecular aspects of embryology; and
- impacts on the efficiency on accelerated breeding programs.

The following sub-headings will be used:

- genotype/selection;
- nutritional history mother during previous 6 months, impact on life long productivity;
- nutrition of feotus and subsequent performance;
- enviroment, toxicology, stress, management stress;
- management strategies to maximize in utero lambs; and
- single/twin implications.

Objective 2. Review factors affecting lamb survival, particularly:

- pre-natal causes of lamb death
- parturition
- post partum factors

The following sub-headings will be used:

- genotype;
- nutritional;
- temperature;
- behavoural;
- predation;
- lactation;
- health status of weaned lambs; and
- management strategies to maximize lamb survival.

EXECUTIVE SUMMARY

SECTION 1. REPRODUCTION IN THE EWE – FROM GERM CELLS TO LAMBING

Chapter 1. Reproductive wastage in Australian sheep flocks

(1). There is large variation both within and between regions in the reproductive performance of flocks.

(2). A number of factors have been identified that contribute to between region variation although there is a paucity of data on flock performance in some regions.

(3). Causes of variation within regions are less obvious although some possible factors are addressed later in this review. Why, for example, can mean ovulation rates in commercial Merino flocks within one region vary between 1.00 – 2.00?

(4). Prioritising research into reproductive wastage needs to be done on a regional basis.

(5). Why can Merino flocks in some regions be induced to cycle (at near 100% efficiency) throughout the year when data and anecdotal evidence indicate that this is not the case in other regions?

(6). Is the ability of the Merino to cycle throughout the year in these regions being fully utilised to give maximum flexibility in terms of time of mating? Is such flexibility being utilised in drought management strategies?

(7). The ability to induce cyclicity throughout the year is dependent on the ram effect. However, in some regions, induction is associated with unique aberrations in subsequent cycles and this problem needs to be better understood if maximum benefit is to be obtained.

(8). Lamb mortality (20 – 30% of lambs born) and embryo mortality (25% of ova) are the major sources of reproductive wastage in the national flock.

(9). Partial failure of multiple ovulations (PFMO) is a significant component of embryo mortality. Benefits to be gained from increases in ovulation rate will be minimal until the problem is better researched and managed.

Chapter 2. Managing the components of reproductive performance

(1). Nutrition during the previous winter/spring can influence the subsequent expression of oestrus (this period can include the time of the previous lactation).

(2). Improvements in lambing performance can occur through one or more of the following pre-mating strategies:

  (a) Improving live-weight and/or body condition.

  (b) Increasing pasture allowance for at least one cycle during the six-week period before mating.

    (c) Supplementing ewes with a high protein diet between 5 – 8 days before ovulation.
    (d) Supplementing (infusing) ewes with branched chain amino acids between 5 – 8 days before ovulation.

(3). Precision feeding before ovulation is more relevant to AI and embryo collection/transfer programs (where the time of ovulation is known) than it is to flock matings. Strategies need to be developed for its use in the latter.

(4). Further research on the role of branched chain amino acids in controlling ovulation is justified including dose rates, method of delivery and influence of prevailing nutritional conditions.

(5). Observations that the pre-ovulatory follicle takes five days to develop provides an unique window in which to focus research to maximise ovum quality at ovulation.

(6). Post-mating management of flock nutrition is important for embryo survival with a high level of nutrition being detrimental. Contrary to convention, ewes should be held on a maintenance (M) or less than M diet for the first two weeks after conception.

(7). Increases in ovulation rate associated with dietary intake are associated with disproportionate increases in PFMO. Strategies to minimise PFMO need to be based on a better understanding of the problem.

(8). When embryos are transferred in groups of two per ewe in embryo transfer programs, they should be transferred bilaterally rather than unilaterally when the opportunity arises. This routine is not currently practised in the embryo transfer industry but could result in significant increases in embryo survival rates.

Chapter 3. Effects of maternal nutrition on the oocyte, embryo and fetus

(1). A low diet (M or less than M) immediately before mating improves the quality of both the ovum and embryo.

(2). A serious dilemma arises in that the optimal nutritional condition for follicle growth (e.g. 1.5M) is not optimal for the production of high quality ova and embryos.

(3). Hence, diets that stimulate multiple ovulations produce ova/embryos of reduced quality. It is speculated that it is for this reason that levels of PFMO are substantially higher in flocks that receive dietary supplements before mating.

(4). A low diet (0.7M) from the time of ovulation in the sheep increases the proportion of trophectoderm cells in resulting blastocysts compared with the proportion obtained with a high diet. This shift in cell allocation may provide an adaptive advantage in placentation. The significance of this finding, in terms of pregnancy rates and birth weights in commercial flocks, needs to be examined.

(5). A low diet in the peri-conceptual period results in changes to the physiology of twin compared with single fetuses which might be detrimental to health in adult life. This finding raises the possibility that peri-conceptual nutrition might influence lifetime productivity in resulting offspring.

Chapter 4. Fetal ovarian development and lifetime performance

(1). The fetal ovary remains an untapped source of germ cells despite many 100,000s being lost during fetal development.

(2). The number of germ cells at birth can not be manipulated by changing maternal nutrition during pregnancy.

(3). On the other hand, the quality of oocytes obtained from new-born lambs is improved by high dietary intakes during specific stages of pregnancy. There is a likelihood that this advantage persists into adulthood.

(4). With the use of *in vitro* technologies, the lamb is a valuable source of oocytes for immediate breeding purposes. High embryo wastage is a constraint to further development of the technology.

(5). Under-nourishment during late fetal development influences adult reproductive performance primarily due to a reduction in prolificacy.

Chapter 5. Nutrition – the master regulator of reproductive performance

(1). Nutrition is the master regulator of reproductive performance by virtue of exerting effects through six phases of the reproductive life-cycle.

(2). These phases correspond with the development of the fetal ovary, the pre-antral follicle, the antral follicle, the pre-ovulatory follicle, the early embryo as well as the pre-implantation period.

(3). There are differential nutritional requirements during these phases with high nutrition improving fetal ovarian development and the number of growing follicles in the adult while lower nutrition improves the quality of the oocyte and embryo as well as improving implantation rates.

(4). The nutritional requirements during pre-antral follicle development are not known but it is hypothesised that nutrition in this period sets the nutritional requirements for subsequent development. Relevant research needs to be conducted on this topic.

(5). The feeding of high protein diets can result in the production of excess ammonia/urea which can pervade all components of the reproductive tract and which is embryo toxic. High protein diets must be balanced with energy content and be fed judiciously.

(6). It is speculated that high embryo wastage/PFMO following high dietary intake is due, partly or wholly, to the production of excess ammonia/urea.

(7). The physiological means whereby nutrition influences reproduction is not known but the insulin-GH-IGF1 axis is strongly implicated.

(8). The role of branched chain amino acids (BCAA) as a physiological link between nutrition and ovulation rate requires further investigation particularly in relation to the role that BCAA might play in determining ovum quality.

Chapter 6. Other environmental determinants of reproductive performance

(1). Environmental factors other than nutrition can influence reproductive performance. These factors operate on a local or regional basis but collectively can significantly reduce the reproductive performance of the national flock.

(2). Heat stress can cause abnormal oestrous patterns, elevated levels of embryo mortality and aberrant patterns of fetal growth.

(3). Environmental toxins, resulting from the use of herbicides and pesticides have the potential to seriously reduce reproductive performance. Routine monitoring of soils/pastures for toxic residues would be a valuable step in monitoring this problem.

(4). Consumption of phyto-oestrogens by sheep in the higher rainfall areas can cause a significant reduction in lamb marking percentages. The problem is not easily detectable in flocks but it is estimated that it affects between 10 – 15 million breeding ewes per year.

Chapter 7. Genetic and epigenetic control of reproduction

(1). Some traits of economic importance are moderately heritable and selection programs have produced annual responses of up to 1.5% in the number of lambs born per year.

(2). Selection for traits of reproduction is not widely practised due, in part, to logistical problems in measuring the desired traits.

(3). Repeatability estimates are sufficient to facilitate flock improvement in some traits.

(4). Gains in flock improvement to be obtained through selection of desired animals are best realised under conditions of optimal flock management.

(5). Epigenetics and, in particular, imprinting patterns might influence oocyte and embryo quality.

(6). It is postulated that the imprinting process is influenced by nutrition and, if so, would provide an additional link between nutrition and reproductive performance.

Chapter 8. Impact of review findings on flock reproduction and controlled breeding programs

(1). A differential nutrition strategy is presented that aims to provide the nutritional requirements of the ewe during the different stages of her breeding cycle.

(2). This strategy aims to maximise the quality of the ovum and embryo and to increase downstream benefits in terms of implantation rates, fertility and fecundity.

(3). This strategy is relevant to flock matings and to AI and embryo transfer programs although it will be easier to implement in the latter two cases because of the control over the time of ovulation.

(4). Future research should concentrate on:

  (a) Nutrition during the development of the pre-antral follicle and its effect on subsequent reproductive performance.
  (b) The role of high protein diets in determining ovum and embryo quality as well as the association between ovum quality and partial failure of multiple ovulations.
  (c) The differential requirements of energy and protein during the different stages of the reproductive cycle.

(5). A demonstration of the expected benefits from the application of the differential nutrition strategy is required.

SECTION 2. NEONATAL MORTALITY AND EARLY GROWTH IN SHEEP

Chapter 9. Neonatal loss in Australian sheep flocks

(1). Reproductive wastage at lambing is high with 20 - 30% of lambs born in commercial Merino flocks dying within a few days of birth. Mortality amongst twins is estimated at 30 - 40%.

(2). Losses at lambing cost the nation an estimated $250m annually.

(3). Mortality in low to medium fecund flocks is mainly due to dystocia and the starvation/mismothering/exposure complex, whereas deaths in prolific flocks are mostly attributed to ante-parturient and prolonged birthing problems.

(4). Reasons for mortality may vary from flock to flock, requiring detailed investigation to determine the major causes.

(5). If lamb losses are large, causes can generally be attributed to one or two single factors but reasons for the core loss may be difficult to reveal.

(6). Most deaths occur within the first few days of life but significant losses can occur thereafter if extreme environmental conditions prevail.

(7). Neonatal adaptations during parturition, such as changes in thermoregulation, mobilisation of energy reserves, alterations in lung fluid dynamics and regulation of hypoxia are required for the newborn to survive. Further understanding of these processes is needed to develop strategies for reducing mortality and morbidity.

(8). Improvement of lamb survival through selection within breeds and through crossing of breeds can be achieved. However, progress through selection within breeds will be slow since heritability estimates for the trait are low.

(9). Indirect selection criteria for enhancing lamb survival have been identified and show promise.

(10). The value of crossing meat breeds with the Merino to increase lamb survival is well known, with contributions to improvement coming from both the ewe and lamb genotype. Early results in pastoral areas indicate that the Damara breed may confer enhanced neonatal survival compared with the purebred Merino.

(12). Selection within a breed for other traits such as body growth rate may be antagonistic to increasing lamb survival.

(13). Nutrition has a major impact on placental and fetal growth starting in early pregnancy and possibly earlier.

(14). Size of the fetus is associated positively with placental mass. Ewes in high body condition at mating and subjected to moderate under-nutrition during early-mid pregnancy may produce placentae of normal size, whereas ewes in poor condition or young growing ewes exposed to poor nutrition during this period will produce small placentae.

(15). Over-nourishment of adolescent ewes during early-mid pregnancy may give rise to small placentae and fetuses.

(16). Further knowledge is required on the physiological basis for growth and function of the feto-placental unit including endocrine and growth factor regulatory mechanisms.

(17). Size of the placenta at the beginning of late pregnancy governs fetal mass at birth and is independent of maternal body condition in ewes fed to appetite during late pregnancy. However, when ewes are underfed during late pregnancy, fetuses at term are heavier in better-conditioned ewes. Ewes having moderate fat reserves throughout pregnancy may reduce insulin sensitivity and partition more glucose to the fetus compared with those from ewes with low fat reserves. Fetuses carried by ewes in better condition may store greater reserves of fat although fetal mass may not increase.

(18). Rapid development of recognition between mother and offspring soon after birth is essential for lamb survival.

(19). Normal parturient behaviour requires both hormonal changes and stimulation of the genital tract via fetal expulsion.

(20). Olfaction then auditory and visual cues become important in establishing the link between mother and offspring. Recognition by the ewe of her lamb(s) develops in the first 2 - 4 hours and identification by the lamb of its mother occurs between 12 - 24 hours of age.

(21). The quality of behavioural interactions between ewe and lamb(s) is determined by breed, parity, litter size and nutrition.

(22). Development of a preferential relationship of the lamb to its mother is established through suckling that has strong rewarding properties and through vocal communication. Colostrum is involved in development of the preferential relationship.

(23). Suckling of the ewe is critical for establishment and maintenance of normal maternal behaviour.

(24). Strength of the maternal bond *per se* and time taken to establish a bond of maximum intensity in twin rearing ewes warrants investigation.

(25). Behavioural characteristics of the Merino ewe and her lamb(s) and their association with physiological parameters during the first 24 hours post-birth deserve further study.

(26). On a national scale, disease is a relatively unimportant factor in causing lamb deaths but can be a significant problem in individual flocks (e.g.Toxoplasmosis).

(27). Transfer of passive immunity via immunoglobulins present in colostrum is essential for lamb survival.

(28). Primary predation can be a significant problem on individual properties; in most cases, current control measures are effective in reducing mortality.

(29). Exposure of ewes to a colder environment by shearing during mid-pregnancy can increase birthweight but responses are variable.

(30). Impairment of non-shivering thermogenesis in lambs delivered prematurely or in lambs born to ewes subjected to nutrient restriction in early-mid pregnancy has implications for survival of lambs in cold environments.

(31). Selection of ewes (and rams) for tolerance to heat may overcome, to some extent, high lamb mortality associated with small fetuses.

(32). The photoperiodic cue that triggers sheep to breed has evolved so that offspring are born during the most favourable time of the year (spring) to maximise survival of offspring. Nutritional and social cues in the Merino can override the entrained photoperiodic cue thus facilitating lambing at less than favourable times of the year.

Chapter 10. Post-natal growth of lambs

(1). Australia’s successful lamb-meat industry has developed a tiered breeding structure based on the Merino.

(2). The majority of lambs slaughtered are second-cross (40%) with significant numbers coming from first-cross (25%) and purebred Merino (20%) sources.

(3). Growth rate differences between purebred Merino and first-cross lambs and between first-cross and second-cross lambs are substantial (15 - 20% and 25 - 30%, respectively).

(4). Large differences exist within breeds for growth rate and fat status. Industry has taken advantage of this genetic variation through the agency of a national scheme (LAMBPLAN) available to both terminal sire and maternal breeding sectors.

(5). Mammary development and subsequent milk yield can be influenced by overfeeding of ewe lambs.

(6). Over nourished post-pubertal ewes can have reduced yield and concentration of colostrum.

(7). The high metabolic challenge placed on the ewe in late pregnancy and early lactation requires a number of metabolic adaptations to meet the demand for nutrients; these are met from both endogenous and exogenous sources. Adaptations involve increased liver gluconeogenesis, reduction in use of glucose by peripheral tissues, non-esterified fatty acid (NEFA) mobilisation from fat deposits with increased use in peripheral tissues, reduced amino acid catabolism but increased muscle proteolysis. Regulation of these adaptations is effected through altered tissue responses to hormones (insulin) and adrenergic substances. Increases in insulin resistance spare glucose and enhance NEFA and amino acid mobilisation.

(8). Placental lactogen appears to have a key role in synergising with steroid hormones, GH and possibly prolactin in promoting mammary development.

(9). GH levels peak at parturition and continue at elevated levels during lactation; GH decreases lipogenesis in adipose tissue possibly through opposing the action of insulin.

(10). Milk yield of first-cross ewes is 25 - 30% greater that that of the Merino, independent of lamb genotype. Even larger differences in milk yield (30 - 35%) have been observed between ewes suckling twins compared with those suckling one lamb. Breed differences in milk composition are thought to be small.

(11). Milk yield of multiple rearing ewes is determined largely before parturition through the effect of increased placental mass and placental lactogen on mammogenesis.

(12). Maintenance of high milk yield during the second and third months of lactation is required for sustaining high lamb growth rates.

(13). An appropriate nutritional strategy for a ewe to achieve adequate milk yield is to supply a minimum protein intake to support efficient rumen flora function. To achieve high milk yields there is a need to supply an increased ME level and a higher ratio of dietary protein to ME concentration.

(14). It has been noted in dairy cattle that feeding high levels of dietary protein may well exacerbate the negative energy deficit through an energy requirement to handle excess urea concentrations and may also incur fertility problems.

(15). Supplementation of ewes with high protein supplements has produced variable responses in lamb growth.

(16). Studies with low and high birthweight lambs have indicated the ease with which carcase composition can be nutritionally manipulated during early post-natal life.

(17). Expression of an immunity acquired through exposure to internal parasites can be influenced by nutrition during the peri-parturient period when function of the maternal immune system is suppressed. Supply of high levels of dietary protein has improved control of incoming parasite larvae.

Chapter 11. Management strategies to improve lamb productivity

(1). Nutrition is a factor that can have a large influence on lamb survival and that can be controlled, to some extent, by management.

(2). Nutrition strategies to optimise lamb survival centre on an understanding of the relationship between mortality and birthweight; the so called “J curve” where extremes of nutrition will result in increased mortality due to either small or large size lambs.

(3). Size of the placenta, under most circumstances, determines growth of the fetus and varying strategies to generate normal size placentae are indicated.

(4). Nutritional requirements of ewes supporting multiple fetuses differ substantially from those carrying singles. The benefits of preferentially feeding multiple bearing ewes on-farm need further investigation, given that such ewes can be identified accurately via scanning on a commercial basis.

(5). Minimal disturbance of the ewe and her lambs, needed to establish a strong bond between mother and offspring, is the key principle governing the development of successful lambing systems. Further studies are required to develop systems of feeding at lambing and to determine the relationships between lamb survival and colostrum production and quality under field conditions.

(6). Choice of lambing paddocks with features conducive to enhancing the microclimate of new born lambs should reduce lamb loss due to cold exposure.

(7). Relationships between stocking density, frequency of lambing, litter size and paddock size *per se* and neonatal loss are not well understood.

(8). Promising indirect selection criteria have been proposed for improving lamb survival but need further development for widespread adoption by industry.

(9). Strategic baiting programs may control primary predation.

(10). Basic and strategic research is required on nutritional management of the Merino ewe rearing twin lambs to enable her to supply adequate milk for her lambs and to recover body reserves needed for the following breeding season.

(11). The most efficient management tools to meet the high nutritional demands of the breeding flock are to match the animals' nutrient requirements with pasture availability, adjustment of stocking rate and attention to major factors governing pasture growth and quality.

(12). Devising nutritional systems for maintaining adequate lamb growth rates when pasture quality declines, amelioration of grass seed problems and control of internal parasites are the major factors that need to be considered during the post-weaning period.

SECTION 3. MOLECULAR EVENTS IN ESTABLISHMENT AND MAINTENANCE OF PREGNANCY

(1). Genes known to be involved from gametogenesis to implantation are described with emphasis on:
    (a) The determinants of the quality of the oocyte at fertilisation.
    (b) Regulation of differentiation in the pre-implantation embryo.
    (c) Function of the trophoblast and uterine epithelium.

(2). Factors responsible for embryo/fetal loss are discussed and are categorised into nutritional, genetic and epigenetic, hormonal, environmental, maternal, paternal and fetal in origin.

(3). It is concluded that there is a large potential for important gene discovery given the array of technologies that are now available. It is recommended that, in the first instance, research be concentrated on the following areas:
    (a) Gene function in granulosa cells during development of pre-antral and antral follicles. This study should also include examination of the thecal cells (theca

interna and theca externa) that immediately surround the oocyte and granulosa layers.

(b) Gene function in granulosa cells of the preovulatory follicle (i.e. during the five days immediately before ovulation) including cells of the corona radiata (the 4-5 cell layer of granulosa cells abutting the zona pellucida).

(c) Gene expression in the pre-implantation embryo with emphasis on the trophectoderm cells from the time of blastocyst formation up to and including the time of implantation.

(d) Gene expression patterns in embryos from single and twin ovulating ewes as a basis for understanding the phenomenon of partial failure of multiple ovulations (PFMO).

SECTION 1

REPRODUCTION IN THE EWE – FROM GERM CELLS TO LAMBING

CHAPTER 1. REPRODUCTIVE WASTAGE IN AUSTRALIAN SHEEP FLOCKS

1.1 INTRODUCTION

Estimates of reproductive wastage in Australian flocks are made difficult by environmental diversity and associated differences in nutrition. Despite flocks performing differently in these environments, detailed information is not available from all relevant locations in the country. Parameters such as the incidence of multiple ovulations, the response of ewes to nutritional change, the response of ewes to the ram effect and the patterns of spontaneous cycling can all differ between these environments. These differences are further confounded by breed and strain effects leading to the need for a multifaceted approach in managing the reproductive performance of the national sheep flock.

In this Chapter, attempts are made to quantify reproductive performance in Merino and crossbred flocks across the country. Data are drawn mainly from studies on reproductive wastage on commercial properties. In some cases (e.g. for estimates on fertilisation rates and embryo mortality) data from experiments flocks are used. One problem with this approach is that detailed “on-farm” studies are only available from Western Australia and South Australia with there being little comparable data from the higher rainfall areas of the eastern states.

1.2 OCCURRENCE OF OESTRUS

The existence of seasonal variation in the oestrous activity of Merino ewes has been demonstrated by many authors (e.g. Kelley, 1937, 1946; Kelley and Shaw, 1943; Stewart and Moir, 1943; Underwood et al., 1944; Radford and Watson, 1955; Oldham et al., 1990). In general, these authors found that there was a period of anoestrus or relative anoestrus in spring and early summer and a peak in oestrous activity in autumn and early winter. There was considerable variation in this general pattern according to age, strain, breed, year and locality. Despite these patterns of spontaneous oestrus, most flocks in southern Australia are joined in the late spring to early summer period (Kelly and Lindsay, 1987: Kleemann et al., 1989).

An important aspect of the expression of oestrus is the way in which the ram effect is managed. Sudden exposure of ewes to rams results in many anoestrous ewes exhibiting oestrus 17 - 29 days after exposure (Radford and Watson, 1957). This stimulation can result in a marked period of oestrous activity as well as reducing the length of the anoestrous period. In the study of Cutten (1980) with South Australian Merino ewes, the incidence of spontaneous oestrus through the year reached a nadir of 20 - 30% in late spring. However, almost all anoestrous ewes were stimulated by the ram to cycle irrespective of the time of year. This study highlights the ability of some

Merino strains, at least in some environments, to cycle with near 100% efficiency throughout the year.

Continuous exposure of ewes to rams results in a more prolonged anoestrous period than in ewes that are suddenly exposed to rams (Riches and Watson, 1954; Barrett et al., 1962). With continuous exposure, Merino ewes display a spontaneous breeding season between approximately January – August (Kelley, 1937, 1946; Underwood et al., 1944; Barrett et al., 1962). The practise of joining flocks in spring (when most ewes are naturally in anoestrus) reflects the need to manage the ram effect properly. Whilst ram exposure is a very effective way of inducing cyclicity in anoestrous ewes, it cannot be assumed that these induced cycles are normal in all respects.

1.3 RESPONSE OF EWES TO THE RAM EFFECT

A conventional response of anoestrous ewes to the ram is to ovulate without oestrus (silent ovulation). Progesterone produced from these "induced" corpora lutea then ensures that the subsequent ovulation is associated with behavioural oestrus and normal fertility. It is for this reason that most ewes display oestrus during the second fortnight when joining occurs in late spring to early autumn.

However, an interesting study in Western Australia indicated that the fertility of ewes mated during the first two weeks of joining is lower than that for ewes mated at other times of the joining period (Knight et al., 1975a). In the study of Oldham et al. (1976), 23% of previously anoestrous ewes ovulated and displayed oestrus simultaneously in the first two weeks after the introduction of rams. These ewes were significantly less fertile than spontaneously cycling ewes that mated in the same period (34% vs 62%, $P<0.01$). Similar results have been reported in other studies (Marshall et al., 1976; Oldham and Cognie, 1980). One important consequence is that a significant proportion of these ewes again became anoestrus during the joining period. In studies on a large commercial flock, 35% of the ewes re-entered anoestrus during the mating period thus contributing significantly to reproductive wastage (Oldham and Cognie, 1980). Hence the ram effect is not necessarily a "switch on and continue cycling" response in the environments studied in Western Australia but the extent to which this phenomenon occurs elsewhere in Australia is not known.

This problem is more serious in the October – November period although it can persist into the spontaneous breeding season (Knight et al., 1975a; Oldham and Cognie, 1980). Its severity can be reduced (but not eliminated) by placing teaser rams in flocks 2 - 4 weeks before joining (Knight et al., 1975a). An alternative strategy is to treat ewes with progesterone (e.g. intravaginal pessary) before the commencement of mating (Oldham et al., 1976).

An additional practical aspect of the ram effect is that the response can be influenced by ram breed with the Romney being the worst and the Poll Dorset the best (Tervit et al., 1977; Knight et al., 1980). There is also evidence of variation in effect between strains and between individual rams (Knight, 1990).

1.4 OVULATORY PERFORMANCE

In a study of 54 commercial Merino flocks, Kleemann and Walker (1992) obtained a mean ovulation rate in mature ewes of 1.41 with a range 1.00 - 2.00. Mean rate varied with age (e.g. maidens 1.25) and these figures were not significantly influenced by season (October – March) or year. Generally, the ovulatory performance of Merino sheep in Western Australia is lower. Chopping and Lindsay (1970) observed ovulation rates of 1.03 - 1.30 in five flocks, Knight et al. (1975a) obtained a mean of 1.19 in 46 flocks, Knight et al. (1975b) obtained figures of 1.00 - 1.21 in four flocks and Marshall et al. (1976) reported a mean of 1.09 in seven flocks. In a study of 53 flocks, Lindsay et al. (1975) reported an overall ovulation rate of 1.14 with a between season (November – April) variation of 1.05 - 1.19. In this study, body weight was significantly correlated with ovulation rate (r = 0.42) indicating an extra 5.0 ovulation per 100 ewes for each 5kg difference in body weight.

In the eastern states, estimates include a mean of 1.36 in 14 Merino flocks at Trangie, New South Wales (Purvis et al., 1988), 1.43 and 1.09 for two flocks (Mattner and Braden, 1967), 1.26 - 1.38 for mature ewes and 1.07 - 1.11 for maiden ewes (Restall et al., 1976) and 1.46 for six-year-old Merino ewes (Edey, 1966). Restall and co-workers obtained comparable figures of 1.28 - 1.52 for Corriedale ewes and 1.77 - 1.89 for Border Leicester x Merino (BL x Mo) ewes. None of the flocks studied were of a commercial nature.

Overall mean figures for the Merino are estimated to be 1.30 in adults and 1.10 in maiden ewes, although the data on Kleemann and Walker (1992) indicate that the Merino can be a more prolific breed. Comparable mean figures for adult and maiden BL x Mo ewes are 1.80 and 1.40 respectively although far less information is available.

1.5 WASTAGE OF OVA

1.5.1 Magnitude of loss

Loss of ova (i.e. ova produced that do not result in lambs) is a major source of reproductive wastage in Australian flocks. This figure is not easy to measure (requiring estimates of at least the number of ovulations per ewe and the number of lambs born per ewe) and, as a consequence, many studies simply report the parameter “ewes mating but failing to lamb”. This figure does not include estimates of either fertilisation failure or partial failure of multiple ovulations

(PFMO; see below). Estimates of “ewes mating but failing to lamb” include 9.3 - 42.4% (Chopping and Lindsay, 1970), 43.3% (Lindsay et al., 1975), 40.3 - 48.7% (Knight et al., 1975a) and 13.4% (Kleemann and Walker, 1992).

Estimates of ova wastage in commercial flocks include 42.4% (Kleemann and Walker, 1992) in South Australia and 34% in Western Australia (Knight et al., 1975a). Estimates from experimental flocks include 29.9 – 51.9% (Edey, 1966), 6 – 48% (Edey, 1969) and 29.1% (Hasnain, 1964). Comparable figures for New Zealand flocks (Romney, Coopworth and Perendale) are 33.6% for the North Island (Knight, 1990) and 24.7% for the South Island (Kelly, 1982).

The two contributing factors to ova wastage are fertilisation failure and embryo mortality and each of these is discussed below.

1.5.2 Fertilisation failure

Fertilisation is generally considered to be an “all or none” phenomenon irrespective of whether ewes have single or multiple ovulations (Cumming and McDonald, 1967; Mattner and Braden, 1967; Restall et al., 1976). Estimates of fertilisation rates from slaughter studies are in the order of 85 - 95% (Robinson, 1951; Averill, 1955; Moore et al., 1960; Quinlivan et al., 1966; Mattner and Braden, 1967). Other studies, involving the collection of ova/embryos by laparotomy, support this figure (84.4% Restall et al., 1976; 88.9 and 89.4% Braden, 1971; 95 and 96% Cumming et al., 1975).

Overall, an estimate of fertilisation rate for the adult sheep population is 87.5% irrespective of breed. A similar figure is estimated for maiden ewes given the figures of Restall et al. (1976) for four maiden Merino flocks of 67.1, 87.0, 91.7 and 92.9%.

1.5.3 Embryo mortality

Embryo mortality (i.e. embryos that do not result in lambs) primarily occurs in the first four weeks of pregnancy with up to 20 - 30% of embryos being lost (Edey, 1966; Quirke and Hanrahan, 1983). Roberts et al. (1990) estimated that 80% of embryonic loss is due to failure of development at the time of implantation and this is likely to be due to abnormal development and function of the placenta (Cross et al., 1995).

Estimates of embryo mortality include 32.7% (Dutt, 1954), 40% (Lafley and Hart, 1959), 9.4 and 28.6% depending on season (Hulet et al., 1956) and 22.8% (Hasnain, 1964). Restall et al. (1976) reported losses of 33% and 18% in BL x Mo ewes, 16% in Corriedale ewes, 46% in mature Merino ewes and 7 - 33% in maiden Merino ewes. Edey (1970a) concluded that, on the basis of all available data, average embryo wastage is 25.5%. Edey (1969) indicated that some reliable estimates of embryo mortality fall well outside the normal range indicating that there might be genetic determinants of pre-natal loss or that basal loss is a highly variable characteristic.

1.6 PARTIAL FAILURE OF MULTIPLE OVULATIONS (PFMO)

The percentage loss of ova from twin ovulating ewes is significantly higher than the loss from single ovulating ewes (Edey, 1966; Cumming, 1972a; Edey, 1970; Geisler et al., 1977; White et al., 1981; Wilkins and Croker, 1990). This source of wastage represents a major loss of potential lambs and can, to a large extent, thwart management practises aimed at improving reproductive performance by increasing ovulation rate (Knight, 1990). On the basis that fertilisation is “all or none” in single and twin ovulating ewes (Restall et al., 1976), it can be assumed that PFMO is due to embryo mortality rather than fertilisation failure.

Estimates of PFMO include 21% (Averill, 1955), 16.5 - 47.5% (Cumming et al., 1975), 18.8% (Kelly, 1982) and 10 and 21% (Mattner and Braden, 1967). In the only studies of PFMO in commercial Merino flocks in Australia, Kleemann and Walker (1992) reported a loss of 12.2% of ova shed and Knight et al. (1975a) reported a 9% figure for Western Australian flocks. In the former study, estimates were as high as 40.0% in individual flocks. Importantly, PFMO on a flock basis increases disproportionately as the mean ovulation rate increases (Smith et al., 1983).

Overall, it is estimated that embryo wastage in Merino flocks with a mean ovulation rate of 1.3 is approximately 25%. There is no convincing evidence that embryo mortality in maiden ewes differs appreciably from that in adults (Restall et al., 1976). A comparable estimate in adult BL x Mo ewes with a mean ovulation rate of 1.8 is 34%. This increased figure, compared with the Merino, is due to an increase in PFMO. The figure is calculated on the basis that there is a linear relationship between ovulation rate and PFMO even though it is known that as ovulation rate increase so there is a disproportionate increase in PFMO. Estimates in maiden BL x Mo ewes would be less due to a reduced occurrence of multiple ovulations compared with adults.

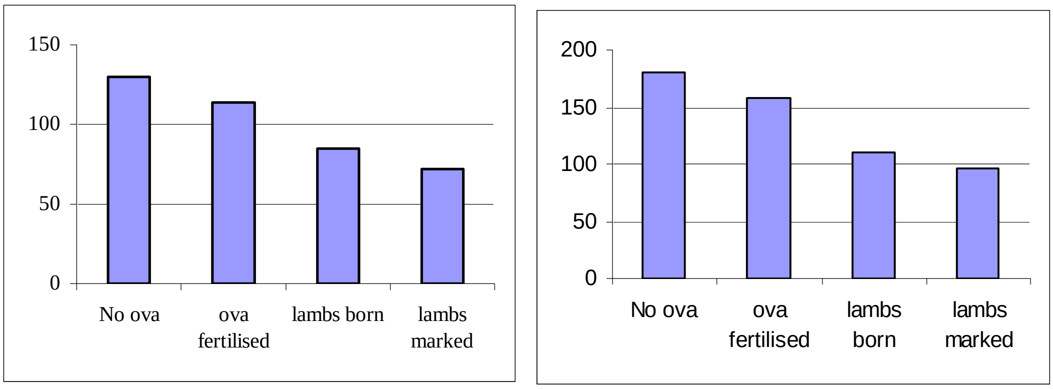
1.7 EWE FERTILITY

Ewe fertility (i.e. ewes lambing of ewes mated) is a product of the processes of ovulation, fertilisation and embryo survival. In South Australian Merino flocks, ewe fertility is estimated to be 0.90 (range 0.53 - 0.99) with the number of lambs born per ewe lambing being 1.27 (range 1.00 - 1.72). Comparable fertility figures in commercial Western Australian Merino flocks are 0.58 - 0.91 (Chopping and Lindsay, 1970), 0.57 (Lindsay et al., 1975), 0.80 (Knight et al., 1975a) and 0.86 (maiden ewes) and 0.93 (adult ewes) (Kelly and Croker, 1990). Fertility figures for New Zealand provide and interesting comparison and include 0.98 and 0.95 (Kelly and Johnstone, 1982) and 0.94 (Kelly, 1982).

1.8 SUMMARY

Estimates determined above for ovulation rate, fertilisation rate and embryo survival and using lamb survival figures determined from Section 2, it is possible to estimate overall productivity figures for Merino and BL x Mo flocks (Figure 1). The calculated lamb marking percentage in Merino flocks of 72% compares with Australian Bureau of Statistics figures of 67.7% for Western Australia (Kelly and Croker, 1990) and 76.9% for South Australia (Walker, 1981). The lamb marking figure for the BL x Mo of 97% compares with 122% obtained in the study of Atkins (1980a).

Figure 1. Estimates of the components of reproduction in adult Merino and Border Leicester x Merino ewes (note different vertical scales).



Estimates of this nature are of limited value in setting research priorities for improving reproductive performance. The large variation in reproductive performance between environments can make overall figures irrelevant to specific areas in terms of both absolute lamb marking percentages and the relative contributions of each component of reproductive wastage. The unique response of ewes to the ram effect in Western Australia, where ewes can “switch off” after one or two cycles and the relevance of PFMO in different regions where flocks have either a relatively high or low mean ovulation are cases in point.

Despite these limitations the important points that arise from this Chapter are:

(1). There is large variation both within and between regions in the reproductive performance of flocks.

(2). A number of factors have been identified that contribute to between region variation although there is a paucity of data on flock performance in some regions.

(3). Causes of variation within regions are less obvious although some possible factors are addressed later in this review. Why, for example, can mean ovulation rates in commercial Merino flocks within one region vary between 1.00 – 2.00?

(4). Prioritising research into reproductive wastage needs to be done on a regional basis.

(5). Why can Merino flocks in some regions be induced to cycle (at near 100% efficiency) throughout the year when data and anecdotal evidence indicate that this is not the case in other regions?

(6). Is the ability of the Merino to cycle throughout the year in these regions being fully utilised to give maximum flexibility in terms of time of mating? Is such flexibility being utilised in drought management strategies?

(7). The ability to induce cyclicity throughout the year is dependent on the ram effect. However, in some regions, induction is associated with unique aberrations in subsequent cycles and this problem needs to be better understood if maximum benefit is to be obtained.

(8). Wastage of ova is the most serious source of reproductive wastage (excluding lamb mortality) with embryo mortality being the major contributing factor.

(9). PFMO is a significant component of embryo mortality. Benefits to be gained from increases in ovulation rate will be minimal until the problem is better researched and managed.

Finally, much of the data reviewed in this Chapter were collected between one and three decades ago. A pertinent question is whether or not progress has been made in the meantime? Kelly and Croker (1990) concluded that lamb marking percentages in 1990 in Western Australia had improved by approximately 10% since the 1970s. Regression analysis indicates that this figure has probably improved by another 3% during the 1990s. Despite such success, there remains considerable potential for further improvements and realisation of this potential will be depend, to a large extent, on a better understanding of the causes of reproductive wastage.

CHAPTER 2. MANAGING THE COMPONENTS OF REPRODUCTIVE PERFORMANCE

2.1 INTRODUCTION

Some components of reproduction are influenced, to varying degrees, by factors that have predictable outcomes on flock performance (e.g. age of animal, genotype). More important to flock performance are those factors that can be manipulated and have larger outcomes on productivity. Nutrition is, without doubt, the most important management tool that producers have at their disposal to manipulate reproductive performance. This statement is reflected in the large amount of research conducted during the 1960s and 1970s into understanding the ways in which nutrition influences the various reproduction processes. This remains particularly important in Australia given the wide seasonal fluctuations in both pasture quality and quantity. Hence, the purpose of this Chapter is to review published information on how best to manage those factors that determine lambing percentages, with particular emphasis on the role of nutrition.

2.2 INCIDENCE OF OESTRUS

As discussed in Chapter 1, season and the ram effect are major determinants of the pattern of cyclicity. Furthermore, genotype is an important influence with British breeds and their crossbreds having a more defined breeding season than Merino counterparts (Allden, 1956; Killeen and Dawe, 1966). An important factor within the Merino industry is the effect of ewe strain on both the spontaneous (Dun et al., 1966) and ram-induced incidence of oestrus. Location of the flock within Australia can influence levels of oestrous activity although this observation is confounded by strain effects (Morley, 1948; Watson, 1956; Dun et al., 1960; Smith, 1960; Barrett et al., 1962).

Irrespective of breed/strain, the occurrence of oestrus is reasonably resilient to changes in live-weight. Ewes need to be in very poor condition before they fail to express oestrus during the breeding season (Lindsay, 1976). However, restricted nutrition during the previous winter/spring can reduce the expression of oestrus in Merino ewes during the following autumn, despite nutrition and live-weight improving during the intervening period (Hunter, 1962; Smith, 1962, 1966). Fletcher (1974) suggested that this is only important when feed in summer is insufficient to allow ewes to return to normal live-weight by autumn. There is no evidence that overweight ewes have aberrant oestrous patterns and loss of weight during mating (as distinct from pre-mating loss) does not appear to influence the incidence of oestrus.

2.3 OVULATION RATE

Ovulation rate can be significantly influenced by several factors including season (see Oldham et al., 1990), breed and strain (Piper and Bindon, 1987; Fahmy, 1996) and the ram effect (Cognie et al., 1980; Oldham et al., 1990). However, it is the response of this trait to nutrition that is particularly relevant to flock management in terms of improving reproductive performance. Original observations by Underwood and Shier (1941) indicated that a rising plane of nutrition six weeks before mating significantly increases the rate of production of twin lambs (the flushing effect). A rising plane of nutrition inevitably led to ewes being heavier at the time of mating leading Moule (1962) to question the effects of body weight *per se* on reproductive outcome. Subsequent experiments by Coop (1966), Killeen (1967), McInnes & Smith (1966) and Fletcher (1971) led to the conclusion that a rising plane of nutrition and absolute body weight are both implicated in increasing the incidence of twins. Coop (1966) coined the phrases “static effect” and “dynamic effect” to describe the influence of body weight *per se* and increases in body weight *per se* on reproductive performance. Many studies were conducted during the 1970s and 1980s to examine these influences on flock performance and their findings largely remain valid today.

2.3.1 Static effect

Both Edey (1968) and Fletcher (1971) demonstrated a strong regression of live-weight on ovulation rate in ewes and this finding was supported by on-farm surveys, where within flock analyses indicated that ovulation rate increased by 0.01 – 0.02 ova per ewe for every 1kg difference in live-weight (Lindsay et al., 1975; Kelly and Johnstone, 1982). The static live-weight effect has been compartmentalised into the effects of body size and body condition, the latter reflecting the ewe’s body reserve status. The significance of the static effect on ovulation rate is debatable but estimates of improvement include 3 - 4% (Brien et al., 1977) and 5 - 10% (Coop, 1966). Pre-joining live-weight is positively associated with improved lambing performance with approximately 0.22 – 1.10 extra lambs per 100 ewes lambing being obtained for each kilogram increase in live-weight. Despite these findings, the value of live-weight as a predictor of the ovulatory response to a nutritional treatment/regime needs to be treated cautiously given the influence of other factors (e.g. genotype, age, season and parity) as well as the specific effects of nutrition that are not associated with live-weight.

2.3.2 Dynamic effect

While the static effect has been found in most studies, there has been a divergence of opinion regarding the dynamic effect (Fletcher, 1971; Morley et al., 1978). In hindsight, this confusion resulted from a lack of awareness of all factors contributing to the dynamic effect – pasture composition, duration of flushing and the specific effects of high protein diets (e.g. lupin supplementation) on ovulation rate. The important elements of the dynamic effect, as determined from pasture grazing studies, are:

(a) Ewes need to be exposed to improved pasture conditions (e.g. an increasing pasture allowance of approximately 6kg DM/ewe/day) for some period during the six weeks before mating commences (Rattray et al., 1978: Rattray et al., 1980 a,b,c).
(b) A three-week exposure period is sufficient to significantly increase ovulation rate, irrespective of when in the six-week period the exposure occurs (Rattray et al., 1980a; Smith et al., 1983). This finding is almost identical to that made many years earlier by Clark (1934).
(c) Further improvements in ovulation rate occur if exposure is increased from three to six weeks (Smith et al., 1983) but the additional increases in ovulation rate may not translate into additional lambs.
(d) The benefits of exposure to improved pasture are “carried over” i.e. the benefits of a three-week exposure continue for an additional three weeks (Smith et al., 1983).

The dynamic effect has a greater impact on ovulation rate than does the static effect with responses of the order of 10 - 50% (Smith et al., 1983; Rattray et al., 1978; Brien et al., 1977). However, many of the additional ova are lost – in some studies up to 50% of twin ovulating ewes gave birth to single lambs (e.g. Smith et al., 1979). Most research into flushing has been conducted in New Zealand where pasture conditions/quality vary considerably from those in Australia. Care has to be taken in applying these practises under Australian conditions, particularly where autumn matings are employed.

2.3.3 High protein supplementation

One factor that confused the understanding of the flushing effect is the ability of high protein diets to elicit comparable responses after very short supplementation periods. Exposure of ewes to lupin-based diets for as little as six days significantly increased ovulation rates (Lindsay, 1976; Oldham, 1980; Oldham and Lindsay, 1984) although there was no “carry over” effect (i.e. the response was specific to the cycle(s) during which supplementation occurred) and nor were there necessarily changes in live-weight (Knight et al., 1975c; Lindsay, 1976; Fletcher, 1981). Subsequent studies identified the critical time of supplementation as being between days 5 - 8 before the next ovulation (day 0) (Nottle et al., 1986; Stewart and Oldham, 1986). Supplementation within five days of ovulation had either no effect on ovulation rate or decreased it (Smith and Stewart, 1990). This latter period corresponds with the approximate time taken for follicles of less than 2mm to develop to the ovulatory stage (Tsonis et al., 1982; Coleman and Dailey, 1983).

It was not initially known if the response to lupins was due to energy or protein content or to a specific nutritional factor. Early studies demonstrated that the response was primarily due to the protein content (Lindsay, 1976) but other studies have implicated both protein and energy intakes (Davis et al., 1981; Smith, 1985; Teleni et al., 1989). Smith (1985) obtained a response to protein intake only above a certain level of energy intake. Subsequent studies indicated that the increase in ovulation rate was correlated with the plasma concentrations of branched chain amino acids (BCAA, valine, leucine and iso-leucine) (Waghorn, 1986; Waghorn and Smith, 1990). The importance of BCAA in controlling ovulation rate has been confirmed by intravenous infusion studies (Downing and Scaramuzzi, 1991; Downing et al., 1995). Lupin supplementation has no advantage over other high protein supplements (e.g. peas, soybeans, protected casein) (Davis et al., 1981).

Not all lupin supplementation studies have resulted in worthwhile increases in ovulation rate leading Rizzoli et al. (1976) to suggest that time of year might be implicated. This notion is supported by Fletcher (1981) who obtained a response to lupin supplementation only when the initial level of feeding supplied insufficient protein and energy. This effect was most noticeable in the months of November and December when pastures had dried.

2.3.4 Benefits of premating supplementation

Lambing percentages may be improved by (1) improving the live-weight of ewes, (2) increasing pasture allowances for at least one oestrous cycle during the six-week pre-mating period or by (3) supplementing ewes with a high protein diet (e.g. lupins, peas) between 5 – 8 days before ovulation. The benefits are in the form of increases in ovulation rate although Knight et al. (1975c), with lupin supplementation, did report an increase in the proportion of ewe lambing that could not be accounted for by increases in ovulation rate.

With these possible sources of improvement, the question of whether pre-mating supplementation is worthwhile is very much dependant on a cost-benefit analysis. In studies reported by Lindsay (1976), involving six flocks supplemented with lupins during joining, a mean increase in ovulation rate of 19.3% was obtained resulting in an increase in ewes lambing of 8.25% (42.7% of the extra ovulations resulted in lambs). However, Kelly and Croker (1990) concluded that only a small proportion of the increase in reproductive performance observed in Western Australia during the 1980s was due to farmers using the lupin response. Never-the-less, the ability to improve ovulation rate by supplementation 5 – 8 days before ovulation is attractive and deserving of further research. This option is more appealing to AI programs where time of ovulation is synchronised than it is to conventional flock mating where synchrony is lacking.

2.4 EMBRYO MORTALITY

Embryo mortality is a serious source of reproductive wastage because of its “double-edged” nature – the loss of an embryo as well as potential barrenness that may result from delays in ewes returning to service. Almost all the mortality occurs within the first 30 days after mating (Edey, 1966) and consequently most nutrition studies have concentrated on this period. However, estimating the effects of nutrition *per se* on embryo mortality is confounded by influences of genotype, body weight, season, temperature and previous nutritional history.

2.4.1 Post-mating nutrition

Some studies have shown that nutritional restriction can result in decreased embryo survival (Bennett et al., 1964; Edey, 1966, 1970a; Blockey et al., 1974) particularly in twin ovulating ewes (Cumming, 1972 a, b) while others have shown no such effect (Hodge, 1966; Killeen, 1967; Edey, 1970 b, c; Bennett et al., 1970; Hulet et al., 1968; Braden, 1971). Differences in response between studies are most likely to have resulted from differences in experimental conditions – duration of treatment, composition of diets, prevailing pasture conditions and breed effects. It is now generally recognised that high levels of nutrition are, in fact, detrimental to embryo survival (El-Sheikh et al., 1955; Foote et al., 1959; Hoxsey et al., 1960; Bellows et al., 1963; Parr et al., 1987). In Merino and BL x Mo ewes fed either 25, 100 or 200% maintenance (M) diets from day 2 - 16 after mating, survival was significantly higher in ewes on the M diet than on the other diets (Cumming et al., 1975). In the same study, there was a significant decrease in embryo survival in Merino ewes on the 200% M diet compared with other treatments. Very short term fasting (for three days at various times during the first two weeks after mating) was also associated with increased pregnancy rates in ewes with twin ovulations (Blockey et al., 1974). On the other hand, nutritional restrictions imposed in the post-mating period but which are initially applied in the pre-mating period can reduce rates of embryo survival (e.g. Gunn et al., 1972; Rhind et al., 1989a).

2.4.2 Post-mating progesterone levels

Evidence that high dietary intake immediately after mating is detrimental to embryo survival is supported by studies on the relationship between feed intake and plasma progesterone levels. There is a strong inverse correlation between dietary intake and these concentrations (Cumming et al., 1971; Parr et al., 1982; Williams and Cumming, 1982; McEvoy et al., 1995; O’Callaghan et al., 2000) due to increased rates of metabolism in the liver at high feeding levels (Parr et al., 1993). Importantly, these higher progesterone levels are associated with improved rates of embryo survival (Parr et al., 1987). There is also evident (Mann et al., 1998) that the level of progesterone is positively correlated with embryonic interferon *tau* levels thus providing a mechanism whereby early post-mating dietary intake is able to influence subsequent implantation rates.

2.4.3 Other factors influencing embryo mortality

(1). *Stress* – Stress is a highly under-estimated and highly under-researched influence on many physiological events in the animal. In the ewe, stress can either increase (Braden and Moule, 1964; Sawyer et al., 1979a,b) or shorten (Przekop et al., 1984) the length of the oestrous cycle, reduce ovulation rate (Doney et al., 1976) and decrease the proportion of ewes mated (McMillan and Knight, 1982). The effects of stress on embryo mortality are generally not known but management procedures such as mustering, yarding and general animal handling during the peri-conceptual period are stressors that could have a detrimental effect on embryo survival. In the study of Van Niekerk et al. (1968), where small numbers of ewes were held in yards (and presumably stressed) and exposed to under-nutrition (e.g. 2 - 4 days) during the first two weeks of pregnancy, embryo survival was reduced despite under-nutrition during this period generally having a positive effect on embryo survival. It appears that embryo viability in sheep is especially vulnerable to stress that occurs within 24h of the onset of oestrus (Dhutt, 1963).

(2). *Genotype* – Cumming et al. (1975) reported that embryo survival in twin ovulating ewes exposed to nutritional stress was significantly higher in BL x Mo ewes than in Merino ewes. Moore (1968) studied reciprocal embryo transfer between Merino and BL ewes and found that the latter had a greater ability to support multiple pregnancies. Bennett et al. (1964) compared the responses of Merino and BL x Mo ewes to under-nutrition after mating and found a significant reduction in lambing rates in Merino but not BL x Mo ewes (this result is confounded by age). Foote et al. (1959) concluded that Columbia ewes lost fewer embryos per head than did Hampshire ewes. Studies in the mouse (Bradford, 1979) indicate that the genotype of the dam accounts for much more of the variation in embryo survival than does the genotype of the embryo.

(3). *Liveweight and body condition* – That live-weight might influence embryo survival can be inferred from the reported increase in barrenness found in ewes of low live-weight (Coop, 1962; Bennett et al., 1964; Edey, 1970a, b). However, Cumming and McDonald (1970) found no significant relationship between live-weight and survival of transferred embryos in ewes of medium and high live-weight. Similarly, Cumming et al. (1975) failed to confirm that live-weight is an important determinant of embryo survival. Edey (1970a) found no consistent regression of pre-natal mortality on body weight at mating.

(4). *Season* – In a study of Merino and BL x Mo ewes, Cumming et al. (1975) concluded that embryo survival in twin ovulating ewes was significantly higher following an autumn mating compared with a summer mating. There was no significant difference in single ovulating ewes.

(5). *Specific nutritional effects* – Increases in embryo mortality have been attributed to specific effects including grain feeding (El-Sheikh et al., 1955), selenium deficiency (Hartley, 1963) and consumption of goitrogenic plants (Williams et al., 1965).

2.4.4 Strategies to minimise embryo mortality

Lindsay (1976) argued that normal fluctuations in pasture nutrition experienced in Australia during and shortly after mating are unlikely to be sufficient to contribute to embryo mortality. However, feed status immediately after mating is important given the demonstration of a relationship between low feed intake and high plasma progesterone levels in association with increases in embryo survival. A pertinent management strategy is thus to restrict feed intake immediately after conception to increase embryo survival rates. Again, this approach is easier in situations where the approximate time of ovulation is known (e.g. as in AI programs).

Progesterone supplementation after mating is also a possibility. In some studies (Pearce et al., 1984; Peterson et al., 1984; Davis et al., 1986), increases in pregnancy rate of 11 – 28% have been achieved using progestagen supplements although other reports have failed to demonstrate such an effect (Smith et al., 1985; Kerton et al., 1986). Differences in pasture quality between these studies might be responsible for these variable results. Time of administration of such a supplement is critical given the role of progesterone in regulating embryo migration. Supplementation is also an additional expense (approximately $2.50 per ewe plus labour) raising the possibility that strategic grazing (i.e. the use of low diets immediately after mating) might be more economical.

2.5 FERTILISATION RATE

Little information is available on factors that affect fertilisation, primarily because of the difficulty in measuring this parameter. Although factors such as genotype, season, fluctuations in nutrition and high ambient temperature can all influence reproductive outcome, there is little evidence of any direct effect on fertilisation rates *per se*. However, Killeen (1972) did report that fertilisation rates in maiden BL x Mo ewes of very low live-weight that mated early in the breeding season were substantially lower than for heavier and/or older ewes. Serious declines in fertilisation rate can occur with problems associated with ram infertility including disease and increases in ambient temperature but these topics are outside the scope of this review.

2.6 PARTIAL FAILURE OF MULTIPLE OVULATIONS (PFMO)

2.6.1 Causes of PFMO

It has long been thought that the most likely cause of PFMO is the occurrence of twin ovulations on one ovary (unilateral ovulations) associated with subsequent impaired migration and/or competition between fetuses after the commencement of implantation. Baier and Rüsse (1968) reported that the incidence of twinning is higher when an ovulation occurs on each ovary (bilateral ovulations) compared with two ovulations on one ovary. Brien et al. (1977) obtained increased survival rates of ova with bilateral compared with unilateral ovulations after lupin supplementation although Edey (1970a) concluded that there were no differences. In a study in cattle, Rowson et al. (1971) observed twinning rates after the transfer of two embryos per recipient, either as two to one uterine horn or as one embryo to each horn and found a significantly higher twinning rate in the latter group (73% vs 45%). These authors concluded that the lower rate with unilateral embryo transfer resulted from failure of migration leading to fewer cotyledons per fetus and ultimately higher mortality. An alternative explanation is that migration does occur but the absence of a corpus luteum on the contralateral ovary ultimately results in death of the embryo or fetus.

Death due to competition between fetuses during the implantation period would be expected to lead to delays in the time taken for the ewe to recycle. There is no evidence that PFMO is associated with such a delay. This indicates that PFMO might be due to other factors, particularly those that are able to influence ovum quality. These factors include (1) intra-ovarian follicle growth regulators that compromise the integrity of ova when more than one dominant follicle develops, (2) disruption to imprinting patterns in the oocyte in association with disturbed pre-antral and/or antral follicle development and (3) increases in ammonia/urea production in ewes in which twin ovulations have been induced by high protein intake. These possibilities are discussed elsewhere in this review (Chapter 8.5).

2.6.2 Response of PFMO to nutrition

The way in which nutrition might influence PFMO is particularly relevant in flocks exposed to a rising plane of nutrition before mating where increases in ovulation rate could be expected. These increases are most often associated with disproportionate increases in PFMO (Smith et al., 1983). In this study, a high diet offered over six compared with three weeks increased ovulation rate by an additional 30% but this increase was not associated with an increase in the incidence of multiple births. Edey (1966) concluded that the loss of ova from single and multiple ovulators was not random in situations where sheep are nutritionally challenged – 20/40 ewes with twin ovulations lost both ova compared with 17/47 single ovulating ewes losing one ovum. Increased embryo wastage in twin ovulating ewes following either pre-mating (Henning, 1939; Cumming, 1972b) or post-mating nutritional stress (Cumming, 1972 a) has been reported although the data of Blockey et al. (1974), obtained from ewes that were fasted at various times during the first fortnight after mating, indicate that PFMO might be reduced by undernutrition. The magnitude of PFMO may also be related to breed. For example, 17% of twin ovulating Coopworth ewes gave birth to single lambs compared with 51% for Perendale ewes (Smith et al., 1979).

2.7 SUMMARY

The important points that arise from this Chapter are:

(1). Nutrition during the previous winter/spring can influence the subsequent expression of oestrus (this period can include the time of the previous lactation).

(2). Improvements in lambing performance can occur through one or more of the following pre-mating strategies:

  (a) Improving live-weight and/or body condition.
  (b) Increasing pasture allowance for at least one cycle during the six-week period before mating.
  (c) Supplementing ewes with a high protein diet between 5 – 8 days before ovulation.
  (d) Supplementing (infusing) ewes with branched chain amino acids between 5 – 8 days before ovulation.

(3). Precision feeding before ovulation is more relevant to AI and embryo collection/transfer programs (where the time of ovulation is known) than it is to flock matings. Strategies need to be developed for its use in the latter.

(4). Further research on the role of branched chain amino acids in controlling ovulation is justified including dose rates, method of delivery and influence of prevailing nutritional conditions.

(5). Observations that the pre-ovulatory follicle takes five days to develop provides an unique window in which to focus research to maximise ovum quality without adversely affecting ovulation rate.

(6). Post-mating management of flock nutrition is important for embryo survival with a high level of nutrition being detrimental. Contrary to convention, ewes should be held on a M or less than M diet during the first two weeks after conception.

(7). Increases in ovulation rate associated with dietary intake are associated with disproportionate increases in PFMO. Strategies to minimise PFMO need to be based on a better understanding of the problem with particular emphasis on the ability to improve ovum quality.

(8). When embryos are transferred in groups of two per ewe in embryo transfer programs, they should be transferred bilaterally rather than unilaterally when the opportunity arises. This routine is not currently practised in the embryo transfer industry but could result in significant increases in embryo survival rates.

CHAPTER 3. EFFECTS OF MATERNAL NUTRITION ON THE OOCYTE, EMBRYO AND FETUS

3.1 INTRODUCTION

Ovulation is the culmination of a series of physiological events that begins primarily with the recruitment of follicles from the primordial germ pool and concludes with the production of ova. The way in which this progression occurs and the role of nutrition in the various physiological events are both poorly understood. Evidence indicates that it takes approximately six months from when the follicle begins to grow until ovulation (Cahill and Mauleon, 1980; Driancourt and Cahill, 1894). During the first 4½ months of this period, the follicle is in the pre-antral phase after which it spends six weeks in the antral phase. In the latter period, the follicle is highly sensitive to hormonal (Moor et al., 1980) and nutritional influences (Cumming, 1972a,b). It is most probable that the quality of the ovum and the resultant embryo are influenced by factors that operate during the entire six-month period and not just during the immediate pre-ovulatory period. It is hypothesised that nutrition, in particular, has a cumulative effect on oocyte development in this period and that this cumulative effect is ultimately reflected in the quality of the ovum produced and, ultimately, lambing performance of the flock.

The purpose of this Chapter is to review the effects of nutrition on the development of the oocyte, embryo and fetus with the primary intention of identifying those periods in development where nutritional management might lead to practical benefits.

3.2 THE OOCYTE

3.2.1 The period of pre-antral follicle development

This period is characterised by follicle recruitment and by the development of gap junctions between the oocyte and granulosa cells thus ensuring that the two are metabolically coupled very early in folliculogenesis (Burghardt and Matheson, 1982). Despite this close association, very little is known of the effects of nutrition during pre-antral follicle development. However, it is the period when many aspects of ovum quality are determined. There is a major reactivation of the oocyte genome, involving increased activity of RNA polymerase-I and 2 in association with the lampbrush state of the chromosomes (Moore et al., 1974; Moore and Lintern-Moore, 1978). The chromosomes are transcriptionally very active and at least 80% of the RNA made in this period is stored as stable ribosomal RNA and mRNA for use in early embryogenesis (Schultz, 1975; Jahn et al., 1976). Furthermore, most oocyte growth is completed in this period even though the follicle continues to grow in size during and after antrum formation (Green and Zuckerman, 1951). The metabolic coupling between the corona radiata cells and the oocyte ensures that the oocyte is able to store an array of metabolites in the cytoplasm in preparation for subsequent development (Donahue and Stern, 1968; Brower and Schultz, 1982). Despite much research, the ability to stimulate or amplify the recruitment process is very low – it is possible to attenuate the process by starvation in the rat (Lintern-Moore and Everitt, 1978) but there is no practical way of enhancing the process.

3.2.2 Nutrition and pre-antral follicle development

In normal flock management practises, this period extends from late lactation until 1½ months before mating and can, for varying periods, coincide with nutritional stress. Little research has been conducted on the effects of nutrition in this period on subsequent lambing performance. The effects of any treatment applied during this period can be influenced by treatments or environmental conditions imposed during the subsequent antral period of development (Killeen, 1967) thus making interpretation of results difficult.

Two parameters that can be affected by nutrition during this period are the incidence of oestrus (Smith, 1962, 1966) and ovulation rate (Fletcher, 1974; Nottle et al., 1997). In a study of Corriedale ewes exposed to maintenance or less than maintenance diets for 5 - 6 months prior to ovulation, Cahill et al. (1984) concluded that the probability of ovulation occurring early in the breeding season was depressed by the low level of nutrition. Lactation imposed an additional “penalty” which was independent of live-weight.

3.2.3 Nutrition and antral follicle development

Unlike the pre-antral follicle, growth of the antral follicle (including the resumption of meiosis) is under the control of LH and FSH as well as the hormones of ovarian origin including progesterone, oestradiol and inhibin (Roche and Diskin, 1995). Nutrition is able to influence the plasma concentrations of at least some of these hormones and, not surprisingly, dietary intake is able to influence follicle development. Information reviewed in Chapter 2 clearly indicates that increased nutrition during the period of antral follicle development leads to an increase in ovulation rate. Despite this long-standing observation, little is known of how nutrition influences the growth characteristics of the follicle or the quality of the developing oocyte.

In the sheep, reduced dietary intake during this period decreases the number of growing follicles in superovulated (Yaakub et al., 1997a) and non-superovulated (O’Callaghan et al., 2000) ewes. However, low dietary intake is associated with improved quality of the ovum. McEvoy et al. (1995) reported that a higher proportion of ova from ewes on a low diet were considered viable when compared with those produced from ewes on a high diet. Similarly, restricted energy intake in cattle before slaughter enhanced the subsequent *in vitro* development of oocytes from small follicles (McEvoy et al., 1997b) and Yaakub et al. (1997b) produced a greater number of blastocysts *in vitro* from cows on a low diet compared with cows on a high diet. **These findings indicate that the preferred conditions for follicle growth differ from those for the production of ova of high quality**. It is postulated that this conflict contributes to the higher than normal level of partial failure of multiple ovulations (PFMO) in ewes that receive high dietary supplements in the lead up to ovulation i.e. more ova are generated but many are compromised and ultimately contribute to embryo wastage.

3.3 THE EMBRYO

Most studies that have examined the effects of nutrition on the quality of the embryo have been conducted with bovine embryos. Generally, short-term pre-ovulatory restrictions have been associated with increased yields of transferable embryos compared with *ad libitum* feeding (Mantavani et al., 1993; Yaakub et al., 1996; Nolan et al., 1998). Nutrition also influences the quality of the ovine embryo. In a study that examined the effects of high (1.5M) and low (0.5M) diets on embryo production and quality in the superovulated ewe, Kakar et al. (2002) reported that under-nutrition imposed from the day of ovulation until six days later significantly increased the number of cells in resulting blastocysts. This increase was entirely due to an increase in trophectoderm cells, thus raising the possibility that under-nutrition might ultimately stimulate the development of a larger (and/or more efficient) placenta and hence improve fetal survival rate. Infusion of superovulated ewe with high levels of glucose (to mimic the effects of high dietary intake) reduced both the yield of good quality embryos (Yaakub et al., 1997c) and pregnancy rates (Rubio et al., 1997). Given the observation that glucose infusion increased ovulation rate (Downing et al., 1995b; Williams et al., 1997), it would again appear that the optimal energy requirements for follicle growth and ovulation differ from those required for embryo development. The effect of high nutrition on embryo quality might simply be a consequence mediated through the ovum (as discussed above). However, high glucose concentrations *in vitro* are detrimental to embryo development (Furnus et al., 1996) indicating that the glucose level *in vivo* might directly influence embryo quality.

Dietary intake can alter the expression of genes involved in early embryo development such as Na/K-ATPase and Cu/Zn SOD (Wrenzycki et al., 1999). It can be speculated from the study of Kakar et al. (2002) that the environment is able to manipulate the embryo to maximize the likelihood of survival when challenged nutritionally. This may be an adaptive advantage that has evolved to increase the odds of survival when starvation is imminent. If the nutritional status continues to decline, ovarian cyclicity is terminated (Rhodes et al., 1996) and/or the embryo/fetus is lost thus minimising the risk associated with an on-going pregnancy at times of life-threatening nutritional deprivation.

3.4 THE POST-IMPLANTATION FETUS

Many factors regulate intra-uterine fetal growth and there are many patho-physiological factors, including nutrition, that can result in perturbations or restrictions to fetal growth (see Robinson et al., 1994). The way in which the fetus is able to adapt physiologically to its suboptimal environment is critical in determining the health and survival of the fetus/newborn. An understanding of this association and its consequences has led to the “fetal origins of adult disease” hypothesis (Barker 1998, 1999). While the detailed nature of this association falls outside the scope of this review, there is evidence that nutrition during the peri-conceptual period can influence the physiological well-being of the fetus.

Edwards and McMillen (2002) investigated the effect of restricted nutrition (0.7M) from 60 days before until seven days after mating as well as restricted nutrition during gestation. There was a differential effect of peri-conceptual under-nutrition on the hypothalamus-pituitary-adrenal axis in singleton and twin fetuses with restricted nutrition specifically increasing plasma ACTH concentrations in the latter. The downstream consequences of this finding are not known but the authors postulated that an increase in the exposure of the fetus to excess glucocorticoid concentrations may be one mechanism whereby under-nutrition before or in early pregnancy results in adverse cardiovascular outcomes in later life (Ravelli et al., 1999; Roseboom et al., 2000).

It has been shown that maternal under-nutrition during the pre-implantation period in the guinea pig (Erwich and Robinson, 1997), rat (Kwong et al., 2000) and sheep (Kakar et al., 2002) alters the allocation of cells to the trophectoderm and inner cell mass of the embryo. It has been postulated that alterations to the development of the trophectoderm may result in changes in the secretion of placental hormones (e.g. PGE_2) that are implicated in the control of ACTH secretion in late-gestation (Hollingsworth et al., 1995).

3.5 SUMMARY

The important points that arise from this Chapter are:

(1). A low dietary (M or less than M) intake immediately before mating improves the quality of both the ovum and embryo.

(2). A serious dilemma arises in that the optimal nutritional condition for follicle growth (e.g. 1.5M) is not optimal for the production of high quality ova and embryos.

(3). Hence, diets that stimulate multiple ovulations produce ova/embryos of reduced quality. It is speculated that it is for this reason that levels of PFMO are substantially higher in flocks that receive dietary supplements before mating.

(4). A low diet (0.7M) from the time of ovulation increases the proportion of trophectoderm cells in resulting blastocysts compared with the proportion obtained with a high diet. This shift in cell allocation may provide an adaptive advantage in placentation. The significance of this finding, in terms of pregnancy rates and birth weights in commercial flocks, needs to be examined.

(5). A low diet in the peri-conceptual period results in changes to the physiology of twin compared with single fetuses which might be detrimental to health in adult life. This finding raises the possibility that peri-conceptual nutrition might influence lifetime productivity in resulting offspring.

CHAPTER 4. FETAL OVARIAN DEVELOPMENT AND LIFETIME PERFORMANCE

4.1 INTRODUCTION

Differentiation of the fetal ovary in the sheep is usually completed by 30 - 35 days after conception. The ovarian follicle cells are derived from two different sources - the primordial germ cells arise from the yolk sac endoderm and the granulosa and thecal cells differentiate from the mesonephric tissue that migrates into the genital ridge. During growth of the fetal ovary, the germ cells and granulosa cells are incorporated into cortical cords (Ohno et al., 1962) and, after a number of mitotic divisions, oogonia begin meiosis in a reasonably asynchronous manner (Gondos et al., 1971). Germ cell numbers reach a maximum of approximately one million per ovary after which degeneration associated with phagocytosis reduces this number to approximately 100,000 per ovary at birth. By day 62, germ cells have normally reached meiotic prophase (Mauleon, 1976). At this stage, the primordial follicle contains a dictyate oocyte, a single layer of squamous granulosa cells and a basal laminar. At birth, the full compliment of germ cells are present, all having been exposed to the uterine milieu during pregnancy. The vast majority of germ cells will remain within the ovary during the animal's lifetime and be exposed to a range of physiological conditions induced by changes in nutrition, age, lactation status and pregnancy.

The aim of this Chapter is to examine the relationship between the development of the fetal ovary and subsequent lifetime performance. Is it possible to manipulate germ cell numbers such that the reproductive performance of the resulting adult is improved? Similarly, to what extent does nutritional deprivation (e.g. conditions of drought) influence development of the ovary and to what extent does it impact on lifetime performance?

4.2 NUTRITION AND GERM CELL NUMBERS IN THE FETAL OVARY

It is not known if germ cells that are lost during development of the fetal ovary are lost because they are inherently non-viable or whether the loss represents a major wastage of reproductive potential. However, potential does exist to manipulate the quantity and/or quality of germ cells by nutritional means. It is known that maternal under-nutrition during fetal life can influence the development of the ovary and, in particular, the time when germ cells commence meiosis. Borwick et al. (1997) examined the effect of low (0.5M) and high (1.5M) dietary intake from the time of mating until either day 47 or day 62 of pregnancy. Under-nutrition did not influence fetal weight, fetal ovary weight or ovarian steroidogenic capacity. However, ovaries of fetuses on the low diet contained significantly more oocytes that those of fetuses on the high diet indicating that the process of oogonial degeneration may have been reduced or delayed in the former group. At day 62, a similar pattern was observed and the authors concluded that the arrest of meiotic activity had been delayed in the low diet group i.e. the development of the fetal ovary was retarded. In a more detailed study, Rae et al. (2001) examined the effect of a high (1.0M) and a low (0.5M) diet during selected windows from the time of conception to 100 days of gestation. These authors concluded that the low diet retarded the onset of meiosis but only when restrictions were applied from the time of mating. Furthermore, under-nutrition did not affect oocyte survival rate (as measured on day 110) but did inhibit the rate of transition of follicles beyond the primordial stage.

These studies were unable to assess the influence of nutrition on the quality of oocytes but it does appear that the potential to increase germ cell numbers in the fetus by maternal nutrition is low. This conclusion is in agreement with the data of Gondos (1978) that indicate that oogonia that are late entering meiosis (e.g. as induced by under-nutrition) have a reduced chance of survival.

4.3 THE LAMB AS A SOURCE OF OOCYTES

Advances in *in vitro* maturation (IVM) and *in vitro* fertilisation (IVF) and the ability to harvest large numbers of oocytes from antral follicles of newborn lambs means that the lamb is a ready source of oocytes. Folliculogenesis begins in the fetal ovary between 65 - 110 days of gestation (McNatty et al., 1995) and antral follicles are present by 135 days. For reasons that are not know, these follicles are particularly responsive to FSH treatment leading to the harvest, in the newborn lamb, of up to several hundred oocytes by follicle aspiration (Kelly and Walker, 2002). Differences exist in the quality of oocytes from adults and lambs in several aspects including time to complete meiosis, protein synthesis, MPF activity, glutamine metabolism and smaller mean size of mitochondria and cortical granules (Kochhar et al., 2002; Ledda et al., 1996; Bogliolo et al., 1997; O'Brien et al., 1996). Despite these differences, compact morulae and blastocysts can be produced *in vitro* from lamb oocytes at relatively high rates. The transfer of these embryos produce between 10 - 20 lambs per donor lamb with up to 50 lambs per donor lamb being obtained in individual cases (Kelly and Walker, 2002). A major constraint in the development of this technology is the high level of embryo wastage that occurs after transfer and this is most likely due to deficiencies in the ability of the IVM system to generate ova of consistently normal quality. Nevertheless, at these levels of performance the technology has the ability to play a significant role in situations where a rapid expansion of sheep numbers is required.

That nutrition plays a role in determining the quality of the lamb oocyte is indicated in studies (J.M. Kelly, unpublished data) in which pregnant ewes were fed either a high (1.3M) or low (0.7M) diet at specific stages of pregnancy. Oocytes obtained from lambs produced in the high treatment groups resulted in significantly higher blastocyst production rates than did oocytes from the low treatment groups with a commensurate increase in the number of lambs born per donor lamb after transfer. It is not known to what extent this nutrition-induced benefit persists into adulthood but it is feasible that accumulation and storage of stable chemicals (e.g. mRNA) in fetal oocyte cytoplasm is one determinant of oocyte quality in the adult.

4.4 NUTRITION *IN UTERO* AND SUBSEQUENT PERFORMANCE

Under-nutrition during late pregnancy can permanently reduce lambing rates of resultant progeny throughout their adult life (Gunn, 1977; Williams, 1984; Gunn et al., 1995). In the study of Gunn et al. (1995), Scottish Blackface ewes were managed to give the following treatments - high nutrition during the last 100 days of pregnancy, high nutrition during the first 100 days of lactation and a low level of nutrition over the same two periods. Lambs produced from ewes that were supplemented during lactation were more fertile than lambs of other treatments and there were more multiple births in lambs produced from the groups supplemented during either pregnancy or lactation. Differences in live-weight in the progeny had disappeared by 18 months of age. The authors concluded that good nutrition during late pregnancy and/or lactation can influence life-time performance of progeny due to a reduction in embryo/fetal loss.

On the other hand, severe growth restriction of Merino lambs after birth (weight of 12 kg at 420 days) followed by normal grazing management did not influence lifetime reproductive performance (Allden, 1979), even though there was a permanent reduction in adult weight. The nutritional treatments imposed in this study were severe by normal management standards indicating that seasonal fluctuations in nutrition during early life would be unlikely to impact on adult performance. In comparison, restricted nutrition for six weeks before lambing until the progeny were 12 months of age, resulted in a reduced reproductive performance during adult life due principally to a reduction in prolificacy (Gunn, 1977). The findings of other studies (Reardon and Lambourne, 1966; Williams, 1984) support this conclusion. Under-nourishment late in pregnancy therefore appears to produce long-term adverse effects in progeny in terms of the recruitment and/or selection of follicles from the growing pool.

4.5 SUMMARY

The important points that arise from this Chapter are:

(1). The fetal ovary remains an untapped source of germ cells despite many 100,000s being lost during fetal development.

(2). The number of germ cells at birth can not be manipulated by changes in maternal nutrition during pregnancy.

(3). On the other hand, the quality of oocytes obtained from new born lambs is improved by high dietary intakes during specific stages of pregnancy. There is a likelihood that this advantage persists into adulthood.

(4). With the use of *in vitro* technologies, the lamb is a valuable source of oocytes for immediate breeding purposes. High embryo wastage is a constraint to further development of the technology.

(5). Under-nourishment during late fetal development influences adult reproductive performance primarily due to a reduction in prolificacy.

CHAPTER 5. NUTRITION – THE MASTER REGULATOR OF REPRODUCTIVE PERFORMANCE?

5.1 INTRODUCTION

The relationship between nutrition and reproduction is complex and variable despite the absolute energy requirements to support follicle growth, ovulation and early pregnancy being only approximately 3MJ of metabolisable energy per day (O'Callaghan and Boland, 1999). Body condition, feed intake, season, stage of lactation and pregnancy are each often quoted as part-determinants of the animal's reproductive performance (e.g. O'Callaghan and Boland, 1999). Additional interacting factors are likely to be lifetime nutrition of the animal as well as the nutrition of the dam and, in some situations, the grand-dam. Interaction of some of these factors, even at moderate levels, is likely to make the investigation of the relationship between nutrition and reproduction very difficult. However, the studies reviewed in the previous Chapters indicate that there are several distinct phases in the reproductive cycle where nutrition has an overriding influence on reproductive outcome. A major dilemma is that each phase has unique nutritional requirements – some phases require a high dietary plane for optimal performance while others require a much lower plane. This dilemma makes flock management for improved reproductive performance difficult given the need to modify diets at specific stages. The dilemma also helps to explain many of the inconsistencies observed between studies on the effects of nutrition on reproductive performance – similar nutritional treatments are often applied across more than one phase thus making interpretation of data difficult.

The aim of this Chapter is to summarise the preferred nutritional requirements for each phase of the cycle as a basis for the development of the differential feeding strategy discussed in Chapter 8. This Chapter also discusses the roles of energy and protein as well as some individual nutrients in determining the animal's response to dietary treatments.

5.2 PHASES OF THE REPRODUCTIVE CYCLE THAT ARE RESPONSIVE TO NUTRITION

From the research reviewed, it is concluded that there are six phases of the reproductive cycle that respond specifically to nutrition. These phases are:

(1). *Fetal development* – This phase is from the time of differentiation of the fetal ovary until delivery of the lamb. High nutrition ensures that the germ cells complete meiosis on time and that the oocytes in the resulting offspring are of high quality. Low nutrition delays the onset of meiosis and reduces oocyte quality. High nutrition during the last trimester of pregnancy also increases the likelihood of the offspring reproducing normally as adults. Reduced nutrition in this period can adversely affect prolificacy in adult offspring.

(2). *Pre-antral follicle development* – This phase is from approximately six months to six weeks before ovulation and is the period characterised by follicle recruitment. Very little is known of the effects of nutrition during this phase. However, it is the time when many aspects of oocyte quality are determined (e.g. production of mRNAs for use in the pre-implantation embryo). Given the duration of the phase (up to 4½ months), it is unlikely that uniform nutrition (either high or low) throughout the period is the preferred option. The early part of this phase corresponds with the time of lactation and a high diet leads to improved fertility in offspring that are suckling at this time. It is postulated that later in this period, more follicles are recruited from the primordial pool in response to moderately low nutrition. Such a response could have evolved to increase the pool of antral follicles so as to maximise the likelihood of downstream reproductive success. There is some evidence that nutritional stress in this period does alter the dynamics of follicle growth. Nottle et al. (1997) studied the effects of restricted nutrition imposed for eight weeks commencing six months before ovulation followed by a period of lupin feeding commencing 10 days before the ovulation of interest. The ovulatory response to lupin feeding was significantly higher in the group that was nutritionally restricted during the pre-antral period compared with unrestricted feeding in the same period. One interpretation of this result is that there were more follicles available for selection in the former group. There is also some evidence that nutritional stress during this period influences subsequent lambing performance (McInnes and Smith, 1966). In this study, ewes exposed to low dietary conditions in the 6 – 12 week period after weaning and then subsequently exposed to high dietary conditions had an improved lambing and twinning performance compared with ewes that were initially exposed to high dietary conditions. Collectively, these results indicate that the nutritional status during the pre-antral period can be manipulated to improve subsequent ovulatory and lambing performance. Results of other studies where nutritional treatments were imposed during the pre-antral period but which continued into the antral period (e.g. Killeen, 1967; Fletcher, 1971; Fletcher, 1981) are not particularly useful in understanding the importance of nutrition during the pre-antral period. It appears that the influences of the antral period mask, at least to some extent, the influences of the pre-antral period.

(3). *Antral follicle development* – This period is from approximately six weeks to five days before ovulation and is the phase characterised by the growth of small to medium antral follicles. In the sheep, high nutrition leads to increases in the number of growing follicles and ultimately in ovulation rate as occurs during flushing. Supplementation with either branched chain amino acids or a high protein diet (e.g. lupins) late in this period (but before the development of the pre-ovulatory follicle) increases ovulation rate, indicating that the supplements influence the selection process. There appears to be no information on whether these short-term treatments influence oocyte quality *per se* but it is hypothesised that high nutrition during this period adversely affects oocyte quality leading to an increase in the incidence of PFMO.

(4). *Development of the pre-ovulatory follicle* – The pre-ovulatory follicle takes approximately five days to grow once selection has occurred. Low nutrition during this five-day period improves oocyte quality whereas high nutrition is detrimental to quality.

(5). *Early embryo development* – Development of the embryo during the first three days of life is largely dependent on metabolites stored previously within the cytoplasm. Much of this storage occurs during development of the pre-antral follicle. Low nutrition from the time of ovulation and during early embryo development improves embryo quality as measured by the rate of blastocyst formation and the number of cells per blastocyst. It is not known to what extent this effect is due to direct effects of dietary components on the developing embryo compared with effects mediated through hormonal/growth factor pathways.

(6). *Pre-implantation period* – The relevant phase is the first two weeks of pregnancy leading up to the time when the embryo produces interferon-*tau* to initiate the implantation process. Low nutrition increases embryo survival rates in association with high concentrations of plasma progesterone.

Generally, high nutrition in the sheep is beneficial during fetal development and antral follicle development but low nutrition improves the quality of the oocyte and embryo as well as improving embryo survival. The nutritional status during the pre-antral follicle period might influence these findings in terms of subsequent follicle growth and oocyte/embryo quality but there are no definitive data. Whilst this possibility remains to be determined, it is clear that nutrition has an overriding but differential influence on the phases of the reproductive cycle. This is a far cry from the sentiments of the 1970s as reflected by Cumming et al. (1975) who concluded that “to achieve the highest lambing percentage, ewes should have maximum live-weight and body condition as governed by economic and management considerations”. There is now a far better appreciation of the role of nutrition in reproduction and further progress is likely to come from an improved understanding of the roles of dietary components and individual nutrients in the reproductive process.

Recommended feeding levels during the reproductive cycle are provided in Chapter 8.

5.3 THE BALANCING ACT BETWEEN DIETARY ENERGY AND PROTEIN

One of the confounding factors influencing the outcome of dietary supplementation is the balance between the energy and protein content of the diet. Of particular interest is crude protein which is hydrolysed in the rumen to ammonia and captured by rumen microbes as a substrate for protein synthesis. Excess ammonia is transported to the liver where it is metabolised into urea or used in the synthesis of amino acids. Urea is a relatively small molecule and consequently is able to move freely through cell membranes and pervade all parts of the reproductive tract, including the follicle and oviduct. Blood urea concentrations will reflect the nutritional conditions of the growing season – generally high in spring and low in autumn.

Excesses of either dietary urea (McEvoy et al., 1997a) or rumen degradable protein (Blanchard et al., 1990) are associated with reduced fertility and embryo loss. These effects appear to be mediated through the oviduct environment or the follicle (Fahey et al., 1998). An important issue, raised by several authors (O'Callaghan and Boland, 1999; Boland et al., 2001), is the possibility that the adverse effects of high crude protein diets only occur when animals are in an energy deficit and microbial synthetic activity is reduced. Gath et al. (1999), in a study in heifers, found that serum urea concentrations were higher when urea was supplemented with a diet containing half the metabolisable energy for maintenance compared with a diet with double the requirement. In that study, there were no significant differences in pregnancy rate between treatments following embryo transfer but this in not surprising given the understanding that high plasma urea concentrations influence the environments of the follicle and/or oviduct rather than the uterus (O'Callaghan and Boland, 1999).

Studies on the effects of nutrition on ovulation rate, embryo survival and lambing performance conducted during the 1960s and 1970s most often provided insufficient information on the composition of diets. Many studies simply reported responses to diets based on energy maintenance rations (without consideration of protein balance) or strategically grazed animals at stocking rates to induce desired changes in body weight. In the study of Davis et al. (1981), high protein supplements fed to ewes before mating increased the ovulation rate above that of ewes receiving less protein but similar energy intake, without increasing live-weight. On the other hand, at similar levels of protein intake, ewes that received high energy supplements for the same period had a higher ovulation rate and higher mean live-weight than those ewes that received less energy. These authors concluded that protein and energy act through separate mechanisms to affect ovulation rate. It is evident that much remains to be learnt about the roles of protein and energy in regulating reproductive performance and, in particular, their influences on ovum and embryo quality.

5.4 STEROIDS AND METABOLIC SIGNALLING AGENTS

5.4.1 Progesterone

As indicated in Chapter 2.4.2, there is a strong inverse correlation between dietary intake and plasma progesterone concentrations (Cumming et al., 1971; Parr et al., 1987; McEvoy et al., 1995). High plasma concentrations are associated with improved rates of embryo survival. Furthermore, high plasma concentrations are reflected in concentrations in the fluid from large follicles (O'Callaghan et al., 1998). Given the role of progesterone in oocyte maturation and early embryo development, it is not surprising that feeding levels experienced by ewes influence oocyte and embryo quality. These effects are in addition to the role that progesterone plays in increasing embryo/fetal survival following dietary restrictions.

5.4.2 Gonadotrophins

Changes in the secretion and circulating levels of FSH and LH could mediate the influence of nutrition on ovulation rate, given that both are essential for follicle growth. However, the effects of nutrition on FSH and LH levels during the cycle are equivocal (Smith et al., 1990) and data on the effects of live-weight on FSH levels are conflicting. Mackey et al. (1997) reported that short-term reductions in the energy content (0.4M) increased plasma FSH concentration in heifers compared with a 2M diet. In sheep, higher plasma FSH concentrations following lupin supplementation have been reported (Brien et al., 1976) although the responses can be variable (Davis et al., 1981). While long-term nutrition restrictions induce anoestrus due to reduced circulatory levels of LH (Rhodes et al., 1996), short-term restrictions (e.g. three weeks) do not influence LH secretion (Abecia et al., 1995) or, at best, produce relatively small reductions in LH pulse frequency (Rhind et al., 1989b).

5.4.3 Glucose, insulin, growth hormones and IGFs

Downing et al. (1995b) reported that the effect of nutrition on ovulation rate in sheep is mediated through an insulin regulated uptake of glucose, a finding supported by the studies of Williams et al. (1997) and Rodriguez Iglesias et al. (1996). The role of IGFs in the interactions between nutrition and reproduction has been reviewed by Monget and Martin (1997). Essentially, the IGF-I concentration is affected by both plane of nutrition and growth hormone levels in circulation and both plane of nutrition (Gutierrez et al., 1997) and GH treatment (Gong et al., 1993) increase the rate of development of small follicles. Furthermore, IGF-I has been shown to increase the sensitivity of granulosa cells to the stimulatory effects of FSH and ewes on a maintenance diet have higher IGF-I concentrations in follicular fluid than ewes on a 0.5M or 2M diet (O'Callaghan et al., 1998). O'Callaghan and Boland (1999) concluded that the insulin – GH – IGF-I axis is at least partly responsible for dietary induced changes in gonadotrophin secretion with downstream changes in follicle growth and ovulation rate.

5.4.4 Branched chain amino acids (BCAA)

As indicated in Chapter 2.3.3, studies into the responses of ewes to protein and energy supplementation produced a correlation between ovulation rate and plasma levels of BCAA. This has led Smith (1991) to speculate that BCAA might be the physiological parameter that (1) is associated with ewe live-weight and condition, (2) is able to respond to changes in both protein and energy intake in the short term and (3) is able to mediate changes in follicle growth. Further studies into the role of BCAA in regulating follicle growth and ovum quality are justified.

5.4.5 Leptin

Evidence indicates that leptin acts as an appetite transducer and as a satiety factor. There is evidence in the mouse that leptin increases the number of follicles (Barash et al., 1996) and may also have a local effect within the ovary to regulate size and possibly oocyte quality. Spicer and Francisco (1997) found that leptin can attenuate insulin-induced steroidogenesis of bovine granulosa cells thus providing evidence that it can act as a metabolic signal in the reproductive system. Thus, leptin may act as a major modulator of reproduction by regulating appetite and food intake and by having direct effects on the reproductive axis (O'Callaghan and Boland, 1999).

5.5 SUMMARY

The important points from this Chapter are:

(1). Nutrition is the master regulator of reproductive performance by virtue of exerting effects through six phases of the reproductive life-cycle.

(2). These phases correspond with the development of the fetal ovary, the pre-antral follicle, the antral follicle, the pre-ovulatory follicle, the early embryo as well as the pre-implantation period.

(3). There are differential nutritional requirements during these phases with high nutrition improving fetal ovarian development and the number of growing follicles in the adult while lower nutrition improves the quality of the oocyte and embryo as well as improving implantation rates.

(4). The nutritional requirements during pre-antral follicle development are not known but it is hypothesised that nutrition in this period sets the nutritional requirements for subsequent development. Relevant research needs to be conducted on this topic.

(5). The feeding of high protein diets can result in the production of excess ammonia/urea which can pervade all components of the reproductive tract and which are embryo toxic. High protein diets must be balanced with energy content.

(6). It is speculated that high embryo wastage/partial failure of multiple ovulations following high dietary intake is due, partly or wholly, to the production of excess ammonia/urea.

(7). The physiological means whereby nutrition influences reproduction is not known but the insulin-GH-IGF1 axis is strongly implicated.

(8). The role of BCAA as a physiological link between nutrition and ovulation rate requires further investigation particularly in relation to the role that BCAA might play in determining ovum quality.

CHAPTER 6. OTHER ENVIRONMENTAL DETERMINANTS OF REPRODUCTIVE PERFORMANCE

6.1 INTRODUCTION

Environmental factors, other than nutrition, can impact significantly on the reproductive performance of the female. These factors are most often site-specific or regional in nature but never-the-less can cause serious problems and reduce the overall performance of the national breeding flock. This Chapter addresses three environmental factors of relevance - two are known to significantly influence reproductive performance (heat stress and consumption of phyto-oestrogens) while the third (toxins associated with animal, pasture and crop management) has the potential to become a serious problem.

6.2 HEAT STRESS

The majority of sheep flocks in Australia are joined between spring – autumn inclusively during which time periods of heat stress can be expected. Heat stress is known to adversely affect male fertility but it can also influence some parameters of female performance.

6.2.1 Incidence of oestrus

Sawyer et al. (1979) indicated that high temperatures can cause disruption to mating activity by blocking behavioural oestrus, reducing the duration of oestrus or by increasing the length of the oestrous cycle. The severity depends on the length of exposure and the stage of the cycle when exposure occurs.

Lindsay et al. (1975) found no significant relationship between mean maximum temperature and the incidence of oestrus for Merino flocks in Western Australia. In South Australia, Kleemann and Walker (1992) observed a significant decline in oestrous activity as mean maximum temperature increased. Moule (1966) also reported an association between the percentage of ewes conceiving to their first oestrus and ambient temperature prior to joining

6.2.2 Embryo mortality

Lindsay et al. (1975) obtained a negative correlation between ambient temperature and lambing performance but only when high temperature coincided with the time of mating or for a few weeks thereafter. These authors concluded that the reductions in lambing performance were due to increases in embryo mortality. Dutt (1963, 1964) and Thwaites (1967) concluded that high temperatures early in the joining period reduced the survival rate of embryos. On the other hand, high post-mating temperatures did not consistently influence embryo mortality (Boyd, 1965; Sawyer, 1979a,b). Effects of high temperature on embryo survival may be mediated through the male. Observations in the mouse (Zhu et al., 1999) indicated that short term exposure of males to high temperature significantly decrease the proportion of zygotes that developed to the blastocyst stage without adversely affecting fertilisation rate.

6.2.3 Fetal development

Heat stress can cause reductions in fetal growth rate and size. Bell et al. (1987) hypothesised that this reduction resulted from heat-induced alteration to the growth and development of the placenta. In this study, heat treatments during days 45 - 120 of pregnancy decreased the placental weight in association with decreased fetal weight. McCrabb et al. (1993b) indicated that the reduction in fetal growth is related to the period over which the body temperature remains elevated.

Skeletal size reductions are also associated with heat stress during pregnancy (Alexander, 1968; Hopkins et al., 1980; McCrabb et al., 1993a).

6.2.4 Parturition and lactation

Heat stress does not appear to alter the duration of parturition for ewes with and without shade and fed at different levels of nutrition (Stephenson et al., 1984). Lactation may be reduced by heat stress during pregnancy (Alexander, 1968).

6.2.5 Variation in response between animals

There is variation between sheep in their ability to tolerate high ambient temperatures (Entwistle, 1973). In this study, rams and ewes of high and low rectal temperature status were selected on the basis of their response to high ambient temperature (>36.0^{o}C). Reduced fertilisation rates were observed when the four groups of animals were mated with each other but the effects were due to ram influences rather than to any detrimental effect on the ewe.

Studies on ewes selected on the basis of their tolerance to heat stress (McCrabb et al., 1993b) indicated that the group selected for lower rectal temperature had higher joining (89% vs 63%) and lamb marking rates (82% vs 50%) than those selected for high rectal temperature.

6.2.6 Strategies to minimise the effects of heat

The likelihood of heat stress occurring should be one factor that determines the season of mating with matings in summer being avoided if possible. Other than the provision of adequate water and shade, there is little that can be done in normal flock management to minimise the adverse effects of heat stress. Some of the studies reported above indicate that there is genetic variation in heat tolerance but the extent to which this trait is heritable is not known.

6.3 ENVIRONMENTAL TOXINS

Both oocytes and pre-implantation embryos are exposed to many environmental factors during maturation and development. With oocytes, the nuclear material is arrested in prophase of the first meiotic division and remains in that state from early in fetal development until just before ovulation. In livestock, this period can be several years so the probability of the DNA being exposed, at some stage, to perturbing environmental factors is reasonably high. Furthermore, it is now recognised that the newly formed embryo is particularly sensitive to changes in its environment (see Young et al., 1998). These changes can induce epigenetic alterations to the phenotype with adverse consequences on adult health.

Of particular interest to livestock reproduction are the residues of pesticides, herbicides, fungicides and insecticides. Prolonged exposure to organochlorine pesticides (e.g. DDT, methoxychlor and chlordecone), organophosphate pesticides (parathion, malathion and diazanon), herbicides and insecticides is associated with reproductive abnormalities in several species (see Sharara et al., 1998). These abnormalities are likely to result, at least in part, from the oestrogenic nature of some of these chemicals. In the human, pesticides have been detected in follicular fluid (Trapp et al., 1984) and epidemiological studies indicate an association between occupational exposure and fetal death, abortion and congenital defects (Nurminen, 1995; Pastore et al., 1997; Arbuckle and Sever, 1998; Shaw et al., 1999).

Inadequate investigations have been conducted globally to determine the effects of chemical residues on livestock reproduction. The organochlorines are known to adversely affect bovine oocyte maturation *in vitro* (Alm et al., 1998), embryo transport through the reproductive tract (Cummings and Perreault, 1990) and embryo development in association with reduced rates of implantation (Cummings and Gray, 1989; Sircar and Lahiri, 1989; Seiler et al., 1994; Hall et al., 1997). The polychlorinated biphenyls, persistent environmental pollutants, also influence embryo development including increasing the rate of polyspermy, affecting mRNA polyadenylation and influencing the dynamics of cortical granule exocytosis (Pocar et al., 2001; Gandolfi et al., 2002). Furthermore, treatment of hamsters with the formamidine pesticide chlordimeform just prior to the preovulatory LH surge delayed the ultimate timing of the surge and impeded the time of ovulation

(Goldman et al., 1993). Despite these effects, there appear to be nothing known of the relationship between acute exposure of embryos and oocytes to these chemicals and long-term developmental consequences.

6.4 PHYTO-OESTROGENS

Phyto-oestrogens are found in many legumes including several subterranean clover and lucerne cultivars. The most important oestrogenic compounds are the isoflavones and coumestans, each of which has a chemical structure that facilitates binding with the oestrogen receptor thereby causing detectable effects.

Bennetts et al. (1946) described severe clinical abnormalities in sheep grazed on highly oestrogenic clover pastures. These symptoms, collectively known as clover disease, include severe ewe infertility, prolapsed uteri and dystocia that in turn can cause an increase in lamb mortality. The severe clinical form of the disease is now seldom seen due to the development of cultivars with low isoflavone content and to the management of pastures to reduce clover content. Instead, a subclinical form of the problem exists, manifested either as temporary infertility or permanent infertility. The former occurs when ewes graze green pastures around the time of mating and is associated with a reduction in ovulation rate (Smith et al., 1979) and, most likely, impaired sperm transport. The incidence of this problem is difficult to assess but the likelihood of ewes grazing green pastures at the time of mating in southern Australia is low. Permanent infertility, on the other hand, results from prolonged exposure of ewes to the pasture and is associated with impaired sperm transport (Lightfoot et al., 1967) due to irreversible changes to the histology of the cervix.

Oestrogenic clovers are widespread in the moderate to high rainfall areas of southern Australia as evidenced by their distribution in South Australia (Figure 2). Adams (1995) estimated that approximately four million ewes are affected in south Western Australia resulting in a reduction in ewe fertility of 7 – 10%. Anecdotal evidence in South Australia indicates that lamb marking percentages can, in individual flocks, be depressed by up to 20 – 30%. It is likely that between 10 – 15 million sheep of the national flock are affected to varying degrees by the problem. Importantly, reductions in flock performance can occur without observable clinical signs making detection of the disease difficult (Adams, 1995). The best form of detection is to assess pastures for their content of oestrogenic cultivars or, alternatively, pasture samples can be assayed for their isoflavone content.

6.5 SUMMARY

The important points that arise from this Chapter are:

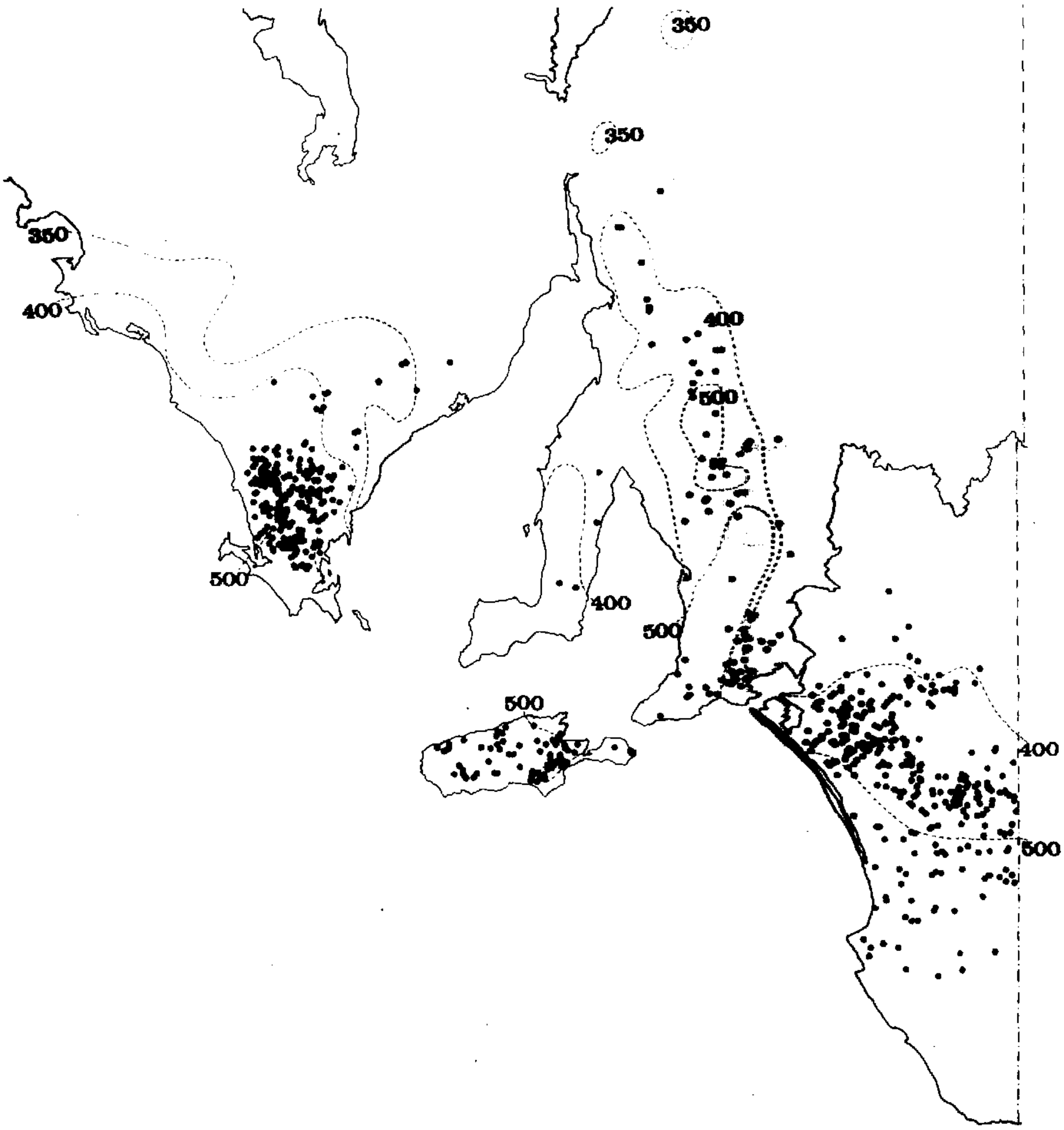
(1). Environmental factors other than nutrition can influence reproductive performance. These factors operate on a local or regional basis but collectively can significantly reduce the reproductive performance of the national flock.

(2). Heat stress can cause abnormal oestrous patterns, elevated levels of embryo mortality and aberrant patterns of fetal growth.

(3). Environmental toxins, resulting from the use of herbicides and pesticides have the potential to seriously reduce reproductive performance. Routine monitoring of soils/pastures for toxic residues would be a valuable step in monitoring this problem.

(4). Consumption of phyto-oestrogens by sheep in the higher rainfall areas can cause a significant reduction in lamb marking percentages. This problem is not easily detectable in flocks but it is estimated that it affects between 10 – 15 million breeding ewes per year.

Figure 2. Distribution in South Australia of oestrogenic cultivars (Dinninup, Dwalganup, Yarloop and Geraldton) of *Trifolium subterranean*. Broken lines are rainfall isohyets; each point represents a randomly sampled property on which at least one oestrogenic cultivar was found [Walker, (1981) adapted from Cocks and Phillips, (1979)]



CHAPTER 7. GENETIC AND EPIGENETIC CONTROL OF REPRODUCTION

7.1 INTRODUCTION

Genetic and epigenetic control of reproduction are both important from a flock management perspective. Several reproductive traits are moderately heritable thus providing the potential for improving flock reproductive performance through quantitative genetic selection. However, logistical constraints, costs and difficulty in measuring the traits of interest detract from this approach. It is also recognised that progress through selection is slow when compared with other management strategies. The significance of egigenetic regulation of reproduction is less obvious and confounded by the fact that the phenomenon is not well understood. However, it is postulated that genomic imprinting (a major form of epigenetic regulation) is responsive to nutritional conditions during the stages of erasure and re-establishment of the imprints. If the postulation is correct, there would be important downstream consequences for both the oocyte and embryo in terms of quality. Furthermore, knowledge of the way in which nutrition influences imprinting would provide a basis whereby dietary intake could be further modified to meet the optimal requirements of the animal.

7.2 GENETIC CONTROL

The breeding objectives for various types of sheep flocks have been reviewed by Ponzoni (1982) who concluded that reproduction is an important trait in the breeding objective irrespective of flock type and changing market trends. However, reproduction rate (the number of lambs weaned or marked per 100 ewes joined) is a complex trait and is a function of component traits, namely fertility, litter size and lamb survival. Each of these component traits, in turn, is determined by other functions. Genetics offer the means of improving reproductive performance by either (1) direct selection depending on the heritability of the trait of interest, (2) culling animals on the basis that the repeatability of the trait is significant or (3) utilising the heterosis that might exist between breeds (cross-breeding).

7.2.1 Estimates of heritability

A summary of estimates is provided in Table 1. There is considerable variation between and within breeds in most functions thus making assessment of genetic variability difficult. Most traits are moderately heritable with ovulation rate being highly heritable in the more prolific breeds of sheep (up to 0.57). However, because of the curvilinear relationship between ovulation rate and litter size, heritability estimates for litter size are substantially reduced.

Heritability estimates for both embryo mortality and lamb survival are both low (0.07 and 0.02 - 0.04 respectively). Given the low heritability for lamb survival and the extended generation interval required to progeny test sires, Piper et al. (1982) concluded that there was little scope for improving lamb survival by direct selection. On the other hand, maternal rearing ability estimates are higher although measurement of this trait can be difficult.

Experiments that have examined the response to selection in the Merino (Atkins, 1980b; Turner, 1978; Mann et al., 1978), Romney (Clarke, 1972) and Galway breeds (Hanrahan, 1984) over 10 - 22 years have given a uniform response in the extra number of lambs born per ewe lambing of 1.5% per year. However, implementation of an effective selection program is not without difficulty. As indicated by Fogarty (1984), selection of rams must be based on information from relatives, testing of rams increases the generation interval, phenotypic expression of many traits is either binomial or at relatively few discrete levels and the collection, recording and analysis of data is expensive. Never-the-less, Fogarty (1984) suggested that there is considerable opportunity for genetic improvement of reproduction rate in sheep.

7.2.2 Estimates of repeatability

Ewe rearing ability has a repeatability of 0.10-0.15 (Fogarty et al., 1982; Piper et al., 1982; Haughey, 1984) indicating that current flock performance can be improved by culling ewes with poor maternal rearing ability. In three flocks analysed by Haughey (1984), ewes that successfully reared lambs at two years of age reared, on average, 2.8 - 14.7% more lambs subsequently than those ewes that failed to rear a lamb at two years of age. Estimates for ovulation rate and litter size are reasonably high – both between cycles (within year) and between years. However, logistical problems in measuring these traits again limit the practical application of this approach.

7.2.3 Variation between breeds

Considerable variation exists between breed in traits such as ovulation rate, fertility at various times of the year, length of the breeding season and lamb survival. Substantial heterosis is expressed for reproduction when cross-breeding is utilized (Nitter, 1978). Heterosis for the various components of reproduction are cumulative and can be in excess of 40% for lambs weaned per ewe joined (McGuirk, 1970; Fogarty, 1984). The development of new breeds by crossbreeding or infusion of new genes into existing breeds may be effective in obtaining desired attributes (e.g. Coopworth, Perendale, Polypay). Of particular interest is the use of breeds carrying genes of major effect. Major genes for ovulation rate have been identified in the Booroola Merino, Romney, Javanese, Cambridge, Belcare, Thoka and Olkuska breeds (see Fahmy, 1996). Each copy of the Fec^B gene from the Booroola adds an extra 1.0 to 1.5 ova per ewe and this translates into an increase in litter size of 0.75-1.00 lambs per ewe in breeds of low to high prolificacy (Piper and Bindon, 1987). However, substantial increases in lamb mortality can nullify benefits expected from increases in ovulation rate (Ponzoni et al., 1984).

7.3 EPIGENETIC CONTROL

Development in mammals is regulated by a combination of genetic and epigenetic factors. One form of epigenetic inheritance is genetic imprinting by which maternally and paternally derived alleles are marked during gametogenesis. The principal form of DNA modification in the germline is the degree of methylation of cytosine in the dinucleotide CpG. Mono-allelic expression is not solely determined by the imprint that is established during gametogenesis but requires an additional series of post-fertilisation and even post-implantation events (see Latham, 1995), which includes changes in imprinted gene methylation patterns (Stöger et al., 1993; Brandeis et al., 1993). In addition to genetic imprinting, there are other forms of epigenetic modification that affect gene expression in the embryo and adult including modifier loci and constituents of the egg cytoplasm that can be specific for maternally and paternally transmitted genes (see Latham, 1995).

7.3.1 Imprinting and reproduction

The imprinting process appears to be highly susceptible to a variety of environmental stimuli with significant implications for its role in congenital growth disorders (Moore, 2001). A schematic outline of how phenotype is the product of genetic and epigenetic influences and the way in which each might be modified by environmental factors is presented in Figure 3.

Disruption to imprinting in livestock has been linked to fetal overgrowth in the sheep and cow (see Young et al., 1998). This is not surprising given the striking number of imprinted genes that act in the fetal growth pathway (IGF2, IGF2r, Grb 10, H19) and in placental development and function (IGF2, IGF2r, H19, PEG3, INS, P57^{kip2}, Gtl 2 and Dlk) (Tilghman, 1999; Tycko and Morrison, 2002). Of interest is the report of Cross et al. (1995) that over expression of a basic helix-loop-helix (bHLH) transcription factor (Hxt) in mouse blastomeres directs their development into trophoblast cells in the blastocyst. As reported elsewhere in this review, nutritional insufficiency in the peri-conceptual period in the superovulated ewe increases the number and proportion of trophectoderm cells compared with inner cell mass cells (Kakar et al., 2002). It is feasible that this consequence is generated through Hxt expression and, if so, would provide a link between nutritional status, epigenetic modification and embryo development.

7.3.2 Imprinting and oocyte/embryo quality

A possible role of imprinting in determining ovum quality and early embryo development cannot be ruled out. Imprints are erased in primordial germ cells and then re-established during gamete development (Chaillet, 1994). During pre-antral follicle development, cytoplasmic signals (in the form of ribosomal and mRNA) are stored within the oocyte. Whilst there is no evidence that nutrition influences these events, it is postulated that ovum quality is, at least partly, a function of its imprinting status and that this characteristic is influenced by the prevailing nutritional conditions during the times of erasure and re-establishment of the imprints. This could include any time period between the development of the primordial germ cells in the fetus and adulthood (Chaillet, 1994).

7.4 SUMMARY

The important points that arise from this Chapter are:

(1). Some traits of economic importance are moderately heritable and selection programs have produced annual responses of up to 1.5% in the number of lambs born per year.

(2). Selection for traits of reproduction is not widely practised due, in part, to logistical problems in measuring the desired traits.

(3). Repeatability estimates are sufficient to facilitate flock improvement in some traits.

(4). Gains in flock improvement to be obtained through selection of desired animals are best realised under conditions of optimal flock management.

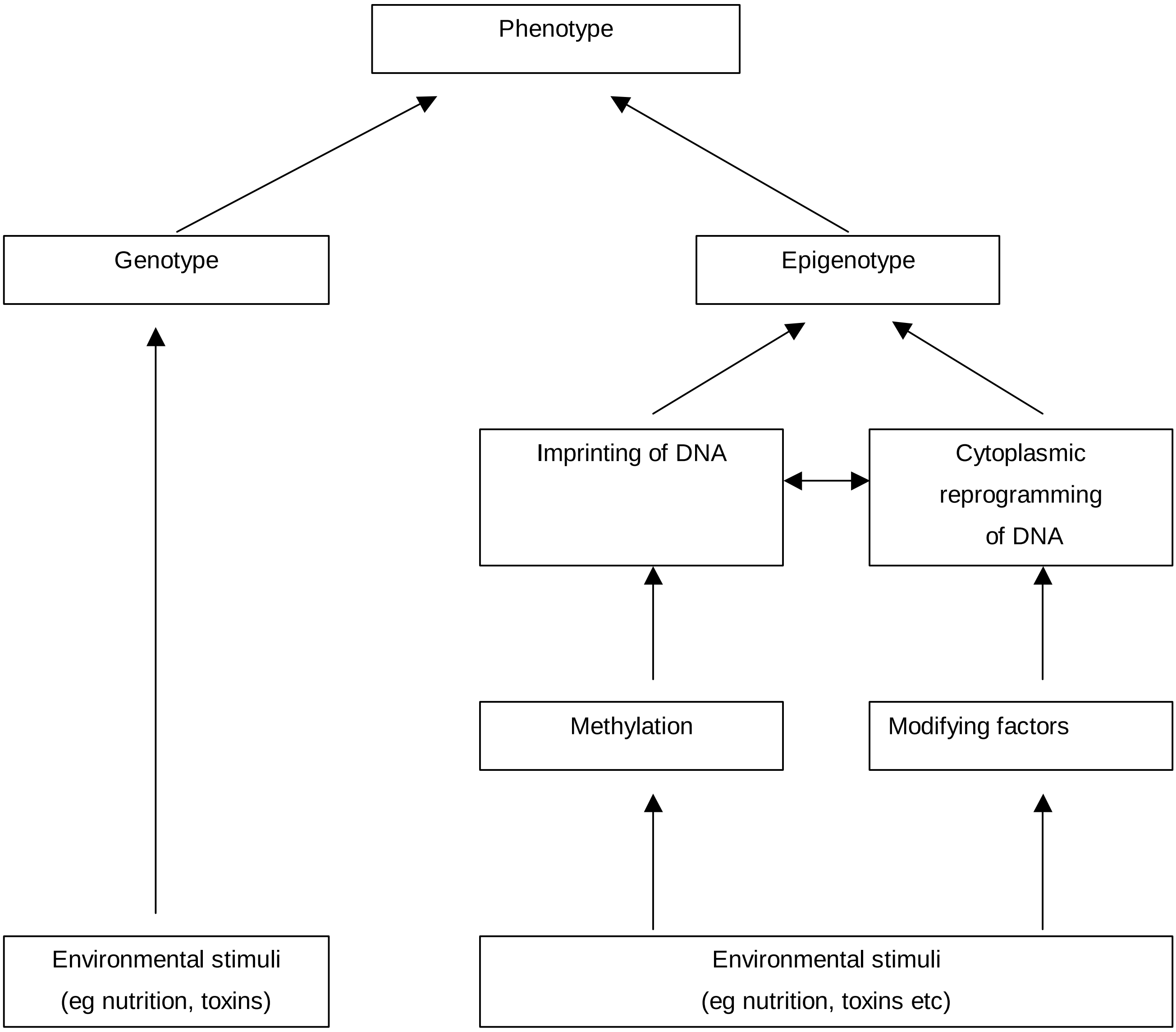
(5). Epigenetics and, in particular, imprinting patterns might influence oocyte and embryo quality.

(6). It is postulated that the imprinting process is influenced by nutrition and, if so, would provide an additional link between nutrition and reproductive performance.

Table 1. Estimates of heritability and repeatability for traits of reproduction in Merino and other breeds of sheep.

Trait	Heritability	Repeatability	Reference
Puberty	0.1 - 0.26	_	Fogarty, 1981,1984; Tierney, 1979; Piper, 1982
No. cycles in first season	0.3	_	Chang and Rae, 1970; Baker et al. 1979
Fertility	0.03 - 0.1	0.08 – 0.09	Fogarty, 1995 Bindon and Piper,1976
Onset of breeding season	0.17 - 0.25	_	Hanrahan and Quirke, 1986; Fahmy, 1990
Ovulation rate	0.05 - 0.57 0.21 (weighted)	0.69 (within year) 0.24 (between year)	Hanrahan, 1980; Piper et al. 1980; Fogarty, 1984,1994; Bindon and Piper,1976
Embryo mortality	0.07	_	Elsen et al. 1994
Litter size	0.04 - 0.26	0.04 - 0.28	Fogarty, 1995; Bindon and Piper, 1976
Lamb survival	0.02 - 0.04		Cundiff et al. 1982; Bindon and Piper, 1976
Rearing ability	0.1	0.1 - 0.15	Piper et al. 1982; Fogarty et al. 1982; Haughey, 1984

Figure 3. Phenotype is the product of genetic and epigenetic factors each of which can be influenced by environmental stimuli.



CHAPTER 8. IMPACT OF REVIEW FINDINGS ON FLOCK REPRODUCTION AND CONTROLLED BREEDING PROGRAMS

8.1 OVERVIEW

Information reviewed indicates that a major cause of reproductive wastage is loss of ova, irrespective of region and breed. Loss of ova is due principally to embryo mortality, a significant proportion of which results from partial failure of multiple ovulations (PFMO). In addition to this major source of wastage, flocks in regions can have their unique problems including varying depths of seasonal anoestrus, variable responses to the ram effect, low ovulation rates and reduced fertility due to the consumption of oestrogenic clovers.

Improving reproductive performance necessitates reducing the impact of one or more of these factors. The greatest gains would come from increases in ovulation rate and/or decreases in embryo mortality. The best management tool to obtain these gains is nutritional management but the dilemma is that the optimal nutritional conditions for increasing ovulation rate actually lead to decreases in embryo survival. Similarly, there are other phases of the reproductive cycle when either high or low dietary intake is desirable. These nutritional requirements have led to the development of a **differential nutrition strategy** that aims to provide preferred level of nutrition during each of the six phases of the cycle (see Chapter 5.2) that respond or are likely to respond to nutrition. Implementation of this strategy largely removes the emphasis on ovulation rate as the key to improving reproductive performance – rather, emphasis in placed on ovum and embryo quality, particularly in those ewes with multiple ovulations.

8.2 THE DIFFERENTIAL NUTRITION STRATEGY

The following provides the recommended feeding levels during the reproductive cycle. These recommendations are deduced from data summarised in Appendix Tables 1 – 9 and, in some cases, these deductions are based on limited information:

(a). Low nutrition during the first two weeks of pregnancy to improve embryo survival rates due to an increase in plasma progesterone concentrations. There is a linear relationship between feed intake and progesterone concentration and it is recommended that ewes be fed a 0.9 – 1.0M diet.

(b). After the first two-weeks of pregnancy, the diet is gradually increased to 1.3 – 1.5M by mid-pregnancy. This diet ensures timely commencement of meiosis in the fetal ovary, proper development of the fetus and improved oocyte quality in resultant offspring.

(c). Nutrition should remain high (e.g. 1.5M) during the first half of the pre-antral period given that it most often coincides with lactation. It is postulated that the level of nutrition should be gradually reduced mid-way through the pre-antral period to reach 0.8 –1.0M towards the end of this period. It is hypothesised that the size of the pool of growing follicles is increased and that the quality of oocytes is improved through this strategy.

(d). Nutrition should be gradually increased as the ewe enters the antral phase of follicle development, rising from a 1.0M diet late in the pre-antral period to 1.5M midway through the antral period. Alternatively, a high protein diet can be fed late in this phase.

(e). The level of nutrition should be gradually reduced from the middle of the antral period to reach 1.0M during the five - day period in which the pre-ovulatory follicle develops to the point of ovulation. This maximises the likelihood of ova of high quality being produced with downstream benefits in terms of both embryo quality and a reduction in the incidence of PFMO.

(f). Ewes should continue to be exposed to a low nutrition level (e.g. 0.7M) during the first three - five days of embryo development to improve embryo quality. This period of low nutrition is then extended to cover the first two weeks of pregnancy as outlined in (a) above.

High dietary intake can have two known adverse physiological consequences. It reduces peripheral plasma progesterone levels and increases the production of ammonia/urea and each can affect reproductive efficiency. It is therefore important that high feed intake be used wisely in normal flock management. It is equally important that changes in the diet occur gradually and this requirement is encompassed within this strategy. Diets need to be balanced in terms of energy and protein content although future research might ultimately demonstrate the need for “unbalanced” diets within particular phases. A major deficiency in this strategy is the lack of knowledge of the effects of nutrition during the period in which pre-antral follicles develop. As reviewed in Chapter 5.2, the little evidence that is available indicates that the nutritional conditions in this period can influence downstream development of follicles and, presumably, oocyte quality.

8.3 MANAGEMENT OF A FLOCK MATING

The following is a list of points, deduced from this review, that are relevant to the management of a flock mating:

(a). Application of the differential nutrition strategy will offer opportunities to increase ovulation rate, reduce embryo mortality and increase implantation rates. It is recognised that application of the strategy will be difficult in a flock mating situation where there is no synchrony of ovulation.

(b). Successful application of the strategy is more likely when matings occur in summer or autumn when it is easier to regulate flock nutrition than in spring when paddock nutrition is generally high.

(c). Reproductive performance of any one animal in any year will reflect the life-time cumulative benefits provided by the differential nutrition strategy given that the strategy is ideally implemented from the time of embryo development and continues throughout the lifetime of the animal.

(d). Overweight animals, particularly those on a rising plane of nutrition, are reproductively inefficient.

(e). A better awareness of the seasonality of the flock will enable maximum flexibility in situations such as drought where early lambing might be desirable.

(f). A better understanding of the ram effect will minimise risks associated with ewes re-entering anoestrus during the mating period.

(g). Pasture assessment in high rainfall areas for the presence of oestrogenic clovers is strongly recommended.

8.4 MANAGEMENT OF A CONTROLLED MATING (E.G. AI, MOET)

The following points are relevant to a controlled mating:

(a). The differential nutrition strategy offers substantial benefits in both AI and embryo transfer programs in terms of improving ovulation rate (in donor animals), improving quality of ova/embryos and maximising embryo survival rate.

(b). Implementation of a controlled breeding program needs long term planning that should extend to the previous lactation.

(c). The ability to implement the strategy in controlled breeding programs is reasonably easy because of the control over the time of ovulation.

(d). Anecdotal evidence indicates that ewes on a rising plane of nutrition but coming from a low base (e.g. store to forward-store condition) are the best performers in both AI and embryo transfer programs. This anecdotal evidence supports the notion that ewes exiting the pre-antral follicle phase ought be on a low plane of nutrition while those in the antral phase ought be on a rising plane.

(e). Overweight animals are poor performers in controlled breeding programs.

(f). When transferring two embryos per ewe in embryo transfer programs, the transfers should be bilateral (one embryo per horn) rather than unilateral (two embryos to one horn) when the opportunity arises.

8.5 FUTURE RESEARCH

The major research issues identified in this review are:

(a). *Nutrition during the development of the pre-antral follicle* – As indicated many times in this review, the lack of knowledge in this period is a major omission in the understanding of the relationship between nutrition and reproductive performance. It is an important period of follicle development and the quality of the oocyte/follicle that exits this period is likely to impact on subsequent developmental potential. This period includes a period of lactation following the previous lambing. It would be logical that a high dietary intake occur during lactation but it is postulated that a low diet thereafter (in the second half of the period) will stimulate more pre-antral follicles to develop into antral follicles. Such a mechanism would provide an adaptive advantage in maximising the likelihood of reproduction occurring in conditions of moderate but not severe nutritional stress.

(b). *Partial failure of multiple ovulations (PFMO)* – PFMO is a major source of ova wastage and, because it increases disproportionately with ovulation rate, can minimise any benefit derived from increases in the latter. It is postulated that PFMO results primarily from one or more of the following - (1) aberrant embryo migration following twin ovulations, (2) reduced oocyte quality as a consequence of intra-ovarian factors produced by the presence of multiple dominant follicles, (3) aberrant imprinting patterns as a consequence of inappropriate nutrition possibly during pre-antral follicle development and (4) excessive ammonia/urea production as a result of high dietary intakes associated with multiple ovulations. There are data from embryo transfer studies to support the first possibility (see Chapter 1.6). The remaining possibilities all relate to the issue of ovum/embryo quality and a better understanding of the relevant determinants is desirable.

(c). *Energy and protein components of the diet* – It is likely that energy and protein requirements for the six phases of the reproductive cycle differ. What are these requirements and how do they differ between the phases? In addition, the role of branched chain amino acids (BCAA) in regulating follicle growth needs to be further researched. It is feasible that a simple administration of BCAA (or any other relevant dietary factor) at relevant stages/phases could provide substantial benefits in terms of oocyte/embryo quality.

(d). *Dietary determinants of embryo quality* – The ability to significantly increase cell number in the trophectoderm of the blastocyst by simple nutritional manipulation at the time of ovulation raises the question of whether such a response is an adaptive mechanism that increases the likelihood of implantation. Research needs to examine (i) what effect nutritional manipulation of this nature has on implantation rates given the changes in embryo quality, (ii) if there is a positive effect on implantation rate, what are the molecular mechanisms involved in redirecting cell allocation in the embryo and (iii) what are the long terms effects on the health and welfare of resultant offspring following nutritional manipulation.

(e). *Demonstration of the benefits of the differential nutrition strategy* – A demonstration of the practical benefits of manipulating nutrition during the different phases is required. This would be a long-term study in which the performances of nutritionally treated ewes are studied over several years.

Appendix Table 1. Experimental data available on feeding levels during the pre-antral period of follicle development that influence the ovulatory response.

Reference	Dietary information	Response
Nottle et al. (1997)	H group maintained weight whereas L group lost 10-20%. Differentially grazed for 8 weeks and then combined for 18 weeks after which half of each group received a lupin supplement (500g/hd/d) for 10 days before ovulation.	Ovulatory response to lupins was greater in the L group than in the H group (0.57 vs 0.29 extra ovulations per 100 ewes).
McInnes and Smith (1966)	H diet = grazing oats; L diet = grazing natural pasture (barley and spear grass). Diets applied for 6 weeks after weaning, for an additional 6 weeks and then 3 weeks before and during joining.	Ewe fertility and proportion of ewes with twin births generally higher with the L nutritional treatment post-weaning.
Fletcher (1974)	Ewes fed either H, M or L pasture diets between March-Sept. Ewes strategically grazed – H (continuous), M (3/4 days) and L (1/2 days). OR recorded in Feb. after mean live weights had come together.	L ewes had the lowest OR (1.28) compared with 1.57 (M) and 1.61 (H). An example of where treatments cut across strategic developmental periods.
Cahill et al. (1984)	Diets provided from July-Sept. and ovulation rate measured in Jan-April. Treatments were M, M/2 and M/3. M ration consisted of crushed barley and lucerne (1:1 w/w) and fed 15g/kg for dry ewes and 37.25g/kg for lactating ewes.	Proportion of ewes with multiple ovulations not affected by diet but onset of breeding activity retarded in the restricted groups.

RECOMMENDATION:

There is a paucity of information available and the experimental designs also limit interpretation. Evidence indicates that in non-lactating ewes, a restricted diet during the pre-antral period can improve subsequent reproductive performance. It appears that sheep need to be losing weight for this response to occur indicating that a less than a maintenance diet is preferred. It is recommended that a 0.8 – 0.9M diet be provided during the pre-antral phase of follicular development.

Appendix Table 2. Experimental data available on feeding levels during the antral period of follicle development that influence the ovulatory response.

Reference	Dietary information	Response
Rattray et al. (1978)	In a 50d pasture trial, ewes offered pasture allowances of 2-10kg DM/hd/d; live-weights changed by –39 to 110g/hd/d.	OR increased from 1.2 to 1.8 and levelled off at approx. 6kg DM/hd/d.
Rattray et al. (1980a)	Heavy and light ewes offered 1.2, 2.5 and 3.9kg DM/hd/d of rye grass/clover pasture 6 weeks before a synchronised mating.	OR levelled off at mid-allowance in heavy ewes (from 1.5 to 1.94 for Coopworth; 1.71 to 1.98 for Perendale) but continued to increase in light ewes (1.22 to 1.82 for Coopworth and 1.29 to 2.08 for Perendale).
Smith et al. (1983)	H (5.0kg DM/hd/d) and L (1.0kg DM/hd/d) diets were fed to 8 groups for varying periods during the 6-week pre-mating period.	H diet gave a 25% increase in OR when offered for 3 weeks at any time in the 6-week period. An additional increase of 30% occurred when offered for all 6 weeks but proportion of multiple births was not increased.
Smith (1985)	A 4 x 4 expt., in which ewes were fed 4 levels of crude protein (12, 15, 18 and 22%) at 4 energy levels (0.5, 1.0, 1.5 and 2.0kg pellets/hd/d containing 11 MJ ME/kg DM). Feeding occurred for 19d.	Ewes with multiple ovulations increased by 1.5% for each extra MJ. The response to increasing protein was discontinuous with preferred level of >125g/hd/d.
Davis et al. (1981)	Expt 1 – ewes fed iso-energy supplements of 500g/hd/d of either peas, lupins, soybean or lucerne/barley pellets for approx. 34d. Expt 2 – ewes fed either iso-protein supplements of peas or lupins or casein for either 17 or 34d.	Expt 1 – ewes fed either lupins or soybean pellets had higher OR. Expt 2 – no difference at 17d but by 34d ewes fed peas had highest OR. Obtained an OR response at moderate energy levels (11.1 MJ ME/hd/d) but not at low levels (6.25 MJ ME/hd/d).
Brien et al. (1977)	Ewes grazed irrigated pasture of perennial rye and white clover or fed hay *ad lib*. Half the ewes received 500g/hd/d lupins from 14d before until 12d after joining.	OR increased significantly in ewes that received lupins but embryo survival was significantly reduced.

Reference	Dietary information	Response
Teleni et al. (1989)	Treatments applied 9d before ovulation and included lupins (750g/hd/d), infusion of glucose (525mM/hd/d), acetate (1122mM/hd/d), glucose and acetate (same conc.), treated casein (100g/hd/d) and lasalocid (150mg/kg feed).	OR increases similar with lupin and glucose + acetate treatments (24-29%).
Knight et al. (1975c)	Several experiments conducted involving the feeding of 0.23-0.45kg/hd/d lupins just prior to and/or during joining.	OR increased by 8-25 ovulations per 100 ewes.
Fletcher (1981)	Five rations based on hay+oats, hay+lupins and hay+peas to give approx. 4 and 8 MJ ME/hd/d and approx. 35-150g/hd/d of crude protein.	OR responses to increased protein intake were only obtained at low energy levels (4 MJ ME/hd/d).
Yaakub et al. (1997a)	Diets of grass meal (ME=12.4 MJ/kg DM; CF=19.3%; CP=16.9%) fed for 28d to provide 0.5, 1.0 and 2.0M. Mean daily intakes were 0.47, 0.94 and 1.91kg DM/d.	Follicle numbers were counted before the LH surge and increased from a mean of 2.4 to 3.5 in non-superovulated ewes and from 7.5 to 12.3 in superovulated ewes.
O'Callaghan et al. (2000)	Ewes fed grass meal diets for 32d before ovulation to provide 0.5, 1.0 and 2.0M. Ewes were unstimulated or superovulated.	Mean follicle numbers in unstimulated ewes were 3.5, 2.4 and 2.4 for 2.0M, 1.0M and 0.5M. Respective superovulation figures were 12.3, 12.0 and 7.5

RECOMMENDATION:

An increasing protein intake during the antral phase increases the number of growing follicles. This increase can be obtained with a three-week feeding period but the increase must be stopped one week before ovulation (see Appendix Table 3). It is estimated that the diet needs to be increased to 1.5M. Alternatively, a high protein diet of lupins (or peas) can be fed in the second week before mating.

Appendix Table 3. Experimental data available on feeding levels during the immediate pre-ovulatory period of follicle development that influence the ovulatory response.

Reference	Dietary information	Response
Oldham and Lindsay (1984)	750g/hd/d of lupins fed to ewes from the day of ram introduction until 24h after the occurrence of mating.	OR increased with lupin supplementation but only after a minimum feeding period of 6d.
Stewart and Oldham (1986)	Lupin diet of 750g/hd/d fed for -d8 to -d5, -d4 to -d1 and -d8 to -d1 before ovulation.	There was a significant increase in OR when lupins were fed between -d8 and -d5 but not between -d4 and -d1.

RECOMMENDATION:

There is very little information available on the importance of nutrition during this period. Evidence indicates that that the follicle is unable to respond to high protein diets within six days of ovulation. The most common interpretation of this result is that the follicle requires six or more days to respond to changes in nutrition. Another interpretation is that the follicle is developmentally competent six days before ovulation and that additional exposure to high nutrition is detrimental to oocyte quality. It is for this reason that the recommended feeding level during this period is 1.0M. This recommendation is made cognisant of the fact that further research is required.

Appendix Table 4. Experimental data available on feeding levels that influence the partial failure of multiple ovulations (PFMO).

Reference	Dietary information	Response
Cumming (1972a)	Control diet of annual pasture with supplements of hay and barley. Restricted diet of 0.2kg/hd/d good quality pasture hay. Restrictions applied 1-7, 8-14 and 15-21d after mating together with all combinations.	Increasing the period of feed restriction decreased embryo survival in twin ovulating ewes.
Smith et al. (1983)	H (5.0kg DM/hd/d) and L (1.0kg DM/hd/d) diets were fed to 8 groups for varying periods during the 6-week pre-mating period.	Feeding the high diet beyond 3 weeks increased OR by an additional 30% (after an initial 25%) but did not increase the incidence of multiple births.
Edey (1966)	Maintenance and sub-maintenance diets (100g lucerne hay/hd/d) applied between 0-7, 6-13, 13-20d after mating.	20/40 ewes with twin ovulations lost both ova compared with 17/47 for single ovulating ewes in the sub-maintenance groups.
Brien et al. (1977)	Ewes grazed irrigated pasture of perennial rye and white clover or fed hay *ad lib*. Half the ewes received 500g/hd/d lupins from 14d before until 12d after joining.	OR increased significantly in ewes that received lupins but embryo survival was significantly reduced.
Cumming et al. (1975)	Diets of 0.25, 1.0 and 2.0M consisting of hay and a sheep concentrate in a 2:1 ratio. Fed from 2-16d after mating.	Embryo survival was signif. reduced at high feeding level.

RECOMMENDATION:

There is an association between PFMO and ovulation rate – as ovulation rate increases so does the incidence of PFMO. High protein diets that increase ovulation rate compromise oocyte quality and this leads to a disproportionate increases in PFMO. Managing the diet is thus a juggling act – increasing the ovulation rate through high nutrition is desirable but the nutrition level must be scaled back as soon as possible to reduce the incidence of PFMO. To reduce PFMO, the recommendation is to provide a 1.0M diet during the pre-ovulatory phase as well as during the pre-implantation period.

Appendix Table 5. Experimental data available on feeding levels that influence peripheral progesterone concentrations.

Reference	Dietary information	Response
Parr et al. (1987)	Diets of 0.25, 1.0 and 2.0M consisting of pelleted lucerne/barley (50:50) with 11.2 MJ net energy/kg DM. Fed between 2-14d after mating.	Prog. conc. significantly reduced as feed level increased. Concentrations of 3.2, 2.0 and 1.5 ng/ml obtained for low, medium and high groups.
McEvoy et al. (1995)	Complete diet of hay, rolled barley, molasses, fishmeal containing 9 MJ ME and 125g crude protein/kg. Oestrus synchronised.	Linear relationship between prog. conc.(Y) and ME intake (X) expressed by the equation Y = 3.1-0.775X. Over 9d period, mean daily prog. conc. = 4.3 and 3.3 ng/ml for L and H groups.
O'Callaghan et al. (2000)	Diets of 0.5, 1.0 and 2.0M consisting of grass meal. Blood samples taken 10-20d after diets offered. Oestrus synchronised.	Prog. levels significantly higher in 0.5 and 1.0M treatments vs 2.0M when measured on day 10.
Yaakub et al. (1997a)	Diets of grass meal (ME=12.4 MJ/kg DM; CF=19.3%; CP=16.9%) fed for 28d before ovulation to provide 0.5, 1.0 and 2.0M requirements. Mean daily intakes were 0.47, 0.94 and 1.91 kg DM/d.	Prog levels did not differ significantly between 0.5 and 1.0M (0.95 and 0.86 ng/ml) but level significantly lower with 2.0M (0.58 ng/ml).
O'Callaghan et al. (1998)	Diets of grass meal provided 0.5, 1.0 and 2.0M requirements offered for 30 days before treatment for superovulation.	Progesterone levels significantly higher in follicular fluid from 0.5M ewes (24.3 ng/ml) compared with those on 1.0M (18.9 ng/ml) or 2.0M (17.7 ng/ml).

RECOMMENDATION:

Embryo survival and peripheral progesterone concentrations are positively associated. Progesterone levels are significantly increased in sheep on M or less than M diets. It is therefore recommended that ewes receive M or less than M diets after mating and up until approximately day 12 of pregnancy.

Appendix Table 6. Experimental data available on feeding levels that influence embryo quality.

Reference	Dietary information	Response
McEvoy et al. (1995)	Complete diet of hay, rolled barley, molasses, fishmeal containing 9 MJ ME and 125g crude protein/kg. Diets of 0.6 and 2.3M and 0.6 and 2.5 M in two separate studies. Fed from the time of 2nd PG injection or 18d before insemination.	A higher proportion of embryos from ewes on the low diets considered viable when compared with higher diets.
McEvoy et al. (1997b)	Study in **cattle**. Diets of 408 (L) and 816 (H) KJ ME/kg $^{0.75}$ with crude protein levels of 20 (low) and 27 (high) g/MJ ME. Treatment time not specified.	Improved *in vitro* development of oocytes obtained from the L diet compared with H diet.
Kakar et al. (2002)	Diets of 0.5M (L) and 1.5M (H) consisting of a manufactured pellet containing 10.5 MJ ME/kg DM and 19% protein.	Undernutrition from the day of ovulation until 6d later significantly increased blastocyst cell number due to an increase in trophectoderm cells.
Nolan et al. (1998)	Study in **cattle**. Diet of silage and grass to give a predicted intake of 28.6 (H) or 9.6 Mcal/kg ME/d (L)	Significantly higher proportion of L vs H embryos developed into blastocysts after culture and total cell number was also significantly higher (superovulation)

RECOMMENDATION:

Embryo quality is improved when animals graze diets of less than maintenance value before conception. However, critical time periods for nutritional influences have not been identified although, as previously indicated, nutrition during the pre-ovulatory period is likely to be important. Consistent with the recommendation for the pre-ovulatory period, the diet after ovulation should be designed to provide approximately 0.7M during the following three to five days.

Appendix Table 7. Experimental data available on feeding levels that influence embryo mortality.

Reference	Dietary information	Response
El-Sheikh et al. (1955)	H diet consisted of 2lb/hd/d of grain mix consisting of corn, whole oats, bran and linseed meal (5:3:1:1) and alfalfa-brome grass hay. L diet of hay only. Commenced sometime before mating and continued through pregnancy.	Embryo mortality at d40 higher with the H diet.
Foote et al. (1959)	Diets as above. Fed for 7 months from weaning age or for two weeks before mating or from mating to up to d40.	H diet increased embryo mortality to d40 compared with L diet.
Hoxsey et al. (1960)	H diet of range fodder + 1lb/hd/d of a 31% protein supplement. L diet of fodder only. Commenced two weeks before mating with various combinations after mating.	H diet gave a 12.5% pre-natal loss compared with 7.6% for L diet.
Bellows et al. (1963)	Diets the same as Foote et al. (1959) and fed for up to 3 months before mating with different combinations after mating.	H diet associated with increased embryo mortality although there was a sire breed effect.
Parr et al. (1987)	Diets of 0.25, 1.0 and 2.0M consisting of pelleted lucerne/barley (50:50) with 11.2 MJ net energy/kg DM. Fed between 2-14d after mating.	Pregnancy rate with 2.0M significantly reduced compared with other treatments.
Cumming et al. (1975)	Diets of 0.25, 1.0 and 2.0M consisting of hay and a sheep concentrate in a 2:1 ratio. Fed from 2-16d after mating.	Embryo survival was significantly reduced at high feeding level.
Blockey et al. (1974)	Control animals paddock-grazed while other ewes fasted in yards for 3 days at various times during the first two weeks after mating.	Pregnancy rate in twin ovulating ewes increased.

Reference	Dietary information	Response
Braden (1971)	Diet of 1:1 lucerne and wheaten chaff. From time of introduction of rams one group fed to maintain weight (15g/kg/d) while another received 1/3 this ration so as to lose weight. Diets applied during mating and for 3-4 weeks thereafter.	Estimated embryo loss was 2.7% in the undernourished group and 7.9% in the control group.
Brien et al. (1977)	Ewes grazed irrigated pasture of perennial rye/white clover or fed hay *ad lib*. Half the ewes received 500g/hd/d lupins from 14d before until 12d after joining.	Lupin supplementation decreased embryo survival in association with an increase in OR.

RECOMMENDATION:

High nutrition is generally associated with an increase in embryo mortality although the magnitude of this loss is related to the time during which the nutritional plane is elevated. Given that most embryo mortality occurs during the first two weeks of pregnancy, it is recommended that ewes be exposed to a 1.0M diet from five days after ovulation until approximately day 12.

Appendix Table 8. Experimental data available on feeding levels during pregnancy that influence fetal ovarian development

Reference	Dietary information	Response
Borwich et al. (1997)	Diets of 0.5 and 1.5M provided 3.9 and 11.7 MJ ME/d. Treatments applied from time of mating and ovaries examined on d42 or d62.	Under-nutrition from time of mating retarded ovarian development.
Rae et al. (2001)	Diets of 0.5 and 1.0M, consisting of pelleted feed and hay, provided 4.0 and 8.0 MJ ME/d. Treatments applied from time of mating and for different windows during gestation.	Under-nutrition during each of the windows studied delayed ovarian follicular development.
JM Kelly, unpublished	Diets of 0.5 (L) and 1.5M (H), consisting of a manufactured pellet containing 10.5 MJ ME/kg DM and 19% protein, offered during different windows of fetal development.	Oocytes harvested from newborn lambs produced from ewes on the H diet from mid-pregnancy were better able to mature and develop into blastocysts *in vitro* than oocytes from other treatments.

RECOMMENDATION:

It is recommended that the nutrition level be gradually increased from 1.0M approximately 12 days after ovulation to 1.5M by mid - pregnancy. This higher level should apply for the remainder of pregnancy.

Appendix Table 9. Experimental data available on feeding levels during pregnancy that influence the performance of resultant offspring.

Reference	Dietary information	Response
Gunn (1997)	Diets applied during last 6 weeks of pregnancy. L diet consisted of pasture + 6h/d on turnips +200g hay/hd/d; H diet consisted of *ad lib* turnips + 450g concentrate/hd/d. Lambs also exposed to H and L diets to 12 months of age.	L diet resulted in reduced reproductive performance in progeny due to a reduction in prolificacy.
Gunn et al. (1995)	Ewes received a H diet during last 100d pregnancy or during the first 100d lactation. Other ewes received a L diet over this combined period. H diet = 525-670g/hd/d commercial pellet + 525-670g/hd/d hay while maintained on perennial rye and white clover. L diet = hill sward + hay.	Nutrition during late pregnancy and lactation influenced lifetime reprod. performance. This effect was mediated through embryo and fetal loss.
Williams (1984)	H and L diets applied for 8 weeks beginning either 2 weeks before or 6 weeks after lambing. H diet = pasture + 500g lupins/hd/d +*ad lib* cereal grain (high protein, high energy diet). L diet = pasture hay to maintain 35kg live-weight (low protein, low energy diet).	Undernutrition delayed onset of puberty that occurred at a lower weight and ovulation rate was also depressed over the first 3 years of life.

RECOMMENDATION:

Consistent with the recommendation of a 1.5M diet applied from mid-pregnancy to improve fetal ovarian development, it is similarly recommended that a comparable diet be applied for the purposes of maximising the productive and reproductive performance of resultant offspring.

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SECTION 2

NEONATAL MORTALITY AND EARLY GROWTH IN SHEEP

CHAPTER 9. NEONATAL LOSS IN AUSTRALIAN SHEEP FLOCKS

9.1 INTRODUCTION

Mortality of the newborn lamb is recognised as a major source of reproductive wastage in Australian sheep flocks (Kelly and Croker, 1990; Kleemann and Walker, 1992; Kilgour, 1992). In addition, growth rate of the neonate during the following 4 - 6 months is important to lamb-meat growers operating their enterprise on pastures that have a limited growing season. Reproductive performance of the ewe and growth rate of the lambs are two of three key factors, the third being annual feed intake of the ewe and her lambs, driving biological efficiency of meat production (Large, 1970; Kleemann et al., 1984).

Chapters 9 – 12 inclusive describe the extent of ovine neonatal mortality in Australia, list the major causes of lamb loss and discuss factors impinging on the fate of the newborn and its subsequent growth. Strategies for improving survival and growth of the lamb are advanced and areas of research are suggested.

9.2 EXTENT OF NEONATAL LOSS

Large scale studies conducted on commercial Merino flocks in Western Australia (Knight et al., 1975) and South Australia (Kleemann et al., 1990a; Kleemann and Walker, 1992) indicated that reproductive wastage was high with 44% and 42% of potential lambs (ova/eggs) lost between ovulation and weaning, respectively. Neonatal mortality was the major source of loss in South Australia (56% of total wastage). In Western Australia, the number of ewes mating but not lambing was the biggest source of wastage. A further census in Western Australia two decades later (Kelly and Croker, 1990) suggested a substantial increase in ewe fertility had been achieved during that time and that lamb mortality had since become the major source of loss. The aforementioned studies and that of Kilgour (1992) in New South Wales on a large number of commercial Merino flocks suggested overall lamb mortality rates of 20 - 30%, with twin lamb loss between 30 - 40%. These estimates are considered to be more reliable than those produced previously since they are based on the number of fetuses scanned versus counts of dead lambs.

The national economic loss due to ovine neonatal mortality is estimated at $250 million annually.

9.3 CAUSES OF MORTALITY

General causes of lamb losses have been the subject of many studies (see Alexander, 1984) with most using the method of autopsy versus direct observation. Most experiments have observed flocks of low to medium fecundity where dystocia and starvation / mismothering / exposure have been the main causes of neonatal losses. In highly fecund flocks examined by Hinch et al. (1986) and Kleemann et al. (1988), causes of deaths were mainly attributed to the prenatal (anteparturient) and prolonged birth categories.

Alexander (1984) has provided a detailed discussion on the following list of causes; a brief updated summary is presented below.

9.3.1 Pre-natal deaths

Included in the pre-natal category are lambs showing evidence of autolysis, the stage of autolysis varying by 2 - 10 days and mummified fetuses. In low fecundity flocks in Australia, Haughey (1981) suggested pre-natal deaths were insignificant (less than 2%). However in prolific Booroola Merino cross flocks (Hinch et al., 1986; Kleemann et al., 1988) pre-natal loss increased with litter size with 15% of quadruplet lambs dying during this period. Maund et al. (1980) observed similar levels of loss in the highly fecund Cambridge and Finnish Landrace breeds. They indicated that intrauterine competition was a possible contributing factor. Detailed observations of Hinch et al. (1986) showed that pre-natal deaths graded from fetuses with autolysed organs (i.e. lungs, kidneys) through to general autolysis and mummification. The incidence of uterine prolapse also increased with pre-natal loss. Clearly the basis for significant pre-natal deaths, particularly in prolific flocks, requires further study.

9.3.2 Dystocia

Difficult births are an important cause of mortality amongst single born lambs (e.g. Haughey, 1981; George, 1976) and are related to high birthweight (e.g. Duff et al., 1982), small pelvis size (e.g. McSporren and Fielden, 1979; Haughey, 1984) or malpresentation. Interestingly, Hinch et al. (1986) reported that single lambs in two prolific flocks that died of dystocia were lighter than single lambs that survived and suggested that dystocic losses in prolific flocks may be due to malpresentation instead of high birthweight and the proportion of malpresentations increased with increasing litter size (Maund et al., 1980). Central nervous system (CNS) haemorrhage is almost always present in dystocic deaths (e.g. Haughey, 1973 a,b) and is usually associated with hypoxia and acidaemia.

A recent study by Holst et al. (2002) indicated that meningeal lesions and presentation difficulties were associated with 67% of all lamb losses. CNS lesions were not related to birthweight or litter size. They hypothesised that progesterone, stored in adipose tissue of overfat ewes, may have caused a hormonal imbalance at parturition leading to a dysfunctional birth process and resultant neural damage to the lambs.

9.3.3 Prolonged birth

The prolonged birth category was of increasing importance as litter size increased (Hinch et al., 1986; Kleemann et al., 1988). Deaths from prolonged births show CNS lesions associated with anoxia and/or hypoxia but signs of difficult birth are not apparent. Factors such as increased length of parturition, growth retardation and poor thermoregulation may be implicated (Hinch et al., 1986).

9.3.4 Starvation and starvation/CNS

Reasons for failure to suckle include poor maternal bonding, accidental separations, delayed milk supply, litter mate competition (see Alexander, 1984) and udder defects (Jordan et al., 1984). The starvation/CNS category include lambs that survive for a shorter period than those in the starvation category and often have typical hypoxia lesions both spinal and cranial (Hinch et al., 1986). Reduced lamb mobility and an affect on the lamb's ability to suckle are suggested consequences of CNS injury (Haughey, 1973b: Eales and Small, 1980; Hinch et al., 1986). Haughey (1991) reported CNS lesion prevalence of 20 - 57% for this category but included exposure.

9.3.5 Exposure

Deaths from cold exposure can be marked (Obst and Day, 1968) and is associated with a combination of low temperatures, wind and rain or prolonged rain (Alexander 1980). Loss of a large number of lambs under these conditions indicates that exposure is a primary cause of death. Lambs that may have died for other reasons such as starvation, low fat reserves, reduced birthweight, may well have succumbed to cold exposure. In this case, cold exposure is defined as a secondary cause of death (Alexander, 1984). Oedema, as an autopsy criterion, is not reliable as cold injury is not observed in many lambs that succumb to cold soon after birth (Alexander et al., 1980).

Heat exposure can be a particular problem in pastoral Queensland where maximum daily temperatures can be >36C for six months of the year (see Kleemann, 1999). Rose (1972) associated high neonatal mortality rates of autumn born lambs to their summer pregnancy and subsequent low birthweight (2.9 kg) compared with spring born lambs (3.7 kg). Hopkins et al. (1980) reported lamb losses due to hyperthermia when ambient temperatures were 42 – 43^{o}C during the neonatal period. Moule (1954) and Smith (1964) found that lambs, exhausted by heat, were unable to follow their mothers to water and were presumably deserted and predated.

9.3.6 Predation

Large lamb losses on an individual property can occur due to predators such as foxes, dogs, feral pigs, eagles and ravens. (e.g. Smith, 1964; Plant, 1981; Lugton, 1993). In recent years communal baiting programs have been instigated to provide better control of predators on a more regional scale and, although lower lamb losses probably have been achieved, the extent of control is difficult to measure. Anecdotal evidence indicates that in grazier communities, where predator control measures have ceased because owners have left the sheep industry, absence of control measures can impact markedly on adjoining properties. Predation is often a secondary cause of death and may be seen by graziers as the prime cause of loss, particularly when potential predators may be present and a significant number of partially eaten carcases are noted (Dennis, 1969).

9.3.7 Minor causes

Minor causes of lamb deaths include infectious diseases, mineral deficiencies such as selenium and copper, fetal malformations, plant toxins and misadventure (Haughey (1983). Alexander (1984) suggested that these sources of loss can be severe for individual properties but represent less than 8% of mortality in large scale studies.

9.3.8 Summary of causes

It is clear that lamb losses will vary from flock to flock and identification of causes is required to ameliorate the problem. Experience of Alexander (1984) indicates that when losses are high (> 15%) single factors may emerge as the cause (e.g. cold weather; Obst and Day, 1968). When losses are < 15%, one or two factors may emerge as the operative cause in a significant proportion of lambs but defining reasons for loss of remaining lambs will not be clear.

9.4 TIMING OF NEONATAL LOSSES

Most lamb deaths occur during the first few days after parturition (e.g. Hight and Jury, 1970) given that starving lambs exhaust their energy reserves after about three days in warm conditions. However, significant lamb and weaner losses can occur after this neonatal period such as under some of the extreme environmental conditions experienced in the pastoral areas of Queensland (see Kleemann, 1999). An understanding of the events that occur during parturition may provide reasons for high losses observed in the first few days thereafter.

9.5 PROCESS OF PARTURITION

It is during the process of parturition that major physiological adaptations need to occur for the lamb to adjust from fetal to adult pathways; changes to respiratory, nutritional, hormonal and excretory functions must be made for the neonate to survive.

9.5.1 Birth process

Mechanisms controlling initiation of labour and expulsion of the fetus are not well understood (Lye, 1996). Myometrial quiescence during pregnancy is maintained by progesterone and inhibitory uterotonins such as relaxin (Challis and Lye, 1994). The switch from a progesterone to an oestrogen dominated system initiates parturition, and it is the increase in the oestrogen:progesterone ratio that begins mobilisation of two biochemical pathways. The first begins activation of the myometrium and sets up responsiveness to uterotonic stimulants and the second stimulates activated myometrium through production of uterotonic agonists. Correct coordination of these pathways results in an efficient delivery (Lye, 1996).

In sheep, the fetus plays a central role in initiating the process of parturition (Challis et al., 2000; Young, 2001). During mid-pregnancy, adrenocorticotrophic hormone (ACTH) circulates in the fetus giving stimulus to the fetal adrenal. In late pregnancy, there is an increase in ACTH release from the fetal pituitary stimulating large increases in cortisol synthesis from the adrenals which act on a placental enzyme enabling metabolism of progesterone to oestrogen. Placental prostaglandins also override the negative feedbacks at the level of the hypothalamus and adrenals on cortisol, enhancing cortisol synthesis (Thorburn and Liggins, 1994; Challis et al., 2000; Whittle et al., 2001). Hence timing of parturition is not only controlled by increasing levels of fetal cortisol (Challis and Brooks, 1989), but is responsible for maturation of the lungs, gut and kidneys required by the fetus to make the transition to post-uterine life (Liggins, 1994).

Effects of maternal nutrition in early pregnancy on *pre partum* activation of the hypothalamo-pituitary adrenal (HPA) axis has been investigated (Edwards and McMillen, 2002) and results indicated that undernutrition altered development of the HPA axis with possible consequences for events occurring during the neonatal period.

9.5.2 Fetal oxygen and asphyxia

Fetal blood gases and pH are reasonably stable during late pregnancy (Randall, 1978) but blood pH values decline 15 - 30 minutes before birth (Randall, 1992) while fetal pO2 may be lower during uterine contractions. These transient periods of hypoxia are compensated such that respiratory and metabolic acidaemia are not measurable during the last hour before term. Adaptive changes include redistribution of circulation away from body muscle favouring the heart and brain and reduction in body movements (Randall, 1992). Muscle survives on anaerobic metabolism, the lactic acid being released following the onset of respiration (Comline and Silver, 1972).

Asphyxia begins when fetus oxygenation is reduced to the stage where fetal adaptive processes cannot maintain homeostasis (Randall, 1978). During anoxia survival depends on anaerobic metabolism and supply of glucose to the brain so mobilisation of glucose from liver and heart stored glycogen and continuation of blood circulation are critical for viability (Dawes et al., 1959). Both respiratory and metabolic acidosis ensues if asphyxia is prolonged resulting in death. Short periods of asphyxia (4 - 6 minutes) is long enough to cause serious brain damage (Randall, 1978). Factors such as feto-pelvic disproportion, malpresentations and assisted births may not only contribute to asphyxia but trauma leading to CNS and possibly abdominal damage and reduced viability (Haughey, 1991).

9.5.3. Lung development and maturation

Fluid secreted from the alveoli epithelium during late gestation helps distend the lungs during fetal 'breathing' and aids lung growth (Liggins, 1984). Proper functioning of the lungs following the first breath is dependent on phospholipids, called surfactant, being secreted into the alveoli. Lung maturation or surfactant synthesis is correlated with ACTH and cortisol concentrations, with the maturational effect of cortisol mediated, in part, by thyroid hormones (Forhead and Fowden, 2002).

9.5.4 Circulatory and pulmonary changes

First breath is stimulated by marked activation of the sympatho-adrenal system and exposure of the fetus to hypoxia, cooling and tactile stimulus. Circulation is changed by the loss of placental blood supply, respiration reduces pulmonary resistance and pO2 increases. Alteration of pressures in the left and right atria closes the foramen ovale (Randall, 1992).

9.5.5 Energy reserves

Particularly during the second half of gestation excess carbohydrate is stored as glycogen mainly in the liver and skeletal muscle. Deposition of glycogen is mainly under endocrine control. Cortisol is involved in glycogen accumulation in the fetal liver (Barnes et al., 1978), while other hormones such as insulin also play a role (Garssen et al., 1983). Release of glycogen from fetal heart, muscle and liver acts as a defence against asphyxia at birth.

Most body fat of sheep at birth is brown fat (Alexander and Bell, 1975). Brown fat, high in mitochondria, nerve and vascular supply, provides direct heat through increased lipolysis in response to sympathetic stimulation due to cold (Hull and Seagull 1965). Stevens and Alexander (1986) suggested that deposition of fetal fat was controlled by lipolytic effect of fetal GH and lipogenic effects of insulin.

9.5.6 Metabolism

A low ambient temperature after birth requires body temperature of the lamb to be maintained through an increase in metabolic rate. Also an infrequent fat rich diet substitutes a placental carbohydrate rich diet. These radical changes in temperature and nutrient supply require mobilisation of glucose from glycogen reserves and activation of the sympathetic system (Randall, 1992). Glucose release from liver glycogen is directly stimulated by sympathetic activity to supply energy before suckling.

Lower ambient temperatures may stimulate sympathetic vascular control of blood away from surface areas and by increasing metabolic rate (Randall, 1992). Shivering and use of brown-fat for thermogenesis are ways of increasing metabolic rate (Alexander and Bell, 1975).

9.6 FACTORS DETERMINING NEONATAL LOSS

9.6.1 Genetic control

Utilisation of genetic material for improvement of reproductive traits is discussed in Chapter 7. For example, improvement in lamb survival could be achieved through (1) direct or indirect selection, (2) by culling animals on current performance and (3) by crossing breeds to take advantage of heterosis. Genetic control of lamb deaths at parturition may differ between individual animals of the same breed or variation may exist between breeds. The genotype of both the lamb and ewe influence lamb survival though the effect may be small compared with the effect of environmental conditions.

9.6.1.1 Selection within breed or strain

Heritability estimates for lamb survival are generally low (Brash et al., 1994; Snyman et al., 1998; Olivier et al*.,* 1998; Southey et al., 2001; Matika et al*.,* 2002) indicating that selection for the trait would result in slow genetic progress. Estimated heritabilities for direct and maternal effects for lamb survival were determined by Burfening (1993); values ranged from 0.002 to 0.051 (direct effects) and 0.052 to 0.112 (maternal effects) derived from three breeds (Targhee, Rambouillet, Columbia). Atkins (1987) reported additive and maternal genetic effects contributed about equally to variation between Merino bloodlines in lamb survival confirming small genetic components exist for lamb survival and that progress through selection for either direct or maternal components is likely to be slow (Cundiff et al., 1982; Piper, 1982; Fogarty, 1984; Atkins, 1987a).

Selection for rearing ability, a component of lamb survival, has been successful. For example, selection of Merino ewes based on multiple rearing ability indicated that lamb survival was improved mainly through increased survival of multiple born lambs; lambs in the high line took less time from standing to suckling than low line lambs and high line ewes tended to stay longer at the birth site (Cloete and Scholtz, 1998).

Indirect selection criteria have been examined by a number of authors for improvement of lamb survival. Cortisol response and an arena test (approach/avoidance test) were measured in a Merino flock selected for rearing ability and a control flock (Kilgour and Szanter-Coddington, 1997). A similar response was observed for cortisol between flocks, whereas differences were observed between flocks for the arena test; total distance travelled, number of bleats and minimum distance from humans varied. Kilgour (1998) also assessed arena behaviour in rams and ewes from the selected flock and compared them with rams and ewes from the control flock at 6, 12 and 20 months of age. He suggested that rapid genetic progress may be achieved given that measurements could be taken early in life and in animals of both sexes.

Rearing performance in Merino, Dorper and South African Mutton Merino ewes and its association with conjugate diameter and area of the pelvic inlet was tested by Cloete (1994). It was concluded that both measurements were positively related to rearing performance, even though failure to rear lambs may be associated with many other factors.

Heritability of maternal behaviour at 0.13 is low to moderate but indicates some potential for improving lamb survival indirectly (Lambe et al., 2001). Although birth coat was highly heritable in the South Australian Merino it was not associated with lamb survival or with ewe reproductive rate (Ponzoni et al., 1997) and as an indirect selection criteria would be of limited value in a genetic improvement program.

Lee and Atkins (1996) examined the relationship between weaning performance of ewes at the first and second lambing with reproductive performance at later ages. Gains could be made in the current flock by culling on low fertility and rearing ability (component of survival). For example, ewes that were dry at the first two lambings reared half as many lambs at subsequent lambings as ewes that had reared lambs at the initial two lambings. This suggested that selection on fertility and survival may increase net reproductive performance in the short term, as indicated by the earlier observations of Donnelly (1982).

Selection for traits other than lamb survival may or may not be genetically associated positively with lamb survival. Selection for lean growth in the Scottish Blackface was associated with an increase in the speed with which lambs stood for suckling and significantly improved neonatal survival (Dwyer et al., 2001), while selection for lean growth in the Coopworth appeared to reduce lamb survival (McEwan et al., 2001). Fogarty and Gilmour (1998) reported an improvement in both lamb survival and lamb growth when selecting Hyfer ewes based on weight of lamb weaned per ewe joined. Single trait selection for fleece weight or liveweight in Romney sheep resulted in a decrease in lamb survival but an increase in litter size for both selection lines (Morris et al., 1996). The net result was an increase in lambs weaned per ewe lambing in both lines, the response being greater in the liveweight line.

9.6.1.2 Variation between breeds

Lamb meat in Australia is produced from a number of breeds but the industry is structured on Merino ewes surplus to wool growing requirements. The Merino is mainly joined to the Border Leicester to generate first-cross ewes or directly to the Dorset for first-cross lamb-meat production. First-cross ewes are predominantly mated to the Dorset. In the last decade, the terminal sire breed, the Texel and, more recently, breeds from South Africa (Damara, Dorper, South African Meat Merino) have entered Australia.

The success of the Border Leicester x Merino ewe as the main maternal breed for prime lamb production was highlighted by the experiments of McGuirk and Bourke (1978) and McGuirk et al. (1978). Estimates of heterosis for lamb survival and growth were 10% and those for ewe fertility and fecundity were 4 - 5%.

Differences in lamb survival have been reported for the traditional breeds of ewe (e.g. Atkins, 1980; Donnelly, 1984; Kleemann et al., 1991; Fogarty et al*.,* 2000b). Survival of offspring from Border Leicester x Merino ewes (Donnelly, 1984; Fogarty et al., 2000b) and Poll Dorset x Merino ewes (Kleemann et al*.,* 1991) is generally higher than those from Merino ewes. While birthweight may account for some between breed variation there is a significant component due to other factors. Survival of Poll Dorset x Merino and Border Leicester x Merino lambs from Merino ewes is usually higher than that of purebred Merino lambs (Fogarty et al., 1972; Kleemann et al., 1983).

Preliminary observations for the Damara x Merino, Dorper x Merino, Dorset x Merino, Rambouillet x Merino and South African Meat Merino x Merino and purebred Merino lambs indicates superior survival of Damara cross lambs compared with all other genotypes in central western Queensland where survival rate of the purebred Merino can be very low (Kleemann et al., 2000).

Studies of Dwyer and Lawrence (1998) indicated significant effects of breed (Scottish Blackface versus Suffolk) in aspects of both maternal and neonatal behaviour that may impact on viability of the lamb. Blackface ewes had shorter labour, required less assistance at birth, were less likely to abandon a lamb and were less aggressive toward a lamb. They devoted more time to grooming their lambs, were more active and made more suckling attempts. Dwyer and Lawrence (1998) suggested that the high level of selection for growth in the Suffolk may have led to deterioration in maternal ability.

9.6.2 Nutrition

9.6.2.1 Early pregnancy

Most experimental studies examining nutritional effects on fetal growth and neonatal well being over the past decades have concentrated on the last trimester of pregnancy when fetal growth is at a maximum. Recent research, however, has indicated that perturbation of environmental factors (nutritional, hormonal, nuclear transfer) during the first few days of embryonic life can have dramatic effects on feto-placental growth and development (Walker et al., 1992; Kleemann et al., 1994; Walker et al., 1996; Holm et al., 1996; McEvoy et al., 1997; Kleemann et al*.,* 2001; Peura et al., 2003). Aspects of this work and its implications for control of reproductive performance have been discussed in Chapter 3.

9.6.2.2 Mid pregnancy

The effects of nutrition on placental and fetal growth have been reviewed by Kelly (1992), Robinson et al. (1995) and Robinson et al. (1999). A summary of experiments is presented in Table 1 (see Appendix). During pregnancies not exposed to overt nutritional stress, there is generally a close relationship between the size of the placenta and the fetus (Mellor, 1983; Greenwood et al., 2000a). The period of sensitivity of the placenta to nutritional changes appears to be between 40 and 80 days, a time of rapid placental growth (Ehrhardt and Bell, 1995). The response of placental growth to nutritional restriction during this period may be influenced by body condition (Russel et al., 1981; McCrabb et al., 1992) and age or maturity of the ewe. Mature aged ewes in good body condition in early pregnancy (score 3.5 or greater) and exposed to moderate undernutrition during mid-pregnancy (loss of 0.5 – 1.0 body condition units) may increase placental and fetal weight (Faichney and White, 1987; McCrabb et al., 1992). Attempts have been made in field studies to demonstrate that moderate nutritional deprivation during mid-pregnancy may improve birthweight and lamb survival but results have been equivocal and any responses found to be small (Holst et al., 1986; Kleemann et al., 1990b, 1993a,b; Fogarty et al., 1992; Holst and Allan, 1992; Holst et al., 1992). Under-nutrition during early-mid pregnancy of ewes in poor condition (score 2 and less) and young ewes that are still growing to maturity is detrimental to placental growth (see Robinson et al., 1999). Heasman et al. (1999) imposed low nutrition (50% maintenance) during early to mid pregnancy and found that placental size was reduced at day 80 but had recovered by day 145 compared with ewes fed adequately in early gestation. While fetal weight did not differ due to treatment, variations were found in fetal dimensions and insulin-like growth factors 1 (IGF-1) concentrations near term.

Nutritional regulation of the glucose transporter 1 (GLUT-1), the main glucose transporter in the placenta, was examined by Dandrea et al. (2001) by restricting maternal intakes between days 28 and 80 of gestation. Placental mass was lower at day 80 but the abundance of GLUT-1 did not differ between the restricted group and the well-fed ewes. At term, placental mass was greater in the restricted ewes and was associated with an increase in GLUT-1 and a heavier fetus.

Overnutrition in adolescent ewes during early and mid gestation reduced placental and fetal growth (Wallace et al., 1996), partly through a decrease in the number uterine caruncles with which the trophectoderm finally attached (Wallace et al., 1997a). These authors noted that progesterone concentrations in the overnourished adolescent ewe were inversely related to dietary intake and that restoration of progesterone concentrations in the high dietary group to levels in the control medium group partially restored fetal but not placental size, and suggested that nutritional effects on progesterone may have influenced development of the inner cell mass (Wallace, 2000). Components of the IGF system are responsive to nutrition (Thissen *et al.,* 1994) and may play a role in placental growth (Wallace, 2000). Angiogenic growth factors (e.g. Torry and Torry, 1997) may be important regulators of placental vascular development and growth. The impact of maternal nutrition and endocrine status, particularly during early pregnancy (Wallace 2000; Kleemann et al., 2001) on the IGF system and angiogenic factors during mid pregnancy placental growth deserves further examination.

9.6.2.3 Late pregnancy

Most studies investigating nutritional effects on fetal growth have focused on this period (see reviews of Mellor, 1983; Robinson, 1992; Symonds and Clarke, 1996; Robinson et al., 1999) since growth of the fetus is at a maximum. Wallace (1948a) indicated undernutrition reduced both placental and fetal weight. Mellor (1983) noted that size of the placenta in late pregnancy limited growth of the fetus and that degree of response to change in nutrition was regulated by size of the placenta. Extending poor nutrition for more than three weeks reduced fetal growth irreversibly. However, in the study of Oddy and Holst (1991) where severe undernutrition (approximately 0.2

maintenance requirements, reduced body score by 1.0 unit) was imposed from day 95 of pregnancy for 4 weeks, little effect was observed on birthweight. Increased voluntary intake during the last week of gestation and increased glucose uptake associated with elevated placental lactogen concentrations appeared responsible for maintaining fetal growth to term.

McNeill et al. (1998a) examined fetal growth response in lean and fat ewes (body condition score 2.0 vs 2.9) given *ad libitum* access to feed during late pregnancy. Lean ewes ate more but fetal weight was not influenced by maternal fat status. Lean ewes partitioned more of their food nutrients into their own body fat than fat ewes. The authors suggested the placenta, of similar size between the groups of ewes at the beginning of the experiment, places an upper limit on the transfer of nutrients to the fetus. In contrast, twin fetuses were 14% heavier in fat ewes than those from lean ewes (with similar size placentae) when underfed (0.6 of total feed requirement) during late pregnancy, with fat ewes mobilising more body fat sparing glucose for fetal growth (McNeill et al., 1999). The advantage of having moderate fat reserves in ewes throughout pregnancy was indicated by the observations of McNeill et al. (1997a). Lean and fat ewes were fed to maintain energy balance throughout pregnancy. A reduction in maternal insulin sensitivity due to higher maternal fatness, partitioned more glucose to the fetus and though fetal weight was similar between treatments fetuses from fat ewes had greater adipose reserves, an advantage for lambs born in a cold climate.

9.6.2.3.1 Long term and short term restrictions on feed supply

An important maternal adaptation to chronic undernutrition during late pregnancy is a natural rise in fetal cortisol and its further response to continued undernutrition (Fowden et al., 1996) and is involved in the retardation of fetal growth. The glycogenic capacity of the fetus is enhanced by cortisol by increasing gluconeogenic enzymes (Fowden et al., 1993).

Severe restrictions of nutrition over short periods during late pregnancy can prevent the fetus returning fully to normal growth when nutritional restriction is no longer imposed (Mellor and Murray, 1982). If under-nutrition is severe and glucose concentrations decline with increased mobilisation of adipose tissue the metabolic disorder of pregnancy toxaemia may develop (Baird, 1981).

9.6.2.3.2 Protein

Requirement for protein is at a maximum during late pregnancy and it appears that microbial protein cannot supply the amino acid needs of the fetuses, particularly cystine, histidine and arginine (Robinson et al., 1985). A study by McNeill et al. (1997b) quantified the effects of maternal protein nutrition on nitrogen accretion and loss in the maternal tissues and fetus in ewes during late pregnancy. They highlighted the ability of maternal tissue to either deposit or mobilise amino acids when variations in dietary protein were imposed.

A number of authors hypothesised that feeding high protein grain to supply the need for rumen undegraded dietary protein during late pregnancy may improve lamb birthweight and survival (Hall et al., 1992; Kleemann et al., 1993a; Murphy and Lindsay, 1996; Hinch et al., 1996; Nottle et al., 1998). Lack of a response to feeding lupin grain (700g/head/day) during the last 1 - 2 weeks of pregnancy in highly fecund Merino ewes may have resulted from inappetence of ewes that had been well fed prior to introduction of high protein grain (Kleemann et al., 1993b). Hall et al. (1992) gained significant increases of 0.2 kg birthweight and 27% increase in twin lamb survival; this was associated with enhanced immunoglobulin scores and colostrum production. Indeed, Hinch et al. (1996) reported a 0.2 kg increase in birthweight and a 15% improvement in survival of lambs in a high fecundity flock fed small amounts of cottonseed meal during mid (80g/head/day) and late pregnancy (80, 160 and 220g/head/day for single, twin and triplet bearing ewes). Nottle et al. (1998) examined the response to feeding lupin grain (500g/head/day) during the last 2 - 3 weeks of pregnancy in a low-medium fecundity Merino flock with lambing synchronised using the ram effect. Lamb survival increased by 7% and improved growth rate to weaning was reported. The growth rate response observed in the latter study could have been due to an improvement in both colostrum production and subsequent milk synthesis (Murphy et al., 1996). Murphy and Lindsay (1996) hypothesised that a positive response in lamb survival to feeding lupin grain 2 - 4 weeks *pre partum* would be associated with increased lamb vigour; however the experimental data did not support their supposition.

9.6.2.3.3 Overnourishment

Excessive feeding during the third trimester increases birthweight, particularly of single fetuses, but increases the incidence of dystocia (e.g. Wallace, 1948b). Fatness of ewes was suggested as a possible cause of a high incidence of meningeal lesions detected in dead lambs (Holst et al., 2002). Overnourishment may also lead to inappetence and pregnancy toxaemia (Baird, 1981).

9.6.2.3.4 Trace element deficiencies

Deficiencies of copper, iodine and selenium in the diet of sheep is usually associated with certain soil types; lack of these trace elements results in fetuses with swayback, goitre and white muscle disease respectively (Haughey, 1991). Mortality can be high but once the particular deficiency is diagnosed treatments can be employed successfully. Responses in lamb survival to administration of zinc have been observed in Merino flocks grazing on Kangaroo Island (D Kleemann, unpublished).

9.6.2.3.5 Leptin, regulation of intake and metabolic adaptations

Leptin is a hormone produced primarily in white adipose tissue (some evidence for its synthesis in BAT and placental tissue) that has a major physiological role in controlling energy balance. Identification of the leptin gene has sparked interest in the control of food intake and its integration with metabolism, energy stores and immunity. The effects of leptin on energy homeostasis and food intake are mediated through neuropeptides of the CNS. It also acts directly on immune cells stimulating hematopoesis, T-cell immunity, phagocytosis and cytokine production (Ingvartsen and Boisclair, 2001).

Marie et al. (2001) examined short-term dynamics of leptin secretion in sheep under changing conditions of photoperiod and availability of food. Plasma leptin was higher in long days than short days and was associated with higher intake, liveweight and condition score. When food was completely deprived, plasma leptin fell to basal levels within 24h. Plasma leptin was positively associated with plasma insulin and negatively with non-esterified fatty acids (NEFA), when fasting and between meals.

Leptin production was investigated in various physiological states of the Karakul ewe (20 - 40 days before breeding, 50 - 60 days of mid pregnancy, 125 - 135 days late pregnancy, 15 - 22 days early lactation) fed to maintain energy equilibrium in maternal tissues. Concentrations of insulin, NEFA and glucose did not vary between physiological states; however, leptin concentrations doubled between pre breeding and mid pregnancy and then declined to early lactation. Changes were explained by alterations in leptin gene expression in adipose tissue (Ingvartsen and Boisclair, 2001).

Reduction in VFI in ruminants around parturition has been ascribed to physical restriction of the abdomen but a metabolic cue may be responsible. For example, a reduction in plasma oestrogen and increased mobilisation of fat could be signals for reduced intake (Ingvartsen and Andersen, 2000). Leptin is unlikely to be responsible for the drop in VFI associated with parturition since plasma leptin levels fall 1 - 2 weeks before term (Ingvartsen and Boisclair, 2001). Ehrhardt et al. (2001) suggest changes in maternal intake and partitioning of nutrients between maternal and fetal components (Thomas et al., 2001) plus placental and mammary functions may be modulated by plasma leptin in earlier pregnancy. Reduced plasma leptin during early lactation could facilitate increased VFI. Another benefit of decreased leptin concentrations at this time is a consistent decrease in the response to insulin of peripheral tissues partitioning glucose to the mammary gland (Bell and Bauman, 1997).

Studies by Mostyn et al*.* (2001, 2002) investigated the influence of acute and chronic administration of leptin to neonatal lambs on responses of colonic temperature, unique uncoupling protein 1 (UCP1) and thermogenesis from BAT. Acute treatment with leptin increased colonic temperature and reduced UCP1 mRNA levels. Chronic treatment over six days post-birth resulted in colonic temperatures similar to controls while UCP1 abundance was lower in leptin treated lambs. The authors concluded that the drop in plasma leptin noted at birth may be a cue to begin enteral feeding. The rapid drop in UCP1 mRNA observed in the first few days of life maybe increased by leptin treatment, perhaps initiating the use of white adipose tissue for heat synthesis while non-shivering thermogenesis via UCP1 in BAT declines.

The energy deficit observed in ruminants, particularly dairy cows, during early lactation is associated with a reduction in plasma leptin and impairment of immune cell functions (e.g. migration, phagocytosis, secretion of immune proteins; Saad et al., 1989).

9.6.3 Behaviour

9.6.3.1 Introduction

Normal behaviour of both mother and offspring are essential for the survival of the species. The main feature of most ungulates is that the offspring are capable of following the mother soon after birth and that litter size is usually small, 1 or 2 in sheep. The other feature is the rapid development of recognition between the young and mother soon after birth. Early recognition of the mother’s own offspring was important from an evolutionary perspective for a species where the parents’ own young were likely to mingle with other neonates, providing a mechanism for investing in their own resources ensuring the survival of their own lambs (Nowak, 2000).

9.6.3.2 Onset of maternal care

Maternal responsiveness is generally associated with the birth process *per se* and physiological changes. Behaviours such as bleating, licking are preceded by a rapid fall in plasma progesterone and an increase in oestradiol and prolactin (Poindron and LeNeindre, 1980). Expulsion of the fetus results in stimulation of the genital tract which is associated with a release of oxytocin (Glatz et al., 1981). Levy et al*.* (1996) indicated that both priming by ovarian steroids plus genital tract stimulation was required for complete maternal behaviour toward the neonate. The same authors found that oxytocin was a major neurotransmitter that increased markedly in the cerebrospinal fluid near birth. They suggested the neurotransmitter can act at different sites, such as at the paraventricular nucleus to stimulate full maternal responsiveness or at the medial preoptic area to induce rejection behaviour toward offspring.

9.6.3.3 Pre-partum maternal interest

Prior to the birth of their own lambs ewes may display interest in recently born lambs of other ewes. Maternal interest may start 8h before birth but mainly occurs within two hours (Arnold and Morgan, 1975). Lamb losses through desertion of the ewe's own lambs is generally small (Arnold and Morgan, 1975) but can account for a high percentage of total lamb losses (20% and 34%; Winfield, 1970). High stocking rates will exacerbate the problem (Alexander et al., 1983a).

9.6.3.4 Isolation

At the onset of parturition there is a tendency for ruminants to isolate themselves from the main flock/herd. In the study of Arnold and Morgan (1975), most ewes isolated themselves to lamb, with a proportion lambing within the flock while fewer ewes lambed subsequent to the flock moving off. Most ewes lambed where birth fluids were spilled. Alexander (1988) commented that the tendency for domesticated sheep to leave their social group before lambing was not always the case. Isolation is considered important to facilitate bonding of the ewe and her lambs, reducing disturbance from other pre-parturient females. Little is known about the features of the environment that parturient ewes may seek; selection of the birth site is an example where ewes have the opportunity to avoid environmental hazards but do not always select to advantage (Stevens et al., 1981).

9.6.3.5 Grooming

Within a short time after parturition the ewe licks the lamb and the fluids expelled on the ground. Placental membranes may be eaten during grooming. Olfaction becomes of primary importance for development of maternal behaviour since fetal fluids become attractive at parturition (Levy et al., 1996). Visual and auditory cues are considered unimportant at this stage (Poindron and Levy, 1990). Grooming is thought to start at the head to remove membranes that could preclude breathing, while general grooming is considered to have advantages of drying the coat to reduce heat loss and stimulate the lamb to begin teat-seeking and also contribute to dam recognition of the lamb's odour for exclusive bonding. Bleating of low intensity may help orientate the lamb with the body of the ewe and begin cues for later recognition of the mother; low sound emitted may avoid attraction of predators and other pre-parturient ewes (Nowak, 2000).

9.6.3.6 Teat seeking

Initially, sight and hearing help orientate the lamb for udder-seeking activity and then olfactory and thermotactile cues help guidance to the teats (Vince, 1993). Tactile stimulus of the face initiates oral exploration; response is greatest from warm smooth surfaces. Fetal fluids on the udder may also stimulate teat seeking activity (Schaal et al., 1995). Back arching suckling posture is adopted by the ewe in response to the lamb touching the area of the udder.

9.6.3.7 Following

Lambs are followers (Ralls et al., 1986) in that they remain close to the mother and join the flock within hours of birth. Following is seen as a means of defence against predators and is part of a migratory existence (Nowak, 2000).

9.6.3.8 Behaviour toward predators

Active defence against predators is reputed for bighorn sheep (Alexander, 1988) and the Damara, whereas Merino sheep have been observed to be undisturbed by foxes (Alexander et al., 1967).

9.6.3.9 Recognition of the lamb

Recognition by the ewe of her own lamb develops within the first 2 - 4 hours after parturition (Poindron et al., 1993). Olfaction is involved in selective nursing but audition and vision develop as cues as early as eight hours after birth (Terrazas et al., 1999). Ewes can be identified by their lambs between 12 and 24 hours of age (Poindron et al., 1993) and the ability to discriminate extends in distance with increasing time.

9.6.3.10 Behavioural interactions

Quality of the behavioural interactions between the ewe and lamb is critical for survival. Primiparous mothers show some delay in development of maternal care which can delay success in first suckling (Poindron et al., 1993). Maternal behaviour can also be influenced by poor nutrition during pregnancy (Putu et al., 1988b); it could be argued that rejection of young when environmental conditions are poor may be a good investment for long term reproductive performance (Nowak, 2000). Maternal care of multiples is a significant problem in particular breeds such as the Merino (Stevens et al., 1982). Although more time is spent licking and stimulating twins compared with singles there is still a delay in bonding. Separation of a twin lamb can be due to a number of factors including disturbance by other parturient ewes, vegetative barriers at the birth site, distance between birth of litter mates and preparedness of the ewe to graze and leave the birth site. Actual time spent on the birth site increases with maternal experience more so than with age (Alexander et al., 1983b,1984). Nowak et al. (1997) suggested a delay in suckling reduces development of a normal relationship with the ewe. Gourasaud and Nowak (1999) indicated that intake of colostrum by the lamb stimulates early establishment of a bond. They suggest the delay in suckling by twins is critical for mother-offspring contact. Twins that have developed recognition of their mother within 12 hours of birth have a better chance of survival (Nowak and Lindsay, 1990).

9.6.3.11 Time spent at the birth site

Parity, litter size and breed have an influence on how long the ewe will spend on the birth site (Alexander 1983b; Stevens et al., 1984) and is of particular importance for survival of twin lambs (Stevens et al., 1982; Alexander et al., 1984; Buttrose et al., 1992). The percentage of separation of twin lambs from their mothers during the first 24 hours after parturition increases as time spent on the birth site decreases; minimal separations (i.e. <5%) were observed in the Merino when time on the birth site was four hours or greater whereas if time was only up to one hour, ewes permanently separating from a twin was as high as 75% (Alexander et al., 1983b).

Penning of ewes with their lambs at the birth site for six hours following birth of the last lamb tended to improve lamb survival and was associated with improved maternal behaviour (Putu et al., 1988a,b). Similar trends were observed for highly fecund ewes rearing triplets (Kleemann et al., 1992). It appears that if the ewe develops a strong bond rapidly with her lambs within the first six hours after birth it not only promotes enhanced maternal behaviour but improves chances of lamb survival. Strength of the maternal bond *per se* and time taken to establish a bond of maximum intensity in twin bearing ewes needs examination (Nowak, 2000).

9.6.3.12 Recognition by the lamb

A majority of lambs can recognise their dams between 12 - 24 hours post parturition (Nowak and Lindsay, 1990; Nowak et al., 1990) but only within less than a metre; this distance extends to several metres within three days of age (Nowak, 1990). Merino lambs take longer to recognise their mothers than do Border Leicester x Merino lambs (Nowak and Lindsay, 1990) and twins take longer than singles (Nowak et al., 1990). Stevens et al. (1984) suggested that crossbred lambs may be more alert at this stage than pure Merino lambs assisting in following the mother during the first 24 hours after birth. Ability to follow the mother over long distances was examined by Oppong-Annane (1991); the proportion of energy expended by the lamb was small indicating a capacity to follow over long distances without apparent exhaustion. Secondly, lambs that were better at recognising their dams also followed at a faster rate. Nowak et al. (1994) concluded that the two key factors in establishing a bond with the mother were early success at first suckling and vocal communication leading to strong motivation of lambs to follow their mother.

9.6.3.13 Other environmental conditions and maternal behaviour

Appropriate feeding during pregnancy influences the quality of maternal behaviour and colostrum yield. Onset of lactation is delayed if ewes are exposed to poor nutrition (Thomson and Thomson, 1949; McCance and Alexander, 1959) with an increase in mortality of singles and twins. Undernutrition during the last six weeks of pregnancy reduces udder development and accumulation of colostrum (Mellor and Murray, 1985). There is evidence that multiple bearing ewes produce less colostrum than single bearing ewes (Hall et al., 1990); twin bearing ewes produce about 50% of the colostrum per unit birthweight as do single bearing ewes (Hall et al., 1992). Delayed onset of lactogenesis may affect initial suckling by the lamb, important for establishing a bond with the ewe (Nowak et al., 1994); suckling promotes release of oxytocin, an important stimulant for maternal behaviour. Feeding high protein grain during the last two weeks of pregnancy increased colostrum production particularly in twin bearing ewes (Hall et al., 1992). This strategy could improve maternal-offspring bonding through ensuring sufficient colostrum for multiple lambs. Putu (1988b) fed high protein grain to ewes during the last few weeks of pregnancy and demonstrated that lamb mortality of twins was reduced and that this was associated with twin bearing ewes spending more time on the birth site. It is not known if maternal behaviour is improved through satisfying the appetite and delaying movement of the ewe from the birth site or whether specific nutritional factors are involved (Nowak, 2000).

Newborn lambs may die without standing or suckling through exposure to cold, wet and windy conditions (Obst and Day, 1968; Alexander et al., 1980). Lamb losses due to cold exposure could be reduced by selecting animals that have a genetic capacity to tolerate cold (Slee, 1981). Another strategy would be to provide shelter but this relies on appropriate ewe behaviour to seek shelter at the time of parturition. Some breeds do seek shelter during poor weather (Windfield et al., 1969) but the Merino ewe generally does not unless shorn and having had experienced cold weather prior to lambing (Lynch and Alexander, 1980; Grosser et al., 1991). Microclimate provided for the lamb is of primary importance and thus the form of shelter should be of primary consideration.

9.6.3.14 Maternal behaviour and genetic selection

9.6.3.14.1 Isolation behaviour

The ability of ewes to remain isolated during and after parturition is indicative of their ability to remain at the birth site for an extended period of time (see Hinch, 1997). Lindsay et al. (1990) suggested reduction of locomotive activity at lambing could have a beneficial effect on rearing. This suggestion was confirmed by Kilgour and Szanter-Coddington (1995) who observed locomotive ability of ewes selected for rearing ability and control ewes in an open arena test; the selected group displayed less movement or quieter temperament. A temperament test was also used by Putu. (1988) and Murphy et al. (1994) in Merino ewes indicating a good association between temperament and time spent on the birth site.

9.6.3.14.2 Grooming behaviour

Licking the lamb identifies it as own through olfactory cues and allows initial bond formation (Levy and Poindron 1987). Lack of grooming reduces lamb activity (Lynch et al., 1992). Evidence for within-breed variation for this trait has been reported but the possibilities for selection to improve maternal care remain obscure (Hinch, 1997).

9.6.4 Disease and immunity

9.6.4.1 Introduction

On a national scale, loss of lambs from disease is a small percentage of total lamb deaths but can be significant for individual flocks or properties. Infections may be acquired before or after birth; the topic has been reviewed by Haughey (1991) and will be discussed briefly.

9.6.4.2 Infections before birth

Congenital infections may be bacterial (e.g. *Coxiella burnetii*, *Camplyobacter fetus*, *Chlamydia spp*, *Brucella ovis*), protozoan (e.g. *Toxoplasma gondii*) or viral (e.g. Akabane) in origin, are present in many flocks but rarely cause significant economic loss (Haughey et al. 1967). At times a large number of fetuses may be aborted in individual flocks. For example, toxoplasmosis is known to be a source of abortion loss (Buxton, 1998) that occurs sporadically in flocks on Kangaroo Island where a feral cat population is the primary host of the protozoon (D Lehmann and G Johnsson, personal communication). *Coxiella* infection (Q fever) is usually localised in the placenta and fetal fluids but can be characterised by abortion and birth of moribund lambs (Brooks et al., 1986). Because an immunity is acquired by the ewe, vaccination may be considered as unnecessary, though a vaccine is available for *Coxiella* (Brooks et al., 1986) and *Toxoplasma gondii* (Buxton, 1998).

9.6.4.3 Infections after birth

Infections acquired after birth are mainly bacterial in origin. Pathogens noted by Haughey (1991) include *Clostridial spp* (gangrene of umbilicus or generalised peritonitis), *Pasteurella spp* (pneumonia or generalised peritonitis), *Staphlococcus aureus* and *Streptococcus spp* (pyaemia), *Eschericia coli* (enteritis, septicaemia). *Clostridial* and *Pasteurella spp* can be controlled by vaccination administered prelambing.

9.6.4.4 Development of the fetal and neonate immune system

Immune system development of the fetus begins with the appearance of hematopoietic cells in embryonic blood vessels (Nesin and Cunningham-Rundles, 2000). The thymus, playing a central role in the immune system through the production of lymphocytes, is first visible in the ovine fetus at 27 - 30 days post mating, begins synthesis of lymphocytes around day 40 and is the major lymphocyte producing organ by day 60. At this early stage lymphocytes, devoid of surface immunoglobulins (Ig), migrate through the blood and lymph systems to the spleen, gut and lymph nodes. By day 100, Peyer's patches are major sites of lymphocyte production but with surface Ig. Maximum development of the thymus and Peyer's patches occurs around three months post birth (Morris et al., 1986).

Recirculation of lymphocytes between the blood vascular system and lymph system is established by day 65 and it is at this time that the fetus is able to respond to antigenic invasion (Morris et al., 1986). Lymphocyte recirculation is maintained postnatally, with the greatest recirculation of lymphatic cells occurring in the gut.

Sheep, unlike primates, develop their antibody repertoire from a small number of genes expressing only IgG with IgD totally absent from ruminants (Butler, 1998). Sheep, as in other ruminants, exclusively use the mammary gland to transfer immunity to offspring via colostrum. This passive immunity is essential for the survival and subsequent vigour of the lamb. There is a high priority for the neonate, therefore, to suckle and ingest colostrum (Varley, 1992). Indeed, there is evidence in pigs that acquisition of effective immunity as a neonate is related to improved immunological competence as an adult (Varley et al., 1987).

9.6.4.5 Maternal immune system and fetal and neonatal survival

The immune system inherited by mammals protects the body against infective organisms by detecting and destroying foreign material. The so-called 'semi foreign' fetus however is protected by development of systems to prevent immune rejection. Reduction of expression of placental major histocompatability complex (MHC) genes is responsible (Bainbridge, 2000). Mechanisms protecting the fetus are beginning to be understood but when defective can result in intrauterine growth retardation (Hanson, 2000).

9.6.5 Predation

It is important for investigators to distinguish between predation that is primary (death of a viable lamb) and secondary (loss of a lamb that would have died for reasons other than predation). Studies have indicated that loss from primary predation is normally low (see Haughey, 1991) but no doubt large losses can be experienced. Foxes, feral pigs, eagles and ravens have all been implicated in primary predation. For example, explosions in feral pig populations in riverbeds of central western Queensland are suspected of significantly reducing lambing percentages; it is now common practice to bait prior to the lambing period. In addition, property owners in Australia are encouraged to bait in neighbouring groups to reduce predators, commonly foxes, on a widespread local scale.

Lugton (1987) concluded from his own observations and from a summary of Australian studies that primary predation of lambs by foxes has a large impact in areas where foxes are common, where ewes lamb outside of the main lambing season and where flocks are isolated from neighbouring flocks. Factors predisposing lambs to predation include fox population density and structure, lamb density, size and vigour of lambs and alternative food sources for foxes (Lugton, 1987,1993). Improvements in lamb marking percentages of up to 30% have been associated with baiting programs over a number of areas in different regions of New South Wales (Lugton, 1987).

9.6.6 Plant toxins

Although a minor cause of lamb deaths, plant toxins are suspected of contributing to lamb mortality and morbidity. Broadmeadow et al. (1984) examined if plant toxins reduced lamb survival when ewes were exposed to a toxin from *Tribulus spp* (a plant forb in north western Queensland) during late pregnancy. They reported that the toxin lowered teat-seeking ability and resulted in high mortality. Cobon and Carter (1994) extended the study to the field and provided evidence that the consumption of a high proportion of the diet from forbs (versus grass) was associated with a reduction in lamb survival.

9.6.7 Exposure

9.6.7.1 Cold

Lambs of low birthweight are particularly vulnerable to cold exposure due to an increased surface area to bodyweight ratio and reduced body reserves. Birthweights can be improved by shearing the dams during pregnancy but responses have varied (Cloete et al., 1994; Morris et al., 1999, 2000; Kenyon et al., 2002 a,b; Revell et al., 2002). Kenyon et al. (2002b) contend that variability of birthweight response to mid-pregnancy shearing could be explained by (a) the fetuses' potential to respond and (b) nutrients of either endogenous or exogenous source must be available for the ewe to respond to the stimulus provided by shearing. Support for this supposition is given for by a number of recent studies including Gate et al. (1999) and Revell et al. (2002). Stimulation of thermogenic activity of BAT and enhanced summit metabolic rate (SMR) in the neonate has been reported by Symonds et al. (1992) for shorn housed ewes. Revell et al. (2002) observed increased SMR in twin lambs, while Kenyon et al. (2002a) found no change in thermoregulatory activity. Revell et al. (2000) examined the metabolic and endocrine responses in ewes shorn in mid pregnancy and housed indoors where ambient temperature was controlled at 9 – 13°C. In this experiment, birthweight of twins increased due to shearing by 1.1 kg, but singles did not respond presumably because control singles had grown to their potential birthweight. Alternatively, Revell et al. (2002) explained that decreased insulin secretion by the shorn twin bearing ewes might have reduced glucose uptake by maternal tissues, sparing glucose for fetal growth. Another hypothesis is that transport of glucose through the placenta was increased through upregulation of glucose transport proteins (Revell et al., 2000). Decrease in maternal IGF-1 and a large increase in IGFBP-1 in shorn twin bearing ewes may have induced a large increase in transfer of IGF-1 to the fetoplacental unit by IGFBP-1 (Revell et al., 2002). Further studies are required to more closely examine the metabolic and endocrine changes in the neonate's mother subjected to cold exposure since this will have important ramifications for lamb survival in the field.

Donnelly (1984) estimated probabilities of neonatal death derived from a chill index (based on wind speed, temperature and rainfall) for single and twin lambs born to Merino and Border Leicester x Merino ewes grazed at low and high stocking rates. The highest probability of death (0.69) was for twins born to Merino ewes with low maternal weight. Maternal weight was important for reducing probabilities of death at high chill index values (1120 kJ m^{-2} h^{-1}) for both breeds. At a low chill index of 850 kJ m^{-2} h^{-1} probabilities for single and twin Merinos were 0.14 and 0.07; corresponding values for Border Leicester x Merino ewes were 0.08 and 0.05. The chill index forms the basis for 'grazier alerts' given by the Bureau of Meteorology (Holst and Cullis, 1982).

The ability of lambs to cope with cold temperatures at birth is reliant on the normal functioning of mechanisms regulating thermogenesis. Premature birth in lambs can compromise heat production through non-shivering thermogenesis in brown adipose tissue (BAT) (Alexander et al., 1972; Clarke et al., 1997). Rapid heat generation is mediated by UCP (Cannon and Nedergaard, 1985) that acts on the electron transport chain of adenosine triphosphate. During pregnancy placental inhibitory factors (adenosine, prostaglandin E2 (Ball et al., 1995) and minimal concentrations of thyroid hormones (Clarke et al., 1997) prevent lipolysis and maintains low fetal thermogenic activity. An important factor controlling temperature of the lamb after birth is method of delivery; normal pelvic delivery results in high thyroid hormone levels and increased sympathetic activity improving heat generation from BAT and maintenance of colonic temperature (Clarke et al., 1997). Delivery by caesarean results in impairment of BAT function and lower colonic temperature. Increased adrenal activity compensates by switching heat production to shivering thermogenesis in caesarean delivered lambs (Clarke et al., 1997). Dexamethasone (corticosteriod) administration two days before day 140 (premature) caesarean significantly improved thermoregulation after birth increasing UCP (Clarke *et al.*, 1998) and prolactin receptor abundance (PRLR) in BAT (Bispham et al., 1999). Maternal nutrient restriction between 28 - 77 days of gestation increased risk of lamb deaths following caesarean delivery but survival is significantly improved if thyrotrophin releasing hormone (TRH) is administered via the umbilicus during delivery. This supports the hypothesis that fetal hypothalamic-pituitary-thyroidal function is impaired in ewes nutrient restricted during early-mid pregnancy (Heasman et al., 2000).

9.6.7.2 Heat

The effect of chronic heat exposure on pre-natal development was examined by Bell et al. (1989). Ewes were exposed to thermoneutral or hot environments between days 64 and 136 to 141 of pregnancy. VFI was not altered by heat treatment until day 120 when VFI then declined. Plasma glucose levels were higher after day 120 due to heat. Placental weight was reduced by 54% and was associated with declines in fetal weight. Progesterone, cortisol and placental lactogen were reduced late in pregnancy while triiodothyronine concentrations were reduced at all stages of pregnancy. The authors indicated that heat induced fetal growth retardation was secondary to primary retardation of placental growth. They suggest that reduction in thyroid hormone could be the responsible mediator. Heat treatment dramatically reduced total cell number and placental size, and only slightly affected cell size (Early et al., 1991).

Hopkins et al. (1980) observed that heat stress during the last month of pregnancy had a greater impact on lamb birthweight (2.3 kg vs 3.4 kg) than did poor nutrition (3.2 vs 3.9 kg). This result was extended to the field where shade was found to be more important than nutrition in autumn lambing ewes in north west Queensland (Stephenson et al., 1984). Heat stress did not alter the duration of parturition for ewes with and without shade and fed at different levels of nutrition (Stephenson et al., 1984).

Studies on ewes selected for their tolerance to heat stress (McCrabb *et al.,* 1993) and exposed to summer gestation in the semi-arid tropics of Queensland indicated that birthweights of lambs from the low rectal temperature group ewes was higher than those from the high rectal temperature group.

9.6.8 Stress

Rousell and Hemsworth (2002) reported that ewes subjected to mild stress, via periodic isolation during the last third of pregnancy, had no effect on ewe liveweight but increased birthweight (4.6 vs 4.1 kg) compared with lambs from control ewes. Basal concentrations of cortisol were higher in prenatally stressed lambs indicating modification of the hypothalamo-pituitary adrenal axis. Confirmation of these results has implications for lamb survival.

9.6.9 Photoperiod

Seasonal changes in temperature and food availability have meant that animals have evolved to reproduce and give birth to their offspring at the most favourable time of the year to minimise neonatal loss.

Although seasonality of reproduction has been lost in some species due to domestication, it operates in most breeds of sheep originating from temperate latitudes (Malpaux et al., 1996). The Merino is thought to have evolved in regions closer to the equator and is less seasonal than temperate breeds. Photoperiod is the main environmental cue determining time of breeding which resumes after a period of short days (autumn) to allow parturition to correspond with the spring months (Ortavant et al., 1985). Light is received by the retina and signals are transmitted via a neuronal pathway to the pineal gland that secretes melatonin during the dark hours. This hormonal secretion regulates the hypothalamo-hypophysial and gonadal axis (Karsch et al., 1988), in particular LHRH, LH and FSH (Thiery et al., 2002). In addition, other functions of seasonal breeders are under photoperiodic control such as metabolic rate that precedes changes in appetite (Argo et al., 1999). Bodyweight changes in sheep that are due to season appear to be mainly found in adipose versus muscle tissues (Faulconnier et al., 2001). Appetite is maximal in grazing sheep in late summer/early winter corresponding with the onset of breeding and is minimal in spring (Clarke, 2001). The increase in appetite is associated with enhanced expression of neuropeptide Y (NPY), an orexigenic agent. Leptin an anorexigenic agent responds to long days and could interact with NPY in controlling appetite. Changes in leptin could provide a link informing the hypothalamus of adipose deposits required for processes of pregnancy and lactation (Theiry et al., 2002).

The photoperiodic cue for reproductive processes are geared for sheep grazing in temperate climates. However, a high proportion of sheep (60%) inhabit latitudes between 35^{o}S and 35^{o}N (Lindsay, 1991) indicating that the annual food supply for sheep within these latitudes may be out of phase with a photoperiodic cued reproductive cycle (Martin et al., 1994). In a Mediterranean environment, spring growth does not extend into summer so that ewes that mate in autumn have been exposed to poor quality pastures for a number of months resulting in low rates of ovulation. Martin et al. (2002) provided evidence that Mediterranean and temperate genotypes (Merino versus Suffolk) elicit different responses to photoperiod and nutritional cues with changes in testicular size. Suffolks were responsive to photoperiod mainly while Merinos did respond to photoperiod but the response can be overridden by nutrition. In addition, the Merino may not only use the nutritional cue to override the entrained influence of photoperiod but also use a social cue such as the “male effect” to induce ewes to breed at any time during the year (Lindsay, 1996). From an evolutionary perspective the “male effect” produces a synchrony of breeding enabled by males that have a large reserve of spermatozoa ensuring that most offspring are born in a short period of time making them less vulnerable to predators (Lindsay, 1996).

The ability of Merino ewes to breed at any time of the year has important implications for nutritional management and the need to grow lambs quickly to maximise product quality and output. This is the subject of the next chapter.

9.7 SUMMARY

The important points arising from this chapter are:

(1). Reproductive wastage at lambing is high with 20 - 30% of lambs born in Australian commercial Merino flocks dying within a few days of birth. Mortality amongst twins is estimated at 30 - 40%.

(2). Losses at lambing cost the nation an estimated $250m annually.

(3). Causes of mortality in low to medium fecund flocks are mainly due to dystocia and the starvation/mismothering/exposure complex, whereas deaths in prolific flocks are mostly attributed to anteparturient and prolonged birthing problems.

(4). Reasons for mortality may vary from flock to flock, requiring detailed investigation to determine the major causes.

(5). If lamb losses are large, causes can generally be attributed to one or two single factors but reasons for the core loss may be difficult to reveal.

(6). Most deaths occur within the first few days of life but significant losses can occur thereafter if extreme environmental conditions prevail.

(7). Neonatal adaptations during parturition, such as changes in thermoregulation, mobilisation of energy reserves, alterations in lung fluid dynamics and regulation of hypoxia are required for the newborn to survive. Further understanding of these processes is needed to develop strategies for reducing mortality and morbidity.

(8). Improvement of lamb survival through selection within breeds and through crossing of breeds can be achieved. However, progress through selection within breeds will be slow since heritability estimates for the trait are low.

(9). Indirect selection criteria for enhancing lamb survival have been identified and show promise.

(10). The value of crossing meat breeds with the Merino to increase lamb survival is well known, with contributions to improvement coming from both the ewe and lamb genotype. Early results in pastoral areas indicate that the Damara breed may confer enhanced neonatal survival compared with the purebred Merino.

(11). Selection within a breed for other traits such as body growth may be antagonistic to increasing lamb survival.

(12). Nutrition has a major impact on placental and fetal growth starting in early pregnancy and possibly earlier.

(13). Size of the fetus is associated positively with placental mass. Ewes in good body condition at mating and subjected to moderate undernutrition during early-mid pregnancy may produce placentae of normal size, whereas ewes in poor condition or young growing ewes exposed to poor nutrition during this period produce small placentae.

(14). Overnourishment of adolescent ewes during early-mid pregnancy may give small placentae and fetuses.

(15). Further knowledge is required on the physiological basis for growth and function of the feto-placental unit including endocrine and growth factor regulatory mechanisms.

(16). Size of the placenta at the beginning of late pregnancy governs fetal mass at birth and is independent of maternal body condition in ewes fed to appetite during late pregnancy. However, when ewes are underfed during late pregnancy, fetuses at term are heavier in better-conditioned ewes. Ewes having moderate fat reserves throughout pregnancy may reduce insulin sensitivity and partition more glucose to the fetus compared with those from ewes with low fat reserves and though fetal mass may not increase, fetuses carried by better conditioned ewes may store greater reserves of fat.

(17). Rapid development of recognition between mother and offspring soon after birth is essential for lamb survival.

(18). Normal parturient behaviour requires both hormonal changes and stimulation of the genital tract via fetal expulsion.

(19). Olfaction then auditory and visual cues become important in establishing the link between mother and offspring. Recognition by the ewe of her lambs develops in the first 2 - 4 hours and identification by the lambs of their mother occurs between 12 - 24 hours of age.

(20). The quality of behavioural interactions between ewe and lambs are determined by breed, parity, litter size and nutrition.

(21). Development of a preferential relationship of the lamb to its mother is established through suckling which has strong rewarding properties and through vocal communication. Colostrum is involved in development of the preferential relationship.

(22). Suckling of the ewe is critical for establishment and maintenance of normal maternal behaviour.

(23). Strength of the maternal bond *per se* and time taken to establish a bond of maximum intensity in twin rearing ewes warrants investigation.

(24). Behavioural characteristics of the Merino ewe and her lambs and their association with physiological parameters manifest during the first 24 hours post birth deserves further study.

(25). On a national scale, disease is a relatively unimportant factor causing lamb deaths but can be a significant problem in individual flocks (e.g.Toxoplasmosis).

(26). Transfer of passive immunity via immunoglobulins present in colostrum is essential for lamb survival.

(27). Primary predation can be a significant problem on individual properties; in most cases current control measures will be effective for reducing mortalities.

(28). Exposure of ewes to a colder environment by shearing during mid pregnancy can increase birthweight but responses are variable.

(29). Impairment of non-shivering thermogenesis in lambs delivered prematurely or in lambs born to ewes subjected to nutrient restriction in early-mid pregnancy has implications for survival of lambs in cold environments.

(30). Selection of ewes (and rams) for tolerance to heat may overcome, to some extent, high lamb mortality associated with small fetuses.

(31). The photoperiodic cue that triggers sheep to breed has evolved so that offspring are born during the most favourable time of the year (spring) to maximise survival of offspring. Nutritional and social cues in the Merino can override the entrained photoperiodic cue allowing them to lamb at less than favourable times of the year.

CHAPTER 10. POST-NATAL GROWTH OF LAMBS

10.1 INTRODUCTION

Lamb-meat produced in Australia comes from a wide range of genotypes and production systems, with most lambs grown on pasture. Early post-natal growth is dependent on a number of factors including the genetic ability of the lamb to grow and the genetic capacity of the ewe to synthesise and provide milk for her young. This chapter will discuss the following aspects: performance of breeds currently used in Australia for lamb-meat production, selection within breeds, physiology associated with lactation and the impact of factors impinging on milk yield and its quality, pre- and post-weaning supplementation and subsequent effects on lamb growth rate.

10.2 PRE-WEANING GROWTH

10.2.1 Breeds

10.2.1.1 Breed differences for growth

The lamb-meat industry has historically relied on a tiered breeding structure where the Merino ewes, usually surplus to wool growing requirements, are joined to produce mainly first-cross ewes (e.g. Border Leicester x Merino) for generation of second cross lambs (40% of lambs slaughtered) or are joined to terminal sires (e.g. Poll Dorset) for production of first-cross lambs (25%) or are slaughtered as purebred Merino (20%). The remaining lambs (15%) are derived from either purebred meat breeds or their crosses or dual purpose breeds (Fogarty, 2000)

Differences in growth rate between first and second lambs [i.e. Dorset x Merino versus Dorset x (Border Leicester x Merino)] is approximately 25% (e.g. Kleemann et al., 1981) while the difference between purebred Merino lambs and first-cross lambs (Dorset x Merino, Border Leicester x Merino) is approximately 15 - 20% (Fogarty, 1972; Kleemann et al., 1983). Lamb genotype and maternal environment contributed approximately equally to the difference in growth rate between first- and second-cross lambs (Kleemann et al., 1981). Success of the Border Leicester x Merino ewe is due to heterosis for fertility and lamb growth (McQuirk et al., 1978).

More recently, Fogarty et al. (2000a,b) concluded that differences between breeds were relatively small for growth when compared with large difference observed between individual sires within breeds. Although high growth rates are important for achieving target carcase weights within a particular time, economic value may also be determined by breed differences in carcase quality. Second-cross lambs [Dorset x (Border Leicester x Merino) and Texel x (Border Leicester x Merino)] and first-cross Border Leicester x Merino lambs were fatter than Dorset x Merino and Texel x Merino (1 mm at GR) and purebred Merino (3mm at GR) lambs, when compared at the same carcase weight (Fogarty et al., 2000b). Further, Kleemann et al. (1991) reported that second-cross lambs [Suffolk x (Poll Dorset x Merino)] were fatter (1.4mm at GR) at the same carcase weight (18kg) than first-cross (Suffolk x Merino) lambs. This indicates that selection within the Dorset and Border Leicester against subcutaneous fat deposition is required to meet consumer demands for lean meat.

Atkins (1980) and McGuirk et al. (1978) provided evidence for breed x year interactions for weaner and hogget liveweights suggesting that Merinos were less responsive to improved environmental conditions than other breeds (e.g. Border Leicester). Specific maternal handicaps such as decreased growth due to maiden status (Kleemann et al., 1983) or twin birth (Fogarty et al., 1972; Atkins, 1980) indicate that the effects are more apparent in breeds of greater growth potential than in that of the Merino (see Woolaston, 1987).

10.2.1.2 Within breed selection for growth

A national program for improvement of all breeds through selection (Banks, 1990) has been well accepted by industry with a major proportion of terminal sires now sold with estimated breeding values for weight and fat and with significant increases observed in the dominant breeds (Fogarty, 2000). Genetic improvements in the maternal breeding sector will be slow given a lack of market signals at point of sale of first-cross ewes and low heritability of some of the traits under selection. Substantial differences between maternal sires in meat production, including lamb growth, from their first-cross daughters has been observed in a national progeny test scheme (Fogarty, 2000).

The ability of existing Australian genetic stock and nutritional strategies to grow lambs to extra heavy weight carcasses (>26 kg) within specified fat requirements, was successfully tested by Shands et al. (2002), using high index LAMBPLAN sires, Merino and Border Leicester x Merino dams and a feedlot system to finish pasture-reared lambs.

10.2.2 Lactation

10.2.2.1 Mammary development

The mammary gland is unusual in that most of its growth occurs during adulthood, undergoing cycles of development, function and differentiation (Knight, 2000). The amount of milk secreted is highly associated with the mass of secretory tissue (Linzell, 1966). In ewes, mass of secretory tissue is correlated with total lamb birthweight; greater than 95% of its growth occurs during gestation (Robinson, 1986). Regulation of mammogenesis via the feto-placental unit is, in part, due to the mammogenic hormone, placental lactogen (Collier et al., 1995). The role of GH and the interaction with IGF-1 in regulating normal mammary development is discussed by Sejrsen et al. (1999).

There is speculation that mammary development and growth of the murine fetus can be influenced by maternal nutrition that affects ductular outgrowth and proliferative activity (Hilakivi-Clarke et al., 1997). It is known that overnourishment of pubertal heifers reduces udder development and milk yield (Sejrsen, 1994; Weber, 1999). Johnsson et al. (1985,1986) established that fast growth rate in ewe lambs during the first five months of life reduced mammary secretory tissue; high plane nutrition during the following four months improved development of secretory tissue. Greater mammary development of low nutrition lambs was associated with increases in GH and reduced concentrations of prolactin. This finding is of relevance to producers generating breeding ewes for lamb-meat production. In contrast, under-feeding during the early post-natal period may be prejudicial to liveweight and reproductive performance of the lambs as adults (Williams, 1984).

Knight and Sorensen (2001) speculated that modulation of fetal mammary development could have a long-term effect through inhibition of ductular growth but demonstration of permanent effects on mammogenesis of peripubertal perturbation of nutrition may be difficult to demonstrate.

Over-feeding adolescent sheep not only affects feto-placental growth (Wallace et al., 1996) but reduces the initial yield of colostrum (Davidson et al., 2000). Total IgG, butterfat, lactose and crude protein available to the lamb was significantly reduced by the overfeeding treatment. Reduced concentration of colostrum, lower colostrum yield and reduced fetal size, lipids and glycogenic stores (Wallace et al., 2000), places lamb viability and subsequent growth at risk. In addition, total amount of IgG consumed is reduced leaving lambs susceptible to both gut and systemic infection through transfer of inadequate antibody supplies (Wallace et al., 2001).

10.2.2.2 Metabolic adaptations before and during early lactation

The high metabolic challenge imposed on the ewe that occurs between late pregnancy and early lactation may not be life threatening to the ewe and fetus/lamb but may compromise lactation and subsequent performance and growth of the lamb. Regulation of nutrient metabolism and adaptation required during the transition from pregnancy to lactation has been reviewed by Bell (1995). In late pregnancy, in ruminants fed to meet energy requirements, liver gluconeogenesis increases but utilisation of peripheral tissue glucose is reduced, NEFA are mobilised from fat depots to a moderate degree, use of NEFA is increased in peripheral tissues along with hepatic 3-hydroxybutyrate, amino acid catabolism is reduced but muscle proteolysis is increased. These metabolic adaptations spare glucose and amino acids for fetal growth while maternal requirements are increasingly met from NEFA and ketones.

Bell (1993, 1995) calculated a substantial shortfall in net energy and metabolisable protein not met from dietary supply of nutrients during the first week of lactation. Endogenous substrates mainly sourced from adipose tissue, peripheral muscle and hepatic tissue are mobilised to meet the shortfall using metabolic adaptations. A massive increase in NEFA observed during the peri-parturient period is achieved in a number of ways; suppression of fat synthesis, promotion of lipolysis and reduction of intracellular reesterification of NEFA. Suppression of lipogenesis and NEFA esterification is involved during late pregnancy in ewes (Vernon, 1981). The high increase in lactose synthesis by the mammary gland requires an increase in carbohydrate metabolism to glucose. The calculated shortfall in glucose substrate is met by a sparing of glucose oxidation in peripheral tissues and mobilisation of amino acids from these tissues (Bell, 1995). The net requirement for protein for animals in negative nitrogen balance is probably derived from a net release of amino acids from skeletal muscle where suppression of protein synthesis outweighs an increase in protein breakdown (Bosclair et al., 1993) and more efficient use of amino acids in the liver (Bell, 1995).

Metabolic adaptations previously mentioned generally start during late pregnancy and change markedly at parturition. Regulation of these adaptations is effected through altered tissue responses to hormones such as insulin and adrenergic substances. Petterson et al. (1993) noted decreased sensitivity of insulin to glucose utilisation, and diminished insulin responsiveness of lipolysis and NEFA mobilisation (Petterson et al., 1994). Therefore during late pregnancy, mobilisation of fat is enhanced by a reduced ability of insulin to store fatty acids and prevent lipolysis. Bell (1995) concluded from several studies that a moderate degree of insulin resistance is observed in adipose and muscle tissue during early lactation, sparing glucose but enhancing mobilisation of NEFA and amino acids.

10.2.2.3 Hormonal control of lactation

Mammary tissue becomes secretory in the second half of pregnancy, and secretions increase massively in both concentration and yield just prior to parturition (Hartmann et al., 1973). Cowie et al. (1980) suggested an important role for placental lactogen in mammary development of ewes. Mammary development does occur in ewes hypophysectomised after day 50 with lactation taking place at parturition. Lactose (detected from day 90 - 100 onwards) and the rise in placental lactogen concentration are positively correlated. In addition, they suggested that although progesterone and oestrogen were essential for normal mammogenesis, the effect of these hormones may be mediated by prolactin by a direct effect of the steroid hormones on prolactin receptors in mammary parenchyma. Tucker (1981) hypothesised that since placental lactogen commences secretion during mid pregnancy and increases to term, that it synergises with the steroid hormones, prolactin and GH to promote mammary development during a normal pregnancy. The role of prolactin promoting mammary growth was not supported by experiments of Schams et al. (1984); bromocriptine (suppresses prolactin secretion) had no effect on mammary development in either ewes or cows. However, initiation of lactation at parturition has an obligate requirement for prolactin (Knight, 2001) and the author suggested that prolactin, primarily secreted from the anterior hypothalamus, is more lactogenic than mammogenic. Indeed, in sheep, decreasing concentrations of prolactin during lactation depresses milk synthesis (Knight, 2001); the effect is only minor in dairy species (goat, cow).

Stelwagen et al*.* (1991) observed that GH administered in late pregnancy stimulated mammary growth and increased milk supply in maiden ewes, a response found in all domestic ruminants (Sejrsen et al., 1999). The mechanism of action probably involves IGF-1, and physiological regulation interplay of the GH-IGF-1 axis with binding proteins, growth factors and receptors involved (Sejrsen et al., 1999).

Pituitary hormone, oxytocin, has a number of roles stimulating milk let-down and also adapting behaviour and physiology to facilitate lactation. Oxytocin stimulates bonding between mother and offspring. As well as having a role in stimulating glucagon release for mobilisation of glucose for milk synthesis, it has an influence on digestive gastrointestinal hormones (cholecystokinin or CCK, gastrin, somatostatin), as shown in rats and also induces antistress effects (Uvnas-Moberg et al., 2001). Suckling versus withdrawal of milk via machine can have long-term consequences for the physiology of the female. For example, a routine incorporating suckling plus milking increased milk production and gave high responses in oxytocin, prolactin, GH and IGF-1 (Bar-Pled et al., 1995). Whether the response was due to teat stimulation or included an interaction between mother and offspring remains to be resolved.

10.2.2.4 Neuroendocrine response to lactation

Adaptations of the female to the demanding physiological state of lactation include initiation of maternal behaviour, large increase in intake of food and water, decline in reproductive cyclicity and increases in oxytocin and prolactin levels. Detailed discussion of how the hypothalamus integrates peripheral messages such as the suckling stimulus and signals indicating energy balance (leptin) during lactation is given by Smith and Grove (2002). In short, the arcuate nucleus Neuropeptide Y (ARH-NPY) system is responsive to the suckling system and a decrease in leptin due to high metabolic demands of milk synthesis and secretion. NPY neurones from the ARH have direct links with GnRH neurones. The system is able to regulate food intake/energy changes in conjunction with reproductive function (Smith and Grove, 2002).

10.2.2.5 Lactational persistency and growth factors

Decline in milk yield in ruminants appears to be mainly due to cell loss but little is known about longevity of individual cells (Forsyth, 1996). Increasing secretory cell renewal or decreasing cell loss during lactation and knowledge of the processes involved are required including an understanding of the control of the cell cycle and the effect of stimulating IGF-1, IGF-2, transforming growth factors, amphiregulin and inhibitory growth factors (Forsyth, 1996).

10.2.2.6 Milk yield and composition

10.2.2.6.1 Breed of ewe and lamb

Milk yield is dependent not only on the breed of ewe but also genotype of lamb. For example, milk intakes of Merino lambs suckling either Merino or Border Leicester ewes were lower than intakes of Border Leicester lambs (Langlands, 1972). Milk yield of the traditional ewe breeds (Merino, Border Leicester x Merino and, to a lesser extent, the Poll Dorset x Merino) were observed in a pen study (Kleemann et al., 1981). First-cross ewes produced 25 - 30% more milk than the Merino, independent of lamb genotype (Suffolk sired first- and second-cross lambs). Second-cross lambs consumed 10 - 15% more milk, independent of dam breed (maternal effects). Differences between breed of dam and between lamb genotypes for milk composition were small and non-significant (D. Kleemann, unpublished).

10.2.2.6.2 Number of lambs suckled

Ewes suckling twins produce more milk than those rearing singles; mean difference calculated from a number of studies was 32% with values ranging from 13 - 77% (see Kleemann, 1983). Milk yield curves for single and twin suckled ewes varied from the start of lactation. Yield from ewes suckling twins is about 50 - 70% greater than that of singles during the first week of lactation (Treacher, 1978) and reaches a peak in the 2 - 3rd week for twin suckled ewes and 3 - 4th week for single suckled ewes (Treacher, 1978; Maxwell et al., 1979). The amount of milk produced then declines with little difference in yield between single and twin suckled ewes at the sixteenth week of lactation (Treacher, 1978; Maxwell et al., 1979).

Nutritional regimes imposed during either late pregnancy (Wallace, 1948b; Maxwell et al., 1979) or during lactation (Gardner and Hogue, 1964) have a smaller effect on milk yield than number of lambs suckled. The greater potential for milk yield in multiple rearing ewes is largely determined before parturition with the number of fetuses having a direct effect on mammary growth (Robinson, 1986) and a positive association with placental lactogen (Butler et al., 1981).

Reports on differences in milk composition between single and twin suckled ewes are variable. Gardner and Hogue (1966), Peart et al. (1979) and Torres-Hernandez and Hohenboken (1979) found that fat and protein content did not vary with number of lambs suckled. In contrast, other studies have shown that fat was higher (Gardner and Hogue, 1964; Peart et al., 1972) and protein was lower (Peart et al., 1972) in twin suckled ewes.

10.2.2.6.3 Milk and solid food intake, and interaction with lamb growth

Early growth of the lamb is largely determined by the amount of milk consumed by the lamb (e.g. Peart, 1968). At about the sixth week of lactation, milk intake still has a large but declining influence on growth of the lamb and influences the rate of solid food intake that, although starting at around three weeks of age (e.g. Langlands, 1977), only becomes significant at 6 - 7 weeks (e.g. Penning and Gibb, 1979). Though twin suckled ewes produce more milk than single rearing ewes, twin lambs ingest less milk than their singles counterparts but are able to partly compensate by eating more solid food (e.g. Gibb and Treacher, 1982).

When ewes have poor milk yields due to low pasture availability, lambs have difficulty in fully compensating by increasing pasture consumption (Williams et al., 1976). In this situation, intake of the lambs was reduced before milk yield declined. Maintenance of high milk yield during the second and third months of lactation is important for sustaining high growth rates in lambs since the efficiency of utilisation of ME for pasture is less than half of that for milk (Penning and Gibb, 1979). This is highlighted by the low growth rate of lambs weaned on to pasture as early as 3 - 6 weeks of age (Langlands and Donald 1975; Gibb et al., 1981).

10.2.2.6.4 Voluntary intake of the ewe

A dramatic increase in the voluntary food intake (VFI) of the ewe has been discussed previously but is pivotal to meeting the nutrient demands of lactation. Maximum VFI is not achieved until peak milk yield has passed at the eighth week of lactation (Foot and Russell, 1979) but may vary with quality of the diet (e.g. Treacher, 1970) but not with number of lambs suckled (Foot and Russell, 1979).

Passage of digesta from the rumen increases with the onset of lactation, water intake increases while efficiency of digestion decreases because feed intake increases markedly (e.g. Weston, 1982; Kaske and Groth, 1997).

10.2.2.6.5 Parity and age of ewe

Milk yields increase from the first to the third lactation, remains steady until the sixth lactation and then declines; this pattern was observed in ewes lactating for the first time at two years of age (Barnicoat et al., 1949).

10.2.2.6.6 Nutrition

10.2.2.6.6.1 Late pregnancy

Very poor nutrition in late gestation can result in a small amount of secretion present before lambing, much reduced mammary tissue and several hours delay in the initiation of lactation post-parturition (McCance and Alexander, 1959). In experiments where bodyweight was maintained during the last trimester of pregnancy, reductions in milk yield were noted for a full lactation (e.g. Treacher, 1970) or early lactation (Bizelis et al., 2000). Other consequences were reductions in birthweight, lamb growth rate and heavy mobilisation of the ewe's body reserves. Other authors (e.g.Thomson and Thomson, 1953; Butterworth and Blore, 1969) have found ewes exposed to under-nutrition in late pregnancy and bearing single fetuses have resulted in reductions in either lamb growth or milk yield.

Body condition at lambing did not affect intake during early lactation (Gibb and Treacher, 1980; Maxwell et al., 1979) but lamb growth was higher in better-conditioned ewes (Gibb and Treacher, 1980) and milk yield slightly higher in ewes starting in good condition (Maxwell et al., 1979).

10.2.2.6.6.2 Lactation

Responses in milk yield to variations in metabolisable energy are curvilinear in response (Treacher, 1979), however interpretation of experiments is rendered difficult because of differences in protein/energy ratio of the feeds and body condition of the ewes at the onset of lactation and number of lambs suckled. Robinson (1978) indicated that (a) there is a minimum protein intake at a particular ME intake, to support efficient rumen flora function; milk yield will decline if protein intake is less than the minimum requirement, (b) ratio of crude protein to ME increases as milk yield increases and (c) if the ewe has not achieved her potential milk yield, increase in dietary protein at the same ME level will increase milk yield.

The lactating ewe looses a large amount of liveweight during early lactation through conversion of fat deposits for milk synthesis. Estimates of the amount of energy derived from bodyweight loss have varied widely (e.g. Cowan et al., 1981) as did estimates of the efficiency of energy use. Cowan et al. (1981) estimated that approximately 10% of total body protein can be used for milk synthesis during the first few week; whereas in other experiments body protein losses were small (e.g. Cowan et al., 1980).

Nutritional strategies designed to stimulate intake and milk yield in dairy cows by supplying a high protein diet can be associated with reduced fertility. This strategy exacerbates the negative energy balance when rations are high in RDP, increasing plasma urea concentrations that compromises normal embryo development (Butler, 2000).

Responses in yield and protein content of milk to dietary protein (e.g. fishmeal, bloodmeal) have been observed (Gonzalez et al., 1982). These protein sources are regarded as not readily degradable, however degradability increases if intake is low and passage of digesta is slow. Wilkinson et al. (2000) investigated the response in milk yield and quality in lactating ewes grazed on pastures and supplied varying proportions of rumen degradable (RDP) and undegradable dietary protein (UDP) from concentrates in rations formulated as isoenergetic. Increasing concentrations of RDP increased milk yield, protein and lactose but decreased fat concentration; effects of UDP were not significant.

Foot and Tissier (1978) fed high quality herbage *ad libitum* indoors to find that VFI rose rapidly during the first two weeks and then reached a threshold at 6 - 8 weeks of lactation. In contrast, Gibb and Treacher (1978) observed high intakes from the start of lactation that gradually decreased during the next 14 weeks. Availability may limit intake if the initiation of lactation corresponds with the onset of the growing season. Matching pasture availability and quality with lactational potential yield to maximise milk yields needs further consideration. If this cannot be achieved experimentation with protein/energy rations may be worth investigating. For example, Penning and Treacher (1981) offered freshly cut ryegrass *ad libitum* with cereal/protein supplements (barley/soyabean meal, soyabean, fishmeal); addition of the protein supplements alone significantly increased milk yield compared with the unsupplemented control.

In Australia lactating ewes are commonly grazed on pasture. There is a lack of information on intake and response in milk yields and milk quality to nutrients ingested while grazing.

10.2.2.7 Supply of protein supplement and lamb growth responses

Although the majority of lambs in Australia are grown on pasture there may be circumstances where supplementation is required to achieve target carcase weights and the need to restore body condition of the breeding ewe before mating. Dove et al. (1984) supplemented Border Leicester x Merino ewes at varying times during late pregnancy and early lactation while ewes were grazing quality pastures. Though birthweight was not affected by supplementation with oats or sunflower meal, subsequent lamb growth rates were higher, increasing the proportion of lambs achieving market weight in both single and twin categories and ewe liveweight was greater.

Brand et al. (1999) ascribed a lack of lamb growth response to supplementation of ewes with lupin seed during late pregnancy and early lactation to high protein degradability of lupin seed. A response was gained in birthweight and ewe liveweight. Liu et al. (1999) determined the effects of diet (canola meal versus lupin seed) on partitioning of nutrients to skin and muscle in dry, pregnant and lactating ewes. Synthesis rate of protein increased in the skin during lactation but was reduced in muscle during lactation compared with during pregnancy, indicating that muscle protein was being lost while skin and wool follicles used nutrients. Canola meal, which had a lower degradability, had a higher rate of synthesis in skin but lower rate in muscle, compared with the lupin diet. Supply of excess nitrogen (ammonia) to the abomasum diverted protein for milk production to body tissues thus influencing partitioning of nutrients in lactating ewes (Malik et al., 1999).

In ewes fed protein supplements (lupin seed, canola meal) during the last three weeks of pregnancy and the first three weeks of lactation, lambs gained slightly more weight on canola meal but birthweight was greater (480g) when lupins were fed (Masters and Mata, 1996). Dixon et al. (1996) provided additional protein supplement for four weeks before and after parturition and concluded that *post partum* feeding was far more effective in promoting milk synthesis and lamb growth than when provided *pre partum*.

10.2.2.8 Post-natal growth of low birthweight lambs

Greenwood *et al.* (1998) investigated post-natal growth characters of low and high birthweight lambs when reared on high quality liquid diet (*ad libitum*) from birth to 20 kg liveweight. This assessment removed the confounding influences of post-natal factors such as behaviour, dam and environment. Under these conditions, low birthweight lambs grew at rates near equivalent to the high birthweight lambs. Small newborns grew relatively slowly during the first two weeks showing a longer period of adaptation than high birthweight lambs and was the critical period of growth for the pre-natal growth retarded lambs. Digestive capacity of smaller birthweight lambs is at a disadvantage (Trahair et al., 1997) and may have needed more time to adapt to an intermittent diet low in carbohydrate but high in fat (Greenwood et al., 1998).

Fat was deposited at a greater rate than protein (muscle) or (ash) in low birthweight lambs reared at either a low or fast rate compared with high birthweight lambs (Greenwood et al., 1998), although the rate of deposition of fat was slower in the low birthweight lambs reared at a low rate than those at a faster rate, highlighting the partition of nutrients toward lean tissue when nutrients are in short supply. In practice, low birthweight lambs are generally twins. When twins are compared with singles at the same carcase weight they have lower amounts of subcutaneous fat (e.g. Kleemann et al., 1991). These experiments highlight the ease with which carcase composition can be manipulated during early post-natal life and requires further research, particularly if reproductive efficiency is to be improved through an increase in litter size.

Greenwood et al. (2000b) also established that low birthweight had a significant, though small influence on muscle weight during post-natal growth; number of myofibres was not responsible but rather DNA content of the muscle (indicative of myonuclei). Although the quantity of nutrients available to low and high birthweight lambs was restored to a similar level postnatally, low birthweight lambs continued to have decreased DNA at an equivalent muscle weight. They concluded that epigenetic influences, such as nutrient supply, during late pregnancy and early post-natal growth, may influence growth potential and mature size of sheep. Current studies by S. Quigley (personal communication) are elucidating the impact of nutritional variation imposed during early, mid and late gestation on gene expression and control of myogenesis and its influence on postnatal carcase attributes. Initial studies provide evidence for nutritional regulation of myogenic regulatory factors and myostatin expression. These experiments and those of Greenwood et al. (2000b) indicate that the lamb-meat industry's desire for lean meat (Speck et al., 1998), may be achieved by developing an understanding of how myogenesis is regulated prenatally and during early post-natal growth.

10.2.2.9 Relative size of twin lambs at birth and glucose homeostasis

Co-twins can vary appreciably in weight at birth affecting the viability of the growth retarded twin, its subsequent growth and glucose homeostasis (Clarke, 2000). Those co-twins significantly lighter than their counterparts (>25%) exhibited differences in glucose homeostasis at one and six months of age, but not at three months. Onset of rumination and endocrine changes associated with adaptation to a high-roughage, low-fat diet (Symonds et al., 1989), maturation of islets of Langerhans and initiation of GH dependent growth (Min et al., 1999) could have differed between treatment groups but remains to be studied. Any affect of light compared with heavier co-twins lambs on glucose homeostasis was transient as glucose tolerance tests in 12-month old lambs were similar between treatment groups.

In another study, glucose and insulin metabolism of Merino lambs was influenced by prenatal nutrient restriction imposed on ewes through varying seasonal conditions of Australia's Mediterranean environment (Cronje and Adams, 2002). Poor pre-natal nutrition reduced glucose response to insulin challenge in lambs at eight months of age.

10.2.3 Periparturient relaxation of immunity (PPR) and nutrition

Acquisition of immunity, that is the process by which the immune system recognises the foreign agent (parasite) and the immunological changes that precede an effective immunological response, is not affected by host nutrition except in the case of severely undernourished animals (Coop and Kyriazakis, 1999). However it is the expression of acquired immunity that can affect parasite establishment, development and survival (Quinnell and Keymer, 1990). In bacterial or viral challenges there is evidence that host nutrition can influence levels of circulating antibodies (Sinclair and Reid, 1999).

Late pregnancy and early lactation are stages that impose a dramatic increase in nutrient requirement. PPR corresponds with this period of nutrient drain toward fetal growth and milk synthesis and, as such, are functions that are prioritised over the expression of immunity. PPR has been attributed to a number of factors; seasonal effects, stress of parturition, immune suppressive effects of hormones such as prolactin, progesterone/oestrogen and corticosteroids (e.g. Barger, 1993). It is only recently that a nutrition role has been proposed and investigated (Coop and Kyriazakis, 1999). For example, Donaldson et al. (1998) fed twin bearing ewes a base pelleted diet plus hay and supplemented with varying levels of fishmeal eight weeks prior to parturition. Six weeks prior to lambing each ewe was infected with two species of parasitic larvae daily. Half of the ewes in each treatment group were challenged with a dose of the two parasitic species approximately two weeks after lambing. Dietary protein supply was negatively associated with worm burdens for both species of parasite, suggesting that (a) higher levels of protein supplementation improved control of incoming larvae and (b) nutrition, and notably protein, was able to influence PPR (Coop and Kyriazakis, 1999). Methods of reducing protein degradation in the rumen and improving protein availability are worthy of study; responses may however vary with breed (Wallace et al., 1995) and may be specific to particular amino acids (Miller et al., 2000).

10.3 POST-WEANING GROWTH

It is most efficient from a production point of view to finish lambs on pasture, however, there may be circumstances where lambs cannot be grown to slaughter weights such as during a seasonal decline in pasture quality. Hence, some form of supplementation may be necessary to grow lambs to achieve a finished weight. It is not the intent of this review to provide an in extensive insight into the requirements to achieve post-weaning growth in meat lambs. Suffice to say that weaner growth and mortality can be impacted on by a number of factors the major ones being parasitism (e.g. Thompson and Callinan, 1981; Brown et al., 1985) and nutritional.

Development of immunity to internal parasites does not usually occur in young grazing sheep until the second year of life (McLeod, 1995) and susceptibility remains a problem. Kahn et al. (2002) examined if oral vaccination with infective *Trichostrongylus colubriformus* larvae and protein supplementation improved development of resistance in lambs. Pen studies confirmed that both treatments increased resistance while protein supplementation only was successful in the field.

Performance of lambs offered various foodstuffs other than green pasture will be discussed in more detail in Chapter 11. Particular aspects are discussed in the literature by the following authors; senescent summer pasture (e.g. Purser, 1981; Kenny, 1984; McLaughlin, 1973; Holst et al., 1996a), legume and cereal crop stubbles (e.g. Mulholland et al., 1977; Hynd and Allden, 1986;

Rowe and Ferguson, 1986), fodder crops and perennial species (e.g. Wheeler, 1981; Kenny and Reed, 1984; Hopkins et al., 1995a,b; Holst et al., 1997; Dove et al., 2002) grain supplements (e.g. Foot et al., 1983; Hynd and Allden, 1986; Dixon and Egan, 2000; Hopkins et al., 2001) and lick blocks (Cottle, 1988). Effects of substitution on efficiency of utilisation of grain and roughage offered to ruminants have been discussed in detail by Dixon and Stockdale (1999). Potential methods for control of acidosis are given by Brown et al. (2002).

10.4 BIOLOGICAL EFFICIENCY AND LAMB GROWTH

Three major determinants of biological efficiency of meat production are the annual feed intake of the ewe, net reproductive rate (lambs slaughtered of ewes joined) and growth rate of the progeny (Large, 1970; Kleemann et al., 1984; Thompson et al., 1985a,b), with growth rate being of lesser importance if lambs attain target slaughter weights before the seasonal decline in pasture quality. In a first-cross lamb enterprise compared with a purebred Merino system, an increase in biological efficiency was due to increased growth rate of the first-cross progeny, while a second-cross lambing enterprise achieved an even higher biological efficiency value due to an increase in both reproductive output and individual lamb weight at slaughter (Thompson et al., 1985a,b).

10.5 SUMMARY

The main points arising from this Chapter are:

(1). Australia’s successful lamb-meat industry has developed a tiered breeding structure based on the Merino.

(2). The majority of lambs slaughtered are second-cross (40%) with significant numbers coming from first-cross (25%) and purebred Merino (20%) sources.

(3). Growth rate differences between purebred Merino and first cross lambs and between first-cross and second-cross lambs are substantial (15 - 20% and 25 - 30%, respectively).

(4). Large differences exist within breeds for growth rate and fat status. Industry has taken advantage of this genetic variation through the agency of a national scheme (LAMBPLAN) available to both terminal sire and maternal breeding sectors.

(5). Mammary development and subsequent milk yield can be influenced by overfeeding of ewe lambs.

(6). Overnourished post-pubertal ewes can have reduced yield and concentration of colostrum.

(7). The high metabolic challenge placed on the ewe in late pregnancy and early lactation requires a number of metabolic adaptations to meet the demand for nutrients; these are met from both endogenous and exogenous sources. Adaptations involve increased liver gluconeogenesis, reduction in use of glucose by peripheral tissues, NEFA mobilisation from fat deposits with increased use in peripheral tissues, reduced amino acid catabolism but increased muscle proteolysis. Regulation of these adaptations is effected through altered tissue responses to hormones (insulin) and adrenergic substances. Increases in insulin resistance spare glucose and enhance NEFA and amino acid mobilisation.

(8). Placental lactogen appears to have a key role in synergising with steroid hormones, GH and possibly prolactin in promoting mammary development.

(9). GH levels peak at parturition and continue at elevated levels during lactation; GH decreases lipogenesis in adipose tissue possibly through opposing the action of insulin.

(10). Milk yield of first-cross ewes is 25 - 30% greater that that of the Merino, independent of lamb genotype. Even larger differences in milk yield have been observed between ewes suckling twins compared with those suckling one lamb (30 - 35%). Breed differences in milk composition are thought to be small.

(11). Milk yield of multiple rearing ewes is determined largely before parturition through the effect of increased placental mass and placental lactogen on mammogenesis.

(12). Maintenance of high milk yield during the second and third months of lactation is required for sustaining high lamb growth rates.

(13). An appropriate nutritional strategy for a ewe to achieve adequate milk yields is to supply a minimum protein intake to support efficient rumen flora function. To achieve high milk yields there is a need to supply an increased ME level and a higher ratio of dietary protein to ME concentration.

(14). It has been noted in dairy cattle that feeding high levels of dietary protein may well exacerbate the negative energy deficit through an energy requirement to handle excess urea concentrations, and may also incur fertility problems.

(15). Supplementation of ewes with high protein supplements has produced variable responses in lamb growth.

(16). Studies with low and high birthweight lambs have indicated the ease with which carcase composition can be nutritionally manipulated during early post-natal life.

(17). Expression of an immunity acquired through exposure to internal parasites can be influenced by nutrition during the periparturient period when function of the maternal immune system is suppressed. Supply of high levels of dietary protein has improved control of incoming parasite larvae.

CHAPTER 11. MANAGEMENT STRATEGIES TO IMPROVE LAMB PRODUCTIVITY

11.1 INTRODUCTION

Economic returns from lamb-meat production are determined by the amount and quality of product generated and by the costs of inputs employed. Lamb survival and growth rate are important components of the amount of product generated and are aspects that can be controlled to some degree by management. This Chapter addresses options that may be considered for reducing neonatal loss and for increasing growth of lambs to achieve target slaughter weights.

11.2 NEONATAL LOSS

11.2.1 Nutrition

Monitoring ewe weight or body condition during pregnancy and adjusting the dietary regime accordingly is worth contemplating since lamb survival, possibly survival of the ewe and potential milk yield, are at stake. Placental growth is positively correlated with growth of the fetus and placental size is mainly determined by the end of the second trimester. Evidence from the literature (see Chapter 9.6.2.2 and 9.6.2.3, and Appendix Table 1.) indicates that ewes that are overfat at mating (body condition score of 4 and greater; Figure 1a) need to loose weight gradually (0.7 – 0.8 of maintenance requirements) during the first 2 - 3 months of pregnancy to achieve a body condition score 3. Ewes that are in low condition (body condition score 2 and less; Figure 1b) require adequate nutrients to gain weight during this period (1.2 – 1.5 of maintenance requirements) to develop normal size placentae. Ewes in good body condition at mating (body condition score 3.0 – 3.5; Figure 1c) and fed near maintence (0.9-1.0) may produce normal size placentae. The body condition and liveweight of these ewes is presented in Figure 2. If ewes are fed to meet their nutritional requirements during late pregnancy; normal size lambs should result. Ewes that are in poor condition at mating (body condition score 2 and less) and are poorly fed during early-mid pregnancy (0.5 – 1.0 of maintenance requirements) may have small placentae and extra feeding during late pregnancy will not give normal size lambs.

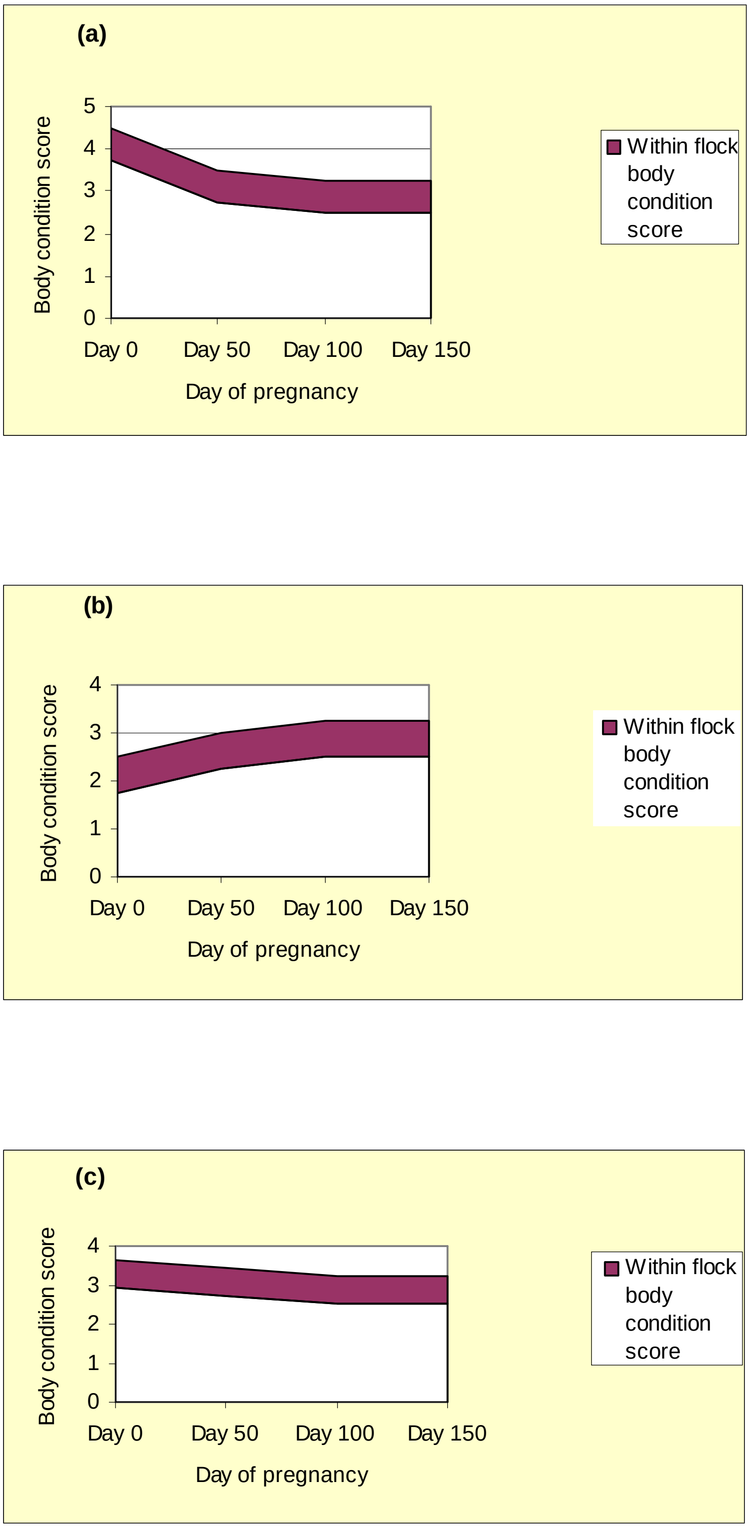

Figure 1. Diagrammatic representation of the relationship between body condition score and stage of pregnancy in the ewe optimising placental growth during mid gestation and fetal growth in late gestation for flocks in (a) overfat (b) poor (c) and good condition at mating.

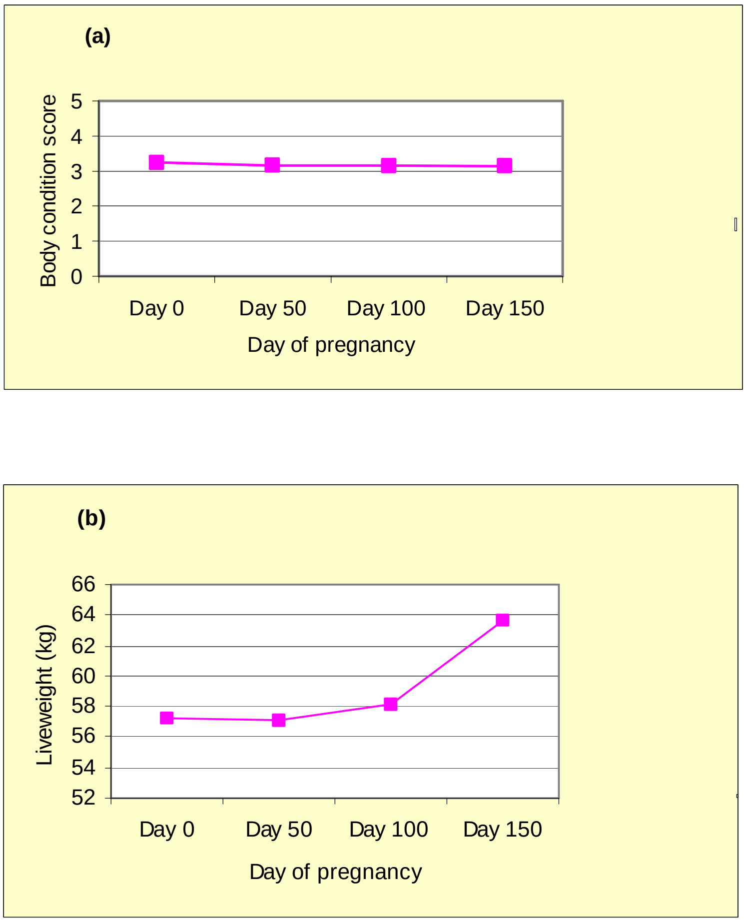

Figure 2. Changes in (a) body condition score and (b) liveweight in South Australian Merino ewes fed to meet nutritional requirements of both the ewe and her fetuses throughout gestation (data modified from Kleemann and Walker 1992; McNeill et al 1997c; Greenwood et al 2000a).

Detection of ewes that are bearing single, twin and triplet fetuses may make nutritional management of these groups easier and may avoid inefficient use of feed resources during late pregnancy and lactation. A short mating period of 5 - 6 weeks will ensure that the majority of ewes will lamb in a reasonable period of time. Detecting those ewes that return to oestrus or by aging fetuses at scanning (Kelly and Newnham, 1989; Sergeev et al., 1990) may further enable refinement of the feeding program.

The value of pregnancy testing and diagnosing litter size will depend on the proportion of non-pregnant ewes and on the proportion of single- vs multiple-bearing ewes. The ability to separate non-pregnant ewes and remate and feed at maintenance level is beneficial when pregnancy rate is low. Similarly, the benefit achieved from managing multiples separately from singles is reliant on a reasonable incidence of multiple bearing ewes. There is little information available defining perceived benefits of increasing lamb survival of singles and multiples and improving lamb growth by managing the groups separately. A preliminary study by Fowler and Thompson (1986) on a limited number of ewes, with twin ewes given preferential nutritional treatment, observed a 22% improvement in twin lamb survival (0.73 vs 0.89) and 10% increase in twin lamb growth. Anecdotal evidence in New Zealand (McAtamney and McAtamney, 1999) indicated that lambing percentage increased by 18% over a five-year period after adoption of real-time scanning to improve management of the breeding flock.

Scanning has been widely accepted in New Zealand as a management tool (Farmer and Davis, 1999) with adoption in a large proportion of commercial flocks. For example, in 1998, 92 ultrasound operators were estimated to have scanned 7 - 8 million ewes (about 40 - 45% of total breeding ewes) on the South Island with almost all properties identifying ewes carrying singles and twin/multiple births with a significant number of properties identifying triplets. Over the last decade, adoption has been facilitated by achievement of highly accurate diagnosis, developed through experience and improved scanning equipment and development of a streamlined system for ewe delivery to the scanning operator.

Perceived benefits of real-time scanning, additional to better use of the feed resource, are reductions in the incidence of ketosis, provision of strategic shelter for the more vulnerable multiple born lambs, reduced dystocia in single lambs and the ability to select ewes and rams of known litter size. The value of real-time scanning was evaluated in Australia using a simulation model that was based on physical data generated in a wool producing system (Bowman et al., 1989). The authors modelled a self-replacing Merino breeding flock based on physical data recorded in western Victoria. Ewes lambing in autumn provided greater economic returns from scanning than from those ewes lambing in spring when pasture in autumn was more likely to be limiting. Economic returns were also greater as the proportion of twins increased in the flock. Farm profit was more sensitive to change in the amount of wool grown than an equivalent variation in the number of lambs sold. The authors also recognised other opportunities that could result from scanning such as increased selection pressure to increase twinning, reduced reproductive wastage and increased wool grown. Given that market conditions and input costs have changed since the study of Bowman et al. (1989) and that the study was directed at the wool growing enterprise, it would be of interest to repeat the study using current data and focusing on sheep meat producing systems.

Indeed Holst et al. (1996a) indicated that where supplement was provided to single and twin bearing ewes grazing together during late pregnancy and early lactation, twin bearing ewes ate more supplement and less pasture than single bearing ewes suggesting that separation under these circumstance may not be beneficial. Further investigations are required under field conditions to ascertain if single and multiple bearing ewes managed separately under various regimes of feeding and shelter give quantifiable responses in neonatal survival and subsequent growth. Physical data of this type is required so that an evaluation of real time scanning can be done using simulation models such as that developed by Bowman et al. (1989).

11.2.2 Lambing systems

Systems of lambing vary from set stocking ewes in large paddocks, typical of those found in pastoral regions, to drifting unlambed ewes into a separate paddock daily, to ewes lambing in pens under close supervision. In industry, the degree of supervision varies from minimal (or zero) to perhaps twice daily inspections rendering assistance to ewes as required (Ferguson, 1982). A drift lambing system improved lamb survival compared with an unsupervised system (87% vs 79%) but in a further study by Tyrrell and Giles (1974) of supervised versus non-supervised, yielded no difference in lamb survival (85.4% vs 84.1%). The degree of supervision will depend on the anticipated problems associated with difficult births that may vary due to breed and seasonal conditions and the proportion of ewes bearing single fetuses. In Merino flocks with a high incidence of twins, however, disturbance of the bonding process between mother and her twin offspring could increase mortalities through permanent separation of the ewes and her lamb/s.

Systems of feeding to reduce disturbance at lambing have not been investigated extensively. A significant proportion of Merino breeding flocks in southern Australia are lambed in the autumn during a period of feed supply that is usually poor in quality and low in quantity; supplementation with hay and/or grain is practised and may lead to significant permanent separation of lambs, particularly twins (Buttrose et al., 1992). Feeding at night, broadcasting lupin grain over the lambing paddock or strategically placing a number of large round bales of good quality hay in the paddock before the onset of lambing (Biggins et al., 1992), synchronising lambing to reduce the feeding period and conserving feed in specifically allotted lambing paddocks are worthy of further investigation. Minimal disturbance of the ewe and her lambs and establishment of a strong bond between them is the key principle behind development of successful lambing systems.

There is evidence that a significant proportion of twin bearing Merino ewes may have insufficient quantities of colostrum to supply their lambs to meet energy requirements (see Lindsay et al., 1990). In addition to insufficient supply of colostrum for twin lambs, immunoglobulin and energy concentrations may vary between ewes (Lindsay et al., 1990). Short-term feeding of high protein grain can stimulate colostrum production though Banchero et al. (2002) have suggested that metabolisable energy rather than protein may play a key role, though a definitive study is required to clarify the issue. Further study of the relationship between colostrum and lamb survival is now possible given that McNeill et al. (1998b) have established an indirect measure of colostrum production (blood lactose).

11.2.3 Provision of shelter

Protection from cold and windy weather in southern Australia and relief from heat exposure in northerly pastoral regions may reduce lamb deaths, particularly among the more predisposed twin lamb offspring. Choosing lambing times to avoid these weather extremes may not always be possible so that consideration should be given to providing lambing paddocks with adequate shelter. Alexander et al. (1990) studied birth site distribution of Merino and British breeds to identify characteristics that may help to locate preferred areas for lambing. Merinos appeared to lamb in a random pattern when paddocks were level or near level but in sloping paddocks, birth sites were commonly aggregated on elevated areas. In contrast, British breeds preferred to lamb along fence lines with less tendency to seek elevated sites. Alexander et al. (1990) suggested that provision of shelter in paddocks at these preferred positions could significantly reduce lamb mortality.

Inducing ewes to seek shelter by shearing them prior to lambing does reduce lamb mortality, particularly among twins (Alexander, 1980). Use of gramineous windbreaks in addition to shearing has also proven beneficial to both single and twin lamb survival (Alexander et al., 1980; Lynch et al., 1980).

Exposure of lambs to hot weather may be unavoidable in the tropical pasture areas where the preferred time of lambing is late summer and treeless grassland areas are the norm (see Kleemann, 1999). However, where shade has been provided, such as during the summer months in Western Australia, lamb survival was improved (Morgan et al., 1972). Placement and distribution of watering points and its influence on lamb survival remain to be studied; responses are more likely to be gained when lambing occurs during hot weather and when ewes are grazing senescent pastures and supplements. Clearly, attention to choice of paddocks with features conducive to improving the microclimate of newly born lambs, such as good pasture cover and strategic placement of shelter in areas that ewes are likely to prefer for lambing could be advantageous for increasing neonatal survival.

11.2.4 Stocking density

Increased density of stocking has been shown to reduce lamb survival due to increased interest of pre-parturient ewes in newly born lambs and resultant stealing and mismothering of lambs (Alexander et al., 1983a), whereas other studies have not observed a relationship between mismothering and stocking density (e.g. Winfield, 1970). Investigations by D. Fowler (see Lindsay et al., 1990) indicated that as lambing rate per day decreased and paddock size increased, survival of twin lambs improved. These aspects, as well as other features of the lambing paddock that may influence stocking density of pre-parturient ewes, are worthy of study.

11.2.5 Training and experience of the ewe

Training of ewes to accept strange surroundings and events has been shown to reduce emotivity and to improve the ewes' ability to mother at lambing (Putu, 1980 see Lindsay et al., 1990). Training consisted of feeding the ewes at weekly or fortnightly intervals, walking amongst the flock and feeding by hand. Though the practicality of adopting a conditioning program may not be possible (Lindsay et al., 1990), nevertheless, opportunities to give positive reinforcement and avoid negative experiences should not be dismissed.

11.2.6 Selection criteria

Heritability estimates of the lamb's own ability to survive are low (Piper et al., 1982; Fogarty, 1995), leaving little scope for increasing lamb viability through direct selection. Indirect selection criteria such as birthweight and birth coat have been advanced as possible candidates; heritability of birthweight is of the order of 0.2 - 0.3 but genetic correlations with survival are small, so genetic improvement using this trait is probably near zero or possibly negative given the curvilinear relationship between birthweight and survival (Piper et al., 1982). Birth coat is highly heritable in the Merino but like birthweight has a low genetic correlation with survival (Ponzoni et al., 1997). There is a good association between resistance to cold stress by the lamb and its udder-seeking behaviour (Slee and Springbett, 1986). Information on genetic correlations between resistance to cold (moderately heritable) and behavioural traits is required (Hinch, 1997).

The second genetic component of lamb survival is that attributable to the mother, an ability to bond and rear her offspring, partly measurable as the number of lambs weaned to the number born (rearing ability). Haughey and George (1982) and a number of other authors have shown that culling of ewes on rearing ability would significantly improve current flock performance. Repeatability estimates given by Piper et al. (1982) for two flocks were 0.16 and 0.15 when two year's records were used versus 0.05 and 0.12 for one record. Heritability estimates for rearing ability were 0.13 and 0.19, suggesting that lamb survival could be improved by selecting for rearing ability. A summary of genetic parameters given by Fogarty (1995) provided a mean heritability estimate of 0.07, which is within the range reported by Ingham and Ponzoni (2000) of 0.00 - 0.08. Accurate measurement of the trait requires identification of lambs with their mother at birth or by constructing maternal pedigrees using DNA technologies. Identification of quantitative trait loci (QTL) for lamb survival may be a possibility in future years though according to the list of traits being searched in sheep, survival is not listed (Crawford, 2002).

In summarising genetic parameters for sheep Fogarty (1995) found that genetic correlations between reproductive component traits (fertility, litter size, lamb survival) were highly variable; fertility and survival –0.07 to 1.00 and litter size and survival -0.73 to 1.00, indicating that selection for a component trait such as fertility would not necessarily change lamb survival. A study of genetic parameters for lamb survival and associated maternal and lamb behaviours indicated that lamb survival to three days was a heritable maternal trait but not that of the lamb. Genetic correlations of note were length and ease of birth in single lambs and incidence of ewe-lamb separation in twins (Alexander and Stevens, 1992; see Hinch 1997).

Indirect selection criteria have been proposed to improve lamb survival since use of direct selection criteria is thwarted by logistical problems associated with data collection on large numbers of animals for a behavioural trait exhibited in the field and because the trait is sex linked. Indirect selection criteria that have been proposed, such as ‘emotive’ tests or ‘temperament tests’, cortisol levels and pelvic conjugate diameter. For example, Murphy et al. (1994) reported high repeatabilities (0.52 and 0.77) for the relationship between maternal behaviour in the Merino and temperament recorded at weaning, while R. Kilgour (personal communication) has proposed that temperament and pelvic conjugate diameter be investigated as indirect selection criteria. Although further studies are required on estimation of genetic parameters these traits can be measured in both males and females and possibly used in selection prior to ewes and rams entering the breeding program. Tests need to be developed that can be widely adopted by industry.

11.2.7 Predation

Although predation can be over-estimated as a source of lamb loss because secondary predation is often confused with primary loss (killing of a viable lamb), flocks on individual properties may loose significant numbers of lambs through primary predation. Where this occurs strategic baiting programs can be instigated and are likely to be more effective if the programs are implemented on a communal basis. For example, baiting for foxes is encouraged by local shire/council authorities.

11.3 POST-NATAL GROWTH

11.3.1 Pre-weaning period

Early growth of the lamb during the first few weeks of life is almost solely dependent on its ability to consume milk and on the mother to provide sufficient milk for nourishment and for the lamb’s acquisition of a passive immunity. These factors are influenced by events that occur before and after parturition at a time when dramatic physiological changes to the metabolic and hormonal environments of the ewe and lamb are invoked. Nutritional adequacy at these times is critical not only for optimising survival of the lamb but for ensuring a good milk supply and high growth rate in lambs destined for the lamb-meat industry. Although the ewe has the ability to buffer nutrient deprivation through mobilising fat reserves and to a lesser extent protein reserves, the metabolic drain on nutrients in ewes supporting multiple fetus is particularly high and requires special consideration when supplying dietary nutrients. Perhaps one of the greatest needs within the lamb-meat industry is to attend to the nutritional requirements of the Merino ewe supporting twin lambs. The ewe's ability to supply sufficient milk for the twin lambs to attain slaughter weight is paramount, as is her capacity to recover body tissue reserves for the next breeding season.

High growth rate in lambs may not always be desired since a strategy to achieve leaner carcases may require lambs to grow to a target weight at an older age (Hegarty et al., 1999; Hall et al., 2001). The merit of using this technique as a management tool will depend on seasonal differences in lamb price and the costs associated with keeping the lambs on the property longer. Preferential treatment can now be given to twin bearing ewes given the advent of real-time scanning. The economic benefits of using this technique in the wool growing industry has been established but monetary value for the lamb-meat industry needs to be tested under a range of grazing and management conditions.

The greatest nutritional needs of the ewe occur several weeks before and after the period of parturition. The most efficient management tool available to meet these nutritional requirements is to match pasture availability with the grazing needs of the breeding ewe and her lambs. For example, in South Australia, Merino ewes are traditionally mated to lamb in the autumn usually on poor quality pasture and cereal stubbles. In less than a decade, more than 30% of Merino breeders decided to delay mating of ewes so that ewes lambed in winter/early spring at a time when nutritional needs of the lambing flock were better matched with availability of paddock feed (Krause et al., 1996; B. Ashton, personal communication).

The optimum time of lambing for any region will be mainly determined by the length of the pasture growing season, though stocking rate can have a large impact on the proportion of lambs attaining marketable weight. The interaction of time of lambing and stocking intensity reported by Kenney and Davis (1974) indicates that early-summer weight of lambs born early but stocked heavily was similar to that of lambs born late but stocked lightly.

Early lambing may not always be indicated for finishing lambs before the end of the growing season as Lloyd Davies (1965) reported that poor early growth of lambs born in the autumn were unable to compensate in growth relative to winter-born lambs; liveweights in January were 26 vs 31 kg respectively. In contrast, Freer et al. (1994) established that an earlier mating in early February versus mid March enabled more prime lambs to reach a target liveweight before pasture quality deteriorated in November. Donnelly et al. (1985) concluded that the major variable controlling lamb production in their studies was the length of time between birth and senescence of the pastures. High growth rate of lambs is best achieved on pastures with a significant proportion of clover versus grass (40%) set stocked at a moderate rate (e.g. Reed 1972; Fitzgerald, 1976).

11.3.2 Post-weaning period

Weaning of lambs constitutes, by definition, a break in contact between the ewe and lamb. Under natural conditions, the social bond is attenuated in a progressive manner. Orgeur et al. (1999) examined hormonal, behavioural and immune consequences of either total or partial separation of Ile-de-France ewes and their lambs. Partially separated animals vocalised more frequently and for a longer period. Cortisol levels were not affected by weaning treatment but excretion of coccidial oocytes increased in totally separated lambs while growth rate of the latter was lower than that of other lambs. Negative consequences were considered to be small.

Weaning can be imposed as early as three weeks of age (e.g. Langlands and Donald, 1975) but lambs require high quality solid foods to survive and to subsequently grow. The consequences of weaning second-cross lambs at various ages (3, 5, 7 and 9 weeks of age) from dams fed drought rations of wheat or oats with varying levels of lupin grain plus hay have been studied (Kenney, 1987). Lupin grain was considered a desirable supplement to cereal grain rations for early weaned lambs but weaning earlier than at five weeks of age was considered undesirable.

In the wool industry, Merino lambs are normally weaned at 3 - 4 months of age but need to grow to a minimum liveweight before entering the summer months if they are to survive while grazing poor quality pastures that may at best maintain or slightly increase liveweight. For these animals, supplementation may not be cost effective as they should exhibit compensatory growth during the following winter/spring flush of pasture. In contrast, lambs grown for meat production may need to be finished during the summer-autumn period and before eruption of the lamb’s permanent incisors (definition of lamb versus hogget) at about 12 months of age or older.

Anthelmintic treatments for control of internal parasites (McLeod, 1995), avoidance of contaminating the lamb’s pelt with grass seed (Holst et al. 1996b) and shearing are important issues to be considered for maintaining growth rate and product quality. Weaners grazing senescent dominant clover pastures in summer may, at best, slowly increase liveweight (e.g. Allden and Geytenbeek, 1984). Conserving good quality pasture hay may maintain its quality over summer and improve growth rates (Kenny, 1984) but regeneration of clover in the following year may be a problem. Regrowth from early cut hay and increased quality of hay may be beneficial to weaners but inclement weather usually prevails during conservation. Cereal and legume crop stubbles, green summer crops such as lucerne and white clover and grain supplements will generally give greater lamb growth rates than those achieved on good quality conserved hay and in situ clover residues. Further discussion on the relative benefits of these sources of feed for lamb growth is beyond the scope of this review.

Variation in consumption of supplements by lambs may be influenced by a number of factors including previous experience, type of supplement, pasture availability, social interactions and method of feeding. These factors have been reviewed by Bowman and Sowell (1997). Financial implications of managing various lamb production systems can be examined using the approach of White et al*.* (2002), generating pasture growth rates with the ProPlus model (McPhee et al., 1998) and animal performance information via Grazfeed (Freer et al., 1997).

11.4 SUMMARY

The important points that arise from this Chapter are:

(1). Nutrition is a factor that can have a large influence on lamb survival, and that can be controlled to some extent by management.

(2). Strategies to optimise lamb survival nutritionally centre on an understanding of the relationship between mortality and birthweight; the so called “J curve” where extremes of nutrition will result in increased mortality due to either small or large size lambs.

(3). Size of the placenta, under most circumstances, determines growth of the fetus and varying strategies to generate normal size placentae are indicated.

(4). Nutritional requirements of ewes supporting multiple fetuses differ substantially from those carrying singles. The benefits of preferentially feeding multiple bearing ewes on-farm need further investigation, given that such ewes can be identified accurately via scanning on a commercial basis.

(5). Minimal disturbance of the ewe and her lambs, needed to establish a strong bond between mother and offspring, is the key principle governing the development of successful lambing systems. Further studies are required to develop systems of feeding at lambing and to determine the relationships between lamb survival and colostrum production and quality under field conditions.

(6). Choice of lambing paddocks with features conducive to enhancing the microclimate of new born lambs should reduce lamb loss due to cold exposure.

(7). Relationships between stocking density, frequency of lambing, litter size and paddock size *per se* and neonatal loss are not well understood.

(8). Promising indirect selection criteria have been proposed for improving lamb survival but need further development for widespread adoption by industry.

(9). Strategic baiting programs may control primary predation.

(10). Basic and strategic research is required on nutritional management of the Merino ewe rearing twin lambs to enable her to supply adequate milk for her lambs and to recover body reserves needed for the following breeding season.

(11). The most efficient management tools to meet the high nutritional demands of the breeding flock are to match the animals' nutrient requirements with pasture availability, adjustment of stocking rate and attention to major factors governing pasture growth and quality.

(12). Devising nutritional systems for maintaining adequate lamb growth rates when pasture quality declines, amelioration of grass seed problems and control of internal parasites are the major factors that need to be considered during the post-weaning period.

CHAPTER 12. OVERVIEW AND RECOMMENDATIONS

The following points provide an overview of the findings from Chapters 9 - 11 on aspects of neonatal mortality and post-natal growth in sheep.

12.1 EXTENT OF NEONATAL LOSS

Commercial Merino flocks in Australia have a high lamb mortality rate of 20 - 30%, with twin lamb mortality estimated at 30 - 40%. This situation is unacceptable for a number of reasons including an estimated annual loss nationally of $250 million.

Recommendation. That strong support is given to conduct research and provide educational programs to reduce lamb mortality in Australia.

12.2 CAUSES OF LAMB MORTALITY

Causes of lamb mortality are many and varied depending on the flock, season, region and standard of management. Major losses may be accounted for by one or two factors (e.g. cold stress) with remaining mortalities caused by a number of factors that cannot be clearly defined. The complex nature of the problem and the need to investigate on an individual flock basis may lead managers to accept that little can be done to reduce mortalities.

Recommendation. Industry support has been given in the past to facilitate educational and investigative programs in regional locations severely affected by lamb mortality. Development of any future programs should build on experiences gained from programs conducted in the past.

12.3 CONTROL OF PARTURITION

Although there is a better understanding of the physiological basis for the initiation of parturition, the practicality of lambing ewes in a synchronous period, on a commercial scale for normal breeding purposes, is difficult to justify.

Recommendation. Basic research on neonatal physiology should be encouraged because the events occurring during parturition are critical for survival of both the lamb and its mother.

12.4 SELECTION FOR LAMB SURVIVAL

Long-term genetic programs required for estimating genetic parameters for lamb survival and other traits have confirmed earlier reports that the values for heritabilities of the maternal component and particularly the lamb component, are low and that progress in selecting for this trait would be slow. Direct selection is currently logistically difficult but further testing of indirect selection criteria suitable for easy adoption by industry is required. Lamb survival of the Merino is low compared with specialist meat-producing ewes and, given its numerical importance to the lamb-meat industry, should be investigated in preference to other breeds. Currently, seedstock suppliers have the opportunity to place some selection pressure on lamb survival when formulating their selection index using the national LAMBPLAN scheme.

Recommendation. That promising research into identification of indirect selection criteria for lamb survival be strongly supported and that investigations be focused on the Merino.

12.5 NUTRITION AND NEONATAL SURVIVAL

Most research until recently has concentrated on nutrition of the ewe during the last trimester of gestation at a time of rapid fetal growth. More recent studies have indicated that nutritional and other perturbations of the embryo’s very early environment may have consequences for placental and fetal development and growth, and viability of the young. Further understanding of mechanisms controlling development and growth of the placenta and fetus and the interaction with nutrition during pregnancy would be rewarding.

Recommendation. That strong support is given to basic research programs that provide further understanding of mechanisms regulating development of the feto-placental unit.

12.6 BEHAVIOUR AND NEONATAL SURVIVAL

The quality of behavioural interactions between the ewe and her lambs is critical for the lambs’ survival. Knowledge in this field has increased substantially but reasons for the sustainability of maternal behaviours and basic reasons for the relative inability of the Merino to successfully bond with two lambs are unknown.

Recommendation. That basic research programs addressing maternal and lamb behavioural inadequacies of the Merino be given strong support.

12.7 REAL TIME SCANNING AND NEONATAL SURVIVAL AND GROWTH

The economic value of real-time scanning as a management tool for giving preferential treatment to multiple bearing ewes (e.g. nutrition, supervision at lambing, strategic shelter) has been evaluated using simulation models for wool growing enterprises but has not been assessed thoroughly for sheep-meat production systems. This technique has been widely adopted by sheep meat producers in New Zealand.

Recommendation. That an economic evaluation be conducted of real-time ultrasound scanning as a management tool to improve lamb survival and growth in Australian lamb-meat enterprises.

12.8 LAMBING SYSTEMS AND NEONATAL SURVIVAL

It is generally accepted that lambing systems and systems of feeding be devised on the principle that the establishment of a strong bond between the ewe and her lambs is dependent on the minimal disturbance at the birth site. Satisfactory systems of deploying feed, distributing water, strategic placement of shelter near preferred lambing areas are worthy of investigation.

Recommendation. That some support be given to evaluating promising lambing systems and methods of feeding to increase lamb survival. Explanation of current principles and knowledge of lambing practices in educational programs would be beneficial.

12.9 SELECTION AND POST-NATAL GROWTH

Selection within the seedstock of all breeds used in the lamb-meat industry for enhanced growth and control of fat status has been facilitated by a national program (LAMBPLAN) with significant improvements observed in the dominant breeds. Potential for further improvements is large.

Recommendation. That strong support is given to facilitate genetic improvement of growth and desired fat status in Australia's sheep meat breeds.

12.10 NUTRITION AND POST-NATAL GROWTH

High growth rates in suckled lambs destined for the lamb-meat trade requires a copious supply of milk achieved through supply of high quality nutrition during mammary development in late pregnancy and during lactation. The most cost efficient supply of nutrients is provided through quality pasture, improvement of which has not been the subject of this review, but is of obvious importance to the lamb-meat industry. The effectiveness of regulating lamb growth rates through a change in stocking rates or by varying the time of lambing is dependent on seasonal conditions and locality of the operation. There may be opportunities within current management systems where short-term feeding of high protein grain say immediately before and during early lactation that may be financially rewarding, particularly if fed to twin bearing ewes at a synchronised lambing.

Recommendation. Further experimentation with short–term supplementation of high protein grain before and after parturition in ewes carrying multiple lambs to enhance post-natal growth is worthy of consideration.

12.11 NUTRITION AND CARCASE COMPOSITION

There is good evidence that nutritional deprivation imposed either pre-natally or in early post-natal life can have significant effects on carcase composition at later ages and that an understanding of how myogenesis is regulated during this early stage of life may help us manipulate muscle growth to advantage in lamb-meat producing systems

Recommendation. That basic studies into nutritional regulation of myogenesis during gestation to ultimately enable manipulation of carcase quality postnatally would be a fruitful area of research.

Appendix Table 1. Summary of experiments that examined placental and fetal responses to varying nutritional regimes imposed on ewes during the three trimesters of pregnancy.

Nutritional treatments imposed during pregnancy	**Responses in placental and fetal growth**	**Source**
High and low nutrition groups fed to increase and decrease liveweight by 25% throughout pregnancy. Approximately 12% liveweight gained or lost by day 90.	At day 90 fetal weight were 490g and 548g for low and high groups; corresponding cotyledon weights were 341g and 490g.	Everitt (1964)
Four treatments using 2 year old Corriedale ewes. Control fed to meet maternal maintenance requirements (MM), and other groups given moderate dietary restriction from day 50-100 (RM), day 100-135 (MR), day 50-135 (RR). Diet was lucerne hay: oat grain (3:2 ratio) pelleted. Maintenance group fed 900g /day (44kg liveweight).	On day 135 weights were: Placenta (g) Fetus (kg) MM 337 3.36 MR 437 3.31 RM 485 3.78 RR 413 3.03 Moderate dietary restriction stimulated placental growth to the extent that fetal growth could be maintained when restriction occurred during the last trimester. Extra placental capacity in ewes restricted in second trimester resulted in enhanced fetal growth when nutrient supply was increased in late pregnancy.	Faichney and White (1987)
Low (L) and high (H) nutritional treatments (L <100kg DM/ha: H > 1000kg DM/ha) imposed on three ewe genotypes (two highly fecund) for 4 weeks starting about day 75 of pregnancy in 2 years.	No effect of treatment on gestation length, birthweight and lamb survival. Beta-hydroxybutyrate level was reduced by the H treatment.	Fogarty et al. (1992)

Ewes with litter sizes 1-4 were group-fed a total mixed ration of 90.0% DM, 2.60 Mcal ME /kg DM, 155g /kg DM CP, 215g /kg DM acid detergent fibre plus 2% mineral mix from day 50-60 of pregnancy. Daily allowance was calculated from estimated requirements for energy and protein based on liveweight, stage of pregnancy and litter size. Measured liveweight and condition score (Russel et al., 1969) at regular intervals to day 135. Aim was to maintain condition score during pregnancy. Ewes gained around 165g/day during 55-130 day period. Twenty one ewes slaughtered at 15 day intervals from day 85 to 130.	If ewes do not suffer overt nutritional restriction during pregnancy, fetal and placental weights were closely associated at any given stage during the final third of gestation, and the strength of this association increases with advancing stage of pregnancy up to 130 days. At any given fetal age, placental size is defined/influenced by the number of placentomes per fetus; which is a function of number of fetuses per ewe and per uterine horn. Given a consistent/adequate nutritional regimen, placental mass is a powerful determinant of fetal weight during the final third of pregnancy. Found evidence that ewe liveweight and body condition in early-mid pregnancy influences placental and fetal growth i.e. condition score is negatively associated with placental mass.	Greenwood et al. (2000a)
Nutritional restriction for 4 weeks from 75-95 days pregnancy. Reduced body condition score by 0.8 to a minimum of 3.0.	Lambs from control and restricted groups had same birthweights and survival. Gestation length increased by feed restriction by 3 days.	Holst and Allan (1992)
Crossbred ewes differentially grazed from days 42-105 and 106-140, giving 4 treatment groups HH, HL, LH and LL. All ewes grazed at H level from day 140 to term. Liveweights (kg) were: Day 42 105 140 HH 50 60 70 HL 50 60 59 LH 50 45 58 LL 50 45 47	Gestation length increased by feed restriction. Feed restriction in last trimester had greatest influence on birthweight, particularly so for twins. Twin lamb survival was lowest for the LL group, with no differences between nutrition groups for singles. Speculated that increased gestation length aids maturity of pulmonary, ingestive and thermoregulatory systems.	Holst et al. (1986)
Dietary restriction imposed on days 79,87,95 for 4 weeks. Pasture availability restricted to <100kg green DM /ha. End of restriction placed on >1000kg/ha. Control was unrestricted group.	At day 144 restricted and control ewes produced lambs with similar birthweights. Compensation included changes in fetal and maternal growth patterns. Increased gestation length of twins due to restriction was part of compensation. Fetal weight was reduced at end of restriction and day 144.	Holst et al. (1992)

Differential nutrition in single and multiple bearing ewes. Treatment (1). Lupin grain offered 300g/head/day from days 30-110 of pregnancy, and 400g/head/day until term. Weight change varied from +0.5 to –4.3kg for 4 plots. Treatment (2). No lupin grain offered from days 30-110 to induce weight loss. Weight change varied from –7.5 to –13.0kg for 4 plots. Triplet bearing ewes were slaughtered at day 90 to measure placental and fetal mass.	In triplet bearing ewes there was no difference between treatments for number of cotyledons but weight of cotyledons and weight of total fetuses was higher for the lupin supplemented group (1159g vs 944 and 1695g vs 1498g). In Merino ewes bearing single and twin lambs, concluded that birthweight was closely associated with changes in liveweight and/or condition score during the first two trimesters of pregnancy.	Kelly and Ralph (1988)
Five groups of ewes fed 0,100,200,300,400g lupin grain /head/day for each of three ewe categories (maiden 1 fetus, mature 1 or 2 fetuses) between 28-108 days of pregnancy. Thereafter feeding was 400-500g /head/day until term. Liveweight and condition score ranged from –8.4kg to +5.0 kg and –1.6 to +1.3 units.	Rate of lupin feeding during mid pregnancy and birthweight were positively related ($P<0.001$). Cotyledon diameters were greater and resistance to placental blood flow was less at day 100 in ewes fed 400g vs 0g ewes. Restricted nutrition reduced nutrient (glucose) supply, decreased placental size and reduced placental vasculature.	Kelly et al. (1992)
Three groups of triplet-bearing Booroola Merino ewes were fed at either (a) maintenance on an oat-pea (4:1) diet (b) unrestricted on the oat-pea diet or (c) unrestricted on lupin grain between 75-102 days of pregnancy.	Placental weight at day 102 was not influenced by nutritional treatment but fetuses of triplet bearing ewes were 11% heavier for the unrestricted lupin fed group compared with the maintenance group while fetal weight for the unrestricted oat-pea group was intermediate. In this model nutrients may have been partitioned toward maintaining placental growth during the latter stages of placental hypertrophy at the expense of fetal growth.	Kleemann et al. (1990b)

Four treatment groups of Booroola ewes heterozygous for the FecB gene; two groups fed low or high during mid pregnancy (days 50-100) and each of these groups split and fed or not fed lupin grain during the last 1-2 weeks of pregnancy. All ewes grazed mature pastures between days 50-100, but the high group was supplemented with an oat, pea, hay ration (800g, 200g, 350g /head/day). Both mid-pregnancy groups received this ration during days 100-135. Lupins replaced oats (700g) in the two high-protein fed groups. Differential feeding during mid pregnancy resulted in liveweight differences of 7.5kg and 5.0kg at days 100 and 135.	Neither birthweight nor lamb survival were influenced by the nutritional treatments. Increased mortality was observed among ewes bearing three or more fetuses when well fed during mid pregnancy. We speculate that overnourishment led to inappetence, ketosis and increased mortality.	Kleemann et al. (1993a,b)
A group of single bearing ewes severely undernourished between 30-96 days of pregnancy lost 8 kg liveweight. Control group fed to gain 100 g/day between 30-140 days. Condition scores (Russell et al., 1969) at days 30, 96 and 140 for restricted and control groups were 2.4, 1.6, 2.3 and 2.5, 2.9, 3.0	Undernutrition increased placental weight by 21% (day 96) and 30% (day 140). Fetal weight not affected by treatment.	McCrabb et al. (1991)
Two experiments of similar design to above experiment (McCrabb et al., 1991). Condition scores Experiment 1 Mating Day 90 Control 2.7 3.1 Restricted 2.6 1.4 Experiment 2 Mating Day 90 Control 2.4 3.1 Restricted 2.6 1.6	Experiment 1 (day 90) Placenta (g) Fetus (g) Control 596 613 Restricted 473 658 Experiment 2 (day 90) Placenta (g) Fetus (g) Control 496 644 Restricted 600 686 Suggested by authors that increase in placental weight in restricted ewes in experiment 2 was due to greater levels of body reserves not detected by condition scores.	McCrabb et al. (1992)

Lean vs moderately nourished (Fat) ewes (body condition score 2.0 vs 2.9) were generated by differential feeding started 75 days before mating and continued to day 55 of pregnancy. Two groups were maintained at these condition levels from 55-146 days. Sub groups were slaughtered at day 110. Aim was to generate two groups differing in condition but with same placental weights. Diet post day 55 was 60% lucerne chaff, 30% lupin grain, 10% barley plus mineral mix (10.9 MJ ME /kgDM and 227g CP/kg DM.	Placental weights at day 110 and 146 did not differ between lean and fat ewes (487g vs. 538g; 434g vs. 502g). Neither did fetal mass differ between groups at day 146, but fetuses from lean ewes had 20% less fat. Suggested that fat ewes are able to partition more glucose to the fetes than lean ewes. This study and that of Greenwood et al (2000a) supports contention that size of the placenta is the main factor controlling fetal weight when ewes are fed to meet dietary requirements in late pregnancy.	McNeill et al. (1997c)
Lean vs fat ewes generated as indicated above (McNeill et al., 1997c). Two groups of ewes maintained at these levels of nutrition to day 110. Then both groups fed the above diet ad libitum during late pregnancy.	Lean ewes increased their VFI by 11%. Lean ewes gained more body tissue but produced fetuses of similar size to fetuses of fat ewes with placentae of similar size. This suggests that the placenta sets an upper limit to nutrient intake by the fetus. Extra nutrient intake of lean ewes was partitioned toward body fat, particularly internal fat reserves.	McNeill et al. (1998a)
Lean vs fat ewes generated as indicated above (McNeill 1997c). Twin bearing ewes used instead of single bearers to increase demand on the placenta to transfer nutrients to the fetuses. Lean and fat ewes both fed to 0.6 of maintenance requirements from day 108. Body condition scores for the groups at day 108 were 2.9 vs. 3.8.	When twin bearing ewes were fed at 0.6 of maintenance requirements during late pregnancy fat ewes produced larger fetal mass (6.65kg vs. 5.83kg). Increasing fat reserves prior to late pregnancy benefits fetal growth if ewes are under-fed during late pregnancy. Fat ewes mobilised 40% more fat than lean ewes as NEFA. NEFA does not cross the placental barrier but spares glucose for fetal growth. Protein was also mobilised; it explained 33% of variation in fetal weight. In this model placental size did not influence fetal weight, but rather fetal weight was determined by nutrient availability.	McNeill et al. (1999)

Experiment 1. Feed was restricted while ewes grazed for 4 weekly periods starting at 79, 87, 95 days of pregnancy. Ten ewes were slaughtered at the end of each 4 weekly period plus 10 ewes on day 144. Groups started at body condition score 4 (Russell et al., 1969) and feed was restricted to achieve score 3 after 4 weeks. Placental lactogen, glucose and progesterone were measured. Experiment 2. Same design as experiment 1 but ewes were fed in pens to determine individual intakes. Diet was lucerne chaff (9.5 MJ ME /kg DM; 30g N/ kg DM) fed ad libitum to all groups including control from day 60-70 to term, except the 3 restricted groups (day 79, 87, 95) were fed 300g/day for 3 weeks, enough to reduce body condition score by 1 unit.	Experiments 1 and 2 Birthweight was maintained despite feed restriction during mid pregnancy, but reduced fetal weight and crown rump length at day 144. Birthweight may have been maintained through (a) increased gestation length or (b) increased maternal feed intake. Increased feed intake in the last week of pregnancy was the most likely reason. Intake of controls declined. Concentrations of progesterone and placental lactogen were inversely related to maternal nutrient intake.	Oddy and Holst (1991)
Two levels of nutrition were imposed on each of two groups of 1.5 year old ewes varying in body condition (2.4 vs. 2.9) between 30-98 days of pregnancy. Ewes were fed 10.6 or 22.0 g/kg/day of a diet containing 8.81 MJ ME /kg DM and 126g CP / kg DM. In both condition flocks the H group maintained liveweight while L lost 5 kg to day 98.	Low condition (2.4) Birthweight (kg) Low nutrition 3.32 High nutrition 3.83 High condition (2.9) Birthweight (kg) Low nutrition 4.96 High nutrition 4.23 The interaction for birthweight indicated that low nutrition during mid pregnancy in high condition ewes was beneficial to fetal growth whereas high nutrition in low condition ewes was beneficial to fetal growth.	Russell et al. (1981)

Adolescent ewes, approximately 60% of adult size (8-12 months of age), were offered a complete diet to promote rapid growth (200-350 g/day) or normal maternal growth (50-75 g/day). Diet contained 10.2 MJ ME /kg DM and 137g of crude protein (CP). The diet was offered during the first 100 days of the gestation period. Between days 100-145, ewes were fed to maintain body condition. Liveweight and body condition diverged significantly by day 35. Switched nutrition in the above model at day 50 (HH, HM, MH, MM). Half of the H adolescent group fed for the first 100 days were given L nutrition (64% of H) during late pregnancy.	High nutritional treatment reduced placental mass and birthweight by 36% and 33%. Placental and fetal masses were highly correlated. Number of cotyledons and gestation length were also reduced in the H group, while neonatal mortality was increased (15% vs 3%) despite intensive care. Results achieved using different amounts of the same complete diet. Balance between protein and energy may play a critical role in the extent of placental and fetal growth restriction. Reduction in maternal intake from H to M in mid gestation stimulated placental growth and increased lamb birthweight. Low nutrition during late pregnancy increased lamb birthweight. This was associated with an increase in fetal cotyledon mass and implies structural remodelling and functional adaptation are involved.	Wallace (2000)

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SECTION 3

MOLECULAR EVENTS IN ESTABLISHMENT AND MAINTENANCE OF PREGNANCY

CHAPTER 13. BACKGROUND INFORMATION ON REPRODUCTION AND MOLECULAR BIOLOGY

From the day of conception and proceeding through prenatal and neonatal development, the most complex of all biological processes are required to establish the mammalian body plan, to create functional tissue and organ systems and to deliver a normal, healthy individual. During this period, a large proportion of the genome is activated, with expression of thousands of genes in each cell type required to specify cell identity and ensuing function. Without accounting for involvement of the recently described non-coding small RNA species (Mattick 2001, Mattick and Gagen 2001), the complexity of cell-specific transcriptomes and proteomes is equalled only by the complexity of the resulting organism. Given these considerations, to identify the molecular mechanisms responsible for subtle or catastrophic events which result in reproductive failure or premature mortality in the ovine fetus and neonate presents a significant challenge. This task is compounded by complexities in both the spatial and temporal specificity of gene expression during development. Despite this, genome-wide scanning and gene expression profiling now possible via high throughput DNA sequencing and microarray hybridisation technologies have begun to provide tools necessary for dissection of the molecular events critical to reproductive and developmental normality and ongoing viability. In these areas, and for analysis of genome function in the ovine, use of these technologies is in its infancy. Indeed, laboratories internationally are striving to advance the bioinformatic resource in this species for the benefit of food and fibre production and to improve understanding of mammalian reproduction, development and growth.

At this point, there is much to discover in regard to the molecular biology of premature mortality of the developing ovine embryo, fetus and neonate and some clues are provided in recent reproduction and biomedical literature, notably in studies of embryonic development in the bovine / ovine / caprine *in vivo* and following *in vitro* manipulation and in studies of pregnancy and neonatal development of the human. Much literature evidence, both from analysis of recurrent pregnancy loss in humans (Lissak et al., 1999; Fatini et al., 2000; Franchi et al., 2001; Uehara et al., 2001) and of pregnancy in livestock species (Martal et al., 1997; Engeland et al., 1997) emphasises the importance of genetics in reproduction, prenatal and postnatal development and survival. These and earlier studies implicate both gross genetic alterations (Evans et al., 1984; Scarborough et al., 1984; Multani et al., 1992; Jauniaux and Hustin, 1998) and altered function of individual genes (Christiansen et al., 1995; Meinardi et al., 1999) as causes of embryo and fetal loss. The impact of the former, aneuploidy, is large in humans, where it accounts for 50 % of all early pregnancy failures (Jauniaux and Hustin, 1998). While pregnancy losses of 25-30% in sheep may partly reflect this, the problem of recurrent fetal loss itself is a heritable trait in ruminants (Engeland et al., 1997).

Bearing this in mind, and that structural differences in the reproductive tract and differences between the mechanisms of pregnancy establishment and maintenance in humans / mice and sheep exist (eg. invasive vs. non-invasive attachment of the conceptus to the uterine epithelium; see Johnson and Everitt, 2000) to follow is a discussion of the molecular events determined to be critical for reproductive success and embryonic and fetal development, with particular attention to factors causing premature loss. To date, differential expression of only a few genes has been conclusively shown to be linked with reproductive advantage in livestock. In comparison, the advent of somatic cell nuclear transfer "cloning" technology has contributed greatly to our understanding of the key events in embryogenesis and fetal development. This review section attempts to indicate that there is a myriad of new gene expression events which could be closely scrutinised with respect to their impact upon embryonic and fetal survival in sheep. As much literature describes the problems for development of the embryo from the single-celled zygote stage up until the early blastocyst stage (see Menezo and Renard, 1993; Holding et al., 2000; Daniels et al., 2000; Niemann and Wrenzycki, 2000; Wrenzycki et al, 2001; Rizos et al., 2002) this review will largely consider the developmental periods before and after this; formation of functional gametes, embryonic development including blastocyst hatching, elongation and implantation, establishment of the feto-maternal connection and immune privilege of the fetus and vascular and endocrine functions critical to fetal growth *in utero*. The text will concern discussion of key molecules and genes identified to date as being involved in major stages of pregnancy establishment and maintenance, with the view that these are genes whose activity may immediately be monitored. With little doubt, they represent only a small subset of genes whose expression could be surveyed through DNA-based microarray screening in an effort to discover reproductively reliable and efficient animals with desirable lambing performance.

CHAPTER 14. MOLECULAR EVENTS FROM GAMETES TO IMPLANTATION

14.1 GAMETOGENESIS AND FERTILISATION

14.1.1 Oogenesis

Formation, maturation and release of oocytes from the ovarian follicles of the ewe is a complex process, first involving cell proliferation by mitosis to expand the number of primordial germ cells (oogonia) during fetal life, meiosis to provide genetic diversity and produce the haploid genome and cell differentiation required in folliculogenesis. Arrest of cell division occurs in the first meiotic prophase when the primary oocyte is formed and when surrounded by flattened mesenchymal cells (granulosa cells) the primordial follicle is established. Oocytes in such primordial follicles stay in this arrested state until recruited for ovulation. Before oocyte release during ovulation, folliculogenesis passes through three stages, known as the preantral (primary) and antral (secondary) stages (also classified into types 1-5; Montgomery et al., 2001) and preovulatory follicle stages. The preantral stage is the longest stage and features an increase in diameter of the primordial follicle but meiotic arrest is maintained. Synthesis of large amounts of ribosomal RNA and messenger RNA occurs within the oocyte, to prepare for later stages of oocyte maturation and the first days of development, along with glycoproteins that form a layer (the zona pellucida) separating the oocyte and granulosa cells. Condensation of ovarian stromal cells immediately outside the granulosa layer forms the bilayered theca and adds also to follicle diameter. Though the granulosa layer is avascular, it supplies the developing oocyte with nutrients via cytoplasmic processes that penetrate the zona and is itself supplied by the vascularised internal layer of the theca.

The antral stage of development proceeds through proliferation of the granulosa cells and appearance of a viscous fluid between the cells. Accumulation and coalescence of droplets of the fluid forms the follicular antrum, a fluid-filled space that swells the follicle and in which the oocyte becomes suspended, attached to the periphery by only a thin stalk of granulosa cells. The oocyte is held in this structure by an enveloping mass of granulosa cells, the cumulus, and continues synthesis of RNA and protein turnover. At this point, follicular atresia (apoptotic death of oocyte and granulosa cells) is prevented by presence of the gonadotrophins, luteinizing hormone (LH) and follicle stimulating hormone (FSH), which act on cells of the theca and granulosa, respectively, through their specific receptors. Growth of antral follicles under the influence of LH and FSH results in production of both androgens (thecal cells only, from progesterone) and oestrogens (thecal and granulosa cells), which are released into the blood but also have an intrafollicular role in cell proliferation. Oestrogen acts on granulosa cells to stimulate its own synthesis and production of further oestrogen receptors, together resulting in a surge of oestrogen in the circulation. LH and FSH also stimulate production of cytokines such as insulin-like growth factor (IGF1), transforming growth factor (TGF)-beta and activin / inhibin.

Progress to the preovulatory stage requires a surge of LH. During this stage, meiosis in the oocyte is reactivated and proceeds through the first division, after which half of the chromosomes and most of the cytoplasm form the secondary oocyte (the rest being discarded as the first polar body). At the second metaphase, meiosis is again arrested and the oocyte is ovulated in this state.

Apart from the molecules described above and considering that the autocrine and paracrine regulation of gene expression within the ovarian cumulus, theca and granulosa cells during folliculogenesis and ovulation has been reviewed in detail recently (Richards et al., 2002), the function of genes acting within these cell types at these times will not be a subject of further discussion here but is presented in the table below.

Along with many in the table, an interesting example which illustrates the importance of single genes to ovarian development and folliculogenesis is that of the genes encoding the caspase proteases, which are involved in selective follicular atresia through programmed apoptotic events required for the production of viable, fertilizable oocytes (reviewed by Johnson and Bridgham, 2002). Reduction of the number of germ cells by about two thirds in the late embryonic-early post-partum phase is essential to remove those unable to support fertilisation and development (Morita and Tilly, 1999). Caspase activity is cell lineage-specific, can prevent (caspase 11; Morita et al., 2001) or promote apoptosis (caspases 2, 3, 7) and different caspases are responsible for germ cell (caspase 2; Bergeron et al., 1998) versus somatic cell (granulosa; caspase 3, 7; Matikainen et al., 2001) apoptosis. The functional importance of these and other caspase enzymes is highlighted by gene knockout models in mice and other species, for example *Caenorhabditis elegans*, where loss of function mutants in the capsase gene homologs feature increased numbers of germ cells, gonadal hyperplasia and decreased numbers of mature oocytes available for fertilisation (Gumienny et al., 1999).

Table 1. Genes involved in ovarian development and folliculogenesis

Gene	Inducer	Expression site	Timing of expression	Specific Function	Reference (* denotes citations not in Richards et al., 2002)
FSH receptor	FSH	Granulosa cells	Maturation of preovulatory follicle	Stimulates cell proliferation	Richards, 1979
Cyclin D2				entry into G1 of cell cycle	Robker and Richards, 1998
IGF1				growth and differentiation	
oestrogen receptor				enhances oestrogen synthesis	Zhou et al., 1997

LH receptor, aromatase	LH	Thecal cells		Stimulates androgen synthesis	Sharma et al., 1999 Johnson and Everitt, 2000
IGFBPs		Granulosa cells	Antral follicle expansion	Attenuate actions of IGFs	*Johnson and Everitt, 2000
Activin	FSH and androgens	Granulosa cells	Early antral follicles	Attenuates the LH-induced androgen rise in thecal cells	*Johnson and Everitt, 2000
Inhibin	Androgens and oestrogens	Granulosa cells	Late antral follicles	Enhances the LH-induced androgen rise in thecal cells and FSH stimulation of granulosa cells	*Johnson and Everitt, 2000
Growth differentiation factor-9b (GDF9b) / Bone morphogenetic protein 15 (BMP15)		Oocyte	Small primary follicles / later	Inducer of Cox-2 and HAS-2 Stimulates growth of primary follicle	Elvin et al., 1999 Yan et al., 2001
BMP1B receptor / ALK-6		Oocyte and Granulosa cells	Primary follicles	Kinase-mediated signalling for TGF-beta members	*Wilson et al., 2001
Cyclooxygenase-2 Prostaglandin E receptor (EP2) Early growth regulatory factor-1 Hyaluronic acid (HA) HA synthase-2 (HAS-2) TNF-stimulated gene-6 serum-derived HABP	LH	Cumulus and Granulosa cells	Cumulus expansion and Ovulation	Prostaglandin synthesis Prostaglandin signalling Transcription factor Forms the cumulus matrix Synthesis of HA Bound to HA in matrix Bound to HA in matrix	Sirois et al., 1992 Hizaki et al., 1999 Espey et al., 2000 Salustri et al., 1999 Weigel et al., 1997 Fulop et al, 1997 Hess et al., 1999
CAAT enhancer BP-beta Progesterone receptor Pituitary adenyl cyclase activating peptide	LH	Granulosa cells	Ovulation	Transcriptional regulator Regulation of cell signalling events Response of granulosa cells to cAMP	Sirois and Richards, 1993 Park and Mayo, 1991; Natraj and Richards, 1993. Park et al., 2000
Activator Protein-1 members	LH	Thecal cells	Ovulation and luteinization	Transcriptional activators	Richards et al., 2002

(c-Fos, c-Jun, Fra2/JunD)				
Steroidogenic acute regulatory protein (StAR)			Supports sterioid synthesis / cholestorol transport into mitochondria	
Matrix metalloproteinases -2, -9, -14 (MMPs)			Proteases that ativate TGF-beta factors	
ADAMTS-1 (a disintegrin and metalloproteinase with thrombospondin-like repeats)	Granulosa cells	Follicle rupture	Proteases involved in matrix degradation, can activate MMP's and cell surface receptors, may restrict angiogenesis during ovulation.	Espey et al., 2000
cathepsin L, other proteases				Robker et al., 2000; Oksjoki et al., 2001

It happens that the genes best characterised with respect to reproductive efficiency in sheep are those shown to affect the ability of the mother to produce oocytes (i.e. ovulation rate; reviewed by Montgomery et al., 2001), namely the autosomal Booroola (FecB) and X-linked Inverdale (FecX^{I}) fecundity genes. It is possible that these and other genes in Table 1 are responsible for reproductive wastage via partial failure of multiple ovulations (PFMO; Chapter 2), especially given that as ovulation rate increases, PFMO increases.

Early evidence from the study of ovaries from heterozygote 3-month old ewe lambs indicated that the Booroola gene has an influence on the composition and function of sheep ovaries before puberty (McNatty et al., 1987). The Booroola (Merino) and Inverdale (Romney) genes, predominantly known to increase ovulation rate in post-pubertal ewes, have a retarding effect upon ovary development and oocyte maturation during fetal development (McNatty et al., 1995). While the FecB mutation has been traced back to the Indian Garole breed and is also found in Javanese thin-tailed sheep, different fecundity loci have been identified in many other sheep strains, including Woodlands Coopworths (FecX2), Cambridge (FecC), Thoka (FecI), Belclare, Lacaune and Olkuska, sheep (Montgomery et al., 2001; Davis et al, 2002). In Olkuska sheep, the increased number of follicles that mature and release oocytes results from differences within the ovaries, and the recruitment of ovulatory follicles lasts longer than in less prolific breeds (Gebarowska et al., 1996). Higher ovulation rate is associated with a lower level of follicular atresia in ewes carrying the Booroola fecundity gene (Mandiki et al., 2000) and homozygous Booroola ewes have a higher level of circulating bioactive FSH (Fleming et al., 1995). Interestingly, Finnish Landrace sheep have developed an increased ovulation rate through reduction in the sensitivity of the hypothalamic-pituitary unit to the feedback effects of ovarian hormones (inhibin and / or oestrogen) which normally suppress FSH production (Baird and Campbell, 1998).

At the gene level, the Booroola FecB mutation has been identified as an autosomal dominant mutation in the Bone morphogenetic protein (BMP) 1B receptor expressed in oocytes and granulosa cells (Wilson et al., 2001), while the Inverdale mutation is in an X-linked gene for the oocyte-derived growth factor BMP15 (Galloway et al., 2000). Preliminary evidence suggests that both gene mutations increase fecundity via effects mediated through the TGF-beta signalling pathway, either through the receptor itself (mutation of the highly conserved intracellular serine-threonine kinase signalling domain of the BMP-1B receptor; McNatty, 2001; Monget et al., 2002) or the ligand (mutation of the coding region of BMP15; Galloway et al., 2002). To dissect the earliest molecular events responsible for oogenesis, expression of genes known to be involved in germ cell development or meiosis and of genes encoding members of the BMP pathway are being assayed during ovine foetal development (Mandon-Pepin et al., 2003). Moreover, roles for the BMP pathway genes in ovulation are being examined in other species (Erickson and Shimasaki, 2003) and are implicated in some human reproductive disease states (eg. polcystic ovary syndrome ; Teixeira Filho et al., 2002).

The other mutations affecting ovulation rate are on the X chromosome (Woodlands) and are autosomal mutations in the Thoka, Olkuska and Lacaune sheep. One other locus for which polymorphisms have been shown to be associated with average litter size in the sheep breeds tested (Rhoenschaf, Merinolandschaf, East Friesian Milksheep and Romanov) contains the beta A-inhibin gene, which is expressed in the granulosa cells and known to be involved in progression of follicles from the large preantral stage through the early antral to the large antral stage (Leyhe et al., 1994; Montgomery et al., 2001). These may be some of the targets of nutritional regulation, as discussed earlier (see Section 1).

As an example to illustrate another level at which gene effects may act, in humans, a study of dopamine D2 receptor gene intron 5 / exon 6 polymorphisms in females showed an association between some haplotypes and reproductive success, through altered levels of secreted gonadotrophins (Legro et al., 1994). Through influences on the hypothalamic control of gonadotrophin release by dopamine, effects of the gene polymorphism include higher LH / FSH and lower prolactin levels and also the associated effects of higher parity and lower miscarriage in the women. Finally, though no specific loci have yet been identified, the high fecundity observed in other ruminant species (eg. the improved boer goat, where around 33% of does give birth to triplets and quadruplet / quintuplet kids (Erasmus, 2000) should be investigated. Expression patterns during reproduction in these animals and expression of the other genes described above all present targets for genetic screening by microarray analysis.

The influence of single genes on ovarian development, folliculogenesis and ovulation is considerable and one must also account for the effect of the environment on the function of these genes. In the case of the developing lamb, nutrition of the mother plays a key role in determination of the "environmental" factors affecting fetal gene function and it has known effects on oogenesis and subsequent development, including improvement of oocyte quality, embryo survival and trophectoderm devlopment / placentation (low nutrition during the pre-antral stage of folliculogenesis, pre-implantation and early post-implantation periods, respectively). While a high plane of nutrition is known to increase ovulation rate in sheep (Munoz-Gutierrez, 2002; others), animal management leading to undernutrition of pregnant ewes in mid-late pregnancy not only has marked effects upon fetal development, but also includes inhibition of fetal gonad development (Gunn et al, 1995, Rhind et al., 1998). The effects of nutrition and other environmental factors on programming of the reproductive axis by the fetus has been reviewed recently (Rhind et al, 2001). Specifically, undernutrition of ewes carrying female fetuses, before and during folliculogenesis, can significantly delay ovarian development in the fetus with a reduction of fetal ovary mass (despite normal total fetal mass), delay germ cell maturation and entry into meiosis and retard ovarian follicle formation (Rae et al., 2001). Further, in a sheep model in which adolescent ewes carry the pregnancy, overnutrition during the first 100 days leads to lowered placental mass, produces growth-restricted male and female lambs and results in an increase in LH-beta expression and a reduction in the number of follicles present (in the resting pool) in ovaries of the developing ewe lambs late in gestation (days 104, 128 and 131; DaSilva et al., 2001, 2002). Results of such experimental under- and over-nutrition suggest that effects on subsequent reproductive attributes of the developing lamb are both maternal age- and gestation stage-specific and that the responses represent many interacting influences governed by multiple gene systems. The nutritive factors, steroid hormones and metabolic signalling molecules involved in mediation of these effects have been described earlier (Chapter 5) and suggest specific targets for genetic analysis by expression profiling.

14.1.2 Spermatogenesis

As in the case of oogenesis, defects in spermatogenesis can contribute to reproductive inefficiency and embryo loss, through reductions in either sperm count or functionality. The literature includes a plethora of information describing pre- and post-natal mammalian testis development and spermatogenesis, with much discovered in rodent models (eg. Cupp and Skinner, 2001; Clarkson and Harley, 2002; Sha et al., 2002; Luedtke et al., 2002; Weber et al., 2002; many others) and the effects mediated by physiological parameters such as hypoxia (Liu and Du, 2002) and maternal nutrition (eg. Meikle and Westburg, 2001) described. With respect to the latter, in sheep, though maternal undernutrition has no apparent effect on the gross anatomy of fetal testes but does upregulate fetal steroidogenesis at day 50 of gestation, the long term effects on fertility are not known (Rae et al., 2002). Interestingly, maternal overnutrition in the adolescent ewe model described in 14.1.1 has also been shown to delay subsequent puberty in male offspring and to reduce peak testosterone concentrations in comparison to male lambs produced by ewes fed moderately (DaSilva et al., 2001).

The importance of proteins involved in many aspects of spermatogenesis, for example sperm chromatin condensation (Zhao et al., 2001) and chromatin remodelling during spermatid maturation (Yan et al., 2002), chromosome movements during meiosis (e.g. as demonstrated for synaptonemal complex protein 3 in mice (Yuan et al., 2000), interaction of sperm and ooocyte (eg. SP22 sperm head protein of the cauda epididymal sperm of rat, known to be predictive of fertilising ability; [Welch et al., 1998] and the spermatozoa of "bouncy" mutant mice which have a severe defect in the sperm-zona interaction [Holland & Orgebin-Crist, 1988]), testis structure (eg. effect of Cystic fibrosis transmembrane conductance regulator (CFTR) gene mutation on the fluid environment of the epidydimal epithelium ; Wong, 1998) have been well documented. These studies and surveys of testis development and spermatogenesis by cDNA array analysis (Sha et al, 2002; Koopman et al, 2002) present many further targets for functional genomic identification of the more reproductively efficient animals, to pinpoint genes which cause either immediate or delayed reproductive failure.

Interestingly, gene mutation leading to defective spermatogenesis does not necessarily incur a reduction in fertility per se (eg. gene knockout to ablate the post-pubertal testis-specific GTP-binding ADP-ribosylation-like factor in mice produces a moderate retardation of germ cell development and reduces sperm count but not fertility (Schurmann et al., 2002). Likewise, there are cases where mutation in hormonal networks regulating the response to androgens has no effect on fertility (eg. mutation of the androgen receptor gene exon encoding the ligand-binding domain (Giwercman et al., 2000). The determination of genetic selection targets is clouded somewhat by exceptions such as these.

In the Australian climate, the effects of hyperthermia on fertility of rams also impacts upon reproductive performance in sheep. Effects of heat upon stages of spermatogenesis have been well documented and implicate the susceptible cell types, the spermatocytes and young spermatids (Lue et al., 1999, Setchell et al., 2002). As is the case for oocyte quality (see below), subtle changes to the chromatin at this stage can cause arrest of blastocyst development and also lead to reduced fertilisation rates *in vitro* and *in vivo* (Jannes et al., 1998). Recently, genes involved in testicular function in mice have been surveyed by microarray expression analysis following testicular heat shock, where spermatocytes were found to be the most susceptible cell type. Expression of genes involved in spermatogenesis, including cell cycle, cell-adhesion, signalling / signal-transduction and apoptosis-specific functions were found to be altered, along with expected changes to heat shock and DNA damage response genes (Rockett et al., 2001).

14.1.3 Quality of the ooplasm at fertilisation

There is no doubt that quality of the oocyte cytoplasm has marked effects upon the developmental potential of embryos and fetuses. It has been established that epigenetic factors laid down in the ooplasm during oogenesis are required for nuclear modification (Gurdon, 1986; see also Prather and First, 1990; Menezo and Renard, 1993) and programming for successful development (Renard, 1998; Christians et al., 1999). Moreover, recent nuclear transfer experiments have demonstrated that factors in the ooplasm are even able to reprogramme the gene transcription profile of foreign nuclei taken from terminally differentiated cells , through chromatin remodelling (eg. fibroblast cell nuclei used as donors in nuclear transfer experiments [DeSousa et al., 1999; Baran et al, 2002; Renard et al., 2002]). However, multiple errors in normal epigenetic mechanisms such as chromatin remodelling for establishment of transcription domains and methylation, each required for the precise control of gene expression (Felsenfeld, 1992; Felsenfeld et al, 1996; Surani, 1998) inevitably result in developmental failure (e.g. Bourc'his et al., 2001; Renard et al., 2002). Considering the complexity of methylation-dependent control of expression of developmental stage-specific genes [eg. control of IGF2 and H19 gene expression by methylation of a CTCF-dependent locus insulator sequence (Bell and Felsenfeld, 2000; Kanduri et al., 2002) through molecules such as CTCF (CCCTC-binding factor; a ubiquitous 11 zinc finger nuclear factor that interacts with transcriptional insulator proteins), downstream developmental catastrophes resulting from insufficiency of this and other epigenetic modifications are not surprising.

To date, monitoring of maternal transcript fate in the pre-transcriptional zygote (Henrion et al., 2000; Oh et al., 2000), activation of the zygotic genome (rRNA synthesis in nucleoli; Hyttel et al., 2000; see also Ma et al., 2001; Schultz, 2002) and translation to produce the first transcription factors (Wang and Latham, 2000) has been followed by conventional analytical methods. However, considering the successes in creation of cDNA libraries from single oocytes and preimplantation embryos (Adjaye et al., 1998; Holding et al., 2000; Hurley et al., 2000] and the latest developments in protein recognition using matrix-assisted laser desorption / ionisation time-of-flight mass spectrometry (MALDI-TOF) proteomics technologies, identification of the molecules vital to ooplasm quality, as related to embryo development, is not far off.

14.2 PRE-IMPLANTATION EMBRYO DEVELOPMENT

After fertilisation to produce the 2n zygote, cell division creates the morula then blastocyst, with subsequent formation of the inner cell mass of embryonic stem cells and the epithelial trophectoderm layer. Timing of the switch from maternal to embryonic gene expression is at the 8 - 16 cell stage in sheep (Crosby et al, 1988; Telford et al., 1990). Zygotic gene expression drives cell lineage development in the inner cell mass to produce the ectoderm, endoderm and mesoderm of the embryo proper (marked by expression of cytokeratin 8, alpha-fetoprotein and vimentin respectively) and in the trophectoderm, which communicates via the ovine trophoblastic protein (oTP; interferan [IFN]-tau) with the uterine epithelium to prepare for implantation (see below). During these very early stages, the pattern of embryonic gene expression reflects both normal programming and inductive environmental and genetic influences, including nutrition, health and age of the mother during oocyte formation and maturation (see Chapter 1) and epigenetic modification of the maternal and paternal genomes. Clearly a critical developmental period dictated by very finely balanced genetic mechanisms, studies in cattle indicate that most embryo loss occurs in the time between fertilisation and implantation (Dunne et al., 2000).

14.2.1 Regulation of differentiation in the preimplantation embryo

Recently, the mammalian POU transcription factor Oct-4 has been suggested to be a master regulator of differentiation in the developing embryo, through its control of totipotency in undifferentiated cells during early cleavage stages of the embryo (Pesce and Scholer, 2001). Achieved through differential regulation of Oct-4 in individual blastomeres (Hansis et al., 2000, 2001) and with effect equating with absolute expression level (Niwa et al., 2000; Kirchhof et al, 2000), maintenance of Oct-4 expression in the inner cell mass and epiblast in the developing blastocyst is accompanied by a lack of expression in the trophoblast (Ezashi et al, 2001). Further differentiation fates are chosen through progressive downregulation of Oct-4 expression as development proceeds (Guo et al, 2002). Though not the only important regulatory factor, the critical nature of Oct-4 has been highlighted by somatic cell nuclear transfer experiments in mice where disturbance of its expression (most likely through incorrect programming of donor nuclei) is sufficient to prevent normal development (Boiani et al., 2002).

14.2.2 Gene expression in the preimplantation embryo

Given that Oct-4 acts somewhat as a gate for development through regulation of the capacity for differentation, the normal pattern of expression of many genes acting downstream of Oct-4 during preimplantation embryonic development has been determined in individual embryos using RT-PCR. Recent surveys of developmental and house-keeping factors expressed in embryos from the single cell stage until the blastocyst stage suggest factors that are critical to cell proliferation and differentiation (Niemann and Wrenzycki, 2000; others). The specific roles of growth factors and cell adhesion molecules (Stewart and Cullinan, 1997; Hardy and Spanos, 2002; Bloor et al., 2002) have been documented. Specific roles of imprinted genes such as IGF2, IGF2R and H19 in embryonic, placental, fetal and neonatal growth have been established (Moore and Reik, 1996) and are known to be influenced by the history of maternal and paternal gamete formation. Another group of genes, the so-called preimplantation embryo development (PED) genes, whose expression is necessary for cell-cell recognition during blastocyst expansion have an influence on cell cleavage rate during blastocyst formation and on subsequent embryo survival (Cao et al, 1999). The genes Q7 and Q9 in mice, induced by IFN-gamma and active at the onset of zygotic transcription, encode a Major Histocompatability Complex (MHC) class Ib cell surface antigen, Qa-2, that is expressed in both the inner cell mass and trophectoderm (McElhinny et al., 1998) and is responsible for the phenotype (Wu et al, 1999; Warner and Paschetto, 2000). In sheep, there are multiple PED gene candidates expressed during this period and we are investigating their expression levels in the developing blastocyst (CJ McLaughlan and CS Bawden, unpublished). In humans, expression of the likely PED-candidate gene human leukocyte antigen [HLA]-G (Cao et al., 1999) is a fundamental prerequisite for establishment of pregnancy as the soluble form facilitates implantation via inhibition of cytolytic natural killer (NK) cells (Fuzzi et al., 2002). Produced by the extravillous trophoblasts, it is a tolerogenic molecule which acts either at the T-cell receptor level or the NK receptor level, to prevent immune cells from attacking self or foreign (fetal) antigens (Gomez-Casado et al., 1999).

To further dissect the critical molecular events occurring beyond this period, analysis of ovine gene expression between blastocyst formation and implantation, both in the conceptus and uterus, is currently being undertaken in many laboratories, including our own. Historically, the involvement of genes in preimplantation development has been investigated via manipulation of preimplantation elongation of the blastocyst and subsequent pregnancy rate through hormonal treatment of the ewe prior to maternal recognition of pregnancy. For example, if human chorionic gonadotrophin (hCG) is given on day 11.5 of the oestrous cycle (onset = day 0), prior to normal luteolysis, blastocyst elongation on d13 of pregnancy is significantly greater and levels of IFN-tau and protein in the uterine fluid are significantly higher (Nephew et al., 1994). Recently, identification and characterisation of new genes involved in the processes of hatching and elongation has occurred through traditional gene isolation strategies based on searches for predicted function, utilising nucleotide sequence information contained in the extensive genomic and EST databases. In mice, implantation serine proteinase-1 (ISP1), a tryptase expressed throughout the murine morula and blastocyst and likely to be involved in blastocyst enzyme-assisted hatching through the zona at the abembryonic pole (the embryonic site of attachment to the uterine epithelium during implantation) has been identified using this approach (O'Sullivan et al., 2001). This approach provides clues to the important embryonic functions and single gene analysis is now complemented by genome-wide expression profiling using cDNA and oligonucleotide array technology, following isolation of mRNA from embryos after various treatment schedules. In the ovine, until appropriate arrays are constructed, this type of analysis could be carried out utilising available bovine and human arrays, considering the high degree of cross-species conservation within coding regions.

14.3 EMBRYO ATTACHMENT TO THE UTERINE ENDOMETRIUM (IMPLANTATION)

Embryonic attachment to the uterine wall begins around day 14 of gestation and is completed by day 35. The process involves establishment of contact between embryonic trophoblast cells and the uterine endometrium. As the embryo completes gastrulation to become a well defined fetus, the chorioallantoic membranes attach to the maternal caruncles (aglandular uterine mucosa). Sites of contact become highly vascularised to produce the fetal cotyledons and together with the caruncles, the placentome units are formed. In sheep and cattle, the feto-maternal connection is of a "non-invasive" type, where penetration of the maternal epithelium does not occur at implantation (c.f. invasive attachment in humans and rodents; Johnson and Everitt, 2000). Later in development, there is some invasion of conceptus cells into the endometrium. At this time, there is a fusion between the binucleate cells of the fetal trophectoderm and the maternal uterine epithelial cells, to form a syncytium and cell-cell junctional complexes are established.

Proliferation, differentiation and attachment of fetal trophoblast cells to the uterine epithelium and subsequent maintenance of the feto-maternal interface is mediated by hormones, growth factors and cytokines (Wooding, 1992; Yamada et al., 2002). A review of the genes regulating implantation and placental development in mice is available (Cross, 2001). Albeit descriptive of invasive placentation, this and other literature make it clear that molecules of both fetal and maternal origin are required to effect a viable connection and that there are dramatic changes in gene expression. *In vivo* models such as the ovine uterine gland knockout model have been used to provide insight into the feto-maternal functions required for implantation (Gray et al., 2002). In this model, ablation of the uterine endometrial glands and observation of conceptus growth revealed that endometrial secretions are not required for conceptus hatching but are crucial for elongation and implantation. In addition to leukemia inhibitory factor (LIF) and calcitonin, which are known in mice to promote conceptus development, uterine receptivity and implantation (Carson et al., 2000), this study implicated molecules of the extracellular matrix of the luminal epithelium, such as osteopontin, in conceptus development via attachment to integrins located on the surface of the trophectoderm (Johnson et al., 1999, 2001). Following a reduction in levels of Muc-1 (a mucin that blocks interactions between glycoproteins on the surfaces of the uterine luminal epithelium and trophectoderm) osteopontin is thought to bridge integrin receptors on the apposing cell surfaces and thus mediate initial conceptus attachment (Johnson et al, 2001). At this time, prolactin-related protein-1 (PRP-1) is expressed in the mononucleate and binucleate cells of the trophoblast that are in direct contact with the uterine endometrial epithelium, and the binucleate cells extend cytoplasmic projections to the uterine epithelium (Yamada et al., 2002), with the aid of the extracellular matrix-degrading enzyme heparanase (Kizaki et al., 2001). In the implantation area, mutinuclear cells of the uterine epithelium also produce PRP-1. Following implantation, formation of the fetal cotyledon structures proceeds, including development of the chorionic villi comprising mostly binucleate cells. Placental lactogen (PL) expression begins and is maintained along with PRP-1 expression during the post-implantation period, when the placentomes form. Although the specific function of PRP-1 and PL is yet to be established, they are thought to have key roles in feto-maternal interactions during the preimplantation period like other members of the prolactin / GH family and are implicated in angiogenesis (Reynolds and Redmer, 1992), immunosuppression to prevent rejection of the fetus and remodelling of the extracellular matrix (Guillomot, 1999).

14.3.1 Role of the trophoblast

In addition to the expression of cell-surface attachment molecules such as the integrin family, the mononuclear trophoblast cells of the conceptus produce large amounts of IFN-tau, a signal for maternal recognition of pregnancy which is used to maintain progesterone secretion by the corpus luteum and to block uterine prostaglandin PGF2-alpha synthesis. IFN-tau is detected by d11 of pregnancy in the elongated blastocyst, peaks at d14 and decreases by d16, with total expression arrest occurring during implantation in regions where the trophoblast has established cellular contacts with the uterine epithelium (Guillomot et al., 1990). An additional consequence of IFN-tau production is the induction of the Myxovirus-resistance (Mx) protein in the uterine epithelium (Ott et al., 1998). Mx is a GTPase with roles in intracellular protein and vesicle trafficking and through interference with the formation of virus particles, has an antiviral activity that is the body's first line of defence against viral infection (Haller and Kochs, 2002). In early pregnancy, IFN-tau upregulates Mx expression throughout the entire uterine wall and also in circulating components of the immune system, the peripheral blood mononuclear cells, which migrate through the endometrial wall (Yankey et al., 2001). Found in all vertebrates, both male and female, it is perhaps not surprising that the two key elements of the blood-testis barrier, the Sertoli and peritubular cells, also express Mx protein, along with other antiviral proteins, 2' 5' oligoadenylate synthetase and the double-stranded RNA-activated protein kinase to provide a testicular antiviral defense system (Dejucq et al., 1997). Considering the recent finding that the chicken Mx gene is highly polymorphic with concomitant reductions in the antiviral activity of mutated forms (Ko et al., 2002), one might expect a similar case in sheep to result in reduced antiviral defense before and during pregnancy and consequent pregnancy failures.

Responses of the conceptus to changing environmental conditions in the uterus are also important in fetal survival. For example, in humans and mice, conditions of low oxygen tension result in a stress response in the trophoblast that leads to the production of PROXY-1 (Protein regulated by oxygen-1; also termed RTP (Xu et al, 1999; Park et al., 2000), a protein believed to protect terminally differentiated cells of the trophoblast from apoptosis (van Belzen et al., 1997). Present in trophoblast-derived cells of the placental syncytium throughout gestation, PROXY-1 may have a specific role in restricting apoptosis normally mediated by the Fas / FasL system (see below) and thus allows progression of the pregnancy.

14.3.2 Role of the maternal uterine epithelium.

In species where placentation is invasive, expression of the high affinity glucose transporters GLUT-1, -3 and -4, by the uterus during decidualisation and implantation and by uterine and embryonic tissues immediately after implantation, is critical to survival and normal development of the fetus because embryonic production of glucose is minimal (Turkay Korgun et al., 2001). In sheep the most predominant glucose transporter is GLUT-1, located in the maternal uterine syncytium. Its level can vary in repsonse to maternal nutrition with resultant increases in placental size and fetal mass (Dandrea et al., 2001). Clearly, differential regulation of the Glut-1 gene plays an important role in fetal development and may provide a basis upon which animals with differing reproductive efficiencies could be distinguished.

Upon fertilisation, zygotes produce platelet-activating factor (PAF; an ether phopsholipid) which causes synthesis of early pregnancy factor and results in recruitment of platelets to the uterus during implantation (O'Niell, 1995). The uterine endometrium also produces PAF (Chami and O'Niell, 2001) and PAF-receptor, whose actions probably mediate the local increase in vascular permability of the endometrium which is known to precede implantation (Tiemann et al., 2001). As described above, the maternal extracellular matrix also synthesises ligands which protrude and interact with trophectodermal surface molecules to provide anchorage of the conceptus.

Purely illustrative of the complexity of regulation of gene expression required to establish pregnancy, in this case implantation, is the recent finding that a spliceosome complex protein, SC35, is increased at implantation sites early in mouse gestation (Nie et al., 2002). Known to be involved in construction of alternatively spliced mRNA isoforms, SC35 is predicted to regulate supply of alternatively spliced mRNAs transcripts of some of the most important implantation-specific molecules (i.e. the oestrogen and progesterone receptors, prostaglandin receptors EP1 and EP3, Cyclooxygenase-2 (involved in prostacyclin synthesis) and the cell surface adhesion molecule CD44).

14.3.3 Establishment of immune privilege of the conceptus

Cytolytic "natural killer" cells, a component of the innate immune system, are found in both the peripheral blood lymphocyte population and among the endometrial epithelial cells of the uterus. The conceptus is protected from the activity of these maternal NK cells via inhibition by progesterone-induced ovine uterine serpin (Tekin and Hansen, 2002). Secreted by the uterine cells, it inhibits protein kinase C and interleukin (IL)-2-mediated events, leading to reduced lymphocyte proliferation. To effect evasion of maternal rejection by the conceptus, both maternal and conceptus-derived factors are responsible for differential regulation of the activity of specific uterine interplacentomal endometrium lymphocyte populations (Majewski et al., 2001). T-cell receptor positive intraepithelial lymphocytes are present only in interplacentomal areas of pregnant uteri, express molecules consistent with a cytotoxic potential (IFN-gamma, tumor necrosis factor [TNF]-alpha, TGF-beta and IL-10) and may be present to control trophoblast invasion of these areas (Fox et al., 1998).

A contribution to immune privilege of the conceptus is made through tight regulation of the adenosine deaminase (ADA) gene within tissues forming the feto-maternal interface during early post-implantation stages of development (Knudsen et al., 1988). Evidence that lymphotoxicity is related to purine metabolism and ADA activity is that a deficiency in humans results in severe combined immune deficiency (SCID), due to defective T-cell receptor signaling (Apasov et al., 2001). In normal pregnancy, a drop in serum adenosine deaminase activity and in the cell-mediated immune capacity of the mother in pregnancy is reflected in lowered levels of cells secreting IFN-gamma. In contrast, women sufffering preeclampsia and hyperemesis gravidarum have elevated levels of ADA activity (Yoneyama et al., 2002a,b). Hence, a potential diagnostic for ewes more able to carry fetuses may lie in determination of ADA regulation in different strains.

Indicators of pregnancy produced around the time of implantation include a number of pregnancy-associated glycoprotein (PAG) molecules that are synthesised by the binucleate trophoblast cells at the placental-uterine surface during pregnancy. (Green et al, 2000). These molecules undergo both glycosylation and phosphorylation and although of unknown function, may have a role in feto-maternal immune interactions.

14.3.4 Fas / FasL system involvement in trophoblast attachment

As outlined above, contact between maternal and embryonic cells would normally elicit an immune response. However, modulation of the maternal immune response to prevent a cell-mediated attack against the attaching embryonic trophoblast cells, in favour of a humoural response occurs, with T helper (Th)-1 pro-inflammatory cytokines (e.g. IL-2, TNF-alpha, IFN-gamma) suppressed and T-helper (Th)-2 anti-inflammatory cytokines (e.g. IL-4, -6 and -10) induced (eg. IL-6 expression has been shown in elongating ovine conceptuses from day 13 - day 25, the period encompassing the stage of attachment of conceptuses to the uterine endometrium [Mathialagan et al., 1992]). Recently in humans, the Function-associated antigen (Fas) / Fucntion-associated antigen lilgand (FasL) system, known to represent a major apoptotic pathway controlling cell proliferation and tissue remodelling, has been shown to be involved in the establishment of immune privilege at sites where placental trophoblasts and maternal immune tissue are in contact. Both Fas and FasL are found in trophoblasts and could activate apoptosis. However levels of Fas, normally upregulated by Th-1, are in fact down-regulated by Th-2, so apopotosis is prevented. Not surprisingly then, in humans, elevated trophoblast expression of Fas has been found in women with preeclampsia (Allaire et al., 2000). Moreover, reduced expression of FasL and RCAS1 (receptor-binding cancer antigen expressed on SiSo cells; shown to have a role in immune evasion by tumour cells) has been shown responsible for activation and infiltration of maternal NK cells into the uterine glands, with resultant destruction of the glands and rejection of the fetus (Oshima et al., 2001).

14.4 FORMATION AND MAINTENANCE OF THE PLACENTA

The fact that placental abnormality has been cited as a major cause of mortality in first trimester somatic cell nuclear transfer (SCNT) clones (Hill et al. 2000) suggests that formation and maintenance of the placenta is the single most critical event for a successful pregnancy and is possibly the most finely balanced and easily perturbed process in development of a pregnancy. Evolutionarily, this makes sense in that if any maternal or embryonic function in cells required for placentation is even slightly compromised, establishment and progression of pregnancy is unlikely. (See also Wilmut et al., 1986.)

Improper (poor or no) vascularisation of the placenta is recognised to be prevalent in SCNT fetuses (DeSousa et al., 2001) and a lack of cotyledonary tissue has also been reported (Stice et al., 1996), again emphasizing the critical nature of earlier events in subsequent differentiation. A powerful stimulator of angiogenesis and vascular permeability, vascular endothelial growth factor (VEGF) is synthesised in both the fetal and maternal epithelia which are interfaced in the placenta. In the pig, which also exhibits non-invasive placentation, VEGF produced in these cells has been shown to bind to VEGF receptors located in the endothelial cells of neighbouring fetal and maternal capillaries (Charnock-Jones et al., 2001) and it is likely that the paracrine action of VEGF produced by the epithelia extends to the subepithelial capillary network present in the placenta. Not only important in the initial stages of establishment of the placenta, VEGF has a role in continued growth of placental blood vessels throughout gestation, to increase the surface area for exchange of nutrients and waste throughout pregnancy.

Upregulation of Cyclooxygenase-1 gene expression in the endothelium of the uterine artery effects vasodilation and increased blood flow to the uterus during pregnancy (Habermehl et al., 2000) via increased prostacyclin (prostaglandin PGI2) synthesis. While improved vascularisation is essential for growth, delivery of growth factors to the developing placenta and fetus also occurs. Only low levels of growth hormone (GH) are found in the maternal circulation in early pregnancy. However GH synthesised by the placenta between days 35 and 50 of gestation, in cells of the trophectoderm and feto-maternal syncytium, is believed to directly stimulate placental and fetal growth through induction of various placental metabolic factors and growth factors and their receptors (Lacroix et al., 1999) and acts via growth hormone receptors expressed within the placenta, uterine endometrium and fetal liver around the same time. Fetal pituitary GH expressed from day 50 onwards is then believed to take on this growth-promoting role.

Illustration of the importance of the embryonic environment and early feto-maternal interactions on embryo and fetal development is provided by experiments where regulation of early events is perturbed by nutritional, hormonal or surgical intervention. For example, administration of progesterone during the first three days of pregnancy causes an increase in volume and surface area of the fetal trophectoderm and of the feto-maternal syncytium (Kleemann et al., 2001) with resultant perturbation of developmental rates of fetal tissues and organs. Moreover, if a 90 % drop in progesterone levels is caused via luteectomy at day 50, spontaneous abortion results. These experiments illustrate the critical requirement for progesterone in pregnancy establishment and maintenance in the first trimester (Al-Gubory et al., 1999).

Placental development and ultimate weight is known to be a primary factor determining birth weight of neonates in many species. The fact that underfed pregnant ewes with poor placental growth in the first 80 days of gestation have larger placentas at 145 days gestation than control pregnant ewes fed a full ration throughout pregnancy (Heasman et al., 1999) suggests a compensatory mechanism controlled by the mother, developing fetus or both, is in operation. Recently, the delicate balance between cell proliferation and programmed cell death in the human placenta has been shown to be regulated through alterations in the ratio of proto-oncogene Bcl-2 (B-cell leukemia / lymphoma-2) and Bax (Bcl-2 associated X protein gene expression), with levels of Bcl-2 low throughout pregnancy and Bax beginning at a low level then increasing late in gestation, coincident with an increase in apoptotic cells (DeFalco et al., 2001).

14.5 MOLECULES INVOLVED IN PRE-TERM LOSS OF PREGNANCY

Just as establishment of pregnancy relies upon the fidelity of multiple and complex gene expression systems, as described above, failure of a single genetic mechanism involved in pregnancy maintenance, whether on the fetal or maternal side, can lead to pre-term pregancy loss. Such failures may be inherent in some sheep breeds and able to be corrected through either pharmaceutical intervention during pregnancy or by genetic modification, with resultant improvements in lambing performance. To follow is a discussion of factors known to contribute to pregnancy loss in ruminants and in other species.

14.5.1 Pregnancy loss in ruminants

Normal progression of an established pregnancy requires on-going communication between the mother and fetus and involves molecules passed from fetal to maternal cells that are in direct contact with each other in the placenta and also molecules passed between mother and fetus via the circulation. While most losses occur in the period between fertilisation and implantation (Dunne et al., 2000) in ruminants, pre-term failure of established pregnancies can result from placental defects. This has been highlighted by pregnancies derived from nuclear transfer embryos (Hill et al., 2000; De Sousa et al, 2001;) where placentae have reduced vascularisation and poor development of cotyledonary structures; in most animals proceeding to term, the physiological consequences are dire. During the period in which the placenta undergoes significant growth (d28-d80 of gestation) the usual expansion in plasma volume associated with pregnancy and mediated through increased activity of the renin-angiotensin system is restricted if nutrition is poor (Dandrea et al., 2002). This suggests again that directed management of maternal nutrition during pregnancy is advisable.

Insufficiency in immune privilege of the fetus is another cause of early pre-term loss but can be prevented by administration of recombinant ovine IFN-tau at the time of implantation (Martal et al., 1997). Acting to ensure early maternal recognition of pregnancy and maintenance through increased progesterone synthesis and prevention of prostaglandin F2 alpha production, it has been separately demonstrated that levels of progesterone produced by the corpus luteum and placenta from day 50 of pregnancy in the ewe must be maintained above a critical level to prevent spontaneous abortion (Al-Gubory et al., 1999). Further, fetal loss in goats is associated with high mean levels of prostaglandin F2 alpha and low mean levels of oestrone sulphate in the blood and indicates that the endocrine fetal-placental function is disturbed (Engeland et al., 1999) and this has also been reported in cattle (Shore et al., 1998). The fact that pregnancy loss is not only recurrent but also heritable in ruminants (Engeland et al, 1997) suggests it may be possible to identify the gene(s) responsible.

14.5.2 Pregnancy loss in other species

Examination of the literature detailing mechanisms of pre-term pregnancy loss in humans and other species provides many incites into causes of pregnancy failure in vertebrates. Remembering there are differences in reproductive structures and mechanimsms between the ruminant and monogastric species, commonly described are failure of immune privilege of the fetus, disorders of the feto-maternal interface and disorders of the blood. For all species, establishment of immune privilege of the fetus is crucial. Failure of the maternal system to actively support pregnancy has recently been demonstrated in mice (Savion et al., 2002) and is caused by production of an inappropriate cytokine network. This study proved the protective action of GM-CSF (granulocyte / macrophage – colony stimulating factor) in immunopotentiation in the mother, leading to increased production of pregnancy-promoting cytokines such as TGF-beta2 which maintain the immunosuppressive phenotype of macrophages, NK and T cells, and reduced production of TNF-alpha, an inducer of cytotoxicity (Yui et al., 1994). In a different study, defective production of LIF, M-CSF (macrophage – colony stimulating factor) and Th-2-type cytokines by T cells at the feto-maternal interface has also been shown to be associated with pregnancy loss (Piccinni et al., 2001). Not surprisingly, and as found in sheep and cattle, defective placental maturation has been shown to lead to late fetal mortality (Stallmach et al., 2001).

Thrombophilic disorders are a common cause of fetal loss in humans and have been reviewed extensively (Brenner and Blumfeld, 1997, Brenner et al., 1999, Brenner, 2000). These include placental thrombosis due to folic acid insufficiency late in pregnancy, caused by mutation of the methylentetrahydrofolate reductase gene (Gris et al., 1999; in"Fetal loss"; see also Lissak et al., 1999), presence of circulating antiphospholipid / antiprotein antibodies leading to arterial and venous thromboses (Gris et al., 2000), protein C (anticoagulant) deficiency in the blood leading to thrombosis (Richards et al., 1997; Brenner et al, 1997) and mutations in the thrombomodulin and endothelial protein C receptor genes in women with late fetal loss (Franchi et al., 2001; in "Fetal loss"). Difficulties in late pregnancy can also arise from malfunction of processes involved in parturition. For instance, an increase in free corticotrophin releasing peptide, normally synthesised during pregnancy but kept inactive (Petraglia et al., 1996), can induce myometrial contractions and lead to pre-term labour (Hillhouse and Grammatopolous, 2002; Challis et al., 2002).

These and other examples to indicate the multiplicity of factors influencing pre-term pregnancy loss and neonatal loss are given below in Table 2.

Table 2 Causes of embryo / fetal / pregnancy loss and mortality in neonates

Cause	First Author	Citation	Affected tissue / stage	Species
Nutritional				
Vitamin B12 deficiency	M Bennett	J Reprod Med 2001	infertility and recurrent fetal loss	human
Elevated Zn / Cu levels in serum	TW Graham	J Reprod Fertil 1994	spontaneous abortion	bovine
Nutrient partitioning	JM Wallace	Proc Nutr Soc 2000	decreased birth weights, neonatal loss	ovine
Genetic - chromosomal / epigenetics				
Inheritance of fetal loss	IV Engeland.	Anim Reprod Sci 1997	fetal loss higher in daughters of affected mothers	caprine
Inherited thrombophilia	B Brenner FE Preston	Curr Opin Hematol 2000 Lancet, 1996	increased fetal loss	human
maternal HLA class II allogenotype markers	OB Christiansen	Eur J Immunogenet 1995	predisposition to fetal loss	human
chromosome translocation	AS Multani	Ann Genet 1992	repeated fetal loss	human
trisomy 21	K Spencer	Prenat Diagn 2001	fetal loss	human
Genetic - specific genes				
Protein Z clotting factor inhibitor deficiency	JC Gris	Blood 2002	Placenta	human
Coagulation factor mutations	I Martinelli.	N Eng J Med 2000	Placenta	human
Methylentetrahydrofolate reductase gene mutation (folic acid deficiency)	JC Gris	Thromb Haemost 1999	Placental thrombosis and late fetal loss	human
MTHFR gene mutations	A Lissak	Am J Obstet Gynecol 1999	recurrent early fetal loss	human
Immunoreglatory role of OX2	RM Gorczynski	Arch Immunol Ther Exp 2001	regulate fetal loss	human

and OX2L (ligand)				
Thrombomodulin and endothelial protein C receptor mutations	F Franchi	Br J Haematol 2001	late fetal loss	human
Angiotensin converting enzyme and angiotensin receptor polymorphisms	C Fatini	Blood Coagul Fibrinolysis 2000	increased first trimester fetal loss	human
factor V Leiden mutation	D Tormene JR Meinardi	Thromb Haemost 1999 Ann Intern Med 1999	increased risk of fetal loss	human
IL-10 and IFN-tau	G Chaouat	J Immunol 1995	prevent fetal loss	murine
IFN-tau	J Martal	Reprod Fertil Dev 1997	improved embryo survival	ovine
Hormonal				
Lowered oestrogen and increased PG2F-alpha	IV Engeland	Anim Reprod Sci 1999	Disturbance of the endocrine fetal-placental function	caprine
Oestrogen concentration at insemination	LS Shore	Theriogenology 1998	fetal loss	bovine
Environment / Toxins				
electromagnetic field exposure	BJ Klauenberg	Am J Epidemiol 1991	fetal loss	human
endocrine changes after dioxin exposure	Y Guo	Biol Reprod 1999	early fetal loss	primate
environmental teratogens	RL Brent	Clin Obstet Gynecol 1994	embryo and fetal loss	human
occupational exposure to antineoplastic drugs	SG Selevan	N Engl J Med 1985	fetal loss	human
Infection				
Human parvovirus B19 infection	M Nyman	Obstet Gynecol	first trimester fetal loss	human
Preparturient infections	BL Munday	Aust Vet J 1966	fetal loss	ovine / bovine
Zoonotic and viral infections	A Sanghi	Br J Obstet Gynaecol 1997	fetal loss after 12 weeks	human
infectious diseases	BC Barr	Vet Clin North Am Food Anim Pract	abortion and fetal loss	bovine
Maternal				
Antiphospholipid (APS) and antiprotein syndromes	JC Gris	Thromb Haemost 2000	Recurrent early fetal loss	human
Thrombophilic disorders	JC Gris	Thromb Haemost 1999	Placenta thrombosis and late fetal loss	human
Antibodies to factor XII	DW Jones	Br J Haematol 2001	recurrent loss / APS	human
Maternal age	AM Nyobo Anderson	BMJ 2000	linkage to fetal loss	human
Breech delivery	S Ong	Ir J Med Sci 1996	birthing difficulties	human
Serum alpha-fetoprotein elevation	JE Maher	Obstet Gynecol 1994	fetal loss	human
Antitrophoblast and	KR McRae	Blood 1993	fetal loss	human

anticardiolipin antibodies				
Coagulaton factor mutations	I Martinelli	N Engl J Med 2000	unexplained fetal loss	human
Peripheral oestrogen concentration at insemination	LS Shore	Theriogenology 1998	early fetal loss	bovine
Interpregnancy intervals	JE Kallan	Soc Biol 1992	preterm birth, fetal loss	human
Twinning	H Rydhstroem	Twin Res 2001	increased fetal mortality	human
Defective placental maturation	T Stallmach	Obstet Gynecol 2001	late fetal loss	human
Biological immaturity	PO Olausson	Br J Obstet Gynaecol 1999	very preterm births, increased neonatal loss	human
Paternal				
benzene and semen quality	S Wang	Zhongua Yu Fang Yi Xue Za Zhi 2000	very early fetal loss	human
Fetal				
Extremes of fetal size	F Lackman	Am J Obstet Gynecol 2001	causes preterm birth	human
Preterm birth	MS Kramer	JAMA 2000	higher loss in infancy	human

CHAPTER 15. POTENTIAL FOR GENE DISCOVERY

15.1 INTRODUCTION

Like all such research, discovery of sheep genes responsible for reproductive inefficiency / failure will rely heavily upon the analysis of gene expression profiles, both at the transcript and protein level, in natural mutants at extremes of reproductive capacity and in experimental animals treated to induce differentials in reproductive capacity. As a result of the immense interest and research effort in human reproduction and due to advances in this medical area, there is a breadth of knowledge and methodology available to experimentally provide the appropriate paradigms of reproductive efficiency. Where studies in animals such as the sheep have contributed much to advances in the human medical sphere, it is now time for the accumulated understanding of reproduction to be applied to the problem of reproductive wastage in livestock species. Below are brief outlines of the tools available for gene discovery and suggested targets for gene discovery programmes aimed at identification of the key determinants of reproductive efficiency and reliability in sheep.

15.2 TECHNOLOGIES AVAILABLE FOR GENE DISCOVERY

In comparison to other vertebrate species, notably the human and mouse, generation of genomic, proteomic and bioinformatic resources for the livestock species has lagged considerably. In the human and mouse, extensive DNA-based arrays have been produced from sequences expressed in individual cell types, specific tissue types, whole organs and in some instances from mixed tissue / organ samples and have revolutionised gene expression analysis. Often made from adult tissues, there are some arrays which have captured ovarian or testis EST's and still others made from preimplantation embryos (e.g. Ko et al., 2000) and fetal tissues. Though not as well advanced, similar information is being generated via high throughput analysis of cell- and tissue-specific protein composition. The paucity in such genomics and proteomics resources for livestock is currently being addressed internationally and the framework for generation of similar analytical capacities in the bovine (e.g. USDA MARC arrays) and ovine (Roslin Institute arrays, U.K.; AgResearch arrays, N.Z.) is progressively taking shape. There are indeed comprehensive cDNA / EST arrays available for gene expression profiling in the bovine, notably with respect to meat production, and some advances have been made in establishing tools for genome-wide analysis of expression in the fetus (e.g. Taniguchi et al., 2001; ESTs from whole bovine fetus).

With respect to the ovine, Australia has yet to formalise its Sheep Functional Genomics / Proteomics programmes and to determine the best alternatives in development of DNA-based arrays covering the areas of prime interest in the sheep production sector, namely meat and wool production. Cleary, with these and the additional interest in reproductive ability, development of arrays should include both fetal and adult material and there is merit in ensuring representation of both male- and female-specific sequences; possible utilising methods such as suppression-subtractive hybridisation approaches in cDNA / EST isolation. The primary effort may be in generating the appropriate "set(s)" of ovine sequences but a secondary effort will be required to establish oligonucleotide-based arrays generated from ovine-specific sequence information. Even in the absence of ovine arrays and considering the high degree of homology within the coding regions of critical molecules across species, much new information about molecules controlling reproductive efficiency in sheep could be gained by utilisation of available human and bovine DNA arrays where conserved coding region sequences are presented for hybridisation.

15.3 TARGETS FOR GENE DISCOVERY PROGRAMMES

In the previous sections, many gene systems are suggested for targetting using the genome-wide approach of expression profiling by DNA microarray hybridisation technology. It appears these fall into five main areas; profiling of gene expression involved in (a) gametogenesis and programmed release of mature gametes in the ewe (b) development of the embryo from fertilisation through to implantation and responses to changing nutrition, (c) establishment and maintenance of the feto-maternal connection, (d) embryonic / fetal responses to the intrauterine environment at different stages during pregnancy and (e) changes in physiological functions in the lamb near to and following parturition.

From this literature survey, it appears that a large proportion of the success of developmental stages referred to above, that allow full-term pregnancy and viability in the post-natal period, is determined by events affecting the gametic contribution of the mother. Consequently, analysis of expression patterns required for successful gametogenesis, specifically oogenesis, is of primary interest and emphasis should be placed on identification of genes that determine egg quality (and hence, to some extent, embryo quality). Gene function in cells and tissues that surround the oocyte during the different stages of follicular development is of central importance. Given that some progress in this area has already been made in other species (reviewed in Richards et al, 2002), key areas of interest in the ovine would be:

(1). Gene function in granulosa cells during development of pre-antral and antral follicles. This study should also include examination of the thecal cells (theca interna and theca externa) that immediately surround the oocyte and granulosa layers.

(2). Gene function in granulosa cells of the preovulatory follicle (i.e. during the four days immediately before ovulation) including cells of the corona radiata (the 4-5 cell layer of granulosa cells abutting the zona pellucida.

(3). Gene expression in the preimplantation embryo with emphasis on the trophectoderm cells from the time of blastocyst formation up to and including the time of implantation.

(4). Gene expression patterns in embryos from single and twin ovulating ewes as a basis for understanding the phenomenon of partial failure of multiple ovulations.

Studies such as these would concentrate on comparisons between high and low nourished ewes at various time-points during egg and embryo development, using the"nutrition model" at the six phases of the reproductive life-cycle where nutrition is known or thought to have an effect on egg/embryo quality (i.e. during fetal gonadal development, early pre-antral follicle development, late pre-antral follicle development, antral follicle development, pre-ovulatory follicle development, pre-implantation embryo development). Determination of mRNA and protein species present in the oocyte cytoplasm (and in other specific cells of the follicle) at different stages of ovum / follicle development could utilise state-of-the-art laser capture microdissection (Emmert-Buck et al., 1996; Sluka et al., 2002). RNA amplification protocols could be used along with cell capture to purify and identify expressed sequences from specific cell types resident in the follicle at all stages.

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