MEAT & LIVESTOCK AUSTRALIA

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MLA LambMax Australia

Towards the Future of Australian Lamb Production – Immediate Targets

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THE UNIVERSITY OF WESTERN AUSTRALIA

Abstract

MLA LambMax Australia was designed to address the decline in the national flock by improving the reproductive efficiency of the dominant maternal genotype, the Merino. It was also designed as a step towards the implementation of 'clean, green and ethical' systems for sheep management. Thus, it tested aspects of focus feeding to improve fecundity and lamb survival, and the non-hormonal management of the timing of lambing.

A series of on-farm and laboratory studies led to the following recommendations:

- Short-term (6-17 days) supplements can be used to increase fecundity and appear to work under all conditions, but may be best in ewes of Condition Score ≤2;
- Supplementation to maintain ewe condition can continue into early pregnancy without a major risk to embryo survival;
- Lambing of maiden and twin-bearing ewes should be managed to maximise neonatal survival (eg, with 'edible shelter');
- Ewes that are to be teased need only be isolated from the males that will be used as teasers, not from all rams;
- Meat & Livestock Australia should fund more R&D in four areas: focus feeding for fecundity, focus feeding for fetal programming, early embryo mortality, and 'teasing'.

These are low-cost ways for farmers to increase lamb output and profitability.

Executive Summary

MLA LambMax Australia was designed to help avoid the decline in the national flock by addressing reproductive efficiency in the dominant maternal genotype, the Merino. It was also designed as a step towards the implementation of 'clean, green and ethical' systems for sheep management. Thus, it tested aspects of focus feeding, lambing management, and non-hormonal management of the timing of lambing. Our initial objective was to respond to the dearth of science teams working in Australia on the broader aspects of sheep reproduction. We thus established the *LambMax Team* – international-quality personnel with essential techniques and equipment who could research the targeted reproductive issues – Dr Carolina Viñoles Gil, Ms Beth Paganoni, and Dr Penny Hawken.

The team connected with local, national and international collaborators on a comprehensive set of studies, including 8 large on-farm experiments. A novel and major dimension to the work on fecundity was the development of the 'first-wave model' in which follicle waves in the ovary are synchronised to increase experimental power. From 3-years work, the *LambMax Team* has thus far produced 22 written reports (refereed articles in journals, books, conferences), presented 17 conference papers, made 9 major communications in industry forums, and had their work aired in mass media on at least 18 occasions. Amongst the **scientific publications** produced to date, two stand out: i) the description of the first-wave model, an experimental protocol that has the potential to revolutionise research on ovulation rate; ii) the discovery that teasers induce the production of new cells in the brain of the ewe in areas that are involved in the formation of memory.

Among the **industry recommendations** produced to date, three need to be highlighted: i) Short-term supplementation (6-17 days) to increase fecundity is only worthwhile in ewes of poor-low body condition (CS≤2), and that supplement can be supplied as lupin grain or as other feedstuffs (eg, soya-maize); ii) In focus feeding for fecundity, it is not necessary to avoid supplementation into early pregnancy to avoid embryo loss; iii) To use teasing to synchonise lambing, it is only necessary to maintain the ewes in complete isolation from the specific males that will be used for teasing. Behind these recommendations is a considerable depth of data, and a range of solid outcomes, that are relevant to the future of the Australian sheep industries and, therefore, to the planning for R&D:

1) Nutrition and Ovulation Rate

a) The importance of nutritional history
Our best advice for short-term supplementation is to restrict it to ewes in low body condition, and not to waste it on ewes in high body condition. However, we may be missing opportunities for this low-cost management tool. On-farm experiments highlight the dynamic nature of body condition and the ways that this dynamism can interfere with responses to, for example, a 6-day lupin supplement. Thus, we are still unsure whether supplements will affect fecundity in ewes that are rapidly losing condition from a high point (eg from CS3) or in ewes that are on a falling plane of nutrition.
The nutritional history of a ewe also stretches back into her embryonic and fetal life and, for too long, we have not been paying sufficient attention to this period of development. The basic biology is now clear: under-nutrition of pregnant ewes can severely compromise the future productivity of their offspring, in terms of fertility, fecundity, and outputs of muscle, milk and wool. More research will be needed in this area before we can make cost-effective recommendations for industry practice.

b) Alternatives to lupins for short-term supplementation
Lupins are remarkable only because they contain very little soluble starch and so can be fed as a short-term supplement without risk of acidosis. Thus, any intelligently designed feed can be used in

their place. For example, fecundity can be improved with soya-maize concentrate, and perhaps with grazed legumes (Lotus, Lucerne). On the other had, a warning comes from the observation that Kasper peas did not affect ovulation rate. We don't yet know why, but hypothesise that, in contrast with lupins, the peas do not provide a physiological stimulus for ovarian follicles. Similarly, we have shown that lupins are ineffective for stimulating colostrum production. It is thus sensible to test all proposed supplements before recommending them for focus feeding.

2) Early embryo mortality

We are now comfortable with extending supplementation into early pregnancy – this is more a cost decision than a risk in reproductive management. On the other hand, our on-farm study has highlighted the massive amount of early embryo mortality in our flocks, re-enforcing the outcome of the review of literature by Walker, Kleemann & Bawden (2003) in their report to MLA.

3) Management of lambing

There is very little value in pursuing the methods for reducing perinatal mortality through field trials because the outcome of trials depends solely on the weather at the time of lambing. Rather, we should accept that the biology is incontrovertible (shelter, ewe retention at birth site, colostrum production) and convince farmers that they should plan conservatively for lambing so as to reduce the possibility of high rates of lamb mortality; this is particularly important for twin-bearers and maidens, for which 'edible shelter' should become standard practice. *This is about risk avoidance for the farmer and for the industry as a whole.* For the same reason, research on lamb survival is still urgently needed, but it should focus on physiology, genetics and behaviour.

4) Management of teasing

The management of lambing is made much easier if the births are synchronised in the flock. We need a tool that is cost-effective for extensively managed flocks and that also compliments our goal of clean, green and ethical production systems. The answer is to be found in 'teasing', at this stage limited to ewe flocks bred before February. Our task is to make this an attractive option for farmers.

a) Simplified management of the isolation of ewes from rams
Our conservative recommendation is that, in the period leading up to teasing, ewes need to be fully separated from teaser males (sound, sight, smell). Our major discovery about olfactory memory in ewes is only the beginning – we need to refine these concepts so we can determine the minimum requirements for isolation. How long do the ewes need to 'forget' a familiar male? Do we need to consider sight and sound? Work is also needed on non-Merino breeds and on maiden ewes.

b) Teasing during the breeding season
A major limitation of teasing is that it cannot be used during the breeding season. This is a very difficult issue, embedded in the core of our understanding of the reproductive system. However, we now know that the reproductive centres in the ewe brain respond to teasers during the oestrous cycle. This cannot directly induce ovulation but it does affect the secretion of major reproductive hormones. Further research may reveal ways to profit from these responses (synchrony, ovulation rate), leading to new, cheap and effective strategies for improving reproductive output.

General Recommendation

Funding uncertainties led to the break-up of the highly effective *LambMax Team* in 2008. Therefore, our view is that MLA R&D Programs need to operate over at least a 5-year time-frame so that high-quality research personnel can be encouraged to remain with the industry.

Contents

1 Background

When this project was being written in 2005, MLA was predicting that Australia would fall 4 million lambs short of demand in the export market over the next 7 years because the number of breeding ewes in the national flock was at its lowest for 50 years. Expanding the flock is difficult because the dominant genotype, the Merino, has a poor reproductive rate. This issue was the focus of MLA Project MS.022, *LambMax WA* (2003-04), from which we developed *LambMax Australia*, with perspectives broadened to be long-term and national. The drafting of this large project led to discussions with MLA and AWI (May 2005) and the formulation of an overall strategy that involved:

i) A Concept Paper written by R Banks (MLA) and I Rogan (AWI), addressing the needs of the industry for research and extension in reproduction, and advocating a joint approach to funding research in the area by AWI and MLA, beginning with a series of reviews and with *LambMax Australia* as a possible model;
ii) A series of reviews completed before the end of financial year 2005-6 so that a full version of *LambMax Australia* (for 2006-2011) could be prepared for consideration by the boards of MLA and AWI before June 2006; the full version of the project was not submitted at that time because it was replaced by the present project (MS.027);
iii) An *Immediate Targets* Research Project (MS.027), comprising the top two priority areas from the full version of *LambMax Australia*, was submitted and supported by MLA so we could continue the momentum set up through the success of MLA's *Primetime Forums*, attract and retain high quality researchers, and prepare for *LambMax Australia* in future years.

The present report concerns only the *Immediate Targets* project, funded for the period 2005-8.

1.1 The Core Business of *LambMax Australia*

LambMax Australia is designed to improve lamb weaning percentage under three goals:

1) The principles of **'clean, green and ethical' production**, recognizing the increasing concerns expressed by consumers and society in general about many aspects of animal production systems. 'Clean' means avoiding drugs, hormones or chemicals; 'green' means that the practices must be environmentally sustainable; 'ethical' means considering animal welfare;
2) Rebuilding the national flock – this is a recognition of the situation we are in with escalating demand for export lamb but a diminishing ewe flock;
3) Finding solutions that are consistent with the needs of reproduction in pasture-fed Merinos – in recognition of the nature of the great majority of sheep production systems in Australia.

1.1.1 The "Clean, Green and Ethical" Package

The core principle underpinning our approach is manipulation of the animals' environment so we can use their normal, internal physiological processes to control their reproductive output. The three environmental factors that we can manipulate are nutrition, socio-sexual signals, and lambing conditions (Figure 1). This is supported by ultrasound, a powerful modern technology that has now become cost-effective, and yet is non-hormonal and non-invasive. Finally, in recognition of the importance of environment x genotype interactions, *LambMax* is linked into companion programs that aim to genetically improve fecundity and lamb survival.

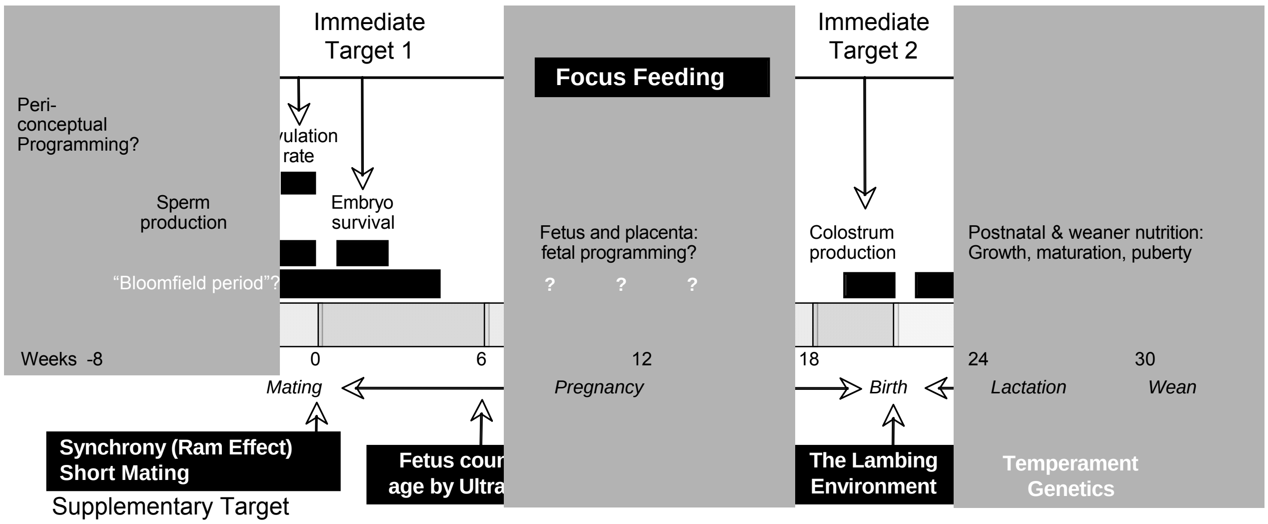


Figure 1. The *Clean-Green-Ethical Package*: periods of focus feeding are used to control the reproductive process and improve the breeding success of the flock. To accurately time the periods of feeding and thus keep costs under control, mating must be brief and controlled (eg, by the 'Ram Effect'), or ultrasound must be used to classify the ewes based on the age of their fetuses. Lamb survival must be maximised by a combination of prenatal feeding for colostrum, a good lambing environment and good maternal genetics. The *Immediate Targets* project initially comprised the two unshaded areas (the highest priority based on the needs of industry and the need to rebuild research capacity), and a Supplementary Target on the 'Ram Effect' was added for Year 3. Redrawn after Martin et al (2004b).

Focus feeding in brief

- In rams, 2 months of high-quality nutrition maximises testicular mass and hence fertility.
- Under-nutrition during the 'Bloomfield Period' can cause premature births.
- A few days of supplementary feed just before ovulation is combined with good genetics to maximise ovulation rate and thus the potential for twin lambs. This is a Target in MS.027.
- Embryo loss is arguably the second biggest source of wastage after neonatal mortality (MLA Review: Walker, Kleemann & Bawden 2003). Under laboratory conditions, overfeeding in the first 2 weeks after mating can reduce embryo survival – this is a Target in MS.027.
- The impacts of nutrition on fetal programming for wool and meat production are poorly understood, let alone quantified, but this area is core business for AWI's *Lifetime Wool* project.
- A high energy supplement in the last week of pregnancy can improve colostrum production and this should promote lamb survival.

Management of Lambing

In addition to prenatal feeding for colostrum, lamb survival can be maximised by a combination of a good lambing environment and good maternal genetics. One critical aspect of maternal genetics is temperament and this is being researched by the Blache-Fergusson MLA project.

The lambing environment involves interactions between this genetic issue and a group of environmental issues: the provision of a maternity ward (shelter, feed, water and calm ambience), and the control of predators. The lambing environment is a Target in MS.027.

1.1.2 Project description

The aim of *LambMax Australia – Immediate Targets* is to increase the weaning percentage of pasture-fed Merino flocks by improving ovulation rate, reducing embryo mortality, and improving the survival of new-born lambs.

1.1.2.1 Immediate Target 1 – Ovulation rate, Embryo survival

To address two major problems (ovulation rate, embryo survival), we needed special expertise in transrectal ultrasound. The highest level of ultrasound technology is essential for tracking the development of ovarian follicles (the process that controls ovulation rate) and for determining the number and timing of embryo losses. We managed to attract to Australia a first class young scientist from Uruguay (Dr Carolina Viñoles), an expert in ultrasound and in the area of nutritional control of ovulation rate in sheep; she operates at the international forefront in this area.

Short-term supplementation (eg, for 4-6 days) increases ovulation rate under controlled conditions but the results are inconsistent under field conditions, where responses range from –18 to +54%. This is a problem area because the risk is unacceptable for many farmers. The scientific essence of the issue is that we cannot solve the problem if we cannot understand the physiological processes involved. After 20 years of painstaking research, we are now beginning to understand how the supplements work, primarily through advances by RJ Scaramuzzi and colleagues (England, France), who have clearly shown that the management of energy by the ovary is involved. For the present project, we worked on three issues that are holding up widespread adoption:

a) Responses to lupin supplements in heavy and light ewes – the problem here is that on-farm results have been variable and unpredictable, perhaps because lupin grain contains large amounts of protein but no starch (glucose); we therefore studied the interactions between lupin grain and factors that affect glucose metabolism, such as changes in body condition score; this should allow us to predict when lupin supplements will be cost-effective;
b) There is increasing evidence that the reproductive performance of an animal depends on its history as a fetus, particularly the nutrition of its mother during pregnancy; we proposed to test whether the ovulation rate of ewe, and its response to lupin supplementation, was affected by its history as a fetus; this may account for much of the between-ewe variation in ovulation rate;
c) Alternative supplements – in many parts of Australia, lupins are not readily available, so we tested other feedstuffs; of major interest were pulses other than lupins.

We hypothesised that the impact of short-term supplements depends on the stage of development of the follicles in the ovary at the time of feeding, as well as on circulating concentrations of glucose and metabolic hormones. All of these factors are affected by body condition. Ewes in high body condition have more gonadotrophin-dependent follicles than ewes in low body condition, an a better ovulatory response to nutritional supplementation has been reported for ewes in high than in low body condition. Thus, the lack of progress with on-farm trials could be due to poor control of the experimental model that is used for studying ovulation rate, particularly body condition and the status of the ovarian follicular waves at the time of nutritional treatment.

1.1.2.2 Immediate Target 2 – Lamb survival

Australia loses about 10 million lambs per year, mostly in the first few days after birth. Most of the losses are among twin-born Merino lambs so this project concentrated on that group. The economic impact of this can be seen simply by comparing the loss with the 4 million extra lambs that MLA estimates is needed to satisfy our export market. In addition, we have a potential disaster awaiting us if our markets decide that that postnatal mortality is preventable and thus an ethical issue.

1.1.2.3 Supplementary Target – Teasing (the Ram Effect)

Tightly synchronized mating is necessary if we are to get efficient reproduction with short mating periods, and implement focus feeding and precision management of lambing. In mid-2007, Dr Penny Hawken was added to the *LambMax* team so we could develop non-hormonal techniques for synchronising conception in sheep flocks. She focussed on improving the practicality of the ‘ram effect’ (or ‘teasing’), in which the sudden introduction of rams induces ovulation in ewes. Because all the ewes in a flock respond at the same time, their oestrous cycles are synchronised. However, the obvious benefits of teasing are not completely realised because of two issues:

a) Understanding and defining *isolation* – it has traditionally been thought necessary to completely isolate the sexes for the ram effect to work. However, ‘isolation’ has never been understood or defined so we have never been able to offer farmers clear advice on how to manage the issue (how far apart and for how long). If *complete* isolation from all ram contact is imposed for months, it creates logistical problems for sheep management on farm. However, it seemed possible that the ram effect would still work if the teasers were different or ‘novel’ as far as the ewes are concerned, the theory being that the novelty of the teaser determines its effectiveness rather than previous absence of all ram contact. This, in turn, infers that ewes can distinguish one ram from another and ‘remember’ them. The ram effect is mostly mediated by pheromones so it seems likely that ewes identify and remember particular males by their odour (‘olfactory learning’). In addition, little was known about the roles of visual and auditory signals, two other factors that may contribute to the definition of ‘isolation’. By clarifying these issues, it should be feasible to offer evidence-based advice about isolation, a simpler way to manage teasing, and more successful outcomes.

b) Use of teasing in the breeding season – currently, teasing can only be used during the non-breeding season (before January) because ovulation can only be induced in acyclic ewes. This is one of the most difficult constraints facing us, but Dr Hawken had evidence that rams could induce a response in the brain systems that control the reproductive axis, so we decided to follow that lead.

A major outcome of this work would be greatly improved flexibility for farmers in the way that they needed to manage teasers. That in itself will help us move closer to the CGE package (Figure 1).

2 Project Objectives

2.1 Project Objectives

2.1.1 Initial Objectives (September 2005)

- To establish the LambMax Team – international-quality personnel with essential equipment that could carry out the research into reproductive issues for the Australian sheep flock.
- To establish the '**first-wave model**', a completely novel experimental approach for studying nutritional factors that affect ovulation rate (eg, glucose drenching, alternative supplements) and early embryo mortality (side-effect of feeding lupins to increase ovulation rate).
- To use the first wave model to assess alternative feed sources for 'focus feeding' that can be used in areas of Australia that do not produce lupin grain.
- To use the first wave model to assess the rate of early embryo loss, and the contribution of nutritional supplementation to the problem.
- To use the first-wave model to assess factors that might interfere with the responses to short-term supplements: body condition, and nutritional history, and fetal programming.
- To field-test the effect on lamb survival in twin-bearing Merino ewes of: a) dietary manipulation of colostrum production, and b) 'edible shelter'.

2.1.2 Supplementary Objectives (June 2007)

- To determine the role of non-pheromonal signals in the ram effect.
- To define the need for isolation of the sexes, and the associated roles of olfactory learning and memory in the ram effect.
- To determine whether the sexual experience and temperament of the ewes affected their responses to the ram effect.
- To determine whether the ram effect is blocked in the breeding season.
- To develop refined advice for farmers about the management of 'teasing'.

3 Methodology

3.1 Methodology – Immediate Target 1

3.1.1 Transrectal Ultrasound

To tackle the issues associated with ovulation rate and embryo survival, we turned to transrectal ultrasound for repeated, direct observation of the follicles in the ovaries. In brief: we used a real-time, B-mode scanner (Aloka SSD 900 Co. Ltd, Insight, Oceania) with a rigid 7.5 MHz transducer modified for external manipulation in the rectum. Ovulation was detected by observing the collapse of a large follicle followed by the presence of luteal tissue at the same site 4 days later. For both ovaries on each day, we noted all corpora lutea (CLs) and the total number, diameter and position of all follicles ≥2 mm in diameter, and recorded their images on videotape. The accuracy and precision of this procedure has been confirmed by analyses of scanned ovaries post-mortem. A follicular wave was defined as one or more follicles growing to at least 5 mm in diameter. Groups of follicles emerging within 48 h were regarded as a single follicular wave. A subordinate follicle was defined as a follicle that reached 3 mm and could be followed by ultrasonography for at least 3 days. The characteristics of follicular waves were described in relation to measures of the largest follicle: day of emergence, maximum diameter, day of maximum diameter, and lifespan (Viñoles et al 2004). The day of wave emergence was the day the largest follicle of the wave was retrospectively identified as being 2-3 mm in diameter.

3.1.2 Ovulation Rate Measured with the 'First-Wave Model'

We coupled the precise measurement of individually identified ovarian follicles with exogenous control of wave development and thus produced the '**first-wave model**', a major scientific contribution from *LambMax*. For the First-Wave Model, 3 injections of 1 mL prostaglandin F2α (PG) analogue (Juramate®, Intervet, Australia) are given 7 days apart (Days -14, -7 and 0) to sychronise the follicle waves of the ewes; the final PG injection is given on the final day of treatment (eg, lupin supplementation); the PG treatment ensures that all experimental ewes are experiencing the first follicular wave of the oestrous cycle, greatly reducing between-ewe variation and increasing experimental power. This model provided consistent responses in ovulation rate when feeding lupins for 6 days, allowing us to use smaller groups of animals (15 instead of 50), a pre-requisite for detailed metabolic and endocrine measurements. The first-wave model was especially effective when it was combined with precision measurement of body condition score (BCS), using a scale of 1-5 (1 = thin and bones easily felt to 5 = obese; derived by excluding the 0 score from the 6-point scale of Russel et al. 1969). This system of BCS assessment has been standardised across Australia in the *AWI Lifetime Wool Project* by Beth Paganoni and colleagues.

3.1.3 Fertility with the 'First-Wave Model'

To test whether the First-Wave Model could lead to fertile cycles, we compared it with a standard method of synchronisation (sponges plus eCG) and evaluated fertility and prolificacy. Merino ewes were treated with intravaginal sponges for 14 days and given 200 IU eCG at sponge removal (n = 100), or were subjected to the First-Wave Protocol (n = 100). Intrauterine AI with 200 million sperm was carried out 49-56 h after the end of the synchronisation treatment. Conception rate and number of fetuses were evaluated by ultrasonography 46 days later.

3.1.4 Embryo Losses

Overfeeding increases progesterone clearance and causes pregnancy failures during the second week after mating. The issue here is that, if we are feeding to increase fecundity, we may lose everything that we have gained by improving ovulation rate if we continue to supplement the ewes after they have mated. The evidence supporting this was derived from studies comparing extreme diets that might not reflect real-world practices, yet the accumulated evidence in the literature (MLA Review: Walker et al 2003) shows that embryo losses are a major component of reproductive wastage. Transrectal ultrasound again comes to the fore by allowing us to quantify embryo losses.

3.2 Methodology – Immediate Target 2

3.2.1 Lamb Survival – Colostrum Production

We know that 7 days of supplementation with maize or barley grain will double colostrum production in twin-bearing ewes and we are sufficiently confident that clear benefits will flow on for lamb survival in the perinatal period that we are encouraging farmers to try it. Nevertheless, we thought that there would be value in demonstrating, at farm level, that feeding for colostrum production actually does improve lamb survival. Work by Goodwin & Norton (2004) has demonstrated this link with multiple-bearing Australian cashmere goats but we need to turn theory into practice for Merino sheep.

3.2.2 Lambing Survival – the 'Maternity Ward'

Two more important factors that contribute to lamb loss also need to be considered: i) poor ewe temperament – this problem is being addressed through testing and genetic selection and is the remit of a sister MLA-funded project (Blache & Fergusson: *Increasing sheepmeat production efficiency and animal welfare by selection for temperament*); ii) the lambing environment – we need to encourage producers to take up the findings of the past 20 years of research, much of it in WA and France, into conditions that promote lamb survival by allowing the development of the ewe-lamb bond. Essentially, producers need to invest in a *maternity ward* where they can keep the ewes near the birth site by providing feed, water and shelter, and by reducing disturbance. This investment will be particularly rewarding for maiden ewes and for mature ewes carrying twin lambs, the two major sources of lamb loss in the national Merino flock.

New ways to provide shelter were included in the research program of the Salinity CRC where deep-rooted, perennial shrubs and forages are being developed to control the water table. The period of biggest risk in many environments is mid-winter and producers are being encouraged to lamb in this season. Here, standing crops might be an option. Effectively, we are working towards the concept of 'edible shelter' that protects the newborn from wind-chill and, at the same time, induces the mother to feed and remain near the birth site. Edible shelter may also promote colostrum production.

3.3 Methodology – Supplementary Target (the 'Ram Effect' or 'Teasing')

'Teasing' is the induction of ovulation in anoestrous females by the introduction of males after a period of isolation of the sexes. The female responds to signals from the male, primarily odours, that trigger brain pathways that stimulate the secretion of gonadotrophins – specifically, there is an increase in the frequency of pulses of luteinising hormone (LH; Fig. 2).

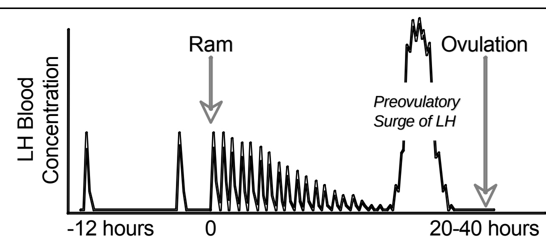


Figure 2. The brain signal that controls reproduction is bioassayed by observing LH pulses in jugular blood. In this schema, a ewe that has a frequency of 2 pulses per 12 h responds to ram introduction with an increase in pulse frequency within minutes. The high frequency pulses stimulate follicle growth, ultimately leading to a preovulatory surge of LH, and ovulation about 2 days later. Review: Martin et al (2004a).

Therefore, the most rapid way to detect the response of the ewe to the teaser stimulus is to take blood samples and measure LH, thus 'bioassaying' the brain signal. In addition, ovulation can be followed, as can oestrus, over the next 20 to 30 days. For the experiments in *LambMax Australia*, we have limited ourselves to the bioassay of the brain response.

3.3.1 The Role of Non-Pheromonal Signals

The hypothesis here is that, in addition to the olfactory ('pheromonal') signals from rams, the ewes also respond to sight and sound. There is evidence that sheep recognize each other by visual cues, particularly facial structures, and this, along with 'olfactory memory' (see below), may also be involved in the recognition, remembering and forgetting of individuals.

Adult Merino ewes that had been previously isolated from ram contact (not within 500 m for a minimum of 2 months) were housed in an animal facility at the University of Western Australia during the non-breeding season. The ewes were fed at maintenance and housed in groups under natural light (9.5L:14.5D). Blood was sampled twice weekly for 2 weeks prior to the experiment to monitor the concentration of progesterone. Two weeks before the experiment began, groups of eight ewes were allocated to treatments, with each group balanced for age (6 years) and weight: Ram (55 ± 1.7 kg); Visual (50 ± 2.5 kg); Audio–visual (55 ± 1.1 kg) and Control (55 ± 1.9 kg). All the four groups were kept in the same animal house and exposed to their stimulus midway through a frequent blood-sampling regimen: the 'Ram' group was exposed to two adult, sexually experienced, Merino rams; the 'Visual' group was exposed to still images of Merino rams; the 'Audio–visual' group was exposed to a video of Merino ewes and rams mating. The 'Control' group remained isolated from ram stimuli for the duration of the experiment. On the day of treatment, blood was sampled every 12 min for 6 h before and 6 h after exposure to the stimulus. The treatment days were staggered to ensure that no group was affected by stimuli from another group: Control ewes were sampled on Day 0; Visual ewes on Day 1; Audio–visual ewes on Day 4 and Ram ewes on Day 6. On the day of exposure to a chosen stimulus, the ewes that were not being treated were moved from the animal house to a paddock out of auditory and visual range (>500 m).

The still images of rams (n = 56) were loaded into presentation software and projected onto a 1.8 m x 31.5 m screen. The images included both frontal and side-on views of adult, Merino rams from the University Farm. Each image remained on the screen for 2 min and was scaled to ensure that it was life-size. The projected images rotated continuously for the second half of the blood-sampling regimen. The audio–visual stimulus was recorded at the University Farm using three sexually active Merino rams and two oestrous Merino ewes. The video was edited so that each 5-minute loop contained an optimal combination of frequent displays of oestrous behaviour by the ewes and mating activity by the rams (about 2.5 mounts/min). After editing, the video was loaded onto a computer video player, sized and projected onto a screen as outlined above for the 'Visual' ewes. The auditory recording of rams was optimised using an external speaker system connected to the computer. The audio–visual stimulus played continuously for the second half of the blood-sampling regimen. The ewes did appear to be aware of both the audio and visual stimuli but no formal observations were made of their behaviour. For the positive controls, the Ram ewes, two adult, sexually experienced Merino rams, were introduced midway through the blood-sampling regimen and had full contact with the ewes for the remainder of the experiment. Negative control ewes remained isolated from all ram stimuli for the duration of the blood-sampling regimen.

3.3.2 The Importance of Isolation of Ewes from Rams

3.3.2.1 The Importance of Male Novelty

Adult multiparous Merino ewes (n = 20) that had been previously isolated from males (i.e., >500 m away from males for a minimum of 2 months) were habituated to vasectomised adult male Merino rams (n = 4) at pasture for about 3 months. Two weeks before the experiment, all ewes were moved into a facility at the University of Western Australia and were allocated to one of the following two groups: familiar (n = 10) or novel (n = 10), balanced for age (mean ± SEM, 2.7 ± 0.3 years for familiar and 2.6 ± 0.2 years for novel) and live weight (55.0 ± 2.2 kg for familiar and 53.0 ± 1.9 kg for novel). The ewes were maintained in group pens under natural light (12.5L:11.5D), and each animal received a daily ration of 150 g of lupin grain, 750 g of rough-cut chaff, and 25 g of minerals. Progesterone pessaries were inserted 11 days before the day of ram exchange (Day 0). Progesterone does not block the neuroendocrine response of ewes to rams but ensures a consistent endocrine milieu within which to monitor LH secretion. The mean ± SEM concentrations of progesterone on Day 0 were 1.30 ± 0.14 ng/ml (familiar) and 1.20 ± 0.09 ng/ml (novel). On Day 0, blood was sampled every 15 min from 6 h before to 6 h after ram exchange to study the effect of ram exchange on LH secretion. At male exchange, the two familiar rams were removed for 15 min and returned (familiar) or replaced with two novel rams (novel).

3.3.2.2 The Role of Cell Division in the Brain

The hypothesis is that the pheromone signal from the ram elicits new cell division in the brain of the ewe and the new cells migrate to the olfactory system to establish new connections that are involved in the recognition of the ‘pheromonal signature’ of the ram that induces ovulation. This would explain why novel rams are needed for the ram effect – any individual ram will need to be ‘forgotten’ before it can induce ovulation in the same ewe a second time.

The production of new cells is detected by labelling them with bromodeoxyuridine (BrdU), an analogue of thymidine that is incorporated into the DNA of dividing cells during the S phase of the cell cycle. The population of cells susceptible to BrdU labelling is dictated by many aspects of the experimental protocol, particularly the dose of BrdU and the time of injection relative to stimulus exposure and to euthanasia. In this study, BrdU (100 mg/kg in 0.9% saline) was injected intravenously into the female sheep immediately before male exposure and at a parallel time point in the control group. BrdU had not previously been used in adult sheep, so the dose was selected based on a rodent study. The short half-life of 2 h, in combination with injection immediately before male exposure, ensures that the only cells labelled with BrdU were those proliferating within hours of male exposure. Approximately 48 h after BrdU injection, all females were heparinized (25000 IU iv), killed (100 mg/kg of sodium pentobarbitone iv), and swiftly decapitated, and the heads were perfused with 2 L of 0.9% saline and 3 L of 4% paraformaldehyde (pH 7.4). The 48-h time point was selected because newborn cells are typically small, irregular in shape, and distributed in clusters and thus are easier to quantify accurately several days after BrdU injection. Blocks of tissue containing the hippocampus and hypothalamus were dissected out and stored in 4% paraformaldehyde for a further 24 h (4°C) followed by 24–48 h in 15% sucrose (4°C). Coronal sections of the hippocampus, hypothalamus, and small intestine were cut at 20 Im on a cryostat, mounted onto charged slides, and stored at –80°C. One in 10 sections (20 μm at 200-μm intervals) through the hippocampus was processed for fluorescence immunohistochemistry to detect BrdU-positive cells.

3.3.3 Teasing During the Breeding Season?

The ultimate form of the ram effect is seen in Merino ewes mated before January-February: the introduction of teaser wethers or rams stimulates the secretion of LH that, in turn, stimulates the follicle(s) in the ovaries and then drives that follicle(s) through growth, maturation and ovulation. During the breeding season, this response to the male is thought to be blocked. However, there was evidence that, during the breeding season, the ram stimulus could still elicit the initial secretion of LH. This may have some beneficial outcome for sheep management … namely, some degree of synchronisation of ovulation among cyclic ewes.

The methodology here involves two experimental models:

a) Ewes out of season (before January) – isolated from males (unless male presence is a treatment), introduction of males (rams or testosterone-treated wethers) or male ‘signals’ (audio, audio-visual), observation of short-term endocrine response (LH pulse frequency, a bioassay of the activity brain reproductive centres) and, where warranted, ovulation, ovulation rate, short cycles (by ultrasound or laparoscopy);
b) Ewes in the breeding season (February-May) – as above, but the ewes are pre-treated with progestogen intravaginal devices, and LH pulse frequency is the only response measured.

4 Results and Discussion

Here, the major results are outlined and discussed in brief. Fuller reports can be found in either the publications listed in Section 8 of this Report, or in the Appendices if no publication is available.

4.1 The LambMax Team

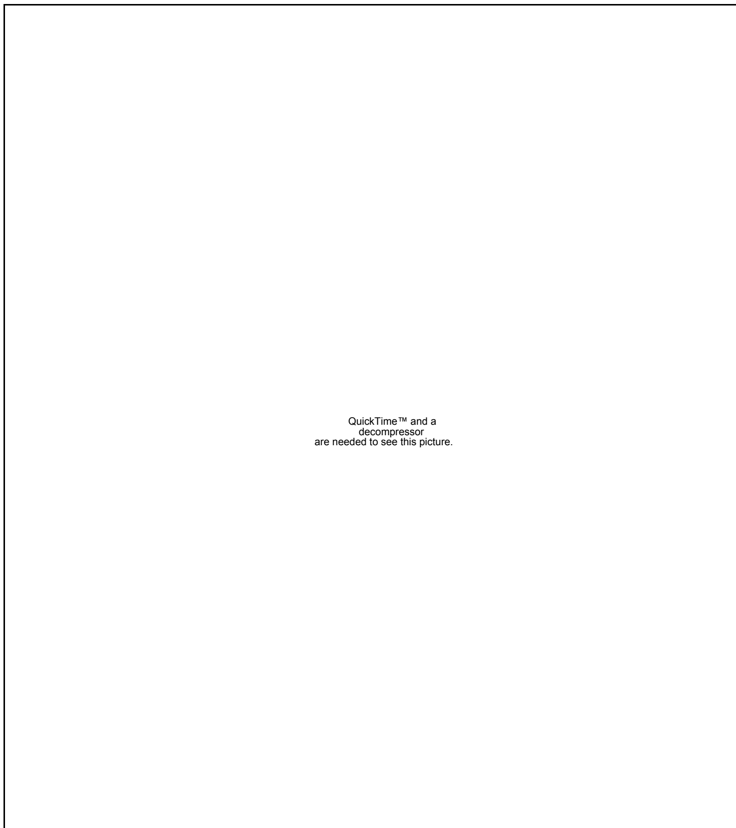
Our initial objective was to respond to the dearth of science teams working in Australia on the broader aspects of sheep reproduction that were relevant to the majority of Australian sheep breeders. This was, and is still, necessary because of the rapidly diminishing national flock. We thus set out to establish the *LambMax Team* – international-quality personnel with essential techniques and equipment that could carry out the research into the targeted reproductive issues.

The LambMax Team (L to R): Dr C Viñoles Gil, Ms Beth Paganoni, Ms Kristy Glover, Dr J Milton.

Dr. Carolina Viñoles Gil (BVetSci, MSc, PhD) was recruited from Uruguay as a Postdoctoral Fellow for the three years of the project, starting 31 October. Her area of expertise is the use of ultrasound to study the responses of the ovary to changes in nutrition, an essential aspect of our research.
Ms Beth Paganoni (BSc Wool Sci, MSc) was recruited as a Research Officer in late 2005, under secondment from the WA Department of Agriculture. She helped forge a strong collaborative link between DAWA and UWA, and brought to us the advantage of a rural background, sheep farming contacts, and the know-how in on-farm studies that she gained through the AWI *Lifetime Wool* Project. For the final year of *LambMax*, she was replaced by Ms Kristy Glover (B Anim Sci).

The other members of the core LambMax Team were Prof Graeme Martin and Dr John Milton. For certain experiments in Targets 1 and 2, we also brought in international collaborators: Dr Ana Meikle (Uruguay), Prof Ken McNatty, (New Zealand), Dr Cecilia Sosa and Dr Alfonso Abecia (Zaragoza, Spain). LambMax also leveraged local resources at the University of WA, particularly the hormone assay laboratory, including the services of Chief Technician, Ms Margaret Blackberry.

Penny Hawken (BAgricSci, PhD) joined *LambMax* for 12 months in late 2007, employed 50% by *LambMax* and 50% by the University of WA. Only her salary was paid by the *LambMax* project because the costs of her research, including a full-time technician (total $100k pa), were covered by the Australian Research Council via a Discovery Project looking into the basic physiology of the ram effect. The UWA side of her position was also linked to *LambMax* – she taught sheep reproduction to undergraduate students (BSc AnimSci; BSc Agric), using the ‘clean, green and ethical’ context. This demonstrates the value of leveraging. By supporting Dr Hawken, the MLA gained: i) external funding for basic but highly relevant research; ii) the services of Ms Trina Jorre de St Jorre, winner of an Australian Postgraduate Award for PhD studies in this part of the *LambMax* project; iii) the inputs of an international expert, Dr Claude Fabre-Nys (CNRS, France) who collaborated with Dr Hawken and Dr Dominique Blache in work that linked *LambMax* to the MLA-funded project on temperament.



Dr Penny Hawken (centre) with two collaborators from France, Dr Claude Fabre-Nys (left) and PhD student Audrey Chanvallon (right), during their experiment on teasing in the Allandale temperament flock (Section 4.5.4).

The productivity of the *LambMax Team* is evident on three fronts:

a) From the list of communications to science, industry and the public (**Section 8**) it can be seen that the team was highly productive – at least 20 peer-reviewed publications will be produced, verifying the quality of the research supported by MLA through this project, and the value gained when a small project such as *LambMax* can leverage other resources;
b) A strong commitment to industry through on-farm trials and high-profile appearances at farmer forums and industry-focussed conferences;
c) The principles of *clean, green and ethical animal production* were widely publicised locally, nationally and internationally (Communications: 2, 10, 11, 12, 16, 34, 35, 44, 48, 58-60). As a consequence, Australia’s sheepmeat industry is now recognised as an international leader and Australia is seen as a focal point for R&D under that umbrella. This has led to numerous invitations to speak on the topic at international conferences, and to *LambMax* personnel running workshops in Thailand (2007) and Uruguay (2009).

4.2 Immediate Target 1a – Nutrition and Ovulation Rate

Communications: 12, 15, 16, 19, 20, 22, 36-39, 42, 43

4.2.1 The First-Wave Model

4.2.1.1 Synchronisation of Follicular Waves

We successfully developed a new experimental model for studying follicular dynamics in the ovaries of female sheep by synchronising the emergence of the first follicular wave of the cycle in all animals. This 'first-wave model' reduces the variation of endogenous patterns that characterise the reproductive endocrine axis among ewes. This permits the examination of ovarian responses to external factors, such as changes in nutrition, with small sample sizes that are compatible with intensive physiological measurements. A full manuscript has been submitted (Communication 19).

4.2.1.2 Fertility and Prolificacy after Artificial Insemination

Pregnancy rate was higher in the group treated with sponges (85/96; 89%) than in the first-wave group (47/100; 47%; P<0.001). Among the ewes that were pregnant, 38% were carrying twin fetuses in both groups. A full manuscript is in preparation (Communication 20).

4.2.2 Short-term Supplements and Ovulation Rate

4.2.2.1 The 'Acute Effect' of Lupins

One hundred and ten Merino ewes were allocated to two groups that were homogeneous for age, body weight and body condition, and they were all subjected to the first-wave protocol. Controls (n = 55) were fed a diet based on hay and the Lupin group (n = 55) received hay plus 500 g lupins/ewe daily for 6 days, from 2 days after the second PG (expected Day 1 before ovulation) until the third PG was injected (expected Day 4 after ovulation). Ovulation rate was evaluated by ultrasonography on Day 10 after the expected ovulation in all the animals. Table 1 shows that ovulation rate was 14% higher in the Lupin group than in the Control group.

Table 1. Effect of feeding lupins for 6 days on ovulation rate in Merino ewes. ab P = 0.06

	Corpora Lutea		Ovulation	Increase
	1	2	rate	(%)
Control	35	20	1.36[a]	
Lupins	25	30	1.55[b]	+14

4.2.2.2 Alternatives to Lupins for Increasing Ovulation Rate

4.2.2.2.1 'Kasper' Peas

In January, 240 Merino ewes (born 2000) were allocated among 3 experimental groups (Control, Lupins, Kasper peas, n = 80) that were homogeneous for body weight (60.8 ± 0.4 kg) and body condition (3.8 ± 0.04 units). The groups were grazed on different paddocks. All the animals were subjected to the first-wave protocol and were fed 50 g peas plus 50 g lupins for 4 days before the beginning of the experimental period.

The availability of dry matter in the three paddocks was evaluated before the introduction of the animals and the stocking rate adjusted accordingly. The control group (n = 80) grazed a pasture with 0.39 tonnes DM available per ewe daily. The groups that received the supplement had 0.3 tonnes DM available per ewe daily. To compensate for differences between paddocks, the Lupin and

Kasper groups were swapped after they consumed half the total amount of supplement for the whole experimental period (10 days). However, because of unplanned rainfall events, there was more 'green pick' (mostly clover) for the Kasper group, so the amount of supplement was reduced in an attempt to provide a diet isocaloric compared to the Lupin group.

A schematic representation of the experimental design is presented in Figure 3. The Lupin group (n = 80) was introduced progressively to the full supplement over 4 days (100 g/head on Day -4; 200 g on Day -3; 300 g on Day -2; and 400 g on Day -1) and were then given the total supplement (500 g lupins/ewe daily for 6 days, from 2 days after the second PG injection until the third PG injection (expected Day 4 after ovulation). The Kasper group (n= 80) were also slowly introduced to the supplement (90 g on Day -4; 180 g on Day -3; 270 g on Day -2; 360 g on Day -1) then received the full supplement 460 g/ewe for the same 6-day period as the lupin-supplemented group. Each animal had 30 cm space in the feeding line. The average daily intake during the 6 days of complete supplementation was 494 g/ewe for lupins and 470 g/ewe for peas.

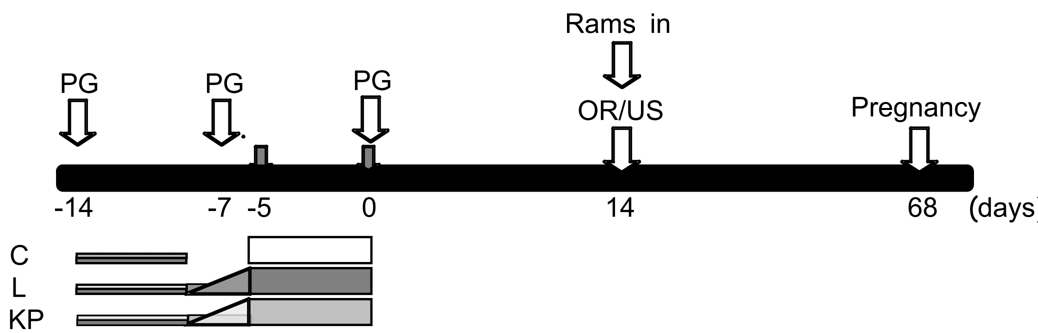


Figure 3. Schematic representation of the experimental design. PG = prostaglandin injection; OR/US = measurement of ovulation rate by ultrasonography; pregnancy = detection of pregnancy and counting of fetuses by ultrasonography. Treatments: C – control; L – lupins; KP – Kasper peas.

Ovulation rate was measured by ultrasonography on Days 10-11 after the expected ovulation. The frequency of animals with 1 or 2 ovulations was analysed using the Chi-square test. Relationships between type of ovulation, body condition score and body weight were analysed by GLMM regression analysis. Since ewes had a high condition score of 3.8, and a carry-over effect of the supplementation has been described for ewes in good condition (Leury et al., 1990), we also tested whether the supplemented ewes would conceive a higher number of twins in the next cycle. The mating period started 14 days after the third prostaglandin injection. Oestrus was expected over Days 19 to 21 after the third prostaglandin injection. Ewes were scanned 54 days after ram introduction and we counted the number of fetuses conceived in the pre-synchronised oestrus.
Table 2 shows that feeding lupins for 10 days increased ovulation rate by 9%. The group that received the lupin supplement had more twin ovulations at all live weights (P = 0.06) and condition scores (P = 0.07) than the control group or the group that received the Kasper pea supplement.

Table 2. Reproductive performance in control ewes and ewes fed either lupins or Kasper peas for 10 days**.** Note: The data for pregnancy are for the cycle following the cycle in which supplements were fed.

		Treatment Cycle				Following cycle			
Treatment	**n**	**1 Ovulation**	**2 Ovulations**	**Ovulation rate**	**(%)**	**Dry**	**Pregnancy rate (%)**	**Singles**	**Twins**
Control	77	46	31	1.08[a]		15	81	26	36
Lupins	77	36	41	1.18[a]	+9	24	68	16	34
Kasper peas	76	47	29	1.05[a]	-3	16	72	25	30

Ewes were mated in the cycle following the administration of the supplement. Pregnancy rate was not significantly different among groups (Table 2, P > 0.05). The increase in ovulation rate observed after the supplementation with lupins was not carried over to the next oestrous cycle.
The hypothesis of a carryover effect of supplementation on ovulation rate during the following cycle was not supported. However, since animals were in a wide range of condition scores within groups, this was perhaps not the ideal experimental design to test this hypothesis.

4.2.2.2.2 Feeding Legume Pasture

In studies done in Uruguay, we tested whether short periods of increased nutrition will improve ovulation rate and prolificacy, irrespective of the method used to synchronise the cycles of the ewes. In Experiment 1, we used 138 Corriedale ewes to evaluate two factors: synchronization treatment (sponges versus a single injection of prostaglandin) and type of pasture (native versus improved with L*otus corniculatus*). Ewes were mated at the end of the grazing period and prolificacy was evaluated at lambing. Grazing *Lotus corniculatus* for 12 days tended to increase the number of twin lambs born (P = 0.09). The percentage of ewes showing oestrus during a 9-day period was similar among synchronization treatments.

4.2.2.2.3 Maize-Soya Supplements

In our second study in Uruguay, we did two experiments. In Experiment 1 (n = 282) and Experiment 2 (n = 288), ewes were allocated to a control group or a group fed a supplement of corn grain and soybean meal for 7 days. Ewes received 2 prostaglandin injections and the supplement was fed from Days 11-17 after the second prostaglandin. Ovulation rate was measured in subsets of 65 (Experiment 1) and 61 (Experiment 2) ewes that were confirmed to have consumed the supplement and that also showed oestrus in a 4-day period. The supplement increased ovulation rate by 14% in both experiments (P < 0.05). We conclude that Corriedale ewes can respond with increases in prolificacy to a 12-day period grazing *Lotus corniculatus* and in ovulation rate to 7 days feeding with a supplement rich in energy and protein.

4.2.3 Nutritional History and the Response to Short-Term Supplements

4.2.3.1 Interactions – ‘Static’ x ‘Immediate’ Effects of Nutrition – Metabolic Physiology

The first-wave protocol was established and nutritional treatments were imposed for 6 days, from 2 days after the second PG injection until the day of the third PG injection. To test whether the ‘static’ and ‘immediate’ effects of nutrition on ovulation rate are additive, we used a 2x2 factorial design with 10 ewes per group (± supplement, Low or High body condition, BC). During the 6-day supplement, LBC ewes received a diet that provided double their requirements for maintenance while HBC ewes received a diet that provided the same increment in energy that the LBC ewes were offered. Follicle development was studied by daily ultrasonography from the day of the second PG injection until the ovulation induced by the third PG injection. Blood was sampled to measure progesterone, FSH, oestradiol, glucose, insulin, leptin and IGF-I. The supplement increased the number of 3-mm follicles ($P = 0.06$) and increased the circulating concentrations of glucose, insulin and leptin ($P < 0.01$). Concentrations of FSH were lower in supplemented than non-supplemented ewes ($P < 0.01$), but oestradiol showed the opposite trend ($P = 0.07$). Compared to LBC ewes, HBC ewes developed more follicular waves, had higher concentrations of insulin, leptin and IGF-I ($P < 0.05$), and tended to have higher FSH concentrations ($P = 0.09$). The changes in metabolic hormone concentrations were affected by the interaction between condition score and supplementation. Leptin and insulin concentrations remained higher in HBC ewes until the end of the supplementation period, but decreased after the third day of supplementation in LBC ewes. In conclusion, the first-wave model has allowed us to show that high concentrations of metabolic hormones in fat ewes are related to the development of more follicular waves. When a supplement is superimposed on this effect, dynamic changes in glucose and metabolic hormones are induced, and more follicles are selected into the wave, perhaps explaining the increase in ovulation rate.

4.2.3.2 Supplementing Ewes on a Decreasing Plane of Nutrition

The objective was to test the effect of a 6-day nutritional supplement on ovulation rate in ewes on a decreasing plane of nutrition. The study began in November with 400 Merino ewes in condition score 2.7 ± 0.02 and live weight 58 ± 0.5 kg. Condition score and live weight were measured every 2 weeks, and 246 ewes that maintained condition score from November to January were selected, and allocated into two groups. The control group (n = 122) remained un-supplemented and the supplemented group (n = 124) received 500 g lupins daily for 6 days. All the ewes were put through the first-wave protocol, and the supplement was fed from Day 2 after the second prostaglandin injection until the day of the 3rd injection. Twelve days after the second injection, ultrasound was used to measure ovulation rate. More control (82/122) than supplemented ewes (59/124) were in anoestrus just before mating ($P < 0.05$). The frequency of singles (30/122 and 42/124) and twins (10/122 and 23/124) were similar among control and supplemented ewes, respectively ($P > 0.05$). The condition score of the ewes fell from 2.7 ± 0.02 to 1.8 ± 0.03 by the time ovulation rate was measured. We conclude that ewes on a decreasing plane of nutrition for 3 months before mating responded to short term supplementation by increasing the frequency of animals ovulating, rather than changing the frequency of ewes having twins.

4.2.3.3 Pre-Supplementation Intake – Follicles, Metabolic State, Ovulation Rate

The aim of this study was to test whether level of feed intake prior to supplementation affected the outcome for follicular development, hormone profiles and ovulation rate in Merino ewes of moderate body condition. Thirty-six Merino ewes of body condition score 3 were subjected to the first-wave protocol then allocated to four groups of 9 ewes in a 2x2 factorial design. Factor 1 was level of feed intake 6 days prior to supplementation: half maintenance (0.5M) or maintenance (1M). Factor 2 was no supplement or a 6-day supplement to twice the previous level. Thus, the 4 groups were: 0.5M-0.5M; 0.5M-1M; 1M-1M; and 1M-2M. The maintenance diet provided 8.9 MJ ME and 101 g crude protein daily. Lupin grain was used as the supplement. Blood was sampled daily for the analysis of glucose and metabolic hormones. Follicular development and ovulation rate were evaluated by daily ultrasound scanning.

Supplementation of the group fed at 0.5M decreased glucose concentrations compared with the non-supplemented group (0.5M-0.5M) ($P < 0.05$). The concentrations of insulin, leptin and IGF-I were higher in the supplemented groups compared to the other groups ($P < 0.05$). FSH concentrations tended to be lower in 0.5M-1M ewes than in 0.5M-0.5M ewes ($P = 0.07$). Ewes fed at half maintenance had fewer 4-mm diameter follicles than 1M-1M ewes and supplementation increased the number of 5 mm follicles ($P < 0.05$). Supplementation tended to increase ovulation rate: fewer double ovulations were observed in 0.5M-0.5M ewes than in 1M-2M ewes ($p = 0.07$). We conclude that acute sub-nutrition inhibits follicular growth, and that this effect can be reversed by a 6-day lupin supplement.

4.2.3.4 Body Condition Score and Responses to Short-Term Supplements

Nutysia Grove, Broomhill (private farm): after their lambs were weaned in October, 700 4-year-old Merino ewes were fitted with electronic tags, weighed and condition scored. Ewes were removed from the experiment if they were less than condition score 2 or greater than condition score 3, or if they had been scanned pregnant with twins for all of their first three matings. The remaining 495 ewes were randomly allocated into 2 groups: Lean (n = 248) and Fat (n = 247).

The Lean ewes were grazed on 50 ha of low quality pasture (FOO = 1909 kg DM/ha) dominated by barley grass and geranium. The Fat ewes were grazed on 60 ha of high quality pasture (FOO = 2286 kg DM/ha) dominated by ryegrass and subterranean clover. The Fat ewes also received 300 g/head daily of “707 Pellets” (Macco Feeds Australia) from early November until shearing in mid December, after which they were grazed on a wheat stubble and the pellets were increased to 600 g/head daily. The Lean ewes received no supplement until after shearing when they were fed 300 g/head daily of 707 pellets. Eleven days after shearing, the Fat group began receiving 800 g/head of pellets daily; this increased 7 days later to 1 kg/head daily. The pellets fed to the Fat group were increased again 7 days later to 1.2 kg/head daily and finally increased another 7 days later to 1.5 kg/head/day. The Lean group began receiving 335 g/head/day of 707 pellets 2 days (Day -16) prior to the first PG injection of the ‘First-Wave Model’. On Day -18 before the first PG injection, the ovaries of 20 ewes from the Lean group were examined using transrectal ultrasonography to check for the presence of corpora lutea (CL), an indicator of cyclic activity.

Experimental ewes were placed under the first-wave protocol, with the final PG injection given on the final day of lupin supplementation (Day 0). Half the Fat ewes were supplemented with lupins at 600 g/head daily and half the Lean ewes with 500 g/head daily for 6 days (Day -5 to Day 0) prior to the final PG injection. Lupin supplementation was based on the last weights prior to supplementation to provide a one times maintenance requirement. Ovulation rate was measured by transrectal ultrasonography over Days 11-13 and the final weights and condition scores were collected on Day 14. The ewes were weighed and condition scored fortnightly. The ewes were fasted overnight before each weighing. Fleeces were weighed for all ewes at shearing in mid-December. The average fleece weight of each group was used to correct ewe live weights measured before shearing.

Ovulation rate was higher in Fat (377/243 = 1.6) than in Lean ewes (333/237 = 1.4; $P < 0.05$).
Table 3: Supplementing Lean ewes with lupins tended to increase ovulation rate by 8% ($P = 0.06$), while it tended to decrease ovulation rate by 4% in Fat ewes ($P = 0.07$).

Our hypothesis, that Fat ewes will have a greater increase in ovulation rate after a 6-day lupin supplement than Lean ewes, is rejected. This conclusion contradicts our finding in Section 4.2.3.1 above, but it is derived from a far more robust design with respect to animal numbers. We conclude that the static and immediate effects of nutrition on ovulation rate are not additive, perhaps because fat ewes have already reached their genetic potential for maximum ovulation rate.

Table 3. Number of CL counted in fat and lean ewes that were supplemented or not with lupins for 6 days between the second and third prostaglandin injection of the first-wave model.

	Fat (CS4)		**Lean (CS2)**	
CL	*Supplemented*	*Non-supplemented*	*Supplemented*	*Non-supplemented*
1	64	51	65	78
2	51	68	53	39
3	6	2	1	1
5	0	1	0	0

Our results are in line with previous findings showing an increase in ovulation rate in lean ewes (Nottle et al., 1997), although the ewes in that study were undernourished rather than fed to meet their requirements for maintenance. Since we have shown that 500 g/head daily of lupins are needed to maintain the condition score of sheep during summer, we suggest that often when lupins are fed this basically allows ewes to only express their average ovulation rate. If ewes are undernourished during summer, they will have an even lower ovulation rate.

4.2.4 Fetal Programming – Nutrition during Fetal Life and Adult Ovulation Rate

The reproductive performance of sheep is reduced when they are exposed to inadequate nutrition during fetal and/or early postnatal life (reviewed; Robinson et al., 2002), a phenomenon known as ‘fetal programming’. It is not known whether the cause is a decrease in ovulation rate or an increase in embryo mortality (Gunn et al., 1995). We hypothesised that inadequate nutrition during fetal and early postnatal life reduces the ability of new-born ewe-lambs to have twin ovulations when they reach reproductive maturity. Furthermore, we expect the ovulation rate of these ewes will not be responsive to the stimulatory effect of a 6-day lupin supplement.

It is difficult to gather data in this area because experimental animals must be maintained for 2 years, beginning with fetal development, before measurements can be made. We have been able to test our hypotheses by making use of a unique flock that has arisen in Victoria from the *Lifetime Wool* project. Our experiment with the progeny of a *Lifetime Wool* flock at Hamilton, Victoria, was done in collaboration with Professor Ken McNatty (Victoria University, New Zealand). He is arguably *the* world expert on follicle development in the ovaries of sheep. Thus, a lot of resources were put into this study because it was a unique opportunity to test these hypotheses. It is difficult to imagine how, in the absence of another project equivalent to *Lifetime Wool*, such animals will ever be generated again. The funding for this work was not envisaged in the original application to MLA so, to take advantage of the opportunity offered through Lifetime Wool, Dr Viñoles applied for and was given $10,000 in extra funding through the University of WA.

4.2.4.1 Ovulation Rate in Daughters of Underfed Mothers

In October 2006, we selected single females that had been born to:

a) Mothers that were in condition score 3 from mating and maintained on high plane of nutrition from Day 90 of pregnancy (HP, 3000 kg/green DM/ha); or
b) Mothers that had lost condition from mating to Day 90 and were maintained on a low plane (LP, 900 kg/green DM/ha) of nutrition from Day 90 of pregnancy to term.

The selected ewes were weighed and body condition scored and 20 HP and 20 LP ewes were chosen for a factorial 2x2 experimental design, the factors being level of feed during pregnancy (HP vs LP), and supplementation (yes or no). All ewes were placed under the first-wave protocol. The supplemented group was fed with enough lupins to cover twice the requirements for maintenance for 6 days, from two days after the second PG injection (Day 0) until the day of the third PG injection. The base diet comprised pellets to cover the requirements for maintenance, starting 2 weeks before the supplementation period. From the day of the second PG injection until sacrifice, the ovaries of the animals were studied by ultrasonography so we could follow follicle development. Blood was sampled daily 1 hour before feeding, or three times a day (-1, 3.5 and 7 h relative to feeding, hour = 0) on Days -1, 3 and 6 of the supplementation period. Plasma was separated and stored at -20°C until assayed for oestradiol, FSH, progesterone, glucose, insulin, leptin and IGF-I.

4.2.4.2 Changes in Follicle Function in Response to a Dietary Supplement

Five of the animals in each group were killed 3 days after the beginning of the supplementation period and the other five were killed 30 h after the last prostaglandin injection. The ovaries were collected and antral follicles ≥ 3 mm diameter were counted and individually dissected under a dissection microscope. Follicles were classified as atretic or healthy based on the colour of theca layer (red, pink or pale) and the numbers of granulosa cells. The follicular fluid was aspirated, the volume measured and 100 µL of assay buffer was added before freezing to await oestradiol assay. After the follicular fluid was collected, 1 mL of media was added to individual dissected follicles and the granulosa cells from the follicular wall were scraped out and counted using a haemocytometer. Granulosa cells were washed and aliquots of 100,000 cells were incubated with eCG and follicle-stimulating hormone (FSH) to establish their responsiveness to gonadotrophins by measuring cAMP production (Henderson et al., 1985). Testosterone (2 ng/mL) was added to a known concentration of granulosa cells before incubation to analyse their aromatase capacity and oestradiol production.

The study is being done in cooperation with Dra Ana Meikle (Universidad de la República Oriental del Uruguay) who will study reproductive tract function. The isthmus of the oviduct (2 cm long) and the middle piece of the uterus ipsilateral to the CL were collected, preserved in 10% formaldehyde, and transferred to 70% ethanol to await embedding in paraffin. Samples of similar size were deep-frozen in liquid nitrogen and preserved at -80°C. Paraffin blocks and frozen samples were transported to Uruguay in dry ice. Progesterone and oestradiol receptors will be analysed using immunohistochemistry and solution hybridisation assays that are specific for ovine mRNAs.

Data will be analysed using the mixed procedure available in the Statistical Analysis System (SAS), considering the fixed factors of nutrition in utero, supplementation, day and their interactions. We are still awaiting some laboratory results, but can present the observations on tract morphology.

Ovulation rate was 25% higher in ewes that received a high plane of nutrition in utero (1.5 ± 0.1) than ewes that received a low plane of nutrition in utero (1.2 ± 0.3; $P < 0.05$). There were more healthy follicles larger than 3 mm diameter on Day 3 (2.0 ± 0.3) than on Day 7 (0.55 ± 0.3). The numbers of healthy 4 mm follicles decreased from Day 3 (1.1 ± 0.2) to Day 7 (0.2 ± 0.2; $P < 0.001$). The numbers of healthy 5 mm follicles increased from Day 3 (0.4 ± 0.1) to Day 7 (1.4 ± 0.1; $P < 0.001$). Follicle numbers were not affected by plane of nutrition in utero, supplementation or the interaction between the two factors. The numbers of healthy 3 mm follicles were higher in non-supplemented (2.7 ± 0.5) than in supplemented ewes (1.3 ± 0.5) on Day 3. Numbers decreased from Day 3 (2.7 ± 0.5) to Day 7 (0.3 ± 0.5) in non-supplemented ewes but remained similar in supplemented ewes (1.3 ± 0.5 and 0.8 ± 0.5; $P < 0.05$). The numbers of healthy 4 mm follicles were lower in supplemented (0.3 ± 0.2) than in non-supplemented ewes (0.95 ± 0.2; $P < 0.05$). The total numbers of healthy follicles were higher in non-supplemented (4.6 ± 0.6) than in supplemented ewes

(2.3 ± 0.6; P < 0.01) on Day 3. Numbers decreased on Day 7 in non-supplemented ewes (2.0 ± 0.6) but remained unchanged in supplemented ewes (2.3 ± 0.6 vs 2.2 ± 0.6; P > 0.05).

The size and number of granulosa cells increased in the largest healthy follicle from Day 3 (4.4 ± 0.2 mm and 2.4 ± 0.2 million) to Day 7 (6.9 ± 0.2 mm and 4.3 ± 0.2 million; P < 0.001). The numbers of granulosa cells were greater in the largest healthy follicle from supplemented (3.7 ± 0.2 million) than in non-supplemented ewes (3.0 ± 0.2 million; P<0.05). However, when expressed as a percentage all follicles, there were similar amounts of granulosa cells (59 ± 0.3%; P > 0.05).

Numbers of 3 mm atretic follicles were similar among groups (0.4 ± 0.2; P > 0.05). On Day 3, the numbers of 5 mm atretic follicles were higher in ewes that received a high plane of nutrition in utero (0.7 ± 0.2) than in ewes that received a low plane of nutrition in utero (nil; P < 0.05).

On Day 3, total numbers of 5 mm follicles were higher in ewes that received a high plane of nutrition in utero (1.2 ± 0.3) than in ewes that received a low plane of nutrition in utero (0.2 ± 0.3; P < 0.01). Total numbers of 5 mm follicles increased on Day 7 in ewes that received a low plane of nutrition in utero (1.7 ± 0.3), but remained unchanged in ewes that received a high plane (1.6 ± 0.26; P < 0.05).
Total numbers of 3 mm follicles were higher on Day 3 in non-supplemented (3.4 ± 0.6) than in supplemented ewes (1.6 ± 0.6; P < 0.05). Numbers of 3 mm follicles decreased on Day 7 in non-supplemented ewes (0.4 ± 0.6; P < 0.01), but remained the same in supplemented ewes (1.0 ± 0.6).
On Day 3, total numbers of 4 mm follicles were lower in supplemented (1.0 ± 0.3) than in non-supplemented ewes (2.4 ± 0.3; P < 0.01). Numbers decreased significantly on Day 7 in non-supplemented ewes (0.4 ± 0.3; P < 0.001) but remained similar in supplemented ewes (0.3 ± 0.3).
Total numbers of follicles were higher on Day 3 in non-supplemented ewes (6.5 ± 0.7) than in supplemented ewes (3.5 ± 0.7; P < 0.01). Numbers decreased on Day 7 in non-supplemented ewes (2.5 ± 0.7) but remained the same in supplemented ewes (2.9 ± 0.7).

Weights of uteri and ovaries were not affected by nutrition *in utero* or by the supplement. The average weights of the CL were lighter in ewes that received the high plane of nutrition in utero (0.205 ± 0.02 g) than in ewes that received the low plane of nutrition (0.289 ± 0.02 g), probably due to the higher ovulation rate. CL were heavier on Day 3 in ewes that received a high plane of nutrition in utero (0.2 ± 0.03 g) than in those that received the low plane of nutrition (0.1 ± 0.03 g, P < 0.03). The average weight of the CL was heavier in supplemented (0.2 ± 0.01 g) than in the non-supplemented ewes (0.16 ± 0.01, P = 0.06).

With the data analysed to date, we support the hypothesis that inadequate nutrition during fetal and early postnatal life reduces the ability of ewes to have twin ovulations when they reach adulthood.

4.2.4.3 Field Trial – Response to a Lupin Supplement

Three hundred and forty one ewes weighing 50.6 ± 0.3 kg and with a condition score of 3.2 ± 0.01 were selected after shearing. Table 4 shows that, in this experiment, we used the progeny from 3 different years, from mothers that had been exposed to a wide range of nutritional situations. The progeny were reared as either singles or twins.

Table 4. Numbers of ewes born in 2001-03, for which their mothers maintained (CS3) or lost (CS2) condition from mating to Day 90 of pregnancy, and were then offered different amounts of feed from Day 90 to term.

Feed offered Day 90 to term (kg DM/ha)	Year born 2001		2002		2003	
	CS 2	CS 3	CS 2	CS 3	CS 2	CS 3
800	8	11	3	10	25	23
1100	12	12	10	10	16	16
1400	12	16	16	17	0	8
2000	12	5	15	5	10	9
3000	13	8	18	6	5	10
Total	**57**	**52**	**62**	**48**	**56**	**66**

Ewes were kept on a maintenance diet, based on dry pastures, and supplemented with pellets. All ewes received the first-wave protocol and were allocated to one of two groups: the Controls received no additional feed, while the Supplemented group were given 500 g lupins/head daily for 6 days, from 2 days after the second PG injection until the day of the third PG injection. Ovulation rate was measured 10 days after the third PG injection by transrectal ultrasonography. Data were analysed after logarithmic transformation using GLMM in GenStat.

The mean ovulation rate of the whole flock was very high (1.7 $\pm$ 0.3). Preliminary analysis suggests that the ovulation rate was higher in progeny from CS3 ewes (2.0 $\pm$ 0.3) than from CS2 ewes (1.28 $\pm$ 0.3; P = 0.06). Ovulation rates did not differ (P = 0.21) between Supplemented (1.7 $\pm$ 0.5) and Control groups (1.5 $\pm$ 0.5). There was no interaction between nutrition *in utero* and supplementation.

4.3 Immediate Target 1b – Embryo Survival

Bibliography: 13, 14, 36, 47 (Appendix 4)

This part of the project also took advantage of the work being done in the laboratory of Dr Alfonso Abecia (Zaragoza, Spain), primarily as the PhD project of Cecelia Sosa. Their research focussed on the effects of under-nutrition on the responsiveness of uterine tissues to steroid hormones. It is not reported here but is cited in the Bibliography.

4.3.1 Effect of Post-Mating Lupin Supplementation

The aim was to determine whether a 6-day lupin supplement before mating increases ovulation rate and if extending supplementation with lupins after mating increases embryo losses. Merino ewes grazed dry pasture without a supplement (n = 116) or were supplemented with 500 g/head/day of lupins for 6 days before insemination (L6, n = 112) or for 6 days pre- and 15 days post-insemination (L6+15, n = 122). All ewes lost weight and condition until Day 5 after insemination (P < 0.001). The L6+15 group regained some weight post insemination and had higher concentrations of insulin, leptin and IGF-I compared to groups L6 and Control (P < 0.001), although concentrations of the metabolic hormones decreased two days after the end of supplementation in the group L6+15. Progesterone concentrations were similar among groups.

Results for reproductive outcomes are shown in Table 5. Feeding ewes for 6 days with lupins did not increase ovulation rate significantly (P > 0.05). Embryo losses from Day 10 to Day 30 were similar among groups (P > 0.05). However, most embryos were lost from Day 17 to Day 30 in the group L6+15 (P < 0.05).

Table 5. Reproductive performance of ewes grazing dry pasture alone (control), supplemented with lupins from Day -7 to -1 relative to AI (Lupin 6), or supplemented with lupins from Day -7 to 15 (Lupin 6+15)

	Control	Lupin 6	Lupin 6+15
Failure to ovulate (%)	11 (13/116)	11 (12/112)	11 (13/122)
Ovulation rate	1.2 (123/103)	1.3 (125/100)	1.3 (138/109)
Conception rate (%)	62 (64/103)	65 (65/100)	65 (71/109)
Pregnancy rate (%)	55 (64/116)	58 (65/112)	58 (71/122)
Embryo losses (%)			
Day 10 to Day 30 (%)	38 (39/103)	35 (35/100)	35 (38/109)
Day 10 to Day 17 (%)	66 (27/39) [a]	66 (23/35) [ab]	45 (17/38) [b]
Day 17 to Day 30 (%)	31 (12/39) [a]	34 (12/35) [ab]	55 (21/38) [b]
Day 30 to Day 60 (%)	20 (13/64)	11 (7/65)	10 (7/71)
Total (%)	**50 (52/103)**	**42 (42/100)**	**41 (45/109)**

We conclude that, for ewes mobilising body reserves before mating, a 6-day lupin supplement does not appear to increase ovulation rate. Extending the period of feeding for 15 days after mating was not detrimental to embryo survival, but the termination of the feeding regime was associated with an increase in embryo losses.

4.3.2 Undernutrition During Early Pregnancy

With our collaborators in Spain, we did two major studies. They have not been described in detail in this report because most of the work was done in Spain and the direct contribution of MLA funding was small. However, there is excellent value in this international link because Prof Abecia and his team are testing hypotheses that are of direct interest to us in the area of embryo mortality.

In brief: the first study showed that undernutrition impairs steroid receptor expression and binding capacity in the uterus. This may alter the uterine environment and help explain the reductions in embryo survival; the second study showed that the Growth Hormone-IGF system was increased by pregnancy in underfed animals but not in adequately fed ewes. These observations make it very clear that undernutrition during early pregnancy can alter the environment of the embryo.

4.4 Immediate Target 2 – Lamb survival

Bibliography: 4, 17, 18, 54, 56, 62-65

Lambing in southern Australia often coincides with winter weather that reduces lamb survival and Merino lambs born as twins have the lowest survival (Kleemann & Walker, 2005). Shelter for winter lambing can increase survival of twins up to 30% (Alexander et al. 1980), but ewes often do not seek the shelter provided (Lynch & Alexander, 1980). This experiment investigated whether twin-bearing Merino ewes lambing on pasture with rows of mallee trees as shelter would have more lambs alive at marking than those lambing on pasture alone and if lambing ewes on a crop of oats as edible shelter would further improve the survival of their twin lambs to marking.

Twin-bearing Merino ewes (n = 520) were stratified at Day 140 of pregnancy into three groups according to age, live weight (mean of 58 ± 2.6 kg) and condition score (3.6 ± 0.16) at joining. Lambing took place in July 2006 at Broomehill (340 km SE of Perth, Western Australia). Fox baits were put out for 4 months prior to lambing and dead lambs were removed to reduce predation by foxes. The season was extremely dry with the property receiving only 35% of the 45-year average rainfall for May and June resulting in feed-on-offer (FOO) being low and variable at the start of lambing (Table 6). Consequently all ewes were supplemented with grain to meet their requirements for late pregnancy and early lactation. Up to 26 June (day 140 of pregnancy) all ewes were fed 300 g/h/d of lupins and on this day they were also fed 50 g/h/d of barley grain. The amount of barley fed was slowly increased to 350 g/h/d by 6 July (day 150 of pregnancy) by substituting barley for lupins. Barley alone was fed at 350 g/h/d for 10 days into lambing and then reduced to 200 g/h/d and held at this level for 20 days until lambing finished. The number of live lambs from each of the six paddocks was counted at marking on 14 August. The lambs were weighed at weaning on 7 October.

Table 6: Survival to marking and weaning and live weight at weaning for twin lambs from Merino ewes grazing pasture, pasture with mallee trees (shelter) or an oat crop (‘edible shelter’) over a 30-day lambing in July 2006.

Treatment	Stocking rate (DSE/ha)	FOO kg/ha (sem)	% Survival to marking (sem)	% Survival to weaning (sem)	Weaning weight kg (sem)
Pasture 1	8.0	552 (241)	80	78	22.4
Pasture 2	8.9	459 (183)	88	87	24.9
Pasture	***8.4***	***505 (160)***	***84 (4.3)***	***82 (4.3)***	***23.6 (1.23)***
Shelter 1	8.0	426 (36)	81	80	20.0
Shelter 2	8.0	498 (80)	77	73	21.9
Shelter	***8.0***	***462 (44)***	***79 (2.2)***	***76 (3.8)***	***20.9 (0.94)***
Edible shelter 1	8.0	597 (165)	74	74	22.8
Edible shelter 2	8.9	881 (257)	91	91	21.4
Edible shelter	***8.4***	***739 (172)***	***83 (8.4)***	***83 (8.9)***	***22.1 (0.73)***
Mean	**8.3**	**552 (88)**	**82 (2.7)**	**72 (3.0)**	**22.2 (0.67)**

The 58 mm of rain during the 30-day lambing was similar to the 20-year average, but most days were sunny and warm. The survival of twin lambs to marking (lambs marked as a percent of lambs in-utero at scanning) did not differ between treatments. However, there was considerable variation in lamb survival between replicates/paddocks (Table 6).

The survival of twin lambs to marking was quite high across all replicates despite the limited and variable FOO. Although not significantly different, with the edible shelter treatment, both FOO and twin survival were numerically greater for Replicate 2 than Replicate 1. Unfortunately (!), the survival of twins was not challenged by the weather, giving results similar to those for lambings under mild conditions in WA in July of 2004 (Oldham et al. 2008) and 2005 (Bibliography 17). It is possible the barley stimulated colostrum production in all ewes (Banchero et al. 2007) and the sunny weather around lambing led to a good balance of soluble carbohydrates and protein to provide quality FOO. Together, these factors would contribute to the lack of difference in survival and live weight of twins at weaning in this study.

4.5 Supplementary Target – The Ram Effect ('Teasing')

Bibliography: 5-9, 23-33, 51-53, 68-73

4.5.1 Teasing in Cyclic Ewes?

Application of the ram effect during the breeding season has been previously disregarded because the reproductive endocrine axis is powerfully inhibited by progesterone during the luteal phase of the oestrous cycle. However, ewes treated with exogenous progestogens respond to ram introduction with an increase in LH concentrations. We therefore tested the hypothesis that ewes at all stages of the oestrous cycle would respond to ram introduction with an increase in LH pulse frequency. We did two experiments using genotypes native to temperate (Experiment 1) or Mediterranean regions (Experiment 2). In Experiment 1 (UK), 12 randomly cycling, North of England Mule ewes were introduced to rams midway through a frequent blood-sampling regime. Ewes in the early (EL; n = 6) and late luteal (LL; n = 6) phase responded to ram introduction with an increase in LH pulse frequency (EL, $P < 0.1$; LL, $P < 0.05$), mean and basal LH ($P < 0.05$). In Experiment 2 (Australia), the cycles of 32 Merino ewes were synchronised using intravaginal progestogen pessaries. Pessary insertion was staggered to produce four groups (n = 8) that could be challenged simultaneously with ram introduction in the follicular (F), early luteal (EL), mid-luteal (ML) and late luteal (LL) phases. Ewes at all stages of the oestrous cycle responded to ram introduction with an increase in LH pulse frequency ($P < 0.01$). EL, ML and LL ewes also showed an increase in mean LH concentration ($P < 0.05$). We conclude that ram introduction stimulates an increase in LH pulse frequency in cyclic ewes, independently of ewe genotype or stage of the oestrous cycle. Interestingly, we found that this also applies to goats (Communication 9).

4.5.2 Are Audio-Visual Stimuli Important?

Stimuli from a prospective mate increases the secretion of luteinising hormone (LH) in sheep. This 'male effect' in ewes and 'female effect' in rams are predominantly mediated by olfactory signals, though it is thought that non-olfactory signals play synergistic or substitutive roles. In this study, we tested whether exposure to visual or audio-visual stimuli from a prospective mate would stimulate an increase in LH in ewes (Experiment 1) and rams (Experiment 2). In Experiment 1, groups of 8 Merino ewes were exposed to one of three stimuli mid-way through a frequent blood sampling regime: full ram contact; still images of rams; a video of ewes and rams mating. Control ewes (n = 8) were completely isolated from rams. Exposure to still images of rams stimulated an increase in mean LH concentrations ($P < 0.05$) and tended to increase LH pulse frequency ($P < 0.1$), but the response was significantly smaller than that observed in ewes exposed to rams ($P < 0.01$). Audio-visual stimuli had no effect on any parameters of LH secretion ($P > 0.1$). In Experiment 2, Merino rams were allocated to either an Exposure (n = 7) or Control (n = 7) group. Exposure rams underwent two exposure periods midway through a frequent blood sampling regime; exposure to still images of ewes and audio recorded during mating of ewes and rams (audio-visual exposure); exposure to oestrous ewes (ewe exposure). Control rams were sampled at the same frequency but remained isolated from ewe stimuli. Exposure of rams to the audio-visual stimuli did not affect any parameters of LH secretion ($P > 0.1$). In contrast, exposure to oestrous ewes increased LH pulse frequency ($P < 0.05$) and advanced the onset of the next LH pulse ($P < 0.05$). In conclusion, visual signals are involved in eliciting the neuroendocrine response of ewes to rams and are of greater importance to this phenomenon in ewes (male effect) than rams (female effect). However, overall the visual and audiovisual signals used in this study were far less effective than stimulus animals, suggesting that these stimuli are less important than olfactory signals, or a combination of olfactory and audiovisual signals.

4.5.3 Is Isolation of the Sexes Necessary for Teasing to Work?

Ewes maintained with rams for an extended period of time become habituated to their presence, thus rendering the ram effect ineffective. The accepted dogma in the field, is that ewes must be isolated from rams for at least one month for ram presence to again stimulate reproductive activity. However, there is some evidence that isolation of ewes from rams is not, in fact, necessary if the rams are unfamiliar or ‘novel’. This is an important distinction as it infers that the ewes are able to differentiate between individual rams, indicating a role of memory in mediating the ram effect.

To test this theory we habituated ewes to rams for 3 months and compared their hormonal responses of ‘familiar’ or ‘novel’ rams. We also evaluated the rate cell proliferation in the hippocampus (a region of the brain vital for learning and memory) of ewes in response to ram exposure. We found that novel but not familiar rams elicited a hormonal response in ewes habituated to ram presence and that ram exposure induced a 100% increase in cell proliferation in the hippocampus. We conclude that the stimulus from novel males switches on the reproductive centres of the brain of female sheep and rapidly doubles the rate of cell proliferation in the hippocampus. The rapidity of this response contrasts with rodents, in which several days of exposure to male pheromones seem necessary for an effect on neurogenesis.

4.5.4 Do Sexual Experience and Temperament affect Teasing?

The response to the ‘ram effect’ can vary greatly among females, even within a breed, so the variability cannot be explained by genotypic differences. On the other hand, it may be explained by diversity in sexual experience or temperament. To assess the role of these factors, we used ewes from the Allandale Flock of Merino in which there had been selection for temperament since 1990. Four groups, each of 15 anoestrous ewes were established: experienced and calm (EC), experienced and nervous (EN), naïve and calm (NC) and naïve and nervous (NN). The sexually experienced ewes were 3-6 years old and multiparous, whereas the inexperienced ewes were 2 years old and had not seen a male since weaning. We studied the changes in pulsatile LH secretion in jugular blood sampled every 15 minutes for 6 hours before and after ram introduction. A catheter had been inserted one day before the experiment began. Further blood samples were collected every 4 hours until 38 hours after exposure to the male to determine the time of the preovulatory LH surge. Six days after ram introduction, ultrasound was used to detect ovulation and count the number of corpora lutea (CL).

The responsiveness of ewes to the ram effect is improved by sexual experience and by calm temperament (Table 7). Experienced and calm ewes appear to be the best combination to permit the development of the follicles (longer delay between the first LH pulse and the surge) and to optimise the endocrine and ovarian response to the ram effect (proportion of ovulating ewes). However, the proportion of ewes ovulating was intermediate in the NN group, so there appears to be an interaction between temperament and sexual experience.

Table 7: Preliminary analysis of data for the effects of the temperament and sexual experience of ewes on their response to the ram effect. ab $p = 0.005$; cd $p = 0.001$; ef $p = 0.019$; gh $p < 0.01$; ij $p = 0.003$

Group	% showing LH Pulses	% showing LH Surge	% LH Surge before 38 h	% Ovulating	Ovulation rate
EC	85	100^{c}	27^{e}	100^{g}	2 ± 1.0^{i}
NC	64	53^{d}	38	40^{h}	1 ± 0.0^{j}
EN	92^{a}	86	67^{f}	47^{h}	1 ± 0.5^{j}
NN	47^{b}	67	60	67	1 ± 0.0^{j}

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5 Success in Achieving Objectives

5.1 Establishing the LambMax Team

We were very successful in establishing a team that became acknowledged nationally and internationally as operating at the frontier of reproductive science as applied to extensive systems of sheep production. In addition, the team established strong collaborative links across Australia (eg, DPI Victoria, Charles Sturt University) and internationally (eg, New Zealand, Spain, France, Mexico, Uruguay), garnering great respect for its experimental approach and technical capabilities. As can be seen from the above report, and from Section 8 below, the team was highly productive, realised its strong commitment to industry through on-farm trials and high-profile appearances at farmer forums and industry-focussed conferences, a made strong gains in re-establishing Australia's credentials in R&D in sheep reproduction.

Unfortunately, this effective, high-quality team broke up as this 3-year project was drawing to its end. Dr Viñoles was lured back to Uruguay, and Ms Paganoni became disillusioned with prospects in sheep research and abandoned the field. She was replaced in the final year of the project by Ms Kristy Glover (BSc AnimSci) who has also now abandoned research. Dr Hawken accepted another position due to funding uncertainties. Thus, we were successful in meeting our primary objective, "To establish international-quality personnel with essential equipment that could carry out the research into reproductive issues for the Australian sheep flock", but it appears the more long-term commitment is needed if we are to escape the stop-start syndrome.

5.2 Immediate Target 1

5.2.1 Establishing and Validating The 'First-Wave Model'

With the '**first-wave model**', we have given the world a completely novel and very efficient experimental approach for studying nutritional factors that affect ovulation rate and early embryo mortality. The combination of the triple-injection regime of prostaglandin with ultrasound, and the focus of attention on the first follicular wave of the cycle, allows researchers to do experiments that were previously thought logistically impossible. They can now test hypotheses that will explain why ovulation rate does not always increase following short-term supplements under field conditions.
We completed all of the hormone assays, including oestradiol, a most difficult steroid to measure in sheep because the concentrations are very low (picomolar) and there are myriad sources of interference. A major paper on the first-wave model has been submitted to a high quality peer-reviewed journal.

The success of the 'first-wave model' hinges on ultrasound – the quality of the machine and the quality of the operator. *LambMax* had an excellent combination but Dr Carolina Viñoles left before we could realise one of our goals – to establish a legacy of high quality ultrasound operators for Australia. All is not lost, however – we have written a chapter for a manual on ultrasound that will be published internationally in 2009 and this will act as a valuable guide for future apprentices.

The fertility of the first-wave cycle is too low for it to have widespread practical application, even with high doses of semen.

5.2.2 Ovulation Rate – Alternative Supplements, Body Condition Score

One of the remits of *LambMax* was to find alternatives to lupins for short-term supplementation because lupins are not readily available outside Western Australia. We tested Kasper peas, but the outcome was inconclusive. On the other hand, we demonstrated ovulation rate can be increased by short periods of supplementation with maize-soya mix, and perhaps even by short periods of grazing on pasture dominated by the legume, *Lotus corniculatus*.

One of the dominant issues that arose when discussing short-term supplementation with farmer groups was whether to use supplements in animals that are already in good condition, and whether the response would be different if animals were losing or gaining condition at the time of supplementation. This project attempted to address these issues in a series of large, on-farm experiments, with animals with a range of nutritional histories. We created animals of high and low static condition score, and the animals that were losing condition, and we looked for interactions between these histories and supplementation with lupin grain. In on-farm studies, it is very difficult to control all of the variables that might impact the outcome of these treatments, so there were some inconsistencies in the results. Thus, some studies were more robust than others with respect ovulation rate, a discrete variable of very limited range, and we are still trying to resolve the issue.

There seems little doubt that little can be gained from a short-term supplement fed to ewes that already have a high condition score (for example, BCS 3) and are on a high level of feed intake. In these animals, the process of follicular growth is accelerated, and all the metabolic signals appear to be in place to encourage maximum ovulation rate – they are probably expressing their maximum and genetic potential for fecundity. We are still analysing the physiological data that have been gleaned from blood samples so, within the next few months, we will be able to verify this conclusion.

On the other hand, fairly consistent responses to supplementation were observed in animals in Condition Score 2 or less. In this situation, there is a clear benefit with respect to fecundity, and the economic benefit from supplementation will depend on the cost of supplementation (for which duration of supplementation is important) and the value of extra lambs born. Obviously, the outcome of this economic analysis will depend on the market situation for supplement and sheep at the time the decision is taken.

Of course, in the real world, animals rarely if ever have a stable body condition – they are either losing or gaining condition at the time of mating. Similarly, they will have either a high feed intake or a low food intake at that time, depending on the quantity and quality of forage on offer. We attempted to test both of these dynamic situations to see whether the response to supplementation would be affected. The results are not completely conclusive, perhaps because on-farm logistics prevented us from doing these experiments under ideal conditions. The larger experiment was done before the breeding season commenced and thus intersected with the onset of ovulation. In this situation, the supplement did not affect ovulation rate but it did improve the proportion of ewes that ovulated and were therefore likely to become pregnant. In the study in which intake was controlled as a factor, we needed to focus on measurement of physiological variables so the numbers of animals were too small to detect changes in ovulation rate. All we were able to observe was a tendency for lupins to increase ovulation rate in animals on low intake. Again, we await the full analysis of the metabolic variables before we draw strong conclusions within this context.

5.2.3 Short-term Supplements and Embryo Survival

This is good news for industry. We can put aside two decades of uncertainty because it seems most unlikely that embryo losses are made worse by feeding supplements to increase ovulation rate. This removes a major hindrance to the introduction of focus feeding for improving twinning rates.

That said, we confirmed that early embryo mortality is very high, an area of reproductive inefficiency may need serious effort in future projects. Our needs could be served by maintaining close links with Prof Alfonso Abecia in Spain because he is clearly at the cutting edge in this field for sheep.

5.2.4 Fetal Programming of Future Fecundity

Inadequate nutrition during fetal and early postnatal life reduces the ability of ewes to have twin ovulations when they reach reproductive maturity. We are still processing samples and data so cannot yet test our hypothesis that the ewes born to underfed mothers are less responsive to a 6-day lupin supplement.

It is too early to offer detailed advice to producers in this regard because it is impractical to supplement pregnant ewes for such a long period. More work is needed to define effective period(s) of focus feeding during pregnancy that will maximise fertility and fecundity and other aspects of future productivity.

5.3 Immediate Target 2 – Lamb Survival

Our lamb survival data have been presented at the biennial meeting of the Australian Society of Animal Production (Brisbane, 2008), where our two papers (Bibliography 17, 18) were provided alongside another paper on the topic (Oldham et al, 2008). This generated a lot of positive interest and feedback. In 2006, we also organised a workshop involving 12 researchers from the Department of Agriculture & Food and the University of Western Australia (UWA) who are working in this area. Finally, our experiences were a major source of input into the formulation of the current Sheep CRC, for which lamb and weaner survival are seen as key objectives.

This is a most difficult area for field trials because whether or not a hypothesis can be tested depends on having weather conditions during the trials that will cause significant lamb mortality in the control treatments. In every experiment that we carried out, the weather was mild and lamb mortality rates were well below average. These experiments are costly and complex, so an alternative approach is necessary, or else we forego the need for field trials – after all, the basic biology explaining neonatal lamb mortality (temperament, shelter, feeding for colostrum) is very solid. The balancing act for the farmer is to decide whether to accept the risk of high mortality or whether to put into place measures that might yield little benefit in years when the weather is mild. Our view is that the unacceptable risk is adverse opinions of farmer behaviour in the market place.

There is anecdotal evidence that focus feeding for colostrum production can disrupt the formation of the ewe-lamb bond because the ewes chase the vehicle that is supplying the supplement. This needs to be addressed in any advice that is offered to industry.

5.4 Supplementary Objectives – The Ram Effect ('Teasing')

The *LambMax* team, with its goal of 'clean, green and ethical production', began to address the need to improve the usefulness of teasing (the male effect). We made major breakthroughs in our understanding of the biology of the phenomenon and this led to new perspectives on how we can manage 'teasing' on-farm.

5.4.1 The Need to Isolate Ewes from Rams

We have clearly demonstrated that complete isolation of the ewes from rams is not essential … all that is needed is for the 'teaser' males to be novel. This has effectively lessened a major management constraint that we have worked under since the 1940s. In addition, if farmers follow the recommended process of using novel testosterone-treated wethers as teasers for 14 days, then exchange the wethers with novel fertile rams for mating over the next 2 weeks, there may be a second stimulus when the fertile rams are introduced. At this stage, we do not know if this happens, or whether it affects the outcomes of mating.

5.4.2 The Role of Memory in the Response to Teasing

The implication from the finding that the novelty of the teaser to the ewe is critical in determining the response of ewes implies and ability to recognise and remember individual males. To prevent teasers from becoming 'familiar' and thus losing their ability to stimulate ewes, we need to find out which characteristics of males are recognised and remembered by ewes – sight, sound or smell?

5.4.2.1 Auditory and Audio-Visual Signals

We tested whether ewes and rams would respond to audio and visual stimuli from a prospective mate with an increase in key reproductive hormones. We found that ewes showed very little, if any, response to auditory or visual stimuli. It seems unlikely that these signals play a major role in teasing. Despite the clear role of vision in the registration and memory of sheep faces, it also seems unlikely that audio-visual signals are involved in memorising individual males with respect to the teasing response. This latter conclusion needs to be tested by direct experimentation.

5.4.2.2 Olfactory Learning and Memory

In a very exciting discovery for the broader field of reproductive biology, we have shown that the socio-sexual signals from the male sheep can induce the production of new brain cells ('neurogenesis') in the memory centres of the female sheep. This happens within a few hours of exposure to the male stimulus. This observation has major implications for the field of neuroscience and also demonstrates clearly the profound role that learning and memory play in the ram effect. Moreover, the sites where we observed the neurogenesis are primarily involved in olfaction, and we know that olfactory signals are more important for teasing than audio-visual signals, so we can conclude that ewes recognise and remember rams through 'olfactory memory'. Presumably, for familiar rams to become novel again, these olfactory memories need to be lost. We are currently in the process of determining how long it takes a ewe to 'forget' an individual ram.

5.4.3 Role of Sexual Experience and Temperament

Sexually experienced ewes, independently of their temperament, exhibited a higher proportion of ewes cycling after ram introduction than sexually naïve ewes. 'Nervous' ewes exhibited a higher proportion of ewes cycling after ram introduction than calm ewes, but only in the sexually naïve group. Thus, temperament has little effect in sexually experienced ewes, and ewes of 'nervous' temperament appear to respond better to the ram effect when sexually 'naïve'. Both sexual experience and temperament need to be taken into consideration when flock management involves the ram effect.

5.4.4 Teasing in the Breeding Season

The 'ram effect' is an excellent tool for managing the onset and synchrony of mating and lambing but it's use to date has been restricted to the non-breeding season, when the animals are acyclic. We tested whether cyclic ewes respond to rams with a hormonal response characteristic of the ram effect at each stage of the oestrous cycle. We found that ewes at all stages of the oestrous cycle responded to rams with an increase in the brain signal that would normally induce ovulation in anoestrous ewes, irrespective of the concentration of progesterone at the time of ram exposure. These observations suggest fascinating potential for the use of the ram effect during the breeding season. It is unfortunate that work in this area has stopped.

6 Impact on Meat and Livestock Industry

Two major outcomes are the extra momentum we have been able to give to the implementation of ‘focus feeding’ for increasing fecundity, and the better guidelines for ease of management of teasing.

6.1 Short-term Supplements for Improving Fecundity

In the **First-Wave Model**, we have established an experimental protocol for future testing of supplements in which detailed physiological and diagnostic measurements can be made so that the causes of failures can be identified. Beyond the direct findings from the current project (6.1.1, 6.1.2), the first-wave model will have little immediate impact on farm practice. However, should the research continue, then 5 more years would see us able to better predict which supplements are unlikely to work on-farm, and under what conditions they are likely to work. We will have been able to evaluate more alternatives more rapidly and cost-effectively. This will make benefit-cost analyses more accurate. On the other hand, progress in this area will be impossible without high-quality technical competence in transrectal ultrasound.

6.1.1 Responses to Short-Term Supplements – Importance of Nutritional History

With the first-wave model, we have begun to unravel the complex interactions among short-term supplementation and changes in body mass and condition score, and how these interactions affect the outcome for ovulation rate. There is little doubt that these issues explain the lack of reliability of on-farm trials with, for example, lupin supplements. Our on-farm studies provide their own evidence. From our initial analysis, four basic conclusions can be drawn, based on data across all on-farm trials and controlled experiments, with respect to decisions about whether to supplement:

i) Ewes appear able to respond to a lupin supplement at almost all levels of condition score and background nutrition; ewes in poor body condition ($CS \leq 2$) appear better able to respond than animals in good body condition ($CS \geq 3$);
ii) More work is needed for animals that are rapidly losing body condition, or on very low or very high background feed intake. In ewes losing condition for the 3 months before mating, the short-term supplement did not affect fecundity but probably would have increased flock fertility because it increased the frequency of animals ovulating;
iii) Short-term supplements are useful but should not replace good nutritional management;
iv) More work is needed with the view to offering solid advice in 5 years time.

6.1.2 Short-Term Supplements – Alternative Feed Sources

Ovulation rate can be improved by some alternatives to lupins:

i) As a concentrate, maize-soya supplements can be used, but we cannot recommend Kasper peas (further assessment is required for a definitive answer); this can be implemented now;
ii) More importantly for an industry based on extensive, pasture-based management, it appears that short periods of grazing a legume supplement can be used to improve fecundity. Our studies are limited to the work in Uruguay with *Lotus corniculatus* in which 12 days grazing increased ovulation rate and, to date, the results for lucerne are less certain, perhaps due to

the vagaries of on-farm trials in areas where drought can interfere drastically with experimental outcomes. Work along these lines is continuing in the *Evergraze* program at Charles Sturt University, for which we provide advice on experimental design and help and laboratory analyses (Dr B King; Dr M Friend; PhD student, Catherine Gulliver).

Thus, for immediate implementation, maize-soya supplements (or an equivalent) and *Lotus* pastures can be used for focus feeding for fecundity. Further recommendations await 2-5 years research with the first-wave model and with the *Evergraze* trials.

6.1.3 Short-Term Supplements do not Increase Embryo Mortality

This is also a critical, positive outcome with immediate application. In advocating 'focus feeding' for improving ovulation rate, including those published by MLA, we have had to err on the side of caution and recommend that farmers to either stop the supplement before oestrus, or at least wind it down during the first 2 weeks after mating (eg, see Communications 44-46, 49). *Now we know that generally there is no need to worry about this.*

With respect to the longer term, however, embryo mortality needs to be addressed. We would focus on these 3 aspects:

i) We have no real feeling for the variability of the losses in the real world – all we have is sparse literature, with observations based on a range of techniques and providing a disturbingly wide range of estimates, with values up to 46% for Merinos (Walker et al 2003);
ii) To diagnose the causes of the problem, we again need high-quality technical competence in transrectal ultrasound;
iii) Embryo mortality that causes large numbers of dry ewes leads to a significant loss of income, increases the amount of greenhouse gas per unit of output (ewes that lose their embryos continue to produce methane), extends generation interval and thus slows genetic progress (perhaps through no fault of the ewe's genetics), and slows down any attempts to manage the decline in the national flock. Importantly, all three of these outcomes apply equally to early mating (maiden ewes) and to postnatal mortality, and the problem with greenhouse gas efficiency comes into play in the assessment of cull-for-age strategies. Therefore, the industry would gain many benefits from investment in this area of research and development.

6.1.4 Future Fecundity is Programmed during Fetal Life

Inadequate nutrition during fetal and early postnatal life reduces the ability of ewes to have twin ovulations when they reach reproductive maturity. Ewes born to underfed mothers may also be less responsive to a 6-day lupin supplement. It is too early to offer detailed advice to producers in this regard because it is impractical to supplement pregnant ewes for such a long period. More work is needed to define that shortest effective period of focus feeding.

6.2 Perinatal Lamb Mortality

It has proven very difficult to test hypotheses concerning the concept of edible shelter in on-farm experiments effectively because it is impossible to organise the weather. This is well illustrated by the two studies that we did in *LambMax* – in both, benign weather led to low lamb losses in the control groups, leaving no room for edible shelter to improve the outcome (Communications 17, 18).

On the other hand, the results were clear in companion studies: "Edible shelter increases survival of twin but not single lambs born to Merino ewes in winter …" (Oldham *et al* 2008).

We therefore see very little value in pursuing the issue through field trials. Rather, we should accept that the biology, presented in peer-reviewed journals, is incontrovertible (shelter, ewe retention at birth site, colostrum production) and convince farmers to implement the 'maternity ward' as insurance, particularly for maidens having their first lamb, and for parous ewes having twins. Future studies can then focus on refining our knowledge about the processes involved rather than expending resources on experiments that require poor weather.

Future R&D should also attempt to resolve issues and formulate recommendations with respect to feeding technique for late pregnancy ewes so that 'ute-chasing' behaviour is avoided.

6.3 The Ram Effect (Teasing)

6.3.1 Isolation of Rams and Ewes – Focus on Male Novelty

For producers wishing to use teasing to breed their ewes (ie, all those who put rams out before February), life is now simpler. For over 60 years, producers have been advised to separate ewes from rams for at least one month for teasing to be effective, a practice that is logistically difficult and adds complexity to an otherwise simple management tool. We now know that it is not necessary to keep ewes completely separate from all rams – all that is needed is for the males used as teasers to be unfamiliar or 'novel'. This will maximise the response to teasing and improve the synchrony of lambing, the management of lamb survival, and the variability in a cohort heading for market.

In 5 years time – our R&D, some of the first in this emerging field of 'male novelty', has wide-ranging implications for the use of teasing as a management strategy. Research in this field will answer such questions as 'how long does it take for a ram to become 'familiar' or to be 'forgotten' by ewes. This, along with other improvements in our understanding of the complexities of communication between ewes and rams, will allow us to improve the flexibility and utility of teasing as a management tool in sheep. Ultimately, we will be able to tackle to thorny issue of using teasing in the breeding season.

6.3.2 Teasing of Cyclic Ewes

One of the major limitations of teasing for the management of reproduction in sheep is that it can only be used during the non-breeding season. However, we now know that that the reproductive control system in the ewe brain responds to teasers at all stages of the oestrous cycle, indicating a potential use of teasing during the breeding season. This does not (and cannot) directly induce ovulation, but it does dramatically affect the concentrations of major reproductive hormones. Further research is needed into the impact of these hormonal changes on oestrous cycle dynamics (synchrony, ovulation rate) because it could lead to the development of cheap and effective strategies for controlling reproductive performance during the breeding season.

7 Conclusions and Recommendations

7.1 Supplementation for Increased Fecundity

7.1.1 The Importance of Nutritional History

- Short-term supplements (eg, 6 days of lupin feeding) appear able to improve fecundity for ewes in a range of body conditions and background rates of feed intake, but are perhaps most reliable when the ewes are in low body condition (CS≤2);
- Soya-maize concentrate can also be used to improve fecundity;
- Grazing legume pastures (Lotus, Lucerne) is not to be recommended for improving fecundity until further research has been done;
- Kasper peas are not recommended for improving fecundity;
- More research is needed to resolve issues around feeding supplements to ewes on falling body condition and feed intake.

7.1.2 Early Embryo Mortality

- There is no need to worry about supplementation extended into early pregnancy – this is more a decision about cost than a problem for reproductive management (embryo mortality);
- More research is needed to solve the massive amount of early embryo mortality.

7.1.3 Feeding of Pregnant Ewes for the Future Productivity of their Offspring

More research should be funded in this area. It is now very clear that underfed pregnant ewes will produce daughters with low fecundity. The literature suggests that ram fertility could be affected in the same way, and that muscle, milk and wool production (quantity and quality) are also at risk.

7.2 Management of Lambing

- Farmers should plan conservatively for lambing so as to reduce the possibility of lamb mortality; this is particularly important for twin-bearers and maidens, for which 'edible shelter' should become standard practice;
- Research on lamb survival is still needed because it is one of the biggest issues in reproductive efficiency and one of the biggest risk factors for the Australian sheep industry. However, research should focus on the basic biology – physiology, genetics and behaviour – not on field trials, the success of which depends on weather events.

7.3 Management of Teasing

Of course, the management of lambing is made much easier if the births are synchronised in the flock. The challenge is to do that with a tool that is cost-effective for extensively managed flocks whilst also helping us to realise our vision for clean, green and ethical production. The answer, in our view, is to be found in 'teasing'. Our task is to make this an attractive option for farmers.

7.3.1 Management of Isolation of Ewes from Rams

- Complete separation of ewes from all males is not necessary, as long as the males (rams, vasectomised rams, testosterone-treated wethers) that will be used for teasing are unfamiliar or novel.
- As a conservative recommendation, ewes need to be fully separated from rams (sound, sight and smell) for them to be deemed 'novel' and suitable for teasing.
- Further research is needed determine how long ewes actually need to be isolated from specific rams for those rams to be 'forgotten' and again become stimulatory and useful for teasing.

7.3.2 Teasing During the Breeding Season

- Further research is needed into the responses of cyclic ewes to teasing, the implications of which are presented as a working hypothesis in Communication 6. We are optimistic that with future research we will be able to make recommendations to producers on how to use teasing during the breeding season.
- Further research is needed to define 'isolation' in the context of ensuring male novelty. Odour is of upmost importance to communication between the sexes, but we still know too little about the respective roles of visual and audio stimuli. Purely visual or audio-visual stimuli were able to induce a hormonal response that matched the response to the full complement of socio-sexual stimuli, but we have not tested interactions and the literature suggests that there may by synergism. Therefore, from a conservative standpoint, we recommend that ewes need to be fully separated from rams (audio, visual and smell) for them to be deemed 'novel' and suitable for teasing.
- Improving our understanding of how 'teasing' works has long-reaching implications to the efficiency of teasing as a management tool. Work needs to continue in this area, with a focus on non-Merino breeds and on maiden ewes.

7.4 General Recommendation

That MLA R&D Programs operate over at least a 5-year time-frame so that high-quality research personnel can be encouraged to remain with the industry.

That MLA consider seriously the return on investment for on-farm trials where experimental control is almost inevitable.

8 Communications

8.1 Book Chapters

1 Blache, D., Chagas, L.M. & Martin, G.B. (2007). Nutritional inputs into the reproductive neuroendocrine control system – a multidimensional perspective. In: *Reproduction in Domestic Ruminants VI* (Eds.: J.I. Juengel, J.F. Murray & M.F. Smith) pp. 123-139 [Nottingham University Press, Nottingham, UK].

2 Martin, G.B. (2008). ‘Clean, green and ethical’ concept in animal production. In: *Clean, green and ethical animal production in Thailand* (Eds.: K. Vadhanabhuti, P.E. Vercoe & D. Blache) pp. 7-15 [Rajamangala University of Technology, Thailand].

3 Martin, G.B., Blache, D. & Williams, I.H. (2008). The costs of reproduction. In: *Resource allocation theory applied to farm animals* (Ed.: W.M. Rauw) Chapter 10, pp. 169-191 [CABI Publishing; Oxford, UK].

8.2 Journal Article (Scholarly Refereed Journal)

4 Banchero, G.E., Perez Clariget, R., Bencini, R., Lindsay, D.R., Milton, J.T.B. & Martin, G.B. (2006). Endocrine and metabolic factors involved in the effect of nutrition on the production of colostrum in female sheep. *Reproduction, Nutrition, Development* 46, 447-460.

5 Delgadillo, J.A., Gelez, H., Ungerfeld, R., Hawken, P.A.R. & Martin, G.B. (2009). The ‘male effect’ in sheep and goats – revisiting the dogmas. *Behavioural Brain Research* 200, 304-314.

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8 Hawken, P.A.R., Esmaili, T., Scanlan, V., Blache, D. & Martin, G.B. (2009). Can audio-visual or visual stimuli from a prospective mate stimulate a reproductive neuroendocrine response in sheep? *Animal* 3, 690-696.

9 Hawken, P.A.R., Esmaili, T., Jorre de St Jorre, T. & Martin, G.B. (2009). Do cyclic female goats respond to males with an increase in LH secretion during the breeding season? *Animal Reproduction Science* 112, 384-389.

10 Martin, G.B. & Kadokawa, H. (2006). “Clean, green and ethical” animal production. Case study: reproductive efficiency in small ruminants. *Journal of Reproduction and Development* 52, 145-152.

11 Martin, G.B., Durmic, Z., Kenyon, P.R & Vercoe, P.E. (2009). Landcorp Farming Limited Lecture: ‘Clean, green and ethical’ animal reproduction: extension to sheep and dairy systems in New Zealand. *Proceedings of the New Zealand Society of Animal Production* 69, 140-147.

12 Scaramuzzi, R.J. & Martin, G.B. (2008). The importance of interactions among nutrition, seasonality and socio-sexual factors in the development of hormone-free methods for controlling fertility. *Reproduction in Domestic Animals* 43 (Supplement 2), 129-136.

13 Sosa, C., Abecia, J.A., Forcada, F., Viñoles, C., Tasende C., Valares, J.A., Palacín, I., Martin, G.B. & Meikle, A. (2006). Effect of undernutrition on uterine progesterone and oestrogen receptors and on endocrine profiles during the ovine oestrous cycle. *Reproduction, Fertility and Development* 18, 447-458.

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8.3 Conference Papers – refereed

16 Blache, D. & Martin, G.B. (2009). Focus feeding to improve reproductive performance in male and female sheep and goats – how it works and strategies for using it. In: *Options Méditerranéennes, Série A (Séminaires Méditerranéens)* No. 85 – Nutritional and foraging ecology of sheep and goats (Eds: T.G. Papachristou, Z.M. Parissi, H. Ben Salem and P. Morand-Fehr) pp. 351-364. Zaragoza: Centre International de Hautes Etudes Agronomiques Méditerranéennes/Food and Agriculture Organization of the United Nations/National Agricultural Research Foundation, Greece).

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18 Paganoni, B.L., Milton, J.T.B., Viñoles Gil, C., Glover, K.M.M. & Martin, G.B. (2008). Shelter does not increase survival of twin lambs born during mild winter weather in Western Australia. *Animal Production in Australia* 27, 89.

8.4 Journal Articles, Book Chapters – In Preparation, Submitted, In press

19 Viñoles, C., Paganoni, B., Glover, K.M.M., Milton, J.T.B., Blache, D., Blackberry, M.A. & Martin, G.B. (2009). The static and immediate effects of nutrition on ovulation rate in ewes – the application of a 'first-wave' model to study ovarian follicular dynamics. *Reproduction* (submitted).

20 Viñoles, C., Paganoni, B., Milton, J.T.B., Driancourt, M.A. & Martin, G.B. Fertility to artificial insemination in ewes after synchronisation with prostaglandin ('first-wave model'), sponges, or sponges with bactericide. *Theriogenology* (in preparation).

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23 Chanvallon, A., Blache, D., Chadwick, A., Esmaili, T., Hawken, P.A.R., Martin, G.B., Vinoles, C. & Fabre-Nys, C. (2009). Sexual experience and temperament affect the response of Merino ewes to the ram effect. *Applied Animal Behaviour Science* (submitted).

8.5 Conference Abstracts

24 Chanvallon, A., Blache, D., Chadwick, A., Esmaili, T., Gray, S., Hawken, P., Martin, G.B., Vinoles, C. & Fabre-Nys, C. (2007). Role of sexual experience and temperament in the response of Merinos ewes to the ram effect. *34ème Congrès de la Société de Neuroendocrinologie* 25-27 Septembre 2007, Tours, France.

25 Hawken, P., Blache, D., Esmaili, T. & Martin, G.B. (2006). Ram introduction stimulates pulsatile LH secretion in cyclic ewes. Proceedings of the *38th Annual Conference, Society for Reproductive Biology* (Gold Coast, Queensland) Abstract 232 (pp. 59-60).

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8.8 Invited Presentations at International Conferences – LambMax Team

2009	*New Zealand*	New Zealand Society of Animal Production ('Landcorp Lecture')
2009	*France*	International Symposium on Ruminant Physiology
2008	*Hungary*	International Congress of Animal Reproduction
2008	*South Africa*	World Congress on Animal Production
2006	*New Zealand*	International Symposium on Reproduction in Ruminants

9 Appendices

9.1 Appendix 1 – Merino Innovation Day, "The Grange" (2006)

Document to support two oral presentations

'Clean, Green and Ethical' Sheep Production

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Animal industries around the world are being challenged by changing attitudes in consumers that are having an impact in the marketplace: there is an increasing demand for products that are 'clean, green and ethical'. What do these three words signify?

Clean

We should look for practices in the industry where drugs, chemicals and hormones are used, and try to find ways to reduce the usage and, perhaps, eliminate it. There is little doubt that, in general, this demand is driven by market forces that are not themselves generated by scientific argument – hormonal treatments rarely leave residues, especially after withholding periods, and it is relatively easy to demonstrate that products from animals that have not been treated with exogenous hormones can contain significant amounts of the same hormone. The positive aspect of the demand for "clean" products is that the demand comes from modern, high-priced markets where farmers can make large profits (eg, the market for 'organic' products).

Green

We should minimise the impact of the industry on the environment so it is more sustainable for the long-term future. On farms, the most important issues are the production of greenhouse gases by ruminants, the production of animal waste (especially for feedlots), and the excessive use of fertilisers to generate animal feeds. This also applies to the allied industries … those that participate in the processing of the products from the farm (eg, transport, abattoirs, processors). If we can say that our industry is 'green', it will help with the marketing in highly developed economies and, at the same time, guarantee the long-term future of the industry.

Ethical

'Animal welfare' is a major concern for all industries that are working in sophisticated markets, where the consumers expect their products to be derived from animals that have been managed sympathetically. This can be a complex issue because, by avoiding the use of antibiotics, for example, we may compromise the welfare of the animals. In addition, the application of ethical judgement needs to be broader than animal welfare: as well as practices in animal management on farms, it should include 'clean' and 'green' aspects of the transport, manufacturing and processing sectors – the processing, packaging and marketing of the products.

Case Study: Reproduction in Merino Sheep

The productivity and profitability of our sheepmeat industry effectively depends on reproductive performance. We can greatly improve reproductive output with exogenous hormone regimens or high-level reproductive technology and molecular genetics. These technologies are remarkable and effective, but we need to find alternatives so we can cope with the changes in consumer sentiment.

In addition, these technologies have little direct, short-term benefit in the extensive management systems that typify the Australian industry.

At the University of WA, we have chosen to concentrate on the natural control systems that the animals themselves use to cope with environmental challenges and ensure reproductive success. Most important here are inputs from the external environment: photoperiod, socio-sexual stimuli and nutrition. Most reproductive responses to environmental factors are coordinated at brain level where all external and internal inputs ultimately converge into a common pathway that controls the secretion of gonadotrophin-releasing hormone (GnRH). This hormone is the ultimate controller of the reproductive system of all animals.

This mix of endogenous inputs into the control of the reproductive system provides us with three major opportunities for management of reproductive efficiency:

1) To control the timing of reproductive events, we can use the ‘ram effect’ (or ‘teasing’) to induce synchronised ovulation in ewes that would otherwise be anovulatory; this allows for short mating periods and concentrated lambings and opens up a wide range of management possibilities, including tightly-focussed supplementary feeding and strategies for reducing lamb mortality;

2) In ‘focus feeding’, we can use the responses of the ewe to nutrition to design supplements that are aimed precisely and specifically at each individual event in the reproductive process, such as egg production, embryo survival, and colostrum production;

3) To maximise the survival and development of the new-born, we can use a mix of environmental management, nutrition and genetic selection:

a) Nutritional input – feeding a high energy supplement for the last week of pregnancy can double colostrum production; this is most important for Merino ewes carrying twins;

b) Genetic selection for calm temperament – improving the temperament of ewes will increase lamb survival;

c) Better management practices at birth – to improve the survival of the new-born lambs, it is important to provide a calm environment, and shelter, feed and water close to the birth site. This is increases the amount of time spent at the birth site and therefore improves the development of the mother-young bond.

Ultrasound

Skilled operators with modern instruments can identify dries, single-bearing and multiple-bearing ewes, allowing the use of specific strategies to manage their nutritional requirements. In addition, accurate estimation of the age of a fetus will allow the use of precisely timed nutritional supplements during pregnancy.

Conclusions

For farmers, ‘clean, green and ethical’ production need not be difficult because, as we work towards a better understanding of the physiology and behaviour of farm animals, we can improve productivity and profitability and, simultaneously, promote a modern image. Understanding the reproductive responses of animals to factors such as photoperiod, nutrition, socio-sexual signals and stressors, can help us develop ‘natural systems’ as replacements for exogenous hormones and drugs for controlling and improving the productivity of our sheep. In addition, we can easily genetically improve our animals (eg, ovulation rate, calm temperament) to greatly improve many aspects of their productivity. The use of such ‘clean, green and ethical’ tools in the management of our sheep flocks can be cost-effective and improve profits, at the same time greatly improving the image of our industries in society and the marketplace. All we need is a little more research and development.

9.2 Appendix 2 – Agribusiness Sheep Updates (2006)

Proceedings of Agribusiness Sheep Updates 2006, pp. 5-8 (WA Department of Agriculture & Food)

Mating – Short and Fast is Better

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Background

Maximising stocking rate is clearly the key short-term driver of profit in wool and sheepmeat enterprises. As we consider the medium and longer terms, however, other factors become increasingly important. First, as the industry generally moves towards increases in stocking rate, there will be a need for more animals and there will be competition for those animals in the marketplace, not only among farmers but also between farmers and meat processors. Second, high rates of reproduction are essential for maximising the gain from genetic selection and for earning a good return on investments in top-class genetics.

In a prime-lamb enterprise, the need for efficient reproduction is self-evident: the ewe flock must generate plenty of lambs because they are the source of income. In a wool-only enterprise, there is less pressure on reproduction because ewes that don't have a lamb still produce wool for sale. Reproduction is usually seen only as providing flock replacements, but genetic improvement is still slowed by poor reproductive efficiency. In any case, wool-only enterprises are becoming rare and the dominant situation is now becoming a mixed meat-wool enterprise based on Merino mothers.

Efficiency can be improved at all stages of the reproductive process so it is necessary to prioritise those stages on the basis of where we can have the greatest impact. Currently, most attention is being placed on lamb survival, and for very good reasons. Lamb losses probably average 30% and are a disastrous waste of investment in labour, pasture and genetics. In addition, the associated ethical issues could lead to closure of our most profitable markets.

Addressing lamb mortality is not easy because the causes are complex, involving genetics, nutrition and management. However, most lambs are lost in the first few days after birth and most of the losses are among twins, so we know where to focus our efforts. The major causes of mortality can be rectified by clever and precise management but this is really only feasible if the mating period is restricted so that the timing of lambing is known and the duration of lambing is short. In this paper, we will look at short matings and begin by considering the arguments for and against it (see the table of “Pros and Cons” below). We aim to show that the arguments against are either not important in the context of the need to improve efficiency or, if they are important, we can manage them so they have minimal impact.

The Arguments For

1) Avoid the effects of the autumn feed gap

The problem with extended autumn matings in WA is that the ewes that attempt to mate late (second or third cycle) are more likely to be losing weight. They might not conceive due to the deterioration in feed quality at that time of the year, especially if the feed gets rained on. They are also less likely to have twins. None of this necessarily reflects their genetic potential … they are less likely to get into lamb or conceive twins simply because of a drop in body weight. The only way to avoid this is an expensive program of feed supplements.

Pros	Cons
Avoid the effects of the autumn feed gap Mate while the ewes are still in good condition *Concentrated, short lambing period* Focus feeding becomes efficient Feeding for twinning Feeding for colostrum Lambing management for lamb survival Marketing simplified (no 'tail') Weaner management simpler (no 'tail') *Labour management at lambing, tailing, drafting* *Increased value from scanning* Separation of dries, singles, twins Lamb survival (focus on twins) Minimise autumn feed costs Manage lambing as 1st and 2nd cycles (survival) Strategy for late start to season *Pressure on genetics of ewe fertility*	*Lost opportunity to conceive, low lambing percentage?* Yes, no 'tail' … but how much is lost? *Need more rams?* Yes, but it can be managed. *Avoid the impact of weather events at lambing?* Yes, but only partially … still a disaster. A substitute for poor management?

2) Concentrated, short lambing period

A short mating period leads to a short lambing period in which intensive activity becomes cost-effective. If the ewes are lambing in a relatively tight pattern, then the lambing environment can be managed and strategies such as 'Focus Feeding' become efficient. This means that we can consider supplements that will promote twinning and increase the production of colostrum. Extra twins, as long as they are gained at minimum cost and then managed correctly at lambing, will improve profitability. Extra colostrum will probably help lamb survival. Lamb mortality can also be reduced by improving the conditions in which ewes lamb, including the provision of feed, water and shelter by, for example, the provision of 'edible shelter' such as a standing oat crop or fodder shrubs. With a combination of a short mating and ultrasound scanning, this investment can be focussed on twin births in the first 10 days of lambing, with cost-effective outcomes.

A concentrated lambing period has additional benefits in that the 'tail' in the lamb crop is avoided, so the cohort is of a more even size. This simplifies marketing into prime lamb supply chains and simplifies the management of weaners over their first summer and autumn.

The difficulties with farm labour in the current economical and social climate, as well as the diverse demands placed on the farmer's time in a crop-sheep enterprise, have led to great interest in the reduction of labour inputs. In turn, this has led to the development of the concept of an 'easy-care ewe"'. Here too, short mating can help because it leads to a short and intensive period of lambing at a known time, so labour inputs can be planned well ahead. Again, ultrasound scanning is a vital source of information for that planning process.

3) Increased value from ultrasound scanning

With a short mating period, all conceptions could be limited to a single cycle, perhaps two. This increases the value of ultrasound scanning because it allows the segregation of ewes into flocks of dries, single-bearers and twin-bearers. The dries can be managed as wethers, or culled to improve fertility or, if they are excessively numerous, mated as a separate flock with a slightly later (but still concentrated) lambing.

This segregation is the first step towards 'focus feeding' for colostrum production, or 'feeding to meet the need', so that supplements and feed resources are not wasted on non-pregnant and single-bearing ewes. This is perhaps most important when there is a very late start to the growing season. Finally, the ability of high quality scanners to detect twins and even differentiate between fetuses conceived in different cycles allows efficient planning of lambing conditions and thus excellent opportunities for reducing lamb mortality.

4) Pressure on genetics of ewe fertility

A short mating period gives the ewe only one chance to conceive. If she fails that test, she faces culling, particularly if it is her second year in succession. Thus, as soon as the results of ultrasound scanning are known, a strategy for genetically improving fertility can be implemented. Clearly, culling for single-cycle fertility needs to be a flexible strategy, with variations made according to ewe body condition and age. It also needs to be matched with an equally careful strategy for ram management (see below).

In contrast to traditional practice, it is important to tighten-up matings with maidens too. Provided they are of adequate weight (above 2/3 mature body weight), not losing weight when mated and have been teased (even for a post February mating), they really should be able to get into lamb with a short mating. If they don't, it may be best to be cull them since they may well become the perennial dries (barren ewes) or ‘laggards’ (never having twins or skipping a lamb every second year) throughout their life. This needs further scientific investigation because we have no solid data for Merino ewes, but the phenomenon is appreciated for cattle so top beef and dairy producers have short matings for their heifers. It nevertheless seems sensible to avoid handicapping maiden ewes and give them every opportunity to quickly get into lamb and cull them if they don't.

The Arguments Against

1) Lost opportunity to conceive, so low lambing percentage?

It is self-evident that a drawn-out mating period will increase the percentage of ewes that conceive, simply because those that mate and fail have a second or third opportunity. Two issues are relevant here. First, as discussed above, the late conceivers may need to be culled because of their poor fertility genetics (assuming all other factors equal, such as ewe body condition and ram performance). Second, the lambs gained in the second and third cycles may actually be a cost rather than a benefit. As well as forming the ‘tail’ of the lamb crop, there may be fewer of them than we realise. This issue is demonstrated in the figures below.

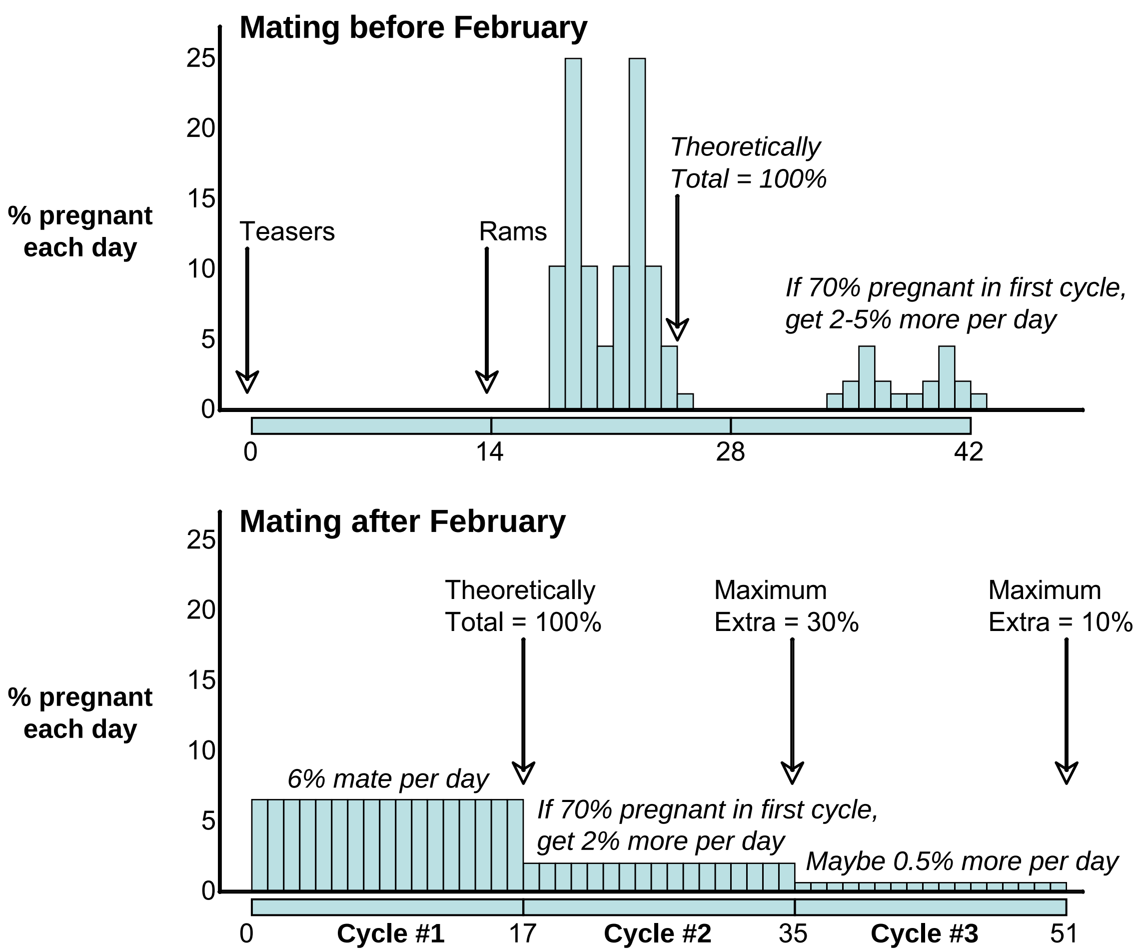
2) Extra rams needed

It is clear that we need to have a higher ram percentage for short matings if we are to offer the ewes a reasonable probability of conception. This is especially important in flocks mated before February because the ewes will be teased and thus synchronised. Instead of the rams having to serve 6% of the ewes every day, as happens during the breeding season (February to May), they will encounter as many as 30% of the ewes in oestrus on some days. In this situation, 4% rams might be needed.

In this light, it is essential for short matings that the rams are managed correctly so that each individual is contributing their best. This means ensuring that they have maximum mass of testes … feed them with 500 g per head per day) for 8 weeks before they go in with the ewes. It also means that they must be anatomically sound, healthy and fit. For matings before February, extra rams are needed and, if this seems excessively expensive, we may need to explore new strategies, such as sharing rams with producers who mate at a different time. This might raise issues such as concern for disease control, but such issues should be addressed not avoided.

3) Avoiding the impact of weather events at lambing

There is no doubt that we are horrified by the impact of a weather event, such as heavy rain with high wind-chill, during lambing. In fact, lambing over 6-8 weeks does not guarantee a good result because most lambs (70% on average) are born within the first 17-day cycle and a random weather event is just as likely to fall during that period as during the second or third cycles when relatively few lambs are dropped. In any case, rather than give in to this risk, we should plan for it and manage the lambing flock correctly. This brings us back to concepts such as 'edible shelter'. The basic fact is that 20 cm of grass or crop is enough for a lamb to drop down out of the wind-chill zone. If the surroundings are managed so that the ewe does not need to wander from the birth site, lamb mortality will not be a major issue.



Conclusion

There are problems with short mating periods, but they are either minor or they can be managed so they have little impact. They are then greatly outweighed by the benefits.

Further reading
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9.3 Appendix 3 – Lambline 8, page 3 (January 2006)

WAMMCO publication

Mating ewes from February onwards – what's important?

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Whilst many rams across WA have completed their annual work load, in other parts of the state where a later lambing is preferred, the next few months are vitally important if producers are to achieve above average lambing percentages.

So what is different about joining from February on? Namely, **mature ewes will cycle spontaneously and not need to be stimulated by teasers**. After February, approximately 6% of ewes in each mob will be displaying oestrus behaviour every day rather than in a teased mob where the majority of Merino ewes have 2 distinct periods of oestrus.

Ideally, **ewes should be in condition score 3 at mating**. In this condition score, the ewes would be "prepared to gamble" on twins (if their genes would allow). However, with mating from February onwards, the ewes are often grazing feed that is declining in nutritive value (particularly after the recent summer rains) and, even if they start in good body condition, losing condition sends panic signals to the reproductive system and the ewes seem to "choose the conservative pathway" and go for a single lamb. If a flock of ewes lose too much condition, some may not even get-in lamb, especially light weight weaners.

Feeding lupins for a few days seems to trick the ewes into thinking they are in good condition so they may again gamble on twins. **To achieve this, feed the ewes (daily) with up to 500g/h/d of lupins for the 7 days before and the 7 days after the rams are put with the ewes.** This fortnight of feeding lupins is cheaper than maintaining ewes above score 3. **Why stop after a fortnight?** Some research suggests that ewes fed at a high level in the second week after mating will lose embryos due to a fall in the level of progesterone - the major hormone of pregnancy.

The age, health, fitness, experience and joining percentage of the rams used in the mating program will all influence the potential lambing percentage.

Although it is getting a bit late for this season, all rams should have a health inspection 8 weeks prior to the start of the planned joining program. Ensure the rams are shorn, jetted, treated for internal and external parasites, that their teeth, testes and penis are all sound and if necessary trim their hooves. **Why 8 weeks?** This is the time it takes sperm production to be completed (ie cell initiation to viable sperm). Any stress during this period can adversely affect sperm production and thus mating efficiency.

Ram testes respond rapidly to nutrition and when correctly fed, their testes grow. The number of sperm produced per gram of testes per day is constant hence the larger the testes at the start of joining, the more sperm there are available for fertilising ewes. **Offer rams daily feeds of 500 – 750 g/h/d of lupins for the 8 week period leading up to joining to maximise testes size.** Keep in mind however that over fat and unfit rams perform poorly.

The aim is to achieve a short and successful mating. **A well prepared, healthy, mature ram should be able to handle 50 mature ewes for a joining from February onwards (ie 2% rams).** However, if the ewes are well synchronised, if joining maiden ewes or if using young inexperienced rams then at least 3% rams is recommended. **Remove rams 35 days after introduction.** This gives the ewes 2 opportunities to mate and the concise mating allows some clever management of ewes later in their pregnancy to maximise lamb survival.

Why pregnancy scan? When a short mating period has been used, **ewes can be scanned 45 days after ram removal.** The scanning will inform you if the mating has been poor, allow you to put a backup plan into action rather than getting a big surprise at marking time. If the mating is successful, scanning allows you to plan resources and timing for lambing. Scanning allows non-pregnant and twin bearing ewes to be identified and managed accordingly. Some scanners can even estimate when a ewe conceived (in the first or second cycle of the joining program) to further refine and plan for lambing.

More information on the managing lambing ewes will be published in future editions of LambLine and John Milton (92425876) or Rob Davidson (0429380195) can be contacted for more information on the above.

9.4 Appendix 4 – Livestock Updates (2007)

WA Department of Agriculture & Food

Embryo losses were not increased when Merino ewes that had lost weight were supplemented with lupins

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ABSTRACT

To determine whether extending supplementation with lupins after mating increases embryo losses, Merino ewes grazed dry pasture without a supplement (n = 117) or were supplemented with 500 g/head/day of lupins for 6 days before insemination (Lupin 6, n = 112) or for 6 days pre and 15 days post insemination (Lupin 6+15, n = 121). All ewes lost weight (3.0 ± 0.5 kg; P < 0.001) and condition (0.2 ± 0.03; P < 0.001) leading up to insemination. The Lupin 6+15 group regained some weight post insemination (1.0 ± 0.5 kg; P < 0.001). Ovulation, conception and pregnancy rates did not differ between supplemented and control ewes. Prolonged supplementation of ewes after insemination did not increase embryo losses.

INTRODUCTION

Supplementing ewes before mating can increase ovulation rate, but extending supplementation to provide twice their requirements for maintenance after mating can increase embryo losses [1]. Lupins are commonly used to supplement ewes to increase ovulation rate, but it has not been established if feeding 500g/hd/day of lupins after mating increases embryo losses under field conditions. This experiment tested the hypothesis that feeding 500g/hd/day of lupins for 15 days after mating would increase embryo losses.

METHOD

In November 2006, 350 Merino ewes aged 2.5 years and weighing 53.8 ± 0.5 kg with a condition score of 2.9 ± 0.02 (0-5) were selected from a flock of 1500. The ovaries of 50 ewes were examined to ensure they were not ovulating. The ewes were synchronised with sponges of medroxi-progesterone (Cronogest®, Intervet, Australia) for 14 days with 200 IU of eCG (Folligon®, Intervet, Australia) injected at sponge removal, two days before artificial insemination (AI, Day 0). The ewes were randomly allocated to three groups on Day -17. The control group grazed dry pasture of predominantly barley grass and capeweed without a supplement (n = 117). The second group grazed a similar pasture in a separate paddock and were supplemented with 500 g/head/day of lupins for 6 days from Day -7 to -2 (Lupin 6, n = 112). The third group were supplemented with 500 g/head/day of lupins from Day -7 to -2 and then again from Day 1 to 15 (Lupin 6+15, n = 121). The second and third groups grazed together until Day -2 when the second group was removed to graze with the control ewes. All ewes were housed in a shed without food and water for 24 hours before AI. The ewes were artificially inseminated 49 to 57 hours after sponge removal. Semen for AI was collected from four Texel rams, evaluated (mass motility >4; concentration >4) and pooled. The ejaculates were diluted 1:3 with UHT skim milk. The concentration of semen was evaluated in a haemocytometer and the insemination volume adjusted to give a dose of 200 million sperm per ewe. The ewes supplemented with lupins each had at least 0.5 m access to the trail of lupins so they could all eat their share of lupins. Live weight and condition score were measured on Days -17, 5 and 17.

Ovulation rate was measured by transrectal ultrasonography on Day 10. Conception rate was calculated as the total number of ewes pregnant as a percent of the ewes that had corpora lutea on Day 10. Pregnancy rate was calculated as the total number of ewes pregnant as a percent of the ewes inseminated in each group, irrespective as to wether they showed corpora lutea or not. The number of actual embryos was determined by trans-rectal ultrasonography on Day 30 and confirmed by trans-abdominal ultrasonography on Day 60. On Day 144 the pregnant ewes were drafted and fitted with a numbered ear tag that was easy to read at lambing. Lambing was observed and recorded for each ewe. Changes in live weight and condition score were analysed by a mixed model procedure using SAS. Ovulation rate and percent embryo loss were analysed after log-transformation of the data. Pregnancy rate, conception rate and total embryo losses were analysed using a chi-square test. Significant differences between groups were accepted at the 95% confidence interval ($P < 0.05$).

RESULTS

Three hundred and forty five of the 350 ewes were inseminated (four ewes lost sponges and one ewe had an abnormal reproductive tract). Thirty-two ewes had no corpora lutea at Day 10 since they did not ovulate in response to synchronisation and these ewes were only included to calculate pregnancy rate. Supplementing with lupins for 6 days increased ovulation rate by only 6% (Table 1). All ewes lost weight and condition from Day -17 (54 ± 0.5 kg, 2.9 ± 0.02) to Day 5 (51 ± 0.5 kg, 2.7 ± 0.03; $P < 0.001$). The Lupin 6+15 group regained some weight and condition from Day 5 to 17 (52 ± 0.5, 2.9 ± 0.03; $P < 0.001$). There were no differences between groups for conception and pregnancy rates (Table 1). Overall, all groups lost more embryos from Day 10 to 30 than from Day 30 to 60. Embryo losses were similar between supplemented and control ewes for both periods (Table 1). The number of lambs born indicated there were no fetal losses after Day 60.

Table 1. The reproductive performance of ewes grazing dry pasture alone (control), ewes supplemented with lupins from Day -7 to -1 relative to AI (Lupin 6) or ewes supplemented with lupins from Day -7 to 15 (Lupin 6+15)

	Control	**Lupin 6**	**Lupin 6+15**
Ovulation rate	1.2 ± 0.04	1.3 ± 0.05	1.3 ± 0.05
Conception rate (%)	63 (65/104)	63 (63/100)	66 (71/108)
Pregnancy rate (%)	55 (65/117)	56 (63/112)	59 (71/121)
Embryo losses			
From Day 10 to Day 30 (%)	42 (53/125)	41 (52/127)	39 (54/140)
From Day 30 to Day 60 (%)	22 (16/73)	13 (10/75)	13 (11/86)

CONCLUSION

In this experiment supplementing Merino ewes with 500g/hd/day of lupins for 15 days after insemination did not increase embryo losses. This may have been because the lupins fed after AI, rather than being a supplement above maintenance, were used to regain some of the weight and condition the ewes lost prior to AI.

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