final report

Project code: B.LSM.0068

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Date published: 01 May 2017

PUBLISHED BY
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NORTH SYDNEY NSW 2059

Anti-leptin vaccination to increase growth rate and fertility in Merinos

Meat & Livestock Australia acknowledges the matching funds provided by the Australian Government to support the research and development detailed in this publication.

Executive summary

The value of sale lambs is of enormous importance to the profitability of sheep production. Ewe reproduction, particularly lamb survival, is identified as a priority field of research because it is critical in underpinning the profitability of lambing systems.

Flock reproduction rate, measured as the number of lambs marked or weaned per ewe joined, is heavily influenced by litter size and to a lesser extent by fertility, both of which are relatively easily improved through nutrition of the ram and ewe. Lamb survival too, can be greatly improved by ewe nutrition. Current extension packages, such as Bred Well Fed Well and Lifetime Ewe Management have been successful in improving the nutritional and genetic status of flocks that both increases the number of lambs born and weaned and also assists in reducing in lamb mortality.

The current extension packages and much of the findings from previous research have demonstrated methods to improve the number of lambs marked or weaned by minimising the highest potential levels of mortality, rather than by lowering the minimum level of mortality, thus opportunities continue to exist for further improvement.

A pilot study was undertaken by co-investigator, Professor Jim McFarlane, which examined the potential for an anti-leptin vaccine to affect ewe reproduction. That study, undertaken over two years, revealed an increase in weaning rates from 102% to 150%.

Similar to the potential of the vaccine against androstenedione, inhibin and bone morphogenetic protein, an anti-leptin vaccine appeared to increase ewe fertility and litter size. Uniquely, lamb survival appeared to improve, too. Given a market already exists for the anti-androstenedione vaccines, but one limited to crossbred ewes and not readily applicable to producers using Merino ewes, it was appropriate that investment was made to further examine the potential of the anti-leptin vaccine, particularly one that may improve lamb survival and can be used in Merino ewe populations.

A study was established to examine the potential for an anti-leptin vaccine to improve ewe reproduction rates. This study used ewe lambs (n=70) and ewe hoggets (n=218), which were mated over two years of reproduction, as lambs and hoggets and in the following year as hoggets and young adult sheep.

Following some evidence of improved weight gain, when comparing laboratory mouse weight gains between control and vaccinates, a mob of wether lambs (n=100) was also studied for an effects on weight gain and carcase qualities. Fleece weight and wool quality attributes were examined on all sheep involved in the study.

In year one of the study, an alteration to the vaccine adjuvant had to be made that would enable the wether portion to be slaughtered and safely enter the food chain.

No significant effects on weight gain, ewe fertility, litter size, lamb survival, ewe or lamb weaning weight or fleece characteristics were observed between the vaccinates and control sheep. There was a tendency for reduced birth weight, lamb survival and weaning rates in vaccinated ewes. The lack of significant difference in weight gain in the wether portion of the sheep led to a change of investment and the decision was made not to slaughter the wethers. Titres of plasma samples collected post-

vaccination revealed high levels of background leptin, indicating limited immune response to the vaccination, suggesting the significant effect on body condition score may be artefactual.

In year two, with no wethers being involved in the study, the decision was made to change the adjuvant again. The adjuvant used in year two was the same as that used in the pilot study, Freund's complete adjuvant. This adjuvant appeared to stimulate an immune response in the vaccinated ewes with large blisters observed in the neck of the ewes at the booster vaccination.

In year two, again, no significant effects were observed for ewe fertility, litter size, lamb survival, weaning rate or lamb or ewe weaning weight. No effects were found on fleece characteristics, although vaccinated ewes were found to be leaner at weaning than control ewes. Titres of plasma samples collected post-vaccination again revealed high levels of background leptin, indicating little immune response to the vaccination.

The conclusions to be taken from this study are that vaccine efficacy needs to be improved; that the vaccine may work in some bloodlines of sheep more than others or; that the anti-leptin vaccine in its current form does not have the potential to affect ewe reproduction. The authors recommend that plot-scale, low investment studies continue in the development of the vaccine because of the potential it may have to improve sheep production in Australia.

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1 Background

The value of Australian sheep and lamb products is estimated at $3.3bn and the value of wool products at $2.7bn (ABS, 2016). Based on these commodity values, for the period 2014-2015, the value of the sheep industry is approximately $6.0bn. Growth in value of the sheep industry is derived largely from the processing of sheep meats, leading to concerns for a declining national flock size. Hence, reproduction has been identified as a key aspect of livestock production requiring improvement (Trompf *et al.*, 2013) not just because of its importance to stabilising or rebuilding the national flock, but also because of the value of surplus sale sheep and lambs.

In examining endemic diseases of the red meat industries, Lane *et al.* (2015) identified that the per annum losses associated with the neonatal period cost the sheep industry around $540M. This is the largest cost of all endemic diseases of the sheep industry. A component of these estimated costs is the loss of ewes to dystocia ($149M) and mastitis ($43M), thus the cost of neonatal lamb mortality may be around $348M. Improving the survival of newborn lambs has been, therefore, identified as a priority for the industry. Mean levels of lamb survival, however, have changed little in more than a century (Plant, 1981) and little in the time passed since that publication.

Over time, and depending on factors such as the value of wool relative to the price of lamb and; the size of the national flock, there has been more or less urgency to improve sheep reproduction rates. When the national flock was near its peak, there was little economic return found by increasing the number of lambs weaned (Young *et al.*, 1990). In more recent times, however, that is no longer the case. Economic analysis of the components of reproduction that offer the greatest returns (Young *et al.*, 2014), shows that improving the survival of twin lambs is the most profitable research investment. In that study, the value for improving twin lamb survival was $515M, while the value in improving conception was $236M and improving weaning rates from ewe lambs was valued at $333M. The scale of the return in that study was influenced greatly by the number of twin bearing ewes, therefore, increasing both the number of twin bearing ewes and the survival of their lambs offers substantial economic returns.

In two separate experiments, Kleemann and Walker (2005) were able to demonstrate the scale of reproductive wastage in South Australian Merino flocks. In both studies, about 60% of the potential reproductive performance was lost between mating and weaning. About half of the loss was due to low rates of twin lamb survival, while the remainder was due to the partial failure of multiple ovulations – the released ova that fail to implant or survive to lambing.

Generally, ewe fertility is not the primary constraint to net reproduction rates, but low ovulation rate is a key limiter (Knight *et al.*, 1975). Hence, much focus has been placed on investigating means to maximise ovulation rates. As understanding of the endocrine system has developed, the disruption of hormones or their pathways became an area of active research. Scaramuzzi and Martin (1984) described this succinctly in stating, “each of the hormones, or substances which influence their secretion, controlling or influencing the oestrous cycle and ovulation in the ewe represent potential sites for manipulation of reproduction”. Whatever such products may be, then and now, important to their successful control of the endocrine system is a long half-life or a lasting effect.

Immunisation against the activity of hormones thus became a useful model to examine the effects of those hormones. For example, through immunising ewes against progesterone, Hoskinson *et al.*

(1982b) and Thomas *et al.* (1984) highlighted the importance of progesterone in the control of oestrus, ovulation and ovulation rate. Another important benefit of that particular vaccine was the ability to obviate the requirement for surgical ovariectomising of ewes to study the same relationships.

Immunisation against the activity of hormones is also being employed to manipulate the natural effects for improvements in reproduction. For example, Wong and Cox (1986) showed that vaccinating against testosterone led to significant reductions in lambing percentage, possibly via an impairment of implantation. Whereas, concurrent immunisation against testosterone, androstenedione and oestrone increased both ovulation and lambing percentage (Wilson *et al.*, 1986). Vaccination against oestrone itself appeared to increase the sensitivity of the ovaries to pregnant mare serum gonadotrophin (Hoskinson *et al.*, 1982a). Vaccination against oestrogen also appears to work well; Corriedale ewes had increased ovulation rate and foetal number at 90 days gestation (Grant, 1982). Other reproduction-oriented hormones that can be immunised against include oxytocin (Sheldrick *et al.*, 1980) and gonodotrophin releasing hormone (GnRH) (Brown *et al.*, 1995).

Immunisation against androstenedione, an ovarian hormone, appears to be an improver of fecundity leading to improved net reproduction, but with variable results demonstrated between flocks and breeds (Scaramuzzi *et al.*, 1983). The causes for the variation in response to androstenedione and oestrone vaccination was not known but suspicions rested on the accelerated development of the stimulated follicles, rather than the factors affecting the timing of ovulation (Scaramuzzi *et al.*, 1982), or ovum pickup (Nancarrow *et al.*, 1985). In the Merino, while ovulation rates do increase following immunisation against androstenedione (nee Fecundin®, Glaxo; currently Ovastim®, Virbac), these appear not to translate into higher fertilisation rates (Boland *et al.*, 1984). When Corriedale ewes were immunised in autumn, Cummins *et al.* (1984) demonstrated an improvement in ovulation and pregnancy rate but little improvement emerged in the weaning rate. Furthermore, in comparing immunisation against the alternative of grain feeding with wheat, no net improvement was found. Wilkins (1997) found feeding lupins to Merinos was more efficient in lifting reproductions rates when compared to vaccination with Fecundin. These results imply that immunisation has some role, but its natural fit with the reproduction cycle may depend entirely on seasonal, genetic or management circumstances.

Inhibin is another hormone that has been studied in depth. A hormone of the ovaries, inhibin specifically suppresses the secretion of follicle stimulating hormone (FSH) in the pituitary and showed some promise as a vaccine alternative. Its ovarian concentration is negatively correlated with blood FSH levels, but mature follicles destined to ovulate may yet dominate inhibin's secretion (Lee and Gibson, 1982). Nevertheless, immunisation against inhibin increases ovulation rate (O'Shea *et al.*, 1982), again with some seasonality behind its improvement potential. Immunisation of ewe lambs appeared to increase the onset of puberty, significantly increasing the number of ewe lambs ovulating at seven months of age (O'Shea *et al.*, 1984). As with the findings of studies into androstenedione, variation between bloodlines, or genotypes, may partly explain variable responses to inhibin vaccination (Cummins *et al.*, 1983). Much research has been dedicated to inhibin, but the product appears to have received little development (Martin and Kadokawa, 2006).

More recently, Bone Morphogenetic Protein 15 has been shown to increase ovulation rate (Juengel *et al.*, 2004). When compared to vaccination against androstenedione, anti-BMP15 treated ewes had lower, higher and not different ovulation rates over three years, with higher ovulation in years two and three compared to control ewes (Juengel *et al.*, 2013). Overall years, mean litter size at mid-pregnancy and at birth was higher for each vaccine group compared to the control, but no improvement was made in comparison to ewes vaccinated against androstenedione. At weaning, ewes vaccinated against androstenedione were rearing more lambs than BMP15 vaccinated ewes (Juengel *et al.*, 2013). More work remains to be done with respect to BMP15, to improve the reliability of the vaccine.

Typically, the vaccination against the action of hormones or their pathways has focussed on the hormones of direct effect. In the adult, leptin acts centrally via receptors within the hypothalamus and caudal brain stem to induce satiety and increase energy expenditure to counteract increases in energy intake (Grill *et al.*, 2002; Barb, 1999). In that respect, vaccination against leptin seems to be a less obvious endocrine candidate for reproduction outcomes, except that factors affecting energy balance may play a role in reproduction and leptin has strong associations with body fatness and possibly GnRH secretion (Blache *et al.*, 2000; Chilliard *et al.*, 2005). Leptin deficient (ob/ob) mice, are infertile, but repeated administration of leptin (recombinant human leptin) corrects sterility and restores ovulation, pregnancy and parturition (Chehab *et al.*, 1996). However, in normal animals leptin appears to have inhibitory effects on ovarian and follicular function (Dupont *et al.*, 2014), particularly when leptin concentrations increase following prolonged hunger or starvation. To do this, leptin must have a role in affecting the hypothalamus-pituitary axis. In sheep, leptin receptors have been identified in the hypothalamus (Hart *et al.*, 2016; Iqbal *et al.*, 2000) and leptin receptor mRNA has also been found in the anterior pituitary of sheep and the expression of the receptor form may interact with the level of nutrition (Dyer *et al.*, 1997). Leptin receptor mRNA is expressed in human, pig and mouse ovaries and may affect follicle (Panwar *et al.*, 2012) and embryo development (Herrid *et al.*, 2014) and implantation (Malik *et al.*, 2001). However, leptin concentrations in some breeds have no relationship with reproduction outcomes (Catunda *et al.*, 2013).

In considering the importance of improving reproduction rates, the number of ewes carrying and rearing multiple foeti is the primary focus of the present study, as well as improving joining, fecundity and weaning rates in ewe lambs. Previously, a University of New England pilot study was undertaken by co-investigator, Professor Jim McFarlane, which examined the potential for an anti-leptin vaccine to affect ewe reproduction. In contrast to expectations, that study, undertaken over two years, revealed an increase in ewe fertility from ~60% and ~90% to 100% and a doubling of the number of twin-bearing ewes (McFarlane *et al.*, 2008). Lamb survival too, was markedly improved, leading to an encouraging lift in lamb weaning rates of 150% compared to control ewes (102%). The present study aimed to progress that pilot study to a small-scale, two-year investigation that examined the potential of the anti-leptin vaccination to increase the reproduction rate of ewe lambs and ewe hoggets. Also examined were the growth rates of wether weaners and fleece characteristics of all vaccinated animals, where the intention was to monitor other aspects of Merino production for other possible effects.

2 Project objectives

The primary objective of this study was to replicate the fertility and fecundity findings of the University of New England pilot study with a greater experimental scale. The pilot study tested 25 ewes over two years, whereas this trial used 240 ewes and 100 weaner wethers.

A secondary objective was to test the relationship in ewe lambs because fecundity is lower in ewe lambs and hoggets when compared to adult ewes, hence ewe lambs were tested and over two years as ewe lambs became hoggets and hoggets became adults.

A tertiary objective was to examine the effects on wool production and fleece characteristics, with the view that the production increases observed in the pilot study may have been due to increased feed intake, which has direct consequences for reproduction improvements of fertility and fecundity.

A quaternary objective was to examine the effects on fat in the live animal and carcase, due to the strong relationships between leptin concentration and fat content. The objective was to slaughter wether hoggets and examine carcase fatness parameters and loin shear force and intramuscular fat content.

3 Methodology

3.1 Site

The study was undertaken at the Cowra Agricultural Research and Advisory Station, Cowra 2794, NSW during the years 2014 and 2015. Annual mean rainfall is 619 ± 175 mm with a relatively uniform monthly distribution (51 ± 14 mm) that is by definition winter dominant. Temperature ranges from warm to hot in summer and cool to cold in winter. Average January temperatures range between 31.9°C and 15.7°C, while July temperatures range between 13.4°C and 2.9°C.

3.2 Animals

The sheep bloodline was a mix of progeny born from Sheep CRC Information Nucleus Merino followers mated to Centre Plus Merino sires (Sheep Genetics DP+ index selected) or are progeny of close to pure Centre Plus ewes mated to Centre Plus sires.

Three classes of sheep were used in the study; 140 ewe hoggets (2 year old ewes, 2012 drop), 100 ewe lambs (one year old sheep, 2013 drop) and 100 wether lambs (one year old sheep). In year one (2014) all sheep were weighed and assessed for body condition score (Russel *et al.*, 1969) and were allocated to treatment or control groups following a stratified allocation based on weight within condition score. Wether lambs were allocated separately to ewe lambs. All ewes were held together for the full duration of the trial, except during late pregnancy and lambing when the sheep were held separately according to pregnancy scanned litter size. Mean ewe lamb weight for the treatment and control groups were 40.7 and 3.06 CS. Mean wether lamb weight for treatment and control groups were 36.1 kg, while body condition score were 2.48 or 2.46 CS, respectively.

Mating occurred during March and April in both years of the study, lasting 5 weeks in duration. In year one, mature rams were mated to the ewe lambs (n=3, 3%) and ewe hoggets (n=4, 2.2%). In year two, a similar cohort of mature rams were used (n=5, 2.2%). All ewes were mated as one mob.

Shearing occurred in November of each year, at which time greasy fleece weight and a mid-side sample were collected. Mid-side samples submitted to AWTA LTD for the measurement of fibre diameter, yield, staple length and staple strength.

3.3 Pastures

Summer pastures generally contained cereal stubbles, lucerne, summer grass weeds, native grasses or senesced stands of mixed perennial grasses such including phalaris, cocksfoot or fescue. Winter pastures were typically a mix of phalaris, cocksfoot and fescue (typically 30-40%) with annual grasses including barley grass, annual rye grass and silvergrass (20-30%). Legume components were subterranean clover with a small component of yellow burr medic (typically 20-40%).

Seasonal conditions were normal throughout the study, with a prolonged dry spell in late 2014 into April 2015, when no pasture growth occurred from early February until mid-April. By March 2015, pasture feed on offer was estimated to be 500 kg DM/ha and a minor supplementary feeding program was undertaken. Ewes were trail fed a mixed ration offering 500 g/h/d containing 85% oats and 15% lupins. The supplementary feed was provided for the two weeks before mating commenced and for four weeks of mating. This situation led to an increase in the range of body condition score (mean 3.08 ± 0.41, range 1 to 4) as some shy feeders lost a considerable amount of weight. This was considered to be better for the trial as it was hypothesised that the effects of the vaccine would be clearer in leaner ewes. After weaning in the previous year, the mean BCS was 3.2 ± 0.43 (range 2 to 4.5), which demonstrates the reduced body condition for all ewes.

3.4 Pregnancy scanning

Pregnancy scanning was undertaken by ultrasound at around 40 days after the removal of the rams. The contractor employed had a long history of scanning at the Cowra Agricultural Research and Advisory Station and is considered to be highly accurate. All ewes were scanned for triplets and foetal aged. After scanning, the ewes were split into two mobs containing single and dry ewes or twin and triplet bearing ewes. These two mobs remained on pastures appropriate to meet their nutritional requirements.

3.5 Lambing

All ewes were placed into their lambing paddocks within 7 days from the expected start of lambing. Ewes were weighed and body condition scored on the day of allocation to the lambing paddock. Single-scanned ewes lambed in one paddock, while twin and triplet-scanned ewes lambed together, in a separate paddock. Dry ewes were separated from all pregnant ewes at this time. After lamb marking, all lambed ewes were joined into one mob and rotationally grazed through to weaning.

Using estimates of feed on offer (FOO), lambing paddocks were selected if sufficient FOO was available to provide for the ewes through to the end of lambing (5 weeks). In both years, minimum FOO available offered 1200 kg DM/ha of green pasture, as lambing paddocks were rested for at least four to six weeks prior to lambing and not grazed heavily beforehand. No supplementation was required during lambing in either year.

3.6 Shearing

Greasy fleece weight including the weight of the belly was recorded at shearing. Also collected was the name of the shearer and a mid-side sample. The site of mid-side sample collection was labelled with a spray mark within 48 hr of the time of shearing. Positioned on the left side of the sheep, at the 12th rib, the spray mark was applied to the tip of the staples. The left side of the sheep is the side of the long-blow and samples collected from this side of the sheep was considered to contain less variation in staple length.

3.7 Vaccine

Producing a vaccine which induces a good titre against leptin in mammals has been difficult. The Beef CRC did a trial some years ago and found similarly to the present study (Ross Tellam, personal communication). Hence antibodies against bovine / ovine leptin have used chickens as a host (Kauter *et al.*, 2000).

In year 1, leptin was crosslinked against itself using glutaldehyde and then diluted in PBS and combined with Freunds incomplete adjuvant in a 50:50 ratio. Ewes received a 100ug primary vaccination in 1ml of emulsified adjuvant followed 4 weeks later with a 25ug booster.

In year 2, the same procedure was used except using Freunds complete adjuvant.

Success of the vaccination was tested using similar procedures to that reported by Kauter et al 2000, briefly 100ng of leptin was coated onto ELSA plates and then incubated at 37C overnight with increasing dilutions of ewes plasma followed by detection with a rabbit anti-sheep IgG conjugated to alkaline phosphatse.

This adjuvant appeared to stimulate an immune response in the vaccinated ewes with large blisters observed in the skin around the neck of the ewes at the booster vaccination.

3.8 Ethics

Approval to undertake this research on animals was provided by the NSW Department of Primary Industries Animal Ethics Committee, Orange. The Animal Research Authority number is ORA 13/16/003.

3.9 Statistical design

Two designs were considered for the trial. The first was to consider the potential for the vaccine to improve ewe reproduction. The second design was to consider the effect on animal growth rate.

Advice has been sought from Remy van de Ven (Senior Biometrician, NSW Department of Primary Industries) to evaluate the power of the study. There were two broad aims: to determine improvements in fertility and litter size from ewes when mated as lambs (7-9 mo) and when mated as hoggets (19-21 mo) and; to determine effects on growth rate of lambs to weaning.

University of New England pilot studies showed improvements in fertility of vaccinated ewes between 10-40% compared to control ewes (90 v 100%; 60 v 100%), while vaccinated ewes also had an increased number of twin lambs born (14 v 26%; 35 v 73%) compared to control ewes. Biometric advice showed that to have sufficient power, 120 ewes were taken for the study, which provided an 80% chance of achieving a significant result when the difference in fertility between the treatment and control was 10%.

The assumed mean wean weights of Merino lambs were 27 kg ± 5.5. kg. To achieve an 80% chance of finding a significant effect ($P<0.05$) on weaning weight, when assuming a difference between the treatment and the control of 8%, requires 102 lambs weaned per treatment.

3.10 Statistical analysis

In each year of the study a different vaccine adjuvant was used and therefore the analyses are treated as if the two years were independent and are analysed separately. All analyses were undertaken using R 3.3.0 (R Core Team, 2016). Significance is determined at 0.05. Two-way interactions were examined; non-significant terms were sequentially dropped from the model except for the main effect of treatment.

The binomial outcome for fertility was tested using logistic regression using the logit link function and significance was tested with analysis of deviance and chi square. The number of lambs born and weaned was analysed using generalised linear models. Models included the main effect of treatment, ewe body condition score (BCS) and ewe weight. For number of lambs born, weight and BCS recorded at mating was used in the model. For the number of lambs weaned, weight and BCS recorded at mid pregnancy was tested. In a separate model, the number of lambs weaned was also tested using weight and BCS that were recorded at late pregnancy.

Lamb birth and weaning weight, growth rate to weaning and total weight of lamb weaned was analysed using likelihood ratio tests using the lme4 package (Bates *et al.*, 2015) in R 3.3.0 (R Core Team, 2016). In doing so, this permitted dam and lambing paddock to be included as random terms. Significance was tested using likelihood ratio tests, comparing against the null model (i.e. comparing the results of two models, one without the treatment). Models included the main effect of treatment, sex, birth type or where appropriate rearing type was used. Lamb survival was analysed using the logit link function.

For models analysing ewe weight or ewe BCS, generalised linear models were used and significance was tested with analysis of deviance and chi square. Fixed effects of pregnancy status or where appropriate, the number of lambs born and the year drop of the dam were included in the model.

The analysis of fleece characters was undertaken for ewes and the wethers separately to allow for the effects of pregnancy to taken into account. The analysis of fleece characteristics used generalised linear models and significance was tested with analysis of deviance and chi square. Base models included treatment, number of lambs weaned (converted to a factor), off-shears liveweight and off-shears BCS and the interactions.

When appropriate, mean values are reported with the standard deviation in parentheses.

4 Results

Raw means for weights, reproduction, fleece and lamb production are tabulated in Appendices 1 through 5.

4.1 Year one

4.1.1 Ewe reproduction

Fertility was not affected by the vaccination of the ewes ($p>0.05$), although vaccinated ewes had a higher fertility (73 v 78%). Weight at mating had a significant effect ($p<0.001$), but body condition score was not significant ($p>0.05$). Table 1 reports all of the ewe reproduction results in 2014. Vaccinated ewes weighed 50.6 kg compared to the control ewes (50.7 kg) and were in the same body condition respectively, 2.97 v 2.95.

The number of lambs scanned was not affected by the vaccination ($p>0.05$, Table 1), while weight at mating ($p<0.001$) was an important factor. Ewe BCS was not a significant ($p>0.05$) factor.

The number of lambs born was not affected by vaccination treatment ($p>0.05$, Table 1). Pregnant, vaccinated ewes gave birth to 1.42 lambs (± 0.66) compared to the control ewes (1.55 ± 0.63 lambs). Weight at mating had a highly significant effect on the number of lambs born ($p<0.001$). Ewe condition score was also significant ($p<0.05$).

Typically, there are losses of potential lambs between pregnancy scanning and the number of lambs born. In year 1, there was a reduction of 17% of scanned foetuses in the Leptin vaccinated group between scanning and lambing, compared to a 5% reduction in the control ewes. Logistic regression for loss (0) or retention (1) of the all scanned foeti revealed no significant effect of vaccination ($p>0.05$) or drop ($p>0.05$), while BCS at mating tended to have had some correlated effect ($p<0.1$).

Lamb mortality tended to be higher in the progeny of vaccinated ewes with some correlated effect ($p=<0.1$, Table 1). Lamb mortality of vaccinated ewes was 30% compared to control ewes (21%). The mean mortality was 25%, where litter size was an important factor ($p=<0.05$), but sex of the lamb had no effect on mortality ($p>0.05$).

The number of lambs weaned (NLW) was tested in two separate models. The first model included weight and body condition score at mid-pregnancy as terms. The number of lambs weaned tended to be lower in the vaccinated group ($p=<0.1$, Table 1). Weight at mid-pregnancy had a highly significant, positive relationship with NLW ($p<0.01$) and ewes that weaned more lambs tended to be leaner ($p=<0.1$). The second model used to test NLW included weight of the ewe in late pregnancy (pre-lambing) and body condition score at that time. In this second model, there was no significant effect of vaccination on NLW ($p=>0.1$), while ewe weight was significant ($p<0.05$), body condition score was not significant ($p>0.05$). The mean number of lambs weaned by vaccinated ewes was 1.00 (± 0.69) compared to control ewes (1.20 ± 0.70).

The total weight of lamb weaned was not significantly affected by the vaccination of the dams ($p>0.05$). Vaccinated ewes weaned 34.1 kg (± 10.5) compared to control ewes (36.5 ± 10.4 kg). Ewes in lighter body condition score weaned heavier lambs. The number of lambs weaned was a significant driver of total weight of lambs weaned, where ewes rearing twins weaned 46.1 kg in lamb, while singled rearing lambs weighed 29.2 kg.

4.1.2 Ewe growth and condition

Weight at mating was different between the year drops ($p<0.001$) but was not different between the vaccinated and control groups ($p>0.05$). Table 1 reports on the results for ewe weight and ewe body condition score for year one of the trial. Vaccinated ewes weighed 50.6 kg compared to control ewes (50.7 kg). Ewe BCS was affected by drop ($p<0.01$) but vaccination had no effect ($p>0.05$).

At mid-pregnancy, ewe weight was significantly affected by year drop ($p<0.001$) and pregnancy status ($p<0.05$), but not by the vaccination ($p>0.05$, Table 1). Ewe body condition at mid-pregnancy was not affected by either vaccination ($p>0.05$) nor pregnancy status ($p>0.05$) and nor drop ($p>0.05$). Vaccinated ewes weighed 60.3 kg compared to control ewes (60.7 kg) and their respective body condition scores were 3.52 and 3.56.

By late pregnancy, pre-lambing ewe weight was affected by pregnancy status ($p<0.001$), ewe drop ($p<0.01$) and the interaction of pregnancy status and drop ($p<0.05$) but not by vaccination ($p>0.05$, Table 1). At late pregnancy, ewe condition score was affected by pregnancy status ($p<0.001$) but not by vaccination ($p>0.05$, Table 1). The interaction of pregnancy status and drop had some correlated effect on pre-lambing BCS, but was not significant ($p<0.1$). Vaccinated ewes weighed 67.3 kg compared to control ewes (68.1 kg) and their respective body condition scores were the same 3.09 and 3.08.

Table 1. Live weight (kg), body condition score (BCS) raw means (± s.d.) collected at mating (M), mid-pregnancy (MP), pre-lambing (L) and off-shears (O/S) and reproduction raw means (± s.d.) for fertility (ewes pregnant per ewe joined), pregnancy scanning percentage (lambs scanned per ewe joined), lambs born per ewe lambing (LB), lamb mortality, number of lambs weaned per ewe joined (NLW) and total weight of lamb weaned (TWWT) for year 2014, reported for vaccinated (Leptin) and control ewes and for the dams' year of birth (Drop)

Group	**M weight (kg)**	**M BCS**	**MP weight (kg)**	**MP BCS**	**L weight (kg)**	**L BCS**	**O/S weight (kg)**	**O/S BCS**	**Fertility (%)**	**Sc (%)**	**LB**	**Lamb mortality (%)**	**NLW**	**TWWT (kg)**
Leptin vaccinates mean (± s.d.)	**50.57 ± 6.21**	**2.97 ± 0.21**	**60.28 ± 7.63**	**3.52 ± 0.25**	**67.3 ± 10.81**	**3.09 ± 0.24**	**61.00 ± 6.97**	**3.2 ± 0.40**	**78%**	**1.59 ± 0.58**	**1.42 ± 0.66**	**30%**	**78%†**	**34.1 ± 10.50**
2012 drop	54.13 ± 5.21	2.88 ± 0.21	65.05 ± 6.12	3.51 ± 0.29	72.81 ± 7.75	3.03 ± 0.21	63.64 ± 7.15	3.29 ± 0.44	63/70 (90%)	1.73	1.56	33%	94%	37.75 ± 9.96
2013 drop	45.60 ± 3.48	3.09 ± 0.15	53.60 ± 3.28	3.54 ± 0.19	59.42 ± 9.65	3.17 ± 0.26	57.40 ± 4.82	3.08 ± 0.30	31/50 (62%)	1.29	1.13	21%	54%	26.98 ± 7.5
Control mean (± s.d.)	**50.74 ± 6.03**	**2.95 ± 0.23**	**60.68 ± 7.60**	**3.56 ± 0.26**	**68.07 ± 9.06**	**3.08 ± 0.27**	**61.05 ± 7.36**	**3.18 ± 0.47**	**73%**	**1.61 ± 0.54**	**1.55 ± 0.63**	**21%**	**87%**	**38.67 ± 10.46**
2012 drop	54.13 ± 5.17	2.83 ± 0.22	65.33 ± 6.03	3.56 ± 0.27	73.22 ± 7.41	3.03 ± 0.27	63.11 ± 8.11	3.22 ± 0.56	64/71 (90%)	1.73	1.68	18%	121%	28.63 ± 5.26
2013 drop	45.74 ± 2.93	3.11 ± 0.14	53.80 ± 3.16	3.55 ± 0.23	60.66 ± 5.30	3.17 ± 0.26	58.07 ± 4.82	3.13 ± 0.28	23/48 (48%)	1.26	1.17	34%	35%	34.11 ± 10.49

*** p<0.001, ** p<0.01, * p<0.05, † p<0.1, when compared to Control ewes (within the column)

4.1.3 Lamb data

Birth weight slightly lower in the vaccinated group ($p=<0.1$, Table 2). Compared to control ewes 3.77 (± 0.95 kg), the mean birth weight of lambs from vaccinated ewes was 3.62 kg (± 0.82 kg) and this difference was due to the higher weight of single-born lambs in the control ewes (4.52 kg) compared to that of the vaccinated ewes (4.00 kg).

Wean weight was not significantly affected by the vaccination of the dams ($p>0.05$, Table 2), where for the vaccinated ewes the average lamb weaning weight was 26.0 kg (± 5.7 kg) compared to the control group (25.9 ± 5.7 kg).

Growth rate to weaning was not significantly affected by the vaccination of dams ($p>0.05$, Table 2), where the mean growth rate from birth to weaning was 235 g/d compared to 237 g/d for the control group.

Table 2. Weight at birth and at weaning raw values (kg ± s.d.) collected in 2014, reported for vaccinated (Leptin) and control groups

2014 drop lambs	**Birth weight**	**Wean weight**
Leptin vaccinate group	3.62 ± 0.82†	26.04 ± 5.69
Control group	3.77 ± 0.95	25.85 ± 5.73
2012 drop dams	3.66 ± 0.87	26.26 ± 5.69
2013 drop dams	3.80 ± 0.94	24.90 ± 5.66

*** $p<0.001$, ** $p<0.01$, * $p<0.05$, † $p<0.1$, when compared to Control ewes (within the column)

4.1.4 Wether weight and body condition score

Weight recorded at the time of mating, was not different between the vaccinated and control wethers ($p>0.05$, Table 3).

Weight, recorded at the same time as the pre-lambing weight recorded on the ewes, was not significantly different between vaccinated and control wethers ($p>0.05$, Table 3). Body condition score of the wethers at this time was also not significantly different between the groups ($p>0.05$, Table 3).

Off-shears liveweight was also analysed separately to the ewe data set, as a means to separate from the effects of pregnancy on ewe off-shears liveweight. Vaccination did not affect liveweight in the wethers ($p>0.05$, Table 3). The vaccinated wethers weight 59.6 kg compared to the control wethers (58.6 kg). Off-shears body condition score was not affected by the vaccination ($p>0.05$, Table 3).

4.1.5 Ewe fleece data

Greasy fleece weight was not affected by the vaccination ($p>0.05$), while off-shears liveweight ($p<0.01$) and off-shears body condition score ($p=<0.1$) were important factors. Table 4 reports all fleece characteristics for the ewes in year one; 2014. Vaccinated ewes produced a 6.9 kg fleece compared to the control ewes (6.8 kg). Yield was significantly affected by the number of lambs, where ewes rearing twin lambs had a higher yield ($p<0.05$), but the vaccination had no significant effect ($p>0.05$). Clean fleece weight was affected by rearing twin lambs ($p<0.05$), off-shears liveweight (p<0.001) and off-shears BCS (p<0.05), but not by vaccination (p>0.05). Clean fleece weight of vaccinated ewes was 5.02 kg and was the same as the control ewes (4.99 kg).

Fibre diameter was affected by off-shears liveweight (p<0.01) and not by the vaccination (p>0.05). The mean fibre diameter of vaccinated ewes was 18.6µm compared to the control ewes (18.5 µm). Staple strength was not affected by the vaccination (p>0.05), but the number of lambs weaned (p<0.05), off-shears liveweight (p<0.05) and off-shears BCS (p<0.01) were all important factors. Staple strength of vaccinated ewes was 36.7 N/ktex, compared to control ewes (36.7 N/Ktex). Staple length was not affected by the vaccination (p>0.05), number of lambs weaned (p>0.05), while off-shears liveweight had a significant effect (p<0.01).

Table 3. Live weight (kg ± s.d.) and body condition score (BCS) raw values (± s.d.) of the wethers throughout 2014, reported for vaccinated (Leptin) and control groups

Group	Weight at time of mating	Weight at time of lambing	Lambing BCS	Off-shears weight	Off-shears BCS
Leptin vaccinates					
mean	34.94 ± 3.53	55.31 ± 5.28	3.40 ± 0.18	59.6 ± 5.81	3.19 ± 0.31
Control					
mean	35.02 ± 4.28	54.83 ± 4.29	3.37 ± 0.15	58.61 ± 5.79	3.18 ± 0.27

*** p<0.001, ** p<0.01, * p<0.05, † p<0.1, when compared to Control ewes (within the column)

Table 4. Fleece and wool quality raw values (± s.d.) collected from the ewes in 2014, reported for vaccinated (Leptin) and control groups

Group	GFW (kg)	Yield (%)	CFW (kg)	FD (µm)	SS (N/Ktex)	SL (mm)
Leptin vaccinates						
mean	**6.88 ± 1.0**	**72.99 ± 4.6**	**5.02 ± 0.8**	**18.62 ± 1.2**	**36.92 ± 8.3**	**122.4 ± 10.4**
2012 drop	6.98 ± 1.1	73.60 ± 4.7	5.13 ± 0.8	18.60 ± 1.1	35.46 ± 7.2	123.4 ± 10.9
2013 drop	6.75 ± 0.9	72.15 ± 4.5	4.86 ± 0.6	18.65 ± 1.2	38.92 ± 9.4	121.1 ± 9.6
Control						
mean	**6.84 ± 0.9**	**73.02 ± 4.6**	**4.99 ± 0.8**	**18.52 ± 1.2**	**35.69 ± 9.1**	**122.2 ± 11.0**
2012 drop	6.90 ± 0.9	74.56 ± 4.7	5.15 ± 0.8	18.55 ± 1.1	35.49 ± 9.2	123.4 ± 11.0
2013 drop	6.74 ± 0.9	70.74 ± 3.4	4.76 ± 0.6	18.48 ± 1.2	36.00 ± 8.9	120.3 ± 10.8

*** p<0.001, ** p<0.01, * p<0.05, † p<0.1, when compared to Control ewes (within the column)

4.1.6 Wether fleece data

Among the wethers, greasy fleece weight (GFW) was not affected by vaccination (p>0.05), with off-shears liveweight tending to affect GFW (p<0.1) and off-shears BCS had an effect (p<0.05). Table 5, reports all fleece characteristics from the wethers, collected only in year 1, 2014. No factors in the model had significant effects on yield (p<0.05). Clean fleece weight was not affected by vaccination (p>0.05), but off-shears BCS (p<0.05) did have an effect.

Fibre diameter was not significantly affected by vaccination (p>0.05) and nor did any other factor (p>0.05). Staple strength was not affected by vaccination (p>0.05). Off-shears BCS did have a significant effect of staple strength (p<0.05). Staple length was not affected by vaccination (p>0.05) or any other factor.

Table 5. Fleece and wool quality raw values (± s.d.) collected from the wether hoggets in 2014, reported for vaccinated (Leptin) and control groups

Group	GFW (kg)	Yield (%)	CFW (kg)	FD (µm)	SS (N/Ktex)	SL (mm)
Leptin vaccinates mean	6.88 ± 1.0	72.99 ± 4.6	5.02 ± 0.8	18.62 ± 1.2	36.92 ± 8.3	122.4 ± 10.4
Control mean	6.84 ± 0.9	73.02 ± 4.6	4.99 ± 0.8	18.52 ± 1.2	35.69 ± 9.1	122.2 ± 11.0

*** p<0.001, ** p<0.01, * p<0.05, † p<0.1, when compared to Control ewes (within the column)

4.1.7 Laboratory titre assays

Antibodies against leptin could be detected but the titres were very low just above background hence suggesting the vaccine effectiveness was limited.

4.2 Year two

In the second year of the trial a new adjuvant was used to stimulate a greater immune response.

4.2.1 Ewe reproduction

Fertility status was not affected by the vaccine used in year two (p>0.05) or mating weight (p>0.05). Effect of condition score at mating however was significant (p<0.01). The mean weight of vaccinated ewes at mating was 58.8 kg compared to control ewes (58.5 kg). Body condition score of vaccinated ewes was 3.05, while control ewes were 3.11. The fertility status of the vaccinated ewes was 93%, compared to the control ewes (91%). Table 6 reports on the reproduction results for the second year of the trial, undertaken in 2015.

The number of lambs scanned was the same between the vaccinated and control ewes (p>0.05, Table 6). While weight at mating was a significant factor (p<0.01), ewe body condition score was not (p>0.05).

The number of lambs born tended to be correlated to weight at mating (p<0.1), but not by BCS (p>0.05) and nor by vaccination (p>0.05, Table 6). The number of lambs weaned was not affected by vaccination (p>0.05) or BCS at mid-pregnancy (p>0.05), but weight at mid-pregnancy was significant (p<0.05). At mid-pregnancy the vaccinated ewes weighed 63.4 kg compared to the control ewes (64.1 kg). The vaccinated ewes gave birth to 1.30 lambs per pregnant ewes, which was similar to the control ewes (1.32).

Survival of lambs to weaning was not affected by the vaccination of the dams ($p>0.05$, Table 6), while survival tended to be correlated with sex ($p=0.06$) and birth type ($p=0.055$). The mean lamb mortality of the vaccinate group was 16% compared to the control group (21%).

Ewe weight at lambing tended to correlated with number of lambs weaned ($p<0.1$), while BCS at lambing was significant ($p<0.001$) but vaccination had no effect ($p>0.05$, Table 6. At lambing, the weight and body condition score of the vaccinated ewes was 82.1 and 3.25, while the control ewes weighed 82.7 kg and were BCS 3.3. The number of lambs weaned by the vaccinated group was 1.06 per ewe mated, compared to the control ewes (1.04).

The total weight of lambs weaned was not affected by vaccination ($p>0.05$, Table 6). The total weight of lamb weaned by the vaccinated ewes was 26.6 ± 15.9 kg, compared to the control group (26.8 ± 15.9 kg).

4.2.2 Ewe growth and condition

In year two, weight at mating was not different between the vaccinated and control groups ($p>0.05$). Year of birth (drop) was significant ($p<0.001$). Both drop ($p>0.05$) and vaccination treatment had no effect on BCS ($p>0.05$) at mating. Table 6 reports on ewe weight throughout the second year of the study.

At mid-pregnancy there were no effects of vaccination on weight of the ewes ($p>0.05$), while pregnancy status and drop were significant factors ($p<0.001$). There tended to be an interaction between vaccination group and fertility ($p<0.1$, Fig. 1), where non-pregnant control ewes were lighter. There was also a significant interaction between fertility and drop ($p<0.05$, Fig. 2), where non-pregnant 2012 drop ewes were lighter in weight than all other ewes. Body condition score at mid-pregnancy was affected by fertility ($p<0.001$) and drop ($p<0.01$), but not the vaccination ($p>0.05$).

Table 6. Live weight (kg), body condition score (BCS) raw means (± s.d.) collected at mating (M), mid-pregnancy (MP), pre-lambing (L) and off-shears (O/S) and reproduction raw means (± s.d.) for fertility (ewes pregnant per ewe joined), fecundity (lambs scanned per ewe joined), lambs born per ewe lambing (LB), lamb mortality, number of lambs weaned per ewe joined (NLW) and total weight of lamb weaned (TWWT) for year 2015, reported for vaccinated (Leptin) and control ewes and for the dams' year of birth (Drop)

Group	M weight (kg)	M BCS	MP weight (kg)	MP BCS	L weight (kg)	L BCS	O/S weight (kg)	O/S BCS	Fertility (%)	Sc (%)	LB	Lamb mortality (%)	NLW	TWWT (kg)
Leptin vaccinates mean (± s.d.)	**57.89 ± 6.50**	**3.05 ± 0.45**	**63.40 ± 5.99**	**3.14 ± 0.32**	**82.11 ± 7.00**	**3.25 ± 0.30**	**68.84 ± 8.93**	**2.91 ± 0.50****	**93%**	**1.37 ± 0.64**	**1.30 ± 0.62**	**16%**	**106%**	**26.58 ± 15.90**
2012 drop	59.74 ± 6.33	3.14 ± 0.42	64.78 ± 6.33	3.18 ± 0.33	63.30 ± 7.02	3.27 ± 0.34	69.16 ± 10.00	2.90 ± 0.54	63/68 (93%)	1.41	1.37	17%	107%	26.44 ± 16.67
2013 drop	55.32 ± 5.88	2.93 ± 0.46	61.49 ± 4.94	3.09 ± 0.30	80.55 ± 6.74	3.22 ± 0.23	68.40 ± 7.27	2.92 ± 0.46	46/49 (94%)	1.27	1.20	14%	104%	26.77 ± 14.94
Control mean (± s.d.)	**58.52 ± 6.64**	**3.11 ± 0.36**	**64.14 ± 6.30**	**3.19 ± 0.30**	**82.7 ± 7.12**	**3.30 ± 0.30**	**70.17 ± 8.35**	**3.09 ± 0.52**	**91%**	**1.35 ± 0.64**	**1.32 ± 0.69**	**21%**	**104%**	**26.75 ± 15.85**
2012 drop	59.76 ± 7.39	3.08 ± 0.41	65.26 ± 7.06	3.24 ± 0.34	84.21 ± 7.83	3.33 ± 0.32	69.79 ± 7.97	3.13 ± 0.53	55/61 (90%)	1.43	1.38	19%	110%	27.66 ± 15.53
2013 drop	56.95 ± 5.21	3.14 ± 0.29	62.73 ± 4.88	3.13 ± 0.23	80.91 ± 5.77	3.27 ± 0.28	70.67 ± 8.86	3.04 ± 0.51	44/48 (92%)	1.29	1.25	25%	96%	25.60 ± 16.33

*** $p<0.001$, ** $p<0.01$, * $p<0.05$, † $p<0.1$, when compared to Control ewes (within the column)

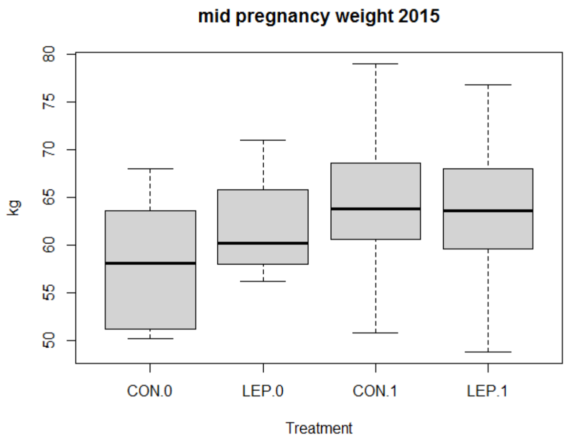

Fig. 1. Weight of ewes (kg) at mid-pregnancy for the treatment group ewes (LEP) and non-vaccinated control ewes (CON) and pregnancy status (0= non-pregnant; 1= pregnant).

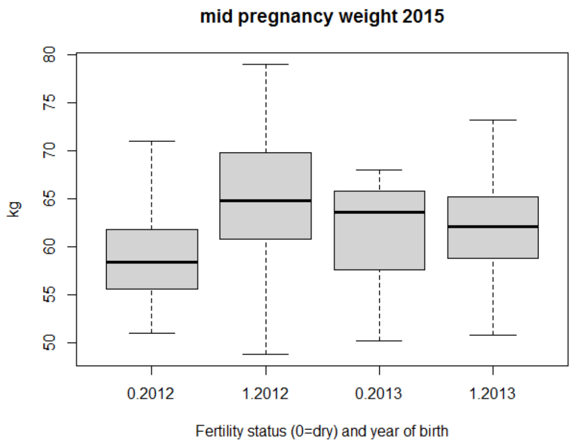

Fig. 2. Weight of ewes (kg) at mid-pregnancy for ewe drop (2012 or 2013 drop ewes) and pregnancy status (0= non-pregnant; 1= pregnant).

At lambing, ewe weight was affected by litter size ($p<0.001$) and drop ($p<0.01$). The vaccination group of ewes were not different in weight compared to the control ewes ($p>0.05$, Table 6). Body condition score was significantly affected by litter size ($p<0.001$) and by drop ($p<0.05$), but not by the vaccination ($p>0.05$, Table 6).

Off-shears weight was significantly affected by the number of lambs weaned ($p<0.001$). Vaccinated ewes did not affect off-shears weight ($p>0.05$, Table 6). Body condition score, assessed off-shears was significantly affected by the vaccination of the ewes ($p<0.01$, Table 6) and by the number of lambs weaned ($p<0.001$).

4.2.3 Lamb data

Birth weight was not significantly affected by the vaccination of dams ($p>0.05$). Lambs from vaccinated dams weighed 4.42 kg (± 1.13 kg) compared to lambs from control dams (4.28 ± 1.14 kg).

Birth weight was affected by sex (p<0.01) and birth type (p<0.001). Table 7 reports on lamb weight at birth and at weaning.

Lamb wean weight was not significantly affected by the vaccination of the dams, although the full model tended to differ from the null model (p=<0.1). Lambs from vaccinated dams weighed 25.1 kg at weaning (± 5.1 kg), compared to lambs from control dams (25.7 ± 5.0 kg). Birth type was a significant factor affecting wean weight (p<0.001), as was sex (p<0.01).

Lamb growth rate to weaning was not significantly affected by vaccination of the dams (p>0.05). Birth type (p<0.001) and sex (p<0.01) were significant factors affecting lamb growth rate to weaning.

Table 7. Weight at birth and at weaning raw values (kg ± s.d.) collected in 2015, reported for vaccinated (Leptin) and control groups

2015 drop lambs	Birth weight	Wean weight
Leptin vaccinate group	4.28 ± 1.14	25.74 ± 5.03
Control group	4.42 ± 1.13	25.10 ± 5.06
2012 drop dams	4.43 ± 1.15	24.86 ± 4.93
2013 drop dams	4.23 ± 1.11	26.25 ± 5.12

*** p<0.001, ** p<0.01, * p<0.05, † p<0.1, when compared to Control ewes (within the column)

4.2.4 Ewe fleece data

Greasy fleece weight was not affected by the vaccination (p>0.05), while off shears live weight (p<0.05) and off-shears BCS (p<0.01) had a significant effect. Yield was not affected by any factor in the model. Clean fleece weight was affected by off-shears weight (p<0.05) and BCS (p<0.05) and vaccination had no impact (p>0.05). Table 8 reports on the fleece characteristics for the ewes in year 2 of the study.

Fibre diameter varied with off-shears live weight (p<0.05) and not vaccination (p>0.05). Staple strength was affected by the number of lambs weaned (p<0.01) and off-shears liveweight (p<0.05) while vaccination had no effect (p>0.05). There were no significant factors affecting staple length, including vaccination (p>0.05).

4.2.5 Laboratory titre assays

Antibodies against leptin could be detected but the titres were very low just above background hence suggesting the vaccine effectiveness was limited.

Table 8. Fleece and wool quality raw values (± s.d.) collected from the ewes in 2014, reported for vaccinated (Leptin) and control groups

Group	GFW (kg)	Yield (%)	CFW (kg)	FD (µm)	SS (N/Ktex)	SL (mm)
Leptin vaccinates mean	**5.90 ± 0.8**	**74.81 ± 4.1**	**4.42 ± 0.7**	**18.54 ± 1.2**	**36.31 ± 12.1**	**108.1 ± 10.3**
2012 drop	5.80 ± 0.7	74.82 ± 4.4	4.35 ± 0.7	18.61 ± 1.2	36.49 ± 12.9	106.3 ± 9.5
2013 drop	6.03 ± 0.8	74.81 ± 3.7	4.51 ± 0.7	18.46 ± 1.3	36.08 ± 11.0	110.5 ± 10.7
	5.87 ± 0.9	75.50 ± 4.6	4.42 ± 0.7	18.70 ± 1.2	36.78 ± 10.7	106.6 ± 10.0
Control mean	**5.77 ± 1.0**	**75.21 ± 4.5**	**4.34 ± 0.7**	**18.71 ± 1.2**	**37.52 ± 11.3**	**105.7 ± 10.8**
2012 drop	6.00 ± 0.8	75.89 ± 4.7	4.54 ± 0.6	18.69 ± 1.2	35.78 ± 9.8	107.8 ± 8.7
2013 drop	5.90 ± 0.8	74.81 ± 4.1	4.42 ± 0.7	18.54 ± 1.2	36.31 ± 12.1	108.1 ± 10.3

*** p<0.001, ** p<0.01, * p<0.05, † p<0.1, when compared to Control ewes (within the column)

5 Discussion

The results of this study do not align with those of the pilot study. Since a limited response was observed in the antibody titres, it is little surprise that no significant phenotypic relationships were found for reproduction. Hence, those results indicating a significant difference between the vaccinated and control animals should be treated with caution.

In year one, three notable effects were found between the vaccinated and control groups and each were interrelated. The number of lambs weaned tended to be lower in the vaccinated ewes, most likely due to the slightly elevated loss of scanned foeti by lambing and lamb mortality rate. Since birth weight tended to be lighter in vaccinated lambs, this is a likely explanation for the higher mortality and lower weaning rate. Buchbinder *et al.* (2001) has observed that fetal leptin concentration was negatively correlated with uterine blood flow, placental weight and fetal weight, but that maternal leptin concentrations did not correlate to fetal concentrations. The findings of Buchbinder *et al.* (2001) do not, however, fit our observations as fetal leptin was not measured.

The development of vaccines against endocrine hormones to increase weaning rate in flocks, has centred on increasing ovulation rate (Juengel *et al.*, 2013; O'Shea *et al.*, 1982; Scaramuzzi *et al.*, 1982). These vaccines have successfully increased ovulation rates but not consistently increased the number of lambs born or weaned, and some vaccines perform better in some maternal breeds compared to Merinos (Scaramuzzi *et al.*, 1983). It is important that all facets of reproduction are improved for the ethical advancement of sheep farming systems (Martin and Kadokawa, 2006). These advancements must lead to, but not be limited by economic benefits.

In year two, there was a tendency for control ewes to be lighter in weight at mid-pregnancy than non-pregnant leptin vaccinated ewes and all pregnant ewes. Since the effect of vaccination was not a significant factor affecting weight at mid-pregnancy, while the pregnancy status was, it is most likely that the interaction reported is due largely to pregnancy status.

By weaning in the second year, the body condition score of the vaccinated ewes was significantly leaner than the control ewes. Vaccinated ewes were about 0.2 condition score units leaner. Given no differences in mean litter size, but a small not-significant ($p>0.05$) improvement in lamb survival was found, the small numerical increase in weaning rate is the likely explanation for the observed difference in condition score.

Investigation into the possible effects of the vaccine on carcase attributes was planned for this study, since reasonable correlations exist between plasma leptin concentration and carcase fatness (Altmann *et al.*, 2005; Altmann *et al.*, 2006). However, a lack of difference in the weight and body condition of the wethers between the vaccinated and control groups led to the cancellation of this aspect of the study.

Targeting hormones whose actions are largely limited to affecting ovulation are a sensible and obvious choice. Finding a vaccine to improve lamb survival seems much less likely, due to the multitude of factors affecting a successful rearing. The field of immunisation against hormones implicated or directly associated with reproduction is a challenging one. Most successful vaccines have demonstrated significant increases in ovulation rates that do not transfer to significant increases in the number of lambs born or reared. This is why the leptin vaccine is attractive, as the pilot study indicated some remarkable responses.

Future work in this field will require on-going investigation. Leptin is a pluripotent hormone, exhibiting relationships with muscle and visceral fat thermogenesis (Henry *et al.*, 2008), implying some potential role for neonatal survival under cold weather conditions. Low cost studies are paramount and investigations must commence with determining the ability to increase ovulation rate or embryo implantation. These reproductive measures can be easily measured via trans-rectal ultrasound, for example sees van Lier *et al.* (2017). More needs to be understood why the vaccine did not create an immune response, as it had done previously (McFarlane *et al.*, 2008). From other studies, the response to vaccines within bloodlines, flocks or breeds are interesting; with some evidence suggesting bloodlines may have some part in the size of the responses observed (Croker *et al.*, 1982; Wilkins, 1997). This is not likely to be the cause for a lack of immune response in the present study, but needs to be part of future investigation.

6 Conclusions/recommendations

The conclusions to be taken from this study are that vaccine efficacy needs to be improved; that the vaccine may work in some bloodlines of sheep more than others or; that the anti-leptin vaccine in its current form does not have the potential to affect ewe reproduction. The authors recommend that plot-scale, low investment studies continue in the development of the vaccine because of the potential this may have to improve sheep production in Australia.

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