Final report

Improving twin lamb survival through melatonin implants: commercial validation

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Abstract

High incidences of lamb mortality cost the industry approximately $450 million per year, with up to 70% of these deaths associated with, or directly attributed to, intra-partum hypoxia, particularly in twin lambs. The objective of this study was to utilise melatonin (commercially available as Regulin®) to increase twin lamb survival. Melatonin is a neuroprotectant and potent antioxidant, which crosses the placenta.

A total of 9,167 twin-bearing ewes were implanted with a single Regulin® implant between day 80 and 100 of gestation, to increase melatonin levels during fetal development and parturition. Ewes were sourced from 17 commercial properties located in multiple agro-ecological zones, accounting for ewe body condition, feed availability and lambing conditions. Control and melatonin treated ewes were only managed separately from lambing paddock allocation, which was standardised for replication, until lamb marking to determine the efficacy of this treatment.

Across the 17 properties, average marking rate was 74% for melatonin treated ewes and 76% for untreated control ewes. Based on these results it was determined that optimal ewe condition at implanting, high quality and abundant feed availability and mild temperatures during lambing resulted in high incidences of lamb survival, which effectively minimised any beneficial effects of melatonin. From this trial, there were no benefits observed in twin lamb survival to marking when the ewes were supplemented with melatonin during mid-late pregnancy. Based on this, future trials are required in subsequent years on sites where environmental conditions and feed availability are likely to impair fetal growth and reduce lamb survival. Interestingly, on three producer sites where weaning weights were recorded, lambs from melatonin treated ewes were on average 1 kg heavier, a finding which also requires further investigation.

Executive summary

Background and Objectives: Each year, $450 million of potential revenue is lost due to high incidences of lamb mortality (Lane *et al.* 2015). Up to 70% of the lamb deaths occur within the first three days post birth (Brien *et al.* 2009; Hinch and Brien 2014) and many can be attributed to intra-partum hypoxia, particularly in twin lambs (Dutra *et al.* 2007). Previous research has demonstrated that supplementing twin-bearing ewes with melatonin (orally) or via a subcutaneous melatonin implant (Regulin®) significantly increased twin lamb survival (14% increase), with the survival of the second born twin most impacted. Melatonin is an effective neuroprotectant and potent antioxidant, crosses the placenta, and has the capacity to promote fetal growth and reduce the impacts of intra-partum hypoxia. The objective of this study was, therefore, to build on previous, positive results, and demonstrate whether implanting twin-bearing ewes with Regulin® would increase twin lamb survival under commercial conditions.

Methodology: Working within 17 commercial properties across 9 different regions across South Australia and Victoria (Upper North SA, North and Barossa SA, South East SA, Murray Mallee region in SA, central SA (pastoral property), the mallee region of Northern Victoria, South-west and west Victoria). A total of 9167 twin-bearing ewes were implanted with melatonin at a single time point between days 70 and 90 from the commencement of joining. Producers were selected based on their ability to identify twin bearing ewes using pregnancy scanning, manage twin bearing ewes separately or identify using EID, provide at least four lambing paddocks (two per treatment: control versus melatonin), determine lamb survival to at least marking, and preferably weaning. Ewes were implanted shortly after pregnancy scanning, with each ewe received a single sub-cutaneous implant of a slow release melatonin capsule (Regulin ®) at the base of the ear.

Results/key findings: The data demonstrated that across 17 farms, a single melatonin (Regulin®) implant between day 70 and 100 gestation did not significantly increase twin lamb survival. This was consistent across a range of lambing times, with at least one producer lambing per month from April to October, and across multiple breeds (Dorper, Suffolk, Merino, First cross and Merino). As reported by the producers, the conditions throughout gestation and over lambing were extremely favourable to ewe condition and lamb survival. Therefore, they experienced some of the highest lamb survival figures in the last 5 years, with four producers recording over 90% survival in their control group. Interestingly, and consistent with recent European data, a positive effect of melatonin (Regulin®) on lamb weaning weights was observed. Across eight different mobs, lambs born to Regulin® treated ewes were 800 grams heavier at weaning compared with the control lambs.

Benefits to industry. Based on previous, positive findings, this study was necessary to quantify the impact of melatonin on twin lamb survival within commercial systems, to justify or otherwise, the adoption of a simple strategy to improve twin lamb survival. By covering multiple regions across South Australia and Victoria, with replication within, and a variety of joining times this creates a robust study to be applicable across majority of producers. At this stage, due to the high cost of melatonin ($7 per animal) there does not appear to be a benefit to using Regulin® at day 70 to 90 of pregnancy to increase twin lamb survival. Even with a potential increase of 800g per lamb the cost of the implant is not justified.

Future research and recommendations. Based on the results from this project, it is recommended to repeat this work utilising the same producer sites for another two lambing seasons. This would allow for the effects of melatonin to be determined across consecutive seasons, strengthening the analysis and possible economic benefits of melatonin. In addition, a closer look at the relationship between

melatonin and weaning weight should be considered in future work, which would likely involve determining the impacts on milk production.

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1. Background

1.1 Project Overview

The project 'Increasing twin lamb survival through melatonin implants' was designed to determine the impact of melatonin supplementation on twin lamb survival across various breeds, lambing times and regions. Results from an existing MLA funded project, novel strategies to increase the weaning rate of the national flock (L.LSM.0015), reported a significant increase in twin lamb survival following maternal supplementation with melatonin. In an intensive experiment conducted at Turretfield Research Centre, (Rosedale, South Australia, Australia) supplementing pregnant ewes with melatonin (oral and Regulin®) during the second half of pregnancy, increased twin lamb survival by 14% (28 more twin lambs weaned per 100 twin bearing ewes). Importantly, maternal melatonin supplementation increased the survival of second born twins, as well as those which experienced an extended parturition. These results were supported by a field study conducted at the Minnipa Research Centre (Minnipa, South Australia, Australia), with maternal melatonin supplementation (delivered via Regulin® implants) increasing survival of twin lambs by 14% in extensive research conditions. These results suggest that melatonin improves twin lamb survival. This can be attributed to melatonin's neuroprotective properties, which increase viability and vigour and reduce the risk of mortality from difficult and prolonged births. Based on previous research, it is proposed that melatonin implants will reduce the welfare and economic costs, which result from lamb deaths associated with dystocia. This is an important hypothesis as it has recently been suggested that dystocia is responsible, directly or indirectly, for the death of 6.9 million lambs per year (Jacobson *et al.* 2020). Further benefits of maternal melatonin supplementation have been observed, with an increase in deposition of brown adipose tissue in neonatal lambs, which can be linked to an increase in their ability to thermoregulate and survive during periods of harsh environmental conditions (Flinn *et al.* 2020b). The outcomes of previous research indicates that implanting twin bearing ewes with melatonin can increase lamb survival by 14 to 15% (Flinn *et al.* 2020a; Flinn *et al.* 2020c), which equates to a return on investment of approximately 5 to 1, as well as a significant step towards increasing the weaning rate of the national flock.

This project was stimulated by interest expressed from producers in the results to date from previous trials that showed positive outcomes of the use of Regulin® in increasing twin lamb survival. This interest from producers was around how Regulin® could be applied into their management systems, and if there are the same benefits seen in commercial setting across a range of breeds, lambing times and production zones.

The purpose of this project was to commercially validate the use of Regulin® to improve twin-lamb survival. The results included within this final report will be used to advise producers on the effectiveness of Regulin® implantation during mid-gestation to improve twin lamb survival. Overall, the project covered multiple agro-ecological zones, a range of breeds, and joining at different times of the year. Feed availability from time of implant through to lambing was considered, as well as ewe body condition scores and the conditions over the lambing period to determine if there were specific circumstances in which the Regulin® implant would be of greatest benefit. By undertaking this research on 17 different properties, including one pastoral property this trial has generated a robust dataset that will be easily digestible to producers across South Australia, and Victoria.

2. Objectives

This project aimed to achieve the following outputs:

1. Establish the benefits of implanting twin bearing ewes with melatonin implants on lamb survival under commercial conditions, across a range of production systems, climatic zones and breeds.
2. Quantify the economic benefits of using melatonin implants to improve lamb survival under commercial conditions
3. Provide a comprehensive package of information to producers, producer groups and consultants describing the impact of melatonin implants on twin lamb survival under commercial conditions, and across a range of production systems. Including
    a. Producer focussed short communication papers
    b. Case studies made available to producer groups and advisors
    c. Presentation of project outcomes at a minimum of one industry event

Project delivery of outcomes

1. Detailed below in the results section, this project established that the benefits of implanting Regulin® during mid-gestation to improve twin lamb survival are not outweighed by the cost of implants. In some specific circumstance, where weather conditions and feed availability were sub-optimal Regulin® was able to improve twin lamb survival. Overall, across almost all agro-ecological zones and production systems producers reported exceptional feed availability throughout mid/late gestation and mild lambing conditions which naturally promoted lamb survival.
2. Based on the results from this project, overall there are no economic benefits of using melatonin implants to improve lamb survival under commercial conditions for the 2022 lambing season. Specifically, one producer in Orroroo saw a notable increase in lamb survival from the Regulin® treated ewes, and in this specific case the increased lamb survival enabled the cost of the implant to be worthwhile.
3. In conjunction with this final report, a comprehensive package of information for dissemination to producers, producer groups and consultants is provided.
    a. A producer fact sheet outlining the overall results from the project
    b. Videos from presentations given throughout the duration of the project for the Sheep Reproduction Strategic Partnership (SRSP) and Lambs Alive
    c. Producer case studies from each of the sites enrolled in this project are included, detailing number of animals, type and use of land, and survival rates
    d. Presentation completed at the 2022 SRSP detailing the results to date of this project

3. Methodology

The project commenced in February 2021 and ended in December 2022. In total, 17 commercial flock owners and just over 9000 twin bearing ewes were included, under University of Adelaide Animal Ethics approval S-2021-006. Flocks which lambed in autumn and winter/spring were included in the project, and the project ran over one lambing season on each site. As outlined in the table below, flocks were included across a range of breeds (Merino, Maternal, Terminal), agro-ecological sheep producing zones (including at least one pastoral flock), season of lambing and ewe parity groups (Figure 1).

Figure 1: Map showing location and the breeds of sheep across the properties involved in the trial.

Ewes were scanned for pregnancy status and litter size, with twin bearing ewes randomly allocated to receive either a melatonin implant on approximately day 90 of pregnancy or act as non-implanted controls. Implanted and non-implanted (control) ewes were managed as a single mob until shortly before lambing.

The rearing status was determined where possible. On sites where accurate mothering-up data could be achieved implanted ewes were managed with control ewes. However, when only wet/dry scoring of udders at marking and marking rates per scanned ewes were achievable then implant and control ewes were managed in separate paddocks. Ewes lambed in small paddocks / mobs, to allow replication across treatment within each site.

Data collection differed between production sites, depending on the management system. However, core data was the same across all sites, with additional data collected where possible.

Core Data: Number of pregnant ewes on trial, number of ewes which lamb, number of ewes which rear a lamb (wet/dry), number of lambs marked and weaned per ewe scanned pregnant, environmental conditions during lambing (BoM data), details of management system (i.e. feed provided, lambing paddock type).

Additional Data: Ewe body condition score, parity, reproductive history, number of lambs born, marked and weaned. Causes of lamb death and timing. Climate data during the lambing period (BoM, and where possible the use of onsite weather station or other onsite recording systems provided by the project team).

Based on the results presented in this final report, a cost benefit analysis was completed to determine the minimum survival increase necessary for a positive return on investment from the purchase of Regulin®.

4. Results

4.1 Lamb survival to marking

Table 1: Lamb survival to marking calculated as potential foetuses scanned from actual lambs present at lamb marking

Breed	Location (Property)	Total Ewes	Regulin® Ewes (n)	Regulin® Lambs (n)	Survival (%)	Control Ewes (n)	Control Lambs (n)	Survival (%)
2021								
Merino early	1	202	100	112	56	102	131	64
Merino late	1	207	104	125	60	103	134	65
Merino	2	488	245	391	80	243	426	88
First Cross	3	495	248	336	68	247	336	68
Suffolk AI	4	121	62	125	101	59	128	108
Suffolk natural	4	415	108	205	95	109	210	96
Suffolk lamb	4	125	64	74	58	61	67	55
Suffolk	5	255	129	230	89	126	214	85
2022								
First Cross	6	646	306	515	84	340	564	83
Merino	7	360	172	214	62	188	262	70
Suffolk x Merino	8	418	209	377	90	209	377	90
Merino	9	165	85	139	82	80	120	75
Merino	9	201	102	168	82	99	171	86
Merino lamb	9	84	44	65	74	40	57	71
Merino	10	381	187	315	84	194	335	86
Merino early	11	274	139	208	75	135	210	78
Merino late	11	271	136	209	77	135	221	82
Composite Mature	12	637	316	557	88	321	575	90
Composite Lamb	12	400	190	249	66	393	533	68
Composite Triplet	12	216	60	114	63	60	120	67

Merino	13	189	94	155	82	95	130	68
Merino	13	163	78	118	76	85	134	79
Merino	14	524	255	335	66	269	412	77
First Cross	15	526	269	315	59	257	355	69
Merino	15	778	390	335	70	388	340	69
Merino	16	190	95	79	42	95	87	46
Merino	16	247	155	199	64	92	124	67
Dorper	17	50	23	29	63	27	36	67
Dorper	17	41	21	23	55	20	28	70

Overall 9,069 ewes were included in the final dataset as number of ewes present at lambing marking. On average lamb survival to marking from Regulin® treated ewes was 73% and lamb survival to marking for control ewes was 75%. This data was collected across seventeen producer sites and 29 different cohorts within these sites.

4.1.1 Pastoral Property data collection

One pastoral property, located with the Australian pastoral zone in central South Australia was used for this trial to assess the efficacy of this treatment in pastoral regions. The survival is based on the number of single and twin bearing ewes allocated to the paddock it was not feasible to separate ewes according to fetal number. An additional two paddocks were included in the trial initially (see tables 2 and 3 for clarity) under the same design, however based on final number of ewes in the paddocks at marking there was significant mixing of animals and therefore unreliable results. There is great value in repeating this trial again in 2023, and are in the process of arranging this with the producer.

Table 2: Paddock allocations for Regulin® and Control ewes on a pastoral property and survival based on potential lambs

Paddock Name	Treatment	single-bearing ewes	twin-bearing ewes	Total ewes	potential	survival
Paddock 1	Control	167	103	270	373	87
Paddock 2	Implant	167	103	270	373	63
Paddock 3	Control	281	103	384	487	69
Paddock 4	Implant	281	103	384	487	70

Table 3: Marking rates based on number of ewes present in each paddock at marking

Paddock Name	Treatment	Ewes present at marking	lambs present at marking	%
Paddock 1	Control	301	324	108
Paddock 2	Implant	252	234	93
Paddock 3	Control	390	335	86
Paddock 4	Implant	388	340	88

4.2 Lamb weaning weights

An unexpected finding from this project was the increase in average weaning weight of lambs born to Regulin® treated ewes. On average Regulin® lambs were 800 grams heavier at weaning compared with lambs born to the control ewes (Table 4). This collection of data was additional to that of the survival to marking data, and was taken when producers had the capacity to provide individual or group average weaning weights.

Table 4: Difference in lamb weaning weights between lambs born from Regulin® treated or control ewes

		Lamb weaning weight (kg per lamb)		
Breed	Property	Regulin®	Control	Difference
Merino	Early Lambing property 1 (2021)	25.7	25.3	0.4 kg
Merino	Late Lambing property 1 (2021)	22.9	22.3	0.6 kg
Suffolk	Property 5 (2021)	34.4	33.7	0.7 kg
Merino Cross	Ewe lambs property 6 (2022)	32.5	31.5	1 kg
Merino Cross	Wether lambs property 6(2022)	38	38	0 kg
Merino	property 10 (2022)	22	21.2	0.8 kg
Merino	Ewe lambs property 7(2022)	25	24	1 kg
Merino	Wether lambs property 7 (2022)	27	25	2kg

4.3 Cost Benefit Analysis

A cost benefit analysis was performed to determine the percent increase in lamb survival required to outset the cost of the melatonin implants. Excluding the additional cost of labour and irrespective of lamb price a 4% increase in lamb survival sees a positive return in income. However, this increase needs to be at 5% or greater before a notable income benefit is seen.

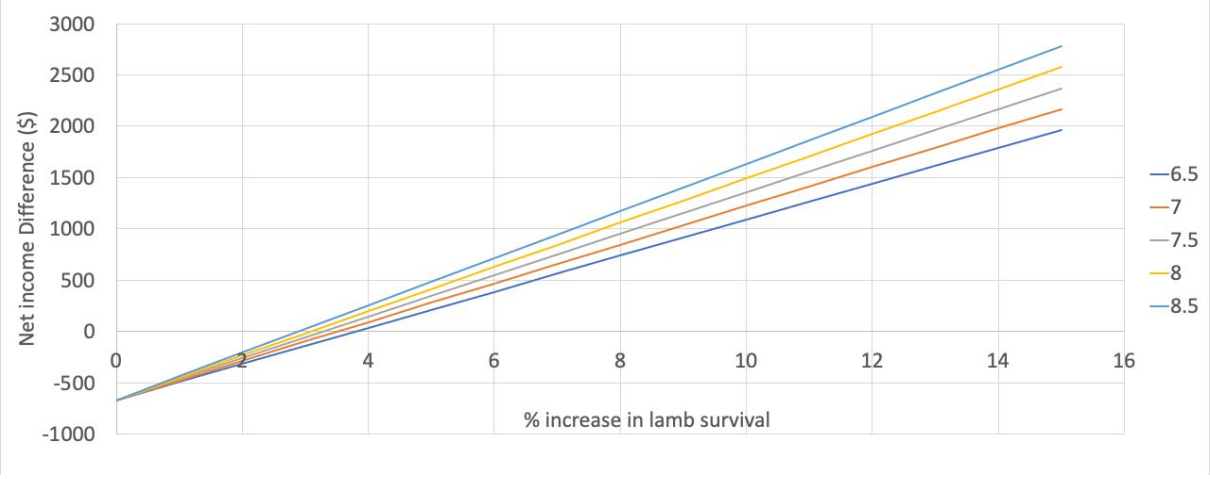

Figure 2: Income benefit from increased lamb survival resulting from Regulin® implant. Based on $ per kg of carcass weight; $6.5, $7, $7.5, $8, or $8.5 per kg.

This was determined using the following assumptions:

Mob size of 100 ewes. 1 lamb per ewe (for the tipping point of genuine survival increase effects)

Additional labour at 10 c per ewe
Lamb live weight at sale -60kg
Lamb dressing percentage at sale- 45%
Regulin® cost at $6.60 + cost of applicator depreciated across 2000 doses ($0.0167)

Table 5: Net income difference (additional lamb sales minus cost of Regulin®) Modelling an increase in lamb survival from 90-100% in 1% increases and at carcass value from $6.50-8.50

		NET INCOME DIFFERENCE				
		$/kg carcase weight				
		6.5	**7**	7.5	8	8.5
% Increase in lamb survival	0	-670	**-670**	-670	-670	-670
	1	-490.75	**-477.25**	-463.75	-450.25	-436.75
	2	-315.25	**-288.25**	-261.25	-234.25	-207.25
	3	-139.75	**-99.25**	-58.75	-18.25	22.25
	4	35.75	**89.75**	143.75	197.75	251.75
	5	211.25	**278.75**	346.25	413.75	481.25
	6	386.75	**467.75**	548.75	629.75	710.75
	7	562.25	**656.75**	751.25	845.75	940.25
	8	737.75	**845.75**	953.75	1061.75	1169.75
	9	913.25	**1034.75**	1156.25	1277.75	1399.25
	10	1088.75	**1223.75**	1358.75	1493.75	1628.75
	11	1264.25	**1412.75**	1561.25	1709.75	1858.25
	12	1439.75	**1601.75**	1763.75	1925.75	2087.75
	13	1615.25	**1790.75**	1966.25	2141.75	2317.25
	14	1790.75	**1979.75**	2168.75	2357.75	2546.75
	15	1966.25	**2168.75**	2371.25	2573.75	2776.25

Table 6: Cost Benefit ratio for the increase in lamb survival from the cost of Regulin® implant

COST:BENEFIT RATIO						
COST	**-666.25**	$/kg carcase weight				
		6.5	**7**	7.5	8	8.5
% Increase in lamb survival	0					
	1	-0.7	**-0.7**	-0.7	-0.7	-0.7
	2	-0.5	**-0.4**	-0.4	-0.4	-0.3
	3	-0.2	**-0.1**	-0.1	0.0	0.0
	4	0.1	**0.1**	0.2	0.3	0.4
	5	0.3	**0.4**	0.5	0.6	0.7
	6	0.6	**0.7**	0.8	0.9	1.1
	7	0.8	**1.0**	1.1	1.3	1.4
	8	1.1	**1.3**	1.4	1.6	1.8
	9	1.4	**1.6**	1.7	1.9	2.1
	10	1.6	**1.8**	2.0	2.2	2.4
	11	1.9	**2.1**	2.3	2.6	2.8
	12	2.2	**2.4**	2.6	2.9	3.1
	13	2.4	**2.7**	3.0	3.2	3.5
	14	2.7	**3.0**	3.3	3.5	3.8
	15	3.0	**3.3**	3.6	3.9	4.2

5. Conclusion

From this project, it can be concluded that the benefits of implanting twin-bearing ewes mid-gestation are influenced heavily by environmental conditions from the time of implant through to lambs. This includes the feed quality and availability as well as the climatic conditions are lambing. As highlighted

throughout this report, some producers reported an increase in twin lamb survival. For some producer sites this increase was not sufficient to offset the cost of the implants. One Suffolk producer reported a 3% increase in twin lamb survival of their ewe lambs, and another Suffolk producer reported a 4% increase in twin lamb survival in the mature mob. Interestingly, both of these producers commenced this trial work in 2021. Comparatively, most other properties enrolled for the 2022 lambing period. For the 2022 lambing season merino ewe lambs in Piangil, Victoria reported a 3% increase in twin lamb survival and both Lameroo and the pastoral property reported a 1% increase in twin lamb survival overall. In these cases, with a Regulin® implant cost of $6.60 per head, this is not economically viable. Conversely, multiple producers reported a 1-3% decrease in twin lamb survival which certainly offsets these other producers. This small range of survival makes it difficult to ascertain whether Regulin®, or the environment is the cause.

It must also be noted that two mixed age merino producer sites reported a 7% and 14% increase in twin lamb survival in Regulin® treated ewes. In both cases, the producers reported ewes in poor condition at time of implant, low feed quality/availability and relatively harsh lambing conditions. This suggests that seasonal conditions will greatly affect the potential of melatonin to increase twin lamb survival.

Our key recommendation is to assess the condition of the ewes and the feed on hand, quality and quantity, at the time of implant. Optimal time of implant is close to pregnancy scanning, or between days 70-90 gestation, depending on labour and time as well. If ewes look well, feed is substantial, and the lambing season is predicted to be mild (as was 2022) the implant may not have additional benefit beyond optimal management. If any of these factors are sub-optimal melatonin implants may prove beneficial. Therefore, it is recommended that future trials are conducted when ewe condition and feed availability are likely to be sub-optimal.

Ultimately, it would have been beneficial to repeat this trial work over the same sites over at least 3-4 years. In this trial various agro-ecological zones, with sufficient differences in lambing times and management were covered; however, the mild weather conditions and exceptional year for feed reduced the possible impact of melatonin. Therefore, focussing additional research on areas where conditions are often harsher, pastoral regions and those outside the Goyder line would see the most benefit from the use of melatonin to increase twin lamb survival.

5.1 Key findings

- Under optimal seasonal conditions, melatonin implants did not improve twin-lamb survival
- On a case-by-case basis, identifying poorer conditioned ewe mobs, poor feed quality/availability, and harsh lambing conditions melatonin did improve twin lamb survival
- Timing the implantation of melatonin to shortly after pregnancy scanning is the optimal time for implanting for management purposes, and aligns within the window of effectiveness determined from our experimental trials
- It would be valuable to repeat this trial at the same locations over an additional 3 lambing periods to truly quantify the relationship between season and melatonin efficacy

5.2 Benefits to industry

It is recommended to implant melatonin in twin-bearing ewes during mid gestation when ewe condition is poor, feed availability and/or quality is low and/or harsh lambing conditions are foreseen.

The results detailed in this project were certainly not anticipated by the research team conducting the trial, broader MLA research teams or by the wider producer community. With such promising experimental results, carrying out this work over 17 producer sites across multiple agro-ecological zones has provided invaluable information around the use of melatonin for twin-lamb survival. This work has provided producers, and MLA, with useful information that will save producers in labour and costs, by ensuring melatonin is used as a tool to mitigate issues that arise due to seasonal conditions.

Interestingly, as stated in numerous producer testimonials, producers in this study believe the conditions impacted the results from this trial. Many of these producers would like the opportunity to perform this trial again when they anticipate a poorer season.

6. Future research and recommendations

This project was successful in covering a broad range of agro-ecological zones, and covering breeds and different management systems. Using a network of producers engaged in research and MLA already was a great way to implement this research, it has also been a useful way of engaging with a broader network of producers through consultants and producer groups. This project has been successful in initiating conversations around the use of melatonin to increase twin-lamb survival, and engaging producers and encouraging research and uptake.

As highlighted throughout, this work should be repeated over multiple seasons. Therefore, future research and development in this area should focus on reliably collecting this data from these same locations (or expanding into more producers) over multiple years.

In terms of practical application, at this stage communication through MLA and with our network of producers, consultants and producer groups developed from this project is key. The main project insight is the importance season has in the efficacy of melatonin implants to improve twin lamb survival.

At this stage, repeating this trial work in harsh environment would be the first stage of development activities. Given the traction this project gained throughout its duration, adoption activities focussed on selecting producer sites struggling with ewe condition, feed or predicted lambing conditions through focussed discussion groups, or through liaising with MLA will be the first step in achieving value from the project findings.

Future research should, therefore, be conducted in the following areas. One, to confirm the benefits of melatonin supplementation of ewes on milk production and lamb growth. Two, to demonstrate whether melatonin supplementation of pregnant ewes improves marking rates when ewes and lambs are managed under sub-optimal conditions, or during periods of low feed and extreme weather.

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