

JANUARY 2023

Sheep reproduction RD&A alert

This sheep reproduction RD&A alert is an initiative of the Sheep Reproduction Strategic Partnership (SRSP).

MLA's Market Information team will be presenting their 2023 forecast for the Australian sheep flock and market in a webinar on **Thursday 16th February at 11.00 am AEDT**. The webinar will present and discuss the forecasts for the flock size, slaughter carcase weights, production and global supply competitors. The team will also present the key macro issues for the sheep industry during 2023. The webinar will include a Q&A session when the team will answer any questions related to the 2023 sheep projections. You can register for the webinar by clicking this [link](#).

Program coordinator

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The SRSP aims to help sheep producers to profitability and sustainably increase lamb production through increasing lamb survival and weaning rates and will coordinate a national approach to improving sheep reproductive performance.

Feature project update

Maximising the reproductive potential of the meat sheep industry by eliminating high-oestrogen clovers, more live lambs on the ground

'Clover disease' was first described in the 1940s. High levels of formononetin, a phytoestrogen, in some older highly-oestrogenic subcover cultivars can cause ewe infertility (both temporary and permanent) and dystocia with subsequent lamb and ewe mortality. The development and adoption of low-formononetin cultivars by the 1990s significantly reduced the incidence of clover disease, however the persistence of highly oestrogenic subterranean clover cultivars which contain unsafe levels of formononetin in their green leaves., continue to negatively impact on the sheep reproductive performance across southern Australia.

Aim

To develop and implement an extension and adoption plan to address oestrogenic induced reproductive failure in meat sheep.

Key findings

Development and circulation of a pasture survey kit and subsequent testing demonstrated that highly oestrogenic subclover pastures are present across southern Australia. Sixty percent of submitted samples contained formononetin levels above that considered safe for livestock (>0.2% of leaf dry weight) with large variation in the level of all three isoflavones that impact on reproductive performance.

The soil seedbank of oestrogenic clovers can be reduced by three years of cropping with in-crop herbicide control of oestrogenic subclover seedlings. Sowing of a low-formononetin cultivar directly following an oestrogenic pasture is likely to result in considerable oestrogenic subclover contamination of future pastures.

This project has increased awareness of the ongoing issue of 'clover disease' among producers through development of resources ([factsheet](#) and [ute guide](#)) and training to facilitate identification of oestrogenic cultivars.

For more information on the oestrogenic clovers project contact Kevin Foster (kevin.foster@uwa.edu.au). For a hard copy of the ute guide, please email Joe Gebbels (jgebbels@mla.com.au).

Review papers

Sperm cryopreservation: current status and future developments

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Reproduction, Fertility and Development, Volume 35(3), January 2023 **OPEN ACCESS**

DOI <https://doi.org/10.1071/RD22219>

Abstract

The cryopreservation of spermatozoa is an important reproductive technology for the preservation of fertility in man and animals. Since the serendipitous discovery of glycerol as an effective cryoprotectant in 1947, sperm cryopreservation has undergone many changes in terms of the freezing methods employed, the rates at which samples are frozen and thawed, and the media used to preserve sperm functionality and DNA integrity. An extensive literature survey has been conducted addressing the cryoprotectants employed for both animal and human semen and the freezing protocols utilised. The results indicate that glycerol remains the dominant cryoprotective agent, usually incorporated into a balanced salt solution containing energy substrates, buffers, osmolytes and protein in the form of human serum albumin (human) or skimmed milk (animal). Realisation that some of the damage observed in cryostored cells involves the generation of reactive oxygen species during the thawing process, has prompted many studies to assess the relative merits of incorporating antioxidants into the cryopreservation media. However, in the absence of systematic comparisons, there is currently no consensus as to which antioxidant combination might be the most effective. Utilising our fundamental understanding of cryodamage to optimise cryopreservation protocols for each species will be important in the future.

Bio stimulation and pheromones in livestock: A review

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Animal Reproduction Science, Volume 248, January 2023

DOI <https://doi.org/10.1016/j.anireprosci.2022.107154>

Abstract

This review examines aspects of the phenomenon of biostimulation in swine, goats, sheep, cattle and deer, to improve the collective knowledge and exploitation of its relevant mechanisms and effects in animal production. The long-term goal is to implement biostimulation strategies that benefit livestock reproduction and production while being both cost-effective and socially acceptable.

Scientific papers

Influence of successive heat waves on the thermoregulatory responses of pregnant and non-pregnant ewes

Messy Hannear de Andrade Pantoja, Jessica Caetano Dias Campos, Douglas Henrique Silva de Almeida, João Alberto Negrão, Gerson Barreto Mourão, Alfredo Manuel Franco Pereira and Cristiane Gonçalves Titto (crisgtitto@usp.br)

Journal of Thermal Biology, Volume 111, January 2023

DOI <https://doi.org/10.1016/j.jtherbio.2022.103420>

Abstract

The frequency of heat waves has increased over the last years, with an impact on animal production and health, including the death of animals. Therefore, the aim of this study was to evaluate the dynamics of thermoregulation and hormonal responses in non-pregnant and pregnant ewes exposed to successive heat waves. Twenty-four non-pregnant and 18 pregnant Santa Ines ewes with black coat color (live weight: 55 ± 9.03 kg; age: 60 months) were used. Weather variables such air temperature, relative humidity, and solar radiation were continuously recorded. The rectal and tympanic temperatures and respiratory rate were measured daily. Serum triiodothyronine (T3) and prolactin were evaluated during the heat wave and thermoneutral periods. The physiological variables were higher under the heat wave conditions and were related to the activation of the thermoregulatory system for maintaining homeothermy ($P < 0.05$). The core body temperature was higher during successive heat waves ($P < 0.05$), as was the tympanic temperature, which are both affected by changes in air temperature ($P < 0.05$). T3 and prolactin levels were not influenced by successive heat waves ($P < 0.05$) and rectal temperature and respiratory rate were highest in non-pregnant ewes ($P < 0.05$). Prolactin was not affected by temperature. The results indicate that the Santa Ines breed overcomes the thermal challenge during a heat wave without showing severe signs of thermal stress regardless of being pregnant or not.

Frequency of feeding during the periconceptual period did not alter reproduction in Merino sheep

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Animal Production Science, Volume 63(1), 3 January 2023 **OPEN ACCESS**

DOI <https://doi.org/10.1071/AN22161>

Abstract

Context: During drought, it is currently recommended to feed complete rations to sheep every second or third day, rather than daily, to reduce labour costs and the incidence of shy feeders. However, the frequency of feeding to ewes in the periconceptual period may influence fertility and fecundity and therefore profitability.

Aims: The study was designed to determine whether the feeding frequency of maintenance energy levels during joining affects reproductive performance and wool production of Merino ewes.

Methods: A group-fed pen study was conducted using two treatments and four replicates. Naturally oestrous-cycling Merino ewes ($n = 800$) were fed barley grain (90%) and wheat straw (10%) from 17 days before ram introduction until Day 30 of a 48-day joining period, either once-daily or on alternate days at maintenance energy levels. Lambing performance was recorded for 505 ewes pregnant by Day 19 of joining. Wool quality was assessed on Day 117 after commencement of joining.

Key results: Clinical acidosis occurred in both treatments, although the rate of mortality was low (4/800). The proportion of shy feeders was not affected ($P = 0.486$) by feeding frequency ($n = 77$, removed from pens). For the remaining ewes, weight loss was reduced by 1 kg ($P = 0.003$) when fed daily. Plasma progesterone concentrations post-mating were reduced ($P < 0.001$) by 18% with alternate-day feeding. Proportions of ewes

mated, returning to service, pregnant or bearing multiple fetuses, lamb survival, lamb weights at marking and ram semen morphology were similar ($P > 0.05$) between feeding groups. Wool fibre diameter, staple strength and yield were similar between treatments. Ewes removed as shy feeders and joined on pasture recorded a 33% lower pregnancy rate but 17% higher rate of multiple fetuses than pen-joined ewes.

Conclusions: Ewes can be fed complete high-grain maintenance rations at 2-day intervals during joining without a reduction in reproductive traits or wool quality compared with daily feeding.

Implications: Producers may save labour with longer feeding intervals; however, greater monitoring and management intervention may be needed to maintain ewe liveweight when fed long term. Impacts in flocks without acidosis, on ram fertility when feeding is prolonged pre-joining, and with different diets require study.

Effect of melatonin and nitric oxide on capacitation and apoptotic changes induced by epidermal growth factor in ram sperm

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Reproduction, Fertility and Development, Volume 35(3), January 2023

DOI <https://doi.org/10.1071/RD22146>

Abstract

Context: Apart from the canonical cAMP-PKA pathway, ram sperm capacitation can be achieved by the MAPK ERK1/2 signalling cascade, activated by epidermal growth factor (EGF).

Aims: This study aims to investigate the effect of melatonin and nitric oxide (NO \cdot) on capacitation and apoptotic-like changes in EGF-capacitated ram spermatozoa.

Methods: In vitro capacitation was induced by EGF in the absence or presence of melatonin (100 pM or 1 μ M). Also, a NO \cdot precursor, L-arginine, or a NOS inhibitor, NG-nitro-L-arginine methyl ester (L-NAME), were added to capacitation media to study the interaction of NO \cdot and melatonin during EGF-capacitation. Sperm functionality parameters (motility, viability, capacitation state), apoptotic markers (caspase activation and DNA damage), NO \cdot levels, and phosphorylated c-Jun N-terminal kinase (JNK) and p38 mitogen-activated protein kinase (assessed by Western blot), were evaluated in swim-up and capacitated samples with EGF.

Key results: NO \cdot levels and the apoptotic-related markers were raised after EGF incubation. Melatonin had a bimodal role on sperm EGF-capacitation, preventing it at high concentration and promoting acrosome reaction at low concentration, but neither of the two concentrations prevented the increase in apoptotic-like markers or NO \cdot levels. However, melatonin at 1 μ M prevented the activation of JNK.

Conclusions: NO \cdot metabolism does not seem to modulate the apoptosis-like events in ram spermatozoa. Melatonin at 1 μ M prevents ram sperm capacitation induced by EGF independently from nitric oxide metabolism, and it could be exerted by limiting the JNK mitogen-activated protein kinase (MAPK) activation.

Implications: This study improves our understanding of the biochemical mechanisms involved in sperm capacitation, and ultimately, fertility.

Ovarian follicle dynamics in ewes treated with intra-vaginal progesterone pessaries. 1. Follicle waves and parameters of the estrous cycle

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Theriogenology, Volume 197, February 2023

DOI <https://doi.org/10.1016/j.theriogenology.2022.11.038>

Abstract

Progesterone treatment for synchrony of estrus is standard in sheep artificial insemination (AI) programs but can be associated with poor outcomes. Potential for improvement exists through a better understanding of the interactions between follicle development, luteal regression, emergence of the ovulatory follicle and timing of estrus. These interactions were examined by comparing progesterone-treated (Day 1 = day of pessary insertion) and naturally cycling ewes (Day 1 = day after estrus) at three times of the year (Autumn, Spring equinox and late Spring). Observations were made from Day 1 until the day of ovulation. Compared with the natural cycle, progesterone treatment (300 mg intra-vaginal pessary for 14 d) reduced the number of follicle waves (2.2 ± 0.18 versus 2.8 ± 0.12 ; $P < 0.05$) and increased the length of the ovulatory wave (8.6 ± 0.45 versus 6.6 ± 0.42 d; $P < 0.05$). The number of follicles per wave, the inter-wave interval and ovulation rate were not affected. However, progesterone treatment induced ($P < 0.05$) an earlier luteolysis (9.7 ± 0.51 versus 15.4 ± 0.49 d after Day 1), an earlier emergence of the ovulatory follicle (7.5 ± 0.48 versus 11.4 ± 0.46 d after Day 1) and an earlier onset of estrus (26.1 ± 2.95 versus 53.3 ± 2.84 h after Day 14). Time of year also influenced the response to progesterone treatment. In Autumn compared with the Spring equinox and late Spring, there was a reduction ($P < 0.05$) in follicle wave number (2.4 ± 0.21 versus 2.5 ± 0.29 versus 3.0 ± 0.20 respectively), follicles per wave (2.6 ± 0.27 versus 3.5 ± 0.25 versus 3.2 ± 0.20 respectively), ovulation rate (1.6 ± 0.12 versus 1.9 ± 0.12 versus 2.0 ± 0.10 respectively) and the inter-wave interval was longer (5.3 ± 0.40 versus 4.0 ± 0.32 versus 3.8 ± 0.27 d respectively; $P < 0.05$). Time of year also influenced ($P < 0.05$) the time of luteolysis (earliest in late Spring), emergence of the ovulatory follicle (earliest in Autumn) and onset of estrus (earliest in Autumn). It is concluded that (1) the effects of progesterone treatment on follicle waves are relatively minor, (2) the effects of treatment on timing of luteolysis, emergence of the ovulatory follicle and onset of estrus are all significant although the effects on AI outcomes remain to be determined and (3) time of year has a minimal effect on follicle waves but a more significant effect on other parameters of the estrous cycle. A better understanding of these complexities will assist in the development of improved protocols for synchrony of estrus.

Short-term energy supplementation before lambing improves maternal behaviour, udder volume, colostrum viscosity and lamb birthweight in ewes under extensive grazing

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Small Ruminant Research, Volume 210, February 2023

DOI <https://doi.org/10.1016/j.smallrumres.2022.106893>

Highlights

- Short-term energy supplementation before lambing improved maternal behaviour.
- Short-term energy supplementation increased udder volume and reduced colostrum viscosity.
- Supplementing ewes with maize and oat before lambing increased lamb birthweight.
- Milk production between weeks 2 and 5 of lactation was not affected by the dietary treatment.

Abstract

The aim of the present experiment was to evaluate the effect of short-term energy supplementation before lambing on maternal and lamb behaviour and colostrum production in a large-scale trial in Merino sheep under extensive grazing. It was hypothesized that prepartum supplementation would improve ewe-lamb bonding at birth, increase udder volume and colostrum production while reducing colostrum viscosity. Milk production between weeks 2 and 5 of lactation was measured to test whether this variable was also affected. A complete randomised designed experiment was conducted over three non-consecutive years (Y1 = 2009, Y2 = 2010, Y3 = 2012) with pregnant adult ewes (Y1: n = 87; Y2: n = 65; Y3: n = 71) from a synchronised mating. Ewes remained under extensive grazing on natural grassland from mating until 5 weeks after lambing. On

day 123 of gestation, ewes were randomly assigned to one of two dietary treatments: grazing ewes (Control) or grazing ewes daily supplemented with 250 g of maize and 250 g of oat per head (SUPPL) until day 144 of gestation. Behavioural and performance variables were measured in ewes and lambs. Data showed that SUPPL ewes returned to their offspring more quickly once the newborn was ear-tagged and weighted than Control ewes ($P < 0.05$). In addition, lambs born to SUPPL ewes tended to suck earlier than Control ($P < 0.10$). Udder volume in SUPPL ewes was 25% greater than in Control ewes ($P < 0.05$). Colostrum produced by SUPPL ewes was less viscous than in Control ewes ($P < 0.05$) and colostrum volume tended to be higher in SUPPL ewes ($P < 0.10$). Overall, birthweight was significantly greater in lambs born to SUPPL ewes compared with Control ($P < 0.05$). Milk production between weeks 2 and 5 of lactation was not affected by the dietary treatment ($P > 0.05$). The present experiment also showed a significant interannual variation in some parameters of interest. A beneficial effect of short-term prepartum supplementation on maternal behaviour, udder volume, colostrum viscosity and lamb birthweight was shown in Merino sheep under extensive grazing. This work also highlights that a practical low-cost supplementation strategy for sheep producers in extensive systems may have clear implications in lamb survival, animal welfare and farm productivity.

In vitro approach points to a chemotactic effect of melatonin on ram spermatozoa

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Theriogenology, Volume 198, March 2023 **OPEN ACCESS**

DOI <https://doi.org/10.1016/j.theriogenology.2022.12.022>

Highlights

- Melatonin increases the ram sperm recovery rate when included in a swim-up method.
- A higher percentage of capacitated spermatozoa are recovered with melatonin 100 pM.
- In vitro capacitation increases the percentage of sperm moving towards melatonin.
- Melatonin effects on ram sperm were observed only during the non-breeding season.

Abstract

Sperm orientation mechanisms, such as chemotaxis, are essential for the sperm to reach the oocyte and fertilize it. Melatonin is secreted by the cumulus cells and is also present in the follicular fluid in mammals. The presence of membrane receptors for melatonin in ram spermatozoa, and its proven involvement in the sperm functionality, may suggest a possible role in the guided movement towards the oocyte. Hence, the objective of the present work is to study the in vitro potential chemotactic action of melatonin on ram spermatozoa, analysing the influence of the season (breeding and non-breeding) and the sperm capacitation state. The first experimental approach consisted in the inclusion of melatonin in the upper layer of a swim-up selection method. During the non-breeding season, the presence of melatonin at 100 pM and 1 μ M concentrations significantly increased the cell recovery rate, and induced changes in the sperm location of the MT2 melatonin receptor, compared with the standard swim-up. Moreover, the selected sperm population with 100 pM melatonin presented a higher percentage of capacitated spermatozoa. The greater recovery rate obtained with melatonin could be due to the stimulation of sperm movement in random directions, i.e., a chemokinetic effect, or due to a guided movement (chemotaxis) towards the gradient of the melatonin. To elucidate this issue, together with the study of the influence of the sperm capacitation status, we performed a second experimental approach which consisted in the use of chemotaxis chambers and an open-source software (Open-CASA) that analyses the sperm trajectories towards the hormone gradient and calculates a chemotaxis index (SL index). There was a significant difference between the SL index in the presence of 1 μ M melatonin and the control without hormone. This effect was only observed in capacitated spermatozoa with cAMP-elevating agents (Cap-CK samples) obtained during the non-breeding

season. These results would point to an in vitro chemotactic effect of melatonin on ram spermatozoa, although chemokinesis cannot be ruled out. Nonetheless, the inclusion of this hormone in the swim-up procedure could enhance the sperm recovery rate.

Metabolic memory determines oviductal gene expression of underfed ewes during early gestation

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Theriogenology, Volume 198, March 2023

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Abstract

Our aim was to investigate the oviduct environment by studying oviduct gene expression after undernutrition in day-5 pregnant ewes with different initial (i) BCS, and its association with the number of embryos recovered. Thirty-six ewes were divided into 2 groups with different iBCS: iBCS ≥ 2.75 (n = 19; high, H) and iBCS ≤ 2.25 (n = 17; low, L), and were randomly assigned to two nutritional treatments for 20 days: 1.5 (control, C) or 0.5 (underfed, U) times the daily maintenance requirements. Thus, the final four groups were: high-iBCS control (HC, n = 9), high-iBCS underfed (HU, n = 10), low-iBCS control (LC, n = 9) and low-iBCS underfed (LU, n = 8). Samples of oviduct were collected and the expression of target genes was quantified using real-time PCR. While high-iBCS control ewes presented more ADIPOR1 mRNA than the high-iBCS underfed group (P < 0.05) and low-iBCS control ewes (P = 0.01), high-iBCS underfed group presented higher ADIPOR2 gene expression than low-iBCS underfed ewes (P < 0.01) evidencing a differential oviductal gene expression for these receptors. In high-iBCS ewes, control animals presented higher IGFBP2 gene expression than underfed ewes (P < 0.05), associated these results with a poor oviductal environment. High-iBCS underfed ewes presented higher IGFBP4 gene expression than high-iBCS control ewes (P < 0.05). Stepwise regression models, using various combinations of data on metabolic and reproductive hormones, and oviduct gene expression as independent variables, identified a set of variables that accounted for 75% of the variation in the number of embryos recovered. In conclusion, the oviductal gene expression depends on body reserves and nutritional treatment, and the effect is gene-specific.

Effects of melatonin on development and hormone secretion of sheep theca cells in vitro

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Theriogenology, Volume 198, March 2023

DOI <https://doi.org/10.1016/j.theriogenology.2022.12.036>

Abstract

Theca cells (TCs) play a unique role in the structure and function of the ovary. They are not only the structural basis of the follicle but also the androgen-secreting cells in female mammals, which can affect the normal development and atresia of the follicle. The results showed that melatonin receptor (MTR) MT1 and MT2 were expressed on sheep TCs. In the present study, the effects of different concentrations of MT at 0, 10⁻¹⁰, 10⁻⁸, 10⁻⁶ and 10⁻⁴ M/L on sheep TCs with regards to the antioxidant levels, proliferation, apoptosis and steroid hormone secretion were investigated. The results showed that in sheep TCs, all concentrations of MT significantly decreased reactive oxygen species (ROS) concentration and BAX expression; increased Cat, Sod1, and BCL-2 expression. The proliferation viability of TCs was significantly inhibited in all groups except for 10⁻¹⁰ M/L MT, and the expression of cyclin D1 and CDK4 was significantly reduced. MT significantly increased StAR expression and progesterone secretion in TCs, but there was no significant effect on androgen secretion

and CYP11A1, CYP17A1 and 3 β -HSD expression in all groups. MT-induced progesterone secretion was completely inhibited by Luzindole (a nonspecific MT1 and MT2 inhibitor) and partially inhibited by 4p-PDOT (specific MT2 inhibitor). MT-induced progesterone secretion can be inhibited by LY294002 (PI3K/AKT pathway inhibitor). This study indicated that MT inhibits apoptosis and proliferation of in vitro cultured sheep TCs, which has implications for slowing ovarian atresia and aging. MT activates the PI3K/Akt pathway to mediate the synthesis and secretion of progesterone by TCs. This study provides a basis for further exploration of the role of TCs on follicle development and ovarian steroid hormone secretion.

Melatonin regulates dihydrotestosterone formation via its membrane receptor in the epididymal epithelial cells of sheep

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Theriogenology, Volume 198, March 2023

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Abstract

Both melatonin and androgen, which affect sperm fertility, are the important factors in epididymis of male animal. In the present study, we confirmed that melatonin regulates the formation of dihydrotestosterone (DHT) in sheep epididymides. Here, we investigated the localization and the expression levels of melatonin keys synthases AANAT and HIOMT, membrane receptors MT1 and MT2, and nuclear receptor ROR α in sheep epididymides and testes. We also cultured epididymal epithelial cells and treated them with different concentrations of melatonin (10⁻¹¹-10⁻⁷ M) and luzindole (10⁻⁵ M) and 4P-PDOT (10⁻⁵ M) to investigate whether melatonin is involved in the regulation of DHT formation and whether these effects are mediated through its receptor pathways. The results showed that AANAT, HIOMT, MT1, MT2, and ROR α were differentially expressed between sheep epididymides and testes. In addition, melatonin is involved in mediating the formation of DHT in epididymal epithelial cells, and its influence on DHT is at least partially regulated by the melatonin receptor pathway. Our findings showed that melatonin regulates the functions of the testes and epididymides through an autocrine mechanism and regulates the formation of androgen in sheep epididymides via the receptor pathway. These results provide a basis for further exploring the regulatory mechanisms of melatonin in animal reproduction.

Upcoming events

Date	Event	Location
28 February 2023	BestWool/BestLamb & BetterBeef Networks Regional Roadshow Ag Victoria, AWI & MLA	Wangaratta, Vic
1 March 2023	BestWool/BestLamb & BetterBeef Networks Regional Roadshow Ag Victoria, AWI & MLA	Sale, NSW
2 March 2023	BestWool/BestLamb & BetterBeef Networks Regional Roadshow Ag Victoria, AWI & MLA	Buninyong, Vic
3 March 2023	BestWool/BestLamb & BetterBeef Networks Regional Roadshow Ag Victoria, AWI & MLA	Dunkeld, Vic

29 March 2023

[Macquarie MLP Final Field Day](#)
AMSEA, AWI & NSW DPI

Trangie, NSW