

FEBRUARY 2024

Sheep reproduction RD&A alert

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

Have your say in shaping the current and future activities of the Sheep Reproduction Strategic Partnership (SRSP) by completing a short Monitoring and Evaluation (M&E) survey.

The SRSP is seeking feedback from our stakeholders regarding current and future initiatives of the SRSP. The short survey will allow the SRSP to evaluate the impact and effectiveness of our current initiatives and gather feedback on future activities the partnership could undertake to achieve our aim of *profitably and sustainably increasing lamb production through increasing weaning rates and decreasing mortality*.

Please click on [this link to the Google form](#) to start the survey. *The survey is anonymous and should take you no more than 10 minutes to complete.*

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.

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Scientific papers

Heat stress and ram semen production and preservation: Exploring impacts and effective strategies

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Journal of Thermal Biology, Volume 199, January 2024

DOI <https://doi.org/10.1016/j.jtherbio.2024.103794>

Highlights

- Heat stress reduces fresh and stored sperm quality in rams.
- Heat stress affects seminal plasma composition in rams.
- Heat stress disrupts the hormonal balance within the reproductive axis of rams.
- Rams exhibit testicular thermoregulation in response to heat stress.
- Mitigation strategies are adopted to counteract heat stress effects on sheep.

Abstract

As global warming persists, heat stress (HS) continues to affect animals, particularly those raised in extensive systems such as sheep. As a result, there is a growing body of research investigating the physiological and biological consequences of HS on these animals. Recent studies have specifically examined the effects of climate change, global warming, and HS on gametes. Heat stress has been shown to affect ram semen

production, resulting in decreased sperm quality and volume in both fresh and stored samples. This is attributed to the effect of heat on hormone production in the testicles, which is critical for successful spermatogenesis. Such effects can have significant consequences on the fertility of female sheep, which could affect the farmers' revenue. Therefore, farmers and researchers are utilizing various strategies and laboratory techniques to mitigate these negative effects. This review aims to comprehensively evaluate the impact of HS on ram semen production and conservation and analyze the different mitigation strategies at various levels, including management and nutritional interventions. The findings of this review will serve as a critical foundation for the development of targeted interventions and sustainable practices in sheep farming, ensuring resilient and profitable operations in the face of ongoing global climate challenges.

Roles of Y-27632 on sheep sperm metabolism

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Journal of Animal Science Volume 102, January 2024 **OPEN ACCESS**

DOI <https://doi.org/10.1093/jas/skae020>

Abstract

To investigate the effect of Y-27632 on low-temperature metabolism of sheep sperm, different concentrations of Y-27632 were added to sheep semen at 4 °C in this experiment to detect indicators such as sperm motility, plasma membrane, acrosome, antioxidant performance, mitochondrial membrane potential (MMP), and metabolomics. The results showed that the addition of 20 μM Y-27632 significantly increased sperm motility, plasma membrane integrity rate, acrosome integrity rate, antioxidant capacity, MMP level, significantly increased sperm adenosine triphosphate (ATP) and total cholesterol content, and significantly reduced sperm Ca²⁺ content. In metabolomics analysis, compared with the control group, the 20 μM Y-27632 group screened 20 differential metabolites, mainly involved in five metabolic pathways, with the most significant difference in Histidine metabolism (P = 0.001). The results confirmed that Y-27632 significantly improved the quality of sheep sperm preservation under low-temperature conditions.

Using lupins and the ram effect to improve reproductive performance in Merino ewes in southern Australia

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DOI <https://doi.org/10.1016/j.livsci.2024.105415>

Highlights

- Ovulation rate and lamb survival are key to improving reproductive performance.
- Short-term lupin feeding with synchronised estrus (ram effect) improves ovulation.
- Variation in lupin response dependent on nutrition months prior to ovulation.
- Lupin feeding with synchronised lambing (ram-effect) improves lamb survival.
- Can a combined mating and lambing strategy give additive increases in weaning rate?

Abstract

The ability of lupins to increase ovulation rate in Merino ewes in as little as four days continues to interest researchers and producers alike. This review summarises work undertaken by us over several years aimed at adapting this response to improving on farm reproductive performance using the ram effect to minimise the number of days required for supplementation in Merino ewes mated in late spring/early summer in southern Australia. The results from these experiments showed that lupin feeding could increase ovulation rate as well

as improve lamb survival. The unanswered question is whether combining both mating and lambing strategies can result in additive increases in weaning percentages of upwards of 20 %.

Manipulation of neonatal ruminal populations at birth results in sustained effects on microbial populations and measures of health and production in Merino and Suffolk lambs

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Highlights

- Inoculations resulted in differences in ruminal bacterial genera.
- Differences were evident in rumen samples at weaning (10 weeks).
- Differences were evident in intestinal samples at slaughter (18 weeks).
- Efficiency and the health of the inoculated animals was affected.
- Effects differ across inoculation and breeds.

Abstract

The efficiency of the microbial-driven fermentation process in the rumen is closely related to the efficiency of the ruminant host. This experiment examined the effect of manipulating the neonatal microbiota on postnatal development, immune function and productivity (liveweight gain and wool growth parameters). The trial examined the differences between naturally inoculated lambs (maternal control, n = 21) and those given ruminal fluid, which had been collected from ruminally-cannulated ewes, fed either a roughage diet (roughage, n = 14), or a grain-based diet (high grain, n = 14). At lambing, newborn lambs were tagged and weighed. Inoculation lambs received 10 ml of rumen fluid per inoculation, daily, for a total of 7 inoculations. Live weight, body condition scores, wool growth (greasy and clean wool weights), wool length, mean fibre diameter, and differential blood cell counts, and IgG and IgA in both blood and saliva) were quantified from birth until week 18 (slaughter). Total feed intake was recorded from weaning. Ruminal fluid was collected by stomach tube from 12 lambs from each treatment group and their dams (n = 7–8 per treatment, with the lower number of ewes coming about through ewes that had twins) at time of weaning (week 10). The pH of the ruminal fluid, the density of ciliated protozoa in ruminal fluid, and the composition of the ruminal bacterial populations were analysed. At slaughter (week 18), ileal samples were taken from the same focal lambs as were used for microbial analysis. The artificial inoculations resulted in significant differences in ruminal bacterial genera to those of naturally inoculated lambs at time of weaning (some 3 months post-lambing). Significant differences were also apparent in ileal bacterial communities at slaughter, between lambs which had received the inoculum from roughage-fed ewes and naturally inoculated lambs. Both the efficiency (growth, condition, feed conversion efficiency, carcass weight) and indices of health (mortalities, red blood cell count, blood haemoglobin concentration, haematocrit, and eosinophil counts) of the inoculated animals, were affected negatively by the inoculations (< 0.05). There was a significant interaction between inoculum source and breed in terms of effects on all parameters measured. The effects of the high grain inoculation were significantly affected by breed, with high grain merinos found to have reduced mean fibre diameter ($P < 0.05$) and increased wool growth relative to the maternal control ($P = 0.035$). This effect was not noted in the suffolks. In summary, we have demonstrated that long-term effects on ruminal and intestinal microbiota can be generated by intervention in the normal process of ruminal inoculation in neonatal lambs. Moreover, there appeared to be genetic differences in response to artificial inoculation. Whilst the effects of artificial inoculation in this trial were negative in terms of indices of health and production, further studies of maternal diet and its effects on the natural establishment of the neonatal microbiota are warranted given the persistence of the effects and the interaction with breed.

Proteomics analysis of pregnancy in ewes under heat stress conditions and melatonin administration

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DOI <https://doi.org/10.3390/ani14030400>

Simple Summary

Heat stress is known to cause disruptions to a variety of physiological processes in sheep, including reproduction and ultimately pregnancy, mostly by promoting reactive oxygen species generation and oxidative stress. It has been proposed that the exogenous administration of melatonin, because of its antioxidant and immunomodulatory properties, could help alleviate adverse effects of heat stress. However, the way that melatonin modulates these activities is unclear, especially during pregnancy under heat stress conditions, as well as during lambing. For these reasons, proteomics analysis was used to elucidate the proteins that are regulated by the presence of melatonin. The findings derived from proteomics analysis indicated that melatonin regulates proteins that are involved in cell cycle division, boosted immune response and protective mechanisms affecting both the maternal organism and the embryo(s).

Abstract

Melatonin is an indoleamine with broad spectrum properties that acts as a regulator of antioxidant and immune response in organisms. In our previous studies, melatonin improved redox status and inflammatory response in pregnant ewes under heat stress conditions. In the present study, using proteomics, the proteins regulated by melatonin during different stages of pregnancy and lambing were assessed. Twenty-two ewes equally divided into two groups, the melatonin (M) (n = 11) and control (C) group (n = 11), participated in the study and were exposed to heat stress during the first months of pregnancy. In the M group, melatonin implants were administered throughout pregnancy, every 40 days, until parturition (a total of four implants per ewe). Blood samples were collected at the beginning of the study simultaneously with the administration of the first melatonin implant (blood samples M1, C1), mating (M2, C2), second implant (M3, C3), fourth implant (M4, C4) and parturition (M5, C5), and MALDI-TOF analysis was performed. The results revealed the existence of 42 extra proteins in samples M2, M3 and M4 and 53 in M5 (sample at parturition) that are linked to melatonin. The biological processes of these proteins refer to boosted immune response, the alleviation of oxidative and endoplasmic reticulum stress, energy metabolism, the protection of the maternal organism and embryo development. This proteomics analysis indicates that melatonin regulates protective mechanisms and controls cell proliferation under exogenous or endogenous stressful stimuli during pregnancy and parturition.

Utilising male stimulus to improve the reproductive efficiency of 8-month-old nulliparous ewes and adult parous ewes

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Theriogenology, Volume 217 March 2024

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Abstract

We tested whether utilising the male effect to stimulate ewes before the mating period can reduce the time to conception following the introduction of entire rams, and increase fertility, prolificacy, and reproductive

rate (number of fetuses per 100 ewes exposed to fertile rams). A retrospective analysis was used to analyse records from 59,716 ewes collected over 34 years (1986–2020) from seven genotypes: Border Leicester, Composite (crossbred), Dorset, Merino, Dorset x Polypay, Rambouillet, White Suffolk. The dataset also included nulliparous young ewes (mated at age 8 months) and adult parous ewes. Vasectomized rams were used to stimulate 20,632 ewes before a mating period that lasted 2 or 3 estrous cycles, and the outcomes were compared with those from 39,084 ewes that had not been stimulated. Independently of genotype, utilising the male stimulus advanced the average conception date by 8 days for young ewes ($P < 0.0001$) and by 1 day for adult ewes ($P < 0.0001$). The male stimulus also increased the proportion of ewes that conceived in their first cycle by 33 % for young ewes and by 6 % for adult ewes ($P < 0.0001$). For the cycle of conception, there were significant ($P < 0.0001$) effects of two interactions: male stimulus x age at mating and male stimulus x live weight at mating. The male stimulus improved fertility in both adult ewes (99.8 % vs 89 %; $P < 0.001$) and young ewes (77.7 % vs 81.3 %; $P < 0.001$). The male stimulus increased the number of young ewes (41.9 % vs 11.1 %; $P < 0.001$) and adult ewes (16.6 % vs 2.7 %; $P < 0.001$) that conceived multiple fetuses in the first 17 days of the mating period. The reproductive rate was improved by the male stimulus in young ewes (129 % vs 135 %; $P < 0.001$) but not in adult ewes (120 % vs 122 %; $P = 0.12$). When all animals for all breeds were included in the analyses, there were improvements in fertility, prolificacy, and reproductive rate as age and live weight increased at mating ($P < 0.0001$). We conclude that, independently of genotype, utilising the male stimulus before the mating period reduces the time to conception and improves reproductive performance in both young and adult ewes.

Effect of antifreeze protein I in the freezing solution on *in vivo*-derived sheep embryos

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Research in Veterinary Science, Volume 168, March 2024

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Abstract

This study evaluated the effects of different antifreeze protein type I (AFP I) concentrations added to a slow freezing solution in sheep *in vivo*-derived embryos. Good-quality embryos were allocated into: AFP-free (CONT); 0.1 µg/mL of AFP I (AFP0.1); or 0.5 µg/mL of AFP I (AFP0.5). After thawing, embryos were *in vitro* cultured (IVC) for 48 h. At 24 h and 48 h of IVC, dead cells and apoptosis, mitochondrial activity, intracellular reactive oxygen species (ROS), and glutathione (GSH) evaluations were performed. At 24 h, evaluated embryos were submitted to RT-qPCR for metabolism (SIRT2, PRDX1, OCT4, CDX2) and quality (AQP3, CDH1, HSP70, BAX, BCL2) genes. The *in vitro* survival rate was 56% (22/39) for CONT, 60% (32/53) for AFP0.1, and 53% (23/43) for AFP0.5 ($p > 0.05$). A tendency ($p = 0.09$) for a higher blastocyst hatching rate was noted in AFP0.1 (62%) compared to AFP0.5 (33%), and both groups were similar to CONT (50%). An increased ($p < 0.05$) mitochondrial activity at 24 h was observed in AFP0.1 compared to CONT. No differences ($p > 0.05$) were observed in oxidative stress homeostasis and viability between treatments. A downregulation ($p < 0.05$) of CDH1 in AFP0.1 and a downregulation of AQP3 in AFP0.5 were observed in comparison to the other groups. An upregulation ($p < 0.05$) was detected in HSP70 and BCL2 on AFP0.5 compared to AFP0.1 group. The addition of AFP I in slow freezing solution can benefit cryopreserved sheep *in vivo*-derived embryos, without affecting embryonic survival.

Exploring AMH levels, homeostasis parameters, and ovarian primordial follicle activation in pubertal infected sheep on a high-protein diet

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Highlights

- The protein level in the ewe lambs' diet altered plasma protein and urea levels.
- Anti-Mullerian Hormone levels measured over time during puberty in infected sheep.
- Unaltered AMH levels proving a reliable marker for pubertal sheep ovarian reserve.
- The interaction Diet/Infection & plasma protein influenced primordial follicle size.
- Protein-supplemented ewe lambs presented a larger oocyte size.

Abstract

The first activation wave of ovarian primordial follicles is part of the onset of puberty and fertility. Abomasal helminth infection may cause an undesirable delay in puberty manifestation. Helminth-infected animals demand a higher amount of protein in their diet to repair the damage caused by the parasite in sheep's tissues, replenish the blood losses, and build the host's immune response. Helminths become resistant to drug therapy shortly after being exposed to a new treatment. Besides, there is the possibility of contamination by anthelmintic drugs in ovine products, possibly affecting human health and the environment. This study's objective was to evaluate if ovarian and clinical parameters can be improved by supplementing their diet with protein, offering a more sustainable management approach than relying on anthelmintic usage. We used a 2 × 2 factorial model where eighteen ewe lambs (*Ovis aries*) between 6 and 7 months old - born to the same ram - were fed one of two diet protein levels (12% or 19%). After 35 days on this diet, they were infected or left uninfected with 10,000 *Haemonchus contortus* L3 larvae. We evaluated Anti-Mullerian Hormone serum levels, blood cells and biochemical parameters at four different time points. Following 42 days of infection and 77 days on the diet, the lambs had their left ovaries removed, and we examined ovarian morphometrics through histological analysis. The groups Supplemented Protein-Infected (n = 5), Control Protein- Infected (n = 5), Supplemented Protein-Not Infected (n = 4) and Control Protein-Not Infected (n = 4) did not differ in their bodyweight gain. In the factorial ANOVA analysis examining the relationship between plasma protein, diet, and infection, the protein level of the diet showed significance (p = 0.02). Primordial follicle size varied with the interaction between diet and infection (p < 0.05), and oocyte size was affected by the level of protein in the diet (p = 0.047). Additionally, to understand how all homeostasis parameters relate to the primordial follicle and oocyte size, we applied an explanatory linear mixed model. In conclusion, serum AMH levels remained stable despite the infection and variations in diet protein levels, indicating its reliability as a marker for ovarian reserve in pubertal sheep. The number of blood cells, biochemical parameters, and primordial follicle activation were affected by both diet and infection.

Upcoming events

Date	Event	Location
7 March 2024	Sticky Beak Day – Containment Feeding AWI, SA Sheep Industry Fund & SheepConnect SA	Lucindale, SA
12 March 2024	Proactive management for sustainable parasite control ParaBoss	Webinar
13 March 2024	Feed budgeting and decision making Agriculture Vic	Ararat, Vic
15 March 2024	Worm Control Workshop	Bungendore, NSW

19 March 2024	NSW Local Land Services Braidwood Effective work management in sheep ParaBoss	Naracoorte, SA
19 March 2024	ProGraze™	Coolah, NSW
21 March 2024	NSW Local Land Services Central West Prograze™	Dubbo & Wellington, NSW
22 March 2024	NSW Local Land Services Central West Winning with Weaners Sheep Connect NSW & AWI	Parkes, NSW
21 March 2024	WormBoss Workshop Leading Sheep & AWI	Goondiwindi, QLD
22 March 2024	WormBoss Workshop Leading Sheep & AWI	Terrica Woolshed, Qld
29 May 2024	Final New England MLP Field Day AMSEA, AWI & CSIRO	Uralla, NSW

Funding calls

Program	Open	Close
Livestock Genetics R&D Project Call Meat & Livestock Australia	November 2023	15 March 2024