

AUGUST 2025

## Sheep reproduction RD&A alert

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

The SRSP's [Shade and Shelter](#) project was recently featured on Landline with an [11 minute video](#) and [accompanying story](#) detailing the impact of shade and shelter on the survival of twin born lambs.

The overall aim of the project is to quantify the impact of shade and shelter on sheep reproduction and welfare. Ewes and rams exposed to heat stress can have reduced fertility and impaired foetal development and lower lamb survival. Likewise, cold stress will increase the mortality of newborn lambs.

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Extreme climatic variation (wind, rain and temperature) poses a significant stress on extensively-managed sheep. Shade and shelter minimise heat and cold stress to improve the production and welfare of sheep as well as having other benefits for pasture production and biodiversity. The University of Western Australia, Murdoch University, CSIRO and NSW DPIRD are investigating and developing interventions to reduce the impact of climatic variation on sheep enterprises.

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.

### Review papers

#### **Mitochondrial regulation of spermatozoa function: Metabolism, oxidative stress and therapeutic insights**

Zhiqian Xu, Qi Yan, Ke Zhang, Ying Lei, Chen Zhou, Tuanhui Ren, Ning Gao, Fengyun Wen and Xiaoxia Li

Animals, Volume 15, Issue 15 August 2025 **OPEN ACCESS**

DOI <https://doi.org/10.3390/ani15152246>

#### **Simple Summary**

Male fertility depends greatly on the quality and function of spermatozoa. Spermatozoa need a lot of energy to move and fertilize the egg, and this energy is mainly produced by mitochondria. However, mitochondria can also produce harmful substances called reactive oxygen species, especially when the balance in the cell is disturbed. While small amounts of ROS are necessary for normal sperm function, too much can damage

sperm quality. In this review, we summarize how mitochondria produce energy in sperm, how excess ROS can lead to problems, and how damage to mitochondrial DNA can affect sperm health. We also discuss promising treatments by antioxidants that specifically target mitochondria to protect spermatozoa from damage. Understanding how to keep mitochondria healthy in spermatozoa could lead to better treatments for male infertility and help improve reproductive success in both humans and animals.

#### **Abstract**

Mitochondria are central to energy production and redox regulation in spermatozoa, supporting key functions such as progressive motility, capacitation, and the acrosome reaction. These processes are essential for successful fertilization and embryo development. However, species-specific differences exist in the reliance on oxidative phosphorylation versus glycolysis. Mitochondria also generate reactive oxygen species, which at physiological levels aid in sperm function but can cause oxidative stress and damage when overproduced. Mitochondrial dysfunction and excessive ROS can impair membrane potential, induce apoptosis, and damage nuclear and mitochondrial DNA, ultimately compromising sperm quality. Sperm mitochondrial DNA is highly susceptible to mutations and deletions, contributing to reduced motility and fertility. Targeted antioxidant strategies have emerged as promising therapeutic interventions to mitigate oxidative damage. This article provides a comprehensive overview of mitochondrial regulation in spermatozoa, the consequences of redox imbalance, and the potential of mitochondria-targeted antioxidants to improve sperm function and male fertility outcomes. The paper aims to deepen our understanding of mitochondrial roles in sperm physiology and contribute to the advancement of strategies for addressing male infertility.

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### **Reproductive challenges in ruminants under heat stress: A review of follicular, oocyte, and embryonic responses**

Danisvânia Ripardo Nascimento, Venância Antonia Nunes Azevedo, Regislane Pinto Ribeiro, Gabrielle de Oliveira Ximenes, Andreza de Aguiar Silva, Efigênia Cordeiro Barbalho, Laryssa Gondim Barrozo, Sueline Cavalcante Chaves, Maria Samires Martins Castro, Erica Costa Marcelino, Leopoldo Rugieri Carvalho Vaz da Silva, André Mariano Batista and José Roberto Viana Silva

Animals, Volume 15, Issue 15 August 2025 **OPEN ACCESS**

**DOI** <https://doi.org/10.3390/ani15152296>

#### **Simple Summary**

Heat stress is the result of the interaction of several climatic variables, including the intensity of solar radiation, air movement, ambient temperature, and humidity. The rise in environmental temperatures has intensified concerns about the negative impact of heat stress on ruminant reproductive performance. This study examines the impacts of thermally unfavorable environments on ovarian follicles, oocytes, hormone production, and embryonic development in ruminants. The data showed that early follicles, from the primary stage onward, suffer the consequences of heat stress, resulting in oocytes with low competence to be fertilized. In antral follicles, the reduction in hormone levels caused by heat negatively affects growth and disrupts the activity of enzymes and proteins responsible for steroid synthesis. With regard to embryonic development, exposure to high temperatures has been associated with a decline in embryo quality. Therefore, developing adaptive mechanisms and thermal management strategies is essential for preserving reproductive efficiency and ensuring the sustainability of animal production under adverse environmental conditions.

#### **Abstract**

This review aims to discuss how heat stress affects ovarian follicles and oocytes, steroidogenesis, and embryo development in ruminants. The literature shows that quiescent primordial follicles appear to be less

susceptible to heat stress, but from the primary follicle stage onwards, they begin to suffer the consequences of heat stress. These adverse effects are exacerbated when the follicles are cultured in vitro. In antral follicles, heat stress reduces granulosa cell viability and proliferation in both in vivo and in vitro models. Oocyte maturation, both nuclear and cytoplasmic, is also compromised, and embryo quality declines under elevated thermal conditions. These effects are linked to intracellular disturbances, including oxidative imbalance, mitochondrial dysfunction, and altered hormonal signaling. The differences between in vivo and in vitro responses reflect the complexity of the biological impact of heat stress and emphasize the protective role of the physiological microenvironment. A better understanding of how heat stress alters the function of ovarian follicles, oocytes, and embryos is crucial. This knowledge is critical to devise effective strategies that mitigate damage, support fertility, and improve outcomes in assisted reproduction for livestock exposed to high environmental temperatures.

### Scientific papers

#### Process mining to identify average time patterns of changes in FAMACHA® scores in ewes and guide monitoring intervals

Matheus Borges de Carvalho, Diógenes Adriano Duarte Santana, Laize Guedes Carmo, Saulo Henrique Weber, Eric R. Morgan, Deborah Ribeiro Carvalho and Cristina Santos Sotomaior ([cristina.sotomaior@pucpr.br](mailto:cristina.sotomaior@pucpr.br))

Veterinary Parasitology Volume 338 August 2025

DOI <https://doi.org/10.1016/j.vetpar.2025.110543>

#### Highlights

- Process mining was used to analyze the time taken for changes in the Famacha scores.
- Ewes maintained F1 and F2 scores for an average of 78.5 and 25.0 days, respectively.
- Ewes in less susceptible physiological states changed from F2 to F3 after 29.6 days.
- For ewes in late pregnancy and lactation, F evaluation should be done every 15 days.
- For less susceptible physiological states, a 30-day interval is sufficient.

#### Abstract

Frequent blanket treatment with anthelmintics (AH) leads to selection of resistant parasites. Targeted Selective Treatment (TST) methodologies were developed to support more discriminating decisions on AH treatment of individual animals. Criteria like FAMACHA® scores (F-scores, a measure of anemia) detect changes in animal health to trigger treatment, but require effort and so the appropriate frequency of monitoring must be considered. Process mining can be used to define optimal intervals for TST application by analyzing flows between states over time. The aim of this study was therefore to evaluate the average time taken for changes in the F-scores of adult ewes using process mining. The study was performed by analyzing a database containing F-score evaluations from 2006 to 2022, using UpFlux® software. From 2017–2022 ewes were categorized based on their physiological state. Ewes in late gestation and lactation were classified as likely to be more susceptible to parasites, while non-pregnant and early pregnant ewes were considered as less susceptible. When considering data for the entire flock from 2017 to 2022, an average of 97 ewes were evaluated weekly, and they spent a mean of 78.5 days at F-score 1 (F1) and 25.0 days at F2. Based on F-score fluctuations, ewes progressed from F1 to F2 in an average of 59.1 days and from F2 to F3 in an average of 26.3 days. Ewes in the more susceptible physiological states maintained F1 and F2 scores for an average of 34.1 and 20.0 days, respectively. Their transition from score F2 (non-anemic) to F3 (anemic) occurred in an average of 19.7 days. Ewes in less susceptible physiological states maintained F1 for 68.2 days and F2 for 24.5 days, while F2 changed to F3 after an average of 29.6 days. Based on the results, evaluations for TST should be conducted more frequently (every 15 days) in ewes during late pregnancy and lactation, while a 30-day interval is sufficient for other physiological states. This method could be used to evaluate

optimal intervals for F-scoring in other settings, to guide efficient animal health monitoring and intervention strategies.

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### Salivary anti-CarLA IgA levels in ewes and their offspring and association with gastrointestinal parasitism and offspring performance in Ontario sheep flocks

Bradley D. DeWolf, Cathy A. Bauma, Paula I. Menzies, Emma A. Borkowski, Richard J. Shaw and Andrew S. Peregrine

Veterinary Parasitology Volume 338 August 2025

DOI <https://doi.org/10.1016/j.vetpar.2025.110543>

#### Highlights

- Carbohydrate larval antigen (CarLA) generates an immune response in sheep.
- Anti-CarLA IgA in ewes is negatively associated fecal egg counts in offspring.
- Salivary anti-CarLA IgA in ewes is positively associated with offspring weight.

#### Abstract

The rate at which pastured lambs develop immunity to gastrointestinal nematodes (GINs) is highly variable and depends on factors such as larval (L3) challenge, nutrition, genetics and overall health. Salivary immunoglobulin A (IgA) against carbohydrate larval antigen (CarLA), found on the L3 epicuticle of GINs, has been shown to be an indicator of immunity to GINs in sheep. This study aimed to measure the salivary anti-CarLA IgA concentrations, considered a moderately heritable trait, of Ontario ewes and their offspring, and examine associations with the health and performance of pastured lambs. In 2022, 98 replacement ewe lambs were randomly selected in Ontario, Canada, following their first grazing season and just prior to breeding. At selection, each ewe lamb had its salivary anti-CarLA IgA concentration, fecal egg count (FEC), and weight measured. In 2023, these ewes were re-sampled 4 weeks after returning to pasture; likewise, offspring lambs ( $n = 107$ ) were identified and sampled 60 days after grazing began. At sampling, 22.4 % (24/107) of offspring lambs had detectable salivary anti-CarLA IgA. Linear mixed models revealed that the 2022 salivary anti-CarLA IgA concentration in dams was positively associated with offspring weight, when age and sex were controlled ( $\beta=0.834$ ;  $p = 0.008$ ). Additionally, the 2023 salivary anti-CarLA IgA concentrations in dams were negatively associated with the FEC of their pastured offspring ( $\beta= -0.191$ ;  $p = 0.044$ ). These data suggest that acquired immunity to GINs develops early in Ontario lambs, and that salivary anti-CarLA IgA concentration of the dam may predict the performance of offspring when exposed to GINs on pasture.

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### Melatonin implantation improves the reproductive performance of estrus-synchronized ewes during seasonal anestrus and enhances the antioxidant and steroidogenic capacities of granulosa and luteal cells

Zengyi Duan, Menghao Liu, Junjin Li, Kexiong Liu, Qi Qi, Zhixuan Yu, Hadia Akber Samoo, Chunxin Wang and Jian Hou

Antioxidants, Volume 14, Issue 7, August 2025 **OPEN ACCESS**

DOI <https://doi.org/10.3390/antiox14070895>

#### Abstract

Seasonal reproduction in sheep reduces reproductive efficiency. Melatonin (MT) plays a crucial role in reproductive processes. The purpose of this study was to assess the effects of a 5-day MT implant pretreatment on estrus synchronization and reproductive performance in sheep during seasonal anestrus. A total of 40 multiparous Mongolian sheep were selected and randomly divided into two groups. In the MT group ( $n = 20$ ), the ewes received an MT implant for 5 days, and then, they were given a progesterone (P4)-

containing vaginal sponge for 14 days with equine chorionic gonadotropin (eCG) administered (330 I.U. per ewe; I.M.) at sponge removal. Control (CON) ewes (n = 20) were similarly treated but did not receive MT implants. The results demonstrated that MT implantation significantly improved serum levels of total antioxidant capacity (T-AOC), superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), and glutathione peroxidase (GSH-Px), increased post-ovulatory luteal diameter and serum P4 levels, and reduced ovarian apoptosis. Compared with the CON group, the MT group showed significantly higher pregnancy (68.23% vs. 50.59%) and lambing rates (63.53% vs. 47.06%; number of lambed ewes/number of total ewes) following cervical-timed artificial insemination. Ovarian transcriptome analysis revealed 522 differentially expressed genes (DEGs) in the MT group compared with the CON group, including 355 upregulated and 167 downregulated DEGs. In addition, MT significantly enhanced proliferation and inhibited apoptosis in cultured granulosa cells (GCs) and luteal cells (LCs) in vitro. Moreover, it enhanced the antioxidant capacity of GCs and LCs probably by activating the NRF2 signaling pathway as well as stimulating steroid hormone synthesis. In conclusion, MT implantation 5 days before applying the conventional P4-eCG protocol enhances ovine reproductive outcomes during seasonal anestrus. MT implantation has a beneficial role on the growth and function of ovarian cells. These findings offer novel evidence supporting the functional role of MT in mammalian reproduction, and would be informative for optimizing estrus synchronization in sheep.

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### **Enhancing sheep vitality through diverse pastures and seaweed bio-stimulants: effects on performance, health, and product quality**

Sagara N. Kumara, Anita Fleming, Fabiellen Pereira, Ashna Khan, Simon Kelly, Gwen-Aelle Grelet and Pablo Gregorini

Agriculture, Volume 15, Issue 16, August 2025 **OPEN ACCESS**

DOI <https://doi.org/10.3390/agriculture15161764>

#### **Abstract**

This on-farm study explored the effects of diverse pasture systems and seaweed bio-stimulants (AgriSea NZ Seaweed Products, Paeroa, New Zealand) on sheep performance, metabolic health, milk composition, and carcass characteristics. A 3 × 2 factorial design was used to compare three pasture systems; ryegrass-white clover (RW), a 23-species diverse mix (DI), and functionally diverse strip swards (ST), with (SW) or without (CO) a seaweed bio-stimulant. Ninety pregnant ewes were stratified by live weight and allocated across six treatment groups (15 ewes per treatment). Lambing occurred on treatment paddocks. At weaning, 90 lambs (15 per treatment) were selected based on body weight and sex balance to continue through to finishing. Pasture chemical composition differed among treatments: ST had lower fibre (neutral detergent fibre, NDF; acid detergent fibre, ADF) than RW and DI, while SW increased dry matter digestibility (DMD) and metabolisable energy (ME), and reduced NDF and ADF ( $p < 0.05$ ). Strip pastures improved lamb average daily gain (ADG) by 17% from lambing to weaning compared to DI, and by 14% from weaning to finishing compared to RW ( $p < 0.05$ ). Seaweed bio-stimulant treatment enhanced lamb ADG by up to 12% and improved carcass traits, including loin and shoulder yields ( $p < 0.05$ ). Ewes and lambs on seaweed-treated pastures exhibited lower serum non-esterified fatty acid (NEFA) concentrations ( $p < 0.05$ ), indicating better energy balance. Milk from ST and/or SW treated ewes had elevated omega-6 fatty acids and essential amino acids, suggesting enhanced nutritional value. These findings demonstrate that combining botanical diversity with natural bio-stimulants can improve animal growth, metabolic health, and product quality, offering a promising strategy for sustainable and welfare-oriented sheep production systems.

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### **Chemical form of trace minerals (Copper, Zinc and Manganese) fed to rams affects plasma testosterone concentrations and semen characteristics**

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Reproduction in Domestic Animals, Volume 60, issue 8

DOI <https://doi.org/10.1111/rda.70115>

### Abstract

This study investigated the impacts of dietary trace mineral supplementation (copper, zinc and manganese) on plasma testosterone concentrations and semen characteristics in Afshari × Booroola merino rams. Twenty rams were equally allocated into four groups and fed for 70 days, as follows: Control (no supplemental minerals); Sulphate (sulphate forms); Hydroxychloride (30% hydroxychloride, 70% sulphate) and Amino chelate (30% chelate, 70% sulphate). Plasma testosterone concentrations, seminal plasma antioxidant enzyme activities, alkaline phosphatase and total antioxidant capacity were assessed on Days 0 and 70. On Day 70, all these end points were higher ( $p < 0.05$ ) in the Hydroxychloride and Amino chelate groups compared to the other groups. Furthermore, the Sulphate group had no significant change from Day 0, whereas the Control group decreased ( $p < 0.05$ ). Ejaculate volume and sperm concentration were measured in fresh semen. Sperm quality was evaluated in fresh and frozen–thawed samples, including motility, morphology, viability and membrane functionality every 14 days. From Day 42, sperm motility, viability and membrane functionality improved ( $p < 0.05$ ) in the Hydroxychloride and Amino chelate groups. These parameters remained stable in the Sulphate group but declined in the Control group ( $p < 0.05$ ). Notably, the Control group had the highest percentage of morphologically abnormal sperm at 70 days ( $p < 0.05$ ). Additionally, the Hydroxychloride and Amino chelate groups had fewer apoptotic sperm and lower malondialdehyde concentrations compared to the Control and Sulphate groups. In conclusion, supplementation with hydroxychloride or amino chelate forms of trace minerals optimised reproductive performance in rams, with higher plasma testosterone concentrations and superior sperm quality compared to sulphate or control diets.

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### Phenotypic and genetic analyses of parasitological, physiological and immunological indicator traits of gastrointestinal nematode infections in German Merino crossbred lambs

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Small Ruminant Research, Volume 250, September 2025

DOI <https://doi.org/10.1016/j.smallrumres.2025.107557>

### Highlights

- Nematode infection indicators were analysed in naïve lambs under natural challenge.
- Nematode infection indicators are heritable in German Merino and its crossbreeds.
- Selection on parasitological, physiological and immunological indicators is possible.
- Varying infection environments hamper the detection of relationships between traits.

### Abstract

Most sheep have access to pasture but thus are exposed to gastrointestinal nematodes (GIN). One sustainable approach with long-term potential to counteract the currently increasing resistance of GIN towards anthelmintics is the breeding of sheep with better immunocompetence against GIN. The present study investigated parasitological, immunological and physiological indicator traits in a German sheep population consisting of Merino and crossbred lambs. The trial was conducted over two years under field conditions on three farms with two different grazing systems (extensive/intensive) per farm in southern Germany. After stable rearing, lambs were kept on pasture where blood and faecal samples were taken after approximately five weeks after natural infection. Depending on the trait, data of 383–1015 lambs were



available for phenotypic and genetic analyses. Estimated heritabilities were  $0.15 \pm 0.02$  for FEC for Trichostrongylidae,  $0.09 \pm 0.02$  for FEC for Nematodirus spp.,  $0.23 \pm 0.03$  for dag score and  $0.13 \pm 0.02$  for faecal consistency score, whereas heritabilities for the total immunoglobulin A (IgA) concentration in plasma or faeces were  $0.63 \pm 0.04$  or  $0.11 \pm 0.06$ , respectively. The heritability of the production trait average daily gain was  $0.09 \pm 0.02$ . Substantial phenotypic variation was observed in all traits. Except for total faecal IgA and average daily gain, heritabilities were significant and low to moderate, hence, indicating clear potential for breeding. The small sample size in combination with the varying infection environments did not allow clear inferences about the relationships between traits despite some significant correlations. However, this is necessary before valid breeding goals can be defined, whereby future studies might benefit from considering the course of infection and antigen-specific immune measures in large samples of purebred sheep.

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### **Innovative protein sources for sustainable sheep farming: Assessing black soldier fly larvae meal in Merino lamb diet**

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Small Ruminant Research, Volume 250, September 2025

DOI <https://doi.org/10.1016/j.smallrumres.2025.107556>

#### **Highlights**

- Black soldier fly larvae meal shows potential as a sustainable protein for ruminants.
- No performance or welfare differences were found with black soldier fly larvae meal diet.
- Black soldier fly larvae meal could replace soybean in lamb feed.

#### **Abstract**

The growing global demand for animal-based food poses challenges to sustainability and animal welfare, especially in intensive production systems. This study explores the potential of black soldier fly larvae (BSFL) meal as a sustainable and innovative protein source for Merino lamb diets, offering an alternative to soybean expeller meal. Over a 50-day fattening period, Merino lambs were fed two dietary treatments: soybean expeller meal (S) and BSFL meal. Key metrics were evaluated, including productive performance (body weight, body condition score, feed intake, average daily gain, and feed conversion efficiency), ruminal fermentation profiles, and some stress biochemical indicators (glucose levels, cortisol levels, and plasma protein concentrations). Notably, no significant differences were found between diets in terms of growth performance, ruminal health, or stress markers, suggesting that BSFL meal could be a viable and competitive alternative. These results highlight the potential of BSFL to enhance sustainability in small ruminant production by reducing reliance on environmentally impactful feed ingredients like soybean expeller meal. This study underscores the relevance of BSFL meal in promoting resource efficiency, environmental conservation, and innovative solutions in small ruminant nutrition. These findings open the door to the inclusion of insect use, specifically black soldier fly larvae, in ruminant diets as a viable strategy to improve the sustainability of production systems.

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### **A comparison of three nutritional models for estimating total metabolisable energy requirements for a ewe, beef breeding cow, lamb, and a calf/yearling in New Zealand's pasture-only system**

Joseph A. Adjabui, Patrick H.C. Morel, Stephen T. Morris, Paul R. Kenyon and Peter R. Tozer

Livestock Science, Volume 99, September 2025 **OPEN ACCESS**

DOI <https://doi.org/10.1016/j.livsci.2025.105766>

## Highlights

- Models may yield different ME requirement values, but the extent of variation matters.
- The AIM (MPI, 2022) model estimated a higher total ME for a ewe, cow, and calf/yearling relative to Nicol and Brookes (2017) and CSIRO (2007) models.
- Differences were mainly due to maintenance requirements estimates.
- Model estimates could yield different stocking rates, carrying capacities, and productivity for a given farm.
- Model estimates could also yield different greenhouse gas emissions profiles for the same farm.

## Abstract

In New Zealand (NZ), the metabolisable energy requirements (MER) of ruminants can be estimated using nutritional models from Nicol and Brookes (2017), CSIRO (2007), and NZ's Agricultural Inventory Model (AIM) of the Ministry for Primary Industries [MPI] (2022). The aim in this study was to calculate the total MER of a ewe, beef breeding cow, lamb, and a calf/yearling in a pasture-only system in NZ under the same assumptions to assess the extent and reasons for variations among the three commonly used nutritional models, and the implications for a given farm/s. The study utilised MER models from the three sources above using a factorial method. This method determines the MER for each physiological phase of an animal, to estimate total MER for each animal. The AIM (MPI 2022) model relative to Nicol and Brookes (2017), and CSIRO (2007) models respectively, estimated 13.4 % and 8.0 % higher MER for a ewe, 16.3 % and 16.6 % for a cow, 1.1 % and 2.0 % lower for a lamb, and 9.2 % and 9.2 % higher for a calf/yearling mainly due to a higher maintenance MER compared to the other models. This has implications for feed budgeting and stocking rates (SR) for a given farm, as the AIM (MPI 2022) model leads to a lower SR than the other two models for a given level of feed available. Energy balances and productivity could be negatively impacted if a model underestimates MER. This also yields different greenhouse gas (GHG) profiles, especially enteric methane, for a given farm and could potentially have financial consequences for farmers if an Emissions Trading Scheme was introduced. For consistency in ME estimates and GHG reporting, further research (feeding trials) is required to compare these model estimates to actual requirements of ruminants under NZ conditions. This could help identify the model that most accurately reflects MER for ruminants in the country.

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## The effects of mitochondria-targeted (MitoQ and Mito-TEMPO) and untargeted (SOD and CoQ10) antioxidants on ram's sperm quality and fertility potential during the cryopreservation process

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Animal Reproduction Science, Volume 280, September 2025

DOI <https://doi.org/10.1016/j.anireprosci.2025.107967>

## Highlights

- The effects of MitoQ and Mito-TEMPO were evaluated as a cryo-additive for ram semen cryopreservation.
- MitoQ and Mito-TEMPO preserved sperm quality parameters during cryopreservation process.
- MitoQ and Mito-TEMPO decreased sperm apoptotic-like changes, DNA fragmentation ROS levels and lipid peroxidation.
- MitoQ and Mito-TEMPO controlled oxidative damage during semen cryopreservation process.

## Abstract

Sperm cryopreservation is a widely used procedure for storing donor semen prior to artificial insemination. However, spermatozoa experience biochemical and functional changes during cryopreservation. The current study aimed to compare the effects of mitochondrial-targeted (MitoQ and Mito-TEMPO) and untargeted



(SOD and CoQ10) antioxidants on the quality traits of ram sperm and reproductive performance. Semen samples were collected and diluted in extenders as follows: the first group served as the control, while the other groups were supplemented with 100 U/mL superoxide dismutase (SOD), 1  $\mu$ M CoQ10 (Q10), 50 and 100 nM MitoQ (MQ50 and MQ100), 50 and 100  $\mu$ M Mito-TEMPO (MT50 and MT100), and 50 nM MitoQ+ 50  $\mu$ M Mito-TEMPO (MQ/MT). After the freezing, sperm quality characteristics and fertility potential were evaluated. According to the current results, the groups MQ50, MQ100, MT50, MT100, and MQ/MT exhibited higher ( $P \leq 0.05$ ) rates of total motility (TM), progressive motility (PM), average path velocity (VAP), membrane integrity (MI), mitochondrial activity potential (MAP), acrosome integrity (AI), viable cells, and fertility potential, as well as lower ( $P \leq 0.05$ ) late apoptotic-like changes, DNA fragmentation, H<sub>2</sub>O<sub>2</sub> concentration, and malondialdehyde (MDA) concentrations compared to the SOD and Q10 groups. Moreover, superoxide dismutase (SOD) concentration increased ( $P \leq 0.05$ ) in the groups of SOD, MT50, MT100, and MQ/MT than in other groups. Furthermore, the MQ/MT group exhibited the highest ( $P \leq 0.05$ ) rates of TM, PM, VAP, MI, MAP, AI, viable cells, and fertility potential, along with the lowest ( $P \leq 0.05$ ) late apoptotic-like changes, DNA fragmentation, H<sub>2</sub>O<sub>2</sub> concentration, and MDA concentrations relative to the other groups. In conclusion, incorporating MitoQ+Mito-TEMPO into the extender during the semen freezing could be a valuable strategy to enhance the success of ram frozen semen.

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### Behavioural study of rams subjected to photoperiod change: sexual, social, vital and group activities monitored by video

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Animal Reproduction Science, Volume 280, September 2025 **OPEN ACCESS**

DOI <https://doi.org/10.1016/j.anireprosci.2025.107969>

#### Highlights

- Photoperiod-stimulated rams shown increased activity, mainly sexual behaviors.
- Behavioral modulation was observed in the mixed group.
- Non-stimulated males increased activity when housed with light-stimulated males.
- BORIS allowed accurate, efficient annotation of large volumes of behavioral video data.
- Animal behavior studies using non-invasive methods enhances animal welfare.

#### Abstract

This study examined behavioural differences in rams exposed to artificial photoperiod stimulation, within the context of the ram effect. Eleven rams were divided into two groups: six received two months of long-day artificial photoperiod (16 h light/day), followed by one month of natural light (9 h light/day, February), while five control rams remained under natural lighting. At the end of the third month, three pens were established: one with photoperiod-stimulated rams (Group L,  $n = 3$ ), one with control rams (Group C,  $n = 3$ ), both with 30 ewes, and one mixed pen with both stimulated (Group L+C,  $n = 3$ ) and control rams (Group C+L,  $n = 2$ ) with 60 ewes. Behavioural observations were conducted over 11 consecutive days. A total of 26 behaviours-classified as sexual, vital, social, and grouping- were manually recorded and analysed using BORIS software. Sexual behaviours were the most frequent across all groups. Rams in Group L exhibited higher frequencies in all behaviour categories ( $p < 0.001$ ). Vital activities had the longest durations, with Group C spending more time on these behaviours than others. In the mixed pen, both stimulated and control rams showed intermediate activity frequencies compared to their respective isolated counterparts (Group L: 36.7 % vs. L+C: 26.6 %; Group C: 17.6 % vs. C+L: 19.0 %;  $p > 0.001$ ).

In conclusion, artificial photoperiod stimulation increased the frequency of key behavioural categories, especially sexual activity. Social interactions in mixed groups modified behaviour, suggesting mutual influence. BORIS software proved valuable for detailed behavioural analysis in ethological research.

## Upcoming events

Date	Event	Location
17 Sep 25	<a href="#">RAMping up Repro Workshop</a> AWI Extension WA	Moora, WA
23 Sep 25	<a href="#">Virtual drought smokos with Dr Jillian Kelly</a> Agriculture Victoria	Online
9 Oct 25	<a href="#">AWI Repro Masterclass</a> AWI Extension NSW	Armidale, NSW
13 Oct 25	<a href="#">RAMping up Repro Workshop</a> AWI Extension WA	Wandering, WA
14 Oct 25	<a href="#">RAMping up Repro Workshop</a> AWI Extension NSW	Grenfell, NSW

## Funding calls

Program	Open	Close
<a href="#">MLA/GRDC Partnership – Producer Demonstration Site (PDS) Projects</a>	25 August 2025	3 October 2025