NOVEMBER 2025

Sheep reproduction RD&A alert

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

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The Sheep Sustainability Framework has released their 2025 Annual Report. The report details the highlights and challenges for the Australian sheep industry for the 2024/25 Financial Year.
The highlights include:

- An increase in the proportion of producers using appropriate pain management for key husbandry procedures which is improving animal welfare outcomes.
- More lambs and sheep are being processed through establishments accredited by the Australian Animal Welfare Certification System (AAWCS), strengthening supply chain trust.
- Greenhouse gas emission intensity in sheepmeat processing declined by 15% and water use per tonne by 7% in the past two years, reflecting improved resource efficiency.

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

Passive immunity establishment through colostral IgG absorption in neonatal ruminants: Foundation for efficient ruminant production

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Animals, Volume 15, Issue 21, November 2025 **OPEN ACCESS**

DOI https://doi.org/10.3390/ani15213093

Simple Summary
Young animals are fundamental to sustainable animal husbandry, yet the management and feeding strategies for young ruminants face significant challenges, particularly high illness and mortality rates in early development. Neonatal hypogammaglobulinemia, a primary cause of this, results from the epitheliochorial placental structure preventing prenatal maternal immunoglobulin transfer. Consequently, neonatal ruminants must acquire essential passive immunity—defined as specific immune protection through passive reception of antibodies—solely via timely colostrum consumption. This review synthesizes current knowledge on the pathways, molecular mechanisms, and influencing factors of passive immunity establishment in neonatal ruminants, alongside its long-term impact on adult production performance. A comprehensive understanding of these aspects provides a scientific basis for optimizing colostrum feeding

strategies and guiding future research into IgG absorption mechanisms, ultimately enhancing ruminant health and productivity.

Abstract

Passive immunity, the acquisition of specific immune protection through external antibodies or immune components, is critically important for neonatal survival. In ruminants, however, neonatal hypogammaglobulinemia, a consequence of their epitheliochorial placental structure preventing prenatal antibody transfer, often leads to high morbidity and mortality. Consequently, neonatal ruminants are entirely dependent on the timely consumption of colostrum to acquire sufficient immunoglobulin G (IgG) for protection. Establishing robust passive immunity is therefore a cornerstone for their survival, healthy development, and future production efficiency. This review synthesizes current knowledge on the establishment of passive immunity in neonatal ruminants. We first outline the fundamental principles of passive immunity transfer, then delve into the specific pathways and molecular mechanisms in ruminants. Key factors influencing this process are subsequently discussed. Furthermore, we highlight the long-term impact of passive immunity on adult production performance. This review aims to provide a scientific foundation for optimizing colostrum management strategies and to stimulate future research into the intricate mechanisms of IgG absorption.

Selenium as a modulator of reproductive immunity: Molecular insights and translational potential in livestock

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Reproduction in Domestic Animals, Volume 60, Issue 11, November 2025 **OPEN ACCESS**

DOI https://doi.org/10.1111/rda.70146

Abstract

The periparturient period represents a critical window of vulnerability in livestock reproduction. Additionally, reproductive performance is often compromised due to a weakened immune system and high oxidative stress. Selenium, an essential micronutrient, emerges as a key element with dual roles in antioxidant defence and immune modulation, making it a cornerstone in maintaining reproductive health in livestock. Selenium exerts its protective effects through incorporation into selenoproteins such as glutathione peroxidase (GPx), which downregulate oxidative stress, support cellular integrity, and regulate inflammation in reproductive tissues. During the periparturient period, selenium deficiency is associated with increased production of β-hydroxybutyric acid (BHBA) and non-esterified fatty acids (NEFA), responsible for triggering lipid mobilisation and activation of the NF-κB (Nuclear Factor kappa-light-chain-enhancer of activated B cells) signalling pathway. This leads to overexpression of pro-inflammatory genes, resulting in uterine infections, mastitis, and other reproductive disorders. Selenium supplementation in organic or nano forms plays a potential role in countering these effects by activating the Nrf2 (Nuclear factor erythroid 2) pathway, boosting antioxidant enzymes, and suppressing the NF-κB pathway. In females, selenium enhances endometrial epithelial repair, hormone regulation, and immune tolerance by regulating the NF-κB signalling pathway. In males, combined supplementation of selenium with vitamin E improves sperm quality, motility, and testosterone levels while preventing lipid peroxidation in spermatozoa. At the epigenetic level, selenium influences histone acetylation to regulate transcription of inflammatory genes such as COX-2 and TNF-α. Recent insights into the role of selenium receptors (LRP8) in ovarian follicular development highlight the applications of selenium in fertility regulation. The efficacy of selenium is highly influenced by its form, dosage, animal species, and physiological state. This review emphasises the need for large-scale, species-specific research trials, nanodelivery strategies, and omics-based biomarkers to improve selenium supplementation strategies and dose rate.

Selenium holds significant translational potential in veterinary reproduction, playing a preventative and therapeutic role against reproductive immunopathologies in livestock.

Scientific papers

Effects of GnRH administration on pregnancy rate and litter size in ewes subjected to a fixed time insemination program during the breeding season

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Animal Reproduction Science, Volume 282, November 2025

DOI https://doi.org/10.1016/j.anireprosci.2025.108010

Highlights

- The effects of GnRH given at various time points of an FTAI program were evaluated in ewes.
- GnRH given at sponge insertion increased the pregnancy rate and litter size in ewes.
- GnRH given at sponge insertion is a simple and effective strategy for increasing fertility in ewes.

Abstract

The objective of this study was to compare the effects of GnRH administration at sponge insertion, insemination, and day 7 post-insemination on pregnancy rate and litter size in ewes subjected to a fixed time insemination program during the breeding season. Estrus was synchronized in 2740 adult ewes at four experimental sites. An intravaginal sponge impregnated with flurogestone acetate was left in the vagina for 12 days followed by an injection of 330 IU of eCG at sponge removal. Each ewe was in seminated twice at 52 h and 64 h after sponge removal. In the Insertion group, the Insemination group, and the Post-insemination group, ewes were given an intramuscular administration of 25 μg of the GnRH agonist triptorelin at sponge insertion, insemination, and day 7 post-insemination, respectively. No GnRH administration was given in the Control group. Pregnancy rates and litter size were calculated after all deliveries were completed. The results showed that the pregnancy rates in the Insertion group were significantly higher compared to those in the Control group, the Insemination group, and the Post-insemination group. The litter size in the Insertion group and the Insemination group were not significantly different and were significantly higher compared to those in the Control group and the Post-insemination group. In conclusion, the fixed time artificial insemination program, in combination with GnRH administration at sponge insertion, is a simple and potentially effective strategy for increasing fertility in estrus-synchronized ewes during the breeding season.

Maternal supplementation with melatonin during mid-pregnancy does not improve the survival of one-year-old ewes and their lambs

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Journal of Animal Science, Volume 103, November 2025 **OPEN ACCESS**

DOI https://doi.org/10.1093/jas/skaf387

Abstract

Mating ewes to lamb at 12 months of age can enhance lifetime productivity but is associated with lower reproductive performance and lamb survival compared to mature ewes. Melatonin, an antioxidant, has been shown to improve placental function and lamb health when mature ewes were supplemented during pregnancy. This study investigated the effect of supplementing one-year-old ewes with melatonin during mid-pregnancy on their survival and the live weight and survival of their lambs. Single- (n = 2711) and twin-

bearing (n = 2605) ewes were randomly assigned to Melatonin or Control groups at seven farms across southern Australia. The Melatonin group received an 18 mg implant of melatonin at 90–100 days after the introduction of the rams, which releases melatonin for 60–80 days, and the Control group were not supplemented with melatonin. Seven farms recorded paddock level information including fetal number, lambs present 200 days after the introduction of the rams, ewes present pre-lambing and 200 days after the introduction of the rams and ewe pre-lambing live weight. Lamb survival and ewe mortality were analysed with analysis of covariance. Three of the seven farms also collected individual lambs' information (n = 4303 lambs) including weight at birth and weaning of lambs, date of birth and weaning, lamb sex and birth type. Individual lamb live weights and growth rate to weaning were analysed with linear mixed models. Supplementation with melatonin had no effect ($P > 0.05$) on ewe or lamb survival. Melatonin supplementation did not impact ($P > 0.05$) individual lamb birthweight, growth rate, or weaning weight, consistent with the observed results in lamb survival. These findings suggest that maternal supplementation with melatonin mid-pregnancy is not an effective strategy to improve the survival or early growth of lambs, or the survival of one-year-old ewes.

MLA project P.PSH.1180 More lambs from ewe lambs through developing and extending best practice

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Breed and season: Key determinants of efficiency in large-scale commercial *in vitro* sheep embryo production

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Animals, Volume 15, Issue 22 **OPEN ACCESS**

DOI https://doi.org/10.3390/ani15223354

Simple Summary

Reproductive technologies like in vitro embryo production (IVEP) allow farmers to rapidly multiply the best genetics in their sheep flocks. A key step is collecting oocytes from valuable donor ewes using a technique called laparoscopic ovum pick-up (LOPU). However, the success of this process can vary. Our study investigated how the sheep's breed and the season affect the entire production chain, from oocyte collection to the birth of a lamb via embryo transfer. We found that the best outcomes are achieved by matching specific breeds to their optimal season. The breeds demonstrated a clear functional division of labor: some were identified as superior oocyte donors, while others showed greater efficacy in supporting pregnancy. Crucially, we show that autumn and winter are the most productive seasons. By aligning breeding schedules with these breed-season combinations, sheep farmers can significantly improve the efficiency and success of their advanced breeding programs.

Abstract

The efficiency of the laparoscopic ovum pick-up and in vitro embryo production (LOPU-IVEP) system in sheep is governed by a complex interplay of genetic and environmental factors. This study comprehensively evaluated the effects of breed, season, and their interaction on oocyte retrieval, embryonic development, and pregnancy outcomes in a commercial setting. A 20-month longitudinal analysis of a large commercial cohort revealed that breed was the dominant factor determining oocyte quantity, whereas season exerted a stronger influence on developmental competence. Specifically, autumn and winter provided optimal conditions, resulting in significantly higher cleavage rate and blastocyst rate ($p < 0.05$). A significant breed-by-season interaction further identified synergistic combinations, such as Black-headed Suffolk in autumn or winter, which achieved superior embryo production outcomes. Importantly, correlation analysis revealed that oocyte quantity was not predictive of developmental potential or pregnancy success. These findings

underscore the necessity of a precision management strategy that aligns LOPU schedules with breed-specific seasonal advantages to maximize annual embryo production efficiency.

Late pregnancy is associated with an increase in regulatory cytokines and a decline in nematode-specific antibody levels in sheep infected with *Teladorsagia circumcincta*

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Veterinary Parasitology, Volume 340, December 2025 **OPEN ACCESS**

DOI https://doi.org/10.1016/j.vetpar.2025.110617

Highlights

- We found a typical peri-parturient rise (PPR) in nematode faecal egg count.
- This was accompanied by declines in nematode-specific antibodies.
- We found levels of the cytokines IFN-γ and IL-4 declined during the PPR.
- In contrast, the regulatory cytokine IL-10 increased around parturition.
- Our results reveal the contribution of cell-mediated immunity to the PPR.

Abstract

The gastrointestinal nematode *Teladorsagia circumcincta* is a prevalent and economically important parasite of sheep. Sheep develop acquired immunity to *T. circumcincta*, characterised by nematode-specific antibody production and a type 2 T-helper (Th) cell response. During late pregnancy and early lactation, ewes experience increased nematode faecal egg count (FEC) known as the peri-parturient rise (PPR). The PPR is associated with a decline in nematode-specific antibodies, but research on the role of Th cell-mediated immunity in the PPR is limited. Here, we characterised the cellular and humoral immune responses of *T. circumcincta*-infected ewes fed at two dietary levels during the peri-parturient period. Diet had a limited effect on any trait, likely due to the high body condition of the ewes entering the study, but all ewes experienced a pronounced PPR. The PPR was associated with a decline in nematode-specific IgG, and antigen-dependent declines of IgA and IgE around parturition. Levels of the Th1-associated cytokine IFN-γ and the Th2-associated cytokine IL-4 showed antigen-dependent declines during the peri-parturient period. In contrast, the regulatory cytokine IL-10 increased around parturition in response to a generic mitogen, nematode antigens and Heptavac® vaccine, suggesting a generalised regulatory immune phenotype. Our results provide a comprehensive view of the immunological changes during the PPR and suggest a possible role for regulatory T cell responses.

Dynamic responses of energy metabolism in ewes during late pregnancy: a meta-regression

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Animal, Volume 19, Issue 12, December 2025 **OPEN ACCESS**

DOI https://doi.org/10.1016/j.animal.2025.101690

Highlights

- This meta-analysis gathers all data on ewe metabolic status during late gestation.
- Litter size and dietary energy affect metabolism of ewes in late gestation.
- The metabolic challenge is increased for late pregnant ewes with larger litter sizes.
- Data on the energy demands of ewes bearing three or more lambs are scarce.
- This study provides data to better adapt dietary energy based on litter size.

Abstract
Insufficient energy intake throughout late gestation in ewes can induce a negative energy balance, leading to hypoglycemia and hyperketonemia, and increasing the risk of metabolic diseases. Previous studies have investigated the changes in key metabolites related to energy metabolism during late gestation in sheep, focusing on glucose, non-esterified fatty acids (NEFAs), and beta-hydroxybutyrate (BHB). The current meta-analysis was undertaken to gather available data on the kinetic of energy metabolites throughout late gestation to investigate the metabolic status of ewes in relation to dietary energy level and litter size. For the construction of the database, dietary energy concentration of each experiment was classified into two categories: diets covering between 60 and 100% (E60) or covering more than 100% (E100) of the metabolizable energy requirement for twin-bearing ewes at 133 days of gestation according to the National Research Council (NRC, 2007). Treatment groups were also categorized according to average litter size: single ([1.0 – 1.5[), twins ([1.5 – 2.5[), triplets ([2.5 – 3.5[), and quadruplets+ ([3.5 – 5.0]). The analysis of the meta-design revealed a quadratic effect of day to lambing for circulating glucose. For both dietary energy levels, glucose concentration decreased from day –42 to reach a nadir between days –28 and –14, depending on litter size group, and then increased until day –1. A greater decrease in glucose concentration was observed with larger litter sizes and was more pronounced for E60 than E100 groups. For each litter size group, when comparing similar days in gestation, E100 groups showed greater glucose concentrations than E60. Circulating NEFA increased linearly throughout late gestation, and a significant interaction was observed between dietary energy level and litter size group. For a similar litter size, NEFA concentrations were higher for E60 compared with E100, and the increase in NEFA concentrations with litter size was more pronounced for E60 than E100 groups, during the last 42 days in gestation. Circulating BHB increased with gestation. The intercept was higher and the slope steeper for E60 compared with E100, as well as for groups of ewes bearing larger litters. The meta-regression developed demonstrate the impact of litter size on energy requirements of ewes in late gestation. The meta-design also highlighted that data on the energy demands of ewes bearing three or more lambs are scarce. Of all the dietary treatments gathered in this meta-analysis, according to NRC (2007), only three provided an adequate amount of energy for triplet-bearing and none for quadruplet+-bearing ewes.

Energy metabolism and ruminal fermentation of prolific ewes in response to scheduled increase, relative to lambing, of dietary energy source and concentration

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Animal, Volume 19, Issue 12, December 2025 **OPEN ACCESS**

DOI https://doi.org/10.1016/j.animal.2025.101691

Highlights

- Influence of high-energy diet on energy balance in late-gestation prolific ewes.
- Prolific ewe intake was positively affected by high-energy diet in late gestation.
- High-energy diet 4 weeks prepartum improved energy balance in prolific ewes.
- Blood beta-hydroxybutyrate was influenced not only by energy balance but also by dietary composition.
- Corn silage can be used to increase energy supply in diets of late-gestation prolific ewes.

Abstract
In late gestation, to prevent metabolic disorders in prolific ewes, energy intake must align with increased energy requirements to support the growth of multiple fetuses. The present study was conducted to evaluate if providing a high-concentrate diet as early as 4 compared with 2 weeks prepartum was more effective to

meet the energy requirements of prolific breeds, and if corn silage could serve as a high-energy feed for late gestational ewes. Eight weeks prepartum, 48 mature F1 crossbred (Dorset × Romanov) ewes were offered grass-legume silage ad libitum. Four weeks later, ewes were randomly assigned to 1 of 3 isoenergetic dietary treatments: a grass-legume silage and ground corn−based total mixed ration (TMR) from week –4 (GC4; gradual increase from week −5) or week –2 (GC2; gradual increase from week −3) or a corn silage−based TMR from week –4 (CS4; gradual increase from week −5). Dry matter intake (DMI), body condition score (BCS), circulating beta-hydroxybutyrate (BHB), glucose, and non-esterified fatty acids (NEFAs) were monitored throughout the last 6 weeks of gestation. Rumen fluid was collected at week –2 to assess fermentation parameters. Inclusion of concentrates at 4 (GC4) compared with 2 weeks prepartum (GC2) resulted in greater DMI and metabolizable energy intake (MEI) from weeks –5 to –3. Overall, during the last 5 weeks of gestation, DMI and MEI of GC4 ewes were 12 and 20% higher compared with GC2, respectively. At week –4, circulating BHB concentrations were greater for GC4 compared with GC2, despite an improved energy balance, as confirmed by lower NEFA and greater glucose concentrations. Except for a decreased DMI and MEI at week –4, resulting in greater NEFA concentrations for CS4 compared with GC4, no other impact of energy source on intake or circulating NEFA and glucose was observed during the prepartum period. Compared with GC4, BHB concentrations were greater for CS4 from weeks –5 to –1. Rumen proportion of acetate decreased by 7%, whereas butyrate increased by 22% for CS4. The current experiment demonstrates that blood BHB concentration was affected by dam energy balance and ingredient composition of the diet. Our study also shows that a high-energy corn silage diet provided from 4 weeks prepartum can prevent the onset of a negative energy balance during late gestation, similar to adding ground corn to a grass-legume silage-based diet.

Cyclic guanosine monophosphate (cGMP) improves freezing tolerance of sheep embryos by reducing lipid content

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Theriogenology, Volume 250, January 2026

DOI https://doi.org/10.1016/j.theriogenology.2025.117685

Abstract

Embryos produced in vitro exhibit heightened cryosensitivity due to excessive lipid accumulation. Previous studies demonstrated that cyclic guanosine monophosphate (cGMP) modulates intracellular lipid metabolism through cGMP-dependent protein kinase (PKG) signaling in various cell types. This study investigated the effects of cGMP on (i) cryosurvival in sheep embryos, (ii) embryonic quality, and (iii)lipolysis-related parameters. Specifically, we quantified lipid droplet content, free fatty acid levels, and hormone-sensitive lipase (HSL) phosphorylation status as key indicators of lipolytic activity. The results showed that cGMP pretreatment (0.5 mM) for 10 min prior to slow freezing significantly improved post-thaw embryo recovery rates and upregulated the mRNA expression of key developmental genes (POU5F1, SOX2, CDX2, and NANOG). cGMP pretreatment significantly upregulated the expression of multiple lipid catabolism genes (ACSL4, HMGCR, HMGCS1, LIPE, LPL, LIPF, and PLIN2), with LIPE (encoding HSL) exhibiting the most pronounced induction (27.10-fold increase vs. control). Following cGMP pretreatment, PKG activation triggered significant increases in the intracellular Ca2+ level and Calcium/Calmodulin-dependent Protein Kinase II (CaMKII) phosphorylation. Given HSL's established sensitivity to calcium fluctuations, this cascade led to markedly enhanced HSL phosphorylation. Concurrently, blastocysts exhibited a significant reduction in lipid droplet content, while transient fatty acid accumulation was observed due to enhanced HSL activity. In summary, pretreating sheep blastocysts with cGMP prior to slow freezing notably elevates their cryotolerance. This enhancement is achieved by triggering the lipolytic activity of HSL via the

PKG/CaMKII/Ca2+ signaling cascade. This pathway specificity underscores cGMP's potential as a targeted cryoprotectant adjuvant for assisted reproductive technologies, particularly for improving the cryosurvival of lipid-rich embryos.

Proanthocyanidin B2 regulates epidermal growth factor receptor (EGFR) and activates the PI3K/AKT pathway to promote the in vitro maturation of sheep oocytes

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Theriogenology, Volume 251, February 2026

DOI https://doi.org/10.1016/j.theriogenology.2025.117726

Highlights

- PCB2 stage-specific optimally enhances oocyte maturation and embryo development at distinct, dose-dependent concentrations.
- 5 μg/mL PCB2 promotes oocyte maturation by activating EGFR-PI3K/AKT and reducing oxidative stress, with normal meiosis.
- 7.5 μg/mL PCB2 maximizes blastocyst formation via potent antioxidant protection against metabolic and oxidative challenges.

Abstract

This study investigated Proanthocyanidin B2 (PCB2), a potent antioxidant flavonoid, using sheep oocytes as a model to assess its effects on oocyte developmental competence, oxidative stress levels, mitochondrial function, and early apoptosis. During in vitro maturation (IVM), supplementation with 5 μg/mL PCB2 significantly increased the first polar body extrusion rate compared to concentrations of 0, 2.5, and 7.5 μg/mL ($P < 0.05$). Further analysis revealed that 5 μg/mL PCB2 significantly reduced reactive oxygen species (ROS) levels while increasing glutathione (GSH) levels ($P < 0.05$). Additionally, this treatment enhanced mitochondrial activity, endoplasmic reticulum function, mitochondrial membrane potential, and cortical granule dynamics ($P < 0.05$). To elucidate the underlying mechanisms, Smart-seq RNA sequencing was employed to identify differentially expressed mRNAs (DEMs) in oocytes treated with 5 μg/mL PCB2. Compared to the control, 585 DEMs (293 upregulated and 292 downregulated) were detected. Transcriptomic analysis suggested that PCB2 promotes cytoplasmic maturation by enhancing EGFR-mediated activation of the PI3K/AKT pathway, thereby mitigating oxidative stress and preserving mitochondrial function.

Proteins as indicators of stress and pain in lambs after castration by a rubber ring with and without pain relief

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Theriogenology, Volume 251, February 2026 **OPEN ACCESS**

DOI https://doi.org/10.1016/j.theriogenology.2025.117741

Highlights

- Cortisol was elevated for an hour in lambs after castration by a rubber ring without pain relief.
- Substance P and thiol oxidation did not significantly differ or change over time among the lambs.
- SWATH-MS analysis quantified 287 plasma proteins in lambs castrated with and without pain relief.

- A significant number of unique proteins were present in lambs after castration without pain relief.
- Meloxicam and Bupivacaine appeared to be effective as pain relief for lambs after castration.

Abstract

The objective assessment of pain in animals is challenging because indicators that are used are complex to interpret, inconsistent in presentation, and unable to distinguish between pain that varies in intensity and severity. The present study evaluated substance P, thiol oxidation, and plasma proteins as candidate indicators of pain in lambs. Merino ram lambs at 10–12 weeks of age were assigned, based on live weight, to one of four treatments: ring castration (n = 13), ring castration with pain relief (Meloxicam and Bupivacaine, n = 14), sham castration (n = 14), or sham castration with pain relief (n = 13). Samples of whole blood and plasma were collected pre-treatment and periodically after the treatment. The plasma concentration of cortisol was increased in the lambs 60 min after castration by a rubber ring without pain relief, while there was no change in the other treatment groups. There was no meaningful difference or change over time in substance P or thiol oxidation among the treatment groups. Proteomic analysis revealed no difference in the plasma proteins that were quantified between the lambs that were castrated by a rubber ring with and without pain relief. However, there was a significant increase in the number of unique proteins present 30 min and 2 h after castration in the lambs that were not provided with pain relief. Proteomics remains a promising avenue and targeted proteomic analysis is warranted to validate the proteins that were identified as indicators of stress or pain in lambs.

Punicalagin enhances cryopreserved sheep sperm quality via activation of the Nrf2/PGC-1α pathway and metabolic reprogramming

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Theriogenology, Volume 251, February 2026

DOI https://doi.org/10.1016/j.theriogenology.2025.117748

Highlights

- Punicalagin ameliorates cryopreservation-induced oxidative damage and improves post-thaw sperm motility and quality in rams.
- Punicalagin's protective efficacy depends on Nrf2 activation, as its benefits were abolished by the Nrf2 inhibitor ML385.
- First evidence that Nrf2 activation is the central mechanism mediating punicalagin's cryoprotective effects in ram sperm.
- Punicalagin shows dual effects: robust antioxidant action via Nrf2, and potential secondary effects when Nrf2 is inhibited.
- Punicalagin is a novel effective natural additive for optimizing sperm cryopreservation in sheep and other ruminants.

Abstract

Cryopreservation-induced damage remains a major limitation in ram semen preservation, particularly impairing sperm motility and structural integrity. Punicalagin, a natural polyphenol with potent antioxidant properties, has demonstrated potential in mitigating oxidative stress. However, its role in ruminant sperm cryoprotection remains incompletely understood. This study investigated the effects of punicalagin on cryopreserved sheep sperm and explored its underlying molecular mechanisms. A comprehensive evaluation of cryodamage indicators was conducted before and after cryopreservation. The effects of varying punicalagin concentrations (0–20 μM) on sperm motility, antioxidant pathways, and mitochondrial function were assessed. Untargeted metabolomics was employed to characterize changes in the sperm metabolite

profile, and the selective Nrf2 inhibitor (ML385) was employed to validate the core signaling pathway. Cryopreservation significantly reduced progressive motility, mitochondrial membrane potential (ΔΨm, MMP), and adenosine triphosphate (ATP) levels while increasing tail bending rate and acrosomal defects ($P < 0.05$). Supplementation with punicalagin (15 μM) significantly decreased reactive oxygen species (ROS) levels, enhanced superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) activities, restored ΔΨm, and downregulated apoptotic markers (Bax/Fas) ($P < 0.05$). Punicalagin activated the Nrf2/PGC-1α pathway, an effect abolished by ML385. Metabolomic analysis revealed punicalagin-regulated lipid metabolism and identified five differential metabolites, including 20-COOH-10,11-dihydro-LTB4, Acylcarnitine 12:2, Acylcarnitine 20:6, Pyridine, and Undecanoic acid, as metabolic biomarkers for cryodamage evaluation (AUC >0.9). These findings demonstrate that punicalagin enhances sperm cryotolerance through dual mechanisms involving antioxidant pathway activation and metabolic reprogramming, providing a novel strategy for improving ruminant sperm cryopreservation.

A novel therapeutic approach: The effect of letrozole-assisted synchronization on reproductive performance in suckling ewes during the non-breeding season

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Theriogenology, Volume 251, February 2026

DOI https://doi.org/10.1016/j.theriogenology.2025.117751

Highlights

- Letrozole supported endocrine priming by increasing pre-synchronization FSH levels.
- Post-mating luteal function improved in response to letrozole pretreatment.
- Fertility during anoestrus improved in suckling ewes following letrozole pretreatment.
- Letrozole given 4 days before synchronization was more effective than at protocol start.
- Letrozole pretreatment may offer a practical alternative to early weaning in ewes.

Abstract

The aim of the present study was to investigate the effect of letrozole treatment on the success of a progesterone-based synchronization protocol and fertility parameters in suckling Awassi ewes during the anoestrus period.

One hundred and five multiparous, suckling Awassi ewes were used. Ewes (n = 105) were randomly divided into three groups as Control (n = 35), LET-4 (n = 35) and LET-0 (n = 35). Four days before the synchronization protocol (D-4), letrozole at a dose of 1 mg/kg was administered subcutaneously (s.c.) to the LET-4 group, while the LET-0 group received it on the day of protocol initiation (D0). The control group received 1 mL of benzyl alcohol s.c. at both time points as a placebo. Subsequently, intravaginal sponges containing 60 mg medroxyprogesterone acetate were inserted into all ewes (D0). The sponges were kept in the vagina for 7 days and 500 IU eCG and 75 μg d-cloprostenol were administered intramuscularly on the day the sponges were removed. Blood samples were taken for measurement of serum E2, FSH and P4 before sponge insertion (D-4 and D-2), on the day of sponge insertion (D0), after sponge insertion (D2 and D4), at mating (DM) and post-mating days (DPM-7, 14, 21, 35 and 50). Pregnancy was diagnosed by transrectal ultrasonography at DPM-21, 35, and 50.

The overall mean serum FSH concentrations, calculated across all sampling days, were significantly higher in the LET-4 group (27.68 ± 1.56 mIU/mL) than in LET-0 (21.85 ± 0.87 mIU/mL) and Control (18.54 ± 0.91 mIU/mL) ($P < 0.001$). Subsequent to the FSH elevation, serum E2 levels at the time of mating were also significantly higher in LET-4 (22.24 ± 1.20 pg/mL) than in LET-0 (14.47 ± 0.92 pg/mL) and Control (10.77 ± 0.69

pg/mL) ($P < 0.001$). Post-mating P4 concentrations were likewise significantly elevated in the LET-4 group (3.79 ± 0.24 ng/mL) compared to LET-0 (3.29 ± 0.24 ng/mL) and Control (2.78 ± 0.24 ng/mL) groups ($P < 0.001$). Estrus rate was highest in the LET-4 group (90.62 %) compared to LET-0 (68.57 %) and Control (57.57 %) ($P = 0.010$). Pregnancy rate on day 50 was significantly greater in LET-4 (59.37 %) than in LET-0 (37.14 %) and Control (27.27 %) ($P = 0.027$). Fecundity rate was also highest in LET-4 (87.50 %) compared to LET-0 (57.14 %) and Control (33.33 %) ($P < 0.001$). No significant differences were observed among groups in litter size, twinning or pregnancy losses rates ($P > 0.05$).

In conclusion, letrozole administration four days before the synchronization protocol significantly enhanced reproductive performance in suckling ewes during the non-breeding season. These results suggest that this approach could be a promising alternative strategy to improve protocol success without resorting to early weaning.