

# Sheep reproduction RD&A alert

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

The <u>Sheep Producer Intentions Survey</u> (SPIS) is now open.

MLA and AWI conduct the SPIS twice each year, in February and October. The focus of the October survey is the breeding ewe population and is seeking information on producers' lamb flocks and their intentions for the remainder of the spring season. The survey will gather information on:

- lamb sales to date
- projected lamb sales to the end of 2024 and,
- forecasted sales for the first half of 2025.

This data will provide the sheep industry with an estimate of lamb supply volumes and timing.

Please click <u>here</u> to complete the survey.

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

#### Unlocking the potential of precision sheep management to improve productivity and profitability

#### Background

Mandatory nationwide implementation of electronic identification (EID) in sheep will begin on 1 January 2025, driven by heightened biosecurity concerns and a desire for more effective animal traceability systems. This presents a major opportunity to exploit the wider range of applications available that EID will enable for precision sheep management (PSM). Despite EID tags and associated technology being readily available in Australia for more than 10 years the adoption of PSM has been relatively low. The overall goal of PSM is to manage mobs of sheep differentially according to their level of production or risk, rather than managing all sheep animals within a flock equally.

#### Aim

To raise awareness of the opportunities in EID usage and PSM based on scientific evidence and a strong economic and social business case and support the adoption of these opportunities utilising existing and novel extension methods.

#### **Project Objectives**

- Awareness, learnings and experiences relating to adoption of EID and PSM to improve weaning rates and weaner growth rates extended to sheep producers and advisors via existing networks.
- Develop defined business cases for the selection and management benefits of investing in the extra EID infrastructure beyond that necessary to meet the statutory requirements.

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- Develop PSM learning modules tailored to engage the continuum of producers with varying technical capability and infrastructure, including piloting these modules with 10 producer groups.
- Support producers at on-farm demonstration sites to implement plans for using EID & PSM technology to monitor interventions for improving reproductive performance and weaner growth rates.
- Quantify reproductive rate, lamb survival, weaner growth rate or wool production responses for different cohorts of animals within flocks from analysis of existing data sets and from on-farm participatory R&D sites and develop rules-of-thumb for optimum differential management of cohorts, based on the identified responses.

#### **Current progress**

This project has recently been contracted. If you would like more information about getting involved in the project, please contact Forbes Brien (0427 004 066 <u>forbes.brien@adelaide.edu.au</u>).

### **Review papers**

#### The effects and mechanisms of heat stress on mammalian oocyte and embryo development

L.I. Lang, Zhen-zhen Wang, Bin Liu, S.H.E.N. Chang-qing, T.U. Jing-yi, W.A.N.G. Shi-cheng, L.E.I. Rui-ling, P.E.N.G. Si-qi, X.I.A.O. Xiong, Z.H.A.O. Yong-ju and Xiao-yan Qiu (<u>qiuxiaoyan168@163.com</u>)

Journal of Thermal Biology, Volume 124 August 2024

DOI https://doi.org/10.1016/j.jtherbio.2024.103927

#### Highlights

- This article offers a systematic overview of recent effects and mechanisms of heat stress on mammalian oocyte and embryo development.
- The article discusses recent measures to ameliorate HS effects, including antioxidant use, mitochondrial function enhancement, gene editing, and environment management.
- This article serves as a valuable reference for enhancing mammalian embryo development and economic benefits under HS in animal production.

#### Abstract

The sum of nonspecific physiological responses exhibited by mammals in response to the disruption of thermal balance caused by high-temperature environments is referred to as heat stress (HS). HS affects the normal development of mammalian oocyte and embryos and leads to significant economic losses. Therefore, it is of great importance to gain a deep understanding of the mechanisms underlying the effects of HS on oocyte and embryonic development and to explore strategies for mitigating or preventing its detrimental impacts in the livestock industry. This article provides an overview of the negative effects of HS on mammalian oocyte growth, granulosa cell maturation and function, and embryonic development. It summarizes the mechanisms by which HS affects embryonic development, including generation of reactive oxygen species (ROS), endocrine disruption, the heat shock system, mitochondrial autophagy, and molecular-level alterations. Furthermore, it discusses various measures to ameliorate the effects of HS, such as antioxidant use, enhancement of mitochondrial function, gene editing, cultivating varieties possessing heat-resistant genes, and optimizing the animals'rearing environment. This article serves as a valuable reference for better understanding the relationship between HS and mammalian embryonic development as well as for improving the development of mammalian embryos and economic benefits under HS conditions in livestock production.

# The potential effect of melatonin on in vitro oocyte maturation and embryo development in animals

Parisa Nadri (<u>parisa.nadri@yahoo.com</u>), Azadeh Zahmatkesh and Azizollah Bakhtari (<u>azizollah.bakhtari@uconn.edu</u>) Biology of Reproduction, Volume 111, Issue 3, September 2024 **DOI** https://doi.org/10.1093/biolre/ioae077

#### Abstract

Melatonin is a hormone mainly secreted by the pineal gland during the circadian cycle, with low levels during the daytime and prominent levels during the night. It is involved in numerous physiological functions including the immune system, circadian rhythm, reproduction, fertilization, and embryo development. In addition, melatonin exerts anti-inflammatory and antioxidant effects inside the body by scavenging reactive oxygen and reactive nitrogen species, increasing antioxidant defenses, and blocking the transcription factors of pro-inflammatory cytokines. Its protective activity has been reported to be effective in various reproductive biotechnological processes, including in vitro maturation (IVM), embryo development, and survival rates. In this comprehensive review, our objective is to summarize and debate the potential mechanism and impact of melatonin on oocyte maturation and embryo development through various developmental routes in different mammalian species.

### **Scientific papers**

# Divergent planes of nutrition in mature rams influences body composition, hormone and metabolite concentrations, and offspring birth measurements, but not semen characteristics or offspring growth

Kerri A Bochantin-Winders, Friederike Baumgaertner, Jennifer L Hurlbert, Ana Clara B Menezes, James D Kirsch, Sheri T Dorsam, Christopher S Schauer and Carl R Dahlen (<u>carl.dahlen@ndsu.edu</u>)

Journal of Animal Science Volume 102

DOI https://doi.org/10.1093/jas/skae207

#### Abstract

Objectives of this experiment were to characterize the effects of ram plane of nutrition on body composition, concentrations of hormones and metabolites, sperm characteristics, and offspring outcomes. Mature Rambouillet rams (n=24, BW=82.9±2.63 kg) were individually housed and randomly assigned to either a positive (POS; n=8), maintenance (MAINT; n=8), or negative (NEG; n=8) plane of nutrition for an 84-day feeding period. Rams were fed a common diet, with daily feed allocations adjusted weekly based on body weight (BW) to achieve the targeted weight gain or loss (approximately 12% of initial BW). On 0, 28, 56, and 84-d, body condition score (BCS) and scrotal circumference (SC) were recorded, and blood and semen were collected. Following the feeding period, rams were placed in pens with 10 ewes each for a 28-d breeding period. Ewes were managed similarly throughout gestation and body weight and measurements were recorded at birth and weaning. Data were analyzed as repeated measures in time where appropriate with the mixed procedure of SAS, and individual ram was the experimental unit for all analysis. Ram BW was influenced by a treatment  $\times$  day interaction (P<0.001), with POS (0.12±0.01 kg) having greater daily weight change than MAINT (0.1±0.01 kg), which was greater than NEG (-0.12±0.01 kg). Ram BCS and SC were influenced by treatment × day interactions (P≤0.01), being similar on day 0 but POS being greater than NEG by day 56. Concentrations of triiodothyronine (T3) and T3:T4 ratio exhibited treatment × day interactions (P≤0.02), as POS had greater values than NEG by day 84 (P≤0.02). Concentration of insulin-like growth factor1 was greater in POS than MAINT and NEG (P $\leq$ 0.02), and non-esterified fatty acids and thyroxine (T4) were influenced by a day effect (P $\leq$ 0.01), but testosterone was unaffected (P $\geq$ 0.09). Minimal differences in semen volume, sperm concentration, motility, or morphology were observed among treatments (P $\geq$ 0.31). A similar proportion of ewes bred by rams in the respective treatments lambed and weaned lambs (P $\geq$ 0.54). Birth weight, chest circumference, and shoulder-hip length were greater (P $\leq$ 0.05) in NEG lambs compared with POS and MAINT; however, no differences were detected in weaning weight and weaning body measurements (P $\geq$ 0.40). Findings suggest paternal nutrition during the period of sperm development may influence offspring outcomes, potentially as a result of in-utero programming of paternal origin.

# Ewe-lamb bond at birth and during lactation in an equatorial semi-arid environment is better in a native than in an introduced breed

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Applied Animal Behaviour Science, Volume 278, September 2024

DOI https://doi.org/10.1016/j.applanim.2024.106362

#### Highlights

- Santa lnes sheep develops stronger mother-offspring bond than Dorper.
- Ewe-lamb bond was weaker in twin than single lambs.
- The local breed (Santa Ines) appears better adapted than the introduced breed.

#### Abstract

Santa Ines and Dorper sheep are the most popular breeds in the Brazilian semi-arid region. The former is of local origin and less intensively selected for productivity, and the latter is an introduced exotic breed selected for meat production. Dorper lambs are heavier at birth than Santa Ines lambs, which might increase the need for birth assistance, negatively influencing the newborn and maternal behaviours. Dorper lambs grow faster, most likely influencing their behavioural strategies during lactation, depending less on their mother than Santa Ines lambs. The aim of this study was to compare the behaviours of Santa Ines and Dorper ewes and their lambs at birth and throughout lactation. A complementary aim was to determine if being single or twin lambs impact differently according to the breed. Twenty-five Santa Ines ewes and their 32 lambs (18 single and 14 twins) and 21 Dorper ewes and their 26 lambs (16 single and 10 twins) were used in the study. Motheroffspring behaviours were recorded from birth until 63 days after lambing. Dorper ewes had a greater incidence of dystocia (P = 0.02) and longer labour (P = 0.0001) than Santa Ines ewes. At birth, Santa Ines ewes displayed a greater frequency of low-pitched bleats (P = 0.0006) and tended to spend more time grooming their lambs than Dorper ewes (P = 0.07). Immediately after birth, Santa Ines lambs shook their head earlier than Dorper lambs (P = 0.003). The litter size effects were unrelated to the breed: ewes of single deliveries groomed their lambs earlier (P = 0.04), and those that delivered twins spent more time grooming the first lamb delivered (P = 0.01). Over the first two weeks after parturition, Santa Ines ewes displayed higher frequencies of head-up postures (P = 0.0001), high-pitched bleats (P = 0.02) and smelled their lambs more times (P = 0.0001) than Dorper ewes. Santa Ines lambs were closer to their mother when grazing (P = 0.0001), while Dorper lambs were closer to their mother when lying down (P = 0.049). In conclusion, Santa Ines sheep shows a stronger ewe-lamb bond than Dorper soon after parturition, and such differences persist at least until 9 weeks after lambing. The ewe-lamb bond was weaker in twin than single lambs, without differences in the degree of its strength between Santa Ines and Dorper sheep.

# Impact of periconceptional and gestational Vitamin D3 restriction on fetal folliculogenesis and anti-mullerian hormone secretion using sheep as a model

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Reproduction in Domestic Animals, Volume 59, Issue 9, September 2024

DOI https://doi.org/10.1111/rda.14717

#### Abstract

Ovarian reserve is a reflection of the overall female reproductive potential. Vitamin D status has been suspected to influence fetal development and female fertility. As maternal diet during pregnancy can affect fetal development and future fertility, we hypothesised that periconceptional and gestational Vitamin D restriction could affect folliculogenesis and AMH secretion in the offspring. Nineteen sexually mature Welsh mountain ewes were randomly assigned to Vitamin D3 deficient (VDD, n = 10) and Vitamin D3 control (VDC, n = 9) diets from 17 days (d) before mating, up to 127–130 days of gestation, when fetal ovaries were collected (3 from VDC and 6 from VDD). Serum 25(OH)D3 concentrations were lower in VDD compared with VDC (p < 0.05). Relative to total follicle number, the percentage of primordial follicles was higher (p < 0.05), while the percentage of primary follicles was lower (p < 0.05) in VDD group compared with VDC group fetal ovaries. The integrated density value and percentage of affected area in TUNEL staining in VDD group did not vary from VDC group fetal ovaries (p > 0.05). Relative expression of AMH mRNA and AMH protein in VDD fetal ovaries were not statistically different compared with controls (p > 0.05). The relative expression of VDR mRNA were lower in VDD compared with VDC group fetal ovaries (p < 0.05). These data indicate that maternal Vitamin D dietary restriction is associated with ovarian tissue stemness and increased primordial follicle number but does not promote normal follicle recruitment or development in sheep fetal ovaries.

## Maternal supplementation with dietary betaine during late gestation increased ewe plasma creatine and lamb thermoregulation under field conditions

Billie-Jaye Brougham, Alice C. Weaver, Alyce M. Swinbourne, Megan R. Tscharke, Amy L. Munn, Jennifer M. Kelly, David O. Kleemann and William H. E. J. van Wettere (<u>william.vanwettere@adelaide.edu.au</u>) Animals, Volume 14, Issue 17 September 2024 **OPEN ACCESS** 

DOI https://doi.org/10.3390/ani14172605

#### **Simple Summary**

Perinatal twin lamb mortality reduces productivity and reproductive efficiency in Australian sheep meat and wool enterprises. This study investigated whether supplementing 4 g of betaine into the diets of twin-bearing Merino ewes during late pregnancy would improve lamb viability measures and survival to weaning under field conditions. The results indicated that dietary supplementation with betaine increased plasma creatine concentrations in ewes at day 130 of gestation and lamb rectal temperature within 4–24 h following birth. While the findings of this study provide some new, promising results, more research in the field is required before supplementation with betaine during late gestation can be used as an on-farm strategy to improve lamb viability and survival.

#### Abstract

Twin lamb mortality is a significant economic problem impacting the Australian sheep industry. Maternal betaine supplementation improved lamb vigour and early post-natal survival when ewes and lambs were housed indoors, suggesting that betaine may be beneficial to feed under extensive pasture systems. This study investigated whether maternal betaine supplementation during late gestation would improve Merino twin lamb live weight, thermoregulation, vigour and survival to weaning under field conditions. Ewes

received dietary betaine at either 0 g/day (CTL; n = 115) or 4 g/day from day 110 of gestation (dG 110) until ~49 days post-partum (pp) (BET; n = 115). Measures indicative of lamb viability and survival were collected within 4–24 h of birth and at ~49 days pp and ~93 days pp. BET ewes had higher creatine and creatinine concentrations at dG 130 than CTL ewes (p < 0.05). BET lambs had a higher rectal temperature within 4–24 h following birth than CTL lambs (p < 0.05). CTL lambs were heavier at ~49 days pp and grew faster from birth to ~49 days pp than BET lambs (both p < 0.05). The time taken after release from the researcher to first suckling was quicker in the CTL lambs than BET lambs (p < 0.05). This study demonstrated that supplementing betaine increased creatine concentration in twin-bearing ewes and thermoregulatory capacity in neonatal lambs under extensive grazing systems.

### Fetal programming in sheep: Epigenetic modifications in offspring from poorly nourished dams

Nicole M Tillquist, Sarah A Reed, Steven A Zinn, Kristen E Govoni Journal of Animal Science, Volume 102, Issue Supplement\_3, September 2024 DOI <u>https://doi.org/10.1093/jas/skae234.121</u>

#### Abstract

Poor maternal nutrition (restricted- and over-feeding) during gestation can negatively impact the efficiency of livestock production by generating offspring with decreased muscle mass, increased adiposity, and impaired metabolism. The mechanisms behind these altered phenotypes are not well-characterized. The effects of poor maternal nutrition during gestation on offspring growth and development begin in utero and contribute to metabolic dysregulation of offspring into maturity and across subsequent generations, demonstrating long-term negative effects on the livestock. Fetal programming results in epigenetic modifications that can involve changes in offspring gene expression and downstream function, with no alteration on the DNA sequence. Using a sheep model of poor maternal nutrition, we have previously shown that F1 offspring from restricted- and over-fed dams are smaller at 10 mo of age relative to offspring from control-fed dams. Additionally, we observed that this phenotype persisted in the F2 offspring from restrictedfed granddams, while F2 offspring from over- and control-fed granddams were of similar body weight. We have recently demonstrated that F1 offspring from poorly nourished ewes have differential methylation patterns and altered gene expression in liver tissue demonstrating a potential mechanism that contributes to persistent altered growth. We have also begun evaluating the effects of maternal nutrient restriction on the development of fetal small intestine, a key facilitator of nutrient transport. Poor maternal nutrition during gestation alters development of offspring small intestine and further understanding of developmental programming in key metabolic tissues can aid in the development of nutritional strategies to improve efficiency of livestock production.

#### Genetic analysis of a maternal assistance score in sheep

K. Hay (<u>kim.hay@sruc.ac.uk</u>), N. Lambe, J. Roden, S. Jarvis and C.M. Dwyer animal, Volume 18, Issue 9, September 2024 **OPEN ACCESS DOI** <u>https://doi.org/10.1016/j.animal.2024.101265</u>

#### Highlights

- An easy to measure maternal assistance score for sheep was assessed.
- The simple score is heritable and repeatable.
- The score was negatively genetically correlated with the number of lambs reared.
- This score could be used as a selection criterion to improve rearing percentage.
- This score could lead to an improvement of lamb welfare by reducing mortality.

#### Abstract

Maternal behaviour is important for lamb survival, as ewes perform many behaviours that affect the chances of a lamb surviving. Collecting maternal behaviour data directly at lambing is time-consuming and not considered suitable for acquiring the large volumes of data that would be required for using as selection criteria within commercial breeding flocks. The aim of this study was to investigate if a simple scoring system is heritable and assesses the expression of behaviours that reduce the probability of lamb mortality. Ewe behaviour was scored on a 3-point Maternal Assistance Score (MAS): (1) the ewe shows a high level of maternal interest (assumed if no intervention required); (2) the ewe shows limited interest in her lamb; and (3) the ewe shows no interest in her lamb. A total of 19 453 MAS were collected over 12 years, across 24 farms (including both indoor and outdoor lambing systems) and 12 different breed lines that make up the Innovis breeding programme. Ewe parity, breed, number of lambs carried, flock, lambing batch, lambing day within flock and pre-mating weight all had a significant effect on MAS (P < 0.05). The maternal assistance score was shown to be heritable ( $h_2 = 0.05$ ) and repeatable (0.10), positively genetically correlated to lambing difficulty (rg = 0.29) and amount of assistance the lamb required to suckle from the ewe (rg = 0.88), and negatively genetically correlated with the number of lambs successfully reared (rg = 0.49). This study shows that an easy-to-measure score can be used by shepherds with large breeding flocks, based on whether the ewe requires further assistance to support her lamb rearing. The score could be used in breeding programmes to select for lamb rearing ability in the future and potentially lead to an improvement in lamb welfare through a reduction in mortality.

#### Genetic parameters for growth and reproductive traits in Santa Inês sheep

Isis Regina Santos de Oliveira, Marisa Silva Bastos, Ana Paula del vesco, Zoila Catalina Rabanal Montalvan, Arnaldo Dantas Barreto Neto and Leandro Teixeira Barbosa (<u>leandro35441@academico.ufs.br</u>)

Small Ruminant Research Volume 239, October 2024

DOI https://doi.org/10.1016/j.smallrumres.2024.107327

#### Highlights

- The economic efficiency of animal farming is significantly influenced by the productive and reproductive traits.
- Few studies evaluate age first lambing and lambing intervals due to difficulty in correct measurements.
- Few estimates of genetics parameters of reproductive traits for sheep exist in Brazil.
- There are few studies that evaluate productive and reproductive traits simultaneously.
- The Bayesian method, although little used, allows for more accurate estimates of the genetic parameters.

#### Abstract

Although Santa Inês sheep demonstrate good adaptation to tropical environments, there is a need for effective breeding programs focused on growth and reproductive traits to harness the breed's full developmental potential. Thus, this study aimed to estimate (co)variance components and genetic parameters for weight at birth (WB), weight at 60 days (W60), age at first lambing (AFL), first lambing interval (FLI), and average lambing interval (AFI) in Santa Inês sheep. The data were obtained from the Sergipe Goat and Sheep Breeders' Association (ASCCO). (Co)variance components and genetic parameters were estimated by Bayesian analysis based on a bitrait model with Gibbs sampling using Multiple-Trait Gibbs Sampler for Animal Models (MTGSAM) software. Heritability estimates (h2) for WB, W60, AFL, ALI, and FLI were 0.11, 0.18, 0.028, 0.25, and 0.30, respectively. Positive correlations were observed between WB and W60, AFL and ALI, and AFL and FLI (0.31, 0.17, and 0.18, respectively). By contrast, negative correlations were obtained for

WB and AFL (-0.55), WB and AFL (-0.42), WB and FLI (-0.39), W60 and ALI (-0.26), and W60 and FLI (-0.37), with magnitudes ranging from low to moderate. Correlation values for ALI and FLI and W60 and AFL were positive and strong (0.97 and 0.98, respectively). These results indicate that the studied traits can be used for indirect selection. Furthermore, the findings underscore the importance of determining genetic parameters when formulating strategies for the genetic improvement of Santa Inês sheep.

# Effectiveness of human recombinant FSH for enhancing reproductive performance of ewes in progesterone injection-based estrus synchronization program

Amir Alijani, Amir Niasari-Naslaji (<u>niasari@ut.ac.ir</u>), Mohammad Baninajjar and Mahdi Ganjkhanlou Small Ruminant Research Volume 239, October 2024 **DOI** https://doi.org/10.1016/j.smallrumres.2024.107340

#### Highlights

- Human recombinant FSH (Cinnal-f) could enhance the prolificacy and the fecundity following estrus synchronization in sheep.
- Human recombinant FSH (Cinnal-f) could increase the tightness of estrus synchrony in combination with progesterone injection-based protocol during the breeding season in ewes.
- Time of laparoscopic AI relative to the time of estrus expression could alter sex ratio of lambs.

#### Abstract

Exploring the safe, cheap, and efficient gonadotropin to enhance reproductive performance of ewe is of worldwide demand. The purpose of this study was to examine the possibility of using human recombinant FSH (hrFSH) at the end of a short progesterone injection-based estrus synchronization program during the breeding season in ewe. Shal ewes (n=184) were assigned randomly into 4 groups using 2 (hrFSH or NohrFSH) by 2 (Mating or Laparoscopic artificial insemination; LapAI) factorial design during the breeding season. On Day 0 of the experiment, ewes received progesterone (37.5 mg; sc) and GnRH analogue (7.5 µg Alarelin acetate; im). On Days 3 and 6, ewes received 25 and 12.5 mg progesterone (sc), respectively. All ewes received prostaglandin F2 $\alpha$  analogue (250 µg Cloprostenol; im) on Day 6. On Day 7, ewes were divided into two equal groups and received hrFSH (75 IU Follitropin alfa; sc) or didn't receive any further treatment. Ewes displaying estrus in each group were assigned for mating for 5 days or LapAI at either 4–10 or 20–24 h after estrous detection. Data were analyzed using GLM and Genmod procedures. The tightness of estrus synchrony within 24 h was greater in hrFSH (89.4 %) than No-hrFSH (63.9 %) group (P<0.0001). The respective prolificacy and fecundity were greater in hrFSH (1.81 and 0.96) than No-hrFSH (1.26 and 0.68) group (P<0.05). The respective lambing rate and fecundity rate were greater for ewes mated (61.9 % and 0.96) compared to LapAI (45.5 % and 0.68; P<0.05). Ewes inseminated between 4 and 10 hours after estrous expression had greater sex ratio toward male offspring (1.89) compared to those inseminated between 20 and 24 hours (0.64, P<0.05). In conclusion, hrFSH could be considered as an effective gonadotropin for enhancing the tightness of estrus synchrony, prolificacy and fecundity in the progesterone injection-based estrus synchronization program in ewe.

### Investigation of chia seed (*Salvia hispanica* L.) extract supplementation to extender on postthawing ram sperm parameters and genes-related antioxidant capacity

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DOI https://doi.org/10.1016/j.smallrumres.2024.107345

#### Abstract

- Chia seed methanolic extract (MCSE) is rich in Omega-3 fatty acids and various phenolic and flavonoid compounds.
- MCSE demonstrated high antioxidant activity in DPPH and FRAP assays.
- MCSE at concentrations of 375 and 500 μg/mL significantly improved sperm parameters after equilibration, thawing, and incubation.
- 500 μg/mL MCSE improved sperm viability and antioxidant capacity, while reducing apoptotic sperm, caspase-3, and MDA in thawed semen.
- Adding 500 µg/mL MCSE to a Tris-soya bean lecithin extender enhances cryopreservation outcomes of ram sperm by acting as a natural antioxidant.

#### Abstract

Ram semen cryopreservation is not so widely used compared to bull semen. This study was carried out to explore the potential of methanolic chia seed extract (MCSE) in ram semen extender on kinematics, acrosome, apoptosis, antioxidant capacity, genes-related antioxidants, and caspase-3 in post-thawing ram semen. Semen from Rahmani rams (n=5) was collected, pooled, and diluted with Tris-extender (15 % egg yolk, 1 % soybean lecithin and 5 % glycerol) supplemented with MCSE (0, 125, 250, 375, and 500 μg/mL). MCSE had 67.5 % linolenic acid, 17.99 % linoleic acid, total phenolic, and flavonoid compounds. MCSE had higher antioxidant activity by DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) and FRAP (Ferric-reducing antioxidant power) assays. MCSE (500  $\mu$ g/mL) significantly improved sperm motility (43.6 vs. 30.0 %), vitality (44.0 vs. 30.7 %), and membrane integrity (43.1 vs. 31.3 %) as well as sperm velocity parameters and acrosome integrity (53.4 vs. 36.2 %) after thawing. MCSE at a level of 500 µg/mL increased viability, total antioxidant capacity level, and decreased apoptotic spermatozoa and malondialdehyde levels in post-thawed semen. MCSE at levels of 250, 375, and 500  $\mu$ g/mL decreased caspase-3 level to 42.8, 35.6, and 30.0 % compared to control (54.6 %) after thawing, and resulted in a clear up-regulation of antioxidant-related genes of superoxide dismutase 1, catalase, glutathione peroxidase 1, and GA-binding protein transcription factor subunit beta-1 (GAPP1). The highest impact was observed for MCSE on GAPP1 as compared to other profiles as well as for MCSE at 500 µg/mL as compared to with other levels. All MCSE levels decreased Caspase-3 mRNA by about 44, 51, 61, and 61 %, respectively. In conclusion, MCSE had higher anti-oxidative activity. Tris-soya bean lecithin extender supplemented with MCSE at a level of 500  $\mu$ g/mL, as a natural antioxidant, has a vital role in maintaining the freezing ability of ram spermatozoa after cryopreservation.

### **Upcoming events**

Date	Event	Location
8 Oct 2024	Picking Performer Ewes Pilot Workshop	Forbes, NSW
	AWI Extension NSW	
9 Oct 2024	Picking Performer Ewes Pilot Workshop	Bathurst, NSW
	AWI Extension NSW	
9 Oct 2024	Mineral supplementation for beef and sheep: What are	Webinar
	the options for mineral supplements and how to best	
	assess cost-effectiveness?	
	Meat & Livestock Australia & Agrista	
9 oct 2024	<u>Sheep eID – what's in it for me?</u>	Cambell Town, Tas
	AWI Extension Tas	
14 Oct 2024	RAMping up Repro	Moora, WA
	AWI Extension WA, Cranmore Merinos & Zoetis	

16 Oct 2024	RAMping up Repro	Gnowangerup, WA
	AWI Extension WA, Barloo Merinos & Zoetis	
17 Oct 2024	Picking Performer Ewes	Webinar
	AWI Extension NSW	
21 Oct 2024	RAMping up Repro	Williams, WA
	AWI Extension WA, Navanvale Merinos & Zoetis	
22 Oct 2024	Sheep Classing Workshop	Oatlands, Tas
	AWI Extension Tas	
24 Oct 2024	Sheep Classing Workshop	Epping Forest, Tas
	AWI Extension Tas	
31 Oct 2024	Yardstick Merino Sire Evaluation Field Day	Katanning, WA
	DPIRD Katanning & WA Federation of Performance Sheep	
	Breeders	