

FEBRUARY 2025

Sheep reproduction RD&A alert

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

[MLA's Productivity & Profitability series](#) presents new and topical information to help southern producers increase the success of their businesses. The March webinar, **An analysis of fertility and fecundity in the Australian sheep flock between 2006 and 2019**, will be presented by Dr Gordon Refshauge (NSW DPIRD) and held on 12 March 2025 from 7pm AEDT.

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PRODUCTIVITY & PROFITABILITY

series

The webinar will cover:

- Identifying the key factors affecting sheep fertility rates.
- Implementing effective nutritional strategies to improve pre-mating conditions for ewes and rams.
- Utilising pregnancy scanning data to enhance your medium-term planning and overall flock productivity

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.

Scientific papers

Genetic and environmental parameters for birth weight and neonatal behaviour of Merino lambs in relation to cold stress

Shannon Steyn, Cornelius L. Nel, Jasper J.E. Cloete, J.H.C. van Zyl, Tertius S. Brand and Schalk W.P. Cloete (schalkc2@sun.ac.za)

Applied Animal Behaviour Science, Volume 282, January 2025 **OPEN ACCESS**

DOI <https://doi.org/10.1016/j.applanim.2024.106491>

Highlights

- This study assessed lamb behaviour in relation to cold stress derived from wind, rain, and temperature.
- Lambs from a line selected for number of lambs weaned per mating (NLW) suckled faster than in a line selected against NLW.
- Increased levels of cold stress resulted in lambs standing sooner but compromised their time to suckling.

- Latency to stand was heritable, but latency to suckle was only affected by dam permanent environment.
- Further research is required to better understand neonatal lamb responses to cold stress.

Abstract

Lamb mortality remains a significant welfare and production issue, constraining ethical and sustainable sheep production. Lamb survival benefits from early suckling and colostrum intake, while it is impaired by inclement weather. The effect of cold exposure on neonatal behaviour and progress to suckling in lambs needs to be understood to curb lamb mortality. This study used historic data to establish the relationship of neonatal lamb behaviour with cold stress and derive genetic parameters for early lamb behaviour. Data of Merino lambs from 1993 to 2002 of the Elsenburg Merino flock were assessed for birth weight (BW) and behaviour latencies (intervals in minutes), namely: from birth to first standing for > 10 sec (LTBS) as well as from standing to first suckling for > 10 sec (LTSS) in relation to a cold index (CI). The CI was derived from a combination of wind, rain, and temperature. The flock consisted of a line selected for number of lambs weaned per mating (NLW; the High or H-Line) and a line selected against NLW (Low or L-Line). Fixed linear and random cubic spline components of LTBS and LTSS on the CI and genetic parameters were derived. Overall, H-line lambs were somewhat heavier at birth and progressed faster from standing to first suckling than L-line lambs ($P < 0.05$). Random spline components as well as interactions of the regression variables with selection line were not significant ($P > 0.05$) and the analyses reduced to modelling the fixed linear component. Increased levels of cold stress resulted in faster progress in LTBS ($P < 0.05$). Expressed relative to mild conditions at a CI of $800 \text{ kJm}^{-2}\text{h}^{-1}$, LTBS was reduced by 17.6 % on the observed scale at a higher CI of $1200 \text{ kJm}^{-2}\text{h}^{-1}$ ($P < 0.05$). In contrast, LTSS was compromised at higher CI values ($P < 0.05$), increasing markedly by 76.0 % from $800 \text{ kJm}^{-2}\text{h}^{-1}$ to $1200 \text{ kJm}^{-2}\text{h}^{-1}$. Direct single-trait heritability estimates were 0.16 ± 0.05 for BW, 0.22 ± 0.07 for LTBS, and 0.06 ± 0.05 for LTSS. The inclusion of the maternal genetic variance ratio resulted in an improvement in the log-likelihood ratio for BW and LTBS, yielding single-trait estimates of 0.37 ± 0.03 and 0.06 ± 0.03 , respectively. The single-trait dam permanent environment variance ratio was 0.09 ± 0.03 for LTSS. Further research is required to better understand the responses to cold stress between LTBS and LTSS.

Ultrasound scanning figures and lambing rates of Merino-type ewes

S.W.P. Cloete (schalkc2@sun.ac.za), W.H. Geerkens, J.E. Cilliers, J. Morris, J.H.C. van Zyl and T.S. Brand
 South African Journal of Animal Science, Volume 54, Issue 6 January 2025 **OPEN ACCESS**

DOI <https://doi.org/10.4314/sajas.v54i6.06>

Abstract

This study assessed ultrasound scanning as a proxy for observed reproduction records, quantified the effects of lambing year and ewe age, and estimated repeatability of traits to predict current-flock gains. Data for number of lambs recorded per ewe scanned, lambs born per ewe lambing, and embryonic losses per ewe scanned were available for 2338 Dohne Merino, 1159 SA Mutton Merino (SAMM), and 138 Merino ewes on the Mariendahl experimental farm of Stellenbosch University, with 7652, 3364, and 240 ewe-year records, respectively. Merino records spanned 1990–1992, whereas the other breeds had lambing records for 1990–2016. Scan records indicated that 89.2%–95.8% of ewes scanned pregnant with multiples also lambing multiples. Embryos lost per ewe at lambing were 0.00–0.05 in all breeds. ASReml was used to fit mixed models to the Dohne and SAMM data. Lambing year and ewe age significantly affected all reproductive traits, except for ewe age effects on embryonic losses. Two-year-old ewes were more likely to be barren than their mature contemporaries, irrespective of breed. Scanning and lambing rates were highly correlated at the ewe level, suggesting that scanning is a good proxy for lambing rate in the absence of full lambing data. Age effects

confirmed that an optimal flock structure contributes to a desirable reproductive output. Results indicated that embryonic losses were random and not meaningfully related to fixed or random effects. Moderate repeatability estimates for reproductive traits support low-to-moderate current-flock gains for scanning and lambing rate. Ultrasound scanning may thus be used to optimise reproduction on farms without detailed reproduction records.

Timing, risk factors, and causes of foetal and preweaning lamb mortality in lowland production systems involving a range of ewe genotypes

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Animal, Volume 19, Issue 2, February 2025 **OPEN ACCESS**

DOI <https://doi.org/10.1016/j.animal.2024.101401>

Highlights

- The neonatal stage (0–3 days) accounted for 75% of all lamb mortality.
- Infection and dystocia accounted for over half of preweaning lamb mortality.
- Lamb mortality causes differ between breeds and flocks even when similarly managed.
- About one-third of the observed lamb mortality was preventable.
- Lamb mortality of 7–8% is achievable in grass-based systems of sheep production.

Abstract

Reducing lamb mortality has production, economic and animal welfare benefits. The timing and causes of death and associated risk factors were investigated in a study conducted over 2 consecutive years (involving 1 103 and 1 038 ewes in 2017 and 2018, respectively) in three prolific (average litter size 1.91) indoor-lambing, lowland flocks (in Ireland) that consisted of a range of genotypes, managed in grass-based systems of production. Data were collected from all foetuses and lambs that died (between ~120 days gestation and weaning at 14 weeks of age); 221 cases in 2017 and 241 cases in 2018. All cases were submitted to a Regional Veterinary Laboratory for necropsy examination using standardised protocols that were developed in advance of case submissions. The majority (60%) of lamb mortality occurred prior to or within 24 h of birth: 46% at or prior to birth and 14% within the first 24 h. Infection (32%) and dystocia (20%) accounted for over half of the mortality. *Chlamydia abortus* was detected more often in lambs from 2-year-old ewes lambing for the first time than in lambs from older ewes. Dystocia accounted for a statistically significant higher proportion of deaths among purebred lambs born to Texel ewes (49.4%, 95%CI (confidence interval) 36.0 – 62.9) compared to purebred lambs born to Belclare ewes (12.8, 95%CI 2.2 – 23.5). More lambs failed to yield a diagnosis of the cause of death when born to Belclare ewes (29.2%, 95%CI 17.8 – 40.6) than to Suffolk-X ewes (7.4%, 95%CI 0.1 – 14.8). About one-third of lamb mortality cases were adjudged to be preventable through more consideration of management factors during pregnancy, parturition and early postpartum. The use of good hygiene practices at lambing time and optimising lamb birth weight should reduce the level of preweaning lamb mortality in indoor lambing flocks.

Long-interval prostaglandin F2 α combined with GnRH improves the estrus synchronization and reproductive performance of sheep during the breeding season

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Animals, Volume 15, Issue 3 February 2025 **OPEN ACCESS**

DOI <https://doi.org/10.3390/ani15030336>

Simple Summary

Estrus synchronization is an important animal reproductive technology, but protocols still need to be refined to enhance its practical use in livestock production. The objective of this study was to improve the efficiency of prostaglandin F₂ α -based estrus synchronization in sheep. We found that addition of an injection of gonadotropin-releasing hormone between the two injections of prostaglandin F₂ α could enhance estrus synchrony and improve the reproductive performance of synchronized ewes, even achieving a comparable efficiency to the conventional progesterone-based method. This modified protocol could provide a promising strategy for estrus synchronization and timed artificial insemination in sheep as well as other domestic animal species.

Abstract

To improve the efficiency of prostaglandin F₂ α (PG)-based estrus synchronization in sheep, this study assessed the effect of the gonadotropin-releasing hormone (GnRH) included in the long-interval PG treatment regimen for sheep estrus synchronization during the breeding season. In experiment 1, 30 multiparous Mongolian sheep (3–4.5 years old) were randomly divided into three groups. In the progesterone (P4)–equine chorionic gonadotropin (eCG) group (P4-eCG, n = 10), the ewes were synchronized with intravaginal P4 sponges for 14 days, and received an injection of 330 IU of eCG at sponge withdrawal. In the PG group (n = 10), the ewes received two doses of 0.1 mg PG with a 14 day interval. In the PG-GnRH-PG group (n = 10), the ewes were synchronized by two doses of 0.1 mg PG with a 14 day interval like that in the PG group, but received 50 μ g of GnRH 7 days after the first injection of PG. It was found that, at the end of treatment, the number of corpus luteum on the ovaries and the concentration of the serum P4 in the PG-GnRH-PG group were significantly higher than that of the PG and P4-eCG groups. In experiment 2, 59 multiparous Mongolian sheep (3–4.5 years old) were assigned to three groups, like in experiment 1 (n = 20, 20 and 19 for the P4-eCG, PG and PG-GnRH-PG groups, respectively). The estrus of ewes in the PG-GnRH-PG group was more synchronous compared to the PG group. After insemination of the estrus ewes, the pregnancy rate was numerically but not significantly higher in the PG-GnRH-PG group than that in the P4-eCG and the PG groups. In a field test, 285 multiparous Hu sheep (3–4.5 years old) were randomly assigned to a P4-eCG group (n = 142) and PG-GnRH-PG group (n = 143). Timed artificial insemination showed no significant differences in the rates of pregnancy and lambing between the PG-GnRH-PG and P4-eCG groups. We conclude that the addition of GnRH in the long-interval PG protocol may improve the efficiency of PG-based estrus synchronization, and would represent a potential alternative to the conventional P4-eCG based protocol during the breeding season in sheep.

Effects of the concentration of plasma platelet on the cryopreservation of ram semen

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Small Ruminant Research, Volume 243, February 2025

DOI <https://doi.org/10.1016/j.smallrumres.2024.107422>

Highlights

- Platelet-rich plasma (PRP) is an option for freezing ovine semen.
- PRP acts as an antioxidant in ovine semen.
- Platelet-poor plasma improved membrane integrity in half of the animals.

Abstract

The objective of this study is to assess the effect of autologous platelet-rich plasma (PRP) and platelet-poor plasma (PPP) on the cryopreservation of ovine semen. Eight rams were used, previously trained for collection using an artificial vagina. The collected semen was separated into five parts and diluted in Botubov[®] (Botupharma, Botucatu, Brazil), and five treatments were tested: Control (pure commercial semen extender);

PRP10 (Extender supplemented with 10 million platelets/mL); PRP20 (Extender supplemented with 20 million platelets/mL); PRP40 (Extender supplemented with 40 million platelets/mL); and PPP (Extender supplemented with PPP in the same volume used for PRP40). The semen was then packaged in French straws, cooled for 3 h at 5 °C, and frozen in liquid nitrogen until evaluation. Kinetic parameters were evaluated using computer-assisted sperm analysis (CASA), immediately post-thaw and after a 3-h thermoresistance test at 37 °C, as well as assessment of plasma membrane and acrosomal integrity (PMAI), mitochondrial potential (MP), superoxide anion (O₂) production, and hydrogen peroxide (H₂O₂) production by flow cytometry. Both PRP and PPP were shown to be safe for the cryopreservation of ram semen, with improvements observed in half of the animals in terms of flow cytometry parameters. When grouped together, it was evident that the PPP group displayed greater integrity of the plasma membrane and acrosome ($P = 0.02$), more stable cells ($P = 0.03$), and increased production of H₂O₂ ($P = 0.05$). In conclusion, PRP and PPP are safe and can be viable additives for freezing ram semen. PPP showed better results for plasma and acrosomal membrane integrity, as well as the number of stable cells in half of the animals. This highlights PPP as a promising antioxidant and cryoprotectant for ram semen.

Heat stress induced by testicular insulation for 24 or 48 h rapidly impairs epididymal sperm quality and reduces spermatogenesis in rams

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DOI <https://doi.org/10.1016/j.smallrumres.2025.107443>

Highlights

- Testicular insulation for 24 and 48 h lead to severe impact in the reproductive soundness of the rams.
- Severe impact was clear for cells in the epididymis (24 h and 48 h post-HS) and spermatids in the testes (7 and 14 d post-HS).
- Testes weight and histology were impacted at 7 and 14 d post-HS, indicating impact on spermatogenesis and forming spermatids.
- Immediate decrease in spermatid number (at 24 h) indicates sensibility of the population and prompt activation of apoptosis.

Abstract

Mammalian testes must be 3–5 °C below body core temperature to produce morphologically normal sperm. The objective was to investigate impacts of heat stress (HS) induced by scrotal insulation on epididymal sperm and temporal aspects of HS on spermatogenesis. We hypothesized that: (1) increased testicular temperature impairs sperm in the epididymis; and (2) spermatids are severely impacted by HS exposure. Testicular HS was induced by scrotal insulation for 24 or 48 h in 20 reproductively sound adult rams, with 5 similar rams designated controls (not insulated). Rams were castrated at 24 h, 48 h, 7 d, or 14 d after the start of insulation (whereas control rams were randomly castrated). Insulation increased scrotal surface temperature by ~5 °C. There were marked decreases ($P < 0.01$) in sperm motility, progressive motility and kinetics starting at 24 h and sustained throughout the study. Percentage of epididymal sperm with normal morphology first decreased at 24 h ($P < 0.01$) with subsequent decreases at 48 h ($P < 0.01$) and 7 d ($P < 0.01$); thereafter, morphology remained stable ($P > 0.05$). At 14 d, there were decreases in testicular weight ($P < 0.05$) and seminiferous tubule diameter (STD) ($P < 0.001$) when compared to all other groups. Regarding seminiferous

tubule integrity (Johnsen's score), a first decrease occurred at 24 h ($P < 0.05$) followed by a more intense decrease at 14 d ($P < 0.001$). In addition, there was an abrupt decrease ($P < 0.05$) in spermatid counts at 24 h that was sustained throughout the study. In conclusion, our hypotheses were supported; testicular HS caused immediate deleterious impacts on epididymal sperm at 24 and 48 h post-insulation as well as developing spermatids at 7 and 14 d, decreasing sperm production and significantly reducing both STD and testicular weight.

Effects of restricted- and over-feeding during gestation on colostrum and milk composition and offspring circulating immunoglobulin G concentrations in multiple generations of sheep

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DOI <https://doi.org/10.1016/j.smallrumres.2024.107423>

Highlights

- Colostrum from restricted-fed ewes had less fat than colostrum from control-fed ewes.
- Maternal diet (over- or restricted-feeding) had no impact on immunoglobulin G concentrations in F0 or F1 ewe colostrum.
- F1 control female offspring had greater IgG concentrations than female offspring of restricted- and over-fed dams.
- Maternal diet may limit IgG absorption in F1, but not F2, offspring in a sex- and diet-specific manner.

Abstract

High-quality colostrum and milk are critical for neonatal growth and immune system development. Immunoglobulin G (IgG), the predominant antibody in colostrum, is vital for passive immune transfer in sheep. It is unknown if poor maternal nutrition during gestation influences colostrum and milk composition, and IgG concentrations over multiple generations. We hypothesized that poor maternal nutrition (restricted- or over-feeding) during gestation would alter F0 and F1 colostrum and milk composition and IgG concentrations in their colostrum and alter serum IgG in F1 and F2 offspring. Multiparous Dorset ewes ($n = 46$) pregnant with twins were fed 100 % (control; $n = 13$), 60 % (restricted; $n = 17$) or 140 % (over; $n = 16$) of National Research Council (NRC) requirements until parturition. Following lambing, all dams were fed 100 % NRC requirements during lactation. Offspring of these ewes ($n = 85$; F1) were managed similarly on 100 % of NRC requirements and are referred to as CON-F1, RES-F1, or OVER-F1, corresponding with dam diet. F1 ewe offspring ($n = 36$) were bred between 16 and 19 mo of age. Offspring of these ewes ($n = 60$; F2) were managed similarly on 100 % of NRC requirements and are referred to as CON-F2, RES-F2, or OVER-F2, corresponding with granddam diet. Colostrum samples from F0 and F1 ewes were collected within 24 h of parturition and milk samples were collected at d 3 and 21 postpartum and analyzed for total solids, crude fat, crude protein (colostrum and milk), and IgG concentrations (colostrum only). Serum samples were collected from F1 and F2 offspring at 7 d of age and analyzed for IgG concentrations. A treatment by time interaction ($P \leq 0.024$) was observed for F0 crude fat where colostrum from restricted-fed ewes had less fat than colostrum from control-fed ewes. However, total fat in d 3 and 21 milk samples were similar across treatments. Colostrum had greater total solids, crude fat, and crude protein compared with milk at d 3 and d 21 ($P \leq 0.01$). Maternal diet had no impact on IgG concentrations in F0 or F1 ewe colostrum ($P \geq 0.46$). A treatment by sex interaction ($P = 0.007$) was observed for F1 offspring serum IgG concentrations where RES-F1 and OVER-F1 ewes and CON-F1 and OVER-F1 rams had between 44.9 – 50.7 % less IgG than CON-F1 ewes. No impact of maternal diet was observed on F2 offspring serum concentration of IgG ($P = 0.40$). In this

experiment, maternal diet had minimal effects on F0 and F1 colostrum and milk composition or IgG concentrations. However, maternal diet may limit IgG absorption in F1 offspring in a sex- and diet-specific manner but does not persist into the F2 generation.

Anti-Müllerian hormone concentrations can be reliably determined by a single measurement, irrespective of cycle, in synchronised ewes during non-breeding season

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Reproduction in Domestic Animals, Volume 60, issue 2, February 2025 **OPEN ACCESS**

DOI <https://doi.org/10.1111/rda.70010>

Abstract

Improvement of yield characteristics in animal breeding is important in terms of increasing animal production and sustainability. Fertility is one of the most important yield traits affecting economic gain in sheep breeding. Anti-Müllerian Hormone (AMH) is widely recognised as a dependable biomarker for assessing ovarian reserves and fertility potential. The aim of this study was to evaluate the dynamics of AMH during different phases of the sexual cycle in Norduz ewes the non-breeding season. Additionally, the study sought to assess the effects of age and body condition score (BCS) on AMH concentrations during these phases. A total of 32 Norduz ewes with a body condition score (BCS) of 3–4.5 and aged between 2 and 4 years were used as animal material in the study. All experimental procedures were carried out outside the breeding season and when the ewes were lactating. In all ewes in anestrus, intravaginal sponges (Esponjavet, 60 mg MAP, Hipra, Turkey) were kept in the vagina for 7 days for estrus synchronisation. Intramuscular injections of PMSG (Oviser, 500 IU, Hipra, Turkey) and PGF2 α analog (Gestavet, 50 μ g, Hipra, Turkey) were administered 48 h prior to sponge removal. Twenty-four hours after sponge removal, ewes were exposed to the ram for estrus detection. Since 5 ewes did not show estrus, blood samples were collected regularly from animals (n = 27) in which estrus was detected at three different stages: one just before the insertion of vaginal sponges (anestrus), another when heat was detected exposing to the ram (estrus), and the final one 10 days after estrus (diestrus). The serum samples were assessed for the levels of AMH and progesterone through the electrochemiluminescence immunoassay technique (ECLIA). The results of the analyses showed that serum AMH concentration did not vary between anestrus, estrus and diestrus phases of the sexual cycle of Norduz ewes outside the breeding season ($p > 0.05$). Furthermore, age and BCS had no effect on progesterone and AMH levels in different phases of the sexual cycle ($p > 0.05$). In conclusion, this study shows that serum AMH levels are constant at any stage of the estrus cycle. This suggests that phenotypic evaluation of ewes can be performed with a single measurement and that AMH is a reproducible and dependable biomarker that can be measured at any stage of the estrus cycle at an arbitrary time point.

Scabiosa arthropurpurea var. *maritima* aqueous extracts improve *in vivo* fertility parameters and *in vitro* granulosa cell steroidogenesis in ewe

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Highlights

- Oral *Scabiosa arthropurpurea* extract (AES) reduces estrus latency *in vivo* in ewes.
- Oral AES increases the duration of estrus and prolificacy rates in ewes.
- AES treatment increases *in vitro* steroidogenesis in ovine granulosa cells.

- AES treatment increases *in vitro* lactate production by ovine granulosa cells.
- AES treatment did not affect ovine granulosa cell viability and proliferation.

Abstract

Scabiosa arthropurpurea, a member of the Dipsacaceae family and *Scabiosa* genus, is renowned for its medicinal properties. In the present study, we investigated the impact of *Scabiosa arthropurpurea* aqueous extract (AES) on the *in vivo* reproductive functions in Queue Fine de l'Ouest ewes, and on *in vitro* ovine granulosa cells. Ewes were synchronized for 14 days with intra-vagina progesterone (P4) devices (FGA, 20 mg) and divided into four groups receiving daily oral doses of 0, 1, 2, and 4 mg of AES/kg Live Body Weight (LBW), respectively. After sponge removal, all ewes received an intramuscular injection of 400 IU of eCG. Estrous behavior parameters as latency and duration, and prolificacy rates, and plasma hormone levels (estradiol and progesterone) were assessed. Estrus latency was reduced and the duration of estrus was increased in ewes that received 1 mg and 2 mg/kg LBW compared to the control. Prolificacy rates were also significantly improved in 1 mg or 2 mg/kg groups compared to the control. Plasma levels of E2 were also higher on 2 mg/kg LBW treated group. Moreover, ovine granulosa cells were cultured and treated with various concentrations of AES (ranging from 0 to 5 mg/ml). While AES did not affect cell viability and proliferation whatever the conditions, it significantly increased basal steroidogenesis (P4 and E2 concentrations) at the concentration of 0.5 and 0.05 mg/ml and in response to IGF-1 but not FSH at the 0.05 mg/ml concentration. These latter data were associated to an increase in the expression of CYP19A1 and STAR genes but not those of CYP11A1 and HSD3B and to an increase in cellular lactate concentration. Taken together, AES extracts enhanced *in vivo* reproductive performance in ewe and this was associated to an increase in *in vitro* granulosa cell steroidogenesis.

The mechanism of Se in regulating the proliferation and apoptosis of sheep Leydig cells through the miR-200a/NRF2 pathway

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DOI <https://doi.org/10.1016/j.theriogenology.2025.01.003>

Highlights

- Se regulates the level of miR-200a in Leydig cells.
- MiR-200a affects the proliferation and apoptosis of Leydig cells by regulating the NRF2 signaling pathway.
- High Se can induce the elevated expression of miR-200a to activate the NRF2 signaling pathway.

Abstract

This study aimed to investigate the mechanism by which Se in regulates the proliferation and apoptosis of sheep Leydig cells via the miR-200a/NRF pathway. The cells were isolated and purified from the testes of 8-month-old sheep via a Percoll density gradient. After the cells were treated with different concentrations of Se (0, 2.0, 4.0, 6.0, and 8.0 $\mu\text{mol/L}$ of Se) for 18 h, the miR-200a levels was detected. MiR-200a mimics and inhibitors were transfected into the cells, resulting in five groups (control, NC mimics, miR-200a mimics, NC inhibitor and miR-200a inhibitor). Cell viability and antioxidant status were measured via CCK8 and antioxidant assays, respectively. The abundances of pro-apoptotic (BAX, CASPASE 3 and CASPASE 8), cell cycle (P21, P27 and CDK1), and NRF2-related (NRF2, HO-1, NQO1 and KEAP1) genes were detected by real-time PCR and Western blot analysis.

The results revealed that miR-200a mimics group presented greater ($P < 0.05$) abundances of NRF2, HO-1 and NQO1 mRNA transcripts and proteins. Compared with those both in the NC mimics and the miR-200a inhibitor groups, the activities of GSH-Px and SOD, as well as cell viability in the miR-200a mimics group were

significantly greater ($P < 0.05$). In contrast, the ROS levels, MDA content and abundances of KEAP1, P21, P27 and apoptosis-related genes mRNA transcripts and proteins were decreased ($P < 0.05$). The highest ($P < 0.05$) miR-200a expression level was detected in the Se6.0 group. Compared with that in the Se (6.0 $\mu\text{mol/L}$) group, cell viability in the Se + miR-200a inhibitor group was lower ($P < 0.05$). The abundances of NRF2, HO-1 and NQO1 in the Se + miR-200a inhibitor group were lower ($P < 0.05$) than those in the Se (6.0 $\mu\text{mol/L}$) group but greater ($P < 0.05$) than those in the inhibitor group, while KEAP1 displayed the opposite trend ($P < 0.05$).

These results indicate that Se can activate the NRF2 antioxidant signaling pathway to regulate the proliferation and apoptosis of sheep Leydig cells and that miR-200a plays a vital role in this process. The regulatory effect of Se on male reproduction and spermatogenesis may be related to the number of Leydig cells. This study aimed to provide experimental data for Se regulation of spermatogenesis.

In vitro study of carbetocin, an oxytocin receptor agonist, and 4-phenylfuroxan-3-carbonitrile, a NO-releasing agent, as cervical dilators in sheep

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Highlights

- Carbetocin increases in vitro cervical MMP-2 activity in cycling ewes.
- DMSO increases in vitro cervical MMP-2 activity in cycling ewes.
- The increase in MMP-2 activity by carbetocin and DMSO is not dependent on PGE2.
- The combination of carbetocin and DMSO has cooperative in vitro effect on cervical MMP-2 activity in cycling ewes.
- Furoxan increases in vitro NO levels without increases in the cervical MMP-2 activity and inhibits PGE2 production in cycling ewes.

Abstract

The aim was to study the effect of 4-phenylfuroxan-3-carbonitrile (Fx), a NO-releasing agent, and carbetocin, an oxytocin receptor agonist, on matrix metalloproteinases-2 (MMP-2) activity and PGE2 production in cervix from cycling sheep. Cervical explants were incubated during 12 h with MEM supplemented with increasing concentrations of Fx in DMSO (2 %) (0 to 300 $\mu\text{g/mL}$) with Cb (100 ng/mL) (Experiment 1, $n = 15$) and DMSO (2 %), DMSO + Cb (100 ng/mL) or DMSO + Fx (30 $\mu\text{g/mL}$) (Experiment 2, $n = 10$), and their respective controls. In the supernatants, activated (A) and latent (L) MMP-2 activities were determined by a SDS-PAGE zymography, PGE2 concentration by immunoassay and NO production indirectly as nitrites by spectrophotometry. Data were analyzed by ANOVA. The Cb treatment increase the A MMP-2 activity in DMSO (Experiment 1 at follicular phase and Experiment 2) or alone (Experiment 2) and increase the L MMP-2 activity (Experiments 1 and 2) ($P < 0.02$). The DMSO treatment also increase the L MMP2 activity (Experiment 2) ($P < 0.0001$). Treatment with Fx + DMSO increased the concentration of accumulated nitrites in the supernatant ($P < 0.0001$) (Experiment 1), but did not affect or decrease the activity of A and L MMP-2 ($P < 0.04$) (Experiments 1 and 2). The PGE2 concentration trend to increase with Cb treatment ($P = 0.0614$) and decrease with Fx+DMSO treatment ($P < 0.0001$) (Experiment 2). In conclusion, Cb and/or DMSO treatment of cervical explants increase the MMP-2 activity through PGE2-independent mechanisms, but Fx in DMSO fail in this, suggesting that the pre-treatment with Cb and/or DMSO could be used to increase cervical dilation in ewes.

Cumulus cells and the TNF-alpha signaling facilitate aging of ovine oocytes

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Highlights

- Post-maturation oocyte aging decreased competence while increasing degeneration and activation sensitivity of ovine oocytes
- The presence of cumulus cells during post-maturation oocyte aging aggravated oocyte aging parameters.
- Post-maturation oocyte aging increased apoptosis, TNF- α production and TNF receptor 1 expression in cumulus cells.
- TNF- α antagonist Etanercept decelerated post-maturation aging of ovine oocytes by increasing TNF- α activity.

Abstract

Post-maturation oocyte aging (PMOA) is known to significantly impair the developmental potential of oocytes; however, comprehensive studies on ovine PMOA remain limited. In mice, cumulus cells (CCs) accelerate oocyte aging by releasing cytokines, but the roles of CCs and cytokines in PMOA of domestic animals are poorly understood. This study aimed to elucidate the involvement of CCs and tumor necrosis factor (TNF)- α in the PMOA of ovine oocytes. Our findings reveal that PMOA significantly reduced blastocyst rates and the expression of development-promoting genes, while increasing oocyte degeneration and activation rates, along with expression of development-inhibiting genes, compared to newly matured oocytes. These detrimental effects were more pronounced in oocytes aged as cumulus-oocyte complexes than as cumulus-denuded oocytes. Additionally, PMOA led to increased apoptotic rates, TNF- α production, and TNF receptor 1 (TNFR1) expression in CCs, coupled with a significant reduction in the expression of anti-apoptotic genes. Mature oocytes expressed TNFR1, with levels decreasing significantly during PMOA. Importantly, the addition of the TNF- α antagonist Etanercept to the aging medium markedly improved parthenogenetic embryo development and the expression of competence-related genes, while mitigating CC apoptosis during PMOA of COCs. In conclusion, PMOA compromises developmental potential while heightening oocyte degeneration and activation sensitivity in ovine oocytes. Cumulus cells exacerbate PMOA through increased TNF- α signaling activity, highlighting the potential of TNF- α antagonists as therapeutic agents to counteract the deleterious effects of PMOA.

Feed efficiency improves sperm quality and viability of fertilisation in Dorper sheep

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Abstract

The aim was to evaluate the influence of residual feed intake (RFI) on the semen characteristics of Dorper sheep after cryopreservation. Six Dorper sheep were used, classified from a population of 64 according to feed efficiency (groups of animals-positive RFI and negative RFI). The ejaculates were harvested three times a week, with a minimum interval of 48-h between collections, being seven collections per animal, totalling 42 ejaculates. Motility, plasma membrane integrity, acrosomal, mitochondrial activity and perivitelline

membrane binding tests were performed. Negative RFI animals presented higher progressive motility (31.28%) than of RFI positive (25.37%). Negative and positive RFI animals present similar qualities in the assessment of plasma and acrosomal membrane integrity and mitochondrial activity, and negative RFI animals presented a higher number of membrane-bound spermatozoa than the positive RFI animals. Negative RFI animals displayed better rates of sperm motility after thawing, and are more efficient in the fertilisation in vitro. RFI did not influence the integrity of the plasma and acrosomal membrane, nor the mitochondrial activity of Dorper sheep spermatozoa after cryopreservation.

Upcoming events

Date	Event	Location
12 March 2025	MeatUp Forum MLA	Naracoorte, SA
12 March 2025	An analysis of fertility and fecundity in the Australian sheep flock between 2006 and 2019 MLA Productivity & Profitability Series	Webinar
18 March 2025	MeatUp Forum MLA	Albany, WA
20 March 2025	PDS Field Day - improving lamb survival on leguminous pastures MLA	Campbell Town, Tas
20 March 2025	Lifting lamb survival field day NSW Local Land Services	Crookwell, NSW
21 March 2025	Lifting lamb survival field day NSW Local Land Services	Binalong, NSW
27 March 2025	Why Wool? Strengthening western Queensland wool businesses Leading Sheep Qld	Longreach, Qld
1 April 2025	Picking Performer Ewes AWI Extension NSW	Curban, NSW
2 April 2025	Picking Performer Ewes AWI Extension NSW	Crookwell, NSW
3 April 2025	Picking Performer Ewes AWI Extension NSW	Cootamundra, NSW