JULY 2021

Sheep reproduction RD&A alert

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

An eclectic collection of journal articles this month from Australian and International research groups. This RD&A Alert includes aspects of ram fertility, animal health, genetic selection, heat stress, shelter and ewe nutrition during pregnancy.

The SRSP aims to help sheep producers to profitability and sustainably increase lamb production through increasing lamb survival and weaning rates and will coordinate a national approach to improving sheep reproductive performance.

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Review papers

Aquaporins: New markers for male (in)fertility in livestock and poultry?

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Animal Reproduction Science, Volume 231, August 2021

DOI https://doi.org/10.1016/j.anireprosci.2021.106807

Highlights

- Selecting the right male for breeding is a prerequisite for maintaining a healthy and fertile herd.
- An increase in the male reproductive disorders among animals prompts a search for new markers of male fertility.
- Aquaporins are widely distributed in the male reproductive organs and sperm.
- Aquaporins play a crucial role in the biology of male reproduction.
- Possibility of using aquaporins for evaluation of male fertility and infertility in livestock and poultry.

Abstract

Improving the methods utilized to facilitate reproduction is associated with a constant need to search for new factors that not only significantly affect reproductive processes, but also create new possibilities when assessing male reproductive potential. Aquaporins (AQPs) belong to a family of small (28–30 kDa) proteins that facilitate the transport of water and other small molecules. There have been 13 AQPs (AQP0-AQP12) discovered in mammals, and these proteins are present in a wide range of cell types. Almost all AQPs, except AQP6 and AQP12 are present in the male reproductive organs and sperm of mammals and birds. Increasing evidence suggests that these proteins are involved in a number of processes responsible for the optimal functioning of the male reproductive system. This review presents the current state of knowledge regarding the abundance and distribution of AQPs in the male reproductive organs and sperm of various livestock and poultry species, including buffalo, cattle, sheep, horses, pigs, turkeys and goose. Furthermore, the possible

physiological and pathophysiological significance of AQPs in male reproduction, as well as hormonal regulation of quantities are discussed. It can be concluded from the studies analyzed in this paper that abundance patterns of AQPs may be considered in the future as specific and universal biomarkers of male fertility and infertility in animal husbandry.

Scientific papers

Chlamydia pecorum detection in aborted and stillborn lambs from Western Australia

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Veterinary Research, 52, Article number 84 (2021) **OPEN ACCESS**

DOI https://doi.org/10.1186/s13567-021-00950-w

Abstract

Lamb survival is an important welfare and productivity issue for sheep industries worldwide. Lower lamb survival has been reported for primiparous ewes, but the causes of this are not well studied. The aim of this study was to determine causes of perinatal deaths for lambs born to primiparous ewes in Western Australia, and identify if infectious diseases are implicated. Lamb mortality from birth to marking were determined for 11 primiparous ewe flocks on 10 farms in Western Australia. Lamb mortality from birth to marking averaged 14% for single-born and 26% for multiple-born lambs. Lamb necropsies (n = 298) identified starvation–mismosthering–exposure (34%), dystocia (24%) and stillbirth (15%) as the most common causes of perinatal lamb death. There was no evidence of exotic abortigenic pathogens in aborted and stillborn lambs (n = 35). *Chlamydia pecorum* was detected by qPCR in 15/35 aborted and stillborn lambs on 5/6 farms. Preliminary molecular characterisation of *C. pecorum* detected in samples from aborted and stillborn lambs (n = 8) using both Multilocus Sequence Typing and ompA genotyping indicated all strains were genetically identical to previously described pathogenic livestock strains, denoted ST23, and dissimilar to gastrointestinal strains. High frequency of detection of a pathogenic *C. pecorum* strains ST23 associated with ovine abortion and stillbirth on multiple farms located across a wide geographic area has not been previously reported. *Chlamydia pecorum* may contribute to reproductive wastage for primiparous sheep in Western Australia. Further investigation to understand *C. pecorum* epidemiology and impact on sheep reproduction is warranted.

Long term genetic selection for reproductive success affects neonatal lamb vitality across cold stress conditions

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Journal of Thermal Biology, Volume 98, May 2021

DOI https://doi.org/10.1016/j.jtherbio.2021.102908

Highlights

- A cold stress index derived from weather data was predictive to lamb viability.
- Genetic selection for reproduction by alleviated symptoms of cold stress.
- Selection line differences were most pronounced at high levels of cold stress.
- A number of factors putatively contributed to the stress-coping ability.

Abstract

Adverse weather conditions are important contributors to mortality in new-born lambs. Previous studies have shown variation between lambs in their ability to cope with circumstances of cold stress, and genetic selection could be a viable option for improving animal robustness. The Elsenburg Merino flock was divergently selected on number of lambs weaned (NLW). This resulted in divergent responses in reproduction and lamb survival. This study evaluated lamb vitality and mortality of positively selected H-Line relative to the negatively selected L-Line in response to cold stress. Traits included lamb rectal temperature (RT), surface temperature (ST), shiver score (SS), lamb vigor score (LVS), breaths per minute (BPM), mortality to three days of age (M3) and to weaning (TM). Cold stress was described by a chill index derived from daily rainfall, wind speed and ambient temperature, and represented as the mean of the one (CI), two (CI-2) or three (CI-3) days since parturition. Overall, H-Line lambs had a higher neonatal RT and were less likely to succumb than L-Line contemporaries. In a significant ($P < 0.05$) interaction, the predicted RT of L-Line showed a non-linear decline with increased levels of CI-2, while H-Line lambs better maintained their core temperature. M3 was also affected by a significant interaction between CI-3 and selection line, further suggesting that observed lower mortality rates in the H-Line depends on H-Line lambs' improved ability to cope with stressful environments. Long term selection for NLW in the H-Line led to improvements in both adaptations associated with lower lamb losses. The continued recording of viability traits to produce larger datasets amenable to genetic analysis is recommended, specifically for rectal temperature.

The effect of tree shade on ambient conditions and heat stress indicator traits of new-born South African Mutton Merino and Dormer lambs: Preliminary results

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Journal of Thermal Biology, Volume 99, July 2021

DOI https://doi.org/10.1016/j.jtherbio.2021.103024

Highlights

- Shade and a temperature-humidity index (THI) were studied on lambs.
- Tree shade tempered the ambient climate experienced by lambs.
- Respiration rate (RR) was at first independent of THI in both treatments.
- RR of unshaded lambs rose faster than shaded lambs above a THI of 77-78.
- Shade benefitted recently born lambs in ethical and welfare terms.

Abstract

This preliminary study investigated the provision of shade on heat stress indicators of South African Mutton Merino (SAMM) and Dormer lambs shortly after birth, during the autumn 2017 and 2018 lambing seasons. Newborn lambs were assessed to determine whether welfare, as assessed by respiratory response and rectal temperature as heat stress indicators, survival and early growth benefitted from the provision of shade. Groups consisting of 4–17 pregnant SAMM and Dormer ewes were randomly allocated to 5–10 paddocks with natural shade from trees and 5–9 paddocks that were directly in the sun with no shade available. The lambs were recorded within 24 h of birth at noon. Climate data were obtained from a nearby weather station. The lambs were also weighed at 12 (SD = 2) days of age at tail-docking. Tree shade had a moderating effect on temperature, resulting in lower maximum daytime and higher minimum night-time temperatures. There was an interaction between a temperature-humidity index (THI) and the treatments (access to shade or no access to shade) for respiration and rectal temperature ($P < 0.01$). Both traits were relatively unaffected by the THI at values below 77. Unshaded lambs exhibited a pronounced upwards trend following a THI-threshold of 77–78. Tailing weight tended to be higher while lamb survival of live-born lambs to tail-docking was lower

in lambs born in shaded paddocks but these trends did not persist to weaning. Shade is needed to enhance animal welfare by alleviating the effect of high THI-values on hot days in an autumn lambing season.

Maternal lysine, methionine and choline supplementation in twin-bearing Merino ewes during mid-to-late gestation does not alter pregnancy outcomes or progeny growth and survival

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Livestock Science, 8 July 2021, *In Press*

DOI https://doi.org/10.1016/j.livsci.2021.104620

Highlights

- Twin lambs have high mortality associated with lower birth weight.
- Nutritional strategies may promote lamb birth weight and survival focus via increasing maternal arginine.
- Supplementing ewes with rumen-protected lysine, methionine and choline did not increase maternal arginine or lamb survival.

Abstract

High twin lamb mortality in Merino sheep is a major cost for the Australian wool and sheep meat industry. Most lambs die within three days of birth due to low birthweight and/or complications arising during parturition. Maternal arginine supplementation can potentially increase birthweight by improving utero-placental haemodynamics; but availability and cost of rumen-protected arginine are barriers. Therefore, we sought to increase circulating concentrations of arginine, lamb birthweight and survival by supplementing twin-bearing Merino ewes with lysine, methionine and choline from day 80 of gestation (dG 80) to parturition. Each day, ewes were individually fed a supplement (barley, pea, pollard and molasses) with addition of either 14 g lysine, 7 g methionine and 7 g choline (amino acid; AA; n = 48); or no amino acids (control; CTL; n = 48). Supplementation did not alter circulating amino acid profiles in ewes at dG 120. Parturition difficulty of ewes and meconium staining of lambs did not differ between AA and CTL groups. Rectal temperatures during the first 24 h of life and serum IgG concentrations (indicative of colostrum intake) at 24 h after birth were similar in lambs born to AA and CTL ewes. AA supplementation did not alter lamb weights from birth to weaning (~three months of age), or lamb survival to weaning (CTL: 75%; AA: 84.8%, P > 0.05). The lack of response to maternal supplementation with this combination of rumen-protected lysine, methionine and choline suggests that alternate strategies are needed to increase maternal arginine abundance and twin lamb survival.

Effects of mid-gestational L-citrulline supplementation to twin-bearing ewes on umbilical blood flow, placental development, and lamb production traits

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Translational Animal Science, Volume 5 (3), July 2021 **OPEN ACCESS**

DOI https://doi.org/10.1093/tas/txab102

Abstract

The objective of the study was to examine how L-citrulline supplementation to ewes during mid-gestation influences placental activity, placental blood flow, lamb body weight, and carcass characteristics. Two studies

were completed. A pharmacokinetic study to compare circulating plasma amino acid concentrations after a single intravenous injection of 155 μmol/kg BW L-citrulline or after an isonitrogenous amount of L-alanine (control; 465 μmol/kg BW). Increases ($P < 0.05$) in circulating citrulline concentrations were detected for 8 h after L-citrulline injection versus the control. Similarly, increases ($P < 0.05$) in circulating arginine concentrations were detected for 24 h after L-citrulline treatment. The second study used 12 ewes with twin pregnancies. Daily intravenous injections of either L-citrulline or L-alanine were administered for 39 d from d 42–45 to 81–84 of gestation. Ewes were limit-fed at 85% daily energy requirements during the injection period. A decrease ($P < 0.0001$) in body weight was observed in both treatment groups during this period. No treatment differences were observed in circulating pregnancy-specific protein B concentrations or placental blood flow during the treatment and post-treatment gestational period. No treatment differences were observed in lamb survival nor in lamb birth, weaning and slaughter weights. Treatment did not influence lamb carcass composition or organ weights. However, there was a tendency ($P = 0.10$) for an increase in antral follicle numbers in ovaries from ewe lambs derived from ewes treated with L-citrulline. In summary, a daily L-citrulline injection increased both circulating citrulline and arginine concentrations in ewes, but daily L-citrulline injections during mid-gestation did not produce any detectable changes in placental activity and blood flow, neonatal and postnatal lamb development, and lamb carcass composition at slaughter. In conclusion, no benefits in placental function and lamb development were observed after providing L-citrulline during mid-gestation in ewes exposed to a mild energy restriction, but there was an indication that follicle numbers in ewe lambs were positively influenced by L-citrulline treatment during fetal development.

Sexual behaviour, semen quality and fertility of young Border Leicester rams administered melatonin during spring

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Animal Reproduction Science, Volume 231, August 2021

DOI https://doi.org/10.1016/j.anireprosci.2021.106804

Highlights

- Melatonin in spring improves reproductive response in young Border Leicester rams.
- Variability in response is due to degree of sexual maturity at time of treatment.
- Most parameters of sexual behaviour and ability were affected by melatonin treatment.

Abstract

This study was conducted to test the hypothesis that between year variability in reproductive response to melatonin treatment of young Border Leicester (BL) rams in spring was related to sexual maturity at the time of treatment. Two variables of sexual maturity (mating behaviour and semen quality) were examined. In Experiment 1, mating activity of melatonin-treated and untreated rams was examined at two sites before a flock mating at one site. In Experiment 2, testosterone concentrations, semen quality and variables of in vitro fertilising capacity were examined. In Experiment 1, melatonin treatment did not alter sexual behaviour (latency to nose, total nosings, courtings including Flehmen expression, attempted mounts, mounts, ejaculations) with the exception of a lesser ($P < 0.05$) Flehmen expression at Site 1 whereas values for most variables were greater ($P < 0.05$) at Site 2. Treatment resulted in a greater pregnancy rate (89% and 82%, respectively; $P < 0.05$) and advanced distribution of pregnancies ($P < 0.001$) at Site 1. Testicular size and testosterone concentrations were also greater ($P < 0.05$) as a result of melatonin treatment in spring whilst the reverse occurred in autumn. In Experiment 2, sperm motility and in vitro fertilising capacity were greater ($P < 0.05$) and acrosome damage less ($P < 0.05$) as a result of melatonin treatment. In conclusion, variability

in age at sexual maturity of young BL rams at spring mating can be reduced with melatonin treatment and is associated with differences in sexual behaviour, testicular growth, testosterone concentrations, and sperm quality.

Spatial behaviour of sheep during the neonatal period: Preliminary study on the influence of shelter

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Animal, Volume 15, issue 7, July 2021

DOI https://doi.org/10.1016/j.animal.2021.100252

Abstract

Effective shelter has been demonstrated to reduce neonatal lamb mortality rates during periods of inclement weather. Periods of high wind speed and rainfall have been shown to influence shelter usage; however, it is not yet known how ewe factors such as breed, age and body condition score influence shelter-seeking behaviour. This study, conducted on a working upland farm in the UK, examined impact of artificial shelter on the biological and climatic factors that influence peri-parturient ewe behaviour. Pregnant ewes (n = 147) were randomly allocated between two adjacent fields which were selected for their similarity in size, topography, pasture management, orientation to the prevailing wind and available natural shelter. In one field, three additional artificial shelters were installed to increase the available shelter for ewes, this field was designated the Test field; no additional artificial shelter was provided in the second field which was used as the Control field. Individual ewes were observed every 2 h between 0800 and 1600 for 14 continuous days to monitor their location relative to shelter. Ewe breed (Aberfield and Highlander), age (2–8 years) and body condition score were considered as explanatory variables to explain flock and individual variance in shelter-seeking behaviour and the prevalence of issues which required the intervention of the shepherd, termed 'shepherding problems'. Any ewe observed with dystocia, a dead or poor vigour lamb or who exhibited mismothering behaviour was recorded as a shepherding problem. The prevalence of these shepherding problems which necessitate human intervention represents arguably the most critical limiting factor for the successful management of commercial sheep flocks in outdoor lambing systems. Overall, ewes in the Test field with access to additional artificial shelter experienced fewer shepherding problems than those in the Control field ($P < 0.05$). A significant breed effect was also observed, with Highlander ewes more likely to seek shelter than Aberfield ewes ($P < 0.001$), and experiencing significantly fewer shepherding interventions ($P < 0.05$). These findings demonstrate the substantial and significant benefits to animal welfare and productivity that can be achieved through the provision of shelter in commercial, upland, outdoor lambing systems in the UK.

Singleton or twin male lambs: Effects on their reproductive development

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Animal Reproduction Science, Volume 231, August 2021

DOI https://doi.org/10.1016/j.anireprosci.2021.106797

Highlights

- There were only slight differences between singleton and twin male lambs with similar birthweights.
- Singleton lambs ejaculated a greater volume than twins in the third ejaculate.
- Overall, twin lambs ejaculated semen with greater sperm concentration.

- Singleton lambs in the first test began courtship behavior earlier than twins.

Abstract

Because intrauterine environment differs between twins and singletons, twin-born lambs are often studied when effects of fetal programming are evaluated. In sheep, fetal programming might have effects on reproductive physiology and behavior after sexual maturation. The aim of this study was to compare sperm output and sexual behavior in developing singleton- or twin-born lambs of similar body weight. Singleton lambs (n = 12) and twin (n = 9) male-male lambs were used. From 5.4 until 9.1 months of age, body weight, scrotal circumference (every 3–4 weeks), sexual behavior (every 14 days) and semen characteristics (every 28 days) were evaluated. In the third ejaculate, singleton lambs ejaculated a larger volume of semen than twins (P = 0.03). Considering a pool of the three ejaculates, twin lambs ejaculated semen with a greater sperm concentration than singleton lambs (P = 0.015). There was an interaction between group and time to the onset of courtship behavior (P = 0.02) and a tendency for an interaction in the number of mount attempts (P = 0.052). Singleton-born lambs, during the first evaluation period began courtship behavior earlier than twin-born lambs (P < 0.0001). In conclusion, there were only slight differences in semen and sexual behavior between male ram lambs born as singletons or twins with similar weight. Male ram lambs born as singletons initiated the courtship behavior earlier than twins during the first sexual behavioral evaluation period, ejaculated a larger volume of semen in the third consecutive ejaculate, and there was a lesser sperm concentration in the three ejaculates.

Upcoming events

Date	Event	Location
5 August 2021	MeatUp Forum Meat & Livestock Australia	Crawley, WA
5 August 2021	Olympic Fever in Spring – Faster, Higher Stronger!! Sheep Connect NSW	Webinar
6 August 2021	Winning With Weaners Workshop Sheep Connect NSW	Hay, NSW
10 August 2021	Winning With Weaners Workshop Sheep Connect NSW	Crookwell, NSW
11 August 2021	Foot issues in sheep Meat & Livestock Australia	Webinar
12 August 2021	Sheep Easy 2021 The Sheep's Back WA	Williams. WA
30 August 2021	Winning With Weaners Workshop The Sheep's Back WA	Wickepin, WA