

AUGUST 2022

## Sheep reproduction RD&A alert

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

**WALRC Comes to Ridgefield Study Tour** was held on 27<sup>th</sup> June and was designed to showcase collaborative livestock research currently underway in WA. A series of short videos of the projects showcased during the day can now be viewed on the [WALRC YouTube channel](#). Presentations included: The impact of crop height on survival of twin-born lambs; Heat stress during joining; Lambing density; Shrub design to improve reproductive performance of sheep; Managing weaners to survive; and thrive and Progressing the melatonin story to drive reproduction. For more information contact Esther Price, WALRC Executive Officer [eo@walrc.com.au](mailto:eo@walrc.com.au).

### Program coordinator

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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

#### Increasing lambing percentages through better use of pregnancy scanning technology

Pregnancy scanning is a vital tool for improving sheep reproductive performance. It enables identification of litter size at mid-pregnancy, so that pregnant ewes can be differentially managed by litter size class to meet recommended condition score targets to enhance lamb and ewe survival. However, a 2018 survey of Australian sheep producers found that 69% do not pregnancy-scan their ewe flocks for multiples and therefore are not able to customise ewe nutrition during pregnancy and lactation according to whether their ewes are carrying singles, twins or more lambs.

#### Project aim

To increase adoption of pregnancy scanning among Australian sheep producers (all regions) by 10% by 2032, with 15% more of the scanned flocks providing customised management to twin/multiple-bearing ewes by 2032 (by scanning for litter size).

#### Project objectives

1. Undertake the following activities with the aim of increasing adoption of pregnancy scanning by 10% (by 2032):
  - a. Conduct an extensive benefit cost analysis on the value of scanning across a range of sheep producing regions and enterprise types.
  - b. Develop detailed business cases for pregnancy scanning.
  - c. Examine the accuracy of pregnancy scanning for pregnancy status and multiples using industry data from the MerinoSelect database.

2. Design and implement strategies to improve the skills base of pregnancy scanners and encourage the trialling and adoption of innovations in scanning technology by:
  - a. Create a scanning image library (videos of real-time assessments) as a tool for benchmarking.
  - b. Create a series of training materials and information resources for pregnancy scanners.
  - c. Conduct workshops for pregnancy scanners.

### **Current progress**

The economic analysis found that pregnancy scanning for multiples and implementing optimal management of empty ewes, differential nutrition and optimal paddock allocation and mob size of pregnant ewes is profitable for Merino and Maternal genotypes, in three production regions and for three times of lambing. The average increase in farm profit was \$5.75/ewe scanned, based on long-term prices (2004 to 2020). Pregnancy scanning for pregnancy status only (i.e pregnant or empty) is generally about half as profitable as scanning for multiples. Business cases detailing the results of these analyses are nearing completion.

A study of approximately 68,000 MerinoSelect records found that scanning for pregnancy status was 97-98% accurate, whereas the agreement between scanning for multiples and the number of lambs born was lower and more variable (85 - 88%) due to 10 - 12% singles in that data observed as born to ewes scanned with twins (from unobserved lamb loss rather than errors at scanning time) and some ewes giving birth to triplets that were scanned as bearing twins.

Two on-line workshops for pregnancy scanners have been held and a library of scanning images (video) and training materials for on-going professional development of pregnancy scanners are nearing completion.

For more information on the pregnancy scanning project, contact Forbes Brien ([forbes.brien@adelaide.edu.au](mailto:forbes.brien@adelaide.edu.au)).

## **Scientific papers**

### **Effects of different feeding systems on growth, fat accumulation and semen quality of Merino-type sheep**

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South African Journal of Animal Science, Volume 51(5)

DOI <https://doi.org/10.4314/sajas.v51i5.3>

#### **Abstract**

In South Africa, supplemental feeding is provided to improve the condition of breeding livestock before the animals are sold by the stud breeders to commercial farmers. This study aimed to evaluate the effects of different ram rearing systems on growth, fat accumulation and semen quality of Döhne Merino (DM), Merino (M) and South African Mutton Merino (SAMM) rams intended for breeding. The ram lambs were stratified according to weight and randomly allocated to one of three treatments, namely an extensive feeding treatment (ET), an extensive-intensive feeding treatment (EIT), and an intensive feeding treatment (IT). Rams in ET grazed for 200 days. Rams in EIT grazed for 73 days, followed by a concentrate diet for an additional 60 days. Rams in IT were fed the concentrate diet for 70 days. Scrotal neck fat was significantly less in IT rams compared with ET and EIT rams. Döhne Merino and M rams in EIT deposited significantly more scrotal fat than DM and M rams in ET. No adverse testicular pathology or differences in semen quality were observed. Regression analyses of pooled data showed extreme functional values for subcutaneous fat and total scrotal fat weight, which, when exceeded, may result in decreased semen volume, percentage normal spermatozoa and mass motility of spermatozoa. Sheep breeders should be cautious when feeding ram lambs in extensive-intensive systems for extended periods since this may affect semen quality adversely.

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## Poor maternal diet during gestation alters offspring muscle proteome in sheep

Sarah A. Reed ([sarah.reed@uconn.edu](mailto:sarah.reed@uconn.edu)), Jeremy Balsbaugh, Xiaomeng Li, Timothy E. Moore, Amanda K. Jones, Sambhu M. Pillai, Maria L. Hoffman, Kristen E. Govoni and Steven A. Zinn

Journal of Animal Science, Volume 100(8), August 2022

DOI <https://doi.org/10.1093/jas/skac061>

### Lay Summary

Poor maternal diet during gestation results in changes in body composition and metabolism in the offspring. Here, we demonstrate that over- and restricted-feeding during gestation alter global protein expression in the longissimus dorsi muscle of offspring during gestation and just after birth. These protein changes are related to protein synthesis and degradation, stress responses, metabolism, and oxidative stress. Proteins related to the initiation of protein translation were increased in offspring of over-fed dams at mid-gestation, while changes in abundance of enzymes associated with metabolism were altered in late gestation and just after birth. In offspring of restricted-fed ewes, proteins relating to cell signaling were increased at mid-gestation, while again, changes in late gestation and birth were related to metabolism, protein degradation, and stress responses. Together, these may provide a mechanism by which poor maternal diet during gestation alters the poor growth and development that occurs in these offspring.

### Abstract

Poor maternal nutrition during gestation can result in reduced offspring muscle growth and altered muscle metabolism. We hypothesized that over- or restricted-nutrition during gestation would alter the longissimus dorsi muscle (LM) proteome of offspring. Pregnant ewes were fed 60% (restricted), 100% (control), or 140% (over) of National Research Council requirements for total digestible nutrients from day 30 of gestation until parturition. Fetal (RES, CON, OVER) LM were collected at days 90 and 135 of gestation, or from offspring within 24 h of birth. Sarcoplasmic proteins were isolated, trypsin digested, and subjected to multiplexed, label-based quantitative mass spectrometry analysis integrating tandem mass tag technology. Differential expression of proteins was identified by ANOVA followed by Tukey's HSD post hoc tests, and regularized regression via the elastic net. Significance was set at  $P < 0.05$ . Over-represented pathways containing differentially expressed proteins were identified by Reactome and included metabolism of proteins, immune system, cellular response to stress/external stimuli, developmental biology, and infectious disease. As a result of maternal diet, a total of 312 proteins were differentially expressed (day 90 = 89 proteins; day 135 = 115 proteins; birth = 131 proteins). Expression of eukaryotic initiation factor (EIF) 2S3, EIF3L, and EIF4G2 was lower in OVER fetuses at day 90 of gestation ( $P < 0.05$ ). Calcineurin A and mitogen-activated protein kinase 1 were greater in RES fetuses at day 90 ( $P < 0.04$ ). At day 135 of gestation, pyruvate kinase and lactate dehydrogenase A expression were greater in OVER fetuses than CON ( $P < 0.04$ ). Thioredoxin expression was greater in RES fetuses relative to CON at day 135 ( $P = 0.05$ ). At birth, proteins of the COP9 signalosome complex were greater in RES offspring relative to OVER ( $P < 0.05$ ). Together, these data indicate that protein degradation and synthesis, metabolism, and oxidative stress are altered in a time and diet-specific manner, which may contribute to the phenotypic and metabolic changes observed during fetal development and postnatal growth.

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## Effect of pre-conceptional nutrition and season on fetal growth during early pregnancy in sheep

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### Abstract

Gestational age in sheep can be closely predicted through ultrasonographic measurement of fetal bones when correlated to standardized fetal growth curves. However, these standardized curves do not account for factors that are known modulators of fetal growth, such as maternal nutrition or health status. Despite being seasonal breeders, and studies reporting an effect of season on birth weight, the influence of season on fetal growth has not been well characterized. In this study, we hypothesized that season of conception will affect fetal growth curves during mid-gestation and that pre-conceptual nutrition would have no effect. We investigated this by provisioning treatments of low, control, and high planes of nutrition during the lactation and flushing pre-conceptual periods to multiparous Dorset x Polypay and Dorset ewes over two seasons (the optimal breeding season [n = 97] and the suboptimal breeding season [n = 104]). Females were mated naturally with mating dates recorded, fetal biparietal diameter measured via ultrasound between gestational days 35–71, and newborn weights recorded at lambing. Pre-conceptual nutritional treatments did not affect fetal biparietal diameter. However, low vs. high nutrition in the pre-conceptual lactation (but not flushing) period resulted in reduced lamb birth weights ( $P < 0.001$ ). Early fetal growth tended to be faster in the suboptimal breeding season than in the optimal breeding season ( $P < 0.061$ ) with lambs being heavier at birth in the optimal breeding season ( $P < 0.001$ ). There was no effect of fetal sex or litter size on fetal biparietal diameter during the first half of pregnancy, however both sex and litter size influenced lamb birth weight ( $P < 0.001$ ) with males being heavier than females and singletons being heavier than twins and triplets. Mating date within the flushing period had a significant effect on lamb birth weight regardless of season and independent of treatment, with ewes that conceived later in the flushing period having heavier lambs at birth ( $P = 0.007$ ). These findings suggest that pre-conceptual under- or overnutrition resulting in substantial changes in body condition does not affect fetal growth during the first half of pregnancy. However, the reduction in lamb birth weight indicates that pre-conceptual maternal nutrition during the previous lactation period may affect fetal growth later in pregnancy.

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### Refeeding ewes ad libitum after a moderate energy restriction during mid gestation did not affect the onset of breeding and ovulating rate of female offspring

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Animal Reproduction Science, Volume 244, September 2022

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### Highlights

- Dams' dietary treatment and litter size have no effect on lambs' milk intake.
- Lambs' milk feed conversion ratio does not differ due to dams' dietary restriction.
- Weaning weight is not affected by ewes' feeding treatments or lamb sex.
- The percentage of ewe lambs cycling is not affected by ewes' dietary restriction.
- The ovulation rate is not affected by ewes' dietary restriction.

### Abstract

Sub-nutrition during pregnancy might affect the offspring's reproductive performance through effects on the development and function of gonads. This study evaluated a maternal energy restriction in ewes from day 48–106 of gestation, on pre- and post-weaning female lambs' performance, onset of female lambs' breeding

and ovulation rate during their first breeding season. Adult Polwarth ewes sired by Finnish rams bearing single or twin lambs were assigned to two dietary treatments from day 48 to day 106 of gestation: restricted (R; n = 60) at 60 % of their metabolizable energy (ME) requirements, or non-restricted (NR; n = 54) at 100 % of their ME requirements. After the restriction period, ewes grazed all together ad libitum until weaning. The ewe lambs were evaluated from birth to weaning, as well as during their first breeding season. Although NR ewes weighed 17.7 % more than R ewes ( $P < 0.05$ ) at the end of the restriction period, the nutritional treatment of the dams had no effect on the weight of the ewe lambs at birth, weaning or final weight ( $P > 0.05$ ). Fat depth, rib eye area, the percentage of ewe-lambs cycling and their ovulation rate during the first breeding season was not affected by the ewe's dietary treatment. Ewes restricted at 60 % of their ME requirements in mid-gestation seemed to have the capacity to compensate any detrimental effects on growth and development as well as the reproductive potential of female lambs if adequate refeeding conditions are provided in late gestation, throughout lactation and post-weaning.

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### **The cost of host genetic resistance on body condition: Evidence from divergently selected sheep**

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Evolutionary Applications, 24 June 2022 **OPEN ACCESS**

DOI <https://doi.org/10.1111/eva.13442>

#### **Abstract**

Trade-offs between host resistance to parasites and host growth or reproduction can occur due to allocation of limited available resources between competing demands. To predict potential trade-offs arising from genetic selection for host resistance, a better understanding of the associated nutritional costs is required. Here, we studied resistance costs by using sheep from lines divergently selected on their resistance to a common blood-feeding gastro-intestinal parasite (*Haemonchus contortus*). First, we assessed the effects of selection for high or low host resistance on condition traits (body weight, back fat, and muscle thickness) and infection traits (parasite fecal egg excretion and loss in blood haematocrit) at various life stages, in particular during the periparturient period when resource allocation to immunity may limit host resistance. Second, we analysed the condition–infection relationship to detect a possible trade-off, in particular during the periparturient period. We experimentally infected young females in four stages over their first 2 years of life, including twice around parturition (at 1 year and at 2 years of age). Linear mixed-model analyses revealed a large and consistent between-line difference in infection traits during growth and outside of the periparturient period, whereas this difference was strongly attenuated during the periparturient period. Despite their different responses to infection, lines had similar body condition traits. Using covariance decomposition, we then found that the phenotypic relationship between infection and condition was dominated by direct infection costs arising from parasite development within the host. Accounting for these within-individual effects, a cost of resistance on body weight was detected among ewes during their first reproduction. Although this cost and the reproductive constraint on resistance are unlikely to represent a major concern for animal breeding in nutrient-rich environments, this study provides important new insights regarding the nutritional costs of parasite resistance at different lifestages and how these may affect response to selection.

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### **Administration of butorphanol with ketamine/xylazine sedation reduces the negative responses to electroejaculation in rams**

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Theriogenology, Volume 191, 1 October 2022

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### Abstract

This study aimed to identify the possible advantages of administering butorphanol (BUT), associated with ketamine/xylazine (KX) sedation or not, for semen collection using electroejaculation (EE) in rams. Fifteen rams received four treatments in a cross-over design using a 2 × 2 factorial arrangement. The treatments were 1) control; 2) received KX; 3) received BUT; and 4) received both treatments. The responses to the procedures regarding heart rate, respiratory frequency, and rectal temperature were recorded, glucose and creatine kinase were measured, and semen quality was determined. More pulses were required when BUT was administered ( $P = 0.0067$ ), but rams vocalized fewer times ( $P = 0.046$ ). The administration of KX also tended to reduce the respiratory frequency ( $P = 0.068$ ) and rectal temperature ( $P = 0.089$ ), with no other effects. The administration of BUT reduced the heart rate ( $P < 0.0001$ ), and there was an interactive effect between the administration of BUT and time on the heart rate ( $P < 0.0001$ ), respiratory frequency ( $P = 0.01$ ), and rectal temperature ( $P = 0.047$ ). The administration of BUT reduced the heart rate increase immediately after EE ( $P < 0.0001$ ). The respiratory frequency following EE was greater when BUT was administered ( $P < 0.0001$ ), but the administration of BUT reduced the rectal temperature at the same time ( $P = 0.002$ ). Glucose concentration was greater when BUT was administered ( $P < 0.0001$ ). The only significant effect on the ejaculate characteristics was from the interaction between KX and BUT on sperm concentration ( $P = 0.004$ ). The administration of KX alone increased sperm concentration ( $P = 0.035$ ), but when BUT was added to KX, sperm concentration decreased ( $P = 0.002$ ). Moreover, sperm concentration was greater when only BUT was administered than when KX and BUT were administered simultaneously ( $P = 0.037$ ). The addition of BUT markedly decreased most negative responses more effectively than sedation with KX alone. In conclusion, BUT appears to be an interesting alternative to reduce the welfare concerns raised by the use of EE in rams; however, its possible effects on the ejaculate characteristics when associated with other anesthesia/sedation drugs require further study.

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### An optimized centrifugation protocol for ram sperm ensuring high sample yield, quality and fertility

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### Abstract

The optimization and implementation of artificial insemination (AI) in sheep is necessary to increase the livestock productivity through enhanced control of reproductive function. Sperm centrifugation is a common procedure in the ejaculate handling in AI and other assisted reproductive technologies (ART), as part of new methods of sperm analysis, selection or preservation. However, our research group previously established that this simple procedure might cause a large sperm loss and induce deleterious effects on the sperm function of the ovine species when high centrifugation forces are employed. To our knowledge, there are no studies on combined effect of extender and different centrifugal forces on ram sperm yield and quality. Furthermore, evidence of in vivo fertility rate using sperm obtained with various centrifugation forces is also lacking in this species. Thus, the objective of this work was to define the ideal conditions for ram semen centrifugation that will achieve the best quantity and quality sample to ensure unaffected fertilization ability



of centrifuged ram sperm. The Experiment 1 evaluated the effect of the centrifugation procedure of two extenders (INRA 96 and Tyrode's) and two cooling protocols (Rapid and Slow Refrigeration  $-35\text{ }^{\circ}\text{C}$  to  $15\text{ }^{\circ}\text{C}$ ) on sperm recovery rate and quality (motility and kinetic parameters, viability, apoptosis and mitochondrial activity). INRA 96 combined with Slow Refrigeration and Tyrode's at room temperature registered the highest sperm recovery and quality values ( $P \leq 0.05$ ). In Experiment 2, the influence of three centrifugal forces (600, 1200 and  $6000\times g$  for 10 min) was assessed immediately after centrifugation on the technical performance and sperm functionality in diluted samples with INRA 96 and Tyrode's at the conditions set out in Experiment 1. The lowest pellet weight ( $P \leq 0.05$ ) without harmful effect on sperm physiological status ( $P > 0.05$ ) was achieved at  $1200\times g$ , since  $6000\times g$  induced sperm motility damage ( $P \leq 0.05$ ) with both extenders. Finally, to ensure the total safety of the centrifugation protocol, Experiment 3 tested in a combined in vitro and in vivo test the effect of these three centrifugal forces on ram sperm quality after dilution (INRA 96) and liquid storage (6–8 h at  $15\text{ }^{\circ}\text{C}$ ). The damage produced by  $6000\times g$  on sperm motility ( $P \leq 0.05$ ) was maintained over time, coinciding with a lower fertility ( $P \leq 0.05$ ). In conclusion, ram sperm can be centrifuged in INRA 96 extender up to  $1200\times g$  for 10 min at  $15\text{ }^{\circ}\text{C}$  as secure values with high recovery rates and without detrimental effects on sperm quality and fertility.

## Upcoming events

Date	Event	Location
1 September 2022	<a href="#">Getting a jump on ewe nutrition this spring!</a> Sheep Connect NSW	Webinar
6 September 2022	<a href="#">RAMping Up Repro</a> Sheep Connect SA	Ettrick, SA
15 September 2022	<a href="#">The lamb marking checklist – are you missing anything?</a> Sheep Connect NSW	Webinar
15 September 2022	<a href="#">RAMping Up Repro</a> Sheep Connect SA	Lochaber, SA
22 September 2022	<a href="#">RAMping Up Repro</a> Sheep Connect SA	Kongorong, SA
14 October 2022	<a href="#">MerinoLink (Final) MLP Field Day</a> AWI & AMSWEA	Temora, NSW
21 October 2022	<a href="#">Pingelly (Final) MLP Field Day</a> AWI & AMSEA	West Pingelly, WA