Sheep reproduction RD&A alert

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

If you missed the SRSP's October **Managing triplet bearing ewes for success** webinar, a recording will soon be available to view from the SRSP website. The triplet's webinar presented the key results and practical outcomes for sheep producers developed from the *Managing fecund flocks to improve survival of triplet dams and their lambs* project.

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.

Program coordinator
Dr Sue Hatcher
M: 0407 006 454
E: sue@makinoutcomes.com.au

Feature project update

Genetics of reproduction and lamb survival

Background
Various nutrition and management interventions are being used by sheep producers to improve their flock's reproduction rate. While some producers have made large gains, others have yielded responses that have been lower than expected. Early results from the Merino Lifetime Productivity project has demonstrated significant variation in reproductive performance between the daughters of industry sires and Sheep Genetics have recently released a suite of new reproduction ASBVs to help producers when making ram selection decisions. These new ASBVs provide an opportunity to demonstrate the role that genetics can play to augment nutritional and management strategies to improve ewe reproductive performance.

Aim
To demonstrate the role that genetics can play in improving ewe reproduction and lamb survival and develop the knowledge, awareness, skills and attitudes of producers involved to enable them to more confidently select rams that will improve the reproductive performance and lamb survival of their flock.

Current progress
Five core sites are underway across in Victoria and Western Australia, with each site aiming to demonstrate the differences between their old ram sources and a new source that has been selected with the intention of improving reproductive rate and/or lamb survival. One of the core sites has demonstrated a significant difference in lamb survival and growth rate as a result of changing their ram source. The remainder of the sites are expected to show differences in the reproductive rate of the daughters from the original sire teams over the coming years. A three-session online training course was held in 2022 to build awareness of strategies to make genetic gain for ewe reproduction and lamb survival.

For more information on the Genetics of reproduction and lamb survival PDS project contact Mark Ferguson (mark@nextgenagri.com).

Review papers

Role of the placenta in developmental programming: Observations from models using large animals

L.P. Reynolds (larry.reynolds@ndsu.edu), C.R. Dahlen, A.K. Ward, M.S. Crouse, P.P. Borowicz, B.J. Davila-Ruiz, C. Kanjanaruch, K.A. Bochantin, K.J. McLean, K.L. McCarthy, A.C.B. Menezes, W.J.S. Diniz , R.A. Cushman and J.S. Caton

Abstract

Developmental programming, which proposes that "insults" or "stressors" during intrauterine or postnatal development can have not only immediate but also long-term consequences for healthy and productivity, has emerged as a major biological principle, and based on studies in many animal species also seems to be a universal phenomenon. In eutherians, the placenta appears to be programmed during its development, which has consequences for fetal growth and development throughout pregnancy, and likewise has long-term consequences for postnatal development, leading to programming of organ function of the offspring even into adulthood. This review summarizes our current understanding of the placenta's role in developmental programming, the mechanisms involved, and the challenges remaining.

Scientific papers

Field demonstration analyzing the implementation of individual animal electronic identification and genetic testing in western range sheep flocks

Julie A. Finzel (jafinzel@ucanr.edu), Austin R. Brown, Roselle C. Busch, Morgan P. Doran, John M. Harper, Daniel K. Macon, Rebecca K. Ozeran, Morgan R. Stegemiller, Karissa Isaacs and Alison Van Eenennaam

Abstract

Adoption of electronic identification ear tags (EID) and DNA testing by commercial range sheep producers in the Western United States has been low, despite the availability of these technologies for over a decade. Jointly, these technologies offer an approach to provide individual animal performance data to improve flock health, genetic and reproductive management. This project involved a collaboration with five California sheep producers representing a broad geographic range, varying levels of pre-project EID adoption, and diverse operational practices. Tissue samples were collected from, and ear EIDs were placed in, a total of 2,936 rams and their potential lambs. We partnered with a commercial packing company, Superior Farms, to genotype the animals. Superior Farms used a targeted genotyping panel to assign parentage, and link individual animal identification (ID) to camera-graded carcass measurements. This enabled the collection of individual progeny carcass data and provided insight into sire performance, providing for the within-flock identification of prolific sires that were producing lambs with significantly more saleable meat as compared to their flock mates. Overall, almost 91% of lambs were successfully matched to their sire, and prolificacy ranging from 0–135 lambs per ram. There was as much as an $80 difference in the average edible product from camera-graded carcasses derived from lamb groups sired by different rams. A partial budget analysis modeling investment in an EID system coupled with an autodrafter and scale to collect individual weights and improve labor efficiency during processing, and a sheep flip chute to improve worker safety during foot

trimmings, yielded a greater than 7:1 return on investment over a five-year time frame. Ideally, the data collection enabled by EIDs and DNA testing would feed into data-driven genetic evaluation programs to enable selection for more productive and profitable animals, and allow the US sheep industry to accelerate the rate of genetic improvement.

Strategies to improve the success of fixed-time artificial insemination in the ewe

Alice C. Weaver (alice.weaver@sa.gov.au), Jennifer M. Kelly, Alyce M. Swinbourne, David O. Kleemann and Simon K. Walker

Abstract
During the sheep breeding season, ovulatory follicles vary widely in age at pessary removal impacting both the timing of oestrus and pregnancy rates following artificial insemination (AI). Ovulatory follicles that emerge between days 7 to 9 of the pessary period are associated with higher fertility whilst those that emerge earlier or later are associated with lower fertility. In this study, two strategies to improve the success of AI by controlling the development of the ovulatory follicle were examined. In the first, ewes were treated with PGF2α at either −12 and/or +6 days (experiment 1) or −27 days (experiment 2) relative to pessary insertion to control the time of emergence of the ovulatory follicle. In the second, ewes were treated with eCG (400 IU per ewe) at either 0 h, −6 h or −12 h relative to pessary removal (experiment 3) to improve the development of young ovulatory follicles. PGF2α administered on day −27 increased the percentage of pregnant ewes by 17.8% and the number of foetuses per 100 ewes inseminated by 33.9%. PGF2α treatment at other times had either no effect or reduced fertility. During the breeding season, treatment with eCG at −12 h improved the synchrony of oestrus, reduced the size of the ovulatory follicle but did not improve pregnancy rate compared with other treatments. Treatment had no effect during the non-breeding season, supporting earlier findings that the quality of young ovulatory follicles differs during the year. In conclusion, PGF2α treatment 27 days before pessary insertion provides a new and cheap strategy to improve the success of fixed-time AI programs.

Ram semen quality after supplementation with gelatin, agar or alginate prior to cooling storage

Juan González-Maldonado (juan.gonzalez.maldonado@uabc.edu.mx), Gustavo Ramírez-Valverde, Raymundo Rangel-Santos, Alfredo Lorenzo Torres, Canuto Muñoz-García and Jorge Alonso Maldonado-Jáquez

Abstract
Adding gelling agents to convert the liquid state of the semen extender to a solid state allows for an increased sperm life span. Gelatin and alginate have been used to study the effects of gelling agents on sperm quality. However, there are other gelling agents that have not been studied, such as agar. In addition, studying different sources of gelling agents or the effect of mixing more than one gelling agent with semen extenders on sperm fertility has received little attention. Therefore, the objective of this study was to evaluate the effect of adding agar and a mixture of gelling agents from different sources to semen extender on ram sperm traits and fertility. The first trial evaluated the effect of the addition of 2.5–3 mg mL−1 of gelatin mixed with 0.5–20 mg mL−1 of agar or alginate to ram semen extender on sperm (motility, progressive motility, live/dead, membrane integrity) and semen (pH) characteristics. The response variables were evaluated 1, 72

and 144 h after storage at 4°C. In the second trial, two sources (feed grade and bacteriological) of gelatin and agar were evaluated on the response variables as in Trial 1. In trial 3, a total of 34 ewes were inseminated with doses supplemented (n = 17) with or without (n = 17) agar and gelatin. The pregnancy rate was diagnosed 40 days after insemination. In general, adding agar and gelatin improves (p < .05) sperm motility, membrane integrity and the ratio of live sperm after 144 h of storage compared to the Control group, regardless of the source (bacteriological or feed grade). However, the pregnancy rate in ewes was not influenced (p ≥ .05) by semen doses stored with agar and gelatin. In conclusion, the addition of agar and gelatin preserves ram sperm motility and membrane integrity after 144 of storage at 4°C without affecting the pregnancy rate in inseminated ewes.

Does size matter? Testicular volume and its predictive ability of sperm production in rams

Rafael Montes-Garrido, Luis Anel-Lopez, Marta F. Riesco, Marta Neila-Montero, Cristina Palacin-Martinez, Cristina Soriano-Úbeda, Juan Carlos Boixo, Paulino de Paz, Luis Anel-Lopez (lanel@unileon.es) and Mercedes Alvarez

Simple Summary
Establishing the most suitable tool and the most accurate testicular volume determination formula could be essential in sperm production prediction. Traditionally, the caliper has been used to perform testicular measurements. In recent years, B-mode ultrasound has also been used as a measurement tool, providing, in addition to greater reproducibility as demonstrated in this work, other advantages such as being a non-invasive and non-ionizing technique that allows for the examination of the organ to see pathological processes. In the ovine species, the demand for seminal doses varies according to the genetic value of the donor males. The frequency of semen collection is high in males of great demand and genetic value. Testicular volume has a different influence on sperm production depending on the frequency of semen collection and the season of the year. This study demonstrated the effect of these factors on donor males to improve their reproductive performance.

Abstract
Over the years, testicular volume has been used to evaluate the reproductive capacity of rams and the effects of different factors related to reproductive performance. The aim of this study was to determine the most suitable tool and formula to calculate testicular volume under field conditions to guarantee a more accurate determination of sperm production. First, testicles from 25 rams (n = 50) were measured in vivo and postmortem using calipers and ultrasonography during the breeding season (BS). The accurate testicular volume (ATV) was calculated through water displacement. In addition, the sexual status of donor rams was evaluated during a period of four years in a reproduction center, and the three most crucial groups in terms of genetic value and seminal collections were studied in the second part of this experiment: ER-NBS (Elite rams during the non-breeding season), ER-BS-S (Elite rams with a standard frequency of seminal collection), and ER-BS-O (Elite rams with a high frequency of seminal collection). The total testicular volume (TTV), testosterone (T), and total spermatozoa obtained from two consecutive ejaculates in the same day (SPERM) were measured, and the relationship between SPERM and TTV and T was analyzed to predict SPERM. Although all published formulas revealed statistically significant differences (p ≤ 0.05) from the ATV, our proposed formula (ItraULE) (Testicular volume = L × W × D × 0.61) did not show significant differences. In the second part of the study, in the ER as a model donor ram for its high genetic value and high demand from farmers, TTV and T showed strong positive correlations with SPERM (r = 0.587, p = 0.007 NBS; r = 0.684, p = 0.001 BS-S; r = 0.773, p < 0.0001 BS-O). Moreover, formulas were established to predict SPERM in these

practical scenarios. In conclusion, the use of ultrasonography and a new formula adapted to rams could improve the prediction of SPERM considering crucial factors such as season and semen collection frequency.

Positive stimulus and sexual behavior of rams

Ana María García-Mahecha (anaga_001@hotmail.com), Rodolfo Ungerfeld and Lorena Lacuesta

Abstract

The reproductive result of commercial flocks is influenced by rams' reproductive performance, including their sexual behavior. Although there are pharmacological strategies for improving their sexual performance, organic production systems require hormone free strategies. Rams can be conditioned to positive stimuli such as food that triggers a response associated with positive affective state that could influence sexual behavior. Thus, a positive affective state could be an alternative strategy to improve sexual behavior in rams. Therefore, the aim of this study was to compare rams' sexual behavior toward an estrous ewe after accessing or not (control) a highly motivating food item (fruit). Initially, the rams' preference to consume bananas or apples was determined, and corneal and rectal temperatures, and heart rate were recorded for 12 rams. The rectal and mean corneal temperature increased after the consumption of the fruits (38.8 ± 0.05 °C vs 39.0 ± 0.05 °C, $P = 0.006$ and 35.2 ± 0.17 °C vs 36.1 ± 0.16 °C, $P = 0.0007$ respectively), without significant effects on heart rate. Thereafter, a test was designed to confirm whether they were enough motivated to consume the fruit, when offered close to an unknown subject making deterring behaviors. All rams responded positively to the test, approaching the fruit and consuming it for at least 30 s uninterruptedly in all the tests. For 10 days, rams were daily conditioned to the place where their sexual behavior would be evaluated, by entering a pen to which they were conditioned, in which the ram remained for 20 min with two ewes in the luteal phase immediately after consuming their motivational fruit. Finally, the sexual behavior of each ram was tested with a previous known ewe (in estrous) in two conditions: in the pen where they were conditioned with prior availability of the motivational fruit or in a similar pen to which they were not conditioned, without access to food. In this way, each ram was tested in both conditions with a crossover design. Rams only tended to display more flehmen after eating the positive stimulus than when tested without positive stimulus ($P = 0.08$). The previous consumption of palatable food did not modify the display of any other sexual behavior. It was concluded that the consumption of a highly motivating food as a positive stimulus did not modify the display of rams' sexual behavior.

Validation of targeted selective treatment (TST) methodology for gastrointestinal parasites of adult sheep in different physiological states

Matheus Borges de Carvalho, Diógenes Adriano Duarte Santana, Caroline Ramos dos Santos, Saulo Henrique Weber, Deborah Ribeiro Carvalho and Cristina Santos Sotomaior (cristina.sotomaior@pucpr.br)

Highlights
- We validated a Targeted Selective Treatment (TST) methodology for adult ewes.
- TST was based on Famacha© (F), body condition score (BCS) and submandibular edema.
- F was the main criterium for anthelmintic (AH) treatment in the TST.
- Ewes that needed AH had lower hematocrit and higher fecal egg count (FEC) means.
- The criteria used for TST could effectively identify ewes that needed AH treatment.

Abstract

We aimed to validate a targeted selective treatment (TST) methodology for treating parasitic gastrointestinal infections in ewes in different physiological states using parasitological and hematological parameters. Forty ewes were monitored from December 2021 to June 2022 and evaluated during various physiological stages in their life cycle. Before starting the experiment, a fecal egg count (FEC) reduction test was performed to evaluate the efficacy of the anthelmintic (AH) treatment. Weekly assessments were performed based on the Famacha© (F) system and body condition score (BCS), and ewes were subjected to AH treatment when necessary, with their physiological states recorded. Ewes were treated when they presented F ≥ 3, BCS ≤ 2.0 (when F = 2), or submandibular edema. Parasitological, i.e., FEC, and hematological, i.e., hematocrit (Ht), parameters were evaluated monthly to determine the efficiency of the TST methodology. Comparisons between the mean Ht and FEC values in ewes subjected to AH treatment and untreated ewes were performed using analysis of variance, followed by Tukey's test. Spearman's correlation was performed to determine the correlation between the variables, i.e., F scores, BCS, Ht, and FEC. All tests were performed at a significance level of 5 %. During the experimental period, 1138 evaluations were performed. The main reason for AH treatment was F ≥ 3. Ewes in early pregnancy, lactation and late pregnancy received comparatively more AH treatments than the other physiological states. Ewes in late pregnancy and lactation exhibited lower mean Ht values (23.5 % and 22.9 %) and higher mean FEC values (3269 and 1426) compared with those in early pregnancy (30.2 % and 727 EPG). In addition, a statistically significant difference was observed in the Ht and FEC values of ewes that presented submandibular edema ($P < 0.001$) compared with those that did not exhibit submandibular edema. The genus Haemonchus sp. showed a 96.4 % prevalence in coprocultures. A positive correlation existed between F scores and FEC ($r = 0.3819$) and a negative correlation between F scores and Ht ($r = -0.4728$). Ewes that needed AH treatment had lower mean Ht values than ewes that did not need the treatment (19.2 % × 29.3 %; $P < 0.001$) and higher mean FEC values (8747 × 1163; $P < 0.001$), confirming that these ewes needed AH treatment. The TST methodology based on F scores, BCS, and submandibular edema could effectively identify individuals in the herd needing AH treatment, identifying 13 % additional cases requiring treatment than using only the F score criterion.

Ovine placental explants: A new ex vivo model to study host–pathogen interactions in reproductive pathogens

Pilar Horcajo, Luis Miguel Ortega-Mora, Julio Benavides, Roberto Sánchez-Sánchez, Rafael Amieva, Esther Collantes-Fernández (esthercf@ucm.es) and Iván Pastor-Fernández (ipastor@ucm.es)

Highlights

- An ex vivo model of the ovine placenta using fresh and cryopreserved tissue was developed.
- Fresh and thawed explants were found to behave similarly and might be useful for studying host–parasite interactions.
- Several markers of explant integrity, viability, and functionality are defined and characterised.
- The model presented has the potential to be applied to several reproductive pathogens and even to other related host species.

Abstract

Reproductive failure is one of the main performance constraints in ruminant livestock. Transmissible agents such as Toxoplasma gondii and Neospora caninum are commonly involved in the occurrence of abortion in ruminants, but little is known about the mechanisms involved. While in vivo models are optimal for the study of abortion pathogenesis, they have a high economic cost and come with ethical concerns. Unfortunately,

alternative in vitro models fail to replicate the complex in vivo placental structure. To overcome the limitations of currently available models, we developed an ex vivo model based on the cultivation of fresh and cryopreserved sheep placental explants, enabling the biobanking of tissues. Reproducible and simple markers of tissue integrity (histology, RNA concentrations), viability (resazurin reduction), and functionality (synthesis of steroid hormones) were also investigated, allowing a clear quality assessment of the model. This work shows that, similar to fresh explants, tissues cryopreserved in ethylene glycol using slow freezing rates maintain not only their structure and function but also their receptivity to T. gondii and N. caninum infection. In addition, the findings demonstrate that explant lifespan is mainly limited by the culture method, with protocols requiring improvements to extend it beyond 2 days. These findings suggest that cryopreserved tissues can be exploited to study the initial host–pathogen interactions taking place in the placenta, thus deepening the knowledge of the specific mechanisms that trigger reproductive failure in sheep. Importantly, this work paves the way for the development of similar models in related species and contributes to the reduction of experimental animal use in the future.

Paternal effects on fetal programming

Carl Robertson Dahlen (Carl.Dahlen@ndsu.edu), Samat Amat, Joel S. Caton, Matthew S. Crouse, Wellison Jarles Da Silva Diniz and Lawrence P. Reynolds

Abstract

Paternal programming is the concept that the environmental signals from the sire's experiences leading up to mating can alter semen and ultimately affect the phenotype of resulting offspring. Potential mechanisms carrying the paternal effects to offspring can be associated with epigenetic signatures (DNA methylation, histone modification and non-coding RNAs), oxidative stress, cytokines, and the seminal microbiome. Several opportunities exist for sperm/semen to be influenced during development; these opportunities are within the testicle, the epididymis, or accessory sex glands. Epigenetic signatures of sperm can be impacted during the pre-natal and pre-pubertal periods, during sexual maturity and with advancing sire age. Sperm are susceptible to alterations as dictated by their developmental stage at the time of the perturbation, and sperm and seminal plasma likely have both dependent and independent effects on offspring. Research using rodent models has revealed that many factors including over/under nutrition, dietary fat, protein, and ingredient composition (e.g., macro- or micronutrients), stress, exercise, and exposure to drugs, alcohol, and endocrine disruptors all elicit paternal programming responses that are evident in offspring phenotype. Research using livestock species has also revealed that sire age, fertility level, plane of nutrition, and heat stress can induce alterations in the epigenetic, oxidative stress, cytokine, and microbiome profiles of sperm and/or seminal plasma. In addition, recent findings in pigs, sheep, and cattle have indicated programming effects in blastocysts post-fertilization with some continuing into post-natal life of the offspring. Our research group is focused on understanding the effects of common management scenarios of plane of nutrition and growth rates in bulls and rams on mechanisms resulting in paternal programming and subsequent offspring outcomes. Understanding the implication of paternal programming is imperative as short-term feeding and management decisions have the potential to impact productivity and profitability of our herds for generations to come.

Upcoming events

Date	Event	Location
1 November 2023	Feeding sheep in dry times NSW Local Land Services	Webinar
8 November 2023	Containment feeding of livestock MLA Productivity & Profitability webinar series	Webinar
13 November 2023	Confinement feeding visit NSW Local Land Services	Mudgee, NSW
14 November 2023	The big decision – Do I keep or sell? NSW Local Land Services	Goulburn, NSW
15 November 2023	The big decision – Do I keep or sell? NSW Local Land Services	Delegate, NSW
15 November 2023	Planning for dry times – early weaning NSW Local Land Services	Webinar
16 November 2023	Winning With Weaners Sheep Connect NSW & AWI	Mudgee, NSW
17 November 2023	Winning With Weaners Sheep Connect NSW & AWI	Coolah, NSW
21 November 2023	RAMping up Repro Sheep Connect NSW, AWI & Zoetis	Bathurst, NSW
23 November 2023	Winning With Weaners Sheep Connect NSW & AWI	Cowra, NSW
23 November 2023	The big decision – Do I keep or sell? NSW Local Land Services	Bega, NSW
24 November 2023	Winning With Weaners Sheep Connect NSW & AWI	Orange, NSW
24 November 2023	The big decision – Do I keep or sell? NSW Local Land Services	Berry, NSW