

### Project Information:

Project Title: Exploring reproductive consequences of gestational heat stress in ewe lambs

Recipient Organization Name: Colorado State University

### Recipient's Project Contact

Name: Caitlin Cadaret

Phone: (970)491-2012

Email: *Caitlin.cadaret@colostate.edu*

### Project Report

Annual Report Type: Semi-annual

Reporting Period                      Start Date:                      2023    End Date: 2024

Performance Narrative: Progress on this project has been continuous with near every proposed activity completed. Overall, work on the project has gone smoothly and supported our hypothesis that reproductive organs are impacted by gestational stress and persist after birth, particularly in the ovary. Lab work for endometrial histology and histotroph assays is completed and data from these are currently being analyzed. RNA samples did not prep well when sent to Novogene for RNA sequencing and thus, we have pivoted to a targeted PCR approach led by co-PI Coleman. We have confirmed that this methodology will be successful for obtaining transcript abundance data to align with the original proposed activities. The manuscript of these findings is in preparation while completing remaining work. We anticipate having all sample and data analysis for this grant completed by the end of the year. This will be incorporated into the current manuscript in preparation, with the goal of submitting it for publication in January of 2025.

### Activities Performed

- Live animal work is completed
- Necropsy has been performed and morphometrics collected
- Tissue distribution to co-PIs is completed
- Ovarian tissue has been isolated and processed
- Uterine tissue has been isolated and processed
- Manuscript is in preparation

Accomplishments

1. Data synthesis, analysis, and results of gross morphometric data is completed
2. Ovarian histology follicle counts are completed, analyzed, and results generated
3. Ovarian PCR analysis is completed, analyzed, and results generated
4. Ovarian immunofluorescence is completed, analyzed, results generated
5. Uterine luminal fluid glucose concentration determination is completed and currently being analyzed
6. Uterine endometrial gland histology is completed and analyzed
7. Endometrial RNA isolation for PCR has been completed
8. Preliminary results presented at the Rocky Mountain Reproductive Sciences Symposium

Estimate the Total Percentage (%) of work Completed on the Project.....

#	Accomplishment/Activity	Relevance to Objective
60%	Live animal work and sample collection completed	Objective 1: treatment application and morphological collection
20%	Ovarian histology and PCR	Objective 1 & 2: Cellular parameters and transcript expression in ovarian tissue of lambs born from heat stressed dams
10%	Uterine histology and histotroph analysis completed – statistics ongoing	Objective 1: cellular and morphological parameters of reproduction

Challenges and Developments

#	Challenge or Development	Corrective Action or Project Change
1	RNA from endometrial tissue sent for sequencing did not prep well in Novagene’s process.	Due to limited sample, we are now performing targeted PCR in house

Outcome and Indicator Results to Date

#	Outcome/Indicator	Quantifiable Results
	IUGR lambs did not appear to have impaired organ specific growth	<ul style="list-style-type: none"> <li>No differences existed in ovary weight or caruncle number</li> <li>At 30d IUGR lambs tended (<math>P &lt; 0.10</math>) to have lower uterine weights despite similar bodyweights</li> </ul>
	Folliculogenesis is impaired in lambs born from heat stressed dams	<ul style="list-style-type: none"> <li>Primordial follicle population reduced (<math>P &lt; 0.05</math>)</li> <li>No differences in primary population (<math>P &lt; 0.05</math>)</li> <li>Increase in secondary population (<math>P &lt; 0.05</math>)</li> <li>Reduced antral follicle counts (<math>P &lt; 0.05</math>)</li> </ul>
	Gene expression and protein concentration for factors important in reproductive cyclicity are impaired in IUGR ewe lambs	<ul style="list-style-type: none"> <li>Fluorescence intensity of AMHR, Er<math>\alpha</math>, Er<math>\beta</math>, and PCNA was reduced in the ovary of IUGR lambs (<math>P &lt; 0.05</math>)</li> <li>Transcript abundance of Er<math>\beta</math> and PCNA were reduced in IUGR lambs while there were no differences in AMHR and Er<math>\alpha</math> (<math>P &lt; 0.05</math>)</li> </ul>

### Upcoming Activities

#	Activities	Anticipated Completion
	Targeted PCR on endometrial tissue and completed data analysis	December 2024
	Manuscript submission	Jan 2024
	Manuscript publication	March 2025

### Project Expenditures to Date