National Sheep Industry Improvement Center
Grant Application

Date Submitted: September 15, 2017

Legal Name of Applicant: Oregon State University

Name: Michelle Anne Kutzler, DVM, PhD

Address: 312 Kerr Administration Building

City: Corvallis
State: Oregon
ZIP: 97331
County: Benton

Congressional District: OR-004

Project Title: PG-600® increases uterine estrogen receptors resulting in increased embryo mortality and reduced pregnancy rates in sheep

Person to be contacted about application: Patricia A. Hawk

Phone: 541-737-4933
Email: sponsored.programs@oregonstate.edu

Project Abstract
The management problem this study aims to resolve is the reduced fertility following estrous cycle synchronization with PG-600®. PG-600® (Intervet) is a single dose injectable product labeled for use in swine, but used off-label by sheep producers. Each dose (5 mL) contains a combination of equine chorionic gonadotropin (400 International Units) and human chorionic gonadotropin (200 International Units). PG-600® is the preferred choice by sheep producers for out-of-season estrus induction over other products because it has a longer metabolic clearance (approximately 72 hours), thereby reducing the number of injections that must be given. However, PG-600® will induce an ovarian overstimulation in sheep, resulting in large un-ovulated follicles as well as an increase in unfertilized oocytes and poorer quality embryos.

Recently, our laboratory demonstrated that PG-600® administered to cycling ewes resulted in significantly elevated serum estrogen concentrations within the first four days after mating and significantly lower 28-day pregnancy rates. Elevated systemic estrogen can increase uterine estrogen receptor expression resulting in enhanced biochemical changes in the uterine endometrium.

We hypothesize that the reduced pregnancy rates observed following PG-600® result from embryo mortality during early pregnancy (4-7 days after breeding), just as the embryos are leaving the Fallopian tubes and entering the hostile uterus environment created by over exposure to estrogen. The objective of this research is to compare the number of uterine estrogen receptors at 4 and 7 days after estrus in ewes treated with 5 mL PG-600® or 5 mL saline (controls). Results from this research will provide a physiologic explanation for reduced pregnancy rates following PG-600® as well as allow for new opportunities for treatment to prevent embryo mortality.

Project Objectives
We hypothesize that the reduced pregnancy rates following PG-600® observed by our research team and reported by other groups result from embryo mortality 4-7 days after breeding. This is a critical stage during early pregnancy because it is the time in which the embryos are leaving the Fallopian tubes and entering the uterus. In addition, we hypothesize that the cause of this early embryo mortality is the result of an increase in uterine estrogen receptors promoting an abnormal inter-uterine environment for the embryo. Therefore, the objective of the proposed research is to compare the concentration of uterine estrogen receptors at 4 and 7 days after estrus in ewes treated with 5 mL PG-600® or 5 mL saline (controls).

Description of efforts – Anticipated results
Our research team has extensive experience in sheep reproductive physiology and estrus induction. In addition, our preliminary results have positioned us well to perform the experiments described in this application. We anticipate that the high estrogen concentration produced from large un-ovulated follicles from the PG-600 will increase uterine estrogen receptors, which will result in greater embryo mortality.

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Amount requested from NSIIC

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GRANT TOTAL

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Provide a qualitative summary, or justification for budget expenditures:
The budget includes the cost for estrus synchronization drugs, blood collection (needles, syringes, blood tubes, storage tubes), tissue collection (surgery packs, anesthesia, suture, post-anesthetic pain management, antibiotics), hormone assays, receptor assays (isotopes, radioactive waste removal, scintillation vials, conical tubes, buffer solutions, protein concentration assay), travel ($1500) to present at the 2018 Society for Theriogenology conference in Milwaukee, Wisconsin, and publication ($500).
Technical Objectives:
Responsiveness to NSIIC Stated Priorities (from web site)
In line with the NSIIC priorities, our proposed research has the potential of identifying
the uterine biochemical basis for early embryonic mortality in sheep and thus
providing information that can be used to develop methods for improving embryo
survival and ultimately overall sheep production in the US.

Prior USDA or other Federal Support
This research has not received previous USDA or federal support. However, it has been
generously supported through two grants from the Oregon Sheep Commission.

Identification and significance of the issue being addressed
The significance of the proposed research is to critically evaluate the fertility outcomes
of PG-600 for estrus synchronization in sheep which, if identified and addressed, could
thereby increase the embryo survival and lambing rate. High doses of PG-600 can
reduce the fertility of ewes because of increasing estradiol production with induction
increasing estrogen receptor concentration within the uterus. High estrogen
concentration may also result in a shortened luteal phase by increasing uterine PGF-2
alpha secretion. A short luteal phase would impair normal progesterone secretion
which in turn negatively affects the pregnancy and then reduces embryo survival and
lambing rate.

What are the goals to be achieved with this grant funding?
The goals from this study are to determine the uterine mechanisms that cause an
increase in the embryo mortality following PG-600 administration. Our working
hypothesis is that high levels of estrogen receptor in the endometrium in the early
pregnancy results in an intra uterine environment that is toxic for the embryo and thus
will have undesirable consequences on embryo survival.

Work Plan – Task, Methodology, Individual responsible, and location where work will
be done.
The experiment will be conducted at Oregon State University. Multiparous ewes (n=24)
will be used in this study. They will be administered a progesterone-releasing vaginal
implant (Eazi-Breed™ CIDR, Zoetis) for 9 days. Progesterone priming is a necessary
component to estrus induction in small ruminants. Two days prior to CIDR removal,
cloprostenol (0.5 mL; Estrumate) will be given intramuscularly to regress any existing
corpora lutea. On the day of CIDR removal (day 0), ewes will be randomly divided into
two groups: PG-600® (5 mL; n=12) and saline (5 mL; n=12). Immediately following
CIDR removal but prior to given the treatment injection, a jugular venous blood sample
will be collected (0 hour). Blood samples will also be collected at 2, 4, 6, 8, 12, and 24 hours, and at 2, 3, 4, 5, 6, and 7 days post treatment. Serum concentrations of estradiol 17-beta and progesterone will be determined from the collected blood samples using an Immulite 1000 immunoassay analyzer (Siemens) as previously described by Kutzler et al (2003). Four days and seven days after CIDR removal, six ewes from each group will undergo general anesthesia. The ewes will be positioned in dorsal recumbency and the ventral abdomen will be aseptically prepared for surgery. A ventral midline incision will be made so that the uterine horn can be exteriorized through the abdominal incision. The ovaries will be examined visually and number of corpora lutea counted. The uterine horn will be incised and approximately 1 gram of intercaruncular endometrial tissue will be removed. The uterine horn will then be sutured closed. The abdominal incision will then be sutured closed. The ewe will be allowed to recover from anesthesia and after 3 days return to the flock. Endometrial estrogen receptor binding affinity will be determined using the nuclear estrogen receptor assay as previously described by Koligian and Stormshak (1977). Hayder Habeeb will administer all of the treatments to the ewes, collect the blood samples and analyze the serum estradiol concentration, as well as perform the estrogen receptor assays. Dr. Kutzler and Dr. Stormshak will perform the surgical tissue collection. All investigators will participate in the data analysis and manuscript preparation.

Related Research or other efforts in this area of which you are aware, including an analysis of the competitive landscape if the award is for a commercial application. Current research; there is no commercial application at this time.

Potential Post application in this area of development of research or commercial endeavor
If acquired data indicate that uterine endometrial concentrations of estrogen receptors are increased in PG-600 treated ewes then experiments may be performed to examine therapies to reduce the level of estrogen receptor and/or their induced products, and effective the therapies on embryo survival.

5 Kutzler MA, Mohammed HO, Lamb SV, Meyers-Wallen VN. Accuracy of canine parturition date prediction from the initial rise in preovulatory progesterone concentration. Theriogenology 2003;60(6):1187-96.
6 Koligian KB, Stormshak F. Nuclear and cytoplasmic estrogen receptors in ovine endometrium during the estrous cycle. Endocrinology 1977;101(2):524-33.
Background and rationale
In sheep, the estrous cycle length ranges between 13-19 days (average of 17 days). The estrous cycle consists of two phases: follicular phase and luteal phase. In the follicular phase, follicles are developing and estrogen from the follicle increases in the blood. Estrogen synergizes with follicle stimulation hormone (FSH) for inducing the final maturation of follicle. Estrogen also changes the pattern of secretion of luteinizing hormone (LH) from high amplitude low frequency to low amplitude high frequency. The new LH pattern results in an LH surge, which initiates ovulation. Once ovulation occurs, the follicular phase ends and the luteal phase starts. During the luteal phase, the ruptured follicle forms into a corpus luteum (CL). With the CL development, progesterone starts to increase until the end of the luteal phase. If there is no pregnancy, PGF-2alpha from the uterus will lyse the CL, resulting in the start of another estrous cycle.

Estrus induction for out-of-season breeding has been used in the sheep industry for decades to increase ewe productivity. To induce ewes to come into estrus outside of their natural breeding season (Fall/Winter), it is necessary to stimulate the ovary with multiple daily FSH injections or a single injection of equine chorionic gonadotropin (eCG) plus human chorionic gonadotropin (hCG) (PG-600®). PG-600® is the preferred choice for estrus induction in sheep because of its ease of use. However, at the dose administered to ewes (5 mL), PG-600® is likely to overstimulate the ovaries, resulting in reduced pregnancy rates (42% when compared to ewes bred during the breeding season).7-8 Our previous research has also found exaggerated ovarian responses and reduced pregnancy rates in ewes treated with PG-600®. Preliminary data in our laboratory has demonstrated that PG-600® (5 mL) given to cycling ewes results in increased systemic estradiol. Elevated serum estrogen levels can increase uterine estrogen receptor expression. We hypothesize that the reduced pregnancy rates observed following PG-600® result from embryo mortality during early pregnancy (4-7 days after breeding), just as the embryos are leaving the Fallopian tubes and entering the uterus. The objective of this research is to compare the concentration of uterine estrogen receptors at 4 and 7 days after estrus in ewes treated with 5 mL PG-600® or 5 mL saline (controls). Results from this research will provide a physiologic explanation for reduced pregnancy rates following PG-600® as well as allow for new opportunities for treatment to prevent embryo mortality.

**Relationship to industry, including technical, economic and social benefit**

Our proposed research will enhance the sheep production and it will provide a reliable method for creating a sustainable supply of lamb for marketing, increase ewe lifetime productivity, and economically increase agriculture production within the United States. Also, our research will provide data to improve the production of lamb meat for cultural occasions during times of the year when lamb is typically not available.

**Cost benefit analysis**

The cost of the proposed research is small in comparison to the knowledge that this research will deliver to the sheep industry and scientific community.

**Policy or decisions affected by results**

A lower dose of PG-600® may be recommended in the future for inducing estrus in ewes outside of the breeding season.

**Financial Feasibility (i.e., Is the budget proportionate to the endeavor, and will the outcome have a financial benefit to the industry in the near term or will additional funding be required?)**

Our proposed research will be financially beneficial to the sheep production industry by providing a reliable method for creating a sustainable supply of lamb for marketing by reducing the embryo mortality, increasing ewe lifetime productivity, and economically increasing agriculture production within the United States. Also, our research may enable production of more lamb meat for cultural occasions during times of the year when lamb is typically not available.

**Business Soundness (i.e., Are project participants qualified and experienced)**

Our research team has extensive experience in sheep reproductive physiology and estrus induction.

**Management Ability (i.e., Are project participants qualified and experienced)**

Our research team has extensive experience in sheep reproductive physiology and estrus induction.

**Potential Industry Impact (i.e., How can the industry be expected to benefit in both qualitative and quantitative measures?)**

Our proposed research will be beneficial to the sheep production industry by providing a reliable method for creating a sustainable supply of lamb for marketing, increase ewe lifetime productivity, and economically increase agriculture production within the
United States. Also, our research will provide for a greater supply of lamb meat for cultural occasions during times of the year when lamb is typically not available.

**Industry Support (i.e., What data or other information is available to substantiate industry’s need or desire for this project?)**

Industry support has been previously substantiated by two successful funding opportunities through the Oregon Sheep Commission.
BIOGRAPHICAL SKETCH

NAME  
Kutzler, Michelle Anne

POSITION TITLE  
Associate Professor

EDUCATION/TRAINING

<table>
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Academic/Professional Appointments

1993-1995  Associate Veterinarian in a private practice, Cold Spring, MN
1995-1997  Associate Veterinarian in a private practice, Kasson, MN
1997-1999  Theriogenology Resident, Department of Clinical Science, Cornell University, Ithaca, NY
1999-2001  Clinical Scientist, Department of Clinical Science, Cornell University, Ithaca, NY
1999-2002  Doctoral Candidate, Department of Biomedical Science, Cornell University, Ithaca, NY
2002-2010  Assistant/Associate Professor (tenured and promoted May 28, 2008), Department of Clinical Sciences, Oregon State University College of Veterinary Medicine, Corvallis, OR
2010-current  Associate Professor, Department of Animal and Rangeland Sciences, Oregon State University College of Agricultural Sciences, Corvallis, OR

Professional Memberships

1989-current  Society for Theriogenology
1989-current  American Veterinary Medical Association
1999-current  American College of Theriogenologists (Certifying Examination Committee; 2012-current)
2000-current  Society for Gynecologic Investigation (Scientific Abstract Reviewer, 2003-current)
2015-current  Society for the Study of Reproduction
Publications Closely Related to the Proposed Project


3. **Kutzler MA**, Ruane EK, Coksaygan T, Vincent SE, Nathanielsz PW. 2004. Effects of three courses of maternally administered dexamethasone at 0.7, 0.75 and 0.8 of gestation on prenatal and postnatal growth in sheep. *Pediatrics* 113(2):313-319.


BIOGRAPHICAL SKETCH

NAME
Habeeb, Hayder Mohammed H.

POSITION TITLE
Doctoral Candidate

EDUCATION/TRAINING

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Academic/Professional Appointments
2002-2003 Agriculture engineer in private farm. (Jar Allah group) Sana'a, Yemen
2003-2004 Biology and chemistry Lecturer, Sana'a - Yemen
2006-2011 Lecturer assistance, Department of Animal Science, Babylon University College of Agriculture, Iraq
2011-2014 Lecturer, Department of Animal Science, Babylon University College of Agriculture, Iraq
2015-current PhD Candidate, Department of Animal and Rangeland Sciences, Oregon State University College of Agricultural Sciences, Corvallis, OR

Professional Memberships
2017-current Society for Theriogenology
2012-current Association of genetic resources conservation and environmental Iraqi

Publications Closely Related to the Proposed Project


BIOGRAPHICAL SKETCH

NAME
Stormshak, Fred

POSITION TITLE
Professor Emeritus

EDUCATION/TRAINING

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Academic/Professional Appointments

1997-current    OSU Distinguished Professor

Professional Memberships

1960- current    American Society of Animal Science
1966- current    Endocrine Society
1967- current    Society for the Study of Reproduction

Publications Closely Related to the Proposed Project

CERTIFICATION

To the best of my knowledge and belief, all data in this application is true and correct. The document has been duly authorized by the governing body of the Applicant and the Applicant will comply with all Grant requirement if the assistance is awarded.

Signature: Lydia Perry				Date: 9/15/17
Acting for:
Name of authorized representative: Patricia Hawk

Title: Assistant Vice President, Office for Sponsored Research and Award Administration

Email: sponsored.programs@oregonstate.edu

Phone: (541) 737-4933