W-112 WESTERN REGIONAL RESEARCH PROJECT
RENEWAL

Project Title: REPRODUCTIVE PERFORMANCE OF DOMESTIC RUMINANTS

Requested Project Duration: October 1, 2001 through September 30, 2006

Statement of Issues and Justification. The past decade has seen dramatic change in basic and applied reproductive biology. The technical revolution that began with artificial insemination and embryo transfer in the last half of the twentieth century reached new heights with the widely reported cloning of sheep and cattle by nuclear transfer from somatic cells. Similarly, less well publicized technical advances, such as in vitro maturation of oocytes, embryo culture, gene modification and transfer, embryo splitting, identification and isolation of X and Y bearing spermatozoa, sexing of embryos prior to transfer, and early pregnancy diagnosis, may also be used to advantage by cattle and sheep producers to increase productivity and ease of management and improve the health and genetic composition of flocks and herds. Although artificial insemination and embryo transfer have been widely accepted and adopted by the dairy industry, these techniques are less commonly used in the beef cattle and sheep industries. However, the improvement in animal health and productivity in the dairy industry has not gone unnoticed and use of these sophisticated reproductive technologies in the beef cattle and sheep industries is expected to increase dramatically during the next decade. One of the objectives of our work in the W112 project is to provide the scientific and technical expertise that will support and encourage development, acceptance, and application of technology-based management tools that will improve the productivity and profitability of livestock producers in the Western states.

Despite recent advances in reproductive technology, cattle and sheep producers are still faced with the persistent problem of low fertility. Indeed, recent work indicates that the fertility of domestic ruminants, even under optimal conditions, is about 50%. That is, at best only one of every two natural or artificial inseminations results in the birth of a healthy calf or lamb. The poor fertility of domestic species is reflective of cumulative loss due to poor fertilization efficiency, high embryo mortality, and spontaneous abortion.

One must also recognize that these statistical estimates of fertility reflect the response noted in animals maintained under highly controlled conditions. The unique and varied environments facing domestic animals on the range lands of the Western states are likely to reduce the efficiency of fertilization and increase embryonic and fetal loss. Indeed, the Western and Southwestern states included in the W112 project encompass a land mass that represents more than 50% of the US total and includes more than 57 million cattle and calves and 6.5 million sheep. Much of the Western range land is included in the high desert plateau, where limited rainfall and a short growing season make feed a limiting factor in animal production. Seasonal and regional fluctuation in feed quality and quantity, climatic extremes, and other environmental factors can markedly affect the efficiency of reproduction.

As noted in Table 1, the inventory of livestock in the Western states represents a significant fraction of the US total. Indeed, the value of sheep and cattle produced by farms and ranches in W112 member states exceeds 32 billion dollars. These figures indicate that livestock production

is a critical component of the economic health of the Western states and, moreover, that the US supply of livestock and products (meat, wool, etc.) is dependent on the production efficiency of Western herdsmen.

Table 1. Cattle and sheep inventory in the Western states and the value of livestock and products sold.

Category[a]	W112 Member States	Fraction of US Total (%)
Number of Beef Cows	20,100,000	60
Number of Feedlot Cattle	21,600,000	80
Value of Cattle and Calves Sold	$31.745[b]	78
Number of Sheep and Lambs	6,550,000	84
Value of Sheep, Lambs, and Wool	$583.5[c]	85

[a] data from USDA 1997 Census of Agriculture, Tables 28, 30, 38, 41, 46.
[b] in billions
[c] in millions

In addition to production of beef cattle and sheep, a phenomenon that has gained momentum in the last decade has been the domestication of exotic ruminant species. This has been particularly true in Texas, Wyoming, Montana, and Alaska, where commercial production of non-traditional species has expanded from bison and white-tailed deer to now include muskoxen, reindeer, elk, and caribou. Management and efficient propagation of these species has added a new challenge to the original mission of our research project.

An additional challenge to livestock producers in the Western states is the changing demographics of our region. Indeed, the unrelenting expansion of our urban populations continues to stretch the boundaries of the urban-rural interface. Issues like air and water quality, waste management, production efficiency, and resource stewardship are becoming of increasing importance as the interface between the rural and urban communities expands. Another issue of concern in the Western states is animal welfare and we are expanding our efforts to develop new management strategies that will improve animal well-being while maintaining or improving productivity.

The members of our research project strongly adhere to the vision of the Regional Research concept. That is, we believe the Western states represent a unique ecosystem that offers opportunities and challenges for producers of domestic ruminants. The challenges that are shared in common among the Western states are best addressed by combining the expertise and resources of representatives from all the states. In addition, we have requested the participation of experts from states outside our region when the advice, experience or knowledge of those experts would enhance our ability to improve the fertility of domestic ruminants in the target region. Thus, membership in the W112 project has not been limited solely to representatives from the Western region, but also includes leading reproductive biologists from Kansas, Michigan, Minnesota, Missouri, Nebraska, Ohio and Texas. We have found the contributions from these distinguished colleagues to be particularly valuable.

To effectively address the critical aspects of reproductive biology affecting reproductive efficiency in the Western states the members of the W112 project have divided into research teams or workgroups. These workgroups place particular emphasis on those components of the reproductive process (follicular maturation, ovulation and fertilization, embryo development, implantation, maternal recognition of pregnancy and maintenance of luteal integrity) that limit fertility. Our research teams also examine the role of stress, season and photoperiod and nutritive status on folliculogenesis and ovulation. In addition, our clinical colleagues are developing vaccines and immunologic strategies to lessen the incidence and severity of diseases that reduce the fertility of domestic species in the Western states.

The **Ovarian Biology Research Group** examines the molecular, cellular, and endocrine mechanisms that control follicular development, ovulation, and subsequent luteal function. The members of this group include representatives from CA, OR, KS, WA, ID, ID [ARS], MT, MT [ARS] WY, CO, NM, AZ, AK, NE [ARS], TX, MN, MI, OH, and MO. The specific charge to this group is to define the endocrine, paracrine and/or autocrine factors that control follicle maturation, with the goal of increasing the number and viability of follicles and improving the synchrony of follicle maturation among animals. The members of this group also examine the cellular and molecular biology of the ovulatory process, with the goal of improving the synchrony between ovulation and estrus. Additionally, members of the Ovarian Biology Research Group examine the process of luteinization, with the goal of reducing the incidence of luteal insufficiency during the post-ovulatory period. The Ovarian Biology Research Group will also examine the effect of stress, photoperiod, season, and nutritive status on follicular development and ovulation.

The **Uterine Biology Research Group** examines the molecular and cellular aspects of embryo development, implantation and maternal recognition of pregnancy. The members of this group include representatives from CA, WA, ID, ID [ARS], WY, CO, OR, HI, TX, and MO. The specific charge to this group is to define the endocrine, paracrine and/or autocrine factors that regulate conceptus and uterine gene expression, signal implantation and contribute to luteal maintenance and maternal recognition of pregnancy, with the ultimate goal of increasing the efficiency of implantation and reducing embryonic and fetal mortality. The members of this group will also examine the maternal and fetal factors that influence placental development and control fetal growth and maturation.

The **Animal Disease Research Group** includes representatives from AK, CA, CO, ID, ID [ARS], NV, TX, WA, and WY. The research conducted by this group is concerned with development of immunologic approaches to lessen the incidence and/or severity of diseases endemic to the Western states. In addition, we will continue to develop management strategies to lessen the severity of bacterial, viral and parasitic disease, with concerted focus on mastitis in animals on the Western range.

The **Reproductive Technology Research Group** includes members from all participating states. The broad membership of this group is reflective of the critical contributions it makes toward attainment of our overall mission; improving fertility of domestic ruminant in the Western region. The charge to this group is the development and optimization of management protocols and treatment regimens that will facilitate and encourage the use of artificial

insemination and other novel reproductive technologies by cattle and sheep producers in the Western states. Another issue of concern in the Western states is animal welfare and we are expanding our efforts to improve animal well-being by seeking to develop effective and efficient immunologic alternatives to castration.

Related, Current and Previous Work. The W112 Regional Research Project was established in 1970 with the goal of creating a cooperative research group that could bring both basic and applied expertise to bear on factors that limit the fertility of domestic ruminants in the Western states. Confining the focus to the Western states was reflective of the unique features of the West (climate, topography, flora, endemic disease organisms, and demographics) and the unique management challenges that these features present to Western livestock producers. Although many of the founding members of the project have since retired, the philosophy and mission established more than thirty years ago continues to be the guiding tenet of our group; that is, *cooperative multi-state research, product and technique development and outreach for the benefit of animal producers in the Western region*.

The list of research achievements, publications, and student theses that are the product of the collaborative work of members of the Project is extensive. Indeed, during the last 5 year period 328 refereed articles, 290 abstracts and 290 technical bulletins and related publications have been generated by research projects conducted with W112 Regional Research support. Although it is not possible to detail each of the significant accomplishments made during the past 5 year period, a summary of the major advances is provided below.

Major Advances of the Ovarian Biology Research Group

Follicular Development Workgroup—During the last 5 year period the Follicular Development Workgroup (which includes representatives from AK, AZ, CA, CO, KS, MN, TX, WY) has identified two intra-ovarian growth factors (GDF9 and BMP15) that regulate the early (gonadotropin-independent) stages of folliculogenesis. Within the ovary, both of these growth factors are expressed exclusively by oocytes. BMP15 is of special interest because it is located on the X-chromosome and has recently been identified as the Inverdale twinning gene in sheep.

Additional studies by the Follicular Development Workgroup have examined the hormonal control of follicular development during the reproductive cycle of cattle, sheep and exotic ruminants (deer, caribou, reindeer, and elk). The results of these studies have demonstrated that inhibin-dependent control of FSH secretion during the follicular phase is a primary determinant of follicular development and ovulation rate. Indeed, this group reports that ovulation rate is increased in animals actively or passively immunized against inhibin. Other studies have examined the factors regulating GnRH receptor expression and LH secretion during the follicular phase. These studies have clearly shown that estradiol secretion from the developing follicle controls gonadotrope responsiveness during the follicular phase of the estrous cycle.

Sexual Behavior Workgroup—Sexual inactivity or poor libido is a common management problem facing sheep producers in the Western states. Indeed, recent studies indicate that the number of rams culled from flocks for mating behavior defects or inadequacies is equal to the number culled for physical limitations or poor semen quality. The Sexual Behavior Workgroup

has examined the neuroendocrine factors that may contribute to insufficient or improper sexual behavior in rams and bulls on the Western range. The considerable achievements of this group over the past 5 year period are due in large part to the strength of cooperative interaction among members from CA, ID [ARS], OR, WA, and WY.

Ovulation Workgroup—Research conducted by the Ovulation Workgroup (which includes representatives from AZ, MI, MO, CO, WY) has demonstrated that proteolytic degradation of the extracellular matrix (ECM) at the apex of ovulatory follicles prior to ovulation is a crucial step in the complex cascade of events initiated by the LH surge. The matrix metalloproteinases (MMPs) digest specific components (collagens, laminin, fibronectin, and proteoglycans) of the ECM and are noted for their role in ECM remodeling. The studies of the Ovulation Workgroup indicate that the MMPs mediate the ovulatory process in domestic ruminants.

Luteal Function Workgroup—Following ovulation, the remnants of the ovulatory follicle (~30 mg) differentiate into the corpus luteum (~600 mg). This rapid and marked growth and functional transformation is associated with dramatic changes in tissue remodeling including cell hypertrophy and hyperplasia and remodeling of the ECM. As noted above, the composition of the ECM is influenced by MMPs. The activity of MMPs, in turn, is regulated by tissue inhibitors of metalloproteinases (TIMPs). TIMP-1 is a mulitfunctional molecule that not only modulates the activity of MMPs, but may also stimulate proliferation of luteal cells and stimulate progesterone production.

The Luteal Function Workgroup includes members from CO, MO, HI and TX. This group has characterized several ovarian proteins that may have important autocrine, paracrine, and/or endocrine roles in the regulation of luteal function. Specific attention has been directed toward characterizing gene and protein expression of ovine TIMP-1. The workgroup has characterized the nucleotide sequence of ovine TIMP-1 mRNA and determined sites of TIMP-1 expression using in situ localization. The group hypothesizes that luteal MMPs and TIMPs play a critical role in development and preservation of a microenvironment that supports luteal formation and progesterone secretion.

In addition, during the current funding period members of the workgroup have 1) cloned the steroidogenic acute regulatory protein (StAR) gene and studied of how its expression is regulated; 2) developed methods to quantify mRNA and protein concentrations of peripheral-type benzodiazepine receptors (PBR) and their natural peptide ligand, endosepine. Although luteal levels of PBR did not appear to be regulated by luteotropic or luteolytic hormones, endosepine was shown to be humorally controlled. Finally, data were obtained which suggested that intra-luteal production of $PGF_{2\alpha}$ may be important for normal luteolysis. Moreover, luteal levels of the enzyme responsible for inactivation of $PGF_{2\alpha}$ were evaluated on day 13 of pregnancy suggesting that intra-luteal metabolism of $PGF_{2\alpha}$ may play a role in maternal recognition of pregnancy.

Environmental Control Workgroup—The reproductive process in domestic and semi-domesticated ruminants is the product of a complex interplay between tissues. The integrity of the pathways of communication and coordination between tissues requires a supportive environment and stress, seasonal changes in photoperiodic inputs, or inadequate nutritional

support can disrupt the endocrine balance required to sustain the reproductive process. The Environmental Control Workgroup (which includes representatives from AK, AZ, CA, ID [ARS], KS, MT, NE [ARS], NM, WY) examines the mechanisms by which environmental variables, such as season, stress and nutrition, affect the efficiency of the reproductive process.

Photoperiod and season control the annual rhythm of reproduction in sheep and semi-domesticated ruminant species (deer, elk, reindeer, caribou, and muskoxen). Research conducted by the Environmental Control Workgroup has examined the effects of season and melatonin on fertility in sheep, with the goal of shortening the period of seasonal anestrus. Recent work by this workgroup indicates that the activity of the thyroid gland triggers the onset of seasonal anestrus. The work of this group has also demonstrated that the route of intracellular movement of the GnRH receptor in gonadotrope cells is altered during the non-breeding season. The physiologic consequence of this change in the pattern of intracellular trafficking is attenuated gonadotrope responsiveness during the non-breeding season.

Another aspect of the work conducted by the Environmental Control workgroup involves the characterization of a newly identified hypothalamic peptide (antigonadotropic decapeptide, AGD) that is a potent inhibitor of GnRH secretion in sheep. Our studies indicate that AGD is an autocrine and paracine regulator of GnRH secretion, modulating activity of the GnRH neuronal network by influencing input of inhibitory neurotransmitter systems. Results also suggest that AGD may function as an inhibitor of GnRH secretion during pubertal development and seasonal anestrus.

Major Advances of the Uterine Biology Research Group

Maternal Recognition of Pregnancy Workgroup—Asynchrony between embryo development and uterine receptivity compromises placental development and results in abortion. In ruminants the incidence of embryonic mortality is 30-35%. Such loss has a significant economic impact on livestock producers in the Western states. Indeed, recent estimates indicate that embryonic and fetal loss costs cattle producers $600,000,000 annually. The Maternal Recognition of Pregnancy Workgroup has hypothesized that maternal recognition of pregnancy involves the production of key uterine proteins that are induced by the conceptus during the preimplantation period. The members of this group have identified several early-onset candidate proteins and postulate that each may play a critical role in the cascade of embryonic and uterine transformations required to sustain pregnancy. The aim of this group is to use the information generated from basic research studies to assist in the development of technology designed to reduce early embryo mortality and improve fertility.

The highly productive program of the Maternal Recognition of Pregnancy Workgroup relies on a coordinated multi-state approach to research, with representatives from WY, ID, WA, and OR taking lead roles in this joint effort. During the past 5 year period this group has identified several uterine proteins that are induced by conceptus-derived interferon tau. One protein (ISG17) covalently attaches to, and regulates, intracellular uterine proteins in a manner similar to that described for ubiquitin. The ISG17 protein may also be found in serum of pregnant ruminants and might be used as a pregnancy test. Another recently identified uterine protein (1-8U) is localized to the membrane of the endometrium and may be involved with adhesion of the

trophoblast in a manner that may block implantation. Members of this research group have also identified the Mx protein as one that is rapidly induced by the conceptus. Interestingly, this uterine protein can be detected in the serum, a characteristic of the Mx protein that may lead to the development of diagnostic procedures that can be used in the field to detect pregnancy loss at a very early stage, allowing for more rapid rebreeding. Detection of this protein in the peripheral circulation may also allow for more accurate diagnosis of the causes of infertility and early abortion.

Placental Development Workgroup—The utero-placental unit is directly responsible for mediating and/or modulating the maternal environment necessary for optimal fetal development. Placental insufficiency results in intrauterine growth restriction (IUGR), whereas altered placental function may also lead to excessive fetal growth. Either scenario results from improper placental development and function. The Placental Development Workgroup includes members from CO, CA, HI, ID, ID [ARS], OR, TX and WY. This group is examining placental development in a sheep model of IUGR. In the past 5 year period this group has examined placental vascular development, and uterine and placental hormone/growth factor production and regulation. Members of the group are currently studying the transcriptional regulation of utero-placental hormones (growth hormone; GH, and placental lactogen; PL). The combined efforts of this collaborative project will provide the basic information needed to reduce pregnancy loss and maintain optimal fetal growth rates.

In addition, members of the Placental Development Workgroup are examining the factors that control placental steroidogenesis during mid-pregnancy. This is particularly important because progesterone is required for continuation of pregnancy and placental tissue becomes the primary source of progesterone during mid- and late pregnancy in domestic ruminants. Recent estimates indicate that 10% of pregnant ruminants undergo spontaneous abortion during mid-pregnancy due, at least in part, to placental insufficiency. The cooperative efforts of scientists in TX, HI, OR and ID have determined that prostaglandins E1 and/or E2 (PGE), not luteinizing hormone (LH), regulate secretion of progesterone by the corpus luteum (CL) at mid-pregnancy in sheep and cattle. Pregnancy specific protein B (PSPB) stimulates PGE synthesis by the corpus luteum. Additionally, the cooperative studies conducted by this group have demonstrated that estradiol regulates uterine/placental secretion of PSPB during mid-pregnancy in sheep.

Additional work by the Placental Development Workgroup has examined the effect of lipid supplementation on uterine production of $PGF_{2\alpha}$ during the estrous cycle and early postpartum period in sheep and beef cattle. Continuing studies of this group have also evaluated the regulatory role of estrogen and progesterone in expression of oxytocin receptors in uterine tissue during the ovine estrous cycle.

Fetal Maturation Workgroup—Collaborative work in CA, CO, ID [ARS] and NE [ARS] has demonstrated that a high percentage of calves that develop from embryos derived by *in vitro* fertilization (IVF) and cloning procedures are much larger at birth than calves that develop from normal embryos. The enlarged calves also exhibit certain developmental defects that increase mortality. The ‘large-calf syndrome’ poses an economic loss to producers and limits the extent to which these new technologies can be applied to practical animal agriculture. The underlying mechanism likely is multifaceted and, therefore, reducing the incidence of the ‘large-calf

syndrome' will require a multidisciplinary approach. Current work of the multi-state team is focused on placental hormones and growth factors. This research group postulates that abnormal placentation of *in vitro*-derived embryos increases production of placental lactogen and other growth factors that accelerate fetal growth.

Major Advances of Animal Disease Research Group

Vaccine Development Workgroup—The Vaccine Development Workgroup includes members from CA, ID [ARS], NV, OR and CO. This group is developing vaccines to provide protection against two pathogens (trichomoniasis and *Haemophilus somnus*) that cause reproductive failure and represent a significant economic concern for cattle and sheep producers in the Western states. The studies of *Haemophilus somnus* are particularly important because wild ruminants, including bison and bighorn sheep, may serve as hosts and facilitate transmission to domestic species. The results of this collaborative effort are expected to lead to a more complete understanding of pathogenesis and transmission of these diseases, as well as development of more reliable vaccines and diagnostic assays for control of disease.

In addition, this multi-state research team is continuing efforts to develop management strategies to control Epizootic Bovine Abortion (EBA). Although the etiology of the disease has not been precisely characterized, EBA induces late-term abortion in cattle. The incidence of EBA is high in the Western states, particularly California, Idaho, Nevada, and Oregon, and cattle producers in those states report significant loss due to late-term abortion. The continuing efforts of this research group will focus on development of methods for culture and identification of the etiological organism, development of molecular and/or immunological probes for rapid diagnosis and identification of exposed animals, and development of vaccines that can provide immunological protection for at risk herds.

Mastitis Control Workgroup—Mastitis in sheep on the Western range is a persistent disease that reduces milk quality and yield, shortens the duration of lactation and, as a consequence, compromises the health, growth and survival of lambs. The high incidence of clinical and sub-clinical mastitis among Western range ewes represents a major economic loss for sheep producers. The Mastitis Control Workgroup (ID [ARS], NV) has used fluorescent staining and flow cytometric analyses to identify the types of inflammatory and somatic cells in the milk of ewes.

Major Advances of the Reproductive Technology Research Group

Immunocastration Workgroup—Previous work of the Immunocastration Workgroup (which includes representatives from AK, CA, ID [ARS], KS, MT [ARS], TX, WA, WY) has demonstrated that active immunization against GnRH markedly reduces testis size and serum concentrations of testosterone in intact bulls. Immunocastration also significantly reduces the aggressive behavior of bulls, while producing carcasses that are bull-like in size, but steer-like in marbling and quality grade. Collectively these results indicate that immunocastration may be an effective and non-invasive alternative to physical castration in the management of bull calves on the Western range.

Moreover, the same immunology-based procedure effectively sterilizes heifer calves and may be used to increase the ease of calf management and reduce the incidence of inadvertent pregnancy in heifers destined for the feedlot. This is an important concern, particularly in the Western states, where more than 25% of feedlot heifers are pregnant on feedlot entry. The resultant cost of abortion and decreased productivity is estimated to be as much as $100 per pregnant heifer. Therefore, we feel that the immunocastration technology may be particularly useful in ensuring feedlot heifers are open and non-cyclic. This is likely to have a significant economic impact, not only due to improved feed efficiency, but also due to cost savings resulting from the absence of pregnancy.

Assisted Reproduction Technology Workgroup—The consensus across states is that expanded use of artificial insemination is crucial to production efficiency and the continued improvement of the quality and genetic composition of beef cattle and sheep. We feel that increased use of artificial insemination will lower the costs of production, increase herd health and improve meat quality, leading to greater consumer acceptance and increased demand for meat and animal products. Additionally, artificial insemination will become a necessary part of the assisted reproduction technology that employs sexed semen and/or gene-assisted selection. We anticipate that the use of artificial insemination by Western beef producers will increase markedly in the next 5 years period. Indeed, we expect that more than 20% of beef cows will be serviced by artificial insemination in 2005.

The Assisted Reproduction Technology Workgroup includes members from all participating stations, united with the common goal of developing management strategies that will increase the effectiveness, and facilitate the implementation, of recent advances in reproductive technology. We feel the multi-state approach is critical to this work due to the wide range of environments and production systems used to produce beef cattle and sheep in the Western states. Comparison of the utility of various approaches across environments and production systems is essential to enhance efficacy and consistency of responses when these approaches are adopted by the cattle industry. Representatives from MT [ARS] are leading our efforts in this critical area and have accepted responsibility for the organization, coordination, and implementation of this multi-state project.

Successful completion of this work will accelerate the genetic improvement of herds and flocks and, thereby, improve the sustainability of the beef cattle and sheep industries.

OBJECTIVES

1. Investigate molecular, cellular, and endocrine mechanisms that limit or control reproductive efficiency in domestic ruminants.

2. Develop and improve assisted reproductive technologies to enhance sustainability of production systems for domestic ruminants.

The research efforts of members of the **Ovarian Biology** and **Uterine Biology Research Groups** will generally address issues most directly relevant to *Objective 1*. Conversely the

studies conducted by members of the **Animal Disease** and **Reproductive Technology Research Groups** are most likely to conform to the goals detailed under *Objective 2*. Despite this apparent segregation of effort, it is important to note that *Objective 1* and *Objective 2* are not necessarily mutually exclusive. Indeed, we anticipate that much of the work conducted by the **Ovarian Biology** and **Uterine Biology Research Groups** will have a practical component that might be consistent with *Objective 2*. Similarly, the applied studies conducted by the **Animal Disease** and **Reproductive Technology Research Groups** are likely to identify new and fundamental questions in reproductive biology that would appropriately fall under *Objective 1*. Irrespective of Objective or Research Group, our unifying long term goal is to apply the latest advances in reproductive biology toward the development and implementation of management strategies and practical tools that will improve the fertility of domestic species in the Western states.

Research Methods of the Ovarian Biology Research Group

Follicular Development Workgroup—A major challenge to current efforts to improve the reproductive efficiency of livestock is the ability to effectively control the ovarian cycle. Development of a healthy dominant follicle is a critical step in the series of physiological events that lead to ovulation. For this reason, studies that focus on the regulation of follicle development and follicular dominance are fundamental to the development of management strategies that result in optimal pregnancy rates in beef cattle, sheep, and other domestic ruminants. Although the majority of bovine follicles undergo atresia, the physiologic mechanisms underlying follicular atresia are poorly understood. Similarly, the factors that contribute to follicular dominance are not precisely defined.

The studies proposed for the next 5 year period will determine the factors that control LH and FSH receptor expression in the developing follicle. Additional studies will assess the genomic and non-genomic effects of estradiol on GnRH receptor expression and LH secretion using pituitary cells in culture. Members of the group will use electron microscopy, in situ hybridization, immunocytochemistry, and molecular biology to examine the mechanisms by which the intraovarian growth factors, GDF9 and BMP15, control the initiation of folliculogenesis. Finally, the potential role of reactive oxygen species (ROS) in follicle atresia will be examined in both in vitro and in vivo studies.

Results of these studies will expand our knowledge of the basic mechanisms that regulate the developmental fate of dominant bovine follicles, and thus contribute to our understanding of the processes that control fertility in domestic livestock.

Sexual Behavior Workgroup—The research group addressing the factors controlling development and expression of sexual behavior in domestic ruminants will use in situ hybridization and immunocytochemistry to identify the neural centers responsible for normal and aberrant sexual behavior. The long-term goal of these studies is the development of diagnostic tests of breeding soundness and libido, and implementation of management strategies that will improve the breeding capacity of bulls and rams and improve overall reproductive efficiency of herds and flocks on the Western range.

Ovulation Workgroup—Maturation and eventual rupture of ovarian follicles and release of

mature oocytes are prerequisites for reproductive success. Development of improved methods to control the ovarian cycle and reduce early embryonic loss require a more precise understanding of the intrafollicular mechanisms that control ovulation and subsequent luteal development. These events in the ovarian cycle are triggered by the preovulatory surge of LH. The long-term goal of the Ovulation Workgroup is to define the intrafollicular processes that are induced by the LH surge and that lead to ovulation and luteal development.

The members of the Ovulation Workgroup propose to determine the effect of a GnRH-induced LH surge on localization, expression and activity of specific MMPs within bovine follicles and developing corpora lutea. Ultrasound-assisted intrafollicular injection will be utilized to examine the specific intracellular pathways that mediate LH surge-induced MMP expression.

In addition, the Ovulation Workgroup proposes to use cDNA microarray procedures to investigate the effect of the preovulatory LH surge on the pattern of gene expression by periovulatory follicular and luteal tissues. Expressed sequence tags (EST) encoding genes differentially expressed during the periovulatory period will be utilized in Northern blot and in situ hybridization procedures to further investigate regulation by the LH surge and to localize expression within preovulatory follicles and corpora lutea. Studies will also be designed to determine the physiological role of the newly discovered gene products induced by the preovulatory LH surge.

The proposed studies will help determine the key proteolytic enzymes and intrafollicular regulatory pathways that mediate follicle rupture and luteal development in domestic livestock. Novel gene products differentially expressed during the periovulatory period will be identified and their regulation and potential contribution to follicle rupture and luteal development determined. A more complete understanding of the cellular and molecular mechanisms involved in follicle rupture and luteal development will set the foundation that will allow us to develop improved methods to control the ovarian cycle in domestic ruminants.

Luteal Function Workgroup—Approximately one-third of conceptions in cattle and sheep are lost during the early part of pregnancy. Most of this embryonic wastage is due to a lack of optimal secretion of progesterone by the corpus luteum. Thus, the mechanisms involved in luteolysis and maternal recognition of pregnancy must be more precisely defined to facilitate development of management strategies that will reduce embryonic loss.

Members of Luteal Function Workgroup postulate that MMPs and TIMPs play critical roles in developing and maintaining a microenvironment that ensures proper formation and function of the corpus luteum. Experiments will be conducted using whole animals, purified preparations of luteal cells or appropriate cell lines. Initially, indomethacin, an inhibitor of prostaglandin synthase (COX-2) will be administered via the ovarian artery to determine the necessity for luteal $PGF_{2\alpha}$ synthesis in the luteolytic process. If intra-luteal synthesis of $PGF_{2\alpha}$ is important for luteolysis, a similar experiment will be conducted using a 15-hydroxyprostaglandin dehydrogenase (PDGH) inhibitor (suphasalazine) to block intraluteal metabolism of $PGF_{2\alpha}$ in pregnant ewes and determine the effects on maternal recognition of pregnancy. If appropriate, the next logical experiment is to elucidate the mechanisms whereby the pregnant uterus induces luteal PDGH expression which likely involves interferon tau and MX protein. Further studies on the role of StAR, PBR and endosepine in cholesterol transport across mitochondrial membranes

will be conducted using fluorescence energy transfer and glutathione S-transferase pull-down assays. Transfer of cholesterol to the inner mitochondrial membrane is the rate-limiting step in steroidogenesis and the step most acutely regulated by second messengers in luteal cells. These studies will be performed in purified preparations of luteal cells and transfected COS-7 cells.

We anticipate that these studies will lead to the development of pharmaceutical products that will effectively stimulate progesterone secretion from the corpus luteum and, thereby, lessen embryonic wastage due to luteal insufficiency. This seems to be a realistic projection, particularly in light of since recent observations suggesting that diazepams, such as valium, bind to PBR and stimulate steroidogenesis in the adrenal gland. Likewise, the short-term regulation of $PGF_{2\alpha}$ synthesis and metabolism in the corpus luteum may be a target that can be regulated by pharmacological agents or novel management strategies.

Environmental Control Workgroup—The Environmental Control Workgroup will continue to examine the mechanisms by which environmental variables, like season, stress and nutrition, affect the efficiency of the reproductive process. The modulatory effect of the thyroid on reproductive function in ruminants will be assessed by treating sheep during the breeding season with propylthiouracil, an inhibitor of thyroid function. In addition, we propose to use immunocytochemistry to examine the pattern of intracellular trafficking of the GnRH receptor during the breeding and non-breeding seasons. The effect of melatonin on synthesis, post-translational processing and intracellular trafficking of the newly synthesized receptor will also be characterized.

Further studies will use antisense cDNA to block expression of the gene for AGD. We anticipate that these studies will define the degree to which this peptide regulates GnRH secretion through combined paracrine and autocrine actions. We will also examine the distribution of AGD mRNA and its association with GnRH neurons in the hypothalamus. Additional experiments will examine AGD regulation of GnRH secretion through steroid-independent neuronal input during seasonal anestrus in the ewe. In these experiments, potential paracrine modulation of serotonergic systems by AGD will also be examined.

Results of these projects will provide basic information concerning the physiologic processes underlying seasonal anestrus. The long-term goal of our work is the development of treatment and/or management strategies that will lessen the impact of season on animal fertility.

Another focus of the Environmental Control Workgroup is the effect of nutritive status on reproductive competence. The abundance and quality of the grasses on the range lands of the Western states varies with season, year, stocking rate, grazing patterns, and management philosophy. But even under the most conservative management, the level of nutrition may, at times, fall short of that required to support full reproductive function. Indeed, replacement heifers grazing native forages rarely are of sufficient weight and size to permit breeding at 12 months of age. We will evaluate the effectiveness of supplemental feeding strategies designed to optimize the reproductive performance of replacement heifers grazing native range.

Additionally, the insulin-like growth factor (IGF) system has been implicated as one mediator of nutritional effects on reproduction. Members of the Environmental Control workgroup have

established that IGF binding proteins (IGFBPs) are expressed in anterior pituitary tissue and may modulate activity of pituitary tissue and, thereby, influence reproductive efficiency. In sheep, low body condition may inhibit the increased secretion of LH associated with the onset of the breeding season by altering relative amounts of IGFBPs within the hypothalamic-pituitary axis. Studies planned for the next 5 year period will examine the role of IGF and the IGFBPs in control of gonadotrope responsiveness.

Research Methods of the Uterine Biology Research Group

Maternal Recognition of Pregnancy Workgroup—The Maternal Recognition of Pregnancy Workgroup will use in vitro (immortalized uterine cells) and in vivo models to characterize the regulation of gene expression in uterine tissue and conceptus during the peri-implantation period, with particular focus on those gene products that control maternal recognition of pregnancy, implantation and placentation, and continued embryo development. Differential display PCR and subtractive hybridization cloning along with library screening will be used to identify and characterize genes up- and down-regulated during early pregnancy. Gene function will be established using RNA interference assays and plasmid-mediated over-expression in immortalized uterine cell lines and primary cells in culture. Genes with established functions will be over-expressed and recombinant protein purified for in vivo studies to enhance or block reproductive processes.

Placental Development Workgroup—The collaborative efforts of the Placental Development Workgroup will provide the basic information needed to reduce pregnancy loss and to maintain an optimal rate of fetal growth. We anticipate that these studies will provide a greater understanding of placental development and function in ruminants. From this base of information we hope to develop strategies to correct abnormal placental development and ensure normal fetal growth. The research proposed by members of this workgroup will examine the role of steroids and oxytocin in regulating uterine synthesis of $PGF_{2\alpha}$ and the mechanism by which prostaglandins promote oxytocin secretion, and ultimately, regression of the corpus luteum.

The long-term objective of these studies is the development of diagnostic procedures that can be used to identify at risk animals at the earliest stages of placental insufficiency and development of corrective therapies to minimize abortion at mid-pregnancy.

Fetal Maturation Workgroup—The proposed research to be conducted by the Fetal Maturation Workgroup will examine the production of placental lactogen and other growth factors from the placental tissue that forms after implantation of *in vitro*-derived embryos. We anticipate that data from this collaborative effort will clarify the role of aberrant placental function in the abnormal fetal growth rate observed in some concepti derived by *in vitro* procedures. Understanding the cause of the 'large-calf syndrome' is essential to the design of corrective management protocols and procedures. New biotechnology procedures can not be optimally applied to animal agriculture until this aberration can be prevented.

Research Methods of the Animal Disease Research Group

Vaccine Development Workgroup—The vaccine development group is developing vaccines to provide protection against two pathogens (trichomoniasis and *Haemophilus somnus*) that cause reproductive failure in domestic ruminants and represent a significant economic concern for cattle and sheep producers in the Western states. In addition, this multi-state research team will continue efforts to develop management strategies to control Epizootic Bovine Abortion (EBA). The continuing efforts of this research group will focus on development of methods for culture and identification of the etiological organisms, development of molecular and/or immunological probes for rapid diagnosis and identification of exposed animals, and development of vaccines that can provide protection for at risk herds.

Mastitis Control Workgroup—Mastitis in sheep on the Western range is a persistent disease that reduces milk quality and yield, shortens the duration of lactation and, as a consequence, compromises the health, growth and survival of lambs. Proposed studies will examine the efficacy of treatment protocols designed to prevent the onset and/or limit the progression of mastitis in sheep on the Western range.

Research Methods of the Reproductive Technology Research Group

Immunocastration Workgroup—The number of heifers going into feedlots each year in the United States is approximately 10 million. These heifers are docked on price paid to the producer for two reasons: 1) the female (heifer) does not grow as efficiently as the castrated male (steer); and 2) a variable percent of the heifers come into the feedlot pregnant. In addition, the cattle industry is receiving a great deal of scrutiny from animal welfare and animal rights groups because of surgical spaying and castration of cattle without anesthesia. A sterilization vaccine would be a less traumatic and less stressful way to spay or castrate our livestock species in the future.

Previous work by members of W112 regional project has validated the concept of a sterilization vaccine against GnRH in heifers. Our continuing efforts are focused on the development of a fusion protein-based vaccine that not only sterilizes the heifer but will be approved by the FDA for use in commercial cattle production. Our goal is to develop a new GnRH vaccine that incorporates a second target hormone, the LHα subunit. The LHα subunit will be the carrier for GnRH thus both of the target hormones have the potential of blocking fertility. This type of double target protein may have the potential of overcoming carrier mediated immune suppression which occurs with other carriers. We also propose to insert a T cell helper epitope into this molecule to eliminate the need for adjuvants.

Assisted Reproduction Technology Workgroup—The consensus across states is that expanded use of artificial insemination and other technologic advances will lower the costs of production, increase herd health and improve meat quality, leading to greater consumer acceptance and increased demand for meat and animal products. We anticipate that the use of artificial insemination will increase significantly during the next decade. A practical key to the use and acceptance of artificial insemination as a viable management tool on the Western range is the development of simple and efficient techniques for estrous synchronization. Joint projects

conducted in MT [ARS], CO, OH, ID, ID [ARS], KS, MO, and AK will examine the efficacy of various synchronization protocols.

Collaboration Between Stations

The members of the W112 Regional Research Project meet annually to present the results of completed studies, review the progress of on-going work, discuss the concerns of the industry, and plan and coordinate the initiation of new studies. In addition, the collegial relationships that are established at the annual meeting are extended and nurtured by regular communication using email or the W112 website. Our goal is to create a network of collaborating scientists focused on a central mission; improving the health and fertility of domestic ruminants in the Western states. The founding members of the W112 Regional Research Project established a tradition of cooperation that the current members strive to match and even surpass. Indeed, in this era of instant and direct communication cooperation and collaboration has never been greater or more significant. The extent of collaboration between stations is extensive and would be impossible to fully enumerate. However, several examples may illustrate the more general theme of extensive station to station collaboration.

One clear example is presented by the cooperative efforts of researchers in CA, ID [ARS], MT, OR, and WY to determine the physiological and biochemical basis for difference in sexual preference in male sheep. This has been a particularly productive program that relies on the animal resource available in ID [ARS] and the technical skills of collaborating researchers in CA, ID [ARS], OR, MT and WY. The success of this program is indicated by the recent receipt of a substantial NIH grant to continue these studies.

Other examples of cooperation are clearly illustrated by the willingness of member scientists to freely share critical reagents with other members of the regional project. Such critical reagents include antisera, vaccines, tissue and serum samples, RNA and DNA tissue libraries, and novel cell lines and cDNA probes. For example, the efficacy of the immunocastration technology was tested on sheep in WY using a vaccine prepared in CA and histochemical techniques developed in CO. Similarly, a unique uterine cell line was developed in WY and used by collaborating researchers in ID, CO, and WA.

Perhaps even more important is the free and unrestrained exchange of advice and information that occurs at the annual meeting and in countless telephone and email conversations that occur during the interval between meetings. Jointly, the members of the W112 Regional Research Project constitute a vast reservoir of experience, knowledge and technical expertise that is an invaluable resource for all members of the project.

Measurement of Progress and Results

Outputs: The results of the proposed research studies will fill critical gaps in our knowledge regarding the reproductive physiology of ruminant animals and, thus, facilitate the development and implementation of management strategies that will optimize animal fertility in the target region.

Outcomes or projected impacts: We anticipate the knowledge gained through the collaborative research approach outlined above will lead to the development and implementation of new management protocols and/or pharmaceutical and nutritional regimens that will increase the fertility of domestic ruminants in the Western region by increasing conception and reducing embryo and fetal loss. We also expect that our collaborative work will result in the development of effective methods of estrous synchronization and, thereby, facilitate the use of artificial insemination in breeding programs of beef cattle and sheep on the Western range. Additionally, we expect that our efforts will result in the development of effective and efficient methods of immunocastration for use in male and female calves and lambs.

Milestones: The overall objective of our work is to improve the fertility, genetic composition and productivity of domestic ruminants in the Western states. To meet this objective we will develop, by 2003, a standard protocol for estrous synchronization and artificial insemination. We will also identify the proteins involved in maternal recognition of pregnancy and develop management techniques to optimize this process and thus reduce embryo loss. We will also develop vaccines for reproductive diseases endemic to the Western region.

Resources. See Appendix E, attached.

Outreach Plan. To disseminate the information obtained from the proposed research studies to academic and industry leaders the results will be published in peer-reviewed scientific journals. Information that is directly relevant to producers will also be presented in industry journals and other, more widely read technical bulletins. In addition, the information obtained from these collaborative studies will presented at national and international conferences, industry meetings, and producer field days. During the final year of the study period we propose to sponsor a meeting of industry leaders and producers to detail the results of the preceding period and receive direct input regarding the areas of concern for the next review period.

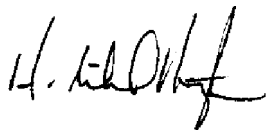
It is also important to note that our representative from MT has created and maintains a WEB site (www.repro.msu.montana.edu/W112) that has proven to be an effective means of communication between stations. The information on the site is regularly updated, allowing livestock producers and other stakeholders to follow our progress toward attaining our long term goals. In addition, the ready accessibility of the site by interested scientists, extension personnel and producers makes the WEB site an effective means of obtaining and conveying information and observations among these various constituencies. The use of email by members of the W112 Region Research Project has also increased dramatically in the past 5 year period. Indeed, the ease of communication between stations has increased and strengthen our collaborative efforts. We expect these means of electronic communication will be of ever increasing importance in the next 5 year period and we plan to use email and the W112 WEB site not only for communication between stations but also as a complement to more traditional vehicles for conveying of information to, and receiving input from, stakeholders.

Organization and Governance. The members of the W112 Regional Research Project receive direction from the Executive Committee, composed of an elected Chairman, Secretary and Member-at-Large. The Administrative Advisor, appointed by the Western Directors, also sits on the Executive Committee. Tenure of each elected officer is one year.

Authorization:

Colin Kaltenbach
Administrative Advisor
May 7, 2001

Approval:



Executive Director
Western Association of Agricultural Experiment Station Directors

Appendix A Projected Participation

Participant Name	E-Mail Address	Institution & Dept	Research							Extension	Objectives				
			CRIS Codes			Personnel					1	2	3	4	5
			RPA	SOI	FOS	SY	PY	TY	FTE	Program					
Milan P. Shipka	ffmps@usf.edu	Univ. of Alaska Plant,Animal & Soil	301	3899	1020	0.20	0.20		0.20	Agriculture		x			
Mark Wise	mwise@ag.arizona.edu	Univ. of Arizona Vet. Sci & Micro	301	3610	1020	0.40	0.30				x				
Adele Turzillo	turzillo@u.arizona.edu	Health Sci Center Physiology	301	3410	1030	0.05					x				
T. E. Adams	teadams@ucdavis.edu	Univ. of California Animal Sciences	301	3310	1020	0.10	0.10	0.20	0.10	Agriculture	x				
G. B. Anderson	gbanderson@ucdavis .edu		301	3310	1020	0.20	0.30	0.50	0.10	Agriculture		x			
Terry Nett	tnett@cvmbs.colostate.edu	Colorado State Univ Physiology	301	3610	1020	0.10	0.25	0.75			x				
Heywood Sawyer	hsawyer@cvmbs.colostate.edu		301	3610	1030	0.10		0.50			x				
Gordon Niswender	kthomas@cvmbs.colostate.edu		301	3610	1020	0.10	0.25	0.75			x	x			
Russell Anthony	ranthony@cvmbs.colostate.edu		301	3610	1050	0.10	0.25	0.50			x				
Charles W. Weems	weems@hawaii.edu Animal Science	Univ. of Hawaii	301	3610	1020	0.30					x				
Troy L. Ott	tott@uidaho.edu	Univ. of Idaho Anim&Vet Sci	301	3610	1020	0.20	0.20	0.20			x	x			
A. C. S. Ward	award@uidaho.edu		311	4010	1040	0.20						x			
J.J. England			311	4010	1100		0.10					x			
B. E. Mamer			311	4010	1090			0.15				x			
G. C. Weiser			135	4010	1040		0.20					x			
David M. Grieger		Kansas St. Univ. Animal Sciences	301	3399	1020	0.30	0.50					x			
Tim Rozell						0.10						x			
George Smith	smithge7@pilot.msu.edu	MSU Animal Sciences	301 301	3410 3410	1020 1040	0.20	0.50	0.50			x				
Jonathan E. Wheaton	wheatoo1@umn.edu	Univ. of Minnesota	301	3310	1020	0.10	0.10				x				
Duane H. Keisler	keislerd@missouri.edu	Univ. of Missouri Animal Sciences	301 301	3310 3610	1020 1020	0.50 0.50	0.10 0.10				x	x			
Michael F. Smith	smithmf@missouri.edu		301 301	3310 3610	1020 1020	0.50 0.50	0.10 0.10				x	x			
James G. Berardinelli	jgb@montana.edu	Montana State Univ Animal & Range	301 301	3310 3610	1020 1020	0.15 0.10					x x				
Mark Hall	hall@med.unr.edu	Univ. of Nevada Vet.& Med.	301	3310	1100	0.20						x			
Don Hanks	dhanks@mail.ag.unr.edu		301	3310	1100	0.25						x			
Dale Holcombe	holcombe@unr.nevada.edu		301	3610	1020	0.25	0.25					x			

Appendix A Projected Participation--Continued

Participant Name	E-Mail Address	Institution & Dept	Research							Extension	Objectives				
			CRIS Codes			Personnel					1	2	3	4	5
			RPA	SOI	FOS	SY	PY	TY	FTE	Program					
Dean Hawkins	dhawkins@nmsu.edu	New Mexico State	301	3310	1020	0.15		0.55			x	x			
		Animal & Range													
Dennis M. Hallford	dhallfor@nmsu.edu		301	3610	1020	0.15		0.15				x			
Michael L. Day	day.5@osu.edu	Ohio State Univ.	301	3310	1020	0.30	0.30	0.30			x	x			
		Animal Sciences		3410	1060										
Fred Stormshak	fred.stormshak@orst.edu	Oregon State Univ.	301	3310	1020	0.60					x				
		Animal Sciences	301	3610	1020	0.20					x				
Ursula Bechert	ursula.bechert@orst.edu	Vet.& Med.	301	3310	1020	0.50					x				
James Brendemuehl	james.brendemuehl@orst.edu		301	3310	1020	0.50					x				
			301	3610	1020										
R. D. Randel	r-randel@tamu.edu	Texas A&M	301	3310	1020	0.10		0.20			x				
		Ag.Res.& Ext													
Jerry Reeves	reevesjj@wsu.edu	Washington State	301	3310	1020	0.30		0.50			x	x			
		Animal Sciences	301	3310	1090										
G. E. Moss	gm@uwyo.edu	Univ. of Wyoming	301	3310	1020	0.10	0.20	0.20			x	x			
		Animal Sciences		3610											
T. R. Hansen	thansen@uwyo.edu		301	3310	1020	0.10	0.20	0.20			x				
				3610											
Tom Geary	tgeary@larrl.ars.usda.gov	USDA-ARS	301	3310	1020	0.20						x			
		U.S. Range Lvstk	302	3310	1020	0.10		0.10			x				
		Exp. Station													
Andrew J. Roberts	roberts@email.marc.usda.gov	USDA-ARS, MARC	301	3310	1020	0.20		0.10			x				
		Reproduction Unit													
J.N. Stellflug	tellflug@pwa.ars.usda.gov	USDA-ARS	301	3310	1020	0.20		0.10			x	x			
		US Sheep Ext.													
TOTALS						9.4	4.6	6.5	0.4						