

V(A). Planned Program (Summary)

Program # 3

1. Name of the Planned Program

Improving Animal Reproduction and Health

V(B). Program Knowledge Area(s)

1. Program Knowledge Areas and Percentage

KA Code	Knowledge Area	%1862 Extension	%1890 Extension	%1862 Research	%1890 Research
301	Reproductive Performance of Animals			14%	
304	Animal Genome			5%	
305	Animal Physiological Processes			17%	
307	Animal Management Systems			8%	
311	Animal Diseases			34%	
312	External Parasites and Pests of Animals			12%	
722	Zoonotic Diseases and Parasites Affecting Humans			10%	
	Total			100%	

V(C). Planned Program (Inputs)

1. Actual amount of professional FTE/SYs expended this Program

Year: 2010	Extension		Research	
	1862	1890	1862	1890
Plan	0.0	0.0	6.0	0.0
Actual	0.0	0.0	6.8	0.0

2. Actual dollars expended in this Program (includes Carryover Funds from previous years)

Extension		Research	
Smith-Lever 3b & 3c	1890 Extension	Hatch	Evans-Allen
0	0	303536	0
1862 Matching	1890 Matching	1862 Matching	1890 Matching
0	0	473408	0
1862 All Other	1890 All Other	1862 All Other	1890 All Other
0	0	4012699	0

V(D). Planned Program (Activity)

1. Brief description of the Activity

A good deal of the research in this planned program related to animal health. Research related to ovarian influences on embryonic survival, genome research that has a goal to understand the crucial elements that result in gamma/delta t cell response to antigens and will help us determine the outcome of vaccines to infectious diseases, the role of serine/tyrosine phosphorylation in capacitation. To become fertilization-competent, mammalian sperm undergo changes in the female reproductive tract termed capacitation. Lastly to be reported on under this planned program is research done on chlamydial major outer membrane proteins which constitute which constitute over 70% of the outer membrane of the bacteria and have been proven over the years to be immunogenic can be displayed in a conformation that will enhance its immunogenicity and therefore serve as a viable vaccine candidate.

2. Brief description of the target audience

This research is important to veterinarians, immunologists, pet owners, meat consumers, farmers and consumers. This research affects meat animals, cows and pets so its audience is broad.

V(E). Planned Program (Outputs)

1. Standard output measures

2010	Direct Contacts Adults	Indirect Contacts Adults	Direct Contacts Youth	Indirect Contacts Youth
Plan	0	0	0	0
Actual	0	0	0	0

2. Number of Patent Applications Submitted (Standard Research Output)

Patent Applications Submitted

Year: 2010
 Plan: 1
 Actual: 0

Patents listed

3. Publications (Standard General Output Measure)

Number of Peer Reviewed Publications

2010	Extension	Research	Total
Plan	0	30	
Actual	0	25	0

V(F). State Defined Outputs

Output Target

Output #1

Output Measure

- # of refereed manuscripts

Year	Target	Actual
2010	8	10

V(G). State Defined Outcomes

V. State Defined Outcomes Table of Content

O. No.	OUTCOME NAME
1	Accurate research on animal reproduction and health made available and shared
2	Accurate research on Animal Genome
3	Accurate research on Pig Sperm Capacitation
4	Accurate research on the outer membrane proteins of the chlamydial membrane which can serve as a viable vaccine candidate

Outcome #1

1. Outcome Measures

Accurate research on animal reproduction and health made available and shared

2. Associated Institution Types

- 1862 Research

3a. Outcome Type:

Change in Knowledge Outcome Measure

3b. Quantitative Outcome

Year	Quantitative Target	Actual
2010	0	0

3c. Qualitative Outcome or Impact Statement

Issue (Who cares and Why)

One of the important questions that we seek to answer is whether oocytes of high producing lactating dairy cows show structural/functional differences with those of non-lactating dairy heifers? Dairy farmers and economists would be the audience for this issue.

What has been done

The importance of this question lies on the evidence that pregnancy rates in high producing cows are lower than those of heifers of the same genetic background. To address these questions, we are examining the first response induced by the sperm upon fertilizing the eggs, which are the Ca²⁺ responses induced by sperm entry. The presence and duration of these oscillations reflect oocyte quality and in the long run developmental competence of the oocyte, or in other word, the capacity of these oocytes to support development to term. We have started to investigate this question by characterizing the function of PLCzeta, the enzyme in the sperm thought to be involved in this Ca²⁺ release. We have also generated a different version of the protein so that we can track its expression and distribution in the bovine egg.

Results

We plan to use these reagents to aid in the activation of eggs fertilized by ICSI, which exhibit poor oscillatory responses after this procedure in the bovine. We also plan via mutagenesis studies to determine what are the amino acids that regulate activity of bovine PLCzeta. Following identification of this site(s), new versions of the protein will be made and used to test impact on activation and embryo development. We have presented this information at the regional meeting and we have shared these reagents with our collaborator at Cornell, Dr. Ronald W. Butler. We hope to now characterize the function of the protein and its mutants in the oocytes of heifers and highly producing cows, so that we start establishing where the causes of infertility in these high producing animals lie.

4. Associated Knowledge Areas

KA Code	Knowledge Area
301	Reproductive Performance of Animals
305	Animal Physiological Processes
311	Animal Diseases
722	Zoonotic Diseases and Parasites Affecting Humans

Outcome #2

1. Outcome Measures

Accurate research on Animal Genome

2. Associated Institution Types

- 1862 Research

3a. Outcome Type:

Change in Knowledge Outcome Measure

3b. Quantitative Outcome

Year	Quantitative Target	Actual
2010	{No Data Entered}	0

3c. Qualitative Outcome or Impact Statement

Issue (Who cares and Why)

This goal is to understand the crucial elements that result in particular gamma delta T cells responding to microbial antigens/components. We hypothesize that the response by these cells determines the ultimate outcome of vaccines and/or infectious diseases. Our research has been to define the number of genes that code for the delta chain of the T cell receptor of cattle. Researchers who are interested in developing protective vaccines against infectious diseases in cattle.

What has been done

To do this we participated in the bovine genome sequencing and annotation project. In addition we are interested in the WC1 co-receptors expressed by the gamma delta T cells of cattle and their relationship to CD163 family molecules. Thus a study of the relationship of the WC1 gene sequences to CD163A, CD163b and CD163c-alpha expressed gene sequences in a number of species was conducted.

Results

We were able to show that cattle, like mice and humans, have only one gene coding for the T cell receptor delta constant domain and four variable families. However we were also able to show

that the T cell receptor delta variable 1 gene family was greatly expanded to include over fifty variable genes. The sequence of these expressed gene products was very similar to one another for many of these genes. However unique characteristics allowed us to divide them into 9 subfamilies. While gaps in the assembly of this region of the bovine genome exist, the knowledge regarding the number of delta variable gene families will allow us to form further hypotheses regarding how bovine gamma delta T cells interact with infectious disease agents. The results of the WC1 and CD163 family relationships showed that these are closely related and WC1 should be considered to be part of the CD163 gene family of scavenger receptors.

4. Associated Knowledge Areas

KA Code	Knowledge Area
304	Animal Genome
311	Animal Diseases

Outcome #3

1. Outcome Measures

Accurate research on Pig Sperm Capacitation

2. Associated Institution Types

- 1862 Research

3a. Outcome Type:

Change in Knowledge Outcome Measure

3b. Quantitative Outcome

Year	Quantitative Target	Actual
2010	{No Data Entered}	0

3c. Qualitative Outcome or Impact Statement

Issue (Who cares and Why)

Although freezing of sperm is the method of choice in other species such as bovine and human sperm, this method has not been successful for pig sperm. The reasons for this are not well understood; nevertheless, it is hypothesized that one or more capacitation-associated signaling events are affected by freezing and thawing. Our hypothesis is that through the understanding of pig sperm capacitation it will be possible to design better media to freeze sperm and/or to improve the fertilization chances of frozen sperm.

What has been done

To become fertilization-competent, mammalian sperm undergo changes in the female reproductive tract termed capacitation. Capacitation correlates with an increase in tyrosine

phosphorylation; however, less is known about the role of serine/threonine phosphorylation in this process. During the last years we have worked in the role of phosphorylation in sperm from different species including mouse, human, horse and pig. In this last year Dr. Leticia Vivani, a DVM from Argentina, has joined our group. Our study involves the identification of proteins that undergo phosphorylation during sperm capacitation. In addition, we are investigating the regulation of the phosphorylation events in horse sperm. During this period, different components of the capacitation medium has been evaluated.

Results

It was determined that the presence of bicarbonate anions is essential for capacitation and for the increase in phosphorylation that correlates with this process.

4. Associated Knowledge Areas

KA Code	Knowledge Area
305	Animal Physiological Processes

Outcome #4

1. Outcome Measures

Accurate research on the outer membrane proteins of the chlamydial membrane which can serve as a viable vaccine candidate

2. Associated Institution Types

- 1862 Research

3a. Outcome Type:

Change in Knowledge Outcome Measure

3b. Quantitative Outcome

Year	Quantitative Target	Actual
2010	{No Data Entered}	0

3c. Qualitative Outcome or Impact Statement

Issue (Who cares and Why)

The overall objective of this project is to test the hypothesis that chlamydial major outer membrane proteins (MOMP), which constitute over 70% of the outer membrane of the bacteria and have been proven over the years to be immunogenic can be displayed in a conformation that will enhance its immunogenicity and therefore serve as a viable vaccine candidate. To do this, we proposed to use the gas vesicles generated by Halobacterium NRC1 species as the display and delivery system.

What has been done

These gas vesicles are themselves highly immunogenic and would therefore attract immune cells, enhancing the delivery of the MOMP antigen which will be displayed as a structural component of

the gas vesicle outer membrane. Our recent data demonstrates that we can indeed manipulate these gas vesicle structures to display chlamydial antigens and that the recombinant chlamydial protein is recognized by both a MOMP-specific antibody as well as immune sera from a Chlamydia-infected individual. This suggests that the epitope has been preserved and is displayed in a conformation that preserves its recognition by these antibodies generated in the course of a normal infection process. These are important milestones in human and animal compatible vaccine design and we currently have over 10 different Chlamydial antigens of interest as vaccine candidates being displayed in this system. Importantly, we have also shown that we can cultivate recombinant gas vesicles with Chlamydia proteins of interest under normal environmental conditions and that the organisms can use the energy from sunlight and grow very well.

Results

This is a major step, since growing a vaccine in large solar salterns or ponds in open fields will significantly reduce cost. It also means that vaccines made in this way will not have to be refrigerated to remain efficacious, further reducing overall cost of the product to the general at risk population. Therefore, farmers would be able to easily afford a chlamydia vaccine made in this way for all of their at risk animals at a minimal cost.

4. Associated Knowledge Areas

KA Code	Knowledge Area
311	Animal Diseases
722	Zoonotic Diseases and Parasites Affecting Humans

V(H). Planned Program (External Factors)

External factors which affected outcomes

- Natural Disasters (drought, weather extremes, etc.)
- Economy
- Appropriations changes
- Government Regulations
- Competing Public priorities

Brief Explanation

V(I). Planned Program (Evaluation Studies and Data Collection)

1. Evaluation Studies Planned

- null

Evaluation Results

{No Data Entered}

Key Items of Evaluation

{No Data Entered}