# IN-DEPTH REVIEW BRIEFING BOOK

## BIOTECHNOLOGY AND GERMPLASM LABORATORY

**Animal and Natural Resources Institute**
**Beltsville Area**
**ARS-USDA**

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# Agenda for In-Depth Review

**Biotechnology and Germplasm Laboratory**
**Wednesday, May 18, 2005**
Morning Session, 8:30 to 12:00
BARC-West, Bldg. 005, Room 021

8:30 AM Executive Session I – Bldg. 005, BARC-West, Room 021
Review Team, Area Management Team, National Program Leaders

9:30 General Session – Opening Remarks
**Thomas J. Sexton**, Animal and Natural Resources Institute

9:40 Overview of BGL Program
**Kurt A. Zuelke**, Research Leader

10:00 Identification and Manipulation of Genetic Factors to Enhance Disease Resistance in Dairy Cattle
(Project 1265-31000-080-00D)
**Robert J. Wall**, Lead Scientist

10:25 Break

10:45 Swine Germplasm Preservation, Propagation, and Embryo Developmental Competence
(Project 1265-31000-082-00D)
**Kurt A. Zuelke**, Lead Scientist

11:05 Analysis of Sperm Storage Mechanisms in Poultry
(Project 1265-31000-083-00D)
**Murray R. Bakst**, Lead Scientist

11:25 Proteomic Analysis of Factors Regulating Egg Production in the Hen
(Project 1265-31000-084-00D)
**John A. Proudman**, Lead Scientist

11:40 Executive Session II
Review Team and Invited Guests (non-ARS invited guests)

12:20 PM Lunch

**Wednesday Afternoon, May 18, 2005**

Interviews with Individual Scientists, National Visitors Center

1:15 Individual Interviews – National Visitors Center (Roosevelt Room)
**Robert J. Wall**

2:00 **David M. Donovan**

2:45 **Le Ann Blomberg**

3:30 Break

3:45 **Neil C. Talbot**

4:30 **Murray R. Bakst**

**Thursday Morning, May 19, 2005**
Individual interviews continued, National Visitors Center

8:15 AM Tour of Laboratories/Facilities

9:15 **Julie A. Long**

10:00 Break

10:15 **H. David Guthrie**

11:00 **John A. Proudman**

11:45 **Non-SY Staff**

12:15 Lunch

**Thursday Afternoon, May 19, 2005**
National Visitors Center

1:00 PM **Kurt A. Zuelke**

1:45 Report Preparation by Review Team

3:15 Executive Session III (Roosevelt Room)
Review Team, Area Management, National Program Leaders

4:15 General Session (Eisenhower Room)

4:45 **Adjourn**

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**Response to 2000 GGPL Program Review Team Recommendations**

**Review Team:**

| | |
|---|---|
| Dr. Robert Kraeling (Chair) | ARS |
| Dr. Robert Peters | NC State University |
| Dr. Billy Hargis | Texas A&M |
| Dr. William Rall | NIH |

**Issue 1:** The Review Team concluded that recruitment actions should begin immediately for the vacant RL (vice L. Johnson) and Research Physiologist (vice A. Donoghue) positions. They recommended that the new RL be an internationally recognized senior level scientist and skilled manager. They also recommended that the Research Physiologist recruitment be directed at someone who could continue Dr. Donoghue's program as well as be able to make significant contribution to the work of Drs. Proudman and Bakst.

**Action:** Dr. Kurt Zuelke was hired as RL and Dr. Julie Long was hired as a Research Physiologist and both reported to duty in April 2001.

**Issue 2:** The Review Team recommended that Drs. Proudman and Bakst be moved from Bldg. 262 to Bldg. 200 as quickly as possible.

**Action:** Renovations to the second floor laboratories in Bldg 200 were completed and Drs. Proudman and Bakst were relocated from Bldg 262 to Bldg 200 in June 2001.

**Issue 3:** The Review Team felt that the Beltsville Sperm Sexing Technology had been developed to the point where private industry should further refine and develop it. It was recommended that further development of this technology within GGPL should be considered a research tool to apply to other GGPL research objectives.

**Action:** The Beltsville Sperm Sexing Technology was transferred to the private sector yet remains in use as a tool in ongoing BGL research programs. The wording 'Develop and improve sperm sexing technology' was deleted from GGPL's mission statement.

**Issue 4:** The Review Team recommended that a new poultry farm be constructed and that renovations of Bldg 203-C for swine proceed as soon as possible.

**Action:** A new poultry facility has been designed and funded and construction bids are pending. Renovation of Bldg 203-C was ultimately deemed too costly and inadequate for current and future needs. Planning for a new swine facility was completed in 2004. Funding to design and construct a new swine facility has not been identified.

**Issue 5:** The Review Team recommended that consideration be given to relocating Dr. Proudman to another laboratory within ANRI where other scientists are conducting research more closely related to his.

**Action:** We did not consider this to be an acceptable recommendation. Dr. Proudman is a highly valued scientist in BGL. Dr. Proudman's research examining the factors that affect egg production may provide a genetic basis to factors that limit reproductive efficiency in poultry. This subject area is well within the mission of BGL. Dr. Proudman's expertise as an endocrinologist, protein chemist and reproductive physiologist is a critical enabling capability across BGL's research portfolio.

**Response to 2000 GEML Program Review Team Recommendations**
**(Comments pertaining to Drs. Talbot and Wall are addressed)**

**LIST OF (1999) GENE EVALUATION AND MAPPING LAB REVIEW TEAM**
Frank Gwazdauskas, VPI, Chair
David Meisinger, National Pork Board
Daniel Pomp, University of Nebraska
John Thompson, Holstein Association

**CRIS 1265-3100-066-00D Molecular and Cellular Strategies to Enhance the Germplasm and Genome of Livestock.**

**Issue 1:** The Review Team felt that Dr. Talbot was a necessary service provider but his role as a support scientist was overemphasized and as a consequence he was having difficulty carving out his own professional niche.
**Action:** Dr. Talbot has clearly defined a program of scientific investigation designed to better understand early embryo development through application of proteomics. His recent publication record and new CRADA project with Hepalife Technologies, Inc. attests to his success in carving out a professional niche.

**Issue 2:** The Review Team suggested that the group needs to become proficient at cloning of cattle prior to considering transgenic somatic cell modifications.
**Action:** The group's cloning prowess has been recently demonstrated with the production of transgenic cattle. The group's ability to produce genetically engineered cattle is similar to that of other laboratories throughout the world as evidenced by producing world's first cloned cattle transgenic with a disease resistance gene.

**Issue 3:** The Review Team was concerned that there was a lack of veterinary expertise to support the cloning project.
**Action:** A new attending veterinarian was hired who is very attentive to the needs of the animals produced by nuclear transfer.

**Issue 4:** The Review Team recommended that Dr. Wall develop an outreach program with industry.
**Action:** See Issue 1 below. Dr. Wall developed and managed a CRADA with Metamorphix, Inc. to make genetic engineering of swine accessible to the pork industry. Though the CRADA has expired, Dr. Wall continues to share research findings with the scientists at Metamorphix.

**Issue 5:** The Review Team suggested that Dr. Wall needed to assume the role of mentor for young scientists.
**Action:** Dr. Wall meets with newly hired scientists and post docs at a regularly scheduled "Friday meeting" to discuss science and recommend strategies for navigating the government bureaucracy.

**CRIS 1265-3100-067-00D Identification and manipulation of factors which alter the efficiency of milk production and the quality of milk.**

**Issue 1:** The Review Team suggested that Dr. Wall develop an outreach program with industry.
**Action:** Dr. Wall has made a concerted effort to discuss the potential benefits of genetic engineering for improvement of livestock production with academic, government and industry policy makers including Ministers or Secretaries of Agriculture from Austria, Belgium, Britain, China, Egypt, Georgia (former USSR member), Israel, and Philippines, French Science Attaché, Deputy Director General, Department of Science, Technology and Education, Ministry of Agriculture, China, Philippine Senate and Congressional members, Assistant Secretary of Agriculture for the Philippines, French Senator, Agricultural Biotechnology and GMO delegations from the United Kingdom, Representative of Danish Minister of Science, Technology and Innovation, Representative of Office of Science and Technology, Consulate General of France, Director Breeding Stocks, Ministry of Agriculture, Japan, Director of the Chinese National Academy of Science. Chancellor of the University of Maryland, Dean of Florida A&M University, faculty and students from Industrial College of the Armed Forces, Penn State Agricultural Engineers, Director, R&D Budget and Policy Program American Association for the Advancement of Science, National Pork Producers Council representatives, Wisconsin Farm Bureau delegation, Arkansas Farm Bureau members, Holstein Association staff, President of Aqua Bounty, and Executive Director of the Federation of Animal Science Societies, Director, Animal Biotechnology, Biotechnology Industry Organization and the Director of the Office of Science and Technology Policy.
**Issue 2:** The Review Team recommended that Dr. Wall needed to evaluate the animal welfare impact of genetic engineering.
**Action:** Dr. Wall designed the new CRIS to specifically address this issue by making the primary focus of the CRIS introduction of genes to combat animal disease.

## BIOTECHNOLOGY AND GERMPLASM LABORATORY

**Mission:** The mission of the Biotechnology and Germplasm Laboratory (BGL) is to improve the genetic, reproductive and productive efficiency of livestock through fundamental research on germplasm, gamete biology and embryology. BGL researchers are pioneering the application of functional genomics and proteomics methods, sperm cryobiology, transgenics, somatic cell nuclear transfer, and related embryo biotechnologies to improve germplasm preservation, oocyte maturation, embryo development and fertility in swine, dairy cattle and poultry.

**BGL History:** In the 1990 Farm Bill, ARS was asked to provide the leadership and assume the responsibilities establishing a National Animal Genetic Resources Program under the ARS National Program 101 Food Animal Production. Beltsville was one of three ARS locations assigned to support this national genetic resources program. To meet this requirement, programs were realigned and two new laboratories were created in 1991 – Germplasm and Gamete Physiology Laboratory (GGPL) and Gene Evaluation and Mapping Laboratory (GEML). During the subsequent 12 years the research programs in GGPL and GEML were enhanced with new funds and the addition of scientists with training in molecular genetics and biotechnology that resulted in significant program strength in gamete preservation, genetic engineering, and genomics. The original GGPL Research Leader Dr. Larry Johnson retired, and poultry physiologist Dr. Annie Donoghue transferred to another ARS location in 2000. The last in-depth review of GGPL was conducted in March 2000. Dr. Kurt Zuelke was hired as GGPL RL, and Dr. Julie Long was hired into the vice Donoghue position in April 2001. In 2003, the bovine genetic engineering and embryo biotechnology expertise within GEML (Drs. Bob Wall, Neil Talbot, and David Donovan) was realigned into the existing reproductive and gamete physiology skill base of GGPL to form the current BGL. Dr. John Dobrinsky resigned from ARS service in April 2004. Dr. Le Ann Blomberg was hired into a newly created SY position in August 2004.

**PROJECT LISTING**

**1265-31000-080**

**Identification and Manipulation of Genetic Factors to Enhance Disease Resistance in Dairy Cattle**

**Investigators:**

Robert J. Wall, Lead Scientist……..…80%
Le Ann Blomberg……100%
David M. Donovan……..…100%
Neil C. Talbot……..…100%
Kurt A. Zuelke……..……...5%

Scientific Staff Years: 3.85
Funding (Net to Location): $2,616,095
National Program: 101, Food Animal Production
OSQR Status: Approved

Start Date: 06/21/2002 Termination Date: 06/20/2007

**Objectives:**

The long term goal of this project is to create dairy cattle resistant to mammary gland microbial infections. Nearly $2 billion is spent in the US annually to combat mastitis, an inflammatory reaction of mammary glands to bacterial infection. This disease not only increases the need to administer antibiotics but reduces milk quality and importantly has a negative impact on animal well-being. Introducing new genetic information encoding antimicrobial peptides into the bovine genome may enhance the ability of a cow to ward off mammary gland infections. This project is designed to test that concept. As first steps, two candidate peptides will be tested, lysostaphin which targets *Staphylococcus aureus,* and a recently cloned rainbow trout lysozyme, which is effective against *Escherichia coli* and acts synergistically with lysostaphin against *S. aureus*. Transgenic mice and cattle carrying these genes will be produced and their ability to lactate normally, produce quality milk and resist bacterial infection will be determined. To enhance our ability to introduce new genetic information into cattle, a series of studies to understand the origin of differences in protein profiles between "normal" (*in vitro*-produced) and nuclear cloned bovine embryos will be conducted. Attempts will be made to alter the *in vitro* environment such that protein profiles of cloned embryos more closely resemble that of *in vitro* produced embryos.

1. Produce and characterize mouse models designed to demonstrate the efficacy of using genetic engineering as a tool to increase resistance to mastitis. Mammary gland function and physical properties of milk from lysostaphin transgenic mice will be characterized. Transgenic mice containing newly cloned rainbow trout lysozyme gene will be produced and characterized as will double lysostaphin / lysozyme mice transgenic. Use of lactoferrin regulatory element will be evaluated as a switch to turn on antimicrobial peptides as needed.

2. Produce and characterize transgenic cows carrying mammary gland targeted lysostaphin. When warranted, based on mouse model studies, transgenic cows carrying other antimicrobial proteins will be produced and characterized.

3. Improve the efficiency of producing transgenic nuclear transfer progeny. Protein expression of nuclear transfer-, in vitro-, and parthenogenic embryos will be compared to identify conditions in somatic cell nuclear transfer that can be altered to better support development to term.

**1265-31000-082**

## Swine Germplasm Preservation, Propagation, and Embryo Developmental Competence

**Investigators:**

Kurt A. Zuelke, Lead Scientist…….....65%
H. David Guthrie….....100%

Scientific Staff Years: 1.65
Funding (Net to Location): $887,150
National Program: 101, Food Animal Production
OSQR Status: Approved

Start Date: 07/24/2002 Termination Date: 07/23/2007

**Objectives:**

The aim of this research is to investigate maternal gamete and embryonic development and develop new enabling technologies for germplasm conservation in swine. Specific objectives are as follows:

1. To analyze the transcriptome of in vivo produced pre-implantation pig embryos using Serial Analysis of Gene Expression (SAGE) and other complementary genomics tools to identify and quantitate the relative abundance of differentially expressed genes within and between critical developmental stages. These benchmark data will describe gene expression profiles of porcine embryos from prior to the maternal to embryonic genome transition (the stage where the embryonic genome takes over from the maternal genome in directing embryo physiology (2/4-cell stage in pigs) through the stage of embryo elongation and maternal recognition of pregnancy. These comprehensive gene expression profiles will provide a public research resource for other investigators performing gene discovery and reproductive biology research.

2. To increase developmental competence of in vitro produced (IVP) porcine embryos by integrating results of functional genomic analyses of early embryonic development into embryo production systems. SAGE analyses will compare the gene expression profiles between in vivo and embryos to characterize how IVP may be compromised at a gene expression level and enable improvement of IVP methods.

3. To develop and improve methods for long-term preservation, hypothermic storage and transfer of swine germplasm and embryos. This objective focuses on improving the technologies currently available for cryopreserving boar semen and porcine embryos. Cellular cryobiological parameters will be characterized and validated for boar semen; these parameters will then be integrated into a 'boar semen freezability index' that will enable customization of semen freezing procedures based upon the cryobiological characteristics of a semen sample. This freezability index will be validated in a

controlled field setting through collaboration with a boar selection and genotyping trial to be conducted at the USDA Meat Animal Research Center in Clay Center, NE. Embryo preservation efforts will focus on improving the viability of frozen-thawed porcine embryos and improving the freezability of in vitro produced embryos.

**1265-31000-083**

**Analysis of Sperm Storage Mechanisms in Poultry**

**Investigators:**
Murray R. Bakst, Lead Scientist.....100%
Julie A. Long.……100%
Vacant……100%
Kurt A. Zuelke..……...10%

Scientific Staff Years: 3.10
Funding (Net to Location): $1,153,482
National Program: 101, Food Animal Production
OSQR Status: Approved

Start Date: 07/26/2002 Termination Date: 07/25/2007

**Objectives:**

The commercial turkey industry relies exclusively on artificial insemination (AI) for fertile egg production. Given that every breeder hen must be inseminated weekly for 24-26 wk, AI is both time and labor-intensive. The turkey industry would benefit by having the capacity to store semen at ambient temperature for 24 h without affecting fertility. Currently, semen is stored for no longer than 6 h and fertility rates slowly drop after 12 wk egg production when using stored semen. This project will address problems associated with the use of stored semen for AI for maximum fertile egg production. To achieve this goal, both in vitro and in vivo mechanisms of sperm storage will be investigated.

1. Determine how and when poultry sperm lose functional competence during liquid and cryogenic storage and use as a basis for developing successful sperm storage methods in vitro.

2. Determine the influence of sperm phenotype (mobility, fertilizing capability) on liquid and cryogenic storage of turkey sperm, as well as phenotypic traits of economic importance in breeder males (feed conversion, body conformation, liveability).

3. Increase the efficacy of oviductal sperm transport to the sperm storage tubule(SST) and the site of fertilization by elucidating the cellular and molecular mechanisms regulating sperm selection and transport in the oviduct.

4. Determine the molecular basis of sperm subsistence in the sperm storage tubules using serial analysis of gene expression (SAGE) and developing an in vitro model (tissue explants) to elucidate molecular and cellular events regulating prolonged oviductal sperm storage and sustained fertility in poultry.

5. Objective 5: An additional objective addresses the isolation and propagation of testicular stem cells ultimately to be the targets of gene insertion and transfer to recipient males.

This work has been approved by the NPL and is part of an anticipated multinational CRADA.

**1265-31000-084**

**Functional Genomics of Growth and Development of Swine and Poultry**

**Investigators:**
John A. Proudman, Lead Scientist.....100%
Kurt A. Zuelke……20%

Scientific Staff Years: 1.2
Funding (Net to Location): $807,171
National Program: 101, Food Animal Production
OSQR Status: Approved

Start Date: 07/26/2002 Termination Date: 07/25/2007

**Objectives:**

1. Implement state-of-the-art techniques for proteomic analysis at the cellular level, including sample preparation, labeling, instrumentation and computer analysis. Through long-standing collaboration with the Laboratory of Neuroendocrinology and Immunological Biotechnology at the Catholic University of Leuven in Belgium, the Lead Scientist has access to state-of-the-art proteomic techniques. Training in these techniques through collaborative experiments to be conducted, in part, in Belgium will provide the basis for future proteomics research in the Germplasm and Gamete Physiology Laboratory.

2. Identify cellular factors in the brain and pituitary that enhance or terminate egg production. Prior research has identified specific brain nuclei and pituitary cells that likely regulate incubation behavior, a major cause of poor reproduction in the turkey hen. Using a differential display technique at the protein level, we will identify factors which differ between superior layers and those that will prematurely terminate reproduction.

3. Develop genomic tools to identify breeders with superior reproductive potential and/or therapeutic intervention to modify reproductive efficiency. Proteins identified by proteomic analysis to be linked to reproductive superiority or failure will provide target molecules for developing genomic tools to identify breeders with superior reproductive potential and/or therapeutic intervention to improve reproductive efficiency.

4. Develop management strategies for prolonging the reproductive season of turkey breeder hens. A photostimulatory daylength both initiates reproduction and programs the termination of reproduction by the onset of photorefractoriness. In this objective, we will attempt to understand the endocrine programming of photorefractoriness with the goal of developing lighting or other management strategies that will extend the reproductive season.

5. Provide hormones, antibodies and techniques to scientists and industry for the improvement of poultry productive efficiency. The Lead Scientist has developed and provided radioimmunoassay reagents for measurement of avian pituitary hormones. The continuation of this service to the poultry research community is an objective of this project.

## EXTRAMURALLY FUNDED PROJECTS

**1265-31000-087-01T** – Hepalife Technologies, Inc.
**Optimization of the ARS-PICM-19 Cell Line for In Vitro Model of Pig Liver Function and Application to an Extracorporeal Liver Assist Device**
Investigator: Thomas Caperna, Neil Talbot
Funding: $803,000
Start Date: 10/01/02
Ending Date: 09/30/07

**1265-31000-083-00D -** Fundación Ramón Areces (Madrid, Spain)
**Developing Semen Cryopreservation Methods for Domestic Poultry and Threatened Raptor Species**
Investigator: Julie Long
Funding: $24,000
Start/End Date: 2003/2004

**1265-31000-083-00D -** Spanish Ministry of Education, Culture and Sport:
**Conserving Avian Germplasm: Developing Semen Cryopreservation Methods for Domestic Poultry and Threatened Raptor Species**
Investigator: Julie Long
Funding: $70,800
Start/End Date: 2004/2006

## SPECIFIC COOPERATIVE AGREEMENTS

**1265-31000-080-01S –**University of Illinois
**Functional Genomics and Proteomics for Improved Cloning Efficiency**
Investigator: Robert J. Wall
Funding: $496,219
Start Date: 05/01/2002
Ending Date: 04/30/06

**1265-31000-080-08S** –University of Connecticut
**Improved Efficiency of Bovine Cloning**
Investigator: Robert J. Wall
Funding: $496,219
Start Date: 05/01/02
Ending Date: 04/30/06

## ACCOMPLISHMENTS BY CRIS PROJECT

### CRIS 1265-31000-080-00D

### Identification and Manipulation of Genetic Factors to Enhance Disease Resistance in Dairy Cattle

1. **Nuclear transfer (cloning) efficiency may be enhanced by a better basic understanding of preimplantation embryo nuclear reprogramming and the development of protein markers of successful reprogramming that would allow prescreen of embryos prior to embryo transfer. Accomplishment:** Primary cultured trophectoderm of bovine nuclear transfer (NT) embryos had lower expression levels of interferon-tau in comparison to the trophectoderm of parthenogenic embryos and embryos created by normal fertilization (IVP). Diagnostic 2-D gel "fingerprints" of NT vs. IVP blastocyst protein expression are being developed by analysis of trophectoderm cell lines derived from NT and IVP bovine embryos. Annexin II protein is greatly reduced or missing in NT trophectoderm cell lines in comparison to IVP trophectoderm cell lines. Targeting the analysis to a specific tissue, the trophectoderm, simplifies the analysis. The trophectoderm is an important tissue to analyze in NT embryos because it is the outermost tissue of the blastocyst and is a critical tissue component of the placenta. Failed placentation is frequently the cause of NT pregnancy failures. Immunocytochemical screening of protein expression in NT embryos vs. IVP embryos will be conducted to increase our understanding of initial developmental difference in embryos produced by fertilization and those produced by cloning. **Role:** Dr. Talbot conceived, coordinated, conducted and interpreted these experiments. **Impact:** Trophectoderm cell lines are an important resource for functional genomic analyses of developing IVP and NT embryos. Differential expression of interferon-tau may prove diagnostic for the successful reprogramming of the nuclei of embryos created by NT. It may also relate to the relatively poor placental formation of NT embryos since interferon-tau expression by the bovine embryo is an important pregnancy establishment signal. Reduced annexin II in NT trophectoderm may negatively impact placentation through the annexin family's reported involvement with prostaglandin production and modulation of blood clotting and immunocyte function at the interface of the cytotrophoblast and uterine epithelium.

2. **Mastitis, the most consequential disease in dairy cattle, costs the US dairy industry billions of dollars annually. Accomplishment:** To test the feasibility of protecting animals through genetic engineering, transgenic mice were produced expressing lysostaphin, a potent peptidoglycan hydrolase, in their mammary glands. Their milk kills *Staphylococcus aureus,* and challenge studies show they are protected from infection. Subsequently, genetically engineered, cloned cattle have now been produced carrying a similar transgene. The transgenic cows are secreting lysostaphin at concentrations ranging from 0.9 to 14 ug/ml in their milk. *In vitro* assays demonstrated the milk's ability to kill *Staphylococcus aureus*. Intramammary infusions of *S. aureus* were administered to three transgenic and ten nontransgenic cows. Increases in milk somatic

cells, indicative of infection, were observed in all of the nontransgenic cows but in none of the transgenic animals. Measures of innate immune responses, such as a rise in body temperature and induction of acute-phase proteins, were observed in nontransgenic animals but not in the transgenics. **Role:** As lead scientist, Dr. Wall developed the conceptual experimental design, established a collaboration and Cooperative Research and Development Agreement (CRADA) with Dr. David Kerr of the University of Vermont, who supplied the initial lysostaphin transgene. Dr. Wall designed and conducted the transgenic mouse experiments, coordinated the effort to build a transgene version suitable for cattle with Dr. Kevin Wells, designed the overall experimental strategy for the cattle project, and now leads the team producing transgenic cloned cattle (that initiated included Drs. Caird Rexroad, Jr., Kevin Wells, Neil Talbot and Vern Pursel). **Impact:** The cattle produced in this study are the first genetically engineered with the intention of improving animal well-being. This transgene appears to be effective at completely protecting cows against S. aureus mammary gland infections when expressed at relatively low (μg/ml) concentrations.

3. **Identifying new antimicrobial peptides to combat additional mastitis causing organisms is critical step for production of disease resistant cattle. A recently identified B30 bacteriophage endolysin has unique properties that make it an ideal candidate . Accomplishment:** Bacteriophage endolysins are acclaimed for their efficient lytic activity, and the absence of resistance development in their target bacteria. The *Streptococcus agalactiae* B30 endolysin gene was obtained from David Pritchard, U. Alabama. A series of carboxyl terminal deletion mutants were designed and constructed in a bacterial expression vector to identify the shortest protein fragment that maintains the lytic activity against Streptococci in order to maximize expression of this bacterial gene in a mammalian system. Plate lysis assays were conducted with bacterial extracts harboring either full length or truncated lysins to show that both the full length and selected truncations maintain their lytic activity against all three of the mastitis causing Streptococci (*agalactiae, dysgalactiae* and *uberis*). Important for downstream milk processing which utilizes Streptococcus thermophilus, experiments were designed and conducted to show that the lysin is inactivated in a milk-like environment by typical pasteurization conditions. Heterologous fusion genes were designed to fuse lysostaphin (*Staphylococcus simulans*) to either the full length or truncated B30 endolysin gene fragments which maintained the lytic activity. The resulting B30 endolysin-lysostaphin fusion proteins kill *S. agalactiae, S. dysgalactiae, S. uberis*, and *Staphylococcus aureus* (the major mastitis pathogen in the USA). This fusion construct is thus predicted to be effective against 50% of the mastitis cases in the USA. The full length endolysin were introduced into a mammalian expression vector and truncated B30-Lysostaphin fusion. There was no detectable lytic activity from the full length construct, but the truncated version was shown via immunohistochemistry to be expressed in cultured mammalian cells and is soon to be tested in a transgenic mouse model. **Role:** Dr. Donovan conceived and coordinated all research, planned all experiments and interpreted all results, in addition to initiating all collaborative efforts. **Impact:** This research is spearheading the novel use of bacteriophage endolysins in animal production and health and has resulted in

Patent Application (Provisional): David M. Donovan; Creating Designer Antimicrobials: Peptidoglycan hydrolase module shuffling., 12/03/04.

**CRIS 1265-31000-082**

**Swine Germplasm Preservation, Propagation, and Embryo Developmental Competence**

1. **Methods to measure physiological activity of individual boar spermatozoa were developed for the purposes physiological research, semen evaluation, and prediction of storage survival and fertility. Accomplishment:** It has been hypothesized that the cooling of spermatozoa for liquid storage or in preparation for cryopreservation causes premature maturation of ejaculated sperm (advanced capacitation or acrosome maturational status) leading to reduced sperm fertilizing capacity and viability. The negative effects of long-term hypothermic storage and cryopreservation may be mediated through membrane lipid peroxidation caused by formation of reactive oxygen active species (ROS). Guthrie developed dual staining fluorescence activated flow cytometry methods to identify and quantify different aspects of sperm physiology on an individual viable cell basis: 1) capacitation status, 2) acrosome maturational status, 3) formation of ROS, and 4) mitochondrial inner membrane potential. Guthrie found that liquid storage and cryopreservation procedures did not cause significant early maturation of viable boar spermatozoa and that cryopreservation procedures did not increase sperm ROS formation compared to fresh semen. **Role:** Guthrie conceived and coordinated research, planned experiments, and interpreted results. **Impact:** These techniques will be valuable in the investigation of basic sperm physiology and to monitor the sperm cell response to experimental changes in hypothermic liquid storage and cryopreservation technology. Guthrie has initiated efforts to apply these technologies to other species. Guthrie will lead an effort to utilize these novel technologies in formation of an index to predict survival of spermatozoa of individual boars before liquid or frozen storage.

2. **Functional Genomic Analyses of Pig Embryo Development**. **Accomplishment:** Pig embryos are highly susceptible to early death as they elongate between gestational days 11 (D11) and 12 (D12). A critical step in developing new strategies for reducing this embryonic mortality is to identify the genes that are switched on and off during this interval in development. Lead scientists from BGL in collaboration with researchers from the Bovine Functional Genomics Laboratory applied a new functional genomics technique, serial analysis of gene expression (SAGE), to investigate fluctuations in gene expression during the D11 to D12 developmental interval in pig embryos. This work led to the simultaneous discovery and identification of over 430 genes that are expressed at significantly different levels between D11 and D12 stage porcine embryos. SAGE databases generated from these D11 and D12 embryo libraries were transferred to the public domain via deposition with the National Center for Biotechnology Information (NCBI) during FY-04. Analysis of differentially expressed genes in these libraries revealed that estrogen synthesis and metabolism, and oxidative stress response pathways appear to play critical roles in embryo elongation. In addition, we validated a new method to amplify mRNA to enable construction of SAGE libraries from embryos at earlier stages of development where quantities of cellular materials are limited. **Role:** Dr. Zuelke conceived and led these experiments in close collaboration with Dr. Le Ann Blomberg and ANRI BFGL scientists Drs. Sonstegard and Van Tassell. **Impact:** This is

a major advance that will now enable us to construct and analyze SAGE libraries that represent all pre-implantation stages of swine embryo development for the first time. The newly developed mRNA amplification methodology now enables direct SAGE comparison between in vitro and in vivo derived pre-implantation stage pig embryos. Combined, these advances demonstrate that SAGE enables simultaneous gene expression analyses at both the metabolic pathway and single gene levels, in embryos from any stage of development, and is thus ideally suited for establishing a novel systems biology approach to investigating swine embryo development.

3. **A modification of the current boar semen cryopreservation protocol by the ARS National Animal Germplasm Program (NAGP) was validated. Accomplishment**: For the ARS National Animal Germplasm Program (NAGP) to efficiently obtain swine germplasm for deposition into its germplasm repository, cooled boar semen must be transported for up to 24 hours from the site of collection prior to cryopreservation at NAGP in Fort Collins, CO. BGL researchers conducted fertility trials to validate whether or not the fertilizing ability of the spermatozoa was maintained after it had been cooled and stored for up to 24 h prior to cryopreservation. The addition of a 24 h cooling period before cryopreservation yielded an average pregnancy rate of 54% compared to 50% for the 3 h cooling period with 6.1 live pigs per sow. **Role:** Dr. Guthrie conceived and coordinated research, planned experiments, and interpreted results. **Impact:** The results are important to swine producers, scientists, and the NAGP because they demonstrate that boar semen can be cryopreserved for the NAGP program with confidence that the spermatozoa will retain fertilizing capacity sufficient to repopulate in the event of a severe population loss or to introduce desired genetics into future swine populations.

**CRIS 1265-31000-083**

**Analysis of Sperm Storage Mechanisms in Poultry**

1. ***Defined the Physiological Impact of Lipid Peroxidation on Turkey Semen Stored In Vitro*** **Accomplishments:** Dr. Long identified a major limitation of conventional turkey semen storage protocols by demonstrating that liquid storage with constant aeration promotes formation of toxic lipid peroxides that adversely affect fertility rates. In the United States, the commercial turkey industry relies exclusively on artificial insemination for production of fertile eggs. Given that each breeder hen must be inseminated weekly during a six-month egg production period, artificial insemination (AI) is both time and labor-intensive. Turkey producers would benefit if freshly collected semen could be stored for 24-48 h without affecting fertility. With current technology, the maximum amount of time that turkey semen can be stored without a significant drop in fertility over the course of egg production is only 6 h. Because turkey spermatozoa utilize aerobic metabolism, most storage protocols involve constant aeration of the semen. **Role:** Dr. Long determined that peroxide formation was characteristically higher for some males than others, and that this propensity for high rates of lipid peroxidation during storage is not evident in fresh semen. She demonstrated that addition of antioxidants to semen extender did not reduce lipid peroxide formation during storage. Dr. Long conceived the study and modified a colormetric lipid peroxidation assay to achieve the project goals. **Impact:** The significance of this discovery is directly related to the industry practice of randomly pooling semen from several males for insemination, in that semen from just one tom producing large amounts of peroxides during storage could greatly diminish the fertility of the entire semen pool. As a result of Dr. Long's research, a major U.S. manufacturer of poultry semen extender (Gobbler's Inc.) has opted not to market antioxidant-supplemented formulations for long-term liquid storage of turkey semen. Dr. Long's lipid research also has led to the development of a potentially patentable new technique to improve the fertility of 24h-stored turkey semen and Dr. Long has at least two manuscripts pending review of the Invention Report.

2. ***Cellular and Molecular Mechanisms Regulating Sperm Transport and Storage in the Hen's Oviduct*** **Accomplishments:** The commercial turkey industry relies exclusively on artificial insemination (AI) for fertile egg production. Due to low success of cryopreserving or storing turkey semen longer than 6 hr AI procedures rely primarily on the use of fresh semen, necessitating the costly maintenance of male breeder flocks. A unique aspect of turkeys is that they have the ability to store sperm in the oviduct of the hen for extended periods of time in sperm storage tubules (SST's). We applied serial analysis of gene expression (SAGE) to characterize gene expression of turkey SST-containing mucosa, and found that over 200 genes were differentially expressed within the mucosa depending upon whether or not sperm were present in the SST. We have since exploited the recent release of the chicken genome sequence to apply proteomics techniques to identify 40 proteins that are expressed in the turkey SST epithelium. Four proteins were uniquely expressed when sperm were absent while three additional proteins were only expressed when sperm were present. Using a complementary candidate gene approach, we discovered the presence of a class of molecules, aquaporins, which regulate

water transport across cells in the turkey epididymis (known site of fluid resorption) and SST in the hen. The aquaporins may function in the release mechanism of sperm from the SST. Procedures using whole mounts of surface mucosa for immunocytochemistry expedited our localization of aquaporins in the SST epithelium, and will facilitate functional analyses of gene candidates identified via SAGE and proteomics analyses. **Role**: Dr. Zuelke conceived and led the SST SAGE and proteomics experiments in collaboration with Drs. M. Bakst and J. Long, and ANRI, BFGL scientists Drs. T. Sonstegard and C. Van Tassell. Dr. Bakst conceived and led the SST candidate gene immunocytochemistry experiments in collaboration with Dr. Long. **Impact**: The fundamental knowledge gleaned regarding oviductal sperm selection, transport and storage will provide a basis for developing novel approaches to *in vitro* semen storage and AI techniques. This is the first reported application of SAGE and proteomics technologies in poultry and these basic findings are facilitating identification of novel gene transcripts and proteins required for maintenance of poultry sperm viability. Having chicken cDNA and genomic sequence data available was critical for successfully applying SAGE and proteomic analyses in the turkey, and thus, these results provide one of the first examples of the power of now having whole genome sequence available from the chicken.

3. ***Impact of Egg Storage on Blastodermal Cell Viability and Embryo Development***
**Accomplishments**: Early embryonic mortality (EEM) in turkeys prior to 72 hr of incubation can be up to 15% of the eggs incubated. Differentiating EEM from unfertilized ova is required to establish the true basis of poor reproductive performance. The inherent difficulty in differentiating EEM from unfertilized ova is probably the basis for the wide-ranging estimates of EEM by industry personnel. We evaluated the gross and microscopic appearance of turkey embryos from fertilization through 36 hr of incubation and observed developmental differences between the chicken and turkey and other species of commercial importance. We showed that turkey egg storage (up to 28-days) had little impact on embryo development, but affected blastoderm morphology and histology and had a negative impact on embryo survival. Based on immunocytochemical probes for cell proliferation, turkey blastodermal cells appear to be arrested in the G1, S or G2 phases of the cell cycle during cold storage and "physiological zero" (temperature that morphogenetic development ceases) is between 20-25C. The magnitude of apoptosis seems to correspond with a decrease in cell number during the first 72 hr after laying. To definitely identify EEM from unfertilized ova in duck, chicken, and turkey eggs before and after egg storage, Dr. Bakst devised a simple, reproducible staining method to highlight blastodermal cells. **Role**: Dr. Bakst conceived and performed these experiments in collaboration with J. P. Brillard (France). Manuscripts and abstracts were a joint effort. **Impact**: Our observations provide criteria for differentiating fertilized from unfertilized ova, and dead embryos and allow an objective assessment of the stage of embryo development in turkey, chicken and duck embryos. The procedures developed are used by poultry scientists and hatchery personnel to better define the biological basis of hatchery losses. We suggest that elevated EEC is, in part, due to the practice of egg storage in combination with the tendency for heavier turkeys to lay fewer eggs with less viable embryos.

**Proteomic Analysis of Factors Regulating Egg Production in the Hen**

1. **The turkey hen has the shortest reproductive season of any commercial poultry species. Accomplishment:** *In vivo* egg production trials that involved manipulation of day length revealed the presence of covert inhibitory neural inputs that lead to the termination of egg production. "Photorefractoriness" is a change in the brain chemistry, thought to be caused by interaction of hormones and day length, that causes the hen to cease laying and undergo regression of the ovary and reproductive tract. The turkey hen was previously thought to only exhibit "absolute photorefractoriness", in which the hen ceases laying because of inhibition of brain peptides, and this inhibition cannot be reversed by increasing day length. This research demonstrated that the turkey hen also exhibits "relative" refractoriness, in which the inhibition is present but not expressed unless day length declines. This type of photorefractoriness can be reversed by increasing day length. Results showed that relative refractoriness is a lesser form of, and precedes, absolute refractoriness; that it is present in some hens but not others early in the reproductive cycle; and that hens which exhibit these inhibitory inputs early in the reproductive cycle are likely to be poorer producers later in the season than those that do not. **Role:** Dr. Proudman jointly conceived the research with collaborator and carried out the *in vivo* studies and hormone analyses. The results were interpreted and manuscripts were prepared jointly with collaborator. **Impact:** This research demonstrated that turkey hens exhibit a range of seasonal reproductive behaviors within a flock that were previously unrecognized. The test reveals the presence of relative photorefractoriness may be useful in selecting breeders with superior reproductive potential by primary breeders, but it is not practical for use at the producer level because of loss of egg production.

2. **An understanding of when and how the end of the turkey hen's reproductive season is "programmed" may allow development of management strategies to extend the reproductive cycle. Accomplishment:** Reproduction in turkeys is a balance between two physiological states, photosensitive and photorefractory. Reproduction starts when a mature, photosensitive hen receives a stimulatory day length. Current theory, developed from studies with wild birds, holds that the termination of reproduction by photorefractoriness is "programmed" at, or shortly after, the onset of photostimulation by a combination of long day length and changes in thyroid hormones, while the subsequent presence or absence of the pituitary hormone, prolactin, may advance or delay its onset. Through numerous experiments comparing hormone levels of hens that do and do not become photorefractory, and by mixing photoperiods in innovative ways, results have shown that programming of the turkey hen for photorefractoriness occurs between 9 and 12 weeks following photostimulation. This is much later than previously thought. **Role:** Dr. Proudman jointly conceived the research with collaborator and carried out most of the *in vivo* studies and hormone analyses. The results were interpreted and manuscripts were prepared jointly with collaborator. **Impact:** This research has demonstrated that turkey hens can be stimulated to maximum egg production early in the reproductive cycle by long day lengths without programming a subsequent reproductive decline. These

findings will stimulate research to design changes in lighting practices that may enhance egg production and extend the reproductive season of turkey breeder hens.

3. **Regulation of the synthesis and secretion of follicle-stimulating hormone (FSH) in birds is poorly understood. The manner in which the secretion of FSH and luteinizing hormone (LH) are independently regulated, and whether by the same or different releasing factors, is controversial in all species. Accomplishment:** Cyclical and pulsatile secretion of LH and FSH is critical for maintenance of the reproductive organs, development of gametes, and production of embryos. In all species except cattle and chickens, the two hormones are produced in the same cell, yet are secreted independently. Three brain peptides, 10-amino acid releasing factors, are known to influence the secretion of one or both of these hormones in some species, but their influence on the secretion of LH and FSH in chickens has not been compared. We found that chicken gonadotropin releasing factor-I (cGnRH-I), the presumed physiological releasing factor for both hormones in birds, does not stimulate secretion of FSH *in vivo*, but that cGnRH-II, a peptide found throughout the vertebrate classes, produces a modest stimulation of FSH secretion. This peptide is not thought to have a physiological role in regulating secretion of either LH or FSH, but this concept remains controversial. Lamprey GnRH-III, proposed as an FSH-specific releasing factor in some mammals and observed in neurons in the chicken brain, had no effect on either LH or FSH secretion in the chicken. **Role:** Dr. Proudman conceived the studies and arranged collaborations for the studies, since we do not have adult chickens at Beltsville. One study was jointly conducted at Texas A&M, while another was conducted by collaborator at Iowa State. Sample and statistical analyses were conducted at Beltsville. **Impact:** This work should stimulate research to better understand the roles of the known releasing factors in the chicken, and to search for other releasing factors (or other mechanisms) that may regulate the secretion of FSH.

## TECHNOLOGY TRANSFER ACHIEVEMENTS

### CRIS 1265-31000-080-00D

**Talbot:** A CRADA was established between ANRI scientists Neil Talbot and Tom Caperna and Hepalife Technologies Inc. (Vancouver), to develop ARS-PICM-19 liver stem cell as a model for hepatic metabolism and to determine its potential for use in a bioartificial liver device. This is a five year (2002-2007) agreement ($805,000) and is currently funding a Research Associate (Ayesha Mahmood), a support scientist (Ryan Willard) and an undergraduate intern (David Hwang).
Pig macrophage culture system was featured on the ARS web site in a "Science for Kids" story. Launch date: July 21, 2000, and was also published in Agricultural Research, Vol. 49, No.3, p. 23. ARS News Service: Scientists Serve Up a Dish of Pig Liver Cells, by Jan Suszkiw, October 19, 2001.

Transferred bovine trophectoderm and endoderm cell lines to Dr. R.D. Shultz, University of Wisconsin-Madison, School of Veterinary Medicine, to Dr. T. E. Spencer of Texas A&M University, College Station, TX, to Dr. Joseph Brewoo, University of Wisconsin-Madison, Madison, WI, to Dr. Wenbin Tuo, USDA-ARS-ADRU, Pullman, WA, and to Dr. Carol Hannah of Texas A&M University, College Station, TX, under five year Material Transfer Agreements (MTA).

In collaboration with Dr. Tad Sonstegard of BFGL, ANRI, isolated and cryopreserved secondary cell cultures of fibroblasts from tissue of elite Angus and Holstein cows selected for whole genome shotgun sequencing for Phase II (SNP Discovery) of the Bovine Genome Sequence project. These cell lines serve as a permanent source of genomic DNA for further sequencing and SNP validation projects by scientists worldwide.

**Wall:** A CRADA between John Bramley of the University of Vermont and ANRI scientist Robert Wall was established to test the feasibility of protecting mice and cattle against *S. aureus* mammary gland infection by introduction of a transgene expressing lysostaphin. Develop resistant transgenic mice and cattle, 1999 – 2003.

Specific Cooperative Agreements were established to formalize collaborative studies to improve the efficiency of somatic cell nuclear transfer between Dr. Wall and Professor Roberts Godke, Louisiana State University, Jerry Yang, University of Connecticut, and Harris Lewin, University of Illinois.

Transgene constructs from various projects have been sent on request, over that last three years, to: Xiuqiong Wang, MD, PhD., Department of Molecular and Cellular Physiology University of Cincinnati, College of Medicine; Jon Neumann, Transgenic Mouse Core, Dept of Molecular Genetics, University of Chicago; Stephan Geley, MD, Institute for General and Experimental Pathology, University of Innsbruck, Medical School, Austria, W.M. (Mac) Grogan, Ph.D., Department of Biochemistry & Molecular Biophysics, Medical Campus,

Virginia Commonwealth University, Richmond, VA; Sadayappan Sakthivel Ph.D, Max-Planck-Institute for Physiological and Clinical Research
Department of Experimental Cardiology, Bad Nauheim, Germany; Anthony J. Zeleznik, Ph.D., Department of Cell Biology and Physiology, University of Pittsburgh School of Medicine, Pittsburgh, PA; Dr. Alexander Belyavsky, Laboratory of Stem Cells, New York Blood Center, New York, NY; P.Y Rescan, SCRIBE-INRA, Campus de Beaulieu
35042 RENNES, FRANCE, Dott. Simona Signorini, Neurobiology Lab, Scientific Institute "IRCCS-Centro S.Giovanni di Dio- Fatebenefratelli," Brescia, Italy; Xiao Xiao, Ph.D., Assistant Professor, Dept. of Molecular Genetics and Biochemistry, University of Pittsburgh School of Medicine, Pittsburgh, PA; Luca Rampoldi, PhD, Human Molecular Genetics Unit - San Raffaele Scientific Institute, Milan, Italy

**CRIS 1265-31000-082**

**Guthrie:** Updated international visitors, including Armenian Minister of Agriculture, representatives of Chinese Academy of Agricultural Sciences, Tianjin Municipal People's Government Vice Major Sun Hailin Delegation, and People's Republic of China Team #6 Pig Farm Management and Disease Control Group on boar semen cryopreservation and gender preselection by sorting sperm on DNA content difference between X and Y chromosome.

**Zuelke:** The SAGE technologies developed for pig embryos have been transferred to researchers at NC State University as part of an NIH-sponsored collaborative study investigating molecular factors affecting mammalian (mouse) oocyte development and maturation.

Organized and served as initial Chair of the Committee for the USDA National Animal Germplasm Program.

Represented BARC functional genomics and proteomics research at BIO 2003 and 2004, MD TEDCO and similar technology transfer showcase events.

Patents:
BGL researchers developed and tested an integrated group of technologies that would enable consistent cryopreservation and subsequent post-thaw viability of swine embryos. This suite of technologies, collectively referred to as the 'USDA Swine Embryo Cryopreservation Technology', was patented in FY 2003 (US Pat. No. 6,503,698).

GenBank SAGE Library Database and Gene Sequence Submissions:

Blomberg, L. and Zuelke, K.A. July 1, 2004. Day 11 ovoid pig embryo SAGE library: GSM GEO Accession No. GSM24470.

Blomberg, L. and Zuelke, K.A. July 1, 2004. Day 12 filamentous pig embryo SAGE library: GEO Accession No. GSM24471.

Blomberg, L. and Zuelke, K.A. Oct. 28, 2004. Sus scrofa steroidogenic acute regulatory protein mRNA. Accession No. AY800265.

Popular Press Publications:

K.A. Zuelke and L. Blomberg (2003). Linking Genetics to Embryo Survival. National Hog Farmer Magazine, December Research Issue.

Guthrie, H. D. (2002). Handling affects fertility of frozen semen. National Hog Farmer. December Research Issue.

Zuelke, K.A. (2002). Applying Serial Analysis of Gene Expression (SAGE) to Uncover Genes Important in Swine Embryo Development. National Hog Farmer. December Research Issue.

**CRIS 1265-31000-083**

**Bakst:** Met with European poultry scientists and industry personnel to discuss semen and fertility evaluation techniques. Consulted with Perdue Poultry (broiler), Wampler Longacre (turkey) and Maple Leaf Farms (duck) addressing fertility and hatchability problems. Discussed poultry research in ARS with representatives from the Navajo Nation. Represented ARS in a meeting with the 10-member Iraqi Chochran Fellows and spoke about poultry research in ARS and academia. Instructed graduate students, faculty, and staff (University of Chicago, University of Maryland, University of Arkansas), and industry representatives (AviGenics, Ross Breeders) on techniques in embryo staging and manipulation of embryos and the preparation of cells and tissues for microscopy. Light and electron micrographs have been given upon request to BBC (UK) for a TV production on reproduction, to Prof. Ruth Bellairs (UK) for text book on avian embryology, Dr. Frank Robinson (Canada) for his text book on poultry production.

**Long:** Identified the causes of significant and persistent decline in fertility rates for a major turkey breeder and proposed recommendations for returning to expected fertility rates (~96%) that resulted in savings of $1,800,000 for the company. Provided protocol for removing the cryoprotectant glycerol from thawed poultry semen to the USDA's National Animal Germplasm Program. Trained Pilgrim's Pride staff to conduct the perivitelline hydrolysis assay in turkey eggs and to evaluate sperm mobility of turkey semen samples. Consulted for Gobbler's Inc. regarding the use of vitamin E as an antioxidant in turkey semen extender. Provided state-of-the-art information about poultry sperm cryopreservation for Perdue Farms in Salisbury, MD. Trained VA Tech staff to conduct perivitelline sperm hydrolysis assay in quail eggs. Presented a hand-on demonstration of poultry sperm storage tubule isolation and function for Senate and House Agricultural Committee Staffers. Provided technical information for Dr. Janet Fulton (Hy-Line International) concerning state-of-the-art poultry semen cryopreservation for Science and Disease Policy Forum. Provided technical advice for Avigenics regarding chicken sperm cryopreservation protocols. Consulted for Pilgrim's Pride Turkey Breeder Division regarding the use of amoxicillin in turkey semen. Provided technical advice for cryopreservation of crane semen for Dr. Larry Anderson, U of MD, College Park, MD and the Baltimore Zoo. Presented

technical seminars on poultry semen storage for Italian poultry farmers and a delegation of Ukranian agricultural officials visiting Beltsville, MD. Submitted an Invention Report entitled 'Novel Extender for 24h Liquid Storage of Turkey Semen' for patent consideration.

GenBank SAGE Library Database and Gene Sequence Submissions:

Zuelke, K.A., Long, E.L., Sonstegard, T.S., Long, J.A., and Van Tassell, C.P. March 24, 2003. Control Treatment Turkey Sperm Storage Tubule SAGE library: GSM GEO Accession No. GSM4899.

Zuelke, K.A., Long, E.L., Sonstegard, T.S., Long, J.A., and Van Tassell, C.P. March 24, 2003. AI Treatment Turkey Sperm Storage Tubule SAGE library: GSM GEO Accession No. GSM4900.

Long, E.L., Zuelke, K.A., and Sonstegard, T.S. September 11, 2002. Meleagris gallopavo avidin mRNA. Accession No. AF545846.

**Major Invitations:**

**Bakst**
Invited to co-author two chapters (oviduct structure and function; fertilization in birds) in *Reproductive Biology and Phylogeny of Aves (Birds)*, Barrie G.M. Jamieson, Editor. 2005

Invited to organize a session on poultry reproduction and presented talk on oviductal sperm storage at the *World's Poultry Science Meeting*, Istambul, Turkey, 2004

Invited talk "Impact of egg storage on turkey egg fertility," *Midwest Poultry Federation*, St Louis, MI, 2003

Invited talk, "Avian oviduct and fertile egg production," AviGenics, Inc., Athens, GA, 2003

Invited talk, "Oviducal sperm storage in birds," *European Society of Reproduction*, Dublin, Ireland, 2003

Invited talk, "The turkey oviduct - sperm storage, fertilization and early embryonic development," *International Symposium on Turkey Reproduction*, Raleigh NC, 2003

Invited talk, "Impact of egg storage on early embryo mortality," *Workshop on Fundamental Biology and Perinatal Development in Poultry*, Berlin, Germany, 2003

Invited talk, "How to maximize fertility and early embryonic development from a biologist's point of view," *25th Technical Turkey Conference*, Manchester, UK, 2002

Invited talk, "Oviducal sperm and fertilization in poultry," *Incubation and Fertility Research Group*, Oxford, UK 2001

**Long**
Invited speaker for the *Poultry Science Association's Ancillary Scientists Symposium* "Conservation of Avian Genetic Resources: Current Opportunities and Challenges", Auburn, AL, 2005.

Invited speaker for the *International Plant and Animal Genome Conference XIII Poultry Symposium* "Addressing the Biological Challenges of Germplasm Preservation in Poultry: New Approaches for Semen Cryopreservation", San Diego, CA, 2005.

Invited speaker for the *Women in Andrology Symposium* in conjunction with the American Society of Andrology Annual Conference, Baltimore, MD, 2004.

Invited speaker for the *National Turkey Federation's Leadership Conference* "Impact of ARS Turkey Research on Commercial Production", Washington, DC, 2003.

Invited speaker for the *USDA-ARS Workshop on Poultry Research*, presented overview of all BGL poultry projects and defined areas of need to continue research success, Orlando, FL, 2003.

Invited speaker for the *International Symposium on Assisted Reproductive Technology for the Conservation & Genetic Management of Wildlife* "Developing artificial insemination for captive management of the cinereous vulture", Omaha, NE, 2001.

## CRIS 1265-31000-084

**Proudman:** Provided reagents to scientists in at least 25 laboratories in Australia, Belgium (3 labs), Canada, China (2), Czechoslovakia, India, Israel, Japan (2), Mexico, Poland, the United Kingdom (2), Cornell University, Iowa State University, Ohio State University, Texas A&M University, University of Arkansas (2), University of Minnesota, University of Nebraska, University of Tennessee, and the University of Washington for use in the detection or measurement of avian hormones in their research programs. Provided instruction and collaboration on the use of these reagents with many of these laboratories, particularly those in Canada, Czechoslovakia, Israel and Poland.

## FUTURE RESEARCH DIRECTIONS

## CRIS 1265-31000-080

**Dr. Blomberg:** Cloning of livestock by somatic cell nuclear transfer (SCNT) remains inefficient and little is known about the molecular mechanisms altered as a consequence of *in vitro* manipulation. In-depth transcriptomic characterization of *in vivo*-and *in vitro* SCNT-derived embryos via SAGE will be utilized to identify alterations in gene expression profiles (candidate genes); the focal points, nuclear reprogramming and trophectoderm remodeling/elongation. Additionally, a comparative analysis of elongating *in vivo*-derived porcine and bovine embryo transcriptomes will be performed *in silico* to identify genes/mechanisms potentially regulating elongation. A new genomics tool, RNAi will be established to examine the function and regulation of candidate genes *in vitro,* utilizing embryonic tissues (embryo-derived cell lines) and ultimately, *in vivo* to correlate gene function and phenotype. The detection of a disruption(s) in crucial developmental mechanism(s) during SCNT embryo production should facilitate the identification of markers for developmental competence, the establishment of non-invasive diagnostics, and improved *in vitro* methodology.

**Dr. Donovan:** The enhanced resistance to infection demonstrated by transgenic cows expressing lysostaphin in their milk suggests this promising approach warrants a continued effort to create transgenes that combat other mastitis pathogens. Additional candidate antimicrobials are needed to broaden the range of mastitis pathogens targeted and to address potential drug resistance issues. Three novel antimicrobial proteins will be investigated. First is the Group B Streptococcus bacteriophage B30 endolysin which kills Streptococcus agalactiae, dysgalactiae, and uberis. Second is the marine fish cationic antimicrobial protein NRC 13 (CAP, small 40 aa proteins) that has shown promise against Streptococcus agalactiae and Staphylococcus aureus. Third is the Staphylococcus aureus bacteriophage K endolysin, the lys K gene. Each candidate will be evaluated and sequence optimized for expression in cultured mammary cells. Transgenic mice will be generated and susceptibility to mastitis tested. Finally those found to be highly efficacious will be introduced into transgenic cattle. Concurrently, intragenic endolysin fusions are being created and tested as a potential means to make antimicrobials that are effective against two or more mastitis pathogens simultaneously, thus saving time and effort in the process described above for making a transgenic cow.

**Dr. Talbot:** Comparative proteomic analysis of cultured trophectoderm tissue from NT, IVP, and parthenogenetic bovine embryos will be undertaken to search for differences in protein expression that reflect incomplete "reprogramming" of the donor nucleus in NT embryos. An emphasis on expanding the number of cell lines derived from each type of embryo will provide for the creation of a cell culture collection for use as a research resource. Fractionation and purification of the trophectoderm cellular proteins will be performed so that proteins expressed at low levels can be identified and compared across embryo types. Comparative proteomic analysis of cultured fibroblasts derived from the in vitro differentiation of the epiblast cells (embryonic stem cells) of NT and IVP bovine embryos will be undertaken. This will afford an opportunity to compare gene and protein expression to the nuclear donor fibroblast cell culture. Finally, a proteomic analysis of in vivo filamentous-stage bovine embryos will be performed for comparison to the in vitro tissue cultures. These initiatives may identify more proteins that are

differentially expressed between NT and IVP embryos and this may lead to means by which nuclear reprogramming sufficiency and embryo quality can be assessed prior to embryo transfer.

**Dr. Wall:** The genetic engineering strategy for improving disease resistance in dairy cattle will further enhanced by incorporation of additional genes into our transgenic cattle model. Two candidate genes, trout lysozyme and bovine CD14, previously screened in tissue culture systems will be further evaluated in transgenic mice. These and other genes, screened in a similar manner, that demonstrate at least 70% protection in a transgenic mouse mastitis model system will become candidates for introduction into cattle. Parallel to the candidate gene screening studies, strategies for improving reliability of mammary gland specific transgenes expression by inclusion of matrix attachment regions into transgene constructions and methods for excising selectable markers will be tested. Several methods for optimally introducing multiple transgenes into the cattle genome will also be evaluated. Approaches such as use of embryonic stem cells as nuclear donors for cloning, transgene introduction through multiple rounds of somatic cell nuclear transfer, assembling multicistronic transgenes, promoter trapping BLG and multigenic large fragement transgenes will be evaluated to determine the most cost effective approach for producing mastitis resistant cattle. The biosafety, nutritional quality and manufacturing properties of milk from the transgenic cows will be evaluated for every new strain of cattle produced.

## CRIS 1265-31000-082

**Dr. Guthrie:** One source of damage to spermatozoa during cryopreservation is osmotic-induced fluctuations in cell volume caused by changes in liquid transport across the plasma membrane. Stopped-flow spectroscopy will be utilized to measure rapid changes in cell volume (changes in cytoplasmic dye fluorescence) during the addition and dilution of cryoprotectants, cooling, freezing, and thawing. These data will be used to mathematically derive water and cryoprotectant permeability coefficients associated with changes in temperature, cell physiology, and genetics. The osmotic tolerance limits of spermatozoa will be determined and these tolerance limits along with permeability coefficients will be utilized to mathematically model new cryopreservation procedures that avoid osmotic induced cell destruction. The new cryopreservation protocols will be evaluated in the laboratory by measuring physiological and functional activity and confirmed in the field by breeding trials in collaboration with boar studs or genetics companies.

**Dr. Zuelke:** SAGE profiling of in vivo derived pig maturing oocytes and blastocyst stage embryos to establish gene expression profiles of each critical preimplantation stage of pig embryo development will be completed. These gene expression profiles from ‘normal’ in vivo embryos will be made available as a public research resource. Concomitant with SAGE profiling, candidate genes critical for development will be identified and functionally characterized. Comparison of SAGE profiles between in vivo and in vitro derived pig embryos will identify gene expression patterns altered during in vitro embryo production to enable development of improved in vitro embryo production systems. The current SAGE profiles have been established from in vivo embryos obtained from conventional large white maternal lines. As candidate genes are identified and functionally characterized, they will be assayed (e.g. via Real-time PCR and/or microarrays) as candidate gene markers in embryos derived from lines

that possess unique reproductive traits (e.g. increased litter size, lower embryo mortality, etc.) as a means of potentially identifying selectable gene markers for these desirable reproductive trait characteristics.

**CRIS 1265-31000-083**

**Dr. Bakst:** We will determine how sperm enter and then egress from the SST in order to provide insight into why some sperm are more successful than others in reaching and entering the SST. Lectin characterization of the SST epithelium and resident sperm will continue in order to determine which glycoprotein/glycolipids on the sperm interact with the surface of the SST epithelium. The immunocytochemical localization of SAGE identified gene products was initiated and will continue as new SAGE data becomes available. These observations will allow us to determine if gene products are being produced only by the sperm storage tubule (SST) epithelium or throughout the oviduct mucosa. The partial purification, culture, and propagation of spermatogonial stem cells will continue. These cells will be transfected and then transferred to recipient sterile males with the ultimate goal of establishing a stable germline transmission of genes of economic importance.

**Dr. Long:** The need for effective semen storage methods will increase in the future for all of the commercial poultry industries (turkey, broiler, layer), although the particular applications for each industry will differ. For example, because of differing production strategies, the layer industry will benefit from long-term semen storage in the form of cryopreserved semen, while the broiler industry will most likely benefit from short-term semen storage similar to the turkey industry. The biological challenges of achieving high fertility rates from stored poultry semen will still require systematic evaluation of sperm physiology under storage conditions in order to develop highly efficient storage methods. To meet the needs of the stakeholders, the future research direction will encompass development of alternative diluents and/or methods specifically for both short-term and long-term semen storage. Ideally, short-term semen storage would utilize new diluents at ambient temperature and maintain sperm fertilizing capacity for 24h or longer. Likewise, semen cryopreservation will utilize new cryodiluents and thawing diluents that maintain a fertilizing capacity of frozen/thawed sperm similar to freshly collected semen.

**CRIS 1265-31000-084**

**Dr. Proudman:** The accomplishments related to photorefractoriness suggest the possibility of developing a new management scheme for lighting turkey breeder hens that would use an initial long photoperiod to maximize onset of lay and peak egg production, followed by a reduced photoperiod that would maintain sufficient photoperiodic drive for continued egg production while remaining below the day length that causes programming for photorefractoriness for some yet-to-be-determined period of time. Such a scheme would be nearly the reverse of current industry lighting practices. Future research will test the efficacy of a new lighting program and fine-tune the details prior to making recommendations to the industry for application to their strains. The identification of cellular factors in the brain or pituitary that enhance or terminate egg production requires implementation and refinement of proteomic techniques for 2-D

Difference In-gel Electrophoresis (DIGE) and for the structural identification by mass spectrometry of post-translational modification that may distinguish identified peptides. Collaboration will continue with the Laboratory of Neuroplasticity and Neuroproteomics, Catholic University of Leuven, Belgium, to perfect and apply these techniques to, first, the identification of neuroendocrine factors that lead to broodiness (incubation) in some hens but not others.

**BGL 2005 BUDGET (ARMPS)**

| | Dollars | |
|---|---|---|
| Net to Location | $5,205,974 | |
| Indirect Research Costs | $ 779,265 | |
| Shared Research Costs | $1,185,158 | |
| Net to BGL | $3,241,749 | |
| Permanent Salaries | | FTE |
| Research Scientists | $1,073,030 | 8.8 |
| Non SY (Support Personnel) | $ 765,053 | 11.4 |
| Bench Dollars/SY | $ 501,769 | |
| Discretionary Funds/SY | $ 48,793 | |

## POSITION STAFFING PLAN

| Employee Name | Position # | Position Title | Grade | Status | FTE |
|---|---|---|---|---|---|
| Zuelke, Kurt | 1B313 | Supvry Res Physiol Anml | GS-14 | PFT | 1 |
| McDougal, Jane | 3B5627 | Molecular Biologist | GS-09 | PFT | 1 |
| Garrett, Wesley | 3B7827 | Physiologist Anml | GS-12 | PFT | .50 |
| Miles, Jeremy[1] | 2B917 | Res Molecular Biologist | GS-11 | TFT | 1 |
| Boyd, Michelyn | 9B226 | Support Services Asst | GS-07 | PFT | 1 |
| Trzupek, Kendra | 9B5566 | Office Automation Asst | GS-05 | PPT | .40 |
| Vacant[3] | 1B???? | Res Physiologist | GS-12 | PFT | 1 |
| Bakst, Murray | 1B220 | Res Physiologist Pltry | GS-15 | PFT | 1 |
| Akuffo, Valorie | 3B225 | Biologist | GS-11 | PFT | 1 |
| Blomberg, Le Ann | 1B710 | Res Molecular Embryologist | GS-12 | PFT | 1 |
| Schreier, Lori | 7B227 | Biologist | GS-09 | PFT | 1 |
| Donovan, David | 1B150 | Res Molecular Biologist | GS-13 | PFT | 1 |
| Foster-Frey, Juli | 3B5433 | Molecular Biologist | GS-11 | PFT | 1 |
| Guthrie, Howard D | 1B316 | Res Physiologist Anml | GS-14 | PFT | 1 |
| Welch, Glenn | 3B324 | Biologist | GS-12 | PFT | 1 |
| Long, Julie | 1B5466 | Res Physiologist Pltry | GS-13 | PFT | 1 |
| Conn, Timothy | 7B408 | Biol Sci Lab Techncn | GS-09 | PFT | 1 |
| Vacant[2] | 2B???? | Molecular Biologist | GS-11 | TFT | 1 |
| Talbot, Neil | 1B311 | Research Animal Scientist | GS-13 | PFT | 1 |
| Graninger, Paul | 7B5915 | Biol Sci Lab Techncn | GS-07 | PFT | 1 |
| Proudman, John | 1B221 | Res Physiologist Pltry | GS-15 | PFT | 1 |
| Wall, Robert | 1B318 | Research Physiologist | GS-15 | PFT | .80 |
| Schulman, Leah | 7B325 | Bio Sci Lab Tech | GS-09 | PFT | .50 |
| Powell, Anne | 3B5343 | Animal Scientist | GS-11 | PFT | 1 |

[1] Research Associate – Kurt Zuelke – In-house funded
[2] Research Associate – Julie Long – Headquarters Funded
[3] Research Physiologist Pltry position to be established - Kurt Zuelke – Permanent Fund Increase

## CONTRIBUTIONS FROM INDIVIDUAL SCIENTISTS

The BGL is well-positioned to be a world leader pioneering and developing novel applications of functional genomics, proteomics and biotechnology research tools in agricultural animal germplasm conservation research. BGL boasts multi-user, high throughput functional genomics and proteomics instrumentation and infrastructure combined with state-of-the-art molecular biology, embryo technology, cell biology, cryobiology, flow cytometry and cell sorting, and confocal microscopy lab facilities. A joint partnership between BGL, the ANRI BFGL and GBL, and the PSI SGIL, established and maintains a joint mass spectroscopy and proteomics lab resource to facilitate agricultural proteomics research across BARC.

**BGL staff has specific strengths and instrumentation in the following areas:**

• Cryopreservation of spermatozoa, embryos, and embryo-derived/somatic cells: Expertise and computerized cryopreservation equipment (IceCube 1810, Minitube of America, Verona, WI) for controlled cooling rate using nitrogen vapor and vitrification is available for research to optimizing cooling rates and cryoprotectant types and concentration to optimize fertility and cell survival of poultry and mammalian germplasm. Producing and recovering large numbers of murine blastocysts for storage of transgenic and knockout lines. (Donovan, Guthrie, Long).

• Fluorescence Activated Flow Cytometry and Cell Sorting: Expertise and instrumentation (Coulter Epics XL flow cytometer, Beckman-Coulter Inc., Hialeah, FL; and MoFlo cytometer, Cytomation Inc., Ft. Collins, CO) is available to perform analysis of cell function and structural components in viable or fixed cells electronically separate or sort viable or fixed spermatozoa populations and somatic cells based on fluorescence activated probes for DNA, lipids, proteins, and membrane electrical potential; and on light scattering characteristics using flow cytometry. (Guthrie and Welch).

• Sperm Motion Analysis: Expertise and a computerized sperm motion analysis system (HTM IVOS 12.1, Hamilton-Thorne Biosciences, Beverly, MA) is available to perform state of the art analysis of motion parameters for mammalian and poultry spermatozoa (Guthrie and Long).

• Cell Volume Analysis: Expertise is being developed to operate a stopped-flow reaction analyzer (SX.18M-R1 Stopped-Flow Spectrophotometer, Applied Photophysics Limited, Leatherhead, UK) to measure changes in cytoplasmic dye fluorescence associated with changes in cell volume (Guthrie and Welch).

• Somatic cell nuclear transfer and transgenic livestock technologies: Expertise in producing cloned cows from fetal fibroblasts is available for studying the process of early development in the bovine and for generating genetically engineered cattle carrying new beneficial genes. (Wall).

• Transgenic and knockout mouse modeling: Expertise in all phases of producing transgenic and knockout mice (i.e. from transgene or knockout construct design through animal production and

testing) to establish models for understanding basic mechanism of gene regulation of physiological processes and for evaluating the efficacy and functionality of genetic alterations for enhancing livestock production. (Wall and Donovan).

• Molecular Biology and Gene Expression (see also Functional Genomics): Expertise and instrumentation spans all aspects of molecular biology including: RNA isolation; cDNA and genomic DNA library construction and screening (homology and expression); genomic DNA isolation; Northern, Western and Southern analyses; SDS-PAGE; gene cloning and plasmid constructs; aRNA; in-situ hybridization and immunohistochemistry; RT- and Q-PCR; nuclear run on; and in vitro transcription, etc. (Blomberg, Donovan, Talbot, Wall, Zuelke).

• Functional Genomics: Expertise is available for utilizing multiple methodologies including serial analysis of gene expression (SAGE), cDNA and oligonucleotide microarrays, suppressive subtractive hybridization, differential display to evaluate and quantify alterations in transcriptome-wide gene expression during embryo and germplasm development (Blomberg, Donovan, Proudman, Zuelke).

• Tissue and Cell Culture: Expertise in primary cell culture and cell line development of cells derived from the early embryos of pigs and cattle. Expertise is available to utilize various types of cell culture (mammalian and insect) to study cellular metabolism and for *in vitro* production of recombinant proteins of interest. Short term turkey embryo cultures to evaluate reversible suppression of the blastodermal cell differentiation and oviductal tissue explants to elucidate sperm oviduct interaction are also utilized. (Bakst, Blomberg, Donovan, Talbot).

• Protein purification, characterization, and development of hormone assay techniques: Expertise in many chromatographic techniques for separating and analyzing proteins that regulate reproduction. Facilities for the radioimmunoassay of avian hormones. (Proudman).

• Light, confocal and electron microscopy: Expertise in several modes of light, confocal, and electron microscopy which allow the visualization of unfixed, live cells and fixed cells and tissues. Immunocytochemistry and histochemical procedures used to demonstrate the presence or absence of specific proteins, glycoproteins, glycolipids and specific stains used to track live cells in embryo cultures, locate sperm in the oviduct, and identify specific cell types. (Bakst, Talbot, Zuelke, and Garrett).

• Protein Separation and Mass Spectroscopy: Expertise in the separating cellular proteins by 2D-gel electrophoresis combined with protein identification via MALDI-Tof and MS-MS mass spectrometry analyses (Talbot, Proudman, Zuelke, Garrett).

# DAVID M. DONOVAN, RESEARCH MOLECULAR BIOLOGIST

## Summary of Research

Dr. Donovan joined ARS in December 2002. He contributes 100% to and is responsible for investigating and conducting research on "Identification and manipulation of genetic factors to enhance disease resistance in dairy cattle." CRIS #1265-31000-080-00D, ARS National Program #101. The broad scope of the research is to identify and develop antimicrobials that are effective against disease causing pathogens in the cow. The specific goals are to identify, characterize, and express as cow mammary gland transgenes, antimicrobials that are effective against the two major mastitis causing pathogens *Streptococcus agalactiae,* and *Staphylococcus aureus*. The current focus is on testing two candidate proteins: 1) a fish cationic antimicrobial peptide (CAP) and 2) a *Streptococcus agalactiae* B30 bacteriophage endolysin. One criterion in choosing an antimicrobial for transgene construction is to first verify the efficacy of the candidate against mastitis causing pathogens. A second criterion is to determine whether or not the antimicrobial is active in a milk environment, and following pasteurization. If so, does it interfere with downstream processing of milk, e.g. effect the milk processing bacteria, such as those used to make yogurt and cheese? A third objective is to employ protein engineering, and gene expression skills to modify the candidate gene in order to obtain high level expression while maintaining antimicrobial efficacy in a secreted form from cultured bovine mammary cells. The successful candidate gene is then moved via transgenesis into a mouse mammary gland for final testing before achieving the final goal, the transgenic cow. The knowledge gained from the study of these prospective antimicrobials is likely to potentiate the development of application(s) of these compounds to other diseases caused by these (or related) infectious agents.

## Publications (2003-present)

Ghorbel, M.T., Sharman, G., Leroux, M., Barrett, T., **Donovan, D.M.,** Becker, K.G., Murphy, D. 2003. Microarray analysis of the dynamics of gene expression in the supraoptic nucleus following dehydration reveals interleukin 6 as a novel secretory product of the hypothalmo-neuorohypophyseal system. J Biol Chem. May 23;278(21):19280-5. Epub 2003 Mar 14.

Cheng-Shui Zhao, Yuan-Xiang Tao, Jill M Tall, **Donovan, D.M.**, Richard A Meyer, Srinivasa N Raja. 2003. Role of μ-Opioid Receptors in Formalin-induced Pain Behavior in Mice. Exp Neurol. 184(2):839-45.

**Donovan, D.M.**, Rowley, D.L., Williams, N. 2004. Microsoft Excel® spread-sheet based animal colony management for genetically altered animals. BioTechniques 37:50-56.

Tao Xie, Liqiong Tong, Una D. McCann, Jie Yuan, Kevin G. Becker, Annis O. Mechan, Christopher Cheadle, **Donovan, D.M.**, George A. Ricaurte. 2004. Identification and characterization of metallothionein-1 and 2 gene expression in the context of (±)3,4-methylenedioxymethamphetamine (MDMA)-induced toxicity to brain dopaminergic neurons. J Neurosci. Aug 11;24(32):7043-50.

H. D. Guthrie, R. J. Wall, V. G. Pursel, J. Foster-Frey, **D. M. Donovan** G. R. Welch, and W. G. Garrett. 2005. Follicular expression of a human □-cell leukemia/lymphoma-2 (Bcl-2) transgene does not decrease atresia or increase ovulation rate in swine. In Press: Reproduction, Fertility and Development.

| **Cooperator** | **Affiliation** |
|---|---|
| Susan Douglas | Institute for Marine Biosciences, NRC, CANADA |
| Sylvain Moineau | Université Laval, Québec, Canada |
| David G.E. Smith | Moredun Research Institute, Scotland |
| Allan Delisle | Univ. Maryland, Dental School |
| Gail Christie | Virginia Commonwealth Univ. |
| Martin Loessner | Institute of Food Science and Nutrition, Switzerland |
| Paul Ross | Dairy Products Res. Ctr., Teagasc, Ireland |
| David Pritchard | Univ. Alabama |
| Brian Wilkinson | Illinois State University |
| Igor Abaev | State Res. Ctr. for Appl. Microbio., Russian Federation |
| David Kerr | Univ. Vermont |
| Bob Wall | BGL, ARS |
| Neil Talbot | BGL, ARS |
| Tad Sonstegaard | BFGL, ARS |
| Le Ann Blomberg | BGL, ARS |
| Harry Dawson | BHNRC, ARS |
| Tony Capuco | BFGL, ARS |
| Carol Keefer | University of MD, College Park, MD |
| Diane VanHekken | ARS, Dairy Processing and Products Research Unit |
| George Somkuti | ARS, Dairy Processing and Products Research Unit |

## ROBERT J. WALL, RESEARCH PHYSIOLOGIST (ANIMAL)

### Summary of Research

Dr. Wall was designated lead scientist for CRIS 1265-31000-080-00D, ARS National Program #101 at its inception and was responsible for overseeing its creation and shepherding it through the review process. Eighty percent of Dr. Wall's time is devoted to addressing objectives 1 and 2, namely using the transgenic mouse model to evaluate potential antiinfective peptides for their ability to protect against mastitis pathogens and to introduce the promising antiinfectives into the cattle genome and evaluate those animals. The genes for three antimicrobial candidates (lysostaphin, lysozyme and CD14) have been cloned, ligated into a mammary gland expression cassette and have been introduced into transgenic mice. It has only been possible to evaluated one candidate to date due to the disruption cause by moving the animals into a new housing facility. Lysostaphin, directed against *Staphylococcus aureus*, has been tested most fully and milk from lysostaphin expressing transgenic mice kill *S. aureus in vitro*. *In vivo S. aureus* challenge studies confirmed that transgenic mice expressing lysostaphin in their milk are resistant to *S. aureus* infection. Very high levels of expression of lysostaphin appeared to retard mammary gland development in the mice. An enhanced version of the transgene, more suitable for use with somatic cell nuclear transfer was fabricated and six transgenic founder Jerseys were generated (3 males and 3 females). We have estimated that employing somatic cell nuclear transfer (cloning) reduced the animal requirements by half to two-thirds of those that would be needed to produce the transgenics by pronuclear microinjection. The three transgenic founder heifers have now completed a first lactation. Two of the heifers express lysostaphin at approximately 1 ug/ml in their milk and the third heifer expresses at approximately 10 ug/ml. The ability of their milk to kill *S. aureus* in *in vitro* assays appears to be related to the measured transgene product concentration. *In vivo* mammary gland challenge studies confirm the *in vitro* results. The highest expressing transgenic appears completely protected against infection. The lower expressing cows show increased resistance, but not complete protection.

### Publications (2000-present)

**Wall, R.J.**, Paleyanda, R.K., Foster, J.A., Powell, A., Rexroad, C. Jr., Lubon, H. 2000. DNA preparation method can influence outcome of transgenic animal experiments. Animal Biotechnology 11:19-32.

Mitra, A., Wells, K.D., Rexroad, C.E., III and **Wall, R.J.** 15-NOV-2000. A new genetic variant of lysozyme from rainbow trout. GenBank Accession number AF321519

Mitra, A., Wells, K.D., Rexroad, C.E., III and **Wall, R.J.** 17-NOV-2000.
Genomic DNA sequence of lysozyme gene from rainbow trout. GenBank Accession number AF322106

Kerr, David E., Plaut, Karen, Bramley , A. John, Williamson, Christine M., Lax, Alistair J., Moore, Karen, Wells, Kevin D., and **Wall, R. J.** 2001. Lysostaphin expression in mammary

glands confers protection against staphylococcal infection in transgenic mice. Nature Biotechnology 19:66-70.

**Wall, R.J.** 2002. Pronuclear microinjection. Cloning and Stem Cells 3:193-204.

**Wall, R.J.** 2002. New methods of gene transfer. Theriogenology 57:189-201.

Mitra,A., Foster, J.A., Wells, K.D., Rexroad, C.E., III and **Wall, R.J.** 29-NOV-2001. Rainbow trout Lysozyme type II gene: longer version. GenBank Accession number AF452171.

Mitra, A., Foster-Frey, J.A., Wells, K.D., Rexroad, C.E., III, Wells, K.D. and **Wall, R.J.** 2003. Molecular Characterization lysozyme type II gene in rainbow trout (Oncorhynchus mykiss): Evidence of Gene Duplication. Animal Biotechnology 14:7-12.

Mitra, A., Hruska, K., Wellnitz, O., Kerr, D.E., Caupuco, A.V., and **Wall, R.J.** 2003. Expression of lysostaphin in milk of transgenic mice affects the growth of neonates. Transgenic Research 12:597-605.

Powell, A.M., Talbot, N.C., Wells, K.D., Kerr, D.E., Pursel, V.G., and **Wall, R.J.** 2004. Cell donor influences the success of producing cattle by somatic cell nuclear transfer. Biology of Reproduction 71: 210-216.

Vernon, P.G., and **Wall, R.J.** 2004. Biotechnology: Transgenic Animals. In: Encyclopedia of Animal Science pp 149-151.

de A. Camargo, L.S., Powell, A.M., Do Vale Filho, V.R. and **Wall, R.J**. 2005 in press. Comparison of gene expression in individual preimplantation bovine embryos produced by in vitro fertilization or somatic cell nuclear transfer. Journal of Reproduction, Fertility and Development (in press).

**Wall, R.J.**, Powell, A.M., Paape, M.J., Kerr, D.E., Bannerman, D.D., Pursel, V.G., Wells, K.D., Talbot, N.C., and Hawk, H.W. 2005. Genetically enhanced cows resist intramammary staphylococcus aureus infection. Nature Biotechnology 23(3): (in press).

| **Cooperator** | **Affiliation** |
|---|---|
| Doug Bannerman | ARS, Bovine Functional Genomics Lab |
| Tom Burdon | Roslin Institute, Scotland |
| Luiz Sérgio Camargo | EMBRAPA, Brazil |
| Jeff Carroll | ARS, Livestock Issues Research Unit |
| Robert Godke | Louisiana State University |
| David Kerr | University of Vermont |
| Harris Lewin | University of Illinois |
| Ian Mather | University of Maryland |
| David R. McCoy | Chr. Hansen, Inc |
| Duane Norman | ARS, Animal Improvement Programs |

| | |
|---|---|
| Max Paape | ARS, Bovine Functional Genomics Lab |
| Belen Pintado | Dpto de Reproducción Animal y C.R.Z, Madrid |
| David Smith | Moredun Institute, Scotland |
| George Somkuti | ARS, Dairy Processing and Products Research Unit |
| David A. Stringfellow | Arburn University |
| Diane VanHekken | ARS, Dairy Processing and Products Research Unit |
| D N Rao Veeramachaneni | Colorado State University |
| Kevin Wells | Revivicor, Inc. (formally PPL Theraputics) |
| Jerry Yang | University of Connecticut |

## NEIL C. TALBOT, RESEARCH ANIMAL SCIENTIST

### Summary of Research

Dr. Talbot contributes 100% to CRIS #1265-31000-080-00D (Objective 3 – Improving efficiency of nuclear transfer) under ARS National Program 101. Dr. Talbot plans, develops, and conducts research on cell-based methods for targeted genetic modification of farm animals and proteomic analysis of nuclear cloned cattle embryos. The overall objective of the research is to improve the efficiency of the somatic cell nuclear transfer (NT) technique. The first goal of the research is to understand better the normal protein expression of early bovine embryos and to find protein markers of successful NT reprogramming that can be used to select competent embryos prior to embryo transfer. This may also allow the rapid evaluation of the various NT methods used to create NT embryos (the only method currently available being the rate of NT calves born). This objective is being accomplished through the comparative proteomic analysis of proteins expressed by IVP or NT embryo-derived cell lines that model placental tissue. The second goal is to establish new cell lines that may be more efficient as nuclear donor cells (i.e., be more easily reprogrammed) by virtue of having "stem cell" properties. The cell lines being established for the first goal may fulfill this second goal as they are early embryo-derived cell lines, and, in addition, efforts are continuing in the establishment of bovine embryonic stem (ES) cell lines.

### Publications (2000-present)

**Talbot, N.C**., Caperna, T.J., Edwards, J.L., Garrett, W., Wells, K.D. and Ealy, A.D. 2000. Bovine blastocyst-derived trophectoderm and endoderm cell cultures: Interferon-tau and transferrin expression as respective in vitro markers. Biology of Reproduction 62: 235-247.

**Talbot, N.C.**, Powell, A.M., Garrett, W., Edwards, J.L., and Rexroad, C.E., Jr. 2000. Ultrastructural and karyotypic examination of in vitro produced bovine embryos developed in the sheep uterus. Tissue and Cell 32: 9-27.

**Talbot, N.C**. and Garrett, W.M. 2001. Ultrastructure of the embryonic stem cells of the 8-day pig blastocyst before and after in vitro manipulation: development of junctional apparatus and the lethal effects of PBS mediated cell-cell dissociation. Anatomical Record 264: 101-113.

**Talbot, N.C.**, Caperna, T.J., Wells, K.D. 2002. The PICM-19 Cell Line as an in vitro Model of Liver Bile Ductules: Effects of cAMP Inducers, Biopeptides and pH. Cells Tissues Organs. 171: 99-116.

**Talbot, N.C**., Garrett, W.M., Powell, A.M. 2002. Spontaneous differentiation of porcine and bovine embryonic stem cell (epiblast) into astrocytes or neurons. In Vitro Cellular and Developmental Biology 38: 191-197.

**Talbot, N.C.**, Garrett, W.M., Caperna, T.J. 2003. Analysis of the Expression of Aquaporin-1 and Aquaporin-9 in Pig Liver Tissue: Comparison with Rat Liver Tissue. Cells Tissues Organs 174: 117-128.

Powell, A.M., **Talbot, N.C.**, Wells, K.D., Kerr, D.E., Pursel, V. G. and Wall, R.J. 2004. Cell Donor Influences Success of Producing Cattle by Somatic Cell Nuclear Transfer. Biology of Reproduction 71: 210-216.

**Talbot, N.C**., Powell, A.M., and Caperna, T.J. 2004. Comparison of colony-formation efficiency of bovine fetal fibroblast cell lines cultured with low oxygen, hydrocortisone, L-carnosine, bFGF, or different levels of FBS. Cloning and Stem Cells 6: 35-45.

**Talbot, N.C.**, Caperna, T.J., Powell, A.M., Garrett, W.M. and Ealy, A.D. 2004. Isolation and characterization of a bovine trophectoderm cell line derived from a parthenogenetic blastocyst. Molecular Reproduction and Development 69: 164-173.

Sohn, E.J., Paape, M.J., Peters, R.P., Fetterer, R.H., **Talbot, N.C**., Bannerman, D.D. 2004. The production and characterization of anti-bovine CD14 monoclonal antibodies. Veterinary Research 35: 597-608.

**Talbot, N.C**., Paape, M., Sohn, E.J., Garrett, W.M. 2004. Macrophage population dynamics within fetal mouse fibroblast cultures derived from C57BL/6, CD-1, CF-1 mice and interleukin-6 and granulocyte colony stimulating factor knockout mice. In Vitro Cell. Dev. Biol. 40: 196-210.

| **Cooperator** | **Affiliation** |
|---|---|
| Tad Sonstegard | ARS, Bovine Functional Genomics Lab |
| Tom Caperna | ARS, Growth Biology Lab |
| Alan Ealy | University of Florida, Gainesville, FL |
| Carol Keefer | University of MD, College Park, MD |
| Jean-Paul Renard | INRA, Jouy en Josas, France |

# H. DAVID GUTHRIE, RESEARCH PHYSIOLOGIST

## Summary of Research

Dr. Guthrie contributes 100% to the Food Animal CRIS # 1265-31000-082. The overall goal of this research is to improve sperm tolerance to frozen and hypothermic low temperature storage to increase the efficiency of reproduction and to increase the rate of genetic improvement. One current objective of the research is to develop methods to quantitatively measure physiological activity and functionality of boar spermatozoa, in terms of absolute change and the proportion of the cell population. These methods will be used to determine the effects of hypothermic liquid and frozen storage of sperm on characteristics that are critical for fertility and viability and to monitor progress the development of new techniques. A number of these measurements may be utilized to develop an index to predict the survival and fertility of sperm subjected to long term hypothermic storage and cryopreservation. Another objective of the research is the improvement cryopreservation protocols for boar semen. This will require development and validation of fluorescence stopped-flow spectroscopy which measures minute changes in cell volume occur in micro-seconds as the external and intracellular environment of the cell change during addition and dilution of cryoprotectants, cooling, freezing, and thawing. These volume changes can be used mathematically to derive water and cryoprotectant permeability coefficients associated with temperature change and specific cell types dictated by their physiology and genetics. These parameters will be utilized to mathematically model and optimize the cryoprotectant type and concentration, cryoprotectant addition and dilution rates, and cooling and warming rates. The new cryopreservation protocols will be evaluated in the laboratory by measuring physiological and functional activity and confirmed in the field by breeding trials in collaboration with boar studs or genetics companies.

## Publications (2000-present)

Garmey, J.C., **Guthrie, H.D.**, Garrett, W.M., Stoler, M.H., Veldhuis, J.D. 2000. Localization and expression of low-density lipoprotein receptor, steroidogenic acute regulatory protein, cytochrome P450 side-chain cleavage and P450 17-alpha hydroxylase/C17-20 lyase in developing swine follicles: in situ molecular hybridization and immunocytochemical studies. Mol Cell Endocrinol. 170, 57-65. (Peer reviewed journal paper)

Garrett, W.M., Mack, S.O., Rohan, R.M., **Guthrie, H.D**. 2000. In situ analysis of the changes in expression of pig ovarian inhibin subunit mRNAs during the early luteal phase. J Reprod Fert. 118, 235-242. (Peer reviewed journal paper)

**Guthrie, H.D.**, Garrett, W.M. 2000. Changes in porcine oocyte germinal vesicle development as follicles approach preovulatory maturity. Theriogenology 54, 389-399. (Peer reviewed journal paper)

**Guthrie, H.D.**, Garrett, W.M., Cooper, B.S. 2000. Inhibition of apoptosis in cultured porcine granulosa cells by inhibitors of caspase and serine protease activity. Theriogenology 54, 731-740. (Peer reviewed journal paper)

Johnson, L.A., Dobrinsky, J.R., **Guthrie, H.D.**, Welch, G.R. 2000. Sex selection in swine: flow cytometric sorting: of X- and Y-chromosome bearing sperm to produce offspring. in swine. In Boar Semen Preservation IV. (Johnson, L. A. and Guthrie, H. D., eds.) Allen Press, Inc., Lawrence, KS pp107-114. (Review paper)

Mack, S.O., Garrett, W.M., **Guthrie, H.D**. 2000. Absence of correlation between in situ expression of cytochrome P450 17alpha hydroxylase/lyase and 3beta-hydroxysteroid dehydrogenase/(Delta5-4) isomerase messenger ribonucleic acids and steroidogenesis during pubertal development in the rat testis. J Steroid Biochem Mol Biol. 73:19-28. (Peer reviewed journal paper)

**Guthrie, H.D.**, Garrett, W.M. 2001. Apoptosis During Folliculogenesis in Pigs. Reproduction (suppl. 58):17-29 (review paper)

**Guthrie, H.D.**, Johnson, L.A., Garrett, W.M., Welch, G.R., Dobrinsky, J.R. 2002. Flow cytometric sperm sorting: effects of varying laser power on embryo development in swine. Mol Reprod Dev. 61:87-92. (Peer reviewed journal paper)

**Guthrie, H.D.**, Liu, J., Critser, J.K. 2002. Osmotic tolerance limits and effects of cryoprotectants on motility of bovine spermatozoa. Biol Reprod. 67:1811-1816. (Peer reviewed journal paper)

Zhu, J., King, T., Dobrinsky, J., Harkness, L., Ferrier, T., Bosma, W., Schreier, L.L., **Guthrie, H.D.**, DeSousa, P., Wilmut, I. 2003. In vitro and in vivo developmental competence of ovulated and in vitro matured porcine oocytes activated by electrical activation. Cloning Stem Cells 5:355-365. (Peer reviewed journal paper)

De Young, R.W., Muller, L. I., Demarais, S., **Guthrie, H.D.**, Welch, G.R., and Engelken, T.J., Gonzales, R.A. 2004. Do Odocoileus virginianus males produce Y-chromosome-biased ejaculates? Implications for adaptive sex ratio theories. J Mammal 85:768-773. (Peer reviewed journal paper)

Kovtun, I.V., Welch, G., **Guthrie, H.D.**, Hafner, K.L., McMurray, C.T. 2004. CAG repeat lengths in X- and Y-bearing sperm indicate that gender bias during transmission of Huntington's disease gene is determined in the embryo. J Biol Chem. 279: 9389-9391. (Peer reviewed journal paper)

**Guthrie, H.D.**, Welch, G.R. 2005. Impact of extender and cryopreservation on membrane and morphological integrity in boar sperm. Theriogenology 63: 396-410. (Review paper)

**Guthrie, H.D.**, Wall, R..J, Pursel, V.G., Foster-Frey, J.A., Donovan, D.M., Dawson, H.D., Welch, G.R., Garrett, W.G. 2005. Follicular expression of a human β-cell leukaemia/lymphoma-2 (*Bcl-2*) transgene does not decrease atresia or increase ovulation rate in swine. Reprod. Fert. Dev. 17: 457–466. (peer reviewed journal paper)

**Guthrie, H.D.**, Welch G.R. 2005. Effects of hypothermic liquid storage and cryopreservation on basal and induced membrane phospholipid disorder and acrosome exocytosis in boar spermatozoa. Reprod. Fert. Dev. 17: 467–477. (peer reviewed journal paper)

| **Cooperator** | **Affiliation** |
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| Bakst, M. R. | USDA/ARS BARC, BGL |
| Critser, J. K | University of Missouri |
| Dawson, H. D. | USDA/ARS BARC, BHNRC |
| Demarais, S. | Mississippi State University |
| De Young, R. W. | Mississippi State University |
| Donovan, D. D. | USDA/ARS BARC, BGL |
| Ford, J. J. | USDA/ARS U. S. MARC |
| Johnson, L. A. | Retired collaborator |
| Long, J. A. | USDA/ARS BARC, BGL |
| Mack, S. O. | Howard University |
| Marshall, C. E. | Select Sires Inc. |
| McMurray, C. T. | Mayo Clinic and Foundation |
| Muller, L. I. | University of Tennessee |
| Pursel, V. G. | Retired collaborator |
| Veldhuis, J. D. | University of Virginia |
| Wall, R. J. | USDA/ARS BARC, BGL |

**J. R. Dobrinsky publications not already included with Guthrie or Zuelke (2000-present) --Which support the objectives of CRIS 1265-31000-082**

**Dobrinsky, J.R.**, Pursel, V.G., Long, C.R., Johnson, L.A. 2000. Birth of piglets after transfer of embryos cryopreserved by cytoskeletal stabilization and vitrification. Biol Reprod. 62:564-570. (peer reviewed journal paper)

King, T.J., **Dobrinsky, J.R.**, Bracken, J., McCorquodale, C., Wilmut, I. 2001. Ovulated pig oocyte production using transcutaneous ultrasonography to determine ovulation time. Vet Rec. 149:362-363. (peer reviewed journal paper)

**Dobrinsky, J.R**. 2001. Cryopreservation of pig embryos: adaptation of vitrification technology for embryo transfer. Reprod Suppl. 58:325-33. (Review).

**Dobrinsky, J.R**. 2001. Cryopreservation of swine embryos: a chilly past with a vitrifying future. Theriogenology. Nov 1;56(8):1333-44. (Review).

**Dobrinsky, J.R**. 2002. Advancements in cryopreservation of domestic animal embryos. Theriogenology. Jan 1;57(1):285-302. (Review).

Zhu, J., Telfer, E.E., Fletcher, J., Springbett, A., **Dobrinsky, J.R.**, De Sousa, P.A., Wilmut, I. 2002. Improvement of an electrical activation protocol for porcine oocytes. Biol Reprod. 66:635-641. (peer reviewed journal paper)

De Sousa, P.A., **Dobrinsky, J.R.**, Zhu, J., Archibald, A.L., Ainslie, A., Bosma, W., Bowering, J., Bracken, J., Ferrier, P.M., Fletcher, J., Gasparrini, B., Harkness, L., Johnston, P., Ritchie, M., Ritchie, W.A., Travers, A., Albertini, D., Dinnyes, A., King, T.J., Wilmut, I. 2002. Somatic cell nuclear transfer in the pig: control of pronuclear formation and integration with improved methods for activation and maintenance of pregnancy. Biol Reprod. 66:642-650. (peer reviewed journal paper)

King, T.J., **Dobrinsky, J.R.**, Zhu, J., Finlayson, H.A., Bosma, W., Harkness, L., Ritchie, W.A., Travers, A., McCorquodale, C., Day, B.N., Dinnyes, A., De Sousa, P.A., Wilmut, I. 2002. Embryo development and establishment of pregnancy after embryo transfer in pigs: coping with limitations in the availability of viable embryos. Reproduction 123:507-515. (peer reviewed journal paper)

Nedambale, T.L., Dinnyes, A., Groen, W., **Dobrinsky, J.R.**, Tian, X.C., Yang, X. 2004. Comparison on in vitro fertilized bovine embryos cultured in KSOM or SOF and cryopreserved by slow freezing or vitrification. Theriogenology 62:437-449. (peer reviewed journal paper)

Gerrits, R.J., Lunney, J.K., Johnson, L.A., Pursel, V.G., Kraeling, R.R., Rohrer, G.A., **Dobrinsky, J.R**. 2005. Perspectives for artificial insemination and genomics to improve global swine populations. Theriogenology. 63:283-299. (review)

Johnson, L.A., Rath, D., Vazquez, J.M., Maxwell, W.M., **Dobrinsky, J.R**. 2005. Preselection of sex of offspring in swine for production: current status of the process and its application. Theriogenology 63:615-624. (review)

## MURRAY R. BAKST, RESEARCH PHYSIOLOGIST

### Summary of Research

Dr. Bakst contributes 100% as the lead scientist to CRIS Project 1265-31000-083-00D *Analysis of Sperm Storage Mechanisms in Poultry* that is covered under National Program 101, Food Animal Production. The broad scope of this research is to develop improved and novel approaches that enhance the reproductive efficiency in poultry. More specifically, Dr. Bakst's research examines the cellular and molecular mechanisms regulating oviductal sperm selection and transport and oviductal sperm storage and sustained fertility. A combination of techniques are used including sperm labeling (fluorescent stains) to locate sperm in the oviduct and on the ovum and various modes of microscopy and immunocytochemistry/histochemisty to discern cells and their molecular characteristics. An additional research objective is a collaboration to develop procedures for the successful transfer of genetically modified spermatogonia (testicular stem cells) to recipient male poultry. This approach includes: the isolation of spermatogonia; their culture and propagation; their transfection with gene(s) of interest; the transfer of donor spermatogonia to recipient testes devoid of germ cells; and, assessment of resulting recipient's sperm and progeny to determine whether the germ-cell transfer was successful. This work has been approved by our NPL and is part of a proposed CRADA currently under development. Other research includes continuing collaborations determining the impact of egg handling and storage procedures used by commercial hatcheries on embryo development. The overall research goal is to improve the management strategies and reproductive efficiency of poultry through decision making by industry based on fundamental and applied research.

### Publications (2001-Present)

**Bakst, M.** 1999. Impact of egg storage on embryo development. Avian Poult Sci Rev 13: 125-131, 2002. Special Issue: Proceedings - Internat. Symp. Avian Fertility and Hatchability,Tours, France, September 22-24.

Freedman, S., Akuffo,V. and **Bakst, M.R**. 2001. Evidence for the innervation of sperm-storage tubules in the oviduct of the turkey (*Meleagris Gallopavo*). Reproduction 121:809-814.

**Bakst, M.R.** 2001. Oviducal sperm and fertilisation in poultry. In: Perspectives in Fertilisation and Embryonic Development in Poultry, Proceedings from the meeting of the Incubation & Fertility Research Group, Oxford, England, September 2001. Ratite Conference Books, Oxford, UK. pp. 1-6.

King, L.M., Brillard, J.P., Garrett,W., **Bakst, M.R.** and Donoghue, A.M. 2002. Segregation of spermatozoa within sperm storage tubules of fowl and turkey hens. Reproduction 123:79-86.

**Bakst, M.R**. 2002. Oviducal Sperm storage in turkeys: The infundibulum as a secondary sperm storage site, Or is it? IN: The New Panorama of Animal Evolution Proceedings XVIII International Congress of Zoology, editors: A. Legakis, S Sfenthourakis, R. Polymeni and M. Thessalou-Legaki, Athens, Greece, pp. 447-450.

McGary, S., Estevez, I., **Bakst, M.R**. and Pollock, D. 2002. Phenotypic traits as reliable indicators of fertility in male broiler breeders, Poult. Sci. 81:102-111.

**Bakst, M.R.** and Vinyard, B.T. 2002. Oviducal sperm storage in turkeys: spatial distribution of sperm within the uterovaginal junction sperm-storage tubules. J. Exp. Zool. 292: 205-209.

Dupuy,V., Nersessian, B. and **Bakst, M.R.** 2002. Embryonic development from first cleavage through seventy-two hours incubation in two strains of Pekin duck (*Anas Platyrhynchos*) Poult. Sci. 81:860-868.

**Bakst, M.R.** 2002. How to maximize fertility and early embryonic development from a biologist's point of view. Proceedings of the 25th Technical Turkey Conference, Turkeys 50:65-68.

**Bakst, M.R**., McGary, S. Estevez, I., and Knapp, T. Using nonsettable eggs to evaluate hen fertility. J. Appl. Poult. Res. 11:402-405, 2002.

**Bakst, M.R** and Holm, L. 2003. Impact of cold storage on carbonic anhydrase activity in the early turkey embryo. Poult. Sci. 82:1193-1197.

McGary, S., Estevez, I., and **Bakst, M.R**. 2003. Potential relationships between physical traits and male broiler breeder fertility. Poult. Sci. 82:328-337.

**Bakst, M.R.** 2004. Sperm Storage in the Hen's Oviduct. Feedinfo News Service http://www.feedinfo.com/console/PageViewer.aspx?page=76347, (Trade publication)

Zaniboni, L. and **Bakst, M. R.** 2004. Localization of aquaporins in the sperm storage tubules in the turkey oviduct. Poult. Sci. 83:1209-1212.

Zaniboni, L., Akuffo, V. and **Bakst, M.R**. 2004. Aquaporins are observed in the duct epithelia of the epididymal region of the Large White turkey. Poult. Sci. 83:1917-1920.

**Bakst, M.R**. and Long, J.A. 2004. Oviducal sperm storage: model for in vitro sperm storage. Proceedings – XXII World's Poultry Congress, Istambul, Turkey, June 8-13, 2004, *CD – "Full-text and Participant List"*; Category, M-3-Poultry Reproductive Physiology.

Noirault, J., Brillard, J.P. and **Bakst, M.R.** Spermatogenesis in the turkey (*Meleagris gallopavo*): quantitative approach in immature and adult males subjected to various photoperiods. Theriogenology (in press, accepted February 2005).

Noirault, J., Brillard, J.P. and **Bakst, M.R**. Effect of various photoperiods on testicular weight, weekly sperm output and plasma levels of LH and testosterone over the reproductive season in male turkeys. Theriogenology (*revised and resubmitted March 2005)*

Chalah, T., Lacroix, F., and **Bakst, M.R**. Viability, morphology, and ultrastructure of Houbara bustard (*Chlamydotis undalata undalata*) sperm. Reproduction (*submitted December 2004*)

| **Cooperator** | **Affiliation** |
|---|---|
| J.P. Brillard | INRA-Tours, France |
| B. Nesessian | Maple Leaf Duck Farms, Indiana |
| D. Pollock | Perdue Farms, Inc., Maryland |
| I. Estevez | University of Maryland-College Park |
| T. Knapp | Pilgrim's Pride, Pennsylvania |
| S. Cerolini | University of Milan, Italy |
| L. Holm | Swedish University of Agriculture |
| N. Anthony | University of Arkansas |
| J. Balog | ARS-Fayetteville |
| G. Fasenko | University of Alberta, Canada |
| S. Pruett-Jones | University of Chicago |

**Summary of Research**

Dr. Long contributes 100% of her time as a research animal scientist to CRIS Project 1265-31000-083-00D, Analysis of Sperm Storage Mechanisms in Poultry, which falls under National Program 101, Food Animal Production. The broad scope of the research is to increase the efficiency of reproduction and germplasm preservation for poultry by improving in vitro semen storage procedures. One objective of this research is to determine how and when poultry sperm lose functional competence during in vitro storage, and use this knowledge as a basis for developing successful sperm storage methods in vitro. For the turkey industry, the emphasis is focused on short-term storage intervals of 24-48h; whereas the chicken industry would benefit more from long-term cryogenic semen storage. The systematic approach for achieving the research goals includes: 1) characterizing changes in sperm biochemistry that occur during in vitro storage, including the degree of lipid peroxidation, changes in membrane glycoprotein and phospholipids content, mitochondrial metabolism, and alterations in cytoskeletal proteins; 2) evaluating the effects of altered sperm biochemistry on sperm function in vitro, including the ability to undergo the acrosome reaction, sperm motility parameters, and binding/penetration of the ovum's perivitelline layer; 3) evaluating the effects of altered sperm biochemistry on sperm function in vivo, including sperm transport to and maintenance in the hen's sperm storage tubules and fertility during the egg production period; and 4) developing strategies to overcome the identified deficiencies in the functionality of stored sperm.

**Publications (2001-present)**

**Long, J.A.**, Kramer, M.H. 2003. Effect of vitamin E on lipid peroxidation and fertility after artificial insemination with liquid-stored turkey sperm. J. Poultry Sci. 82:1802-1807.

Long, E., Sonstegard, T.S., **Long, J.A.**, Van Tassell, C.P., Zuelke, K.A. 2003. Serial analysis of gene expression in turkey sperm storage tubules in the presence and absence of resident sperm. Biol. Reprod. 69:469-474.

Bakst, M.R., **Long, J.A.**, Zuelke, K.A. 2004. Sperm storage in the hen's oviduct. Feedinfo News Service www.feedinfo.com (electronic only).

Bakst, M.R., **Long, J.A.** 2004. Oviducal sperm storage: model for in vitro sperm storage. Proceedings – XXII World's Poultry Congress, Istambul, Turkey, June 8-13.

Donoghue, A.M., Blanco, J.M., **Long, J.A.**, Wildt, D.E., Gee, G. 2004. Comparative semen cryopreservation of poultry and non-domestic avian species: characterization of sperm freeze damage and development of applicable semen preservation procedures. Foreign Agricultural Service Technical Report.

**Long, J.A.**, Kulkarni, G. 2004. An Effective Method for Improving the Fertility of Glycerol-exposed Poultry Semen. J. Poultry Sci. 83:1594-1601.

**Long J.A**., Guthrie H.D. 2005. Validation of a Rapid, Large-Scale Assay to Quantify ATP Levels in Spermatozoa. Theriogenology; under review.

Blanco J.M., **Long J.A.**, Gee G., Wildt D.E., Donoghue A.M. 2005. Comparative Cryopreservation of Avian Spermatozoa: Benefits of Supplemental Osmoprotectants and ATP on Turkey and Crane Sperm Viability and Motility. Journal of Poultry Science; under review.

**Other Publications (2001)**

Moreland, R.B., Richardson, M.E., Lamberski, N., **Long, J.A.** 2001. Characterizing the reproductive physiology of the male southern black howler monkey, *Alouatta caraya*. J. Androl. 22:395-403.

**Long, J.A.**, Willis, M.J., Czekala, N., Gryzbowski, K., DeMatteo, K., Diebold, E.N., Gee, G. 2001. Developing artificial insemination for captive management of the cinereous vulture. Assisted Reproductive Technology for the Conservation & Genetic Management of Wildlife, P. 187-190.

Bertschinger, H.J., Asa, C.S., Calle, P.P., **Long, J.A.**, Bauman, K., DeMatteo, K., Jochle, W., Trigg, T.E., Human, 2001. A. Control of reproduction and sex related behavior in exotic wild carnivores with the GnRH analogue deslorelin: preliminary observations. J. Reprod. Fertil. Suppl. 57:275-83.

| Cooperator | Affiliation |
|---|---|
| Kramer, M.H. | ARS Biometrical Consulting Service |
| Long, E., | BGL (formerly GGPL) |
| Sonstegard, T.S. | BFGL (formerly GEML) |
| Van Tassell, C.P. | BFGL (formerly GEML) |
| Zuelke, K.A. | BGL |
| Bakst, M.R. | BGL |
| Donoghue, A.M. | PPPSR |
| Blanco, J.M. | Center for Studies on Iberian Raptors, CERI Toledo, Spain |
| Wildt, D.E. | National Zoological Park, Smithsonian Conservation & Research Center, Front Royal, VA |
| Gee, G. | Patuxent Wildlife Research Center, USGS |
| Kulkarni, G. | ADOL |
| Guthrie H.D. | BGL |

# JOHN A. PROUDMAN, RESEARCH POULTRY PHYSIOLOGIST

## Summary of Research

Dr. Proudman is Lead Scientist on, and contributes 100% to, the Food Animal Production CRIS #1265-31000-084-00D. Current and future research will be both applied and basic. The applied research will attempt to improve the reproductive efficiency of current breeder hen stocks by manipulating the lighting management to extend the reproductive season through delaying the onset of photorefractoriness. The basic research will identify cellular factors in the brain and pituitary that differ between superior layers and poor layers present in today's breeding stock. Average egg production of turkey breeder hens ranges from 55 to 100 eggs before they become unprofitable in about 24 weeks of production. However, some hens within a flock will lay 135 eggs in 24 weeks of production, indicating that existing genetic stocks have the potential for much higher egg production. Earlier research has revealed that changes within the brain and pituitary initiate incubation behavior in some hens, but not others. Research will focus on proteomic analysis of pituitaries from hens initiating and maintaining incubation behavior in comparison to superior layers that do not express this behavior.

## Publications (2001-present)

**Peer-reviewed journal publications:**

Weatherly, K. L., Ramesh, R., Strange, H., Waite, K.L., Storrie, B., **Proudman, J. A.**, and Wong, E. A. 2001. The turkey transcription factor Pit-1/GHF-1 can activate the turkey prolactin and growth hormone gene promoters *in vitro* but is not detectable in lactotrophs *in vivo*. Gen. Comp. Endocrinol. 123:244-253.

Puebla-Osorio, N., **Proudman, J. A.**, Compton, A. E., Clements, K. E., Decuypere, E., Vandesande, F., Berghman, L. R. 2002. FSH- and LH-cells originate as separate cell populations and at different embryonic stages in the chicken embryo. Gen. Comp. Endocrinol. 127:242-248.

**Proudman, J. A.**, and Siopes, T. D. 2002. Relative and absolute photorefractoriness in turkey hens: Profiles of prolactin, thyroxine, and triiodothyronine early in the reproductive cycle. Poult. Sci. 81:1218-1223.

**Proudman, J. A.** 2002. Cysteamine on turkey growth and body composition. Effects of cysteamine on growth hormone, weight gain, feed consumption, and body composition of turkeys. Meiji Univ. Agr. Res. Bul. 131:37-46.

Siopes, T. D., and **Proudman, J. A.** 2003. Photoresponsiveness of turkey breeder hens changes during the egg-laying season: Relative and absolute photorefractoriness. Poult. Sci. 82:1042-1048.

**Proudman, J. A.**, Clerens, S., van den Bergh, G., Garrett, W. M., Verhaert, P. F., Vandesande, F., and Berghman, L. R. 2003. Immunohistochemical localization of chromogranin A in gonadotrophs and somatotrophs of the turkey and chicken pituitary. Gen. Comp. Endocrinol. 132:293-303.

Paczoska-Eliasiewicz, H. E., Gertler, A., Proszkowied, M., **Proudman, J.**, Hrabia, A., Sechman, A., Mika, M., Cassy, S., Raber, N., and Rzasa, J. 2003. Leptin affects ovarian function in fasted hens (*Gallus domesticus*). Reproduction 126:739-751.

Kuenzel, W. J., Abdel-Maksoud, M. M., Elsasser, T., and **Proudman, J. A.** 2004. Sulfamethazine advances puberty in male chicks by effecting a rapid increase in gonadotropins. Comp. Biochem. Physiol. Part A 137:349-355.

McElroy, A. P., Caldwell, D. J., **Proudman, J. A.**, and Hargis, B. M. 2004. Modulation of in vitro DNA synthesis in the chicken ovarian granulosa cell follicular hierarchy by follicle-stimulating hormone and luteinizing hormone. Poult. Sci. 83:500-506.

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**Invited Book Chapters:**

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| **Cooperator** | **Affiliation** |
|---|---|
| Berghman, L.R. | Texas A&M University |
| Clerens, S. | Catholic University of Leuven, Belgium |
| Kirby, J.D. | University of Arkansas |
| Kuenzel, W.J. | University of Arkansas |
| Paczoska-Eliasiewicz, H. E. | Crakow Agricultural University, Poland |
| Ramesh, R. | Pennsylvania State University |
| Robinson, F.E. | University of Alberta, Canada |
| Scanes, C.G. | Mississippi State University |
| Siopes, T.D. | North Carolina State University |

**KURT A. ZUELKE, DVM, Ph.D.**
**SUPERVISORY RESEARCH PHYSIOLOGIST (ANIMAL)**

**Leadership:** Dr. Zuelke became Research Leader of GGPL in April 2001 with a vision to leverage GGPL's existing strengths in animal germplasm biology and reproductive physiology by integrating biomedical advances in functional genomics and proteomics into the lab's research mission. BGL's current research portfolio was composed through realigning former GEML transgenic animal and embryo biotechnology research programs with GGPL's germplasm biology expertise in 2003. BGL now boasts multi-user, high throughput functional genomics and proteomics labs in addition to world-class embryo technology, cell biology, cryobiology, flow cytometry and cell sorting, and confocal microscopy lab facilities. In partnership with three other research units, BGL has established a joint mass spectroscopy and proteomics lab to facilitate agricultural proteomics research across BARC. BGL is poised to be a world leader pioneering and developing novel applications of functional genomics, proteomics and biotechnology research tools in agricultural animal germplasm conservation research.

**Personal Research:** Dr. Zuelke conducts primary research in two projects: 65% (Lead Scientist) to Swine Germplasm Preservation, Propagation, and Embryo Developmental Competence (1265-31000-082-00D) and 10% Analysis of Sperm Storage Mechanisms in Poultry (1265-31000-083-00D). The balance of Dr. Zuelke's research effort is split 5% each to Identification and Manipulation of Genetic Factors to Enhance Disease Resistance in Dairy Cattle (1265-31000-080-00D) and 20% Proteomic Analysis of Factors Regulating Egg Production in Hens (1265-31000-084-00D) to provide comparative physiology and molecular biology advice.

Research objectives are to use Serial Analysis of Gene Expression (SAGE) and proteomics methods to increase basic knowledge of porcine embryo development and poultry sperm selection and storage mechanisms. BGL is one of the first labs in the world to apply SAGE to animal germplasm research. This work has identified several hundred differentially expressed genes during critical stages of porcine embryo development or prolonged sperm storage in turkeys. Future research will characterize the potential functional significance of these differentially expressed genes and ultimately lead to developing new methods to decrease porcine embryo mortality and improve liquid and cryogenic storage of poultry semen.

**Publications (2000 – present)**

Lewis, I.M., McClintock, A.E., French, A.J., **Zuelke, K.A**., Harford, B.A. and Trounson, A.O. 2000. Cloning and transgenesis in farm animals – an Australian perspective. Aust. Vet. J 78:694-697.

**Zuelke K.A**., Blomberg L.A., Long E.L., Dobrinsky J.R., Van Tassell C.P., and Sonstegard T.S. 2002. Serial analysis of gene expression (SAGE) in preimplantation stage swine embryos. IN: Biological Resource Management in Agriculture: Mammalian Embryo Genomics. Ed, Andrew Watson, OECD Publications, Paris, France, pp 47-55.

**Zuelke, K.A**., Jeffay, S.C., Zucker, R.M., and Perreault, S.D. 2003. Glutathione (GSH) concentrations vary with the cell cycle in maturing hamster oocytes, zygotes and pre-implantation stage embryos. Mol. Reprod. Dev. 64:106-112.

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Blomberg, L., and **Zuelke, K.A.** 2004. Serial analysis of gene expression (SAGE) during porcine embryo development. Reprod. Fertil. and Devel. 16:87-92 (review).

**Zuelke K.A**., and Blomberg LA. 2004. Serial analysis of gene expression (SAGE) during porcine embryo elongation. International Embryo Transfer Society Newsletter 22(3):10-14 (short communication).

Lewis I.M., French, A.J., Tecirlioglu, R.T., Vajta, G., McClintock, A.E., Nicholas, K.R., **Zuelke, K.A**., Holland, M.K., and Trounson, A.O. 2004. Commercial aspects of cloning and genetic modification in cattle. Aust J of Exp Agriculture 44:1105-1111.

Blomberg, L., Long, E.L., Sonstegard, T.S., Van Tassel, C.P., Dobrinsky, J.R., and **Zuelke, K.A.** 2005. Serial Analysis of Gene Expression (SAGE) During Elongation of the Peri-Implantation Porcine Trophectoderm (Conceptus). Physiol. Genomics 20(2):188-94. (E-pub Nov 09, 2004).

Blomberg, L., and **Zuelke, K.A.** 2005. Expression Analysis of the Steroidogenic Acute Regulatory Protein (STAR) Gene in Developing Porcine Conceptus. Mol Reprod Dev (submitted).

Agency Abbreviations

| | |
|---|---|
| AAD | ASSOCIATE AREA DIRECTOR |
| AAO | AREA ADMINISTRATIVE OFFICER |
| ABFO | AREA BUDGET & FISCAL OFFICER |
| AC | ADMINISTRATORS COUNCIL |
| ACS | AREA COMPUTER SPECIALIST |
| AD | AREA DIRECTOR OR AGRICULTURE DEPARTMENT; E.G., AD-332 |
| ADA | ASSOCIATE DEPUTY ADMINISTRATOR |
| ADO | AUTHORIZED DEPARTMENTAL OFFICER |
| ADODR | AUTHORIZED DEPARTMENT OFFICER'S DESIGNATED REPRESENTATIVE |
| ADOL | AVIAN DISEASE & ONCOLOGY LABORATORY |
| ADP | AUTOMATED DATA PROCESSING |
| AE | ARCHITECTURAL ENGINEER |
| AES | AGRICULTURAL EXPERIMENT STATION |
| AFM | ADMINISTRATIVE AND FINANCIAL MANAGEMENT |
| APHIS | ANIMAL PLANT HEALTH INSPECTION SERVICE |
| APP | ANNUAL PERFORMANCE PLANNING |
| ARIS | AGRICULTURAL RESEARCH INFORMATION SYSTEM |
| ARMPS | ANNUAL RESEARCH MANAGEMENT PLANNING SYSTEM |
| ARS | AGRICULTURAL RESEARCH SERVICE |
| ARSITS | AGRICULTURAL RESEARCH SERVICE INVENTION TRACKING SYSTEM |
| ASST AD | ASSISTANT AREA DIRECTOR |
| B&F | BUDGET & FISCAL |
| BA | BELTSVILLE AREA |
| BARC | BELTSVILLE AGRICULTURAL RESEARCH AND DEVELOPMENT |
| BARD | BINATIONAL AGRICULTURAL RESEARCH AND DEVELOPMENT |
| BPA | BLANKET PURCHASE AGREEMENT |
| BPMS | BUDGET PROGRAM MANAGEMENT STAFF |
| BRDC | BIOTECHNOLOGY RESEARCH & DEVELOPMENT CORPORATION |
| CAD | CONTRACTING & ASSISTANCE DIVISION |
| CAT | CATEGORY |
| CD | CENTER DIRECTOR |
| CEPS | CLUSTER ENVIRONMENTAL PROTECTION SPECIALIST |
| CFC | COMBINED FEDERAL CAMPAIGN |
| CNRC | CHILDREN'S NUTRITION RESEARCH CENTER |
| COB | CLOSE OF BUSINESS |
| CR | CIVIL RIGHTS |
| CRADA | COOPERATIVE RESEARCH AND DEVELOPMENT AGREEMENT |
| CRAS | CRIS RESOURCE ALLOCATION SCHEDULE |
| CRIS | CURRENT RESEARCH INFORMATION SYSTEM |
| CS | CONTRACT SPECIALIST |
| CSREES | COOPERATIVE STATE RESEARCH EDUCATION EXTENSION SERVICE |

| | |
|---|---|
| CSRS | CIVIL SERVICE RETIREMENT SYSTEM |
| CTAP | CAREER TRANSITION ASSISTANCE PROGRAM |
| CWU | CRIS WORK UNIT |
| CY | CALENDAR YEAR |
| DA | DEPUTY ADMINISTRATOR |
| DAD | DEPUTY AREA DIRECTOR |
| DAEA | DESIGNATED AREA ETHICS ADVISOR |
| DE | DATA ENTRY |
| EAP | EMPLOYEE ASSISTANCE PROGRAM |
| EEAC | EQUAL EMPLOYMENT ADVISORY COUNCIL |
| EEO | EQUAL EMPLOYMENT OPPORTUNITY |
| EOD | ENTER ON DUTY |
| EPF | EMPLOYEE PERFORMANCE FOLDER |
| ERB | EMPLOYEE RELATIONS BRANCH |
| ERRC | EASTERN REGIONAL RESEARCH CENTER |
| ERS | ECONOMIC RESEARCH SERVICE |
| FAS | FOREIGN AGRICULTURAL SERVICE |
| FEGLI | FEDERAL EMPLOYEE'S GROUP LIFE INSURANCE |
| FEHB | FEDERAL EMPLOYEE'S HEALTH BENEFITS |
| FERS | FEDERAL EMPLOYEE'S RETIREMENT SYSTEM |
| FMD | FINANCIAL MANAGEMENT DIVISION |
| FOIA | FREEDOM OF INFORMATION ACT |
| FPL | FULL PERFORMANCE LEVEL |
| FTE | FULL TIME EQUIVALENT |
| FY | FISCAL YEAR |
| FYI | FOR YOUR INFORMATION |
| GBL | GOVERNMENT BILL OF LADING |
| GCP | GRADE/CATEGORY PROBLEM |
| GOV | GOVERNMENT OWNED VEHICLE |
| GPO | GOVERNMENT PRINTING OFFICE |
| GPRA | GOVERNMENT PERFORMANCE & RESULTS ACT |
| GS | GENERAL SCHEDULE |
| GSA | GENERAL SERVICES ADMINISTRATION |
| HNRC | HUMAN NUTRITION RESEARCH CENTER |
| HPRL | HIGH PRIORITY REQUIREMENTS LIST |
| HQ | HEADQUARTERS |
| HRD | HUMAN RESOURCES DIVISION |
| IDP | INDIVIDUAL DEVELOPMENT PLAN |
| IR | INVENTION REPORT |
| IRC | INDIRECT RESEARCH COSTS |
| IS | INFORMATION STAFF |
| LAO | LOCATION ADMINISTRATIVE OFFICER |
| LC | LOCATION COORDINATOR |
| LD | LABORATORY DIRECTOR |
| LOTS | LOCATION OBLIGATION TRACKING SYSTEM |
| LS | LEAD SCIENTIST |
| LWOP | LEAVE WITH OUT PAY |
| MAP | MODERNIZATION OF ADMINISTRATIVE PROCESS |

| | |
|---|---|
| MARC | MEAT ANIMAL RESEARCH CENTER |
| MSA | MID-SOUTH AREA |
| MU | MANAGEMENT UNIT |
| MWA | MIDWEST AREA |
| NAA | NORTH ATLANTIC AREA |
| NADC | NATIONAL ANIMAL DISEASE CENTER |
| NAL | NATIONAL AGRICULTURAL LIBRARY |
| NAS | NATIONAL AGRICULTURAL STATISTICS SERVICE |
| NCAUR | NATIONAL CENTER FOR AGRICULTURAL UTILIZATION RESEARCH |
| NCRPIS | NORTH CENTRAL REGIONAL PLANT INTRODUCTION STATION |
| NFC | NATIONAL FINANCE CENTER |
| NFMP | NATIONAL FACILITIES MANAGEMENT PLAN |
| NPA | NORTHERN PLAINS AREA |
| NPL | NATIONAL PROGRAM LEADER |
| NPPC | NATIONAL PATENT PROGRAM COORDINATOR |
| NPS | NATIONAL PROGRAM STAFF |
| NSAC | NATIONAL SECRETARIAL ADVISORY COUNCIL |
| NSRC | NATIONAL SWINE RESEARCH |
| NSRC | NATIONAL SWINE RESEARCH CENTER |
| NSTL | NATIONAL SOIL TILTH LABORATORY |
| NTE | NOT TO EXCEED |
| OA | OFFICE OF THE ADMINISTRATOR |
| OCI | OFFICE OF COOPERATIVE INTERACTIONS |
| OGC | OFFICE OF THE GENERAL COUNSEL |
| OGE | OFFICE OF GOVERNMENT ETHICS |
| OICD | OFFICE INTERNATIONAL COOPERATION & DEVELOPMENT |
| OIG | OFFICE OF INSPECTOR GENERAL |
| OIRP | OFFICE OF INTERNATIONAL RESEARCH PROGRAMS |
| OPM | OFFICE OF PERSONNEL MANAGEMENT |
| OSQR | OFFICE OF SCIENTIFIC QUALITY REVIEW |
| OTT | OFFICE OF TECHNOLOGY TRANSFER |
| OWCP | OFFICE OF WORKERS' COMPENSATION PROGRAM |
| PA | PROGRAM ANALYST |
| PAA | PROGRAM ANALYST ASSISTANT |
| PAO | PROCUREMENT ASSISTANCE OFFICER |
| PASTG | PROGRAM ADMINISTRATIVE SUPPORT TASK GROUP |
| PC | PERSONAL COMPUTER |
| PCMS | PURCHASE CARD MANAGEMENT SYSTEM |
| PD | POSITION DESCRIPTION |
| PFT | PERMANENT FULL TIME |
| PI | PRINCIPLE INVESTIGATOR |
| PIADC | PLUM ISLAND ANIMAL DISEASE CENTER |
| PIP | PERFORMANCE IMPROVEMENT PLAN |
| PM | PROGRAM MANAGEMENT |
| POV | PERSONAL OWNED VEHICLE |
| PPT | PERMANENT PART TIME |
| PSP | POSITION STAFFING PLAN |

| | |
|---|---|
| PWA | PACIFIC WEST AREA |
| QSI | QUALITY STEP INCREASE |
| R&D | RESEARCH & DEVELOPMENT |
| R&M | REPAIR AND MAINTENANCE |
| RAP | RESEARCH APPRENTICESHIP PROGRAM |
| RARC | RUSSELL AGRICULTURAL RESEARCH CENTER |
| REE | RESEARCH EDUCATION & ECONOMICS |
| RGEG | RESEARCH GRANDE EVALUATION GUIDE |
| RIF | REDUCTION IN FORCE |
| RIG | REMAIN IN GRADE |
| RL | RESEARCH LEADER |
| RPB | REE POLICY BRANCH |
| RPES | RESEARCH POSITION EVALUATION SYSTEM |
| RPS | RESEARCH PROJECT STATEMENT |
| RSA | RESEARCH SUPPORT AGREEMENT |
| RSB | REE SERVICES BRANCH |
| RU | RESEARCH UNIT |
| SAA | SOUTH ATLANTIC AREA |
| SAMS | SALARY ALLOCATION MANAGEMENT SYSTEM |
| SCD | SERVICE COMPUTATION DATE |
| SEC | SECRETARY |
| SES | SENIOR EXECUTIVE SERVICE |
| SEU | SPECIAL EXAMINING UNIT |
| SIP | SUMMER INTERN PROGRAM |
| SIR | STATUTORY INVENTION REGISTRATION |
| SLP | SALARY LAPSE POLICY |
| SOP | STANDARD OPERATING PROCEDURES |
| SPA | SOUTHERN PLAINS AREA |
| SRC | SHARED RESEARCH COSTS |
| SRRC | SOUTHERN REGIONAL RESEARCH CENTER |
| ST | SCIENTIFIC AND TECHNICAL POSITIONS |
| STEP | STUDENT TEMPORARY EMPLOYMENT PROGRAM |
| STP | STRATEGIC PLAN CODES |
| SY | SCIENTISTS |
| T&A | TEMPORARY FULL TIME |
| TPS | TARGET PERCENT IN SALARIES |
| TSP | THRIFT SAVINGS PLAN |
| UPG | UPGRADE |
| USDA | UNITED STATES DEPARTMENT OF AGRICULTURE |
| USDFRC | U.S. DAIRY FORAGE RESEARCH CENTER |
| WGI | WITHIN GRADE INCREASE |
| WHNRC | WESTERN HUMAN NUTRITION RESEARCH CENTER |
| WRRC | WESTERN REGIONAL RESEARCH CENTER |
| WSB | WESTERN SERVICES BRANCH |

# *BARC "CORE" Research FACILITIES*

(10/04/04)

**Bioinformatics**

8-processor Linux server that provides a number of services, including campus access to GCG, 32-processor Linux cluster running accelerated Blast algorithms and Sun Grid Engine for general computing, and a Timelogic Decypher server for hardware accelerated genomics comparison algorithms including Blast, Smith-Waterman, and Hidden Markov Models.

Contact: Curt Van Tassell, ANRI, Animal Improvement Programs Laboratory and Bovine Functional Genomics Laboratory, Bldg 200, BARC-E, Tel (301) 504-6501 curtvt@aipl.arsusda.gov

**DNA sequencing and fragment analysis**

The Bovine Functional Genomics Laboratory (Building 200, Rm 2) houses an automated 96-capillary DNA analyzer (ABI-3730). Researchers in the BA can access use of this instrument for either DNA sequence or fragment analysis (STS and AFLP marker). A minimum of 96 samples most be submitted, and access to GeneMapper analysis software is provided for determining marker genotypes.

Contact: Tad Sonstegard, ANRI, Bovine Functional Genomics Laboratory, Bldg 200, BARC-E, (301) 504-8416 tads@anri.barc.usda.gov

**Electron Microscopy Unit**

Hitachi S-4100 Scanning Electron Microscope with Oxford CT-1500 Cryostage, Hitachi S-4700 Scanning Electron Microscope with Polaron (Quorum) Polarprep 2000T Cryopreparation Chamber and Cold Stage and Hitachi H-7000 Transmission Electron Microscope with AMT 2K X 2K Digital Image Acquisition System and STEM capabilities. In addition specimen preparation equipment, including American Optical-Reichart Ultracut-Ultramicrotomes, Denton DV-502 Vacuum Evaporators, and Tousimis Samdri 780A Critical Point Dryer, RMC MF 7500 Ultrafreeze Freeze Substitution Devise, Ted Pella 345 Laboratory Microwave Fixation Devise, consultation and collaborative support is available.

Contact: Eric Erbe, PSI, Soybean Genomics and Improvement Laboratory, Bldg 465, BARC-E, Tel (301) 504-8046 erbee@ba.ars.usda.gov

**Microarrays and bioinformatics**

Cartesian Technology PixSys 5500 pin microarray printer, GSI Lumonics ScanArray 4000 chip reader, BioDiscovery ImaGene software, microarray hybridizing chamber, and SPOT™ software available on a collaboration basis. Bioinformatics support is provided with microarray fabrication and data collection.

Contact: Benjamin F. Matthews, PSI, Soybean Genomics and Improvement Laboratory, Bldg 006, BARC-W, Tel (301) 504-5730 matthewb@ba.ars.usda.gov

**Proteomics**

Thermo Finnigan Deca ion trap mass spectrometer coupled with a Surveyor quaternary pump and an Applied Biosystems Voyager-DE STR MALDI-TOF mass spectrometer and a 7 processor Dell server with Mascot software for processing data.

Contact: Kurt Zuelke, ANRI, Biotechnology and Germplasm Laboratory, Bldg 200, BARC-E, (301) 504- 8545 at ANRI kzuelke@anri.barc.usda.gov

Ciphergen PS2B ProteinChip Reader (a SELDI-TOF mass spectrometer) for protein profiling and biomarker discovery.

Contact: Thomas Linke, BHNRC, Phytonutrients Laboratory, Bldg 307C, BARC-E, (301) 504-8270 linke@bhnrc.arsusda.gov.

**Nuclear Magnetic Resonance Spectroscopy**

Bruker QE 300 NMR 5mm dual $^{1}H$-$^{13}C$ probe used extensively for solving structural elucidation problems, run on very user friendly Aquerius software. Single pulse and multi-plus 1D experiment including APT and DEPT and multi-pulse 2D experiments including COSY, NOESY, and HETCOR. Technical support is available as needed. Bruker DMX 400 NMR, $^{31}P$ 20mm solutions probe, $^{1}H$ 5mm proton solution probe, 10 mm multi-nuclear solution probe, and 7mm multi-nuclear solids probe: its operation requires specialist expertise. A nominal fee is charged per hour of NMR time, used to cover the cost of Helium and Nitrogen cryogens and maintenance.

Contact: Ute Klingebiel, or Walter F. Schmidt, ANRI, Environmental Quality Laboratory, Bldg 012, Room 1-6. BARC-W, (301) 504-5030 KlingebU@ba.ars.usda.gov

**Polymer and Polymer Composite Processing and Characterization**

Brabender Prep-Center with internal mixing head and 25:1, ¾" single screw extruder. Slit and capillary dies and automated pelletizing capability for mixing, compounding, and extruding polymers. Carver Press Autofour/30 Model 4394 compression molder for molding polymers. Misonix Sonicator 3000 for mixing polymer solutions. Retsch ZM 1000 centrifugal grinder and Retsch PM400 planetary ball mill for grinding fillers. TA Instruments DSC910s for thermal characterization of polymers. Com-Ten Industries 95 RC Test System with compression and tensile fixtures for mechanical property characterization. Affiliated with NMR facility (QE 300 MHz and Bruker 400 MHz with solids capability) and FT-IR laboratory (Thermo Nicolet Avatar 370) for advanced characterization of polymers and composites.

Contact: Justin Barone, ANRI, Environmental Quality Laboratory, Bldg. 012, Rm. 1-3, BARC-W, Tel (301) 504-5905, baronej@ba.ars.usda.gov