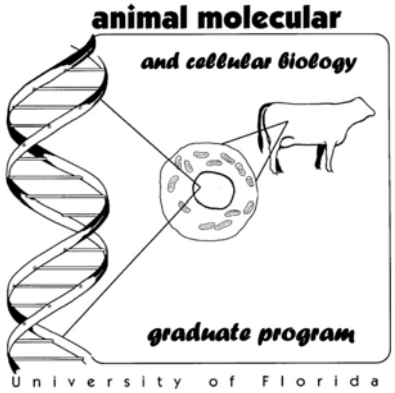
# SIXTH ANNUAL RESEARCH SYMPOSIUM

# ANIMAL MOLECULAR AND CELLULAR BIOLOGY GRADUATE PROGRAM

## UNIVERSITY OF FLORIDA


**Cedar Cove Beach and Yacht Club**
**Cedar Key, Florida**
**March 28-29, 2008**

UF | UNIVERSITY of FLORIDA




# Animal Molecular and Cellular Biology Graduate Program

# Sixth Annual Research Symposium

## WELCOME

It is our great pleasure to welcome you to the Sixth Annual Research Symposium of the Animal Molecular and Cellular Biology Graduate Program of the University of Florida. The sixth anniversary of our symposium brings us to a new locale – Cedar Key – famous for fishing, shellfish, and art festivals. We trust the site will be conducive to science and fellowship.

As we hold our 6th symposium, we will have completed our first year as a formal graduate program. The AMCB was founded in 1993 as an Interdisciplinary Graduate Concentration. In 2007, the AMCB was approved as a formal graduate program by the Board of Governors of the State University System. The change in status has brought new resources into the program and raised its visibility.

The program has continued to evolve since the last symposium. Two students, Jeremy Block and Luiz Augusto de Castro de Paula, completed their Ph.D. degrees. Dr. Lokenga Badinga has started service as AMCB Co-Director. Drs. Dan Sharp and William Buhi have retired and a new faculty member, Dr. Geoffrey Dahl from Animal Sciences, has joined the AMCB as have several new students.

To new students and faculty, we welcome you to the premier scientific event in the AMCB calendar. To new and veteran students and faculty, we trust the Sixth Symposium will be marked by good science, good fellowship, and good memories.

Pete Hansen, AMCB Director
Lokenga Badinga, AMCB Co-director

# ACKNOWLEDGEMENTS

***The faculty and students of the Animal Molecular and Cellular Biology Graduate Program thank the following for support of the 6th Annual Research Symposium***

Dr. Kirby Barrick, Dean for Academic Programs, IFAS, University of Florida

Dr. Mark McLellan, Dean of Research, IFAS, University of Florida

Dr. George J. Hochmuth II, Associate Dean for Research, IFAS, University of Florida

Dr. Winfred Phillips, Vice-President, Research and Graduate Programs, University of Florida

***Appreciation is also expressed to those who have supported the AMCB throughout the year***

Dr. Kirby Barrick, Dean for Academic Programs, IFAS, University of Florida

Ms. Joann Fischer, Program Assistant, Dept. of Animal Sciences, University of Florida

Dr. Joel H. Brendemuhl, Professor, Dept. of Animal Sciences, University of Florida; Graduate Coordinator, AMCB

***Appreciation is also expressed to the Symposium Committee***

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|---|---|---|
| Pete Hansen (chair) | Karen Moore | Lokenga Badinga |
| Luciano Silva | Lilian Oliveira | Kathleen Pennington |
| Maria Padua | Flavia Cooke | Izabella Thompson |

***Special thanks to Kathleen Pennington for her work to organize the Symposium***

## 2008 Animal Molecular and Cellular Biology Distinguished Lecturer

### Dr. Eckhard Wolf

Professor Dr. Eckhard Wolf is an international authority on use of genomic tools for studying the biology and genetics of reproduction. He is currently Head of the Institute for Molecular Animal Breeding and Director of the Laboratory of Functional Genomics at the Gene Center, Ludwig-Maximilians-Universität München. Dr. Wolf received his veterinary and doctoral degrees at LMU-Munich. Following a postdoc at the same institution, Dr. Wolf worked as Group Leader at the Institute for Animal Breeding and Genetics, University of Veterinary Medicine, Vienna in 1994 before returning to LMU-Munich in 1995. A major focus of the Wolf laboratory has been elucidating important processes in the biology and biotechnology of reproduction. Using the bovine as a model system, his group analyzes the cross-talk between pre-implantation embryos and their maternal environment by using state-of-the-art transcriptomics and proteomics technologies. Results of this project are being used to define new parameters for the improvement of reproductive performance by genetic selection. An additional goal is to use information regarding the specific requirements of early embryos to improve the efficacy of reproductive biotechnologies such as in vitro production of embryos and generation of embryos by somatic cell nuclear transfer. The latter technique is used as a model to understand basic mechanisms of nuclear reprogramming, to define the roles of epigenetic mechanisms during this process, and to experimentally evaluate maternal cytoplasmic effects on the phenotype of resulting embryos, fetuses and offspring. Dr. Wolf's group has also successfully established lentiviral gene transfer in farm animals as a tool for candidate gene validation. Dr. Wolf has been recognized for his work several times. Among his honors, he has been elected as a Member of the Deutsche Akademie der Naturforscher Leopoldina and received the Erwin Schrödinger-Award in 2000 for interdisciplinary collaboration.

## Guest Lecturer

## Dr. Mary B. Brown

Dr. Mary B. Brown is Professor in the Department of Infectious Diseases and Pathology at the University of Florida College of Veterinary Medicine. She received degrees from the University of South Carolina (BS), University of Florida (MS) and University of Alabama-Brimingham (PhD) before joining the faculty at Florida in 1985. Dr. Brown's research, which has resulted in 75 peer-reviewed publications, has focused on the pathogenic mechanisms by which mycoplasmas cause respiratory and urogenital infections. Her group has worked with a number of host species including rodents, food and fiber animals, environmentally threatened gopher and desert tortoises, and humans. The inherent mechanisms by which mycoplasmas colonize the host and induce chronic disease are surprisingly similar, despite the wide range of hosts. Among the highlights of her research has been the elucidation of the etiologic agent, characterization of clinical disease, and diagnosis of respiratory mycoplasmosis in two environmentally threatened species of tortoises. In experimental transmission studies, she demonstrated that *Mycoplasma agassizii* was the etiologic agent of upper respiratory tract disease (URTD in both gopher and desert tortoises. Her group has developed serological diagnostic tests (ELISA) as well as a PCR based diagnostic tests to allow epidemiological surveys of large natural populations. Another area of research uses genital mycoplasmosis as a model for studying the impact of intrauterine infection on pregnancy outcome. Her laboratory is also studying the host immune response to *M. bovis* infections of dairy calves, control by vaccination, and the microbial virulence factors of this microbe. Finally, Dr. Brown has developed an animal model for urogenital tract infections with *U. urealyticum.* This model is being used to identify potential genes involved in genetic susceptibility to infection.


## CEDAR KEY

Cedar Key is located on the Gulf Coast of Florida on the island of Way Key south of the mouth of the Suwannee River. The area was originally frequented by pirates and a treasure chest was discovered from nearby Fowler's Bluff on the Suwannee in the late 1800s. The city, which was founded in 1842, was originally located on Asenta Otie Key. A hurricane on September 23, 1896 wiped out the town and what was left was floated by barge to Way Key where the town was reestablished.

In the late 19th Century, Cedar Key was one of Florida's largest cities with a population of 10,000. Cedar Key is named after trees growing in the area that were used by the Faber and Eagle pencil companies. Other major industries were fishing, oyster harvesting and shipping. Cedar Key was a deep-water port and was used as a major shipping point for goods into and out of Florida. The first railroad built in Florida (completed in 1861) connected Cedar Key with Gainesville and with Fernandina Beach on the Atlantic coast.

In the Civil War, the town was occupied by Federal forces and used as a base for raids up the Suwanee and the rail line. While the town survived the war and the temporary closing of the railroad, the 1896 hurricane was a major blow and the town went into a long period of economic decline. The constitutional amendment to ban net fishing passed in 1995 destroyed the local commercial fishing industry. Many fishermen became engaged in clam farming and this industry, along with tourism, remains a major business today. There are two festivals held each year in Cedar Key: the Spring Sidewalk Art Festival and the Fall Seafood Festival.

## SCHEDULE

**Friday morning, March 28, 2008**
**Auditorium, McKnight Brain Institute**
**AMCB Distinguished Lecturer Series**

9:00 – 10:00 AM Eckhard Wolf, Lehrstuhl für Moleulare Tierzucht und Biotechnologie, Genzentrum der LMU-München. Genetically modified large animals in biomedical research

**Friday afternoon, March 28, 2008**
**Cedar Cove Beach and Yacht Club**

**Session 1. Research Reports (Kathleen Pennington, chair)**

1:00 – 1:15 PM Padua, M. B., and P. J. Hansen. Dept. of Animal Sciences, University of Florida. Uterine serpins: molecular phylogeny and sequence conservation in Laurasiatheria.

1:15 - 1:30 PM Oliveira, L., and P. J. Hansen. Dept. of Animal Sciences, University of Florida. Characterization of macrophages and γδ+ T cells in the endometrium during pregnancy in cows.

1:30 - 1:45 PM Silvestre, F.T., T. S. M. Carvalho, C. Crawford, J. E. P. Santos, C. R. Staples, W. W. Thatcher. Depts. of Animal Sciences and Small Animal Clinical Sciences, University of Florida. Effects of differential supplementation of calcium salts of fatty acids (CSFAs) to lactating dairy cows on plasma acute phase proteins and leukocyte responses: phagocytic and oxidative burst, CD62L and CD18 expression and cytokine production.

1:45 – 2:00 PM Thompson, I. M., R. L. Cerri, I. H. Kim, J. E-P. Santos, and W. W. Thatcher. Dept. of Animal Sciences, University of Florida. Current status in development of a resynchronization platform to improve reproductive efficiency and evaluation of pregnancy associated glycoproteins (PAGs) as a monitor of pregnancy and embryo/fetal development in lactating dairy cows.

2:00 – 2:15 PM Cooke, R.F., J.D. Arthington, D. B. Araujo, G. C. Lamb, and A. D. Ealy. University of Florida – IFAS, Gainesville. Effects of supplementation frequency on growth, reproductive, and metabolic responses of Brahman-crossbred heifers.

BREAK 2:15 - 2:30 PM

**Session 2 – Guest Lecture (Lokenga Badinga, Chair)**

2:30 – 3:15 PM Mary Brown Dept. of Infectious Diseases and Pathology, University of Florida. *Mycoplasma bovis*: Lighting up the genomes.

3:15 – 3:45 PM GROUP PICTURE

**Session 3 – Research Reports (Joe Kramer, Chair)**

3:45 – 4:00 PM Pennington, K.A., and A.D. Ealy. Dept. of Animal Sciences, University of Florida . Uterine-derived fibroblast growth factors: do they regulate interferon-tau production during early pregnancy?

4:00 – 4:15 PM Yang Q., S. E. Johnson, and A. D. Ealy. Dept. Animal Sciences, University of Florida. Protein kinase C delta mediates fibroblast growth factor 2-induced expression of interferon-tau in bovine trophectoderm.

4:15 - 4:30 PM Cooke, F.N.T., and A.D. Ealy. Dept. of Animal Sciences, University of Florida. Facing the challenges of achieving normal post-hatching bovine embryo development in culture.

4:30 – 4:45 PM Loureiro, B., L. Bonilla, J. Block, J. Fear, A. Bonilla and P. J. Hansen Dept. of Animal Sciences, University of Florida. Effect of granul-ocyte-macrophage colony stimulating factor on development and post-transfer survival of bovine embryos produced in vitro using sexed semen.

4:45 – 5:00 PM Kramer, J.M., A. Evans, K. Drury, and K. Moore. Dept. of Animal Sciences and Dept. of Obstetrics and Gynecology, University of Florida. Premature chromosome condensation of bovine blastomeres using the serine/threonine phosphatse inhibitor calyculin-A.

5:00 – 5:15 PM Zhang, K., E. Hughes, and K. Moore. Dept. of Animal Sciences, University of Florida. Characterization of DNA methylation status in bovine cloned and in vitro produced embryos.

5:15 - 5:30 PM Klein, C., M. H .T Troedsson, and A.D. Ealy. Dept. of Large Animal Clinical Sciences and Dept. of Animal Sciences, University of Florida. Deciphering embryo-maternal communication in the horse – a genomic approach.

Check into Rooms
Volleyball
Cookout
Karaoke (starring Izabella Thompson and the AMCB-tones)

**Saturday morning, March 29, 2008**
**Cedar Cove Beach and Yacht Club**

7:45 – 8:45 AM Breakfast

**Session 4 – Distinguished Lecture (Pete Hansen, Chair)**

8:45 - 9:30 AM Eckhard Wolf, Lehrstuhl für Moleulare Tierzucht und Biotechnologie, Genzentrum der LMU-München. Structural, molecular and functional views on oocytes, embryos and their maternal environment.

**Session 5 – Research Reports (Qien Yang, Chair)**

9:30 – 9:45 AM Silva, L.A., and D. C. Sharp. Dept. of Animal Sciences, University of Florida. Role of the equine conceptus in endometrial angiogenesis and architecture.

9:45 – 10:00 AM Reed, S.A., and S.E. Johnson. Dept. of Animal Sciences, University of Florida. Optimization of culture conditions for equine umbilical cord blood derived stem cells.

10:00 – 10:15 AM Esterman, R. D., J. V. Yelich, and S. E. Johnson. Dept. of Animal Sciences, University of Florida. Investigation of myostatin gene mutations in common cattle breeds.

10:15 – 10:45 AM BREAK

**Session 6 – Research Reports (Regina Esterman, Chair)**

10:45 – 11:00 AM Predmore, B.L., M. J. Alendy, K.R. Olson, and D. Julian. Department of Zoology, University of Florida, Gainesville. Indiana University School of Medicine, South Bend. Inhibition of NO, $K_{ATP}$, and prostanoids decreases hypoxia-induced, but not $H_2S$-induced, contractions in rat aorta .

11:00 – 11:15 AM Hendricks, K., and P.J. Hansen. Dept. of Animal Sciences, University of Florida. Effect of preincubation of sperm at 38.5ºC or 40ºC before insemination on developmental competence of bovine embryos derived from in vitro fertilization.

11:15 – 11:30 AM Fear, J., and Hansen, P.J. Dept. of Animal Sciences, University of Florida. The Yin-Yang of apoptosis: developmental changes in the concentration of Bcl-2 and Bax in preimplantation bovine embryos.

11:30 – 11:45 AM Bonilla, A. Q. S., and P.J. Hansen. Dept. of Animal Sciences, University of Florida. Developmental changes in actions of insulin-like growth factor-I in the preimplantation bovine embryo – receptor expression, Akt activation and thermoprotection.

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| 11:45 – 12:00 PM | Moss, J. I., and P.J. Hansen. Dept. of Animal Sciences, University of Florida. Insulin-like growth factor-1 reduces the anti-development effects of menadione on development of bovine preimplantation embryos. |

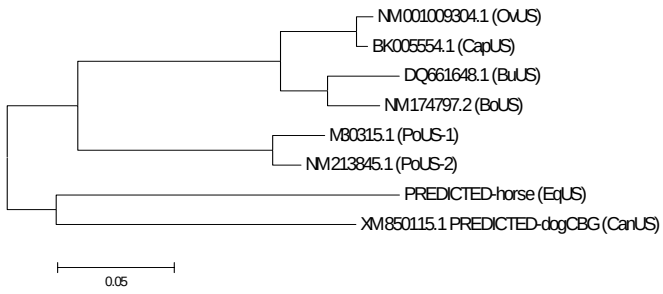
ADJOURNMENT

# ABSTRACTS

**UTERINE SERPINS: MOLECULAR PHYLOGENY AND SEQUENCE CONSERVATION IN LAURASIATHERIA**

Maria B. Padua and Peter J. Hansen
Department of Animal Sciences, University of Florida, Gainesville, Florida

Uterine serpins (US) are a unique group of proteins that are produced by the endometrial epithelium of the sheep, goat, cow and pig during pregnancy. Unlike most members of the serpin superfamily, US have functions distinct from anti-proteinase activity. The species in which uterine serpin genes have been identified are members of the Ruminantia and Suidae orders of the Laurasiatheria superorder of mammals; all have epitheliochorial placentation. The objective of the present study was to identify novel US genes in species within and outside of Laurasiatheria to determine whether the US gene is limited to species with epitheliochorial placentation in this superorder. A search performed in the nucleotide collection (blastn) of the NCBI website, using the coding sequence of ovine (Ov) US as a query, identified coding sequences of known US, caprine (Cap), bovine (Bo) and porcine (Po-1 and 2) and also coding sequences for new uterine serpins, the recently identified and unpublished US gene in the water buffalo (BuUS) and a novel US gene in the dog (CanUS). The US gene was localized on chromosome 21 for the cow and 8 for the dog. There are two small untranslated regions (UTR) in exons 1 and 6 respectively of the BoUs gene but none in the CanUS gene. A genomic blast performed on complete genomic sequences using OvUS as a query failed to identify US in mammals such as human, chimpanzee, rhesus monkey, mouse, rat, opossum, and duck-billed platypus (all outside Laurasiatheria) and in other vertebrates like chicken, puffer and zebra fish. However, a genomic blast of the non-completed whole shotgun genomic sequence of the horse identified four stretches of nucleotides that matched with the coding sequence of OvUS with 68, 75, 86 and 79% identity respectively. Construction of a phylogenetic tree using the MEGA 4 program indicated that the CanUS and the predicted US for the horse are closely related and they diverged from a common ansector of pig and cow/buffalo (see Figure). The Selecton program was used to calculate the ratio of non-synonymous/synonymous substitutions at each codon site and determine regions in the gene with purifying and positive selection. There were a few positive selected sites in the US gene and the likelihood ratio test for positive selection was significant ($P<0.001$). The finding that US has been identified in the dog, a species with endotheliochorial placentation, suggests that this gene is not restricted to species with epitheliochorial type of placenta. The absence of US in other mammals and vertebrates suggests that the gene diverged within Laurasiatheria. Nevertheless, experiments are warranted to verify expression of the US gene in the pregnant uterus of the horse and dog. The existence of positive selection suggests that the functionality of the gene may be species-dependent.



## CHARACTERIZATION OF MACROPHAGES AND γδ+ T CELLS IN THE ENDOMETRIUM DURING PREGNANCY IN COWS

Lilian Oliveira and P.J. Hansen
Department of Animal Sciences, University of Florida, Gainesville, Florida

Pregnancy involves cross-talk between the mother and conceptus to promote maternal immune tolerance to fetal alloantigens. In a previous study, we demonstrated a decrease in the number of circulating $CD68^+$ cells (i.e., monocytes) in periparturient cows as compared to non-pregnant cows and an increase in circulating $\gamma\delta^+$ T cells. In the sheep, pregnancy is associated with an accumulation of $CD68^+$ macrophages and $\gamma\delta^+$ T cells in the uterine endometrium and it is possible that the changes in circulating concentrations of leukocytes in cows described above reflects trafficking between the blood and uterus. In particular, we hypothesize that the periparturient decrease in numbers of circulating $CD68^+$ cells may occur because of recruitment of macrophages to the uterus and that the increase in circulating $\gamma\delta^+$ T cells may represent recirculation of these cells from the uterus to the periphery. The purpose of the present study was to determine changes in numbers of endometrial macrophages and $\gamma\delta^+$ T cells associated with pregnancy. Endometrium from pregnant (n=20; estimated fetal ages ranging from 54-240 d of pregnancy) and nonpregnant (n=7; ipsilateral to corpus luteum) bovine uteri were used for immunolocalization studies by immunohistochemistry (for anti-CD68) and dual-color immunofluorescence (anti-CD68 and anti-CD14; anti-CD68 and anti-MHC class II; anti-CD68 and anti-IL-10; anti- γδ+ T TCR and anti-MHC class II). Additional labeling was performed with isotype controls. Cells positive for CD68 were localized primarily in the shallow stroma and they were higher in number in pregnant cows (Fig. 1). Using two-color immunofluorescence, CD68+ cells were found to also express the macrophage marker CD14. Preliminary results indicate that expression of MHC class II and IL-10 on CD68+ cells was low suggesting that uterine macrophages are not activated via the classical pathway and are not involved of inhibition of lymphocyte function through IL-10 secretion. Intense IL-10 expression was found in the luminal epithelium. The $\gamma\delta^+$ T cells were found in small numbers only in the subepithelium and periglandular stroma or in the luminal and glandular epithelium. Numbers were greater for pregnant endometrium than endometrium from nonpregnant animals. Uterine macrophage and $\gamma\delta^+$ T cells were thought to be a source of IL-10 at maternal fetal interface. IL-10 is a potent immunosupressive and anti-inflammatory molecule which seems to be important to pregnancy outcome. Perhaps, the maternal secretion of IL-10 by the luminal epithelium is a consequence in response to fetal mechanisms to prevent activation of macrophages and other immune cells present at maternal fetal interface in the cow. Also the presence of small numbers of $\gamma\delta^+$ T cells leads us to reconsider the hypothesis on how these cells would play a role in the maintenance of maternal immune system tolerance, because it does not seem to be an indirect by cytokine secretion but may be more related to cell-cell contact and fetal antigen recognition.

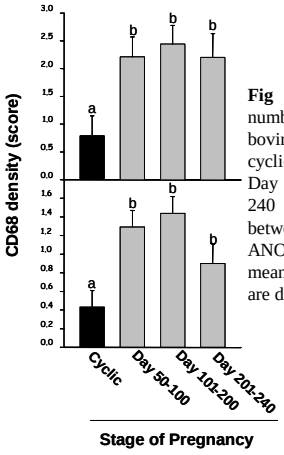



**Fig 1**. Effect of pregnancy status on numbers of $CD68^+$ macrophages in the bovine endometrium. Results are shown for cyclic cows (n=7) and for pregnant cows at Day 50-100 (n=7); 101-200 (n=8) and 201-240 of pregnancy (n=5). Differences between treatments were determined by ANOVA. Data are least squares mean ± S.E.M. Bars with different letters are different from each other ($P < 0.05$).

**EFFECTS OF DIFFERENTIAL SUPPLEMENTATION OF CALCIUM SALTS OF FATTY ACIDS (CSFAs) TO LACTATING DAIRY COWS ON PLASMA ACUTE PHASE PROTEINS AND LEUKOCYTE RESPONSES: PHAGOCYTIC AND OXIDATIVE BURST, CD62L AND CD18 EXPRESSION AND CYTOKINE PRODUCTION**

F.T. Silvestre, T. S. M. Carvalho, C. Crawford[1], J. E. P. Santos, C. R. Staples, and W. W. Thatcher
Dept of Animal Sciences and [1]Small Animal Clinical Sciences Univ. of Florida, Gainesville

The objective was to characterize immune responses to different CSFAs supplemented during -32 to 30 days postpartum (dpp; CON [Palm oil, 47% C16:0] vs LN [Safflower oil, 64% C18:2n-6]) and from 30 to 80 dpp (CON [C16:0] vs FO [Fish oil, 11% of C20:5n-3 + C22:6n-3]). Supplementation was at 1.5% of CSFAs in the diet. Plasma samples were collected daily from 0 to 10 dpp for measurements of $PGF_{2\alpha}$ metabolite (PGFM) and thrice weekly until 30dpp for haptoglobin (HP), fibrinogen (FB) and ceruloplasmin (CE). At -32, 0, 4, and 7 dpp, blood samples were collected to evaluate phagocytic and oxidative burst of neutrophils in whole blood (100µl) using a dual color flow cytometry method and expression of the adhesion molecules CD62L and CD18 utilizing monoclonal antibodies against bovine CD62L (L-selectin) and canine CD18 (β2-integrin). Mouse control antibody was used to correct for non-specific binding. All antibodies were conjugated with a goat anti-mouse IgG1:RPE. Flow cytometry was used to acquire and analyze the neutrophil and mononuclear cells populations for CD62L and CD18 mean fluorescence intensity (MFI). At 30 and 80 dpp, neutrophils also were isolated, suspended in RPMI, and added ($5x10^5$ in 100µl) to 96 well plates in duplicate for non-or LPS (20µl of 1mg/ml) stimulation, volume was completed to 200µl with RPMI for incubation (18h at 37ºC and 5% $CO_2$), and TNF-α subsequently measured in supernatant. Plasma concentration of HP and FB were higher ($P<0.05$) for LN (0.034 OD and 248.8 mg/dl, n=17) compared with CON (0.02 OD and 205.3 mg/dl, n=15). PGFM and CE were not affected by diets (206 pg/ml, 8.8 mg/dl; n=32). Percent neutrophils that phagocytized with oxidative burst did not differ between diets for samples challenged with *E. coli* (49%, n=47) or *S. aureus* (33%, n=39), but increased ($P<0.01$) from 0 (38%, 23%) to 4 (53%, 38%) and 7 (57%, 34%) dpp, respectively. Neutrophil MFI of CD62L was higher in LN at 4 ($P<0.05$) and 7 ($P<0.08$) dpp and increased ($P<0.05$) from 0 to 4 and 7 dpp in cows across diets (n=45). The MFI of CD62L in mononuclear cells and CD18 in neutrophil and mononuclear cells were not affected by diet or dpp. However, number of mononuclear cells positive for CD62L was higher in LN at 4 ($P<0.01$) and 7 ($P<0.01$) dpp and increased more in the LN (diet*dpp; $P<0.05$). Number of mononuclear cells positive for CD18 was not affected by diet, but decreased ($P<0.01$) from 0 to 4 and 7dpp. Concentration of TNF-α from neutrophil cultures for non-stimulated and LPS at 30dpp was higher ($P<0.05$) for LN (52.7 and 107 pg/ml, n=17) compared with CON (30 and 63.2 pg/ml, n=16); however, the incremental increase did not differ. Concentration of TNF-α from neutrophil cultures at 80dpp for non-stimulated samples was not affected by diets (36.58 pg/ml, n=18), but LPS stimulation and the incremental increase were lower ($P<0.01$) for FO (42.5 and 4.6 pg/ml, n=14) compared with CON (82.7 and 47.5 pg/ml, n=14), respectively. In conclusion, differential immune responses to n-6 or n-3 FAs in dairy cows may serve as a mean to enhance or suppress inflammatory responses according to stage of lactation.

**CURRENT STATUS IN DEVELOPMENT OF A RESYNCHRONIZATION PLATFORM TO IMPROVE REPRODUCTIVE EFFICIENCY AND EVALUATION OF PREGNANCY ASSOCIATED GLYCOPROTEINS (PAGs) AS A MONITOR OF PREGNANCY AND EMBRYO/FETAL DEVELOPMENT IN LACTATING DAIRY COWS**

I.M. Thompson, R.L.Cerri, I.H. Kim, J. E-P. Santos, W.W. Thatcher
Department of Animal Sciences, University of Florida, Gainesville, Florida

Synchronization of ovulation protocols for timed AI (TAI) have improved pregnancy rates by increasing submission rates, but little increase in conception rates has been achieved. Progesterone can regulate both ovarian and uterine function and is essential for maintenance of pregnancy. Pregnancy associated glycoproteins (PAGs) are a multigene family temporally expressed in trophoblast cells during gestation. Aim of this study is to implement a Presynchronization/Modified Ovsynch/Re-synchronization (Resynch) program to enhance both embryo survival to 1st TAI and pregnancy rate of resynchronized non-pregnant cows to 2nd TAI. Cows (n=1600) are pre-synchronized with two injections of $PGF_{2\alpha}$ given at 42±3 and 56±3 days post partum. At 14 days after the second $PGF_{2\alpha}$, GnRH is injected followed 7 days later with an injection of $PGF_{2\alpha}$. A second injection of GnRH, at 56 hours after $PGF_{2\alpha}$, is followed 16 h later with a TAI (day 0). The treatment resynchronization group, Re-synch CIDR/Modified Ovsynch, is based upon insertion of a CIDR insert on day 18 after the first TAI. The CIDR insert is removed 7 days later concomitantly with an injection of GnRH on day 25. Ultrasonographic diagnosis of pregnancy occurs one week later on day 32, and nonpregnant cows receive an injection of $PGF_{2\alpha}$. At 56 h after $PGF_{2\alpha}$ injection, GnRH is injected and cows are re-inseminated 16 h later on day 35 after first TAI. The control resynchronization group (i.e., cows that did not receive a CIDR insert), Re-synch Control/Modified Ovsynch, are diagnosed for pregnancy at day 32 after insemination, and if nonpregnant will receive a GnRH injection followed by a $PGF_{2\alpha}$ injection 7days later (day 39). At 56 h after $PGF_{2\alpha}$ injection, GnRH is injected and cows are TAI 16 h later (day 42). All day 32 pregnancies are re-confirmed at day 60. PAGs in plasma will be evaluated as a marker for both detection of pregnancy and developmental status of the embryo/fetus following first TAI in Sub-set I of 105 cows. Serial blood samples will be collected between days 18 to 60 after first insemination, and both progesterone and PAG concentrations will be measured for both resynchronization groups. Ovarian responses to GnRH (i.e., presence of follicles and formation of CL) will be monitored in Sub-set II of 150 non-pregnant experimental cows. Ovaries are examined by ultrasound on days 25 and 32 in the Re-synch CIDR/Modified Ovsynch treatment group and on days 32 and 39 of the Re-synch Control/Modified Ovsynch group. Preliminary estimates of pregnancy rates on day 32 after 1st TAI (n= 707) are 39.6% and 41.4% for the Re-synch CIDR/Modified Ovsynch and Re-synch Control GnRH groups, respectively. Pregnancy losses at 60 days for the respective groups are 3.61% and 4.81%.

## EFFECTS OF SUPPLEMENTATION FREQUENCY ON GROWTH, REPRODUCTIVE, AND METABOLIC RESPONSES OF BRAHMAN-CROSSBRED HEIFERS

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Energy supplementation is a common practice in beef cow-calf systems because growth and reproductive development of heifers are influenced positively by energy intake. The labor expenses associated with supplement feeding contribute significantly to the fixed costs of cattle operations; therefore offering supplements 3 times or once weekly instead of daily are typical strategies to decrease costs of production. However, decreasing the supplementation frequency of energy-based feeds to cattle consuming low-quality forages can be detrimental to animal performance. The objective of this experiment was to investigate the effects of supplementation frequency on BW gain, concentrations of plasma metabolites and hormones, mRNA expression of liver and muscle genes associated with metabolism and growth, and reproductive performance of yearling heifers grazing low-quality forages. Fifty-six Brahman x British heifers (avg. age = 10 mo) were stratified by initial BW and age, and randomly allocated to 14 bahiagrass (*Paspalum notatum*) pastures. Pastures were randomly assigned to receive an energy-based supplement daily (S7) or 3 times per wk (S3), at a weekly rate of 18.2 kg of DM per heifer. Heifer shrunk BW was obtained before the start (d -11) and at the end of the experiment (d 109) to calculate BW gain. Blood samples were collected weekly to determine onset of puberty via progesterone concentration. From d 0 to 45, blood samples were also collected on four consecutive days, every other wk, starting at 4 h after feeding time for determination of glucose, blood urea nitrogen (BUN), insulin, and IGF-1 concentrations. On d 35 or 36 of the experiment, heifers within pasture were randomly assigned to either muscle or liver needle biopsy starting at 4 h after feeding time. Following RNA isolation, quantitative real-time RT-PCR was used to assess mRNA expression of IGF-1, IGFBP3, pyruvate carboxylase (PC), cytosolic phosphoenolpyruvate carboxykinase (PECK-C), and mitochondrial PEPCK (PEPCK-M) in liver samples, and IGF-1, IGF1BP3, IGF1BP5, and myostatin in muscle samples. On d 46, heifers were re-allocated by treatment into two bahiagrass pastures and exposed to mature Angus bulls for a 60-d breeding season. Pregnancy was determined via trans-rectal ultrasonography 70 d after the end of breeding season. Mean BW gain was greater ($P < 0.05$) for S7 compared to S3 heifers (0.41 and 0.33 kg/d, respectively; SEM = 0.02). Treatment x sampling day interactions were detected ($P < 0.01$) for all blood measurements. Heifers provided S7 had reduced daily variation in concentrations of BUN, glucose and insulin, and frequently had greater ($P < 0.05$) concentrations of IGF-I compared to S3 heifers. Expression of liver IGF-I mRNA was greater ($P < 0.05$) whereas expression of liver PEPCK-M mRNA tended ($P < 0.10$) to be greater for S7 compared to S3 heifers. Treatment x day interactions were detected ($P \leq 0.05$) for mRNA expression of liver IGFBP-3, gluconeogenic enzymes, and muscle myostatin because the expression of these transcripts was greater ($P < 0.05$) for S3 heifers when both treatment groups were supplemented, but were similar or greater (PEPCK-C; $P < 0.05$) for S7 heifers when only these were supplemented. Attainment of puberty and pregnancy were hastened ($P < 0.05$) in S7 compared to S3 heifers. In conclusion, offering an energy-based supplement daily instead of 3 times weekly to Brahman-crossbred heifers grazing low-quality forages improved their nutritional and metabolic status, resulting in enhanced growth and reproductive performance.

**UTERINE-DERIVED FIBROBLAST GROWTH FACTORS: DO THEY REGULATE INTERFERON-TAU PRODUCTION DURING EARLY PREGNANCY?**

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Conceptus-derived signals are required for establishing and maintaining pregnancies in most mammals. In bovids, the first maternal recognition of pregnancy signal is interferon-tau (IFNT). This protein is produced by trophectoderm and interacts with the endometrium to limit pulsatile secretions of prostaglandin F2 alpha, thus preventing luteolysis. Uterine secretions are implicated in regulating IFNT gene expression during early pregnancy. One such factor, fibroblast growth factor 2 (FGF2), stimulates IFNT production upon supplementation to bovine blastocysts and a trophectoderm cell line, CT1. Multiple FGFs, notably FGF1, 2 and 10, are produced by the endometrial epithelium in sheep and cattle. The overall goal of this work was to determine if uterine-derived FGFs regulate IFNT expression in bovine trophectoderm. In the first series of studies, CT1cells were exposed to recombinant (r) bovine (b) FGF1, rbFGF2, or r human (h) FGF10 (0.5 to 500 ng/ml) to determine whether multiple FGFs stimulate IFNT mRNA and protein production. When RNA was extracted 24h-post treatment, IFNT mRNA concentrations were greater ($P<.05$) in CT1 cells exposed to $\geq$ 50 ng/ml rbFGF1 , $\geq$ 5 ng/ml rbFGF2, and 500 ng/ml rhFGF10 than vehicle-only treated controls. Similarly, conditioned medium derived from CT1 cells 48h post-treatment contained greater ($P<.05$) quantities of IFNT protein when cells were exposed to $\geq$50 ng/ml rbFGF1 or 2 or $\geq$500 ng/ml rhFGF10 compared to controls. It remains unclear if endometrial production of FGF1, 2 and 10 has any physiological impacts on conceptus development and IFNT production during early pregnancy. Interestingly, FGF1, 2 and 10 are expressed in cultured ovine endometrial epithelial cells and recent findings suggest that secreted products of these cells control IFNT production. In a preliminary study, medium was conditioned in ovine luminal epithelial (oLE) or ovine glandular epithelial (oGE) for 72h before supplementing it to CT1 cultures to determine if cell secretions affect trophectoderm production of IFNT. After 24h, extracted CT1 RNA samples contained more IFNT mRNA when CT1 cells were incubated in oLE or oGE conditioned medium than when they were incubated in non-conditioned medium. Our plan for continuing to describe this conditioned medium phenomenon and determining if FGF1, 2 and/or 10 are vital components of the conditioned medium will be discussed in the presentation. To summarize, current findings indicate that several FGFs can influence IFNT production in trophectoderm, and at least a subset of these factors are produced in a manner that permits them to interact with the pre-attachment bovine conceptus. Current work is aimed at identifying the functional implications of these findings. Project supported by CSREES NRICGP # 2003-35203-15382.

**PROTEIN KINASE C DELTA MEDIATES FIBROBLAST GROWTH FACTOR 2-INDUCED EXPRESSION OF INTERFERON-TAU IN BOVINE TROPHECTODERM**

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Interferon-tau (IFNT) serves as the signal for maternal recognition of pregnancy in cattle and sheep. Various developmental and uterine-derived factors potentially regulate trophectoderm IFNT expression. This laboratory discovered that fibroblast growth factor-2 (FGF2) is produced by the bovine endometrial epithelium and released into the uterine lumen during early pregnancy. Supplementation with recombinant bovine FGF2 stimulates IFNT mRNA and protein concentrations in bovine blastocysts and a bovine trophectoderm cell line (CT1). The objective of this work was to identify the intracellular signaling modules utilized by FGF2 to regulate IFNT expression in bovine trophectoderm. CT1 cells were treated for 2 h with either a chemical inhibitor of protein kinase C (PKC) isoforms (0.5 μM, Calphostin), extracellular signal-regulated kinases (ERK) 1/2 (50 μM, PD98059), p38 mitogen-activated protein kinase (MAPK) (25 μM, SB203580) or vehicle only prior to FGF2 supplementation for 24 h. After cell lysis and RNA extraction, quantitative RT-PCR revealed a 10.70 ± 1.93 fold increase (P=0.001) in IFNT mRNA abundance in response to FGF2. Suppression of ERK1/2 or p38 activity did not alter the FGF2 response but exposure to the pan-PKC inhibitor limited (P=0.01) the ability of FGF2 to increase IFNT mRNA abundance (2.42 ± 1.13 fold-induction over controls). In a follow-up study, supplementing medium with a PKC activator, phorbol 12-myristate 13-acetate (100nM PMA) increased IFNT mRNA levels similar to that of FGF2 To specify the PKC isoform used for this FGF2 and PMA response, CT1 cells were incubated with chemical inhibitors of two classical PKCs (α, βI; 5μM Gö6976) or two novel PKCs (δ, θ; 5μM rottlerin). Treatment with Gö6976 did not affect FGF2- or PMA-mediated increases in IFNT mRNA abundance. By contrast, inhibition of PKCδ/θ attenuated (P<0.01) the PMA and FGF2 responses. In a related study, supplementation with a myristoylated pseuodosubstrate inhibitor specific for PKCθ did not affect PMA or FGF2 induction of IFNT mRNA abundance. In conclusion, present findings suggest that PKC, and specifically PKCδ is required for FGF2-mediated stimulation of IFNT expression in bovine trophectoderm. Additional studies utilizing different approaches for manipulating PKCδ activity are forthcoming to confirm this and other roles for PKCδ during bovine conceptus development. This work will provide valuable insight into how the uterine environment promotes the establishment and maintenance of pregnancy in ruminants at the molecular level. Project supported by CSREES NRICGP # 2003-35203-15382.

**FACING THE CHALLENGES OF ACHIEVING NORMAL POST-HATCHING BOVINE EMBRYO DEVELOPMENT IN CULTURE**

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Blastocyst formation is a routinely used indicator of bovine embryo quality and post-transfer survivability in various scientific and commercial settings. The emergence of the first differentiated extra-embryonic tissue, the trophectoderm, and the formation of its fluid compartment, the blastoceol, represent key distinguishing features of blastocysts. However, despite their microscopic similarities, molecular variations between *in vivo*, *in vitro*, and nuclear transfer-derived bovine blastocysts generate disparate pregnancy outcomes. Therefore, although blastocyst formation represents a crucial stage of embryo development, its use to predict survival potential following embryo transfer can be inaccurate. Post-hatching embryonic development in vitro may be a better suited endpoint of embryo competency. *In utero*, endoderm, ectoderm and mesoderm germ layers emerge from the inner cell mass (ICM) between days 8 and 16 post-fertilization. Also, trophectoderm proliferation generates an elongated conceptus mass between days 13 to 15 of pregnancy. Replicating these events in vitro is not possible when conventional medium and environment conditions are provided. Embryos can remain viable in extended culture until days 11 to 14 post-fertilization. Under standard culture conditions, hatched blastocysts either attach to the bottom of plates and form trophectoderm and extra-embryonic endoderm outgrowths or continue to grow in a spherical appearance. Elongation does not occur in either case. Morphological elongation can be induced by culturing post-hatched bovine embryos inside agarose gel tunnels. Trophectoderm development continues in some embryos but the rate and extent of this growth is severely reduced when compared to their *in utero*-developed counterparts. Moreover, extensive embryonic disc degeneration occurs as culture time continues. A similar scenario occurs in uterine gland knock-out ewes during early pregnancy. In these studies, hatched embryos fail to elongate and embryonic disks fail to develop normally when the number and overall functional capacity of uterine glands is reduced. In summary, previous investigative studies illustrate the limitations of achieving post hatching bovine embryo development *in vitro*. It is becoming clear that uterine factors are required for normal conceptus development, including elongation.

**EFFECT OF GRANULOCYTE-MACROPHAGE COLONY STIMULATING FACTOR ON DEVELOPMENT AND POST-TRANSFER SURVIVAL OF BOVINE EMBRYOS PRODUCED IN VITRO USING SEXED SEMEN**

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Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a multifunctional cytokine originally recognized as a regulator of cell proliferation and differentiation. GM-CSF has also been implicated in the regulation of preimplantation embryonic development in several species. In cattle, bovine GM-CSF has been reported to improve the proportion of embryos that become blastocysts in vitro (Moraes and Hansen, Biol Reprod 57:1060-1065, 1997). It is possible that these blastocysts are better able to survive after transfer as shown for mouse embryos (Sjoblom et al., Endocrinology 146:2142-2153, 2005). The objective of the present study was to test whether addition of GM-CSF to culture medium enhances post-transfer survival of in vitro produced bovine embryos. Holstein cumulus-oocyte-complexes (COC) shipped overnight in a portable incubator in oocyte maturation medium were fertilized with X-chromosome selected sperm from Holstein bulls. Embryos were cultured in KSOM-BE2 alone or with 100 ng/ml of modified insulin-like growth factor-1 (IGF-1) added at day 1 after insemination, 10 ng/ml recombinant BoGM-CSF added at day 1 after insemination, or 10 ng/ml recombinant GM-CSF added at day 5 after insemination. Morulae and blastocysts were collected at Day 7 after insemination and transferred to lactating dairy cows at day 7 after ovulation. Ovulation was synchronized using a modified OvSynch protocol. The percent of oocytes that became transferable morulae or blastocysts at Day 7 was greater for embryos treated with $Arg^3$-IGF-1 (7.9 $\pm$ 0.7%, $P<0.07$), GM-CSF at Day 1 (8.5 $\pm$ 1.0%, $P<0.06$) and GM-CSF at Day 5 (8.4 $\pm$ 0.7%, $P<0.03$) compared to controls (6.2 $\pm$ 0.5%). The pregnancy rate, as determined by pregnancy diagnosis between ~ day 30-35, was higher ($P=0.06$) for cows receiving embryos treated with GM-CSF at Day 5 (47 of 107 cows; 43.9%) than for control cows (36/103; 35.0%), cows receiving embryos treated with $Arg^3$-IGF-1 (24/75; 32.0%) or cows receiving embryos treated with GM-CSF at Day 1 (12/39; 30.8%). These data suggest that embryo competence for post-transfer survival can be enhanced by treatment with GM-CSF at Day 5 after fertilization. Research supported by USDA Grant 2006-55203-17390, BARD Grant US-3986-07 and the Southeast Milk Dairy Checkoff Program. BL was supported by a CAPES (Brazil)/Fulbright Fellowship.

**PREMATURE CHROMOSOME CONDENSATION OF BOVINE BLASTOMERES USING THE SERINE/THREONINE PHOSPHATSE INHIBITOR CALYCULIN-A**

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Chromosome analysis of preimplantation embryos, such as assessment of chromosome copy number or evaluation of translocations, has traditionally required interphase fluorescence in situ hybridization (FISH). Unfortunately, this technique is hindered by the low mitotic index of blastomeres, combined with technical limitations of interphase FISH and therefore does not allow for a comprehensive assessment of all chromosomes. Premature chromosome condensation (PCC) is a potential method that could overcome the limitations of interphase FISH; however, chemical approaches including okadaic acid, vinblastin, and colcemid or cell fusion approaches to induce PCC have proven to be laborious and inefficient. Our objective was to evaluate the potent serine/threonine phosphatase inhibitor, calyculin-A, as an alternative to induce PCC in blastomeres from bovine embryos. In vitro produced (IVP) bovine 8-cell stage embryos were cultured with either calyculin-A, colcemid (0.1 µg/ml) or vehicle (DMSO). Blastomeres were washed in hypotonic solution, loaded onto slides, and fixed in methanol:acetic acid (3:1). The degree of chromatin condensation and quality of chromosome spreads were determined by 4',6-diamidino-2-phenylindole (DAPI) staining or by fluorescent in situ hybridization (FISH) and visualized on an epifluorescence microscope. Blastomere cell cycle phase was determined by examining chromatids. Blastomeres that contained chromosomes with single chromatids were scored as G1, double chromatids were scored as G2/M and pulverized chromatin were scored as S phase cell cycle. Data were analyzed using chi-square test, with significance deemed $P<0.05$. Experiment 1 tested calyculin-A durations of 0, 60, 120, and 180 min to induce PCC in bovine blastomeres. More blastomeres that underwent PCC had chromosomes suitable for cytogenetic analysis if treated for 120 min and 180 min compared to shorter durations ($P < 0.005$). Experiment 2 compared a dose titration of calyculin-A (0, 10, 50, and 100 nM) to induce PCC in blastomeres from bovine IVP embryos. Calyculin-A (50 nM) induced condensation suitable for cytogenetic analysis in the greatest number of blastomeres when compared to other doses ($P < 0.005$). Finally, experiment 3 evaluated the effectiveness of calyculin-A under optimal conditions (50 nM for 120 min) to induce PCC suitable for cytogenetic analysis in bovine IVP embryos. Whole chromosome paint probes for X and Y were successfully hybridized to chromosomes obtained from calyculin-A treated blastomeres allowing detection of gender and ploidy of individual blastomeres. In each experiment, the majority of blastomeres containing analyzable chromosomes came from cells that were in either G1 or G2/M phases of the cells cycle. Chromatin from S phase blastomeres often appeared pulverized or chaotically condensed and was not suitable for cytogenetic analysis. In conclusion, our data demonstrate that the degree and quality of condensation was affected by dose and duration of calyculin-A as well as blastomere cell cycle phase. These results suggest that calyculin-A can be used to rapidly induce PCC in blastomeres from bovine embryos, but the degree of chromatin condensation may not always be suitable for detailed cytogenetic analysis from a single blastomere.

## CHARACTERIZATION OF DNA METHYLATION STATUS IN BOVINE CLONED AND IN VITRO PRODUCED EMBRYOS

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In the last ten years since the birth of 'Dolly', somatic cell nuclear transfer (SCNT) has been successfully used in a number of mammals to pursue its application in domestic animal breeding, as well as therapeutic cloning. However, a plethora of aberrant phenotypes occur in the fetus and placenta of SCNT-derived animals, such as large offspring syndrome. These may be due to the abnormal epigenetic reprogramming of the transferred somatic donor cell from a differentiated status to a totipotent state. Altered global DNA methylation has been observed in bovine SCNT embryos when compared with bovine in vitro produced (IVP) embryos and in vivo produced counterparts during preimplantation stages of development. Given that DNA methylation is related to genetic silencing, imprinting and X-chromosome inactivation, DNA hypomethylation or hypermethylation could contribute to inappropriate gene expression during preimplantation development. Two variants of the DNA maintenance methyltransferase exist that impact the cloned embryo, specifically the somatic form, Dnmt1s and the oocyte form, Dnmt1o, which could methylate newly synthesized DNA. Normally, the paternal and maternal genome of the zygote are rapidly demethylated after fertilization; however the SCNT process may add Dnmt1s from the transferred donor cell to the resultant cloned embryo, possibly explaining their higher global methylation profiles. Therefore, the central hypothesis of our study is that introduction of the somatic form of DNA methyltransferase, may be associated with higher DNA methylation and aberrant gene expression in cloned embryos. We have tested two genotypically identical fibroblast cell lines that differ epigenetically (high and low Dnmt1s transcripts, respectively) for use in the production of cloned embryos. We have previously evaluated global DNA methylation patterns in IVP, high Dnmt1 and low Dnmt1 cloned embryos by immunocytochemistry (ICC). Elevated global DNA methylation was observed in the high Dnmt1 cloned embryos when compared to IVP embryos at all three stages. Low Dnmt1 clones had reduced global DNA methylation similar to IVP embryos, but not until the 8-cell and blastocyst stages. As the next step to understand chromatin remodeling, we are assessing methylation patterns at 4 loci (IGF2, Oct-4, SatI, and αSat) across three stages of development (2-cell, 8-cell, and blastocyst) to determine whether donor cell Dnmt1s affects chromatin remodeling at specific euchromatic and heterochromatic loci. Bisulfite treatment converts unmethylated cytosines to uracils, while methylated cytosines remain unchanged, and are evaluated by sequencing amplimers within CpG islands at each loci of interest. We expect clones produced from high Dnmt1s donor cells will have higher DNA methylation at these loci than clones produced from low Dnmt1s donor cells, and IVP control embryos. The present study will improve our understanding of the epigenetic reprogramming of cloned bovine embryos, and will provide for the further optimization of SCNT, allowing reprogramming to occur more normally, reducing abnormalities in cloned offspring and improving survival to term. Current research developments will be discussed. (Project supported by National Research Initiative Competitive Grant no. 2006-35203-16620 from the USDA Cooperative State Research, Education, and Extension Service).

## DECIPHERING EMBRYO-MATERNAL COMMUNICATION IN THE HORSE – A GENOMIC APPROACH

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Establishment of pregnancy requires elaborate embryo-maternal interaction to prolong luteal lifespan and prepare the uterine environment for embryo fixation and implantation. Unlike other species, such as ruminants and pigs, the underlying physiology of maternal recognition of pregnancy still remains to be elucidated in the horse. Microarray analyses as a tool to describe global gene expression have proven suitable to study reproductive processes and to promote more detailed understanding at a molecular level. The present work therefore uses transcriptional profiling of endometrial samples derived from pregnant and non-pregnant mares to study early pregnancy in the horse. We specifically hypothesize that the identification of transcripts differentially expressed at Day 13.5 of pregnancy (compared to Day 13.5 of diestrus) will reveal biochemical pathways in the endometrium crucial to an intact embryo-maternal communication in the horse. To construct an equine specific microarray, high-throughput 454 sequencing on normalized Day 13.5 pregnant and non-pregnant endometrial cDNA was performed. This sequencing approach yields approximately 100 million base pairs of sequencing information in a single run. One-half to two-third of the sequencing information obtained by the initial titration run represent new expressed sequence tags (ESTs). The results of the large scale production are pending. The information obtained through sequencing will be complemented with the 18,342 mRNA reference sequences available in the NCBI public databases to design probes for micorarray production. The resulting microarray will be hybridized to fluorescently labeled cDNA obtained from pregnant and non-pregnant animals to detect differentially expressed transcripts. The subset of transcripts showing a different expression level between pregnant and non-pregnant animals will be further analyzed regarding their biological function and their putative role during early pregnancy. To our knowledge this is the first study exploiting large scale transcriptional profiling of endometrium in the horse. This work will provide valuable insight into how the uterine environment responds to the presence of an embryo during early pregnancy. Research supported by the Grayson-Jockey-Club Research Foundation.

## ROLE OF THE EQUINE CONCEPTUS IN ENDOMETRIAL ANGIOGENESIS AND ARCHITECTURE

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Uterine architectural and vascular bed modifications are crucial for conceptus survival during early gestation. Many uterine adaptations have been described by B-mode and color-Doppler ultrasonography. The spherical embryonic vesicle moves throughout the entire uterine lumen many times a day and reaches its maximal mobility on Day 13-14. The mechanisms of maternal pregnancy recognition are still unknown but the mobility has been shown to be necessary. During the mobility phase, an increase in endometrial blood flow accompanies conceptus presence and a disproportional increase in the dorsal portion of the endometrium compared with the ventral portion have been described. Fixation of the conceptus in the base of one uterine horn occurs around Day 16 and a rampant increase in blood flow and dorsal endometrial encroachment around the conceptus is progressively observed. More intense changes are observed in the horn containing the conceptus and it leads us to our main hypothesis that endometrial angiogenesis and architecture changes are mediated by the presence of the conceptus. Histomorphology evaluation, immunolocalization of VEGF and its receptors (1 and 2) and Ki-67 proliferative marker, and quantitative gene expression analysis of VEGF and its receptors in the endometrium will be accomplished. Eleven pregnant ponies on Day 21 and six each for estrus and diestrus phases were used for endometrium biopsies in both uterine horns. For histomorphological evaluation, percentage of area with uterine glands, stroma, and blood vessels were calculated. In the pregnant group, a larger area with glands was observed closer to the luminal epithelium (*stratum spongiosum*) in the uterine horn ipsilateral to the conceptus when compared with the contralateral horn. The area occupied by blood vessels near the lumen was approximately 3-fold greater in the ipisilateral horn compared with the contralateral horn and in the deeper regions (*stratum basale*) was approximately 2-fold greater in the ipsilateral horn. Furthermore, the area occupied by blood vessels in the deeper regions was approximately 2-fold greater than in the luminal region. As a result, the deeper areas presented smaller areas of glands and larger area of stroma. Overall, results suggest elevated glandular secretory activity for histotrophic nutrition of the conceptus. The total number of glands did not differ between uterine horns in the pregnant group suggesting glandular hypertrophy or cellular proliferation. Mares in estrus presented smaller area of uterine glands in all endometrial areas when compared with pregnant and diestrus mares. During estrus, endometrial edema likely due to elevated estradiol levels is easily observed by ultrasonography and is the likely cause of stromal area increase. No differences were detected between uterine horns in glandular and stromal areas in estrous and diestrus mares indicating that differences observed between horns in pregnant mares are conceptus mediated. Blood vessel areas were 2 to 3.5-fold less in deep areas and 4 to 8-fold less near the luminal epithelium in mares in estrus and diestrus compared with pregnant mares. Area of blood vessels was similar between mares in estrus and diestrus. Partial results of immunohistochemistry and immunofluorescence analysis in the endometrium of the pregnant group indicates strong expression of VEGFR-2 (KDR) in the luminal epithelium, glandular epithelium, and endothelium. Staining in stromal cells was also observed although with less intensity.

**OPTIMIZATION OF CULTURE CONDITIONS FOR EQUINE UMBILICAL CORD BLOOD DERIVED STEM CELLS**

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Equine umbilical cord blood derived stem cells (eUCB) are naïve cells with great potential as a therapeutic aid. One of the challenges of *in vitro* stem cell research is expansion and maintenance of a malleable population of stem cells throughout the duration of culture. Thus, the objective of this work was to identify a set of culture conditions that optimized the maintenance of the naïve eUCB stem cell. Three substrata (uncoated plastic, gelatin, and fibronectin) and four media (FBS only: 10% fetal bovine serum, FBS, in Dulbecco's Modified Eagle Medium; Modified FBS: 10% FBS, 1X Nonessential Amino Acids, 10 ng/ml basic fibroblast growth factor, 1% Insulin-transferrin-selenium in DMEM, McGuckin: 10% FBS, 50 ng/ml Flt3, 10 ng/ml thrombopoietin, 20 ng/ml c-Kit; and eUCB conditioned medium) were chosen from current human and mouse literature. Cell number was recorded daily until 100% confluency was obtained. Population doubling times were calculated as $N=(N_0)(2^{t/dt})$ where N is the final cell number, $N_0$ is the initial cell number, t is time in hours and dt is doubling time. Total RNA was isolated and RT-PCR performed for Oct4, Nanog, Sox2, Klf4, and c-Myc, markers of stem cell pluripotency. GAPDH was included as a control. eUCB stem cells seeded on uncoated plastic had the longest doubling time (44.69 ± 2.33 hours). Both fibronectin and gelatin coatings decreased doubling times in all animals compared to uncoated plastic ($p<0.01$; 30.20 ± 1.14 and 29.52 ± 1.07 hours, respectively). On all substrata, the McGuckin and conditioned media resulted in a severe depression in cell growth (no change or loss in cell number over five days of culture) and were excluded from further experiments. FBS only media promoted cell growth over the modified FBS media ($P<0.01$, 31.19 ± 1.74 and 38.42 ± 2.14 hrs, respectively). However, RT-PCR and immunocytochemistry results showed no differences in Oct4, Nanog, Sox2, Klf4, or c-Myc expression between these two media on any substrata. eUCB population growth is enhanced by culture on fibronectin or gelatin. Pluripotency markers are maintained on these substrata in both FBS only and modified FBS media. Therefore, eUCB culture in either FBS media on a gelatin or fibronectin coated plate optimizes expansion rate while maintaining markers of stem cell pluripotency.

## INVESTIGATION OF MYOSTATIN GENE MUTATIONS IN COMMON CATTLE BREEDS

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Myostatin, also known as growth and differentiation factor 8 (GDF8), is a unique negative regulator of muscle growth in the TGFβ superfamily. Myostatin is expressed in both embryonic and adult skeletal muscle in mice, humans, cattle, and fish. Myostatin exhibits a large number of polymorphisms, some of which cause truncation of the protein product, and therefore, a loss of function. Previous work has demonstrated *myostatin*$^{(-/-)}$ mice have a dramatic increase in skeletal muscle mass. In cattle, disruption of the myostatin gene can result in bovine muscular hypertrophy syndrome (***mh***). Cattle with ***mh*** have greatly increased muscle development (often termed double muscling) and often have faster growth rates and improved carcass traits. However, ***mh*** cattle have significantly less fat in their carcass and marked decreases in intramuscular fat. Cattle with ***mh*** have a greater incidence of dystocia and therefore, the economical value of them is dependent on the level of management available. The phenotype of animals with *myostatin* mutations can vary greatly in intensity among and within different breeds of cattle. Some mutations within the myostatin gene are non-disruptive and are seen across many breeds of cattle, resulting in a wild type phenotype. The frequency of disruptive mutations among breeds is much lower, but is seen in breeds such as Belgian Blue, Piedmontese, Charolais, and Limousine. However, some breeds, such as the Aubrac, have non-disruptive mutations but display a greater phenotypic influence than would be expected for conservative mutations. From a production standpoint, one *myostatin* mutation has been correlated with an intermediately muscled animal with desirable carcass characteristics. The mutation results in substitution of phenylalanine by leucine at position 94 (F94L) of the protein sequence. The F49L mutation has been identified in Angus, Aubrac, Charolais, Limousine, and Longhorn cattle. Carcasses of Jersey x Limousine divergent reciprocal backcrosses containing at least one copy of the F94L mutation demonstrated a 5.5% larger ribeye area, as well as a 2.3% increase in total meat percentage compared to the wild type. Another study also showed more dramatic effects on carcass characteristics when two copies of the same mutant allele were present. Fat percentage was decreased in these cattle, but meat quality (as measured by pH and tenderness) was not affected by the mutant allele. Birth weight and pelvic area of F94L cattle were similar to wild type cattle, suggesting that dystocia would not be as prevalent as cattle with the ***mh*** phenotype. Development of a simple method to identify the genotypes of specific animals would allow breeders to make more informed breeding decisions within their herds. Selection for cattle exhibiting greater carcass values without the negative aspects of a true double muscled animal would allow for more efficient beef production. The objectives of our studies are to 1) identify what mutations are present in Aubrac cattle, 2) test compound mutations *in vitro*, and 3) determine if we can generate compound mutations in the live animal.

**INHIBITION OF NO, $K_{ATP}$, AND PROSTANOIDS DECREASES HYPOXIA-INDUCED, BUT NOT $H_2S$-INDUCED, CONTRACTIONS IN RAT AORTA**

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Hypoxia and $H_2S$ elicit similar contractile responses in every vertebrate smooth muscle thus far tested, but the mechanisms are poorly understood. In vascular smooth muscle, blockade of nitric oxide (NO), ATP-sensitive potassium channels ($K_{ATP}$), and prostanoid production have each been shown to modulate aortic responses to hypoxia or $H_2S$, but no study has tested whether the effect of blockade on the responses to hypoxia and $H_2S$ are similar in the same tissue preparation. We tested this with aortic rings from Fisher 344 rats using standard vascular myography techniques. We found that both hypoxia and $H_2S$ elicit a tri-phasic, contract-relax-contract response. Hypoxia-induced contractions were significantly reduced by the NOS inhibitor L-NAME ($P < 0.029$; ANOVA, Fisher LSD), the $K_{ATP}$ inhibitor glibenclamide ($P < 0.020$), and by a cocktail of glibenclamide, L-NAME and the prostanoid inhibitors esculetin, clotrimazole and indomethacin ($P < 0.002$), indicating a role for NO, $K_{ATP}$ and prostanoids in modulating hypoxia signaling. In contrast, $H_2S$ signaling was not significantly affected by any of these inhibitors ($P > 0.134$; ANOVA). Therefore, the mechanisms by which hypoxia and $H_2S$ elicit a contract-relax-contract response in rat aortic smooth muscle appear to be different. It remains to be tested whether this is true for other smooth muscle tissues. Research support: NSF IBN-0422139.

**EFFECT OF PREINCUBATION OF SPERM AT 38.5ºC OR 40ºC BEFORE INSEMINATION ON DEVELOPMENTAL COMPETENCE OF BOVINE EMBRYOS DERIVED FROM IN VITRO FERTILIZATION**

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The ability of the preimplantation embryo to develop depends in part on genetic and non-genetic inheritance from gametes. Here, the hypothesis was tested that aging of ejaculated sperm would affect embryo competence for development. Oocytes were inseminated with frozen/thawed sperm maintained for 4 h at 38.5ºC or 40ºC. Control oocytes were inseminated with sperm not subjected to 4 h incubation. The percent of cleaved embryos that became blastocysts at Day 8 after insemination was used to assess embryo competence. In Exp. 1 (n=9 bulls), oocytes were fertilized for 22 h and the resultant embryos cultured in 5% $CO_2$ in air. The percent of oocytes that became blastocysts and percent of cleaved embryos that became blastocysts was lower for oocytes inseminated with preincubated sperm than for oocytes inseminated with control sperm ($P<0.01$).There were no differences between oocytes inseminated with sperm at 38.5ºC when compared to 40ºC. Exp. 2 (n=13 bulls) was carried out as for Exp. 1 except that embryos were cultured in 5% $O_2$. The percent of oocytes that cleaved (66.1 ± 3.2, 60.2 ± 3.2% and 74.5 ± 3.2% for 38.5°C, 40°C and control; $P<0.05$), that became blastocysts (23.6 ± 2.3, 14.9 ± 2.3% and 27.9 ± 2.3%, $P<0.01$) and the percent of cleaved embryos that became blastocysts (32.9 ± 2.9, 22.2 ± 2.9%, and 37.4 ± 2.9%, $P<0.05$) was lower for oocytes inseminated with preincubated sperm. Similarly, the percent of oocytes that became blasts ($P<0.05$) and the percent of cleaved embryos that became blastocysts ($P<0.05$) was lower for oocytes inseminated with sperm at 40ºC than for oocytes inseminated with sperm at 38.5ºC. A high cleavage rate in a parthenogenesis group (38.9 ± 3.2%) made interpretation of data difficult because of the possibility that fertilization rate was overestimated. Accordingly, Exp. 3 (n=8 bulls) was carried out with fertilization reduced to 8 h. There were no differences between oocytes inseminated with sperm preincubated at 40ºC compared to oocytes inseminated with sperm preincubated at 38.5ºC. Results indicate that embryos produced with aged sperm can have reduced competence for development although aging effects were independent of temperature within the range of 38.5-40 ºC. Support: USDA NRICGP 2007-35203-18070 and BARD US 3986-07.

**THE YIN-YANG OF APOPTOSIS: DEVELOPMENTAL CHANGES IN THE CONC-ENTRATION OF BCL-2 AND BAX IN PREIMPLANTATION BOVINE EMBRYOS**

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Preimplantation embryos undergo apoptosis in a stage-dependent manner. Spontaneous and induced apoptosis is blocked in 2-cell and 4-cell embryos (Matwee et al., Zygote 8:57-68, 2000; Paula-Lopes and Hansen, Biol Reprod 66:1169-1177, 2002). Capacity for apoptosis is restored at ~ the 8-16 cell stage, a stage coincident with embryonic genome activation. The long-term objective is to determine the molecular and cellular basis for developmental regulation of apoptosis in the preimplantation embryo. Induction of apoptosis by heat shock involves depolarization of the mitochondria as an early signal in the caspase cascade leading to apoptosis. Previous results indicate that the mitochondrion is resistant to depolarization by apoptosis-inducing signals (Brad et al., Reproduction 134: 789-797; de Castro et al., Mol. Reprod. Dev., in press). Whether depolarization of the mitochondrial membrane occurs in response to a pro-apoptotic signal depends upon the complex balance of anti- versus pro- apoptotic proteins. Here, we hypothesized that 2-cell embryos have higher amounts of anti-apoptotic proteins and lower amounts of pro-apoptotic proteins when compared to embryos $\geq$16-cells. The anti-apoptotic protein examined was Bcl-2 while the pro-apoptotic protein examined was Bax. Concentrations in embryos were determined by immunoflourescence microscopy. Briefly, bovine embryos were produced in vitro and cultured in KSOM-BE2. Two-cell embryos were collected between 28 to 30 h post-insemination and embryos $\geq$16-cells were collected at Day 5 post-insemination. Antibodies for Bcl-2, Bax, or nonspecific rabbit IgG were labeled with the Alexa 488 Zenon Labeling Kit (Invitrogen). Embryos at the 2-cell stage and the $\geq$16 cell stage were incubated with either Bcl-2:Alexa 488, Bax:Alexa 488, or rabbit IgG:Alexa 488 for 1 h. Embryos were counterstained with Hoechst 33342. Individual mean pixel intensity was measured on a per embryo basis using Zeiss Axiovision software. Representative images are shown in Figure 1. Average mean pixel intensities for IgG control embryos were subtracted from the values of Ab-stained embryos to compensate for background and autofluorescence. Amount of immunofluoresence caused by reaction with anti-Bcl-2 was greater at the 2-cell stage than for embryos $\geq$ 16 cells (585 $\pm$ 84.7 vs 131 $\pm$ 86.4; $P<0.0001$) (n=83). Preliminary results for Bax indicate that this protein is also more abundant at the 2-cell stage (164 ± 26.8 vs 25 ± 30.0; $P<0.01$) (n=18). These data suggest an overall loss of proteins of the Bcl-2 family during early development. This loss may be part of a global reduction in protein content (Thompson et al., Mol. Reprod Dev., 50:139-145, 1998) or may reflect a loss of mitochondria during this period (May-Panloup, Reprod. Biol. Endocrinol. 3:65, 2005). Given that both pro- and anti-apoptotic proteins declined from the 2-cell to $\geq$ 16 cell stage, it is unclear whether changes in Bcl-2 proteins are responsible for developmental changes in apoptosis. Research supported by USDA NRICGP 2007-35203-18070.

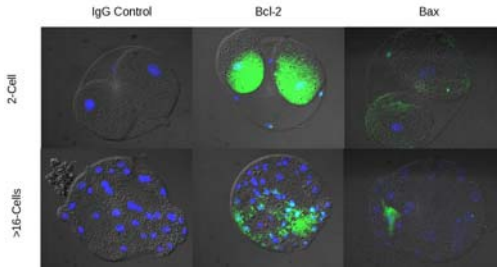


Figure 1. Representative images for immunofluorescent labeling of embryos. Green=antibody; blue=DNA. Note that embryos were also photographed under light microscopy using differential interference contrast.

**DEVELOPMENTAL CHANGES IN ACTIONS OF INSULIN-LIKE GROWTH FACTOR-I IN THE PREIMPLANTATION BOVINE EMBRYO – RECEPTOR EXPRESSION, AKT ACTIVATION AND THERMOPROTECTION**

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Insulin-like growth factor-I (IGF-I) can affect function of the preimplantation bovine embryo by increasing the proportion of cultured embryos that become blastocysts, reducing effects of heat-shock on development and apoptosis, and enhancing the survival of embryos transferred into heat-stressed recipients. It is not known how early in development that IGF-I can affect embryo physiology. By Day 5, the embryo is sensitive to IGF-I as determined by activation of the phosphatidylinositol 3-kinase/Akt pathway and activation of thermoprotective mechanisms. Experiments are ongoing to determine whether the two-cell embryo is also responsive to IGF-I. In the first study, the presence of the IGF-I receptor (IGF-IR) was evaluated by immunofluorescent techniques using a rabbit polyclonal antibody against a synthetic peptide from human IGF-IRβ subunit. Rabbit IgG was used as a negative control. The Zenon labeling method (Invitrogen) was used to label antibody. In this method, antibody is labeled with Fab fragments of anti-rabbit IgG conjugated with Alexa 488. Using this technique, specific labeling for IGF-IR was observed for two-cell embryos (n=20) and embryos $\geq$ 16 cells collected at Day 5 after insemination (n=17). Currently, two experiments are underway. The objective of the first is to test the ability of IGF-I to activate Akt. Two-cell embryos and Day 5 embryos are treated with 100 ng/mL IGF-I or vehicle. After 15 min, embryos are fixed and processed for immunofluorescence using a rabbit polyclonal antibody specific for phosphorylated Akt (at amino acid S473). In the second experiment, the thermoprotective actions of IGF-I are being determined at the two-cell stage and Day 5. In particular, it is being tested whether the negative effect of culture at 41$^{o}$C for 15 h on development to the blastocyst stage is blocked by incubation with 100 ng/mL IGF-I. (Support: USDA NRI 2007-35203-18070 and BARD US-3986-07).

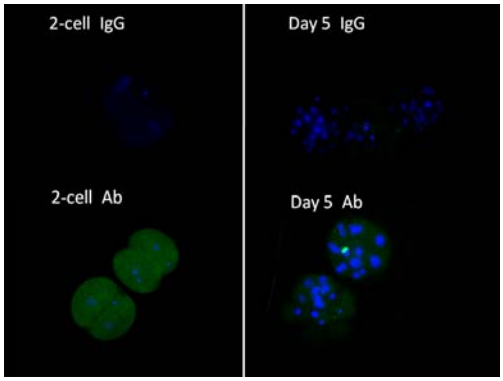


**Figure 1. Labeling of two-cell and Day 5 bovine embryos with anti-IGF-IRβ subunit (green) and DNA (blue; shown only for two-cell embryos). The top panel represents embryos incubated with Zenon labeled IgG while the bottom panels represents embryos incubated with anti-IGF-IRβ subunit.**

**INSULIN-LIKE GROWTH FACTOR-1 REDUCES THE ANTI-DEVELOPMENT EFFECTS OF MENADIONE ON DEVELOPMENT OF BOVINE PREIMPLANTATION EMBRYOS**

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Insulin-like growth factor-1 (IGF-1) can reduce effects of heat shock on development of preimplantation bovine embryos. The present objective was to determine whether IGF-1 can block other adverse environmental conditions affecting embryonic development. The approach was to evaluate effects of IG-1 on embryos treated with menadione. This polycyclic aromatic quinone serves as a precursor for vitamin K2 and can inhibit development of mouse embryos (Toxicology 191:77). Bovine embryos were produced in vitro and cultured in KSOM-BE2 medium. At Day 5 after fertilization, embryos $\geq$ 16 cells were placed in fresh drops $\pm$ 100 ng/ml of recombinant human [Arg3]-IGF-1 (analog with reduced affinity for IGF binding proteins). Menadione was added 1 h after addition of IGF-1 at a final concentration of 0, 1, 2.5 and 5 µM. Embryos were cultured for 24 h, washed, and then cultured in fresh KSOM-BE2 medium until Day 8. The experiment was replicated 14 times with 120-204 embryos per treatment. The percent of embryos that became blastocysts at Day 8 was affected by the menadione x IGF interaction ($P<0.001$). Menadione reduced development in a concentration-dependent manner and IGF-1 reduced effects of 1 and 2.5 µM. Percent of embryos becoming blastocysts was 50.1 $\pm$ 2.4%, 36.2 $\pm$ 2.9%, 5.8 $\pm$ 2.9%, and 0 $\pm$ 2.6% for 0, 1, 2.5 and 5 µM in the absence of IGF-1 and 37.0 $\pm$ 2.5%, 46.3 $\pm$ 2.5%, 13.0 $\pm$ 2.5% and 0 $\pm$ 2.6% in the presence of IGF-1. In conclusion, the anti-developmental action of menadione on development of bovine embryos can be blocked by IGF-1. Research supported by BARD US-3986-07 and USDA NRICGP 2007-35203-18070.

## NOTES

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## History of the AMCB Research Symposium

| Year | Location | Distinguished Lecturer |
|---|---|---|
| 2003 | Whitney Laboratory, St. Augustine, FL | Randy Prather, University of Missouri |
| 2004 | Chinsegut Hill, Brooksville, FL | John Dobrinsky, USDA-ARS, Beltsville, MD |
| 2005 | Chinsegut Hill, Brooksville FL | Doug Stocco, Texas Tech University |
| 2006 | Lake Wauberg & Animal Science Bldg, UF, Gainesville, FL | Ida Dobrinski, Univ. Pennsylvania |
| 2007 | Whitney Laboratory, St. Augustine, FL | Douglas Bannerman, USDA-ARS, Beltsville, MD |
| 2008 | Cedar Cove Beach and Yacht Club, Cedar Key, FL | Eckhard Wolf, Genzentrum der LMU-München |