ELEVENTH ANNUAL RESEARCH SYMPOSIUM

ANIMAL MOLECULAR AND CELLULAR BIOLOGY GRADUATE PROGRAM

UNIVERSITY OF FLORIDA

Harbor Front Hampton Inn & Suites
Fernandina Beach, Florida
April 5-6, 2013

WELCOME

The Animal Molecular and Cellular Biology (AMCB) Symposium Committee would like to welcome faculty and students to this year's Research Symposium held at the seaside resort of Fernandina Beach, FL. Fernandina was the last city founded by Spain in the Western Hemisphere (1811) and has seen 8 flags fly over it including that of France, Spain, Great Britain, East Florida Patriots, Green Cross, Mexico, Confederate States of America and the USA.

On behalf of the faculty of the AMCB, we welcome you to our 11th symposium and wish you hearty portions of good science, exceptional fellowship and lasting memories.

Pete Hansen, Director

ACKNOWLEDGMENTS

The faculty and students of the AMCB Program thank the following for support of the 11th Annual Research Symposium

Dr. John Hayes, Dean for Research, IFAS, University of Florida

Dr. David Norton, Vice President for Research, University of Florida

Special thanks to Jeremy Block and Ovatech LLC, Gainesville, Florida for financial support

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

Drs. Adegbola Adesogan, Graduate Coordinator, Animal Molecular and Cellular Biology Graduate Program, University of Florida

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

Special thanks to Jim Moss and company for preparing the Friday night meal

2013 AMCB DISTINGUISHED LECTURER

Martin Sheldon, BVSc PhD FRCVS
Institute of Life Science
School of Medicine, Swansea University

Professor Martin Sheldon was educated at Bradford Grammar School and the University of Liverpool. He qualified as a veterinary surgeon in 1984 and worked in clinical practice for 14 years in West Wales, becoming a partner in 1986. Martin was awarded the Diploma in Bovine Reproduction from the University of Liverpool in 1992; became a Royal College of Veterinary Surgeons Specialist in 1993; and was awarded the Diploma in Cattle Health and Production from the Royal College of Veterinary Surgeons in 1997.

Sheldon joined the Royal Veterinary College in 1998, where he taught veterinary reproduction and completed a PhD with Professor Hilary Dobson in 2002 through the University of Liverpool. He was awarded the James Bee Educator Prize twice by the Royal Veterinary College. Research project funding from the Wellcome Trust and BBSRC formed the foundation for studying the mechanisms of microbiology, infection and immunity in the female genital tract. He spent some time on an OECD sabbatical in Cornell University, USA; as a visiting fellow at the University of Bologna, Italy; and, on the Frontiers in Reproduction Course at the Marine Biological Laboratory, Woods Hole, USA.

Sheldon was awarded a 3-year BBSRC Research Development Fellowship in 2006 to develop a full-time research career and in 2008 he moved to a new Chair at the Institute of Life Science at the School of Medicine, Swansea University. He has given keynote presentations across the World, including USA, Canada, Europe, New Zealand and Japan.

Professor Sheldon's team are engaged in research on host-pathogen interactions and how microbes are sensed by the innate immune system. Program and project grant funding is from BBSRC, industry and the EU.

AMCB FACULTY

Lokenga Badinga, Department of Animal Sciences
Mary B. Brown, Department of Infectious Diseases and Pathology
Geoffrey E. Dahl, Department of Animal Sciences
John Driver, Department of Animal Sciences
Daniel A. Hahn, Department of Entomology and Nematology
Peter J. Hansen, Department of Animal Sciences
Kwang Cheol Jeong, Department of Animal Sciences
David Julian, Department of Biology
Maureen Keller-Wood, Department of Pharmacodynamics
Christopher Mortensen, Department of Animal Sciences
Jose E.P. Santos, Department of Animal Sciences
Stephanie Wohlgemuth, Department of Animal Sciences
Charles E. Wood, Department of Physiology and Functional Genomics

Emeritus Faculty

William C. Buhi, Departments of Obstetrics & Gynecology, Animal Sciences
Kenneth C. Drury, Department of Obstetrics & Gynecology
Michael J. Fields, Department of Animal Sciences
Daniel C. Sharp, Department of Animal Sciences
William W. Thatcher, Department of Animal Sciences

CURRENT AMCB STUDENTS

PhD Students

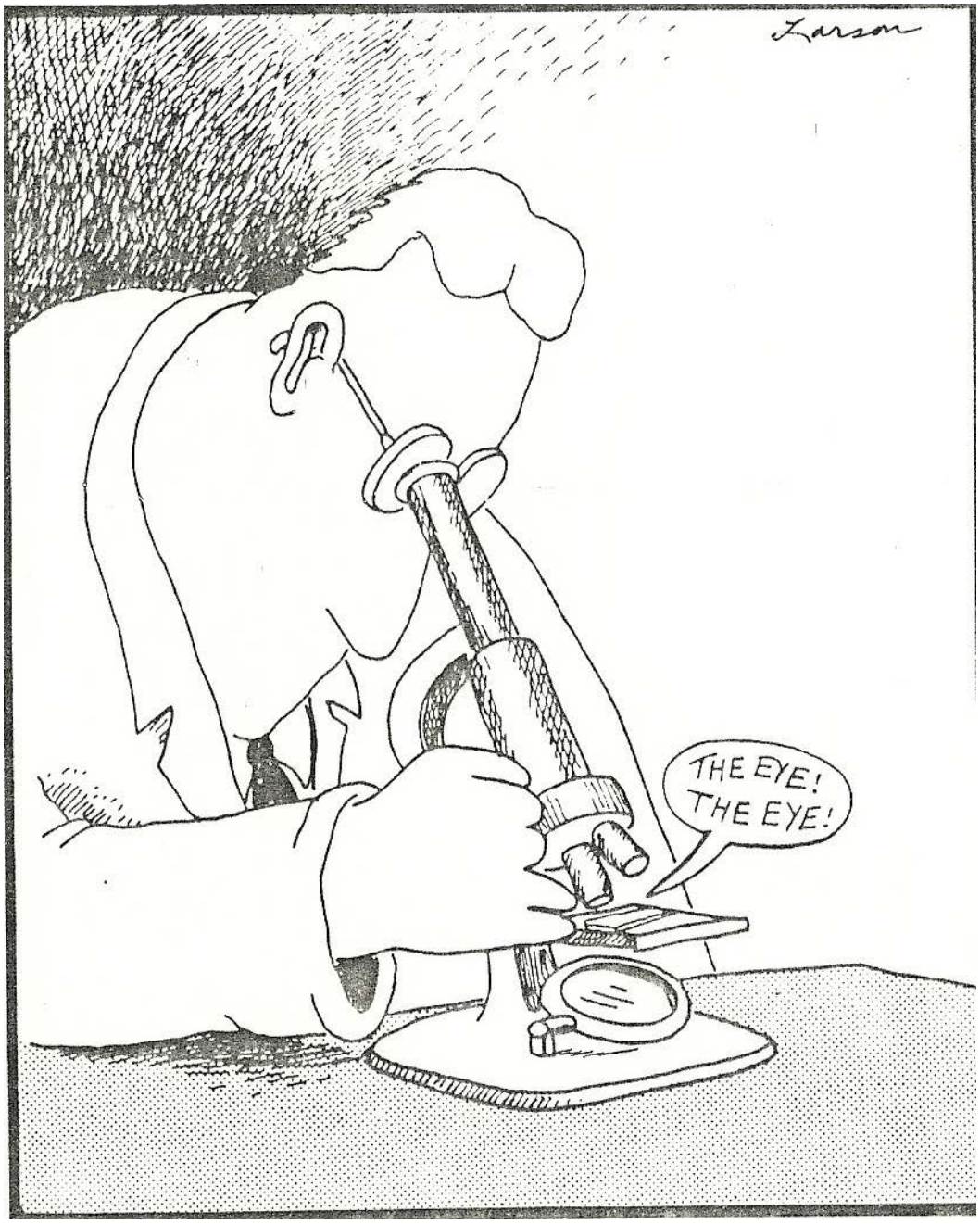
Sarah Cochran (Advisor: PJ Hansen)
Anna Denicol (Advisor: PJ Hansen)
Kyle Dobbs (Advisor: PJ Hansen)
Ashley Grapes (Advisor: C Wood)
Leandro Greco (Advisor: JEP Santos)
Dale Kelley (Advisor: C Mortensen)
Martha Sofia Ortega (Advisor: PJ Hansen)
Eduardo Ribeiro (Advisor: JEP Santos)
Leticia Del-Penho Sinedino (Advisor: JEP Santos)
Christina Vasquez (Advisor: D Julian)

MS Students

Gabriel Carvalho Gomes (Advisor: JEP Santos)

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 Wauburg Gainesville, FL	Ina Dobrinski University of Pennsylvania
2007	Whitney Laboratory St. Augustine, FL	Doug Bannerman USDA-ARS, Beltsville, MD
2008	Cedar Cove Beach & Yacht Club Cedar Key, FL	Eckhard Wolf LMU Munich, Germany
2009	Plantation Golf Resort and Spa Crystal River, FL	Dean Betts University of Western Ontario
2010	Whitney Laboratory St. Augustine, FL	Marc-Andre Sirard Laval University
2011	Steinhatchee Landing Resort Steinhatchee, FL	Kimberly Vonnahme North Dakota State Univ.
2012	Holiday Isle Oceanfront Resort St. Augustine, FL	Rocio Rivera University of Missouri
2013	Harbor Front Hampton Inn Fernandina Beach, Florida	Martin Sheldon Swansea University


Larson
THE EYE!
THE EYE!

SCHEDULE OF EVENTS

FRIDAY, APRIL 5

12:45 PM Pete Hansen
Welcome, introductory comments

Session 1: Embryonic and Fetal Development
Leticia Del-Penho Sinedino, Chair

1:00 PM Kyle Dobbs, Animal Sciences
Dynamics of the methylome during early development of the preimplantation bovine embryo

1:15 PM Anna Denicol, Animal Sciences
Exposure of bovine embryos to WNT antagonist dickkopf-1 and to colony-stimulating factor 2 enhances embryo survival and pregnancy rate following embryo transfer in lactating dairy cows

1:30 PM Rafael Bisinotto, Animal Sciences
Effects of follicular wave and progesterone concentration during follicle growth on conceptus and endometrium global gene expression in dairy cows

1:45 PM Sofia Ortega, Animal Sciences
Changes in expression of CWC15 during preimplantation development in the bovine embryo

2:00 PM Antonio Ruiz, Animal Sciences
Effects of addition of forskolin, L-carnitine, trans 10, cis 12 conjugated linoleic acid and phenozine ethosulfate during embryo culture on lipid content, abundance of genes involved in lipid metabolism and embryo survival following cryopreservation

2:15 PM Maria Padua, Physiological Sciences
Disruption of ovarian and testicular development in mice by the conditional deletion of the genes encoding the transcription factors GATA4 and GATA6

2:30 BREAK

Session 2: 2013 AMCB Distinguished Lecturer Presentation
Pete Hansen, Chair

3:00 PM Professor Martin Sheldon
Institute of Life Science, School of Medicine, Swansea University
Mechanisms of infection and immunity in the female genital tract of cattle

4:00 PM Break

Session 3: Microbiology and Immunology
Sofia Ortega, Chair

4:15 PM Soojin Jeon, Animal Sciences/Emerging Pathogens Institute
Animal genetic and physiological factors contribute to the prevalence of E. coli *O157 in cattle*

4:45 PM Won-Sik Yeo, Animal Sciences/Emerging Pathogens Institute
Paralogous non-LEE effectors of enterohemorrhagic Escherichia coli *target disparate loci in host cells and cause cell damage*

5:00 PM Raes Mir, Animal Sciences/Emerging Pathogens Institute
Is cefotaxime resistance endemic in farm animals?

5:15 PM Fabio Lima, Animal Sciences
Effects of intrauterine infusion of Trueperella pyogenes *on endometrial mRNA expression of genes associated with luteolysis in dairy cows*

5:30 PM Bianca Libanori Artiaga, Animal Sciences
Targeting natural killer T cells to enhance immunity: A novel swine model

5:45 PM Dale Kelley, Animal Sciences
Characterization of MHC Class I conserved locus and Locus II mRNA in three Equus africanus somaliensis *jennies*

6:00 PM Check into rooms, break

7:00 PM Cookout (location to be announced)

SATURDAY, APRIL 6

6:00-10:00 AM Hot breakfast, second floor café.

Session 4: *Adverse Environments*
Gabriel Carvalho Gomes, Chair

9:00 AM Ashley Grapes, Physiology and Functional Genomics
Effect of triclosan on placental gene expression in the late gestation sheep

9:15 AM Dana Schreffler, Animal Sciences
Effects of PPARγ agonist rosiglitazone on cardiac tissue and cultured cardiomyocytes

9:30 AM Christina Vasquez, Biology
Multiple stressor interactions delay horseshoe crab embryo development

9:45 AM Maria Von Chamier, Infectious Diseases and Pathology
Impact of combined infection and prenatal nicotine exposure on postnatal outcome

10:00 AM Sha Tao, Animal Sciences
Effect of in utero *heat stress on insulin response of calves after weaning*

10:15 AM Gabriel Carvalho Gomes, Animal Sciences
Effects of evaporative cooling prepartum and vitamin E supplementation on performance and conceptus development in Holstein cows during summer in Florida

10:30 AM Break

Session 5: *Dairy Cattle Management*
Ashley Grapes, Chair

10:45 AM João Henrique Jabur Bittar, Large Animal Clinical Sciences
Effects of induction of ovulation early in lactation on uterine health and fertility in dairy cows

11:00 AM Leticia Del-Penho Sinedino, Animal Sciences
Effect of early or late resynchronization on reproductive performance of dairy cows observed for estrus

11:15 AM Miriam Garcia Orellena, Animal Sciences
Does supplementing essential fatty acids in the late gestation and the preweaning periods influence future productivity of Holstein heifers?

11:30 AM Natalia Martinez-Patino, Animal Sciences
Effect of induced subclinical hypocalcemia on clinical parameters and immune function in dairy cows

11:45 AM Eduardo Ribeiro, Animal Sciences
Low doses of recombinant bovine somatotropin (rbST) enhance fertility of dairy cows

12:00 PM Closing (and very brief) remarks, Pete Hansen

ABSTRACTS

(Arranged alphabetically by first author)

Targeting natural killer T cells to enhance immunity: A novel swine model

B.L. Artiaga[1], P.M. Mercadante[1], and J.P. Driver[1]

Department of Animal Sciences, University of Florida, Gainesville, FL[1]

Natural killer T (NKT) cells are a thymus-dependent T-cell subset that recognize glycolipid molecules presented by the major histocompatibility complex (MHC) class I-like molecule CD1d. NKT cells stimulated by glycolipid-CD1d complexes induce profound effects on the immune system in part by secreting large quantities of immune signaling molecules known as cytokines. It is believed that activated NKT cells play an important immunomodulatory function that prevents a host of immune disorders including multiple types of infectious disease. Much interest is currently focused on improving immunity in humans using synthetic glycolipid therapeutics including the NKT cell superagonist α-galactosylceramide (α-GalCer). Unfortunately, studies using mice have been difficult to translate to humans because NKT cell frequency, function and subsets differ dramatically between these two species. For this reason, we initiated studies to determine if pigs, a species much more immunologically similar to humans, provide a suitable model to determine how NKT cells may be harnessed for immunotherapeutic purposes. Analysis of peripheral blood, spleen, lymph nodes, liver and thymus indicate that the frequency and subsets of NKT cells in pigs is more similar to humans than mice. Also, NKT cells in pigs, like humans and mice, were found capable of rapidly producing the cytokines IFNγ and IL-4 upon stimulation and may be expanded in culture with α-GalCer and IL-2. One method NKT cells may be utilized to improve immunity is by administering NKT cell superagonists such as α-GalCer with vaccines. NKT cells activated with α-GalCer release cytokines with strong adjuvant effects that boost immunity to microbial antigens contained within the vaccine that prevents infection when an animal later encounters the live pathogen. To establish whether NKT cells can be harnessed to improve immune responses to foreign antigens, pigs were treated with the neoantigen hen-egg-lysozyme (HEL), alone or in combination with α-GalCer. Later analysis of serum from these animals demonstrated that NKT cell activation at the time of vaccination greatly enhanced antibody responses to HEL compared to control. Collectively, our results indicate that swine NKT cells share many similarities with those from humans and that swine NKT cells may be targeted to enhance immune responses against microbial pathogens. Future efforts will focus on exploiting our model system to establish how NKT cell therapeutics may be utilized to improve human immunity as well as the health of commercial swineherds.

Effects of follicular wave and progesterone concentration during follicle growth on conceptus and endometrium global gene expression in dairy cows

R.S. Bisinotto[1], D. Taylor-Rodriguez[1], E.S. Ribeiro[1], L.F. Greco[1], F.S. Lima[1], N. Martinez[1], R.L.A. Cerri[2], W.W. Thatcher[1], J.E.P. Santos[1]

[1]University of Florida, Gainesville. [2]University of British Columbia, Vancouver

Low progesterone (**P4**) concentrations during the growth of the ovulatory follicle impair fertility in dairy cows, whereas supplemental P4 reversed this negative impact. Objectives were to evaluate the effects of wave of the ovulatory follicle and P4 concentrations during follicular growth on subsequent conceptus and endometrium global gene expression. Non-lactating Holstein cows were induced to ovulate a follicle of the first (**FW**) or second follicular wave (**SW**), during which endogenous P4 is low and high, respectively. A third group of cows was induced to ovulate FW follicles and received exogenous P4 (**FWP4**). All cows were inseminated and then slaughtered 17 d after insemination. Total mRNA was extracted from recovered concepti and endometrium representative of the pregnant horn. Global gene expressions in conceptal and endometrial samples were evaluated using Affymetrix GeneChip Bovine Genome arrays (FW: n=5; FWP4: n=7; SW: n=4 from each tissue type). Probesets were summarized and normalized using GCRMA. Expression data were analyzed by robust regression using the LIMMA package in Bioconductor for R. Correction for multiple testing was performed using FDR. Genes with adjusted *P*-value $\leq$ 0.05 and fold change $\geq$ 1.5 were considered to be differentially expressed. The length of the concepti evaluated in this study were 14.9±3.0, 14.8±2.6, and 14.9±3.4 cm for FW, FWP4, and SW cows, respectively (*P*=0.99). Based on the number of genes differentially expressed, manipulating the wave of the ovulatory follicle and concentrations of P4 during follicular growth did not result in a major shift in gene expression in the endometrium of pregnant cows and concepti on d 17 after insemination. In the concepti, a greater number of genes were affected for SW vs. FW (n=27) and SW vs. FWP4 (n=27), whereas P4 supplementation (FW vs. FWP4) altered the expression of 18 probesets. Concepti from FW cows had increased expression of genes linked with cell metabolism and tissue differentiation, such as SLC5A1 (glucose transporter) and BEX5 (nerve tissue differentiation) compared with SW and FWP4. Conversely, DHX8 (nuclear export of spliced mRNA), B2M (component of MHC-I), and MYL4 (embryonic muscle) were upregulated and EMILIN2 (pro-apoptotic glycoprotein) was downregulated in SW compared with FW and FWP4. Fatty acid desaturase 3 (FADS3) was greater in SW than FWP4, indicating possible changes in fatty acid metabolism in pre-implantation embryos. The results from endometrium followed a similar pattern, with most differentially expressed genes observed for SW vs. FW (n=24), followed by SW vs. FWP4 (n=12), and FW vs. FWP4 (n=10). Endometrial expression of STAR was greater in SW than FW and FWP4. In addition, all immune-related genes (FCRL3, CD79A, and CD5L) differentially expressed for SW vs. FW were downregulated in the latter group. Noteworthy, these genes were not downregulated in FWP4 compared with FW. Further analyses to contextualize differentially expressed genes into biological processes are to be performed.

Effects of induction of ovulation early in lactation on uterine health and fertility in dairy cows

J.H.J. Bittar[*]; K.E. Hencken[*], P. Pinedo[*], C.C. Barbosa[*], M.Gobikrushanth[*], S. Croyle[*], C.A. Risco[*], A. Vieira-Neto[†], J.E.P. Santos[‡], and K.N. Galvão[*]

[*]*Department of Large Animal Clinical Sciences, College of Veterinary Medicine; University of Florida, Gainesville, FL, 32610, USA;* [†]*Departamento de Medicina Veterinária, Centro de Ciências Agroveterinárias (CAV) Universidade do Estado de Santa Catarina (UDESC), Lages, SC – Brazil;* [‡]*Department of Animal Sciences, University of Florida, Gainesville, FL, 32610, USA*

Holstein dairy cows have the first wave of follicle growth starting two weeks postpartum with about 30% of these cows ovulating the dominant follicle of the first follicular wave within 21 Days In Milk (DIM). Another 30 to 50% of the cows will ovulate follicles from subsequent follicular waves from 30 to 50 DIM and about 20 to 40% remain anovulatory by 60 DIM. Resumption of ovarian cyclicity before the first artificial insemination (AI) has been shown in several studies to be a positive determinant of first service pregnancy/AI, particularly cows that start cycling early in lactation. The objective was to evaluate the effects of GnRH early postpartum on induction of ovulation, uterine health and fertility in dairy cows. Lactating Holstein cows without a CL at 17±3 DIM (n=255) from the University of Florida Dairy Unit were randomly assigned to receive 100µg intramuscularly of GnRH agonist (n=128) at 17±3 DIM and at 20±3 DIM or to remain as controls (n=127). Cows had their ovaries scanned by ultrasonography (US) twice a week for a total of 4 US. Ovulation was characterized by the appearance of a Corpus Luteum (CL) ≥20mm in any US or when a CL<20mm appeared in two consecutive US. Clinical (CE) and subclinical (SCE) endometritis were diagnosed at 35±3 DIM. Data were analyzed using the LOGISTIC or PHREG procedures of SAS adjusting for any effect of parity, calving related problems, metabolic problems, or metritis. Cows receiving GnRH had increased ovulation (71.1 vs. 43.3%; $P< 0.001$). GnRH treatment (GTRT) did not affect the prevalence of CE (26.2 vs. 20.8; $P= 0.41$) or SCE (30.9 vs. 32.8; $P= 0.29$). Cows having calving problems (39.7 vs. 17.5%; $P=0.004$) and metritis (39.7 vs. 16.2%; $P< 0.001$) had increased prevalence of CE. Metritis (39.7 vs. 16.2%; $P< 0.001$) also increased the prevalence of SCE (50.7 vs. 23.5%; $P< 0.001$). Interaction between GTRT and ovulation after GTRT (GTRTxOv) showed that treated cows that ovulated had decreased SCE compared to cows that did not ovulate (25.6 vs. 43.2%; $P= 0.05$) while there was no difference ($P= 0.88$) in control cows. GTRT did not affect conception rate (CR) at 32 (42.2 vs. 43.3%; $P= 0.74$) or 74 d after AI (37.5 vs. 35.4%; $P= 0.26$), or pregnancy loss (11.1 vs. 18.2%; $P= 0.30$). Interaction GTRTxOv showed that treated cows that ovulated had increased CR at 74 d compared to cows that did not ovulate (42.9 vs. 24.3%; $P= 0.05$) while there was no difference ($P= 0.57$) in control cows. Interaction GTRTxOv showed that treated cows that did not ovulate had decreased hazard of pregnancy up to 300 DIM compared to cows that ovulated (HR= 0.53; $P= 0.01$), or control cows that did (HR= 0.56; $P= 0.03$) or did not ovulate (HR= 0.53; $P= 0.01$). GnRH treatment early postpartum increased ovulation however it did not improve uterine health or fertility.

Exposure of bovine embryos to WNT antagonist dickkopf-1 and to colony-stimulating factor 2 enhances embryo survival and pregnancy rate following embryo transfer in lactating dairy cows

Anna C. Denicol, Dale E. Kelley, Jeremy Block, Ky G. Pohler, Christopher J. Mortensen, and Peter J. Hansen

Department of Animal Sciences, University of Florida, Gainesville, FL and Department of Animal Sciences, University of Missouri, Columbia, MO.

Activation of the WNT signaling pathway stimulates cell proliferation and maintenance of pluripotency. Activation of WNT signaling at day 5 after insemination in *in vitro*-produced bovine embryos decreases blastocyst development at day 7. Colony-stimulating factor 2 (CSF2) has been shown to improve pregnancy rate following ET and to decrease expression of WNT-related genes in day 6 morulae. It was hypothesized that dickkopf-1 (DKK1), an antagonist of the WNT pathway, would increase embryo survival and pregnancy rate after embryo transfer (ET). Holstein embryos were produced *in vitro* using female sex-sorted semen. At day 5 after insemination, embryos were treated with vehicle, DKK1 (100 ng/ml) or CSF2 (10 ng/ml). At day 7, blastocysts were transferred to lactating Holstein cows (n=251). Pregnancy was diagnosed by rectal ultrasonography at day 32 of gestation (day 25 after ET). Blood samples were collected from a subset of 153 cows at the time of pregnancy diagnosis for analysis of plasma concentrations of pregnancy-associated glycoproteins (PAGs) using a monoclonal-based ELISA. Blastocyst development at day 7 was not affected by treatment but culture of embryos with DKK1 or CSF2 tended to improve (P=0.078) pregnancy rate as diagnosed by ultrasonography at day 32 of gestation (DKK1=38%; CSF2=37%, control=26%). Cows (n=153) were also classified as pregnant or non-pregnant based on PAG concentration in plasma at day 32 (cut-off for pregnancy > 0.922 ng/ml). Pregnancy rate was 51% for DKK1, 54% for CSF2, and 34% for control (control versus DKK1+CSF2, P = 0.04). Doppler ultrasonography was performed at day 34 in a subset of 20 pregnant cows. Embryos exposed to DKK1 (n=8) tended to be longer (1.5 cm ± 0.06, P = 0.08) compared to CSF2-treated (1.4 ± 0.06, n=8) and control embryos (1.4 ± 0.08, n=4). Mean resistance index in the uterine artery supplying the gravid horn was increased in cows carrying embryos exposed to DKK1 (1.1 ± 0.06, P = 0.05) compared to CSF2 (0.9 ± 0.06) and control (0.9 ± 0.09). Pregnancy loss at 64 days of gestation was 16% for cows receiving a control embryo, 19% for CSF2, and 13% for DKK1 embryos (P = 0.8). In conclusion, exposure to DKK1 and CSF2 from days 5 to 7 of embryo development improved embryo survival to day 32 of gestation. DKK1 positively affected embryo growth as evidenced by greater embryo length at day 34 of gestation. Treatment with DKK1 also altered vascular dynamics in the uterine blood supply. Support: USDA 2011-67015-30688.

Dynamics of the methylome during early development of the preimplantation bovine embryo

Kyle B. Dobbs[1], Marlon Rodriguez[1], Mateus J. Sudano[1,2], M. Sofia Ortega[1] and Peter J. Hansen[1]

[1]Department of Animal Sciences, DH Barron Reproductive and Perinatal Biology Research Program, and Genetics Institute, University of Florida, Gainesville, FL; [2]Universidade Estadual Paulista, Campus de Botucatu, São Paulo, Brazil

DNA methylation is an important epigenetic modification during embryogenesis. Preimplantation development in the mouse is characterized by a period of passive and active demethylation followed by *de novo* methylation mediated by DNA methytransferases 3a and 3b. The exact sequence of demethylation and methylation in bovine embryos has not been established. Nor is it known whether genetic factors, such as gender, or maternal signals that regulate development, such as colony stimulating factor 2 (CSF2), change DNA methylation during the preimplantation period. These questions were addressed here. In the first experiment, DNA methylation was assessed using immunofluorescent labeling with anti-5-methylcytosine for embryos produced with X-chromosome sorted sperm. Fluorescent intensity decreased from the 1-cell stage to the 6-8 cell stage (intensity=1.13±0.12 and 0.61±0.09 respectively, $p<0.0001$) before increasing thereafter and peaking at the blastocyst stage (intensity=1.25±0.09; $p<0.0001$). A subsequent experiment was performed using similar procedures except embryos were separately fertilized with X- and Y-sorted sperm. The developmental pattern was similar to the first experiment but there was stage x gender interaction ($P=0.0007$) because methylation was greater for females at the 8-cell stage (0.76 vs 0.51) but greater for males at the blastocyst stage (0.95 vs 1.08). In experiment 3, treatment with 10 ng/ml bovine CSF2 from Day 5-7 had no effect on labeling for DNA methylation at the blastocyst stage. Inner cell mass (ICM) was distinguished from trophectoderm (TE) by labeling with anti-CDX2; fluorescent intensity was less ($p<0.0001$) for ICM than TE (0.52 vs 1.17). In Experiment 4, high resolution melting analysis was used to assess methylation of a CpG rich region in an intronic region of *DNMT3B*. Methylation percent decreased between the 6-8 cell and the blastocyst stage (95.4% and 39.3% respectively, $p=0.004$) but there was no difference in methylation between ICM and TE. In Experiment 5, steady-state mRNA for *DNMT3B* was determined by real-time PCR. Expression decreased from the 2-cell stage to a nadir for Day 5 embryos >16 cells and then increased at the blastocyst state ($p<0.0001$). In conclusion, the methylome of the bovine embryo undergoes dynamic change during the preimplantation period with initial demethylation followed by *de novo* methylation. Gender affects the pattern of methylation with female embryos having less global methylation compared to males at the blastocyst stage. The ICM is less methylated than TE, which could be a requisite of maintenance of pluripotency. Changes in methylation status of the embryo are correlated with changes in expression of *DNMT3B*, which suggests an important role for this gene in developmental regulation of methylation. Moreover, developmental regulation of expression of *DNMT3B* may itself be determined, at least in part, by regulation of DNA methylation because the increase in *DNMT3B* expression at the blastocyst stage is accompanied by a reduction in DNA methylation for a region of the *DNMT3B* gene. Support: USDA 2011-67015-30688.

Does supplementing essential fatty acids in the late gestation and the preweaning periods influence future productivity of Holstein heifers?

M. Garcia*, L.F. Greco, W.W. Thatcher, J.E.P. Santos, and C.R. Staples

Dept. of Animal Sciences, University of Florida, Gainesville, Florida, USA

Information on the effect of feeding of long chain fatty acids (LCFA) on future offspring productivity is lacking. Holstein cows (n = 96) were fed 1) no fat supplement (control; 2.3% dietary fat), 2) hydrogenated LCFA (SFA, Energy Booster 100, Milk Specialties, Eden Prairie, MN; 1.7% of dietary DM), or 3) Ca salts of LCFA containing essential LCFA (EFA; Megalac-R, Arm and Hammer Animal Nutrition, Princeton, NJ; 2.0% of dietary DM) from dry-off to calving. Heifers (n = 56) born from these cows were fed a milk replacer (MR) of low linoleic acid (LLA. 0.56% LA, DM basis) or high LA (HLA, 1.78% LA, DM basis) during the first 60 d of life. A single grain mix was offered between 31 and 60 d of life. Performance of replacement heifers that went on to complete at least 290 DIM (n = 33) was evaluated. Diets did not affect culling rate. Body weight at birth was not influenced by dam diet but ADG for the first 60 d of life was greater for heifers born from dams fed SFA vs. EFA (0.45, 0.48, and 0.42 kg/d for heifers born from control, SFA-, and EFA-fed dams, respectively). Sixty-d intake of LA averaged 6.2 and 12.1 g/d for LLA- and HLA-MR fed heifers, respectively. Type of MR did not influence ADG (0.45 vs. 0.45 kg/d) during the first 60 d of life. After weaning and during the first lactation, heifers were fed diets formulated to meet nutritional requirements. Heifers born from dams supplemented with either fat source (control vs. (SFA + EFA)), tended to have a greater number of AI at first conception (2.6 vs. 1.7, $P = 0.06$) and were older at first calving (24.2 vs. 22.9 mo, $P = 0.02$). Heifers born from dams fed fat prepartum produced more ($P = 0.02$) mature equivalent milk during their first lactation (10,605 ± 458, 11,745 ± 486, and 12,559 ± 568 kg for heifers born from control (n = 13), SFA- (n = 11), and EFA-fed dams (n = 9), respectively). Heifers fed HLA MR did not produce more milk than those fed LLA MR (12,011 ± 423 vs. 11,262 ± 403 kg). Fat supplementation during the last 2 months of pregnancy can "program" calves in utero to produce more milk during their first lactation.

Effects of evaporative cooling prepartum and vitamin E supplementation on performance and conceptus development in Holstein cows during summer in Florida

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Objectives were to evaluate vitamin E (**VitE**) supplementation above NRC recommendations to cows managed in noncooled (**NC**) or cooled (**C**) environments during the last 4 weeks of pregnancy on performance, immune function and embryo development. Holstein cows (36 primiparous and 34 multiparous) were blocked by parity, milk yield, and body weight, and assigned randomly to 1 of 4 treatments arranged in a 2 x 2 factorial. Prepartum cows were provided only shade (NC) or shade plus evaporative cooling with fans and sprinklers (C). After calving, cows were housed together in free-stall facility equipped with fans and sprinklers. All-rac-alpha-tocopherol (DSM, Belvidere, NJ) was top-dressed daily at 1,000 IU prepartum and 500 IU postpartum for moderate VitE (**M**) or 3,000 IU prepartum and 2,000 IU postpartum for high VitE (**H**) resulting in treatments: NCM, NCH, CM, and CH. The study lasted from 4 weeks pre- to 15 weeks postpartum. Measurements included intake of dry matter (**DMI**), yields of milk and milk components, body weight, prepartum respiration rate and four times hourly measurement of vaginal temperatures for 7 d. Cows had the estrous cycles synchronized and were inseminated at 46 and 64±3 d postpartum and had their uteri flushed for conceptus collection 16 d after AI. Endometrial tissue was collected by biopsy immediately after uterine flush. Conceptus morphology and conceptus and endometrium RNA were extracted for analyses of gene expression. Continuous data were analyzed by ANOVA for repeated measures with the PROC MIXED of SAS. Binary data were analyzed with the PROC Logistic of SAS. Results were considered statistically significant if $P<0.05$. During prepartum, temperature and humidity index (**THI**) averaged (±SD) 74.8±4.9, and cows were exposed to THI>70 during 85% of the day. Providing prepartum evaporative cooling reduced body temperature during 7 h in the afternoon by 0.36ºC (39.42 vs. 39.07ºC) and respiration rate (69 vs. 43 breaths/min). Cows in C had increased prepartum DMI (8.9 vs. 10.3 Kg/d), yields of milk (31.0 vs. 33.8 Kg/d), energy corrected milk (30.9 vs. 34.5 Kg/d), 3.5% fat-corrected milk per Kg of DMI (1.49 vs. 1.69), and milk fat percentage (3.60 vs. 3.79%) compared with NC cows. Additional VitE increased milk fat (3.62 vs. 3.76%) and protein (2.96 vs. 3.02%) percentages. Response to VitE was dependent on parity as primiparous M produced more milk than H, whereas for multiparous those in H produced more milk than M. Thirty-three concepti were recovered (28% of flushes). Embryonic disc was observed in 80.7% of the concepti and 93.8% were elongated, and neither prepartum cooling nor level of dietary VitE influenced those responses. Conceptus length tended to be larger ($P=0.08$) for NC than C cows (45.7 vs. 23.1 mm), and an interaction ($P<0.01$) between cooling and VitE was observed for conceptus size. Additional VitE increased conceptus length in H (NCM = 28.2 vs. NCH = 63.1 mm), but not in C cows (CM = 37.7 vs. CH = 8.4 mm). Providing evaporative cooling during the last 4 weeks of gestation improved lactation performance of dairy cows. Supplementation with VitE above NRC recommendations increased fat and protein content of milk, but did not influence yields of milk and milk components.

Effect of triclosan on placental gene expression in the late gestation sheep fetus

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In pregnant sheep, estrogens circulate in high concentrations and peak dramatically 1-2 days before parturition. Most of the estrogens that circulate during gestation are synthesized in the placenta, where there are high transcript levels of estrogen sulfotransferase (STF). This enzyme sulfo-conjugates estrogen, allowing the hormone to circulate in blood plasma at much higher concentrations than unconjugated estrogens. These sulfo-conjugated estrogens are poorly understood, but may act as a reservoir for rapid deconjugation by estrogen sulfatase (STS) in late gestation. Estrogens augment fetal corticotropin (ACTH) secretion in the pituitary, causing a downstream secretion of cortisol in the adrenal glands and further estrogen synthesis in the placenta. It is this interplay between the placenta and fetal brain that have led some to believe that estrogens play an important role in activating the fetal hypothalamic-pituitary-adrenal axis, a pivotal event in parturition initiation and fetal maturation. The estrogen conjugation enzymes, STS and STF, are therefore of interest as they may be a key player in regulating estrogen action at HPA tissues and the fetal-placental interface. Triclosan, an antibacterial biocide found in everyday household items like soap and toothpaste, has been shown to be an endocrine disrupter. Specifically, Triclosan has been shown to inhibit STF (gene name SULT1e1) and estradiol in the ovine placenta and can affect reproductive success in various organisms. In this study, we hypothesized that triclosan would disrupt normal pregnancy via its action on estrogen biosynthesis and metabolism and that the transcriptomic effect of triclosan would significantly overlap the transcriptomic effect of exogenous estrogen. We infused Triclosan, estradiol sulfate (E_2SO_4), and saline into 120 day sheep fetuses for three days and took terminal tissue samples. We then analyzed triclosan's effect on gene expression in the placenta via microarray analysis. We found 829 genes and 767 genes to be differentially expressed ($p \leq 0.05$) in the E2SO4 and triclosan infused animals, respectively. Interestingly, only 128 of these genes overlapped, which suggests that triclosan may have some non-estrogenic actions. Triclosan targets lipid synthesis, and many genes involved in steroid biosynthesis such as CYP1A1 and STAR were greatly down-regulated by triclosan in the ovine placenta. Such inhibition could prove detrimental to fetal development, and our next steps in the triclosan study are to assess other fetal tissues, including those involved in the HPA axis, for differential expression.

Animal genetic and physiological factors contribute to the prevalence of *E. coli* O157 in cattle

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The prevalence of *Escherichia coli* (*E. coli*) O157:H7 in cattle herds is positively correlated to outbreaks of this pathogen, causing severe diseases in humans. Controlling the prevalence of *E. coli* O157:H7 in cattle at the pre-harvest level is critical to reduce outbreaks of this pathogen. We hypothesized that animal factors contribute to the prevalence of *E. coli* O157:H7 in cattle. Rectal anal junction swab samples were collected from 91 cattle. The swab samples were resuspended in 2 ml of tryptic soy broth and serially diluted in 0.1% (w/v) peptone. The diluted samples were plated on CT-SMAC to isolate and determine the number of *E. coli* O157. Multiplex PCR targeting *stx1, stx2, hly,* and *rbfE* was conducted to confirm *E. coli* O157. For subtyping, pulsed-field gel electrophoresis (PFGE) was performed. Farm-to-farm transmission of *E. coli* O157 was confirmed by strain tracking through use of multiplex PCR and PFGE methods. The lowest number of *E. coli* O157 was observed in the Brahman breed among an Angus-Brahman multibreed herd, and bulls excreted more *E. coli* O157 than steers in the pens. We also found that the high shedding of *E. coli* O157 is related to the presence of a super-shedder, defined as cattle excreting $>10^5$ CFU/rectal anal swab. The data suggest that animal factors such as genetic difference and physiological characteristics (castration) influence the prevalence of *E. coli* O157 and super-shedders play a pivotal role in the high prevalence and transmission of *E. coli* O157 within/between farms. This study provides insights for the development of mitigation strategies to reduce *E. coli* O157 at the pre-harvest levels.

Characterization of MHC class I conserved locus and locus II mRNA in three *Equus africanus somaliensis* jennies

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MHC diversity has been implicated in species ability to adapt to new and emerging diseases. *Equus africanus somaliensis* are the most endangered equid and consequently have a reduced population which posses the risk of reducing MHC diversity. The objectives of this pilot study were to determine if MHC class I conserved locus and locus 2 mRNA from *Equus africanus somaliensis* can be sequenced using *Equus Caballus* primers and compare those sequences to *Equus caballus* and E*quus asinus*. Blood samples were taken from *Equus caballus* (*n*=3), *Equus asinus* (*n*=3) and *Equus africanus somaliensis* (*n*=3) and RNA was isolated using the LeukoLOCK™ Total RNA Isolation System (Life Technologies) followed by reverse transcription. The presence and approximate size of amplified products were determined by electrophoresis in an agarose gel (1.2% (w/v)) containing ethidium bromide (100ng/mL) and visualized on an u.v. light box. Amplicons were purified using Purelink™ PCR Micro Kit (Invitrogen), sequenced in both directions at the University of Florida DNA Core Facility and aligned using GENtle program (v. 1.9.4, University of Cologne, Germany) to the *Equus caballus* genome (BLAST). Nucleotide sequences from *Equus asinus* and *Equus africanus somaliensis* closely aligned to horse MHC class I genes. Both MHC class I nucleotide sequences from two related Somali wild ass closely matched (> 90%) horse haplotypes equine leukocyte antigen (ELA)-A9 and ELA-A5. Nucleotide sequences from the third Somali wild ass matched (> 90%) horse haplotypes ELA-A4 and ELA-A10. Donkey nucleotide sequences closely matched (> 90%) the following horse haplotypes; ELA-A1, ELA-A7, ELA-A9, ELA-A10, and ELA-11. One horse exactly matched a 60 nucleotide deletion in the MHC class I Locus 2 gene sequences of all three Somali wild ass. This data demonstrated that *Equus africanus somaliensis* MHC class I conserved locus and Locus 2 mRNA can be sequenced using *Equus caballus* primers and the sequences appear to be conserved between species. Further work is needed to determine whether other MHC class I gene loci follow the same pattern.

Effects of intrauterine infusion of *Trueperella pyogenes* on endometrial mRNA expression of genes associated with luteolysis in dairy cows

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Trueperella pyogenes (former *Arcanobacterium pyogenes*) is one of the most relevant pathogens involved in uterine diseases because of the high environmental prevalence, persistence in the uterus, severity of lesions on the endometrium, resistance to treatment, and synergistic action with gram-negative anaerobes. Intrauterine infection with *T. pyogenes* after ovulation led to regression of the newly formed CL in 50% of the cows (Kaneko and Kawakami, Theriogenology, 71:858). The objective of the current study was to determine the effects of intrauterine (**IU**) infusion of *Trueperella pyogenes* on endometrial mRNA expression of genes affecting the luteolytic cascade. Fifteen early postpartum healthy Holstein cows had the estrous cycle synchronized and on day 4 after ovulation were allocated randomly to receive one of three treatments**: TP** (n=5), IU infusion of 10 mL of saline solution containing 10^9 cfu/mL of *T. pyogenes*; **TNF** (n=5), IU infusion of 10 mL of saline solution containing 1 μg of tumor necrosis factor α (TNFα); and **Control** (n = 5), IU infusion of 10 mL of saline solution. Uterine biopsies were collected at 6, 12 and 24 h after treatment to evaluate the endometrial mRNA expression of TNF-α, Interleukin (**IL**) 1β, IL6, IL8, prostaglandin E synthase (**PGES**), prostaglandin F synthase (**PGFS**) and oxytocin receptor (**OXR**). RT-PCR was used to measure mRNA expression. Mitochondrial ribosomal protein S15 (**MRPS15**) was used as house keeping gene. The MIXED procedure of SAS was used for statistical analysis. Gene expression of IL1β and IL6, PGES and PGFS was not affected by treatment, time or treatment by time interaction. However, TP cows had higher ($P< 0.05$) mRNA expression of IL1β and IL8 than TNF cows at 24 h. TNFα mRNA expression was lower ($P<0.05$) for TP cows than TNF cows at 6 h. Overall OXR mRNA expression was higher ($P=0.03$) for TP cows than Control cows. In conclusion, IU infusion of *T. pyogenes* did not consistently increase endometrial mRNA expression of genes involved in the luteolytic cascade; however, the endometrial expression of OXR was increased for cows infused with *T. pyogenes*.

Effect of induced subclinical hypocalcemia on physiological parameters and immune function in dairy cows

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Objectives were to create a model to induce subclinical hypocalcemia [**SCH** blood ionized calcium (Ca^{2+}) <1.0 mM], and to study the effects on clinical parameters and function of immune cells in cows. Ten nonpregnant Holstein dry cows were blocked by lactation and assigned randomly to normocalcemia (**NC**, 0.9% NaCl i.v. plus 43 g of oral Ca at 0 and 12 h) or induced SCH [**SCHI**, 5% ethylene glycol tetraacetic acid (**EGTA)** at pH 7.4, i.v.] in a crossover design. The infusion lasted 24 h. The sequence of treatments was either NC-SCHI or SCHI-NC. A 6-d period between treatment administrations was used to minimize carryover effects. Heart and respiratory rates, rectal temperature, and rumen contractions were measured during and after infusion at 6 to 12-h intervals. Blood ionized Ca, K, Mg, HCO_3^- and pH were evaluated at 0 h, hourly during the infusion period, and at 24, 48 and 72 h after the infusion to monitor Ca^{2+}. In addition, DMI, leukocyte differential count and neutrophil function were evaluated at 0 h and at 24, 48 and 72 h after treatments. Data were analyzed using PROC GLIMMIX of SAS. Infusion of a 5% EGTA solution successfully induced SCH in SCHI cows (0.78 ± 0.01 vs. 1.27 ± 0.01 mM Ca^{2+}, and 1.74 ± 0.04 vs. 2.08 ± 0.04 mM total Ca) during the infusion period. There were no differences in heart and respiratory rates, rectal temperature, and leukocyte differential count between SCHI and NC cows. On the infusion day, SCHI cows had lower ($P < 0.01$) K (2.92 ± 0.07 vs. 3.47 ± 0.07 mM) and higher ($P < 0.01$) Mg (0.94 ± 0.03 vs. 0.68 ± 0.03 mM) in blood compared with NC cows. The decrease in blood Mg was likely caused by supplemental oral Ca in NC. Interactions ($P < 0.01$) between blood pH and treatment, and HCO_3^- and treatment were observed because cows with SCHI had lower blood pH and HCO_3^- during the first 12 h of the infusion period, followed by a compensatory increase in the last 12 h compared to NC cows. SCHI cows had reduced ($P < 0.01$) DMI on the day of infusion (5.1 vs. 10.0 kg/d) and decreased ($P = 0.01$) number of rumen contractions every 2 min (1.7 vs. 2.7) in the second half of the infusion period. In addition, cows with SCHI had a reduced ($P < 0.01$) percent of neutrophils with phagocytosis (79.9 ± 8.8 vs. 119.2 ± 13.0, % baseline) and oxidative burst (80.2 ± 17.9 vs. 140.3 ± 17.9, % baseline), evident at 24 h after the end of the infusion. These findings demonstrate that 5% EGTA successfully induced SCH in dairy cows and corroborate the detrimental effects of SCH on DMI, rumen contraction and neutrophil function observed in early lactation dairy cows.

Is cefotaxime resistance endemic in farm animals?

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Antibiotic resistant microorganisms (ARMs) are a growing concern in animal and public health due to the slow development of new antibiotics and lack of alternative therapies for bacterial diseases. Extended spectrum β-lactamase (ESBL) producing microorganisms are resistant to third-generation cephalosporins and present a new challenge to the food animal industry. Here we report the cefotaxime resistant microorganisms from animals at 9 different food animal production establishments. Samples were plated on MacConkey agar containing cefotaxime. 16S rRNA gene sequencing was conducted to identify the resistant microorganisms. Seventeen different species of microbes including animal, human and plant pathogens as well as soil bacteria were identified. The prevalence of cefotaxime resistant microorganisms in cattle showed farm to farm variation, ranging from 5.2% to 100%. Animals reared in loose housing systems show lower prevalence of ARMs compared to animals in intensive housing systems indicating animal-to-animal transmission plays a key role in ARM transmission. Thus, our findings provide the first occurrence of cefotaxime resistance in animals and shows that development of antibiotic resistance is a continuous natural process and cefotaxime resistant microorganisms might have originated from nature as well as the use of antibiotics.

Changes in expression of *CWC15* during preimplantation development in the bovine embryo

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Recently, a nonsense mutation in *CWC15* was identified as a likely causative mutation affecting fertility in Jersey cattle. Although the carrier frequency in the population is relatively high (23.4%), no homozygous animals have been identified, leading to the conclusion that this mutation is associated with embryonic or fetal loss. *CWC15* encodes for a protein associated with the spliceosome, but little is known about its role in splicing of pre-mRNAs. The purpose of this experiment was to determine whether *CWC15* is expressed in the preimplantation embryo. If so, it is possible that inheritance of the nonsense mutation could lead to early embryonic death. Embryos were produced *in vitro* from slaughterhouse oocytes and bulls from *Bos taurus* and *B. indicus* breeds. For each of the four replicates, pools of 30 matured oocytes or 30 embryos at the 2 cell [28-32 h post insemination (hpi)], 3-4 cell (48 hpi), 5-8 cell (57-60 hpi), 9-16 cell (72 hpi), morula (120 hpi) and blastocyst (168 hpi) stages were collected. The RNA was purified and subjected to real-time PCR analysis. The expression of *CWC15* was measured with the delta delta Ct method and *YWHAZ, GAPDH* and *SDHA* were used as housekeeping genes. Amounts of mRNA for *CWC15* were affected by stage of development (P<0.0001). Relative to the oocyte, expression remained constant through the 9-16 cell stage and then declined thereafter (Figure 1). Given that the embryonic genome becomes activated at the 8-16 cell stage, it is unlikely that *CWC15* is one of the genes that are upregulated in the period through development of the blastocyst. Further research with embryos at later stages of development will clarify when *CWC15* becomes crucial for embryonic survival. Support: AFRI Grant No. 2013-68004-20365 from USDA NIFA.

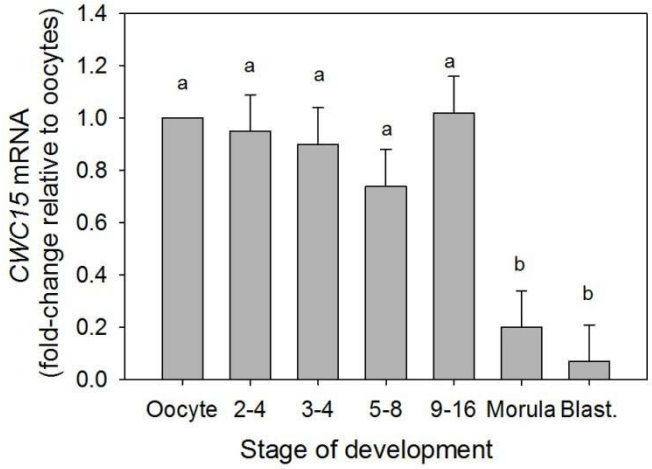

Figure 1. Changes in expression of CWC15 during preimplantation development. Data are least-squares means ± SEM. Bars with different superscripts differ (P<0.05).

Disruption of ovarian and testicular development in mice by the conditional deletion of the genes encoding the transcription factors GATA4 and GATA6

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The zinc finger transcription factors GATA4 and GATA6 are prominently expressed in the mammalian gonads, both during development and in the adult. The importance of GATA4 in testis differentiation has been reported previously, where the absence of *Gata4* expression results in animals with small testes, testis cord defects and sterility. Moreover, DMRT1, a protein required for postnatal testis differentiation, is not expressed in the somatic cells of *Gata4* mutant testis during embryogenesis. Similarly, the role of GATA4 in ovarian development and function has been recently elucidated. The loss of *Gata4* expression leads to a drastic reduction in the number of developing follicles shortly after birth with subsequent formation of hemorrhagic follicles, ovarian cysts, and sterility. In contrast to GATA4, the involvement of GATA6 in gonadogenesis is much less understood. It was hypothesized that a functional overlap exists between the two proteins. Here, we examined the roles of GATA4 and GATA6 proteins in sexual differentiation during embryogenesis and in adults by ablating simultaneously both genes in mice. Deletion of both genes resulted in striking phenotypes during gonadal development of both sexes. In the double mutant *Sf1Cre; Gata4*$^{floxed/floxed}$*; Gata6*$^{floxed/floxed}$ female, immunofluorescence (IF) analysis showed a marked decrease in the expression of the granulosa cell marker, FOXL2, as early as E15.5 in double mutant ovaries. Moreover, postnatal ovaries were considerably smaller, with very few FOXL2-positive cells when compared to controls, suggesting a complete and early block in follicular development. Meiosis appeared to initiate normally, but clusters of germ cells never progressed to the follicular stage and were lost by postnatal day (PND) 9. In the male *Sf1Cre; Gata4*$^{floxed/floxed}$*; Gata6*$^{floxed/floxed}$ double mutant, testes were notably smaller, with irregular seminiferous tubules as compared to controls. Neither DMRT1 nor GATA1 expression was detected in the Sertoli cells by IF. However, the expression of Anti-Müllerian Hormone (AMH) was persistent in the Sertoli cells of double mutant testes long beyond PND7 when it is down-regulated in the control animals. Moreover, ectopic expression of FOXL2 in the seminiferous tubules at PND30 suggested that male developmental program could not be maintained in the absence of GATA4 and GATA6 expression and somatic cells of the double mutant testis embark on the female pathway instead.

Low doses of recombinant bovine somatotropin (rbST) enhance fertility of dairy cows

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Objectives were to evaluate the effects of a single or two low doses of rbST on plasma hormone concentrations and fertility in dairy cows. Lactating Jersey and crossbred cows (n=1,493) from two farms were inseminated for first AI postpartum on estrus. On the day of AI (study d 0), cows were blocked by parity and assigned randomly to receive a single placebo injection (control) at AI, a single treatment injection with 325 mg of rbST (S-bST) at AI, or two treatment injections with 325 mg of rbST (T-bST) administered at AI and 14 d later. Blood was collected twice weekly and plasma analyzed for concentrations of growth hormone (GH), insulin-like growth factor 1 (IGF-1), insulin, progesterone, and pregnancy-specific protein B (PSPB). Pregnancy was diagnosed on d 31 and 66. Ultrasonographic morphometry of conceptuses were performed on d 34 and 48 from a subset of 151 cows. Data were analyzed using PROC GLIMMIX of SAS and fitting adequate data distribution. Treatment with bST increased plasma concentrations of GH (control: 3.5 vs. S-bST: 6.4 vs. T-bST: 7.6 ng/mL; $P < 0.01$) and IGF-1 (control: 64.1 vs. S-bST: 77.4 vs. T-bST: 98.5 ng/mL; $P < 0.01$) from d 3 to 31, and increments were extended in T-bST. Treatments did not affect concentrations of insulin, progesterone and PSPB. However, a distinction in plasma PSPB between pregnant and non-pregnant cows ($P < 0.01$) occurred earlier for bST-treated cows (on d 21) than for controls (on d 24). Pregnancy per AI, amniotic vesicle and embryo/fetus sizes were all increased by T-bST compared with control and S-bST (Table 1). In conclusion, administration of 325 mg of bST on d 0 and 14 relative to AI increased concentrations of GH and IGF-1, enhanced conceptus size, and improved fertility of dairy cows.

Table 1. Effect of bST treatments on fertility and conceptus morphometry

	Treatment			
Item	Control	S-bST	T-bST	*P*
Pregnant, %				
d 31	35.6^{b}	37.5^{ab}	43.1^{a}	0.08
d 66	29.9^{b}	29.2^{b}	38.0^{a}	0.01
Pregnancy loss, %	12.6^{ab}	19.1^{a}	8.6^{b}	0.04
Amniotic vesicle length, mm				<0.01
d 34	12.5 ± 0.6^{B}	12.5 ± 0.6^{B}	13.9 ± 0.6^{A}	
d 48	29.3 ± 0.8^{b}	28.5 ± 0.8^{b}	31.8 ± 0.8^{a}	
Embryo/fetus crown-rump length, mm				<0.01
d 34	10.6 ± 0.5^{B}	10.5 ± 0.6^{B}	12.0 ± 0.6^{A}	
d 48	23.6 ± 0.8^{b}	22.5 ± 0.8^{b}	26.1 ± 0.8^{a}	

Different superscripts within row: ^{a,b} $P < 0.05$, ^{A,B} $P < 0.10$

Effects of addition of forskolin, L-carnitine, *trans 10, cis 12* conjugated linoleic acid and phenozine ethosulfate during embryo culture on lipid content, abundance of genes involved in lipid metabolism and embryo survival following cryopreservation

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Widespread commercial application of in vitro embryo transfer in cattle production systems is limited by the increased susceptibility of in vitro produced (IVP) embryos to cryopreservation. Altered lipid metabolism is one reason for the reduced cryotolerance of IVP embryos, thus a potential strategy for improving the survival of IVP embryos following cryopreservation is to alter lipid metabolism during embryo culture. The objective of the present set of experiments is to determine the effects of addition of the metabolic regulators forskolin (FK), L-carnitine (LC), trans-10, cis-12 conjugated linoleic acid (CLA) and phenozine ethosulfate (PES) during embryo culture on lipid content, abundance of genes involved in lipid metabolism and survival following cryopreservation. Four experiments are planned. In experiment 1, embryos will be produced in vitro using abattoir-derived oocytes. Following fertilization, presumptive zygotes will be randomly assigned to culture treatments in a 2x2x2x2 factorial design. At day 7 after fertilization, grade 1 and 2 blastocyst and expanded blastocyst stage embryos will be harvested from culture and cryopreserved in 1.5 M ethylene glycol using a controlled rate freezing machine. Embryos will be thawed and cultured in vitro for 72 hours and re-expansion and hatching rates will be recorded at 24, 48 and 72 hours post-thaw. For experiments 2 and 3, only treatments from experiment 1 that have significant effects on cryosurvival will be tested. In experiment 2, cell number and allocation and lipid content will be evaluated in grade 1 and 2 blastocyst and expanded blastocyst stage embryos harvested at day 7 after fertilization using differential staining and the Nile Red assay, respectively. In experiment 3, the relative abundance of genes involved in lipid metabolism in grade 1 and 2 blastocyst and expanded blastocyst stage embryos will be determined using real-time quantitative RT-PCR. In experiment 4, the treatment from experiment 1 that improved cryosurvival most significantly will be tested in a 2x2 factorial design. Embryos will be produced in vitro and randomly cultured with or without the optimal combination of metabolic regulators. At day 7 after fertilization, grade 1 blastocyst and expanded blastocyst stage embryos will be harvested and randomly assigned to be transferred either fresh or following cryopreservation in 1.5 M ethylene glycol. Recipient animals will be synchronized for timed-embryo transfer. At day 7 after presumptive ovulation, the ovaries of all recipients will be palpated to confirm the presence of a corpus luteum. A single embryo will be transferred to the uterine horn ipsilateral to the ovary with a corpus luteum. Pregnancy will be diagnosed at days 30 and 60 of gestation. It is expected that embryo survival following cryopreservation, both in vitro and in vivo, will be increased by the addition of a combination of either FK, LC, CLA or PES during embryo culture and that this improvement in cryotolerance will be associated with reduced embryo lipid content and alterations in the abundance of genes regulating lipid synthesis.

Effects of PPARγ agonist rosiglitazone on cardiac tissue and cultured cardiomyocytes

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The transcription factor peroxisome proliferator-activated receptor γ (PPARγ) is activated by synthetic ligands known as Thiazolidinediones (TZD's), which induce expression of genes associated with insulin sensitivity, fat oxidation, mitochondrial biogenesis, and fat cell maturation [1]. This led to wide application of TZDs for the treatment of Type II diabetes. Recently, the TZD rosiglitazone has been associated with adverse effects such as cardiac hypertrophy, myocardial infarction and hepatotoxicity, and has been taken from the market in the US [2,3]. In the liver, ischemia-reperfusion was associated with PPARγ activation and increased autophagy, an effect that appeared age-dependent [4]. Low concentrations of rosiglitazone have been shown to reduce mitochondrial function and possibly activate the AMPK pathway [5]. Besides direct interaction with PPARγ, TZDs also exhibit receptor-independent actions such as activation of the anti-inflammatory pathways [5]. In the present study, we investigated the effect of rosiglitazone (Rosi) on mitochondrial function and content, as well as its possible role on the cellular housekeeping mechanism autophagy, in rat cardiac tissue and AC-16 cells, a human ventricular cardiomyocytes cell line. Our overall hypothesis was that Rosi impairs mitochondrial function and induces autophagy. To test our hypothesis, we measured markers of mitochondrial function (respiration and enzyme activities) and autophagy (expression of regulatory proteins), in rat cardiac tissue and AC-16 cells. We collected whole hearts from Fischer 344 rats of different ages (5, 12 and 18 months) that had been administered 10mg/kg/day of Rosi for 19 days prior to tissue collection. Enzymatic activity of mitochondrial cytochrome *c* oxidase and citrate synthase in heart tissue was determined using spectrophotometric enzyme activity assays. Cultured AC-16 cells were exposed to varying concentrations of Rosi (10 μM to 200 μM) for 0, 4, and 24 hours. Resting (routine) and activated mitochondrial respiration of Rosi-treated and control cells was analyzed using high resolution respirometry (HRR) with a titration protocol using activator (ADP), mitochondrial complex inhibitors (Rotenone, Antimycin A, Oligomycin), and uncoupler (Carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone , FCCP). To assess expression of proteins involved in mitochondrial biogenesis and autophagy, lysates of rat cardiac tissue and AC-16 cells, respectively, were subjected to Western blot analysis. Our results show that neither control rats nor rats administered 10mg/kg/day Rosi for 19 days exhibited an increase in heart weight with age. We furthermore found that this dose of Rosi did not affect cardiac mitochondrial content and activity, which both decreased with age, independent of the drug. Cultured AC-16 cells exposed to 200 μM Rosi *in vitro* displayed a reduction in mitochondrial routine and leak respiration as well as in electron transport chain capacity compared to control cells and cells exposed to 10 μM Rosi. This effect was independent of exposure time. Preliminary data analysis of expression of autophagy-regulatory proteins in cardiac tissue and AC-16 cells suggested no significant effect of Rosi on autophagy. Future experiments will assess the effect of Rosi on 1) mitochondrial fatty acid utilization and 2) on AC-16 cells that are oxidatively stressed (tBOOH) using HRR and cell viability assays.

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Effect of early or late resynchronization on reproductive performance of dairy cows observed for estrus

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The aim of this study was to evaluate reproductive performance of dairy cows subjected to early (ER) or late resynchronization (LR) after nonpregnancy diagnosis. Lactating Holstein cows (n=972) were subjected to the Ovsynch protocol (d0 GnRH, d7 PGF2α, d9 GnRH, d10 AI) for first artificial insemination (AI) at 68 d in milk (DIM). Weekly cohorts of cows were blocked by parity and assigned randomly to ER, based on nonpregnancy diagnosis using pregnancy associated glycoprotein (PAG) in blood, or LR based on palpation. ER cows received GnRH 2 d before PAG testing between 27 and 33 d after the previous AI, and not reinseminated nonpregnant cows continued on the Ovsynch for timed AI. LR cows had pregnancy diagnosed by transrectal palpation between 36 and 49 d after AI and those not reinseminated nonpregnant were resynchronized with the Ovsynch starting on the day of nonpregnancy diagnosis. After the first AI, all cows were observed for estrus based on removal of tail chalk and those in estrus were inseminated on the same day. The study lasted 70 d for ER and 112 d for LR to allow a maximum of 2 resynchronized timed AI for each treatment in cows not observed in estrus. A cow was considered pregnant at the end of the study based on palpation at 36 to 49 d after AI. Categorical and continuous data were analyzed with the GLIMMIX procedure of SAS fitting the proper distribution. Time to pregnancy was analyzed using the Cox's proportional hazard model with the PHREG procedure of SAS. The sensitivity (Se), specificity (Sp), positive (PPV) and negative (NPV) predictive values of using PAG for diagnosis of pregnancy were calculated at different intervals after AI. Pregnancy per AI (P/AI) at first AI did not differ between treatments and averaged 28.9%. Cows in ER tended (P=0.09) to become pregnant faster after the first AI than LR cows (adjusted hazard ratio =1.25; 95% CI=0.96-1.65), resulting in median days open of 133 and 141 for ER and LR, respectively. The proportion of cows not pregnant to first AI resynchronized with timed AI was greater (P<0.01) for ER than LR (29.9 vs. 8.5%). P/AI after the first AI tended (P=0.09) to be greater for cows reinseminated on estrus than resynchronized with timed AI for both ER (18.3 and 14.0%) and LR (15.7 and 10.4%). A total of 2,129 test diagnostics were evaluated. Se (true pregnant) and Sp (true nonpregnant) of PAG for pregnancy diagnosis of dairy cows, according to days after AI, were: ≤ 27 d: Se = 94.6%, Sp = 89.9%; 28-30 d = 96.1%, Sp = 90.7%; 31-35 d = 98.7%, Sp = 88.1%; > 35 d: Se = 94.4%, Sp=85.2%. Overall, Se was 95.1% (95% CI = 93.6-96.3), Sp was 89.0% (95% CI = 86.9-90.8), PPV was 90.1% (95% CI = 88.3-91.8), NPV was 94.5% (95% CI = 92.8-95.8), and accuracy was 92.1%. Using PAG for reproductive management would result in overall 4.9% iatrogenic abortion. Cows diagnosed as pregnant with PAG require additional re-evaluation as 11% are either not pregnant or lose their pregnancy in the following 2 to 3 weeks, which might delay re-insemination. In summary, early diagnosis of nonpregnancy based on PAG with ER increased submission to timed AI, but tended to reduce interval to pregnancy in cows observed for estrus. The benefits of early resynchronization with a negative PAG diagnosis need to offset the 4.9% iatrogenic abortion.

Effect of *in utero* heat stress on insulin response of calves after weaning

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Heat stress during the dry period not only negatively impacts subsequent lactation in the cow, but may also impact the calf postnatally. Previous studies suggest that calves born to cows heat-stressed during late gestation have lower birth weight but similar overall weight gain during the pre-pubertal period compared with normothermic conditions in utero. However, it is unclear if insulin sensitivity of peripheral tissues, and thus metabolism, of calves is altered in their postnatal life after in utero heat stress. The aim of present study was to examine the effects of maternal heat stress during the dry period on insulin response at peripheral tissues of calves after weaning. Calves (10/treatment) were born to cows exposed to heat stress (HT) or cooling (CL) when dry. Calves were immediately separated from their dams and fed 3.8 L of high quality colostrum within 1 h after birth and then 1.9 L 12 h later. All calves were fed 1.9 L to 3.8 L pasteurized milk in the morning and afternoon from 2 to 42 d of age and then only in the morning until weaning at 49 d. Calf starter and water were offered ad libitum starting at 2 d of age. All calves were managed in the same manner throughout the study. A glucose tolerance test (GTT) and an insulin challenge (IC) were performed on all calves at 55 d of age. Gestation length was not affected (HT: 277 ± 1.8 d; CL: 279 ± 1.8 d) by heat stress during the dry period, but HT calves were born lighter (40 ± 1.4 vs. 45 ± 1.4 kg, $P = 0.03$) compared with those cooled in utero. Both groups of calves had similar weaning weight (HT: 68 ± 3.2 kg; CL: 71 ± 3.3 kg) and body weight gain from birth to weaning (HT: 28 ± 2.2 kg; CL: 26 ± 2.3 kg). Relative to those cooled in utero, HT calves had similar insulin response to GTT and insulin clearance during IC but faster glucose clearance during GTT and stronger glucose response to IC. In conclusion, in addition to impaired fetal growth, maternal heat stress during the dry period enhances the insulin response at peripheral tissues of calves after weaning, which may suggest a possibility of accelerated lipogenesis and fat deposition in early life.

Multiple stressor interactions delay horseshoe crab embryo development

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Fertilized eggs of the American horseshoe crab, *Limulus polyphemus,* are buried in shallow nests above the high tide line, where they are exposed to variations in abiotic conditions during early development. We examined whether the rate of embryonic development is affected by exposure to environmentally-relevant combinations of three factors: temperature (T; 25°, 30° and 35° C), salinity (S; 5, 15 and 34 ppt), and dissolved O_2 (DO; 5%, 13% and 21% O_2). Newly fertilized eggs collected from nests of individual mating pairs were returned to the lab and incubated under fully-factorial stressor combinations for 14 d, then placed in "control" conditions (30° C, 34 ppt, 21% O_2) for an additional 14 d. Growth rate was measured every 2 d throughout the experiment. We assessed 8 embryos from each of 6 mating pairs at each of the 27 treatment combinations (1296 eggs). We found that although the effect of isolated stressors (high T, low S or low DO) on development was minimal, stressor combinations showed stronger effects with evidence of complex interactions. For example, whereas high T and low S in isolation each had no effect, they were lethal in combination, and although low T in isolation slightly decreased the rate of development, it reduced the negative effects of low S and/or low DO. Furthermore, low DO increased the effect of high T, but it did not affect the response to low S. Low DO also appeared to pause development, which then resumed upon return to control conditions, but only after a 9 d lag. These data demonstrate that complex, synergistic interactions among environmentally-relevant levels of abiotic stressors can substantially alter the development of a coastal invertebrate in ways that may not be predicted from the effects of the stressors in isolation.

Impact of combined infection and prenatal nicotine exposure on postnatal outcome

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Preterm birth is a major public health problem in obstetrics, occurring in 10% of all pregnancies in the US and accounting for up to 75% of perinatal mortality and over half of all neurologic morbidity. Although both smoking and intrauterine infection are among the two greatest risk factors for adverse pregnancy outcome, no study to date has addressed the interactions of these two key risk factors. The central hypothesis is that prenatal nicotine exposure lowers the threshold for microbial infection to establish maternal and fetal infection with a subsequent detrimental effect on perinatal growth and development. To test this hypothesis, we have developed a model of moderate nicotine exposure during pregnancy and *Mycoplasma pulmonis (Mpul)* infection in pregnant rats to examine the patterns of infection and inflammation in fetal and prenatal maternal tissues, as well as patterns of infection and inflammation in the neonatal rat. At gestation day (GD) 6, an osmotic minipump was inserted subcutaneously for continuous infusion of nicotine tartrate (6 mg/kg) or vehicle (saline). This nicotine (Nic) dose induces maternal plasma levels of 20-25 ng/ml, a concentration similar to that induced by moderate cigarette consumption. Based on previous dose response studies, rats were infected intravenously with 10^5 CFU *Mpul* or sterile broth at GD 14. Treatment groups were sham controls, Nic only, *Mpul* only, and Nic + *Mpul.* The 10^5 CFU *Mpul* dose caused consistent colonization of the placenta but was lower than the threshold required for 100% colonization. At GD 18, dams were necropsied, and maternal tissues and fetal units (placenta, amniotic fluid, fetus) were collected for culture and histology. Dams that received Nic had significantly more *Mpul* isolated from the fetal units [placenta (0.0383), amniotic fluid ($P<0.0001$), and fetus (0.0051)] than did dams that did not receive Nic. There was no difference in the colonization of the dam tissues. The gross placental lesions were more severe in rats that received both Nic and *Mpul.* Suppurative exudate in the uterus was observed only in rats that received both Nic and *Mpul.* We observed a clear effect of Nic on infection, both lowering the threshold for infection and increasing severity of clinical disease in both fetal and prenatal maternal tissues.

Paralogous non-LEE effectors of enterohemorrhagic *Escherichia coli* target disparate loci in host cells and cause cell damage

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Proficient delivery of multiple “effectors” via a type III secretion system (T3SS) into host cells is a widespread infection mechanism in many Gram-negative bacterial pathogens. These events are critical steps to trigger signal transduction pathways leading to a multitude of host cell responses, ultimately causing disease in humans. A recent study of genome-wide bioinformatics and proteomic analysis in enterohemorrhagic *Escherichia coli* (EHEC) reveals that it possesses a large number of non-LEE (the locus of enterocyte effacement)-encoded effectors of which a set of effectors are paralogous proteins. However, only few cases have been elucidated their biochemical roles, and the functional specificity of paralogous non-LEE effectors are poorly understood during EHEC infection. We determined that many effectors exhibited cytotoxicity when expressed in host cells ectopically. In contrast, cytotoxicity was decreased when an effector gene was deleted in the chromosome, indicating that each non-LEE effector might have a distinct role during infection. A set of paralogous effectors fused to green fluorescence protein (GFP) localizes in different host cell compartments, suggesting that subtle differences in amino acid sequences of paralogous effectors determine the destination in host cells. Thus, our findings provide an example whereby paralogous non-LEE effectors have evolved in a diverse manner to manipulate host signaling pathways by targeting different cell compartments and reflect the functional specificity toward their host targets during infection.

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