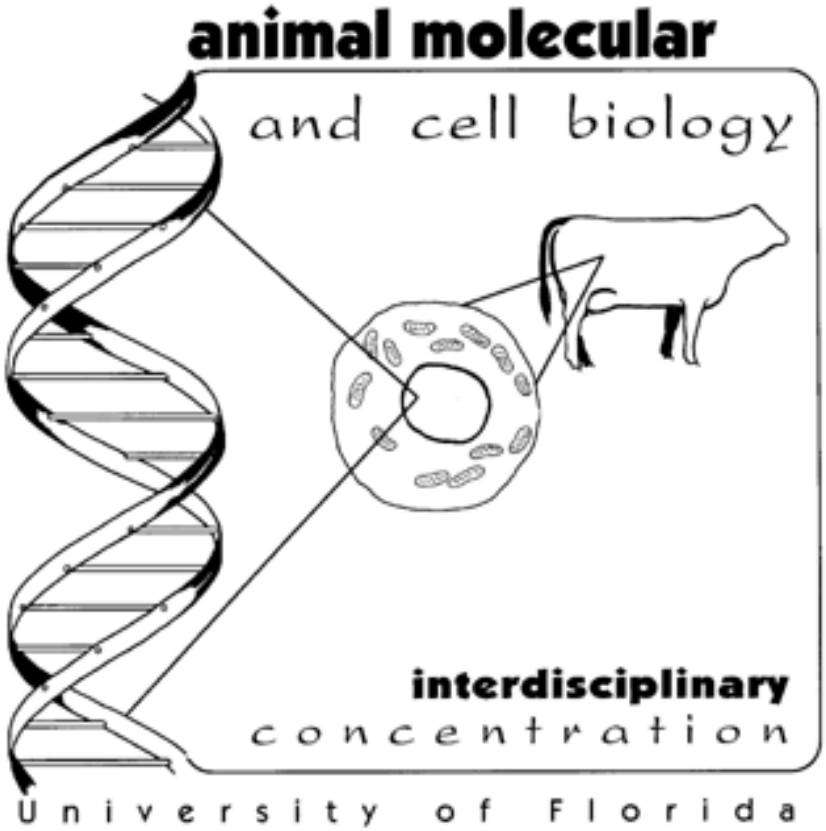
University of Florida

Animal Molecular and Cellular Biology

First Annual Research Symposium



Whitney Laboratory, FL

May 2nd and 3rd, 2003

Animal Molecular and Cellular Biology
First Annual Research Symposium

WELCOME

It is our great pleasure to welcome you to the First Annual Research Symposium for the Animal Molecular and Cellular Biology Interdisciplinary Graduate Program, University of Florida, held at the Whitney Laboratory. We've planned this two-day event away from the University to give everyone a break from their hectic schedules, to build camaraderie and to share science. The program is a mixture of student presentations of proposed, ongoing and completed research, as well as invited presentations from Dr. Judith Ochrietor, a scientist at the Whitney Labs, and our Distinguished AMCB Lecturer, Dr. Randall Prather, from the University of Missouri. Additionally, we have planned Friday evening social events and a barbecue to allow participants time to relax and enjoy the area. We hope you find this symposium enlightening and enjoyable and we look forward to this becoming an annual tradition for the AMCB.

Pete Hansen, AMCB Director
Karen Moore, AMCB Co-director

ACKNOWLEDGEMENTS

The AMCB faculty and students are grateful to the following sponsors for their support of the First Annual Research Symposium:

Dr. Bill Buzzi, Whitney Laboratory; http://www.whitney.ufl.edu

Dr. Richard Jones, Dean of Research, IFAS

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

Dr. Jimmy Cheek, Dean of Teaching, College of Agricultural and Life Sciences

Dr. F. Glen Hembry, Chair, Department of Animal Sciences

Dr. Mike Fields, Interdisciplinary Reproductive Biology Group

The Whitney Laboratory

The Whitney Laboratory is located in the town of Marineland, on the border between St. Johns and Flagler counties in northeast Florida. The five-acre campus is situated on a narrow barrier island, bounded by the Atlantic Ocean and the Intracoastal Waterway, both of which are just a few hundred feet away.

The area provides a great variety of habitats: acres of salt marsh and mangroves with countless tidal creeks and other estuarine areas; a shoreline of sand beach and shallow surf including a unique and extensive outcropping of coquina rock. The nearby Matanzas Inlet connects the intracoastal estuarine basin with the benthic and pelagic habitats of the Atlantic. This habitat diversity ensures that many species of marine organisms, both invertebrates and fishes, will be available locally throughout the year. Several additional habitats, though distant, are accessible: the great grassflats of the Mosquito Lagoon near Cape Canaveral, and the rich animal resources of the Gulf coast and the subtropical Florida Keys.

The origins of the Whitney Laboratory stem from the 1930's, when Cornelius Vanderbilt Whitney's long-standing interest in the natural history of marine animals provided the basis for his founding of Marineland, the world's first oceanarium. This enterprise, launched in 1938, included a small research laboratory that immediately attracted the attention of many academic biologists.

When the University of Florida College of Medicine opened in Gainesville in 1956, researchers from that institution came to the modest facility at Marineland to study physiological adaptations of marine animals - both fish and invertebrates.

In the early 1970's, prompted by the possibility that experimental studies of marine animals could bring about medical advances, C.V. Whitney donated to the University of Florida over three acres of land adjacent to Marineland, as the site for a new marine biological research facility. Whitney provided about half of the construction costs. The Laboratory opened its doors on January 30, 1974. Two years later a second building, Whitney Hall, containing dormitory rooms, apartments, and a conference center, was constructed with funds provided by Cornelius and Marylou Whitney.

SYMPOSIUM AGENDA

Friday, May 2

9:00 AM – AMCB Seminar. Speaker: Dr. Randall Prather, *Transgenic Pigs for Medicine and Agriculture*
10:00 AM - Departmental van leaves Animal Sciences for the Symposium
1:00 PM - Welcome and Introductory Comments
1:30 PM – Invited Speaker, Dr. Judith Ochrietor, The Whitney Laboratory
Expression of 5A11/Basigin family members in the developing mouse retina
3:00 PM - Break
3:30 PM - Student Presentations Session 1

3:30-3:45	Dean Jousan
3:45-4:00	Lynda Miller
4:00-4:15	Leslie MacLaren
4:15-4:30	Nicole Nichols
4:30-4:45	Rocio Rivera
4:45-5:00	Joseph Kramer

5:00 PM - Volleyball
6:00 PM - Social Hour
7:00 PM - Barbecue on the Beach (Hosted by AMCB Faculty)

Saturday, May 3

7:00AM – Continental Breakfast (Hosted by AMCB Faculty)
8:00 AM - Dr. Randall Prather, Distinguished AMCB Lecturer
Swine and Bovine Genomics Research with a Focus on Female Reproduction
9:30 AM - Break
10:00 AM – Student Presentations Session 2

10:00-10:15	Sarah Balaguer
10:15-10:30	Todd Bilby
10:30-10:45	Maria Padua
10:45-11:00	Aydin Guzeloglu
11:00-11:15	Andria Desvousges
11:15-11:30	Stacey Goicoa

11:30 AM - Wrap Up
12:00 PM – Adjourn

INVITED SPEAKERS

2003 Distinguished AMCB Lecturer, Dr. Randall Prather, University of Missouri; *Swine and Bovine Genomics Research with a Focus on Female Reproduction*

Dr. Prather obtained his Ph.D. in Endocrinology-Reproductive Physiology at the University of Wisconsin-Madison under the direction of Drs. N.L. First and G.P. Schatten. He is currently a Distinguished Professor of Reproductive Biotechnology at the University of Missouri-Columbia, Department of Animal Sciences. Dr. Prather's research has focused on the early mammalian embryo for the past 21 years. He is best known for his work on cattle and pig cloning by nuclear transfer and is considered an expert in the areas of oocyte activation and nuclear remodeling. Recently his group has created miniature pigs that have the alpha 1,3 galactosyltransferase gene knocked out. Descendants of these pigs may prove very useful for xenotransplantation (the transfer of pig organs into humans). In addition, he and his collaborators are conducting two EST projects with the final goal of transcript profiling of reproductive tissues in pigs and cattle. Other projects in his laboratory continue to describe the cellular and molecular program of events that occurs during development of the preimplantation pig embryo.

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AMCB Invited Speaker, Dr. Judith Ochrietor, The Whitney Laboratory; *Expression of 5A11/Basigin family members in the developing mouse retina*

Dr. Ochrietor obtained her Ph.D. from Ohio State University in 1998 in Biochemistry, studying the expression of Mouse Pentraxin Genes during inflammation. She has been employed at The Whitney Laboratory, University of Florida since 1999 and is currently a senior research scientist in the laboratory of Dr. Paul J. Linser, where they are investigating the role of 5A11/Basigin family members in the development and degeneration of the vertebrate retina.

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ABSTRACTS

INDUCTION OF APOPTOSIS IN 2-CELL AND DAY 5 BOVINE EMBRYOS USING STAUROSPORINE AND HEAT SHOCK

F.D. Jousan* and P.J. Hansen
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Heat-induced apoptosis in bovine embryos is not observed until the 8-16 cell stage. The present objective was to determine if 2-cell embryos have the inherent machinery for apoptosis by testing whether the protein kinase inhibitor staurosporine (ST) induces apoptosis. In vitro-produced embryos cultured in modified KSOM supplemented with IGF-1 (100 ng/ml) were randomly assigned at the 2-cell stage or at d 5 to: 1) 10 µM ST for 24 h at 38.5°C; 2) heat shock (41°C) for 9 h followed by 38.5°C for 15 h (HS); or 3) 38.5°C for 24 h (CON). Embryos were analyzed by TUNEL procedure to determine total apoptotic nuclei. CON embryos at the 2-cell stage had more total cells than ST embryos (8.1 ± 2.1 vs. 2.2 ± 1.9, respectively; $P<0.04$) but were not different from HS embryos (5.7 ± 1.7). At d 5, total cell number was lower for ST (28.5 ± 2.2 cells) than for HS and CON (42.8 ± 2.1 and 54.7 ± 2.4 cells, respectively; CON vs. HS and ST $P<0.001$; HS vs. ST; $P<0.001$). ST embryos had a higher percentage of apoptotic cells ($P<0.001$) than HS and CON embryos at the 2-cell stage (75.1 ± 3.1 vs. 9.7 ± 2.9 and 12.0 ± 3.5, respectively) and at d 5 (51.3 ± 3.7 vs. 9.2 ± 3.6 and 6.2 ± 3.9, respectively). Observations suggest that the machinery for apoptosis is present in the 2-cell embryo. Failure of heat shock to induce apoptosis at d 5 may reflect anti-apoptotic effects of IGF-1.

IMMUNOCYTOCHEMICAL DETECTION AND LOCALIZATION OF SPERM PROTEIN 22 (SP22) IN FRESH AND CRYOPRESERVED EQUINE SEMEN

L.M.J. Miller*[1,] M.H.T. Troedsson[1], L. Duoos[2], G.R. Klinefelter[4] and K.P. Roberts[3]

Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida[1], Gainesville, FL, Clinical and Population Sciences, College of Veterinary Medicine[2], and Department of Urologic Surgery, School of Medicine[3], University of Minnesota, Minneapolis, MN, Reproductive Toxicology Division, US EPA[4], Research Triangle Park, NC

Localization of SP22 (sperm protein 22KDa) on spermatozoa is correlated with fertility in rats, but has not been investigated in stallions. The objective of this report was to identify SP22 on equine spermatozoa, and to determine its localization in fresh and cryopreserved semen. Eighteen semen samples from 3 fertile stallions were divided into two treatment groups: (a) fresh semen, and (b) cryopreserved semen according to a standardized protocol. Spermatozoa were immunocytochemically stained using a primary SP22-antibody and FITC-conjugated secondary antibody. SP22 localization was determined using fluorescence microscopy. Localization patterns within each group were analyzed using Kruskal-Wallis one-way ANOVA, and different patterns between the treatments were analyzed using a Wilcoxon signed rank test. SP22 was detected on both fresh and frozen spermatozoa. Four significantly ($p<0.01$) different localization patterns of SP22 were observed in fresh semen: (1) overlying the acrosome and equatorial region (**AER**; 69% ±3), (2) overlying the acrosome (**A**; 13% ±2), (3) equatorial region (**ER**; 13% ±3), and (4) neck (**N**; 5% ±1). There was a significant difference in the localization pattern of SP22 between fresh and frozen/thawed semen ($p<0.01$). The most common localization pattern in frozen/thawed samples was **ER** (47% ±3), followed by a fifth pattern, scattered over the head, (**S**; 18% ±4), **A** (16% ±6), **AER** (15% ±3), and the least common was **N** (5% ±1; $p<0.05$). The localization of SP22 on sperm is altered in frozen/thawed samples suggesting that the sperm plasma membrane has been altered or that SP22 has been redistributed during the freeze/thaw process.

THE FATTY ACID RECEPTORS: PEROXISOME PROLIFERATOR-ACTIVATED RECEPTORS (PPARs) IN BOVINE ENDOMETRIUM AND CULTURED ENDOMETRIAL (BEND) CELLS

L.A. MacLaren*[1], A. Guzeloglu[2], T.R Bilby[2], F. Michel[2] and W. W Thatcher[2]

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The peroxisome proliferator-activated receptors (PPARs) are a family of orphan nuclear receptors activated by specific fatty acids, eicosanoids and peroxisome proliferators. Previous studies have shown that they are involved in the regulation of genes affecting steroid and prostaglandin synthesis. The objectives of the current study were to determine which, if any, PPAR isoforms were present in bovine endometrium and whether expression of endometrial PPARs changed in response to supplements known to improve reproductive performance in lactating dairy cows. Northern blot analyses indicated that PPARα and PPARδ, but not PPARγ, mRNAs were expressed in endometrium from cyclic and pregnant Holstein cows at day 17 following estrus. Similarly, the PPARα and PPARδ isoforms were expressed in a cultured bovine endometrial (BEND) cell line. Treatment of BEND cells with phorbol 12,13 dibutyrate (PDBu) increased steady state levels of PPARδ mRNA ($P<0.05$), an effect that was augmented by addition of 100 μm eicosapentanoic acid ($P<0.05$). Omega-3 fatty acid treatment suppressed both prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and E_2 (PGE_2) production ($P<0.05$). Addition of 50 ng/ml interferon- τ to PDBu-stimulated BEND cells increased PPARδ mRNA levels ($P>0.05$) and suppressed production of both $PGF_{2?}$ and PGE_2 ($P<0.05$). Expression of PPARα mRNA was not affected by PDBu or omega-3 fatty acid supplementation ($P>0.05$), but was increased by growth hormone ($P<0.05$). The results support a role for PPARs in the regulation of endometrial prostaglandin production by omega-3 fatty acids and growth hormone. Preliminary experiments to determine the effect of PPAR activation using synthetic analogues will be presented.

EXPRESSION AND LOCALIZATION OF LUTEAL OXYTOCIN AND RELAXIN-LIKE FACTOR mRNA AND PROTEIN DURING THE PERI-IMPLANTATION PERIOD OF BOVINE PREGNANCY

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The peri-implantation period of pregnancy has been identified as a time of high embryonic loss for the cattle industry. The objective of this study was to examine the expression of two genes, relaxin-like factor (RLF) and oxytocin (OT), and localize their corresponding proteins in an effort to better characterize the peri-implantation period of bovine pregnancy. Northern blot analysis was performed using corpora lutea of days 7, 14, 18, 22, 30, 40, and 50 of pregnancy as well as day 0 (estrus) and 18 of the estrous cycle. Expression of RLF was highest on day 14, though overall expression levels did not differ significantly between each day studied. Oxytocin expression was significantly higher on days 7 and 14 of pregnancy as compared to days 18, 22, 30, 40, and 50 ($p < 0.01$). Likewise, corpora lutea obtained on days 18 and 22 of pregnancy expressed significantly more OT than those representing days 30, 40, and 50 ($p < 0.05$). In terms of the estrous cycle, no difference in RLF expression was detected in corpora lutea of days 0 and 18 while OT expression was significantly higher on day 18 versus day 0 ($p < 0.01$). Immunohistochemistry was used to localize the OT protein in the large steroidogenic cells of the corpus luteum. Despite repeated efforts, we were unable to localize the RLF protein. In conclusion, the high luteal expression of RLF and OT during early pregnancy may suggest a role for luteal hormones during implantation.

EFFECTS OF A PHYSIOLOGICALLY-RELEVANT HEAT SHOCK ON THE CYTOSKELETON OF 2-CELL BOVINE EMBRYOS

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Development of cultured preimplantation embryos is inhibited by exposure to temperatures similar to those experienced by heat-stressed cows. Exposure to a physiologically-relevant heat shock also causes ultrastructural changes including swelling of mitochondria and redistribution of organelles away from the plasma membrane. The latter is suggestive of cytoskeletal rearrangement due to the association of cytoskeletal proteins and filaments with organelles. In the present study, inhibitors of microfilament (latrunculin B) and microtubule (rhizoxin) polymerization were used to determine if movement of organelles caused by heat shock was a result of cytoskeletal rearrangement. Approximately 28 h post-insemination, 2-cell bovine embryos were harvested and cultured for 6 h at either 38.5° (homoeothermic temperature of a cow), 41.0° (characteristic body temperature of a heat-stressed cow), or 43.0°C (severe heat shock), in the presence of either 1 µM latrunculin B, 10 nM rhizoxin or vehicle (0.1% ethanol). Embryos were fixed immediately after heat shock and subsequently dehydrated, embedded, sectioned, and examined for organelle distribution at the light microscopy level. Based on preliminary data, it was found that heat shock caused redistribution of organelles away from the plasma membrane. Rearrangement was blocked by rhizoxin but was not affected by treatment with latrunculin B. It is suggested that the movement of organelles from the periphery of the cell is the result of rearrangement of microtubules and not of microfilaments. Future studies will be conducted to verify effectiveness of inhibitors in causing depolymerization and to characterize effects of heat shock on distribution patterns of microtubules in the cell.

DEVELOPMENT, PREGNANCY, AND SURVIVAL TO TERM OF IN VITRO PRODUCED BOVINE EMBRYOS CULTURED IN MEDIUM SUPPLEMENTED WITH A SERUM REPLACER

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INTERACTIONS OF BOVINE SOMATOTROPIN AND INTERFERON-TAU IN THE CONTROL OF PROSTAGLANDIN PRODUCTION IN CATTLE

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Our laboratory has shown previously that bovine somatotropin (bST) attenuates phorbol ester-induced $PGF_{2\alpha}$ production in bovine endometrial (BEND) cells. The first objective of this study was to examine the effect of supplemental bST on endometrial expression of genes encoding estrogen receptor-alpha (ERα), oxytocin receptor (OTR) and cyclooxygenase-2 (COX-2) in cyclic lactating dairy cows. About 12 h before expected ovulation, cows were assigned randomly to receive bST (500 mg; n=11) or serve as untreated controls (n=10). Cows that ovulated were divided within treatment to be sacrificed on day (d) 3 or 7 postovulation. In spite of small tendencies, short-term bST treatment had no detectable effects on endometrial ERα, OTR and COX-2 mRNA concentrations. However, endometrial COX-2 protein content was lower in bST-treated than control cows on d 7 of the estrous cycle. The second objective was to examine the molecular mechanisms by which bST may interact with IFN-τ to control phorbol ester-induced $PGF_{2?}$ production in BEND cells. Serum-deprived BEND cells were incubated with or without bST or recombinant bovine IFN-τ (rbIFN-τ) for 3 h and then treated with phorbol 12,13-dibutyrate (PDBu) for an additional 6 h. PDBu induced COX-2 and PGE synthase mRNA transcripts within 6 h. The bST had no detectable effects on COX-2 or PGE synthase mRNA response to PDBu. In the absence of PDBu, cultured BEND cells expressed a single OTR mRNA transcript (4.8 kb), which was unaffected by bST and IFN-τ treatments. Results indicate complex mediation of uterine prostaglandin biosynthesis by bST and IFN-τ in cattle.

EFFECTS OF BOVINE SOMATOTROPIN (bST) ON THE INSULIN-LIKE GROWTH FACTOR SYSTEM IN NONLACTATING CYCLIC AND PREGNANT DAIRY COWS ON DAY 17 AFTER OVULATION

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Nonlactating cows were used to examine the effects of exogenous bST on uterine protein secretions and gene transcripts encoding components of the IGF system. Following a Presynch/Ovsynch protocol, cows received either bST or no bST on d0 (i.e., at time of +/- AI) and d11. A total of 78 cows (23 cyclic and 55 TI) were slaughtered on d17, and uteri of all cows were flushed to verify presence of a conceptus. Conceptuses were recovered from 9/33 bST (27.2%) and 14/22 (63.6%) control cows ($P<0.01$). Blood samples were collected from d0 until d16. Endometrial tissues were collected from 14 cyclic and 16 pregnant (PR) cows for Northern blot analyses. BST treatment stimulated ($P<0.01$) plasma concentrations of IGF-1 ($618 \pm 37 > 369 \pm 37$ ng/ml). Ligand blots revealed IGFBP-3, 4, 5 and molecular weight protein 28-29 in uterine flushings from 19 cyclic and 18 PR cows. IGF-binding protein-3 (IGFBP-3) was higher in bST treated pregnant cows ($P< 0.05$) compared to pregnant control cows. IGFBP-4, 5 and molecular weight 28-29 proteins were higher in cyclic versus pregnant cows ($P< 0.001$) regardless of bST treatment. The mRNAs encoding IGF-I, IGF-II and IGFBP-3 were increased in bST treated cyclic cows ($P< 0.01$). An increase in IGFBP-2 mRNA was detected in bST treated cows compared to control cows ($P< 0.01$). The growth hormone receptor (GHr-1A) mRNA was undetectable in all cows. In conclusion, differential uterine responses of the IGF family were detected due to bST and pregnancy status in nonlactating Holstein cows.

EFFECT OF REMOVAL OF UTERINE GLANDS ON THE IMMUNOSUPPRESSIVE EFFECT OF PROGESTERONE ON UTERINE FUNCTION IN EWES

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Progesterone plays an important role in inhibition of uterine immune responses during pregnancy. It has been hypothesized that progesterone acts through induction of ovine uterine serpin (OvUS), a 55 – 57 kDa protein secreted by endometrial glands that can inhibit immune responses. Development of endometrial glands is a postnatal event; ewes without uterine glands (i.e., the uterine gland knockout ewe; UGKO) can be produced by postnatal exposure to norgestomet. The present experiment was conducted to determine whether eliminating uterine glands blocks the immunomodulatory effects of progesterone on skin allograft survival in the uteri. Ovariectomized ewes were treated for 30 days with either corn oil vehicle (n=4 for control and n=4 for UGKO) or 100 mg/day progesterone (n=8 for control and n=8 for UGKO). An allograft and an autograft were then placed in each uterus; treatments were continued for an additional 30 days before grafts were examined for survival. All autografts survived and had a healthy appearance after histological analysis. Allografts were rejected in 4/4 control ewes and 2/4 UGKO ewes treated with vehicle. Allografts were present in the uterus for all progesterone-treated control and UGKO ewes. While allografts persisted, histological analysis indicated that tissues were necrotic. Results indicate that presence of uterine glands is not a requirement for graft-promoting effects of progesterone. Thus, progesterone may inhibit uterine immune responses through pathways that do not involve uterine glands. The fact that some allografts survived in UGKO ewes treated with vehicle suggests that uterine immune function may be compromised in UGKO ewes.

INTERFERON-TAU (IFN-t) DECREASES STABILITY OF PROSTAGLANDIN H SYNTHASE-2 (PGHS-2) mRNA IN BOVINE ENDOMETRIAL (BEND) CELLS THROUGH A TRANSCRIPTION DEPENDENT MECHANISM

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Objectives were to: 1) demonstrate if PGHS-2 mRNA stability is regulated by p38 MAPK, and 2) determine if IFN- τ regulates stability of PGHS-2 mRNA. Experiment 1: Following a 3h PdBu stimulation of PGHS-2 mRNA expression, Actinomycin D (Act D; 1 μg/ml) was added (+/-) for an additional 1.5h. PGHS-2 mRNA concentration did not differ between cells treated with or without Act D. Simultaneous addition of a p38 MAPK inhibitor, SB203580 (1 μM) with Act D to cells at 3 h caused a decline in PGHS-2 mRNA at 4.5h ($P < 0.01$). Following 3.5 h of PdBu stimulation, addition of IFN- τ (50 ng/ml) for 1h reduced PGHS-2 mRNA at 4.5h ($P < 0.01$). However, if Act D was present then IFN–τ failed to reduce PGHS-2 mRNA concentration. Experiment 2: After a 3h PdBu stimulation of cells, SB203580 (+/-) was added followed by addition of IFN- τ (+/-, 5 ng/ml) at 3.5h. At 4.5h, PGHS-2 mRNA was decreased in an additive manner in response to SB20380 and IFN-τ ($P < 0.01$). Experiment 3: Treatment of cells with PdBu, IFN- τ and PdBu +IFN- τ for 10 min induced phosphorylation of p38 MAPK compared to control ($P < 0.01$). Activation of p38 MAPK is required to increase stability of PGHS-2 mRNA induced by PdBu. In contrast, IFN- τ negatively regulates stability of PGHS-2 mRNA through a mechanism that requires gene transcription.

THE RELATIONSHIP BETWEEN STEROIDOGENESIS AND TISSUE REMODELING ENZYMES IN OVULATION OF CYCLING PONY MARES

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Ovulation involves tissue remodeling events involving Matrix Metalloproteinases (MMPs) and their inhibitors (TIMPs). We evaluated follicular steroidogenesis and tissue-remodeling enzymes near ovulation in mares. Exp. 1, 10 mares were assigned to groups (Saline 2.5cc IV; G1, n=5) and (hCG 2500IU IV; n=5), at a 30 mm follicle; 24 hours later the follicle was aspirated. MMP's were analyzed by gelatin zymography; steroids by RIA (E2/P4). Exp. 2, 30 mares were assigned to groups, at a 30 mm follicle, and follicles were aspirated at 4, 9 or 24 hrs post injection. Groups 1-3, (saline 2.5cc IV n=5 per group) and Groups 4-6 (hCG 2500IU, IV n=5 per group). Exp. 3, mares were assigned to groups (G1 saline 100µl, n=4), or MMP-2/9 inhibitor (G2 100µl, n=4) intrafollicularly, at a 30mm follicle; 48 hours later the dominant follicle was aspirated. Experiment 4, 7 mares were assigned to groups (G1 sesame oil IM; n=4) or (G2 500µg RU486 IM; n=3), at a 30mm follicle. Mares were treated 2X- 48 hours apart; aspirated 48 hours later. In Exp.1 both P4 and MMP-2 levels ($p<0.0001$) were elevated in G2. Exp. 2, P4 was elevated in G3-6 ($p<0.0001$) and time trend analysis of MMP-2 indicated that treatment responses were nonhomogeneous. Exp. 3, P4 ($p<0.005$). and MMP-2 levels ($p<0.0008$) were reduced in G2. Experiment 4, P4 ($p<0.0007$) and MMP-2 ($p<0.0006$) levels were reduced in G2. We found an intrafollicular response by steroidogenic and tissue remodeling pathways to gonadotropin and inhibitor administration, indicating P4 and MMP-2 intimate involvement.

NUCLEAR REPROGRAMMING IN CLONED BOVINE EMBRYOS

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Animal Molecular & Cell Biology

The goal of the AMCB Interdisciplinary Graduate Program is to educate graduate students towards an understanding of the principles of molecular and cell biology and the application of this knowledge to contemporary problems of animal production. This is achieved by providing extensive training in molecular and cell biology in topic areas under each faculty member's area of research focus. Graduates are granted M.S. or Ph.D. degrees in their respective home department with specialization in Animal Molecular and Cell Biology. The major professor, who serves as Chair of the student's Supervisory Committee, has responsibility for directing the student's research and, along with the Supervisory Committee, provides advice for a required plan of formal coursework. Fellowships and assistantships are available. For additional information go to http://www.animal.ufl.edu/amcb.

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